JASN Abstract Supplement
ASN Kidney Week 2011
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Abstract Publication
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TH-OR001

The Kidney Defends the Urinary System from Infection by Secreting NGAL

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Background: NGAL is a critical component of innate immunity because it binds catecholate-siderophores which microorganisms require to capture iron. Urinary(u) NGAL is expressed at mg/L levels after either septic or aseptic diseases of the kidney and it has two potential functions, epithelial growth and/or bacteriostasis. Here we examined the activity of uNGAL in the growth of CFT073, a uropathogenic E. coli (UPEC).

Methods: To examine the role of uNGAL in bacteriostasis. (1) We designed a conditional allele of NGAL. (2) We created a bioluminescent mouse that releases Luciferase and mCherry when the NGAL locus is activated.

Results: We found NGAL significantly inhibited UPEC growth in vitro, which could be rescued by the addition of iron. In a mouse pyelonephritic model, the intensity and timing of urinary CFUs was mirrored by uNGAL, including a decrease in uNGAL coincident with the resolution of infection. To determine the source of uNGAL, we made a NGAL reporter mouse NGAL-Luc2/mC to visualize kidney expression in vivo and we found that UPEC detritus introduced into the bladder induced NGAL-Luc2/mC expression distantly in the kidneys. Reporter expression was consistent with the bladder and kidney Ngal expression according to QPCR. By high power in situ hybridization we located Ngal to alpha intercalated cells. To determine the physiological role of uNGAL, we made a global NGAL KO and found that UPEC infections had delayed resolution. We verified by knocking out Ngal in the intercalated cells by HoxB7-cre of the CD. To determine whether UPEC signaling directly activates kidney epithelia, we developed an in vitro assay using the CDs of Ngal-Luc2/mC kidneys. These cells express Ngal-Luc2/mC in response to co-culture with UPECs and expression was reversed by antibiotics. Additional studies suggest that TLRs are the critical local sensors of infection.

Conclusions: uNGAL is essential for clearance of a UPEC in a model of acute UTI. The kidney responds to infections localized to the bladder by secreting NGAL. These findings provide an explanation for the massive secretion of NGAL from the kidney in both septic and aseptic diseases, demonstrating that the kidney defends the urinary system via exocrine delivery of NGAL.

Funding: NIDDK Support

TH-OR002

Proximal Tubule Specific Expression of Heme Oxygenase-1 Is Protective in Acute Kidney Injury

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Background: Heme Oxygenase-1 (HO-1), an anti-oxidant enzyme is induced during oxidative stress and has cytoprotective properties. HO-1 deficient mice are highly sensitive to acute kidney injury (AKI) secondary to ischemia, rhabdomyolysis and nephrotoxins. Although HO-1 is cytoprotective, it has been suggested that generalized HO-1 overexpression might lead to harmful effects due to excessive amounts of its reaction-products: iron, carbon monoxide, biliverdin and bilirubin.

Methods: Proximal tubules are the target of maximal injury in models of AKI and are also the site where HO-1 induction is most abundant. Hence, we hypothesized that targeted overexpression of HO-1 in the proximal tubule segment of the kidney will confer cytoprotection against AKI. Therefore, we generated proximal tubule specific HO-1 overexpressing (PEPCK-HO-1) mice using the cre-lox system and tested them in models of AKI.

Results: Compared to age and sex matched HO-1wildtype littermates, PEPCK-HO-1 mice demonstrated significant renal protection (structural and functional) in two different intrinsic models of AKI: cisplatin nephrotoxicity and glycerol-induced rhabdomyolysis. Following rhabdomyolysis, HO-1 wildtype mice demonstrated significantly higher mortality compared to PEPCK-HO-1 mice, along decreased tubular casts and necrosis and better preserved kidney function (BUN-control: 145 ± 17; PEPCK-HO-1: 42.8 ± 6; $P < 0.001$, creatinine-control: 1.55 ± 0.25; PEPCK-HO-1: 0.3 ± 0.14; $P < 0.001$). In the cisplatin model of AKI, PEPCK-HO-1 mice had significantly fewer casts, necrotic tubules and better-preserved kidney architecture along with preserved renal function (BUN-control: 106.67 ± 13.5; PEPCK-HO-1: 32.5 ± 3.8; $P < 0.001$, creatinine-control: 0.63 ± 0.1; PEPCK-HO-1: 0.28 ± 0.04; $P < 0.05$).

Conclusions: These studies demonstrate for the first time that proximal-tubule specific HO-1 overexpression alone is sufficient to protect against AKI and targeting the HO-1 system may serve as a novel therapeutic strategy.

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TH-OR003

Endothelial HIF2, but Not HIF1 Modulates Inflammation and Protects from Renal Ischemia-Reperfusion Injury

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Background: Hypoxia inducible factors (HIF)-1 and -2 are basic helix-loop-helix transcription factors that regulate cellular responses to hypoxia. Pharmacological activation of HIF signaling prior to injury protects kidneys from ischemia-reperfusion injury (IRI). However, it is unclear which HIF homolog, cell type, and which HIF target genes confer cytoprotection.

Methods: To address this question, we inactivated HIF-1 (HIF-1eKO), HIF-2 (HIF-2eKO) or both (HIF-1/HIF-2eKO) in endothelial cells using VEcadherin-driven Cre recombinase. KO mice and Cre-negative littermates were subjected to unilateral or bilateral IRI. Renal injury was assessed by histologically, mRNA and protein analysis, and by analysis of inflammatory cell infiltration at 2 hours, day 1 and 3 days after reperfusion.

Results: At day 3 following IRI renal injury was exacerbated in HIF-1/HIF-2eKO mice compared to control as determined by BUN levels (1.4-fold increase), histological injury score (1.5-fold increase), Kim-1 expression (1.6-fold increase) and inflammatory cell infiltration (8.5-fold increase). To determine which HIF homolog was cytoprotective, we subjected HIF-1eKO and HIF-2eKO mice to unilateral IRI. Injury in HIF-2eKO IRI kidneys was increased, whereas renal injury between HIF-1eKO and littermate controls was not different. Increased presence of CD45-positive cells in IRI kidneys from HIF-1/HIF-2eKO and HIF-2eKO correlated with enhanced VCAM-1 expression, whereas E-selectin and ICAM-1 did not change significantly. To examine whether endothelial HIF modulated HIF-mediated ischemic preconditioning, we activated HIF prior to injury with a prolyl-hydroxylase inhibitor (PHI). Although PHI treatment was cytoprotective in wild type mice, cytoprotection was not found in HIF-1/HIF-2eKO mice compared to vehicle-treated KO mice.

Conclusions: Our data suggest that a) endothelial HIF-2 but not HIF-1 is cytoprotective in renal IRI and that b) endothelial HIF-2 exerts its cytoprotective effect partly by suppressing IRI-associated inflammation. Furthermore, we provide evidence that endothelial HIF is required for HIF-mediated ischemic preconditioning.

Funding: NIDDK Support

TH-OR004

Critical Role of Sphingosine Kinase-1 Signaling in A_1 Adenosine Receptor-Mediated Renal Protection

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Background: Acute kidney injury (AKI) is a devastating clinical problem without effective therapy and renal ischemia reperfusion (IR) injury is a major cause of AKI. We previously demonstrated that activation of renal A_1 adenosine receptors (ARs) attenuated multiple pathways of cell death including necrosis, apoptosis and inflammation after renal IR.

Methods: Here, we tested the hypothesis that renal A_1AR activation protects against IR injury by induction of sphingosine kinase-1 (SK1) and sphingosine-1 phosphate (S1P) synthesis.

Results: In cultured human proximal tubule epithelial (HK-2) cells, a selective A_1AR agonist 2-chlorocyclopentyladenosine (CCPA) significantly induced SK1 (without changing SK2) mRNA and protein expression and increased the synthesis of S1P. CCPA also induced SK1 and S1P in mouse kidney cortex. Further supporting a critical role of SK1 in A_1AR-mediated renal protection, A_1AR agonist failed to protect $SK1^{-/-}$ mice (Cr=2.7±0.1mg/dL, N=6) but protected $SK2^{-/-}$ mice (Cr=1.2±0.2mg/dL, N=6, $P<0.01$) subjected to 30 min of renal ischemia and 24 h of reperfusion. In addition, *in vivo* gene knockdown of $S1P_1$ receptors with small interfering RNA (Cr=2.2±0.2mg/dL, N=6, $P<0.01$) or a selective $S1P_1$ receptor antagonist W146 (Cr=2.4±0.2mg/dL, N=6, $P<0.01$) completely abolished the renal protection provided by CCPA (Cr=1.2±0.2mg/dL, N=5). Finally, mice specifically deficient in proximal tubule $S1P_1$ receptors ($S1P_1R^{flox/flox}PEPCK^{Cre/-}$, Cr=2.3±0.2mg/dL, N=6) were not protected against renal IR with CCPA. Mechanistically, A_1AR activation increased HIF-1a nuclear translocation in HK-2 cells and in mouse kidney cortex. Furthermore, 2-methoxyestradiol (a selective HIF-1a inhibitor) blocked A_1AR-mediated induction of SK1 in HK-2 cells and in mouse kidney cortex.

Conclusions: Taken together, our data show that induction of proximal tubule SK1 and subsequent $S1P_1$R activation is critical for A_1AR-mediated renal protection. Selective renal proximal tubular induction of SK1 and $S1P_1$ receptor signaling after IR may provide a novel therapeutic approach for the prevention and treatment of AKI.

Funding: NIDDK Support

TH-OR005

Activation of Proximal Tubule Sphingosine 1 Phosphate Receptor-1 (S1P1) Ameliorates Cisplatin Induced Nephrotoxicity in Mice

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Background: Sphingosine 1-phosphate (S1P), a sphingolipid that is the natural ligand for a family of five G-protein coupled receptors (S1P1-5Rs), regulates cell survival and lymphocyte circulation. The pan S1PR agonist, FTY720 (fingolimod) attenuates ischemia-reperfusion injury (IRI) by activating S1P1 on proximal tubule-PT cells directly, an effect previously thought to be due predominantly to their canonical effects of S1P1 activation on B and T cells leading to lymphopenia.

Methods: FTY720 reduced cisplatin-induced AKI, therefore in the current study we sought to determine whether the protective effect was mediated by proximal tubule (PT) S1P1. We used conditional renal PT S1P1 null (*PEPCK-CreS1pr1$^{fl/fl}$*) and control mice (*PEPCK-Cre)* to assess renal injury by monitoring plasma creatinine (mg/dl), flow cytometry to analyze inflammatory cell infiltration and Real Time RT-PCR for changes pro-inflammatory cytokines.

Results: Compared to control mice (0.79±0.02) cisplatin induced more injury in PT S1P1 null mice (1.22±0.13, $p<0.05$) and FTY720 reduced injury in control mice (0.47±0.06, $p<0.01$) but not in PT S1P1 null mice (1.32 ±0.22, p=n.s.). There were no differences in

circulating lymphocytes counts in FTY720 treated control and S1P1 null mice. Control or PT S1P1 null mice treated with cisplatin have significantly higher levels of pro-inflammatory cytokines (CXCL1, MCP-1, TNF-α and IL-6) compared to vehicle-treated mice. Treatment of control mice with FTY720 significantly attenuates the mRNA levels of afore mentioned cytokines. Decrease in cytokine production with FTY720 results in less neutrophils and macrophages infiltration at day 3 in kidneys.

Conclusions: In summary, S1P1 expressed in PT attenuates cisplatin-induced AKI. We conclude that FTY720 administration might represent a novel strategy in the prevention of cisplatin-induced AKI.

Funding: NIDDK Support

TH-OR006

IL-2/anti-IL-2 Antibody Complexes Attenuate Renal Ischemia-Reperfusion Injury through Expansion of Regulatory T Cells Myung-Gyu Kim,[1] Han Ro,[1] Curie Ahn,[2] Jaeseok Yang.[1] *[1]Transplantation Center, Seoul National University Hospital, Seoul, Republic of Korea; [2]Division of Nephrology, Seoul National University Hospital, Seoul, Republic of Korea.*

Background: Regulatory T cells (Tregs) can contribute to suppression of immunologic damage or facilitation of the recovery process following renal ischemia-reperfusion injury (IRI). However, isolation and expansion of rare Tregs are practically hard for clinical application. Recently, complexes (IL-2C) of interleukin-2 (IL-2) and anti-IL-2 antibodies have been shown to control various inflammatory diseases by inducing expansion of Tregs. Therefore, we investigated whether IL-2C can control renal IRI.

Methods: C57BL6/J mice underwent bilateral renal ischemia. IL-2C or vehicles were administered for 3 consecutive days from 5 days before or 1 day after renal IRI. Renal function was assessed with infiltration of inflammatory cells, tissue cytokines and tissue recovery. Anti-CD25 antibodies (PC61) were administered to assess the effect of DC depletion on impacts of IL-2C. We also performed IRI using IL-10 knockout mice to clarify the role of IL-10.

Results: IL-2C induced a 3-5 fold expansion of Tregs in both spleen and kidney. IL-2C treatment before renal IRI significantly improved renal function, attenuated histological renal injury and apoptosis. IL-2C significantly decreased expression of pro-inflammatory cytokines (MCP-1, IL-6) and also reduced infiltration of both neutrophils and macrophages. IL-2C treatment after IRI significantly increased tubular cell proliferation in recovery phase. Depletion of Tregs using PC61 abrogated the beneficial effects of IL-2C. When the IL-10 knockout mice were used, the beneficial effects of IL-2C were abrogated, and these results suggested that IL-10 might mediate the renoprotective effect of IL-2C.

Conclusions: IL-2C attenuated acute renal damage and also facilitated renal recovery in renal IRI, by inducing Tregs with an IL-10-dependent manner. Because IL-2C are easy to manipulate as well as effective for renal IRI, IL-2C is promising for clinical application to renal IRI.

TH-OR007

Pentraxin-3 Suppresses Postischemic Acute Kidney Injury by Inhibiting P Selectin-Mediated Renal Leukocyte Recruitment Maciej Lech,[1] Christoph Roemmele,[1] Regina Groebmayr,[1] Cecilia Garlanda,[2] Alberto Mantovani,[2] Bernd Uhl,[3] Christoph A. Reichel,[3] Fritz Krombach,[3] Hans J. Anders.[1] *[1]Klinische Biochemie, Medizinische Poliklinik der LMU, Munich, Germany; [2]Instituto Clinico Humanitas, Rozzano, Italy; [3]Walter Brendel Centre of Experimental Medicine, Munich, Germany.*

Background: Ischemia-reperfusion injury is a neutrophil-dependent sterile inflammation that is unable to control the causative trigger and therefore induces unnecessary tissue damage. Little is known about the factors that limit postischemic inflammation, e.g. in acute kidney injury, a common medical problem associated with poor survival. The long pentraxin PTX3 regulates multiple aspects of host defense and tissue inflammation therefore we hypothesized that PTX3 would be involved in ischemia-reperfusion injury.

Results: PTX3 induction in postischemic kidneys was largely limited to CD11c+ renal dendritic cells. Lack of PTX3 aggravated postischemic acute renal failure and kidney injury as evidenced by massive tubular necrosis and TNF or IL-6 release. Remarkably, neutrophil and macrophage infiltrates were also massively increased in Ptx3-deficient mice although intrarenal chemokine CXCL2 and CCL2 expression were independent of the PTX3 genotype. PTX3 rather modulated leukocyte recruitment via interacting with P-selectin as P-selectin inhibition completely abrogated the enhanced leukocyte recruitment and tissue injury in postischemic kidneys of Ptx3-deficient mice. In-vivo microscopy revealed increased leukocyte adhesion and transmigration in postischemic microvessels of Ptx3-deficient mice. Finally, intravenous injection of recombinant PTX3 shortly after surgery suppressed renal leukocyte recruitment and prevented postischemic kidney injury.

Conclusions: Together, PTX3 is an endogenous suppressor of ischemia reperfusion injury by blocking P-selectin-mediated adhesion of leukocytes to activated endothelia of postischemic tissues, a mechanism that can be mimicked with recombinant PTX3 for therapeutic purposes. Vice versa, Ptx3 loss-of-function mutations may predispose to ischemia-reperfusion injuries such as postischemic acute renal failure.

TH-OR008

TRPM2 Channels Mediate Hypoxic Cell Death and Contribute to Ischemic AKI William Brian Reeves, Weiwei Wang, Guofeng Gao, Wenyi Zhang, Barbara A. Miller. *Departments of Medicine and Pediatrics, Penn State College of Medicine, Hershey, PA.*

Background: TRPM2 channels belong to the TRP family of non-selective cation channels. TRPM2 channels are activated by TNFα and by oxidant stress, and TRPM2 expression enhances susceptibility to cell death in certain in vitro settings. However, the role of TRPM2 in cell injury or cell death in vivo is unknown. Since TNFα and oxidant stress have been implicated in the pathogenesis of AKI, we evaluated the role of TRPM2 channels in AKI.

Methods: We created mice with the TRPM2 gene flanked by loxP sites and then bred them with Ella-cre mice which express Cre recombinase ubiquitously to create a global TRPM2 knockout mouse. The resulting mice developed normally and had normal kidney function and histology. Ischemic AKI was induced in the TRPM2 KO mice and control littermates by clamping both renal arteries for 28 minutes. Renal function was assessed by measurements of BUN and creatinine. Histology and histochemistry of kidney sections were used to measure structural injury, apoptosis and neutrophil infiltration. Gene expression was determined using quantitative RT-PCR.

Results: Ischemia-reperfusion injury in TRPM2 KO mice

		BUN			Creatinine	
	0 hrs	6 hrs	24 hrs	0 hrs	6 hrs	24 hrs
WT	19±1	59±2	118±19	0.27±.03	0.64±.03	1.1±
TRPM2 KO	20±1	47±4	69±10	0.26±.03	0.47±.04	0.60±.05
P value	0.5	0.01	0.002	0.9	0.001	0.02

BUN and creatinine levels at 6, 24 and 48 hrs (not shown) after ischemia were significantly lower in the TRPM2 KO mice compared to littermates. Likewise, tissue injury scores, apoptosis and kidney neutrophil infiltration at both 24 and 72 hrs post ischemia were all significantly lower in TRPM2 KO vs WT mice. Analysis of gene expression showed >2-fold increases in certain pro-survival genes, e.g. akt, peroxiredoxin-2 and BCL2L10, in kidneys of KO vs WT mice. Primary cultures of proximal tubule cells prepared from WT and TRPM2 KO kidneys were subjected to hypoxia by incubation in a GasPak chamber for 24 and 48 hours. At both time points, cell survival in the TRPM2 KO cells exceeded that of the WT cells (<0.001).

Conclusions: These results point to an important role for TRPM2 channels, likely via direct effects in renal epithelial cells, in the pathogenesis of ischemic AKI.

Funding: Pharmaceutical Company Support

TH-OR009

Mice with Absent Heat Shock Protein Induction Are Protected Against Ischemic Renal Injury Rajasree Sreedharan, Shaoying Chen, Melody A. Miller, Dipica Haribhai, Calvin B. Williams, Scott K. Van Why. *Pediatrics, Medical College of Wisconsin, Wauwatosa, WI.*

Background: Heat Shock Factor 1 (HSF1) regulates inducible heat shock protein (HSP) expression, and HSPs protect against renal cell injury *in vitro*. Whether HSPs provide protection against ischemic renal injury *in vivo* is unclear.

Methods: To determine whether inducible HSPs protect against ischemic renal injury *in vivo*, inducible HSP70 and 25 expression and renal injury from bilateral renal ischemia was studied in HSF1 functional knock-out mice (HSF KO) and compared with HSF wild type (WT).

Results: There was no difference between WT and HSF KO in baseline renal HSP70 or 25 levels. WT kidneys had the expected induction of HSP70 and 25 after 45 min of bilateral ischemia and 24 hr reflow, but KO mice had no induction of either HSP. Baseline serum creatinine was also comparable (0.22 mg/dL WT versus 0.19 mg/dL KO). Serum creatinine at 24 hrs reflow in WT was 2.1 mg/dL compared with 0.9 mg/dL in HSF KO (p=0.0002).

Flow cytometry was used to study mononuclear cells isolated from kidneys from both strains. In sham operated kidneys, there was no difference in number of CD4+ and CD8+ T cells in WT compared with HSF KO. However, after 45 min of bilateral ischemia and 1 hr reperfusion, CD4+ and CD8+ cells in WT kidneys increased by 58% (p=0.02) and 75% (p=0.08) respectively from uninjured controls. There was no significant change in either CD4+ or CD8+ cells (p=0.6 and 0.77 respectively) in the HSF KO kidneys after ischemia compared to uninjured controls. In addition, Fox p3+ T regulatory cells decreased by 40% in WT kidneys after ischemia, but did not change in HSF KO (p=0.02 WT versus KO).

Conclusions: This study demonstrates 1) HSP induction is completely absent in HSF1 KO mice kidneys subjected to ischemia, 2) absence of HSP induction, contrary to expectation, is associated with protection against ischemic renal injury *in vivo*, and 3) HSF1 KO mice kidneys have altered T-cell infiltration immediately following ischemia. So, inducible HSPs may contribute to early ischemic renal injury by facilitating T cell response.

Funding: NIDDK Support, Private Foundation Support

TH-OR010

miR-21 Contributes to Renal Protection Conferred by Delayed Ischemic Preconditioning Alison J. Kriegel,[1] Xialian Xu,[1,2] Yong Liu,[1] Kristie Usa,[1] Domagoj Mladinov,[1] Yi Fang,[2] Xiaoqiang Ding,[2] Mingyu Liang.[1] *[1]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Division of Nephrology, Fudan University Zhongshan Hospital, Shanghai, China.*

Background: Delayed ischemic preconditioning (IPC) effectively protects kidneys from ischemia-reperfusion (I/R) injury. We examined the in vivo role of microRNA miR-21 in the renal protection conferred by delayed IPC in C57BL/6J mice.

Methods: Mice were subjected to 15min ischemic preconditioning and, 4 days later, 30min bilateral renal ischemia and 24h reperfusion. Tail vein injection of locked nucleic acid(LNA)-modified anti-miR (10 mg/kg) was used to knock down miR-21 in vivo. Renal injury was evaluated by the plasma creatinine, histological change, and TUNEL assay. A miR-21 target protein was analyzed by Western blot.

Results: A 15 min renal IPC substantially attenuated I/R injury induced 4 days later in mice, as indicated by decreases of plasma creatinine and histology damage score ($P<0.05$). Renal abundance of miR-21 expression was increased in the delayed IPC + I/R group by 179% ± 17% compared to the Sham + I/R group ($p<0.05$; n=6/group). Time-course analysis indicated that miR-21 was upregulated at 4 h after IPC compared to the Sham mice, and remained significantly higher 4 days after IPC (n=6/group each time point; $P<0.05$). Administration of LNA anti-miR-21 at the time of IPC decreased miR-21 levels in the kidney detectable by real-time PCR by 98% ($p<0.05$; n=5-6/group), and significantly exacerbated functional and histological damage of subsequent I/R injury in the mouse kidney ($P<0.05$). Knockdown of miR-21 resulted in significant upregulation of PDCD4 protein, a pro-apoptotic target gene of miR-21 (arbitrary unit, 89 ± 27 vs. 20 ± 10, $P<0.05$), and a substantial increase in tubular cell apoptosis in the mouse kidney as determined by TUNEL staining (proportion of positive cells, 16% ± 3% vs. 3% ± 1%, $P<0.05$). In the absence of delayed IPC, knockdown of miR-21 did not significantly affect I/R injury in the mouse kidney.

Conclusions: These data support a novel mechanism in which upregulation of miR-21 contributes to the protective effect of delayed IPC against subsequent renal I/R injury.

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TH-OR011

Anti-TGF-β Antibody (1D11) May Attenuate High-Turnover Renal Osteodystrophy by Stimulating Sost Expression Shiguang Liu, Joseph H. Boulanger, Wenping Song, Susan Ryan, Brian S. Kelley, Russell Gotschall, Wen Tang, Yves Sabbagh, Susan Schiavi. *Genzyme Corporation.*

Background: TGF-β1 plays an important role in normal bone remodeling. Its elevated expression in the bone and bone marrow of patients with high-turnover renal osteodystrophy suggests that this growth factor may also contribute to the pathogenesis of bone disease in the setting of chronic kidney disease. We have previously reported that anti-TGF-β antibody (1D11) prevents the onset of high bone turnover in an adenine induced rat model of CKD-MBD.

Methods: To further explore the potential efficacy and mechanism of action of 1D11 on high-turnover renal osteodystrophy, we administered 1D11 to Jck mice, a genetic model of CKD-MBD that had established high-turnover renal osteodystropy. 1D11 was injected IP at doses of 0.5, 2 or 5 mg/kg three times a week for 7 weeks.

Results: Bone histomorphometric analysis indicated that 1D11 significantly suppressed the elevated bone turnover in a dose-dependent manner. To explore the possible mechanism of 1D11's action on bone, we examined expression of genes associated with key pathways regulating bone turnover using a TaqMan Low Density Arrays (TLDA). We have previously shown that expression of the Wnt antagonist, SOST is elevated in early CKD in Jck mice and humans, but this increase is attenuated by rising PTH levels late in disease. In this study, Sost expression was significantly suppressed in Jck relative to wild-type littermate control mice at 17 weeks of age (late in CKD). Importantly, 1D11 treatment resulted in a significant increase in Sost expression in parallel with reduction in bone turnover despite elevated serum PTH levels.

Conclusions: Our data suggest that antagonism of TGF-β attenuates high-turnover renal osteodystrophy independent of changes in serum PTH levels and the effect of 1D11 on bone may be at least partially mediated by stimulation of Sost expression.

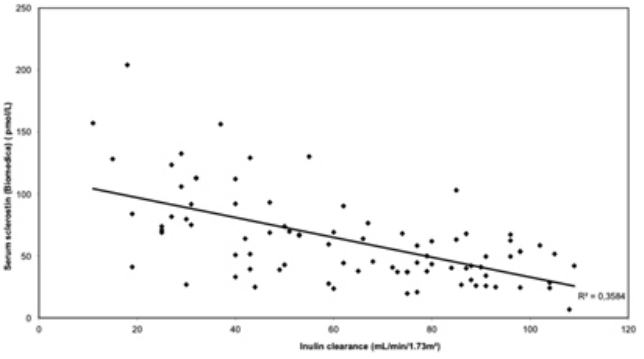
TH-OR012

Sclerostin, a Bone osteopenic Factor, Is Increased in Chronic Kidney Disease Solenne Pelletier,[1,3] Aoumeur Hadj-Aissa,[3] Laurence Dubourg,[3] Denis Fouque.[1,2] *[1]Nephrology, Hopital Edouard Herriot, Lyon, France; [2]U1060, INSERM, Lyon, France; [3]UJERNE, Hopital Edouard Herriot, Lyon, France.*

Background: Sclerostin is a small peptide secreted by osteocytes. Recent experimental data suggest that sclerostin blocks bone formation and better predicts bone turnover than parathormone. We assessed the relationship between serum sclerostin and glomerular filtration rate (GFR) in CKD patients

Methods: A prospective cohort of 86 patients (43 women, 7 diabetic) was studied. Glomerular filtration rate was measured by the gold standard inulin clearance. Serum sclerostin was measured by EIA (Biomedical, Wien). Normal values were 46.4 ± 23.3 pmol/L

Results: Mean age was 51 ± 19 years, GFR 63 ± 27 mL/min/1.73 m2, sclerostin 68.8 ± 36.1 pmol/L, and serum phosphate 1.11 ± 0.24 mmol/L. Sclerostin was strongly and inversely correlated with GFR ($r=-0.6$, $p< 0.001$), positively with age ($r=0.38$, $p< 0.001$) and with serum phosphate ($r=0.25$, $p=0.02$).



By multiple regression, only GFR was associated with the observed sclerostin increase

Conclusions: We found a new and strong inverse relationship between sclerostin and GFR in CKD patients (Fig 1). Since sclerostin reduces bone turnover, and accumulates in CKD, this may suggest a role on renal osteodystrophy. As in postmenopausal osteoporosis, for which sclerostin antagonists have been developed, sclerostin might soon become a new player in CKD mineral and bone disease.

TH-OR013

Decreased Trabecular Tissue Mineralization Is Associated with Fracture in Patients with Kidney Disease Thomas L. Nickolas, Xiaowei Sherry Liu, Chiyuan Amy Zhang, Donald J. McMahon, Elizabeth Shane. *Columbia University.*

Background: In renal osteodystrophy (ROD), abnormal Vitamin D metabolism and remodeling cause defects to tissue mineral density (TMD), which affects tissue-level mechanical properties. Abnormal TMD may contribute to high fracture (Fx) risk in CKD patients. Previously, invasive iliac crest bone biopsy was required to evaluate mineralization. We have now noninvasively measured TMD on high resolution peripheral QCT (HRpQCT) scans of the distal radius (DR) and tibia (DT). We hypothesized that trabecular (Tb) TMD is lower in CKD patients with fractures (Fx) than both CKD patients without Fx and healthy Controls. We also hypothesized that TbTMD is inversely related to calciotropic hormones and bone turnover markers (BTM).

Methods: We performed HRpQCT (Scanco Medical; voxel size 82μm) of the DR and DT in 47 patients with CKD (n=23 Fx; 24 nonFx) and 21 age, sex and race matched Controls without Fx. By digital topological analysis (DTA), Tb bone voxels were classified into one of 3 envelopes: surface (s), central (c) and intervening (middle (m); between surface and central). TMD was calculated for each envelope. Serum iPTH, 25OHD and bone formation (BSAP, P1NP) and resorption (CTX, Trap5b) markers were measured.

Results: CKD-Fx, CKD-nonFX, and Controls were well matched for age, sex and race (all p=NS). TbTMD was significantly lower in CKD-Fx than Controls. At the DR, s- and m- TMD were lower by 2.6% and 3.0%, respectively (all $p<0.05$). At the DT, s-, m- and c- TMD were lower by 2.2%, 2.6% and 3.1%, respectively (all $p<0.05$). TMD in patients with CKD-nonFx did not differ from that of either CKD-Fx or Controls. At the DT, each SD decrease in s-, m- and c- TMD was associated with 1.7%, 1.4% and 1.1% increased odds of Fx ($p<0.05$ for all). Higher levels of iPTH, BSAP, P1NP and CTX were significantly and inversely correlated with s-, m- and c- TMD; r values ranged from -0.40 for P1NP vs DT sTMD ($p<0.009$) to -0.53 for iPTH vs DR sTMD ($p<0.0005$).

Conclusions: CKD patients with Fx have low TbTMD that is likely related to SHPT and high bone turnover. HRpQCT may provide a noninvasive approach to measurement of TbTMD in CKD; validation against iliac crest bone biopsy is needed.

Funding: NIDDK Support

TH-OR014

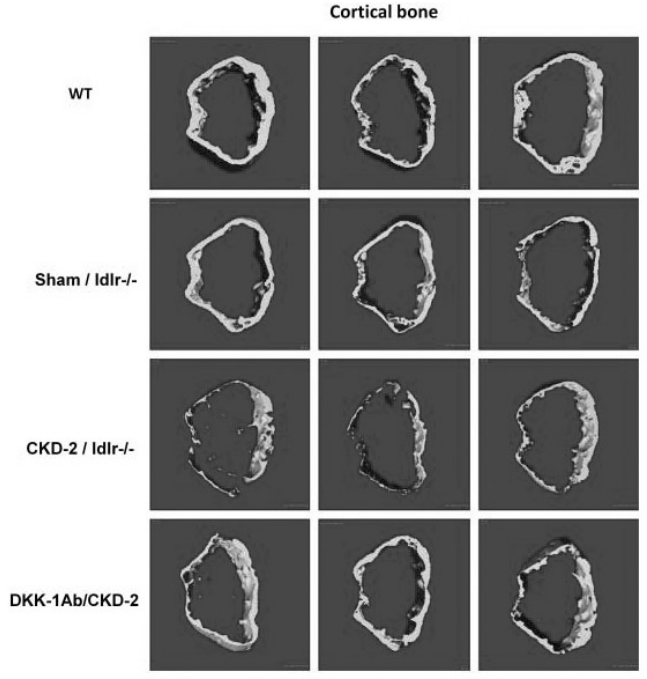
The Pathogenesis of the Early CKD-MBD in Stage 2 CKD Keith A. Hruska, Yifu Fang. *Pediatrics, Washington University School of Medicine, St. Louis, MO.*

Background: CKD induces the CKD-MBD associated with increased mortality by stage 2. Vascular calcification (VC) is a strong risk factor in CKD. The CKD-MBD in stage 2 CKD consists of stimulation of VC, the onset of osteodystrophy, and an increase in FGF23 levels, while Ca, Pi and PTH levels remain normal. The increase in FGF23 serves as a biomarker of the CKD effect on the skeleton. We have linked the skeleton in CKD to stimulation of VC. The pathogenesis of the initial stimulus for osteocytic secretion of FGF23 is unclear. Early changes in phosphate homeostasis, changes in bone remodeling, and PTH are the candidate factors under investigation. Here we examine the actions of either modifying Pi balance through intestinal Pi binding or a skeletal anabolic factor (a neutralizing monoclonal antibody to a Wnt inhibitor, DKK1) on the early CKD-MBD.

Methods: Ldlr deficient mice fed high fat diets were subjected to partial kidney ablation at 12 weeks of age to create an early CKD-MBD mouse model. Treatment protocols with Vehicle, CaAc (3% w/w mixed in diet), or the DKK1 mab (30 mg/kg tiw IP) were conducted from 14-22 wks or 22-28 wks.

Results: The reduction in the GFR measured by inulin clearance was 75% of normal (stage 2 CKD). Aortic Ca levels were increased by CKD at 22 weeks of age

and progressively increased to 28 wks. The serum levels of BUN, Ca, Pi and PTH were normal, but a transient elevation of PTH at 14 weeks was discovered. MicroCT of the femurs revealed cortical but not trabecular bone loss which was corrected by the DKK1 mab but not by CaAc therapy.



FGF23 levels were decreased by CaAc but not by the DKK1 mab. Vascular Ca levels were decreased by the DKK1 mab but not by CaAc therapy.

Conclusions: We conclude that an early osteodystrophy in CKD may be causatively associated with the stimulation of VC, and that the stimulus to FGF23 secretion is independent and may be Pi or PTH.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-OR015

Effect of Calcium Carbonate Supplement on Phosphate Balance and Homeostasis in Patients with Stage 3 and 4 Chronic Kidney Disease

Kathleen M. Hill,[1] Berdine Martin,[2] Sharon M. Moe,[3] George P. McCabe,[4] Connie M. Weaver,[2] Munro Peacock.[1] *[1]Endocrinology, Indiana University School of Medicine, Indianapolis, IN; [2]Foods and Nutrition, Purdue University, West Lafayette, IN; [3]Nephrology, Indiana University School of Medicine, Indianapolis, IN; [4]Statistics, Purdue University, West Lafayette, IN.*

Background: In chronic kidney disease (CKD), calcium carbonate ($CaCO_3$) given with meals is used to bind dietary phosphate (Pi) and thus lower plasma Pi. Data on the effect of $CaCO_3$ on Pi balance and homeostasis in CKD are limited.

Methods: Three patients, of a projected eight, with stage 3/4 CKD participated in two 3wk balance studies using a randomized crossover design. Patients received 500 mg elemental Ca as $CaCO_3$ or placebo with meals 3x/24h. Diets consisted of 957 mg Ca/24h and 1500 mg Pi/24h. Wk 1 was outpatient equilibration period. Patients were inpatients wk 2-3, and all 24h urine and feces were collected and analyzed for Ca & Pi. Ca & Pi balances were calculated as diet (mg/24h) – fecal (mg/24h) – urine (mg/24h). Fasting blood and urine were collected for biochemistries at the end of each week. Repeated measures ANOVA for crossover designs was used to test differences between $CaCO_3$ and placebo.

Results: As compared to placebo, net and percent Pi absorption were lower with $CaCO_3$ (495 mg/24h, 32% v. 710 mg/24h, 45%); urinary Pi excretion was lower with $CaCO_3$ (650 v. 877 mg/24h); fecal Pi excretion was higher with $CaCO_3$ (1061 v. 852 mg/24h); and Pi balance was similar (-155 v. -167 mg/24h). Ca balance was higher with $CaCO_3$ (404 v. 57 mg/24h). Biochemistries did not differ between $CaCO_3$ and placebo: serum Ca 9.4 & 9.3 mg/dL, Pi 4.0 & 4.1 mg/dL, Cr 2.5 & 2.4 mg/dL, PTH 70 & 78 pg/mL, FGF23 77 & 74 pg/mL, 25(OH)D 21 & 22 ng/mL, $1,25(OH)_2D$ 46 & 48 pg/mL, osteocalcin 21 & 23 ng/mL, bone alkaline phosphatase 36 & 33 U/L, urine N-telopeptide crosslinks:Cr 54 & 38 nM BCE/mM, urine Ca:Cr 0.07 & 0.05, Pi:Cr 0.29 & 0.39.

Conclusions: A $CaCO_3$ supplement providing 500 mg elemental Ca given with meals as a Pi binder lowers through put of Pi by only 200 mg/24h and does not change Pi balance or fasting serum biochemistries of Ca & Pi in early CKD.

Funding: Pharmaceutical Company Support

TH-OR016

Reduced Fracture Risk with Early Corticosteroid Withdrawal after Kidney Transplant: An Analysis of the USRDS

Lucas Nikkel,[1] Sumit Mohan,[1] Chiyuan Amy Zhang,[1] Donald J. McMahon,[1] Stephanie Boutroy,[1] Geoffrey K. Dube,[1] Bekir Tanriover,[1] David J. Cohen,[1] Lloyd Ratner,[1] Christopher S. Hollenbeak,[3] Mary B. Leonard,[2] Elizabeth Shane,[1] Thomas L. Nickolas.[1] *[1]Columbia University; [2]Children's Hospital of Philadelphia; [3]Penn State Hershey.*

Background: Co-administration of calcineurin inhibitors (CNI) with corticosteroid (CS) after kidney transplant (KTx) results in severe bone loss and very high fracture (Fx) risk. Early CS withdrawal (ECSW) has not been associated with bone loss in comparison to CS based immunosuppression (CSBI) after KTx. We hypothesized that Fx rates in KTx recipients managed with ECSW are lower than with CSBI.

Methods: Using the United States Renal Data System (USRDS), 77,625 adults undergoing first KTx between January 1, 2000 and December 31, 2006 were identified. CS use was determined from UNOS immunosuppression forms completed at KTx in an intent-to-treat analysis. Fx after KTx was identified from hospitalization discharge ICD9 codes. Continuous characteristics (age, BMI, HLA mismatches) were compared by Students t tests and binary characteristics were compared by chi-square. Time to first Fx was modeled after KTx using Kaplan-Meier and Cox methods.

Results: Patients on ECSW differed slightly from those on CS in age (49.9 vs. 48.2 years; $p<0.001$), gender (62% male vs. 60%, $p<0.001$), race (69% white vs. 67%, $p<0.001$), BMI (27.1 vs. 26.6 kg/m2, $p<0.001$), and live donor (48% vs. 39%; $p<0.001$). 2395 Fx resulting in hospitalizations were observed during follow-up (307662 person-years); median (IQR) follow-up was 1448 (808-2061) days. There were 5.8 and 8.0 Fx per 1000 patient-year for ECSW and CSBI, respectively. Fx site distribution was similar between groups. Multivariate Cox models demonstrated a 31% ($p<0.001$) Fx risk reduction for ECSW versus CSBI after adjustment for other risk factors for Fx. By 24-months after KTx, Fx risk was significantly lower in the ECSW group.

Conclusions: ECSW is associated with a 31% reduction in serious Fx risk after KTx. Fx risk begins to decrease in ECSW patients by the second year after KTx. There is a need for prospective studies to understand how ECSW affects bone and mechanisms through which Fx risk is decreased.

Funding: Private Foundation Support

TH-OR017

Effect of Steroid Withdrawal on Growth, FGF23/Klotho and IGF-I/IGFBP3 Axis in Pediatric Kidney Transplantation

Luis Michea,[4] Angela Delucchi,[1] Magdalena Gonzalez,[4] Paulina Salas,[2] Viola Pinto,[2] Francisco Cano,[1] Veronica Mericq.[3] *[1]Pediatric Nephrology, Luis Calvo Mackenna Hospital, Santiago, Chile; [2]Pediatric Nephrology, Exequiel Gonzalez Cortes Hospital, Chile; [3]IDIMI, Santiago, Chile; [4]ICBM. Centro de Estudios Moleculares de la Celula, Falculty of Medicine. University of Chile, Santiago, Chile.*

Background: Successful renal transplant improves growth in pediatric patients. Steroids immunosuppression has adverse effects on growth, bone metabolism and GH/IGF-I axis. The Fibroblast Growth Factor 23 (FGF23) is a phosphatonin produced by bone cells, which is active with its co-receptor Klotho. FGF23 reduces osteoblastic activity and matrix mineralization by autocrine/paracrine mechanisms. We hypothesized that steroids increase the FGF23/Klotho axis activity.

Methods: A prospective, randomized, multicenter study, steroid withdrawal on growth, FGF23/Klotho and IGF-I/IGFBP3 levels in pediatric kidney recipients (2-15 years) was evaluated. Six days post-Tx patients receiving tacrolimus (TAC) and mycophelolate mofetil (MMF) were assigned into two groups: steroid withdrawal (SW, n=9) and steroid control (SC, n=10). The ethics committee approved the protocol, written informed consent was obtained. Growth and biochemical parameters were measured up to 12 months post Tx. Data were presented as means±SD, student-t test, Mann-Whitney and Wilcoxon tests. Regression analysis for repeated measurements (mixed models) was performed.

Results: A significant improvement in height Z-score at 1 year post Tx in SW vs. SC group (-1.02±1.0 vs.-2.25±1.0; p=0.02) was observed. The SW group showed lower FGF23 levels as compared to SC (SW=5.8±3.1pg/mL vs. SC=18.6±11.5 pg/mL; $p<0.003$) at 1 year post Tx; that were similar to those of healthy controls. Delta zIGF-I was higher in SW 1.6±1.2 vs.SC 0.2±2.1;p=0.03. No significant changes in delta IGFBP3 levels between groups were found. An inverse relationship between FGF-23 levels and growth was observed (R^2:0.4 p=0.004).

Conclusions: These results suggest that steroids withdrawal improve growth due to less GH resistance, improvement of IGF-I and/or FGF23/Klotho axis function.

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Funding: Government Support - Non-U.S.

TH-OR018

Ezrin Is Essential for the Phosphate Reabsorption in the Renal Proximal Tubule Ryo Hatano,[1] Atsushi Tamura,[2] Hiroko Segawa,[3] Ken-Ichi Miyamoto,[3] Sachiko Tsukita,[2] Shinji Asano.[1] *[1]Department of Molecular Physiology, Ritsumeikan University, Kusatsu, Shiga, Japan; [2]Laboratory of Biological Science, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University, Suita, Osaka, Japan; [3]Department of Molecular Nutrition, University of Tokushima Graduate School, Japan.*

Background: Ezrin is a crosslinker between membrane proteins and actin cytoskeleton and also known to be related with the membrane fusion of gastric vesicles in stomach. In the kidney, ezrin is mainly expressed in the apical membrane of proximal tubules and interacts with scaffold protein as NHERF1, which is essential for membrane localization of some transporters and receptors. However, the physiological roles of ezrin in the kidney are not still unraveled. Therefore, we used ezrin knockdown ($Ezrin^{kd/kd}$) mice as a model for the analysis of the physiological roles of ezrin in the kidney.

Methods: Ezrin knockdown mice was constructed as previously reported (Tamura A *et al. J Cell Biol*. 2005.). Male mice at 4 to 8 week age were kept in the metabolic cages for 7 days and daily urine was collected for the analysis of urinary excretion of phosphate and other substrates. These mice were sacrificed and kidney was collected for the immunohistochemistry and western blot analysis. Right tibiae were also collected for the bone analysis.

Results: Whereas there is no apparent difference in the morphology of the kidney between adult wild type and $Ezrin^{kd/kd}$ mice, $Ezrin^{kd/kd}$ mice showed hypophosphatemia and abnormal bone formation caused by significant urinary loss of phosphate but not calcium. These phenotypes were shown even in the prepubertal mice (4wk). Furthermore, we determined a decreased apical membrane localization of Na^+/phosphate cotransporter, NaPi2a in the proximal tubules by western blot and immunofluorescence analysis.

Conclusions: These results suggest that ezrin is essential for the membrane localization of NaPi2a at the apical membrane in the proximal tubules and functional or genetic disorder of ezrin might be related with the onset of hypophosphatemic rickets.

TH-OR019

NHERF1 Modulates Intestinal NaPi Transporter NaPi2b Expression in Apical Microvilli Yupanqui A. Caldas,[1,2] Hector Giral-Arnal,[1] Luca Lanzano,[3] Enrico Gratton,[3] Moshe Levi.[1] *[1]Department of Medicine, Anschutz Medical Center, University of Colorado Denver, Aurora, CO; [2]Department of Toxicology, Laboratory of Molecular Toxicology, University of Zaragoza, Spain; [3]Laboratory for Fluorescence Dynamics, University of California Irvine, Irvine, CA.*

Background: The regulation of Pi homeostasis is maintained by the coordinated function of the renal and intestinal phosphate transporters. In the intestine the type II sodium-phosphate (NaPi) co-transporter NaPi2b, is considered to be the main mediator of sodium dependent transcellular Pi transport. The PDZ (PSD-95/discs large/ZO-1 homologous) domain proteins, including NHERF1, PDZK1, ShanK2, and PIST play an important role in the regulation of the renal NaPi transporters. However their potential role in regulation of the intestinal NaPi2b transporter is not known.

Methods: For this purpose we performed studies with mouse small intestine, and in cell culture to determine a potential role for NHERF1 in regulation of NaPi2b.

Results: We found that the human intestinal cell CaCo2BBE cells expressed the endogenous NaPi2b transporter, and we used them to study the regulation of NaPi2b. Expression of GFP-NaPi2b was observed in the microvilli, and along of a single microvillus with the novel multitracking technique (MT), from which we measured the diffusion coefficient (D=0.2 um2/s). Cells transiently co-transfected with GFP-NaPi2b and mCherry-NHERF1 were analyzed for molecular interaction using the FLIM-FRET technique which revealed significant FRET between NaPi2b and NHERF1. The functional significance of this interaction was further confirmed in NHERF1 KO mice, where we found that adaptation of NaPi2b was markedly impaired in the KO mice.

Conclusions: Our results therefore indicate a novel and a critical role for NHERF1 in modulation of NaPi2b expression in the microvilli of the mouse intestine.

Funding: NIDDK Support

TH-OR020

Novel Non-Systemic NaP2b Inhibitors Block Intestinal Phosphate Uptake Marc Navre,[1] Eric Daniel Labonte,[1] Christopher Carreras,[1] Andrew G. Spencer,[1] Joanne Marks,[2] Deborah Black,[1] Jason G. Lewis,[1] Jeffrey Jacobs,[1] Dominique Charmot.[1] *[1]Ardelyx Inc., Fremont, CA; [2]University College London, London, United Kingdom.*

Background: The inhibition of intestinal phosphorus (P) uptake via the blockade of NaP2b-mediated transport emerges as a novel approach for P management in CKD. Novel classes of non-systemic NaP2b (SLC34A2) inhibitors were identified and their pharmacodynamic profiles evaluated in various preclinical models.

Methods: Compound IC50s were measured in a 33P uptake assay in NaP2b transfected cells, in a rabbit jejunal brush border membrane vesicle (BBMV) assay, and an everted jejunal sleeve P uptake technique. Pharmacodynamic response was tested by monitoring the uptake of 33P in ligated rat jejunum or by oral gavage in rats. P balance studies were performed where urine P was measured upon treatment with test compound for 4-7 days. NaP2b levels in gut were monitored by immunoblot.

Results: NaP2b inhibitors were evaluated for potency, non-competition with P (to ensure post-prandial inhibition of P absorption), systemic exposure and metabolic stability. Two classes of compounds are represented by NTX116 and NTX2128 with IC50s vs. rat NaP2b of 0.16 and 3.4 μM, respectively. In the BBMV and everted sleeve techniques, NTX116 inhibited sodium-dependent P uptake with an IC50 of ~5 μM. Orally dosing rats with 100 mg/kg NTX2128 resulted in a >50% reduction in serum 33P.

Using these NaP2b inhibitors and a novel Pit-1 (SLC20A1) inhibitor in the BBMV, everted sleeve, and ligated jejunum techniques, Pit-1 was found to contribute less than 10% to jejunal P uptake.

NTX2128 induced a reduction in urine P similar to Renvela but at 1/8th of the dose. The combination of NTX2128 and Renvela was additive in terms of reduction in urinary P, an observation consistent with their complementary mechanisms of action; i.e., intestinal NaP2b blockade and phosphate binding, respectively. In rats, NTX2128 was found to be minimally systemic with a high recovery of intact drug in feces (~95% of dosed material).

Conclusions: NaP2b inhibitors such as NTX2128 suppress active P transport in the gut and may offer a new treatment modality for P management in CKD and ESRD patients.

TH-OR021

Molecular Consequences of the SLC34A3 Mutations of Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) Akiko Ohi,[1] Sakiko Haito Sugino,[1] Mikiko Ito,[2] Yuji Shiozaki,[1] Kengo Nomura,[1] Yuri Kusaka,[1] Shohei Sasaki,[1] Saori Ohnishi,[1] Seiichi Yamaguchi,[1] Shinsuke Kido,[1] Sawako Tatsumi,[1] Hiroko Segawa,[1] Ken-Ichi Miyamoto.[1] *[1]Molecular Nutrition, Institution of Health Bioscience, University of Tokushima Graduate School, Tokushima, Japan; [2]University of Hyogo, Himeji, Japan.*

Background: The majority of the renal handling of phosphate (Pi) occurs via two sodium-dependent Pi cotransporters, SLC34A1 and SLC34A3. Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a metabolic disorder due to homozygous loss-of-function mutations in the SLC34A3 gene. SLC34A1 (NaPi-IIa) transporter is the most extensively characterized in terms of function, structure, and regulation, but such information for SLC34A3 (NaPi-IIc) is limited.

Methods: Mutations in the SLC34A3 protein (S138F, G196R, R468W and R564C) are responsible for HHRH. We studied the function and cellular localization of the mutant transporters in Xenopus oocytes and OK cells.

Results: In Xenopus oocytes, the levels of sodium-dependent Pi co-transport activities in G196R and R468W mutants were significantly decreased when comparing with those in wild type transporter. In both mutants, biotinylation assays revealed that lack of cell surface expression was associated with abolition of mature complex glycosylated NaPi-IIc. Immunohistochemical analysis showed that R468W and G196R co-localized with the ER marker protein, demonstrating that they are retained in the ER. In R468W and G196R, cell treatment with proteasome or lysosomal inhibitors failed to restore the loss of complex-glycosylated NaPi-IIc, further eliminating the possibility that the G196R and R468W mutants were processed by the Golgi apparatus. The G196R and R564C mutant proteins were sensitive to Endo H digestion. Reduction of the temperature overcomes trafficking defect of the mutant proteins. The analysis of blue native polyacrylamide gel electrophoresis showed the difference in the complex states between WT and G196R/R468W mutant.

Conclusions: The present data suggest that the G196R and R468W mutants cause the ER retention by protein folding defect, while S138F and R564C mutants stimulate its degradation in renal proximal tubular cells.

Funding: Government Support - Non-U.S.

TH-OR022

Effect of Conditional PiT-1 Transporter Deletion on Phosphate Uptake and Calcification in Cultured Mouse Vascular Smooth Muscle Cells Matthew H. Crouthamel, Cecilia M. Giachelli. *Bioengineering, University of Washington, Seattle, WA.*

Background: Elevated serum phosphate (Pi) in chronic kidney disease patients is recognized as a major risk factor for cardiovascular events as it predisposes to vascular calcification (VC). *In vitro*, elevated Pi levels stimulate vascular smooth muscle cell (VSMC) calcification. The sodium-dependent uptake of Pi into VSMC is mediated by the membrane bound type III sodium-dependent Pi (NaPi) cotransporters PiT-1 (SLC20A1) and PiT-2 (SLC20A2).

Methods: Using SM-22α driven Cre-recombinase transgenic mice, we have recently generated a conditional, smooth muscle specific PiT-1-deficient mouse ($PiT\text{-}1^{\Delta sm}$) to study the role of PiT-1 in VC. VSMCs were isolated and cultured from aortae of control ($PiT\text{-}1^{fl/fl}$) and $PiT\text{-}1^{\Delta sm}$ mice. RealTime rt-PCR, Pi uptake, and calcification assays were performed with these cells.

Results: RealTime rt-PCR confirmed the absence of full length PiT-1 mRNA in $PiT\text{-}1^{\Delta sm}$; however, PiT-2 expression was increased 2-fold in $PiT\text{-}1^{\Delta sm}$ compared to control VSMC. Pi uptake at normal Pi concentrations (1 mM) showed no significant difference between $PiT\text{-}1^{fl/fl}$ and $PiT\text{-}1^{\Delta sm}$. Interestingly, at hyperphosphatemic levels (2.6mM), while sodium dependent uptake was present in the $PiT\text{-}1^{fl/fl}$ VSMCs, it was absent in the $PiT\text{-}1^{\Delta sm}$ VSMCs. Addition of phosphonoformic acid did not alter Pi uptake, indicating that type II NaPi cotransporters were not contributing to Pi uptake in these VSMCs. Also, $PiT\text{-}1^{\Delta sm}$ VSMCs were larger and grew more slowly than controls. To assess the propensity for calcification, the VSMCs were cultured for 12 days in media containing 1 or 2.6 mM Pi. Final calcification levels were not significantly different between the $PiT\text{-}1^{fl/fl}$ and $PiT\text{-}1^{\Delta sm}$ genotypes.

Conclusions: Deletion of PiT-1 in mouse VSMCs did not cause a change in calcification in high or normal Pi media, or a difference in Pi uptake below 1mM Pi. However, at 2.6 mM Pi, Pi uptake was reduced in $PiT\text{-}1^{\Delta sm}$ VSMCs. These results may be due to PiT-2 compensation, which is currently being investigated.

Funding: Other NIH Support - HL081785, HL62329, T32 HL07828

TH-OR023

Upregulation of the Ca^{2+} Channel TRPV5 by Uromodulin – A Potential Mechanism for Hypercalciuria with *UMOD* Mutations Matthias Tilmann Florian Wolf, Chou-Long Huang. *Pediatrics and Medicine, UTSW Medical Center, Dallas, TX.*

Background: *Uromodulin* (*UMOD)* encodes Tamm-Horsfall protein. *UMOD* mutations result in tubulo-interstitial nephropathies. A genome-wide association study linked nephrolithiasis with a *UMOD* allele. Mouse models of human *UMOD* mutations have hypercalciuria, which exceeds the degree of Na^+ wasting, suggesting defective transcellular Ca^{2+} reabsorption in the distal nephron. We studied if UMOD regulates TRPV5, an apical Ca^{2+} channel in DCT and CNT critical for transcellular Ca^{2+} reabsorption.

Methods: We transfected TRPV5 and UMOD in HEK293 cells and analyzed TRPV5 current by whole cell patch-clamping.

Results: Coexpression of TRPV5 and UMOD showed increased TRPV5 current density (492+/-118 vs 294+/-104 pA/pF for UMOD vs vector; p=0.005). UMOD upregulates TRPV5 extracellularly: supernatant from UMOD - but not from vector-expressing cells - increased TRPV5 current density in TRPV5-transfected cells (856+/-148 vs 446+/-129 pA/pF for UMOD vs vector supernatant; p=0.0005). Biotinylation showed increased surface expression of TRPV5. Half-maximal effective concentration (EC50) for extracellular Tamm-Horsfall protein treatment in TRPV5-expressing cells was approximately 100 ng/ml, which is 100x lower than in human urine, underlining physiological significance. Cotransfection with disease-mutant $UMOD^{C150S}$ resulted in significantly less TRPV5 current compared to wild type UMOD (344+/-98 vs 852+/-121 pA/pF for mutant vs wild type UMOD; p=0.0005). We analyzed if UMOD regulation of TRPV5 is dependent on TRPV5 N-glycosylation by cotransfecting UMOD with glycosylation-defective N358Q-TRPV5 or with wild type TRPV5. We found that UMOD did not increase N358Q-TRPV5 (453+/-111 and 422+/-104 for N358Q+vector and N358Q+UMOD, respectively; ns) while it increased wild type TRPV5 (446+/-90 and 802+/-110 for WT+vector and WT+UMOD, respectively; p= 0.0001).

Conclusions: We show TRPV5 upregulation by extracellular UMOD at physiological levels. TRPV5 upregulation requires N-glycosylation of TRPV5, and is impaired by *UMOD* mutations. We suggest that in *Umod* mutations reduced UMOD secretion from the TAL decreases TRPV5-mediated Ca^{2+} reabsorption in the distal nephron contributing to hypercalciuria.

Funding: NIDDK Support, Other NIH Support - Pediatric Scientist Development Program

TH-OR024

Kidney-Specific Calcium-Sensing Receptor (CaSR) Deficient Mice Display PTH-Independent Hypocalciuria and NKCC2 Activation Hakan R. Toka,[1,2] David B. Mount,[1] Gary C. Curhan,[1] Martin R. Pollak.[2] [1]*Nephrology, Brigham and Women's Hospital, Boston, MA;* [2]*Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: The calcium-sensing receptor (CaSR) is a central player in regulating calcium homeostasis. Various studies have shown that rare loss-of-function mutations in the CaSR can lead to decreased urinary calcium (Ca2+) excretion in the context of relative hyperparathyroidism and hypercalcemia. In addition to playing a central role in regulating PTH release, evidence suggests that CaSR is directly involved in regulating calcium handling independently of PTH. The current study was designed to specifically examine the role of CaSR in the renal tubule.

Methods: We generated a conditional CaSR knockout (KO) mouse with targeted inactivation of exon 3 using the Cre/Lox system. A nephron-specific knockout of CaSR was generated by crossing CaSR flox/flox mice with animals expressing Cre recombinase driven by the Six2 promoter, targeting tubular epithelial cells throughout the entire nephron except for the collecting duct. Loss of CaSR in the kidneys of the resulting homozygous CaSR floxed mice was confirmed at both the RNA and protein level.

Results: Homozygous CaSR flox/flox mice were viable without gross phenotype. Interestingly, while baseline serum Ca2+, PTH and 1,25 Vitamin D levels showed no significant difference, kidney-specific CaSR KO mice showed a tendency to decreased urinary calcium excretion compared to controls. This effect was significantly increased when mice were challenged with increased dietary Ca2+ (1.5% Calcium water) and disappeared when animals were treated with furosemide. Western blotting analysis from whole kidney lysates with NKCC2-phospho antibody showed increased expression in Six2-Cre animals compared to control animals.

Conclusions: The CaSR expressed in renal tubules regulates Ca2+ re-absorption independently of PTH through the TAL. Further studies analyzing gastrointestinal Ca2+ re-absorption and addressing bone metabolism in these mice will be important to understand Ca+ metabolism regulated by CaSR in the kidney.

Funding: Other NIH Support - Program Project

TH-OR025

Claudin -16-Mediated Renal Magnesium Transport Is Transcriptionally Inhibited by 1,25($OH)_2$VitD Via a Calcium Sensing Receptor-Dependent Mechanism Orly Kladnitsky,[1,2] Julia Rozenfeld,[1,2] Edna Efrati,[1,2] Israel Zelikovic.[1,2] [1]*Lab of Developmental Nephrology, Faculty of Medicine-Technion;* [2]*Pediatric Nephrology, Rambam Medical Center, Haifa, Israel.*

Background: The role of 1,25$(OH)_2$VitD in renal Mg^{2+} handling is obscure. The bulk of filtered Mg^{2+} is reabsorbed in the TAL via the tight junction protein, claudin-16. We have shown that Mg^{2+} depletion increases and 1,25$(OH)_2$VitD inhibits claudin-16 gene (CLDN16) transcription. We aimed to further explore the molecular mechanisms underlying the effect of 1,25$(OH)_2$VitD on claudin-16.

Methods: Adult mice received **1.** 1,25$(OH)_2$VitD or **2.** 1,25$(OH)_2$VitD plus low Mg^{2+} diet for 3 days, and kidneys were harvested. HEK293 cells were exposed to treatments **1.** and **2.** for 24 hours. A luciferase reporter vector containing 2.5 kb human CLDN16 (hCLDN16) 5' flanking DNA sequence, was transfected into CaSR-devoid HEK293 cells (HEK293), HEK293 cells transfected with CaSR (HEK-CaSR), CaSR-harboring OK cells (OK), and OK cells transfected with a dominant negative CaSR construct (OK-DNCaSR).

Results: Treatments **1.** and **2.** in mice increased CaSR mRNA in kidneys. Treatment **1.** decreased claudin-16 mRNA and protein in kidneys but had no effect on claudin-2 mRNA. Treatment **2.** reversed the expected increase in claudin-16 mRNA and protein in Mg^{2+} depleted animals. HEK293 cells treated with **1.** and **2.** showed the same pattern of changes in claudin-16 mRNA as in mice, and no influence on mRNA of the Mg^{2+} channel, TRPM6 . Exposure of transfected HEK293 cells to 1,25$(OH)_2$VitD minimally decreased hCLDN16 promoter activity but markedly inhibited it in HEK-CaSR cells. A 1,25$(OH)_2$VitD induced inhibition of hCLDN16 promoter activity in OK cells was completely abolished in OK-DNCaSR cells. Furthermore, 1,25$(OH)_2$VitD decreased hCLDN16 promoter activity in Mg^{2+} depleted HEK293 cells.

Conclusions: In conclusion, 1,25$(OH)_2$VitD inhibits paracellular, claudin 16 mediated renal Mg^{2+} transport at the transcriptional level via a CaSR dependent mechanism. 1,25$(OH)_2$VitD dampens the Mg^{2+} depletion -induced stimulation of CLDN16 transcription. The 1,25$(OH)_2$VitD-induced repression of renal Mg^{2+} transport may serve as an adaptive mechanism to the 1,25$(OH)_2$VitD-induced increase in intestinal Mg^{2+} absorption.

Funding: Government Support - Non-U.S.

TH-OR026

1,25$(OH)_2$D Is Essential for PTH Stimulation of FGF23 Bing Dai, Valentin David, Aline Martin, Hua Li, Leigh Darryl Quarles. *Medicine, University of Tennessee Health Science Center, Memphis, TN.*

Background: FGF23 is an bone-derived hormone involved in an endocrine loop where 1,25$(OH)_2$D stimulates FGF23 and FGF23 suppresses 1,25$(OH)_2$D. FGF23 is elevated in CKD, where its relationship to hyperparathyroidism is controversial. FGF23-mediated reduction in 1,25$(OH)_2$D should lead to elevations in PTH, but PTH is reported to variably stimulate FGF23.

Methods: To investigate the role of 1,25$(OH)_2$D and PTH in the regulation of FGF23, we transferred $Cyp27b1^{-/-}$ and $Gcm2^{-/-}$ onto $Col4a3^{-/-}$ mouse CKD model and analyzed the resulting phenotype.

Results: Single mutant $Col4a3^{-/-}$ mice develop progressive renal failure along with elevations of serum PTH and FGF23 levels. $Gcm2^{-/-}$ exhibit low PTH, 1,25$(OH)_2$D and calcium levels. $Cyp27b1^{-/-}$ exhibit high PTH, low calcium and very low 1,25$(OH)_2$D levels. FGF23 levels are reduced in $Gcm2^{-/-}$ mice compared to WT. We have reported that administration of 1,25$(OH)_2$D increases FGF23 in $Gcm2^{-/-}$ mice. In contrast, FGF23 is undetectable in $Cyp27b1^{-/-}$ mice, in spite of high PTH levels. Thus, 1,25$(OH)_2$D can elevate FGF23 in the setting of low PTH, but PTH does not stimulate FGF23 in the setting of low 1,25$(OH)_2$D. Low PTH in compound $Col4a3^{-/-}/Gcm2^{-/-}$ mice and low 1,25$(OH)_2$D in compound $Col4a3^{-/-}/Cyp27b1^{-/-}$ mice are associated with reduced serum FGF23 concentrations. Factors other than 1,25$(OH)_2$D and PTH may also contribute to increased FGF23 in CKD, since FGF23 levels are higher in $Col4a3^{-/-}/Cyp27b1^{-/-}$ than $Cyp27b1^{-/-}$ mice.

Serum Biochemistries

Genotype:	WT	Col 4a3-/-	Cyp27b1-/-	Cyp27b1-/-	Gcm2-/-	Gcm2-/-
[Serum]				Col4a3-/-		Col4a3-/-
FGF23 (pg/ml)	137.3 ± 9.5	1248 ± 188 *	0.00 ± 0.0*	98 ± 34.5	24.1 ± 12.3*	130.2 ± 10.9
PTH (pg/ml)	32.2 ± 3.8	1772.1 ± 452.9*	2504± 530	2802± 459	5.2 ± 0.21	6.1 ± 0.1
1,25(OH)2D (pM)	89.6 ± 13.9	138.9 ± 21.6	6.7± 3.58*	3.1 ± 1.6*	30.1 ± 2.7*	32.6 ± 6.3*
Calcium (mg/dl)	8.7 ± 0.20	9.4 ± 0.3	5.1 ± 0.30*	5.1 ± 0.3*	4.5 ± 0.4*	5.4 ± 0.7 *

Data as mean±SEM. n≥7 per group, *p<0.05 vs. WT.

Conclusions: 1,25$(OH)_2$D plays a dominant role to PTH in regulating FGF23. Further studies are needed to determine the mechanisms for the cross-talk between 1,25$(OH)_2$D and PTH and the variable effects of PTH to regulate FGF23 expression.

Funding: NIDDK Support

TH-OR027

Activation of the Calcium-Sensing Receptor Induces Tight Junction Formation in MDCK Cells Francois Jouret, John P. Geibel, Michael J. Caplan. *Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT.*

Background: The calcium-sensing receptor (CaSR) belongs to the G-protein-coupled receptor superfamily, and plays a critical role in divalent ion homeostasis, as well as in cell growth and differentiation. Since extracellular Ca2+ (Ca2+e) is essential for the development of stable epithelial tight junctions (TJ), we hypothesized that CaSR contributes to the assembly of TJ during epithelial cell polarization.

Methods: First, we assessed the level of CaSR expression in Madin-Darby Canine Kidney (MDCK) cells following manipulations that modulate TJ assembly. Next, we examined the effects of CaSR agonists and antagonists on TJ assembly.

Results: Real-time RT-PCR and immunoblotting analyses showed that switching MDCK cells from low (5μM) to normal (1.8mM) Ca2+e concentration significantly increases CaSR expression. Exposure of MDCK cells to both type I, i.e. neomycin (1mM), gadolinium (100μM), and type II, i.e. R-568 (800nM), CaSR agonists facilitated TJ formation under normal Ca2+e conditions, as measured by the relocation of Zonula Occludens 1 (ZO-1) and occludin. Moreover, CaSR activation in the absence of Ca2+e initiated TJ assembly, and this effect occurred without inducing the phosphorylation of AMP-activated protein kinase. Co-immunoprecipitation studies further showed that CaSR activation increases the interaction between ZO-1 and the F-actin-binding protein, afadin-I. By contrast, CaSR inhibition by NPS-2143 (800nM) significantly decreased ZO-1/afadin-I interaction and reduced ZO-1 deposits at cell surface following exposure to culture media containing a 200 μM Ca2+e concentration.

Conclusions: These results suggest that CaSR plays a role in the regulation of TJ assembly during epithelial cell polarization.

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TH-OR028

PINCH-1 and -2 Are Essential for Podocyte Adhesion, Shape Modulation and Maintenance of Glomerular Filtration Barrier Function Jeffrey B. Hodgin,[1] Hong Qu,[3] Xingqun Liang,[4] Simone M. Blattner,[2] Ka Chen,[3] Courtenay M. Vining,[2] Stephanie Wylie,[2] Masashi Nishio,[2] Ju Chen,[4] Chuanyue Wu,[3] Matthias Kretzler.[2] *[1]Pathology, University of Michigan, Ann Arbor, MI; [2]Nephrology, University of Michigan, Ann Arbor, MI; [3]Pathology, University of Pittsburgh, PA; [4]Medicine, University of California, San Diego, CA.*

Background: PINCH proteins are 5 LIM domain-only adaptor proteins that have a key role in cell-extracellular matrix adhesion and communication by linking integrins to the actin cytoskeleton. Two PINCH proteins that share high homology, PINCH-1 and PINCH-2, are expressed in most cells including podocytes. Podocyte-specific PINCH-1 knockout (KO) or global PINCH-2 KO mice exhibit no basal renal phenotype, which suggests a redundant role for these proteins in the kidney.

Methods: Therefore we crossed podocyte-specific PINCH-1 KO with global PINCH-2 KO mice to generate mice doubly homozygous null for PINCH proteins in podocytes. In addition, PINCH-1 and PINCH-2 expression in human podocytes in culture was 'knocked-down' using siRNA technology.

Results: Though normal at birth, double KO podocyte-specific PINCH-1/2 mice displayed severe proteinuria by 4 weeks of age. Morphologically they display focal segmental and global glomerulosclerosis and ultrastructurally, podocytes display diffuse foot process effacement. By 8 weeks proteinuria and glomerulosclerosis has progressed to encompass most of the nephrons (end-stage renal disease) and death follows. In vitro, single knockdown of either protein resulted in minor defects in cell spreading and cell monolayer permeability to albumin. Double knockdown of both PINCH-1 and PINCH-2 demonstrated more severe defects in cell spreading and permeability, and also showed significant decrease in cell adhesion to fibronectin, suggesting that PINCH proteins are critical for optimal podocyte adhesion and shape modulation.

Conclusions: These results demonstrate essential roles for PINCH proteins in maintenance of podocyte integrity and the glomerular filtration barrier, highlighting the importance of studying the role of PINCH proteins in human glomerular disease.

Funding: NIDDK Support

TH-OR029

Loss of Talin 1 in Podocytes Results in Severe Progressive Albuminuria and Kidney Failure Xuefei Tian,[1] Keita Soda,[1] Rena Zheng,[1] Roy Zent,[2] Shuta Ishibe.[1] *[1]Internal Medicine/Nephrology, Yale University, New Haven, CT; [2]Internal Medicine/Nephrology, Vanderbilt University, Nashville, TN.*

Background: Talin 1 (Tln1) is a focal adhesion protein, linking beta integrins to the actin cytoskeleton. The importance of Tln1 in podocyte biology is poorly understood.

Methods: Using the Cre-LoxP system, we generated podocyte specific Tln1 KO mice. Albuminuria and creatinine were measured by ELISA. Kidney histology was analyzed by H.E, PAS, trichrome, and electromicroscopy (EM). Apoptosis was assayed by TUNEL. Primary podocytes were isolated by Dynalbeads and sieving. Adhesion was assayed by crystal violet staining. Immunofluorescence was performed for phalloidin and actin related proteins.

Results: Tln1 KO mice were born in the expected mendelian frequency. Compared to wild type littermates, KO showed failure to gain weight by 3 weeks (gms. WT: 9.7±0.4, KO: 7.3±0.4. n=9. P<0.01), progressive albuminuria which started 2 weeks after birth (ug albumin /mg creatinine (WT vs KO): 2 weeks: 21.46±3.29 vs 942.10±69.42; 4 weeks: 16.60±0.97 vs 1357.60±72.08; 6 weeks: 15.68±2.19 vs 1798.60±115.60; 8 weeks: 18.04±3.23 vs 3021.60±469.93. n=4) , and kidney failure (serum creatinine (mg/dl) (WT vs KO): 2 weeks: 0.25±0.15 vs 0.72±0.33; 4 weeks: 0.21±0.13 vs 0.96±0.25; 8 weeks: 0.24±0.12 vs 1.25±0.35. n=6) . The majority of Tln1 KO mice were dead by 8 weeks. Histological examination in Tln1 KO mice revealed glomerular capillary loop dilation which progressed to glomerulosclerosis, and interstitial fibrosis. EM demonstrated foot process effacement and basement membrane thickening. TUNEL staining of kidney sections demonstrated no significant difference in podocyte apoptosis between KO and WT mice at P14. Isolated podocytes from KO demonstrated modest reduction in adhesion on type I collagen and beta 1 integrin activation (OD: WT: 0.28±0.03, KO: 0.19±0.02, n=6, p<0.01). However, Tln1 KO podocytes had a major reduction in stress fiber formation compared to WT.

Conclusions: Loss of Tln1 specifically in mice podocytes results in severe albuminuria progressing to kidney failure and death by 8 weeks of age, suggesting its fundamental role in regulating the structural integrity of the glomerulus.

Funding: NIDDK Support

TH-OR030

Requisite Role for Nephrin Tyrosine Phosphorylation in Podocyte Morphology and Response to Injury Laura A. New,[1] Bozena Samborska,[1] Vera Eremina,[2] Susan E. Quaggin,[2,3] Nina Jones.[1] *[1]Molecular and Cellular Biology, University of Guelph, ON, Canada; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [3]Division of Nephrology, St. Michael's Hospital, Toronto, ON, Canada.*

Background: Nephrin is a key component of the kidney slit diaphragm that contributes to podocyte foot process morphology and filtration barrier function. In addition to its structural role, nephrin also serves as an intracellular signalling scaffold through its ability to undergo tyrosine phosphorylation and recruit adaptor proteins, such as Nck, to the slit diaphragm, where they link nephrin to the actin cytoskeleton. However, the physiological significance of nephrin tyrosine phosphorylation and its role in the development of glomerular disease is not well understood.

Methods: To investigate the role of nephrin phosphotyrosine-based signalling in vivo, we have generated mice in which the tyrosine residues at all three complementary Nck binding sites have been altered to phenylalanine to prevent their phosphorylation (herein referred to as nephrinY3F mice).

Results: NephrinY3F mice with disrupted nephrin signalling develop progressive and significant proteinuria beginning at approximately three months of age, and this is accompanied by glomerulosclerosis and widespread foot process effacement. Furthermore, several podocyte signalling proteins appear to be mislocalized from a linear into a granular pattern in nephrinY3F mice. Finally, prior to the onset of proteinuria, nephrinY3F mutant mice display an enhanced susceptibility to podocyte injury induced by nephrotoxic challenge.

Conclusions: Taken together, this model demonstrates that nephrin signalling through these conserved tyrosine motifs is required for maintenance of the glomerular blood filtration barrier. Our findings are consistent with the observation that nephrin phosphorylation is reduced in some glomerular diseases, and they suggest that phosphotyrosine-based signalling pathways at the kidney slit diaphragm provide highly dynamic control over foot process morphology.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-OR031

GLEPP1 Reduces Endocytosis of Nephrin Eva Koenigshausen, Sarah Grabowski, Laura Gerbaulet, Ivo Quack, Magdalena Woznowski, Paul Probst, Sebastian Alexander Potthoff, Lars C. Rump, Lorenz Sellin. *Nephrology, Heinrich Heine University, Duesseldorf, Germany.*

Background: GLEPP1 is a receptor tyrosine phosphatase expressed in the apical membrane of foot processes. GLEPP1 expression is reduced in proteinuric kidney diseases. Previously we could show that the interaction of nephrin with podocin and β-arrestin2 and endocytosis of nephrin is regulated by src family kinases. This observation led to the concept of the dynamic regulation of the glomerular slit diaphragm. GLEPP1 was implicated to be a protective factor for the glomerular slit. We hypothesize a protective regulatory role for GLEPP1 in respect to nephrin endocytosis.

Methods: We used cells expressing GLEPP1 and nephrin, podocin, src, fyn, β-arrestin2 or paxillin. After cell lysis, co-immunoprecipitation with subsequent western blot analysis was performed. For immunofluorescence, cells expressing GLEPP1 and paxillin.GFP were fixed and permeabilized thereafter. GLEPP1 was visualized by immunofluorescence.

Results: GLEPP1 interacts with the slit diaphragm proteins nephrin and podocin as well as with the src family kinases src and fyn. Furthermore, GLEPP1 enhances the interaction of nephrin with podocin and reduces the interaction of nephrin with β-arrestin2. GLEPP1 dephosphorylates src and fyn at their regulatory Y527. The interaction of src/fyn with a substrate trapping mutant of GLEPP1 is enhanced compared to the wild-type phosphatase which indicates that src and fyn are indeed substrates of GLEPP1. GLEPP1 reduces nephrin endocytosis. In addition, GLEPP1 expression induces cytoskeletal rearrangement.

Conclusions: The integrity of the glomerular slit diaphragm is regulated by src kinase mediated tyrosine phosphorylation of the nephrin C-terminus. We have substantial evidence that GLEPP1 activates src family kinases through interaction and dephosphorylation of src family kinases at their regulatory domain. Activated src family kinases consequently

increase nephrin tyrosine phosphorylation at its C-terminus. Additionally, GLEPP1 seems to force cytoskeletal changes which might be of importance maintaining the delicate shape of healthy foot processes. Via this mechanism, GLEPP1 may support the integrity of the slit diaphragm.

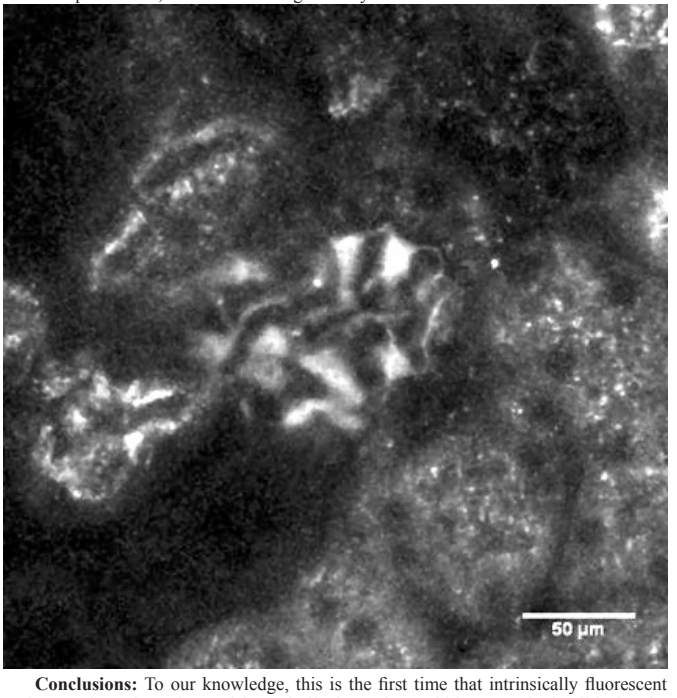
TH-OR032

Intravital Multiphoton Imaging of Mouse Podocytes Mark Fadi Khayat,[1] Charbel C. Khoury,[1] Tet-Kin Yeo,[1] Petr Pyagay,[1] Weiming Yu,[2] Sheldon C. Chen.[1] *[1]Nephrology/Hypertension, Northwestern University, Chicago, IL; [2]Physiology, Northwestern University, Chicago, IL.*

Background: Multiphoton intravital microscopy (MPIM) is an ideal methodology, due to its minimal phototoxicity, to image podocytes in their native glomerular environment. However, most glomeruli in the mouse lie beyond the maximum imaging depth of MPIM in the kidney, which is 150 to 200 μm. We intend to show that intravital imaging of murine podocytes is now feasible with the nephrin knockout/green fluorescent protein (GFP) knock-in mouse (Nphs1tm1Rkl/J).

Methods: The Nphs1tm1Rkl/J mouse was genetically designed to have the nephrin promoter restrict GFP expression to the podocyte in the kidney. Under anesthesia, the mice were perfused with 500-KDa Texas Red dextran via the retro-orbital sinus, using a 28G insulin syringe. The left kidney was then exposed and imaged through a custom-built window on an upright multiphoton microscope.

Results: Optical sections of the renal cortex revealed green fluorescent podocytes, clearly resolvable down to their primary processes, as they lined the glomerular capillaries perfused with Texas Red dextran. In the female Nphs1tm1Rkl/J mouse, the glomerular depth was as shallow as 60 μm. This allowed detailed intravital microscopy of an intact kidney, avoiding the need for surgical sectioning of the renal cortex and enabling repeat imaging in the same mouse. Multiple time-lapse series of different glomeruli were collected, typically for hours per session, while maintaining viability of the mouse.



Conclusions: To our knowledge, this is the first time that intrinsically fluorescent podocytes have been imaged intravitally in an intact kidney, made feasible by the proximity of the glomeruli to the kidney capsule. Our technique offers the opportunity to add a visual and temporal component to the study of podocytopathies in the multitude of mouse models.

Funding: NIDDK Support

TH-OR033

Lack of Functioning HVPS34 in Podocytes Disrupts Cellular Homeostasis and Causes Significant Proteinuria Jianchun Chen, Raymond C. Harris, Jian-Kang Chen. *Medicine, Vanderbilt University, Nashville, TN.*

Background: Podocyte injury is a leading factor of many glomerular diseases and renal failure. Recent studies suggest that autophagy is a key mechanism maintaining the integrity of podocytes. HVPS34 (the homologue of yeast vacuolar protein sorting defective 34 in mammalian cells) has been implicated in the regulation of autophagy, but its role in podocytes has not previously been explored.

Methods: We generated a HVPS34-floxed mouse ($HVPS34^{flox/flox}$) and crossed with Podocin-Cre mice to produce podocyte-specific HVPS34 knockout mice ($HVPS34^{pdKO}$) and compared them with their $HVPS34^{flox/flox}$;Podocin-$Cre^{(-)}$ wild type littermates (*WT*).

Results: $HVPS34^{pdKO}$ mice were smaller (11.86 ± 0.72g, N=7 vs. *WT*: 20.55 ± 0.95g, N=6-7; P<0.0001) without any differences in kidney weight/body weight. $HVPS34^{pdKO}$ developed significant proteinuria at the age of 6 weeks (Alb/Cre (mg/mg): *WT*: 4.020 ± 1.264, vs. $HVPS34^{pdKO}$: 127.9 ± 16.16, N=6-7; P<0.0001). There was striking vacuolization of podocytes in the glomeruli as well as numerous protein casts. Electron microscopy indicated the vacuolated podocytes were filled with numerous autophagic vacuoles and enlarged lysosomes, with foot process effacement/fusion. The GBM was also irregular and thickened. Immunofluorescent staining for the lysosomal marker protein, LAMP1, confirmed that the majority of the vacuoles in the podocytes were LAMP1-positive. Podocin protein levels of the podocytes were markedly down-regulated, revealing a key role for HVPS34 in maintaining the cellular homeostasis of the podocytes.

Conclusions: These novel findings indicate an essential role for HVPS34 to protect the normal cellular metabolism, structure and function of podocytes.

Funding: NIDDK Support

TH-OR034

Inhibition of mTOR in Mice Alters Autophagic Flux in Podocytes and Other Renal Cell Types Davide Pietro Cina,[1,2] Tuncer Onay,[2] Aarti Paltoo,[2] Javier De Arteaga,[3] Chengjin Li,[2] Susan E. Quaggin.[1,2] *[1]Faculty of Medicine, University of Toronto, ON, Canada; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [3]Division of Nephrology, Hospidal Privado, Cordoba, Argentina.*

Background: Inhibitors of the mammalian target of rapamycin (mTOR inhibitors) belong to a family of drugs with potent immunosuppressive, anti-angiogenic and anti-proliferative properties. Although they are approved for the treatment of a number of renal diseases, the use of mTOR inhibitors has been associated with a significant incidence of de novo or worsening proteinuria.

Methods: Here we explore the mechanism of proteinuria induced by mTOR inhibition through the generation and characterization of mice carrying a podocyte-selective knockout (mTOR pod-KO) or global postnatal knockout of the mTor gene resulting in a loss of both mTORC1 and mTORC2 functions.

Results: Our results show that while mTOR is dispensable in developing podocytes, mTOR pod-KO mice develop nephrotic-range proteinuria by 3 weeks of age, and progress to endstage renal failure by 5 weeks of age. Human podocytes treated with the mTOR inhibitor rapamycin accumulate autophagolysomes, damaged mitochondria and increased levels of reactive oxygen species. In vivo, podocytes of mTOR pod-KO mice exhibit an accumulation of the autophagosome marker LC3 (rat microtubule-associated protein 1 light chain 3), autophagosomes, autophagolysosomal vesicles and damaged mitochondria. Global postnatal deletion of mTOR affects all renal cells leading to marked enhancement of renal injury, demonstrating that mTOR function is not only important for podocytes, but also for the tubular epithelium.

Conclusions: Taken together, our results suggest that disruption of the autophagic pathway may play a role in the pathogenesis of proteinuria in patients treated with mTOR inhibitors and that multiple renal cell types may be affected.

Funding: Government Support - Non-U.S.

TH-OR035

Translational Profiling of Podocytes in Focal Segmental Glomerulosclerosis Ivica Grgic,[1] Giulio Genovese,[2] Martin R. Pollak,[2] Benjamin D. Humphreys.[1] *[1]Brigham and Women's Hospital, Boston, MA; [2]BIDMC, Boston, MA.*

Background: Identifying new biomarkers and therapeutic targets for podocytopathies such as focal segmental glomerulosclerosis (FSGS) requires a detailed understanding of the earliest events in disease pathogenesis. Transcriptional profiling has already provided important insight into gene expression changes in FSGS. Yet, a key limitation of this approach to define the podocyte transcriptome in FSGS has been the analysis of whole glomerular mRNA rather than podocyte-specific mRNA.

Methods: We have engineered a novel transgenic mouse line that allows extraction of podocyte-specific mRNA from whole kidney by translating ribosome affinity purification (TRAP). In this model, a GFP-tagged ribosomal protein (eGFP-L10a) was expressed under the control of the collagen 1α1 promoter.

Results: Expression of eGFP-L10a was restricted to podocytes in kidney cortex. Expression analysis of TRAP-isolated RNA by whole genome arrays confirmed robust enrichment of podocyte-specific mRNA (podocin: ~60-fold, nephrin: ~32-fold, MYH9: ~47-fold compared to whole cortex), permitting establishment of a podocyte expression fingerprint. We next crossed col1α1-L10a mice with actn4+/- and mutant actn4+/K228E mice to study expression profiles of podocytes in a genetic form of FSGS. RNA was extracted by TRAP from cortex of bigenic col1α1-L10a;actn4-/- and col1α1-L10a;actn4+/K228E and single-transgenic col1α1-L10a littermate controls at 2 and 6 weeks of age. Regression analysis comparing podocyte expression profiles between genotypes with age as a covariate identified significantly up- and downregulated genes, including Cxcl1 (~12-fold up, p<0.0001) and Gadd45b (~5-fold up; p<0.001) for actn4-/- vs actn4+/+ and DMPK (~5-fold up; p<0.0001) for actn4+/K228E vs. actn4+/+. We confirmed some of the largest changes in gene expression at the protein level by immunostaining.

Conclusions: In conclusion, we have developed a new approach to define podocyte-specific gene expression in vivo. We used this technique in a model of FSGS and identified new candidate genes that hold promise in elucidating the earliest signaling events in FSGS, and thereby provide potential new therapeutic targets for FSGS.

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

TH-OR036

Multiphoton Imaging of the Development of Glomerulosclerosis In Vivo
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Background: The development of podocyte injury/dysfunction and albuminuria in glomerulosclerosis (GS) are still incompletely understood due to technical limitations in studying the glomerular filtration barrier (GFB) in its native environment in vivo. We aimed to directly visualize the early morphological and functional changes in GFB using in vivo multiphoton microscopy (MPM) and the puromycin (PAN) model of focal segmental GS.

Methods: Munich-Wistar-Fromter rats (200 g) were given 100 mg/kg body weight PAN ip and surgically instrumented for MPM 4-10 days later. The plasma was labeled using albumin-Atto565, and Lucifer yellow (LY) was infused into the carotid artery to visualize GFB elements.

Results: Hypertrophy and cystic dilatations of the sub-podocyte space developed in several, but not all podocytes 4 days after PAN. The largest podocyte cysts, as the core structure, formed small, focal adhesions of the glomerular tuft to the parietal Bowman's capsule (synechiae) 7-10 days after PAN. The thin podocyte wall of these core cysts were highly permeable to albumin indicated by the intense red fluorescence of cyst fluid. Intact, healthy podocytes (normal shape and no albumin permability) were also observed making contact with parietal cells in other glomerular regions, but never developed synechiae. Numerous new, densely packed small cells that appeared to be healthy and migrating were observed on the outside of the core cysts, forming a continuous cell layer (bridge) between podocytes and parietal cells. Interestingly, most synechiae were positioned next to a collecting duct segment; intense infiltration of immune cells with high albumin uptake was visible in this region of the periglomerular interstitium. Similar results were obtained in 3-6mo old MWF rats, a model of spontaneous FSGS.

Conclusions: In several animal models of GS, high albumin permeability is focal, restricted to the most injured podocytes and to the area of synechiae. The formation of synechiae appears to involve multiple intra- and extraglomerular cell types. Signs of podocyte repair (replacement) were evident, but their mechanism needs further study.

Funding: NIDDK Support

TH-OR037

Novel Concepts in Nephrotic Syndrome: Angiopoietin-Like 4 Induced Hypertriglyceridemia Results from a Multisystem Effort To Reduce Proteinuria
Lionel C. Clement, Camille E. Mace, Sumant S. Chugh. *Medicine/Nephrology, University of Alabama at Birmingham, AL.*

Background: Hypertriglyceridemia is a cardinal feature of nephrotic syndrome. A recent study (Clement Nature Medicine 2011) shows that podocyte secreted, but not circulating Angiopoietin-like-4 (Angptl4) also noted in minimal change disease, induces proteinuria. Here, we investigated the role of circulating Angptl4 in nephrotic syndrome.

Results: Circulating Angptl4 is known to inhibit endothelium bound lipoprotein lipase, which reduces tissue uptake of triglycerides from circulation, resulting in hypertriglyceridemia. We induced the gamma2-NTS and LPS models of proteinuria in Angptl4 -/- and +/+ mice. Angptl4 -/- mice did not develop hypertriglyceridemia despite significant residual proteinuria, suggesting that Angptl4 is the key determinant of hypertriglyceridemia in nephrotic syndrome. Next, we studied albuminuria in adipose tissue specific Angptl4 transgenic rats (aP2-Angptl4), that have hypertriglyceridemia due to increased circulating Angptl4, and noted reduced albuminuria in transgenic compared to wild type rats ($P<0.05$). Induction of PAN in these rats caused lower levels of proteinuria (Day 8 wild type 315±2 mg/18 hours, transgenic 194±25 mg/18 hours, mean + SE, $P<0.05$). Immunogold EM showed binding of Angptl4-V5 to glomerular endothelial surface. Cultured rat glomerular endothelial cells were protected from oxidative stress by recombinant neutral pI Angptl4 similar to that present in the circulation ($P<0.001$). By inducing puromycin nephrosis in podocyte specific Angptl4 transgenic rats (NPHS2-Angptl4), we determined that beyond the initial stages, the bulk of the circulating Angptl4 is contributed to by peripheral tissues, predominantly adipose tissue. In addition, injection of recombinant neutral pI Angptl4 reduces proteinuria in multiple animal models of proteinuria.

Conclusions: Our studies suggest that in later stages of nephrotic syndrome, adipose tissue secretes Angptl4 into the circulation to reduce proteinuria by glomerular endothelial stabilization, but this increase in circulating Angptl4 also results in hypertriglyceridemia.

Funding: NIDDK Support

TH-OR038

Acute Podocyte VEGF-A Knockdown Disrupts αVβ3 Integrin Signaling in the Glomerulus
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Methods: To study the molecular mechanism involved in the pathogenic effects of podocyte VEGF-A knockdown, we developed a mouse model that silences all VEGF-A isoforms in the podocyte using an inducible shRNA approach, and we generated an immortalized podocyte cell line ($VEGF^{KD}$) that downregulates VEGF-A upon doxycycline exposure.

Results: *Tet-O-siVEGF:podocin-rtTA* (*siVEGF*) mice express VEGF shRNA in podocytes in a doxycycline-regulated manner, decreasing VEGF-A mRNA and VEGF-A protein levels in isolated glomeruli to ~20% of non-induced controls and urine VEGF-A to ~30% of control values a week after doxycycline induction. VEGF knockdown in adult *siVEGF* mice causes acute renal failure and proteinuria, associated with decreased glomerular volume, mesangiolysis and microaneurisms. Glomerular ultrastructure revealed endothelial cell swelling, GBM lamination and podocyte effacement. VEGF knockdown downregulates podocyte fibronectin and glomerular endothelial αVβ3 integrin in vivo, as determined by immunoblotting and dual-labeling immunohistochemistry. Co-immunoprecipitation showed that VEGFR2 interacts with β3 integrin and neuropilin-1 in the kidney in vivo and in cultured podocytes. Podocyte VEGF knockdown disrupts αvβ3 integrin activation in glomeruli, as determined by WOW1 Fab immunolabeling, while VEGFR2-αvβ3 integrin interaction and phosphorylation remain intact. VEGF silencing in cultured $VEGF^{KD}$ podocytes downregulates fibronectin and disrupts αvβ3 integrin activation cell-autonomously.

Conclusions: 1) *In vivo* podocyte VEGF-A regulates αvβ3 integrin signaling in the glomerulus; 2) decreased autocrine and paracrine VEGFR2 signaling induced by podocyte VEGF knockdown disrupts VEGFR2 - αvβ3 integrin crosstalk and damages the three layers of the glomerular filtration barrier, resulting in proteinuria and acute renal failure. The present studies uncover a specific molecular mechanism mediating VEGF-A requirement in the adult glomerulus, i.e. activation of αvβ3 by VEGFR2 signaling.

Funding: NIDDK Support

TH-OR039

TGF-β1 Signals through Smad4 to Diversely Regulate Renal Inflammation and Fibrosis by Impairing TGF-beta/Smad3 and Smad7 Transcriptionally
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Background: TGF-β1 plays distinct roles in renal inflammation and fibrosis, but signaling mechanisms by which TGF-β1 diversely regulates these processes remain largely unclear. The present study tested the hypothesis that Smad4 may be a key regulator of TGF-β signaling in renal disease.

Methods: We first generated the kidney-specifically conditional Smad4 KO mouse by crossing $Smad4^{F/F}$ to KspCre mouse in which Smad4 was specifically deleted from tubular epithelial cells. To determine the functional role of Smad4 in renal inflammation and fibrosis, a mouse model of unilateral ureteral obstructive nephropathy (UUO) was induced in conditional Smad4 KO mice. Furthermore, role and mechanisms of Smad4 in regulating renal inflammation and fibrosis were investigated in vitro in macrophages and fibroblasts that lack Smad4.

Results: Disrupted Smad4 significantly enhanced renal inflammation by a much greater CD45+ leukocyte(25% ↑) and F4/80+ macrophage infiltration (60%↑) and upregulation of IL-1β, TNFα, and MCP-1 in the UUO kidney and in IL-1β-stimulated macrophages (all $p<0.05$). In contrast, deletion of Smad4 inhibited kidney fibrosis and TGF-β1-induced collagen I expression by fibroblasts ($p<0.01$). Further studies revealed that loss of Smad7 and its responsive promoter activity, thereby inhibiting IκBa expression while enhancing NF-κB activation, were a central mechanism by which disrupted Smad4 promoted renal inflammation. Interestingly, impaired Smad3 transcription such as Smad3 responsive promoter activities and the binding of Smad3 to collagen promoter (COL1A2), but not Smad3 activation, was an underlying mechanism whereby disrupted Smad4 blocked fibrosis in vivo and in vitro.

Conclusions: TGF-β1 acts by stimulating Smad4 to diversely regulate renal inflammation and fibrosis. Transcriptionally impaired Smad7-dependent anti-inflammatory activity and Smad3-mediated fibrogenesis may be key mechanisms by which disrupted Smad4 enhances renal inflammation while inhibiting fibrosis in vivo and in vitro.

Funding: Government Support - Non-U.S.

TH-OR047

Dot1 Deficiency Facilitates Derivation of Renal Intercalated Cells from AQP2-Positive Cells and Increases Water Excretion Hongyu Wu,[1] Lihe Chen,[1] Qiaoling Zhou,[2] Mary R. Reisenauer,[1] Stefan Berger,[3] Günther Schütz,[3] Dennis Brown,[4] Hua Ann Jenny Lu,[4] Yang Xia,[1] Wenzheng Zhang.[1] *[1]University of Texas Medical School at Houston; [2]Central South University; [3]German Cancer Research Center; [4]Massachusetts General Hospital and Harvard Medical School.*

Background: The mammalian kidney has three distinct types of collecting duct cells: principal cells (PC), α-intercalated cells (α-IC) and β-intercalated cells (β-IC), which are responsible for sodium and water balance, acid secretion, and bicarbonate secretion, respectively. The epigenetic players regulating differentiation of these cells remain elusive. Histone H3 K79 methyltransferase Dot1 may regulate this process as it is expressed in PC in mouse kidney.

Methods: To test this hypothesis, the Cre-LoxP system was used to develop a mouse model lacking Dot1 in AQP2-expressing cells (Dot1f/f AQP2Cre). Double immunofluorescence staining and metabolic analysis were performed to identify the cellular and renal phenotype.

Results: With AQP2 and V-ATPase subunits B1/B2 as PC and IC markers, respectively, we found that the mutant mice had ~20% fewer PC in cortex, outer medulla and inner medulla vs controls. This change was coupled with a similar increase in α-IC, featured by AE1 expression. Dot1 deletion in PC abolished histone H3 K79 methylation and had no effect on total H3 or methylation of all other H3 residues tested. Unexpectedly, 68-75% of ICs in Dot1 mutant mice also had no detectable H3 K79 methylation. At least some of these IC also expressed other IC markers (V-ATPase subunit A and carbonic anhydrase II) and/or the β-IC marker pendrin. In contrast, all PC and IC in controls had H3 K79 methylation. The mutants had significantly increased urine volume (by 40%), 18% decreased urine osmolarity, and 12% lower urinary [Na+] vs. controls.

Conclusions: We show here that Dot1 is solely and specifically responsible for H3 K79 methylation in mouse kidney, and at least some α-IC and β-IC may be derived from AQP2-expressing progenitor cells or can be derived from mature PC. We have, in conclusion, identified Dot1 as a new regulator of PC and IC differentiation and, thus, water, Na+ and possibly pH regulation.

Funding: NIDDK Support, Private Foundation Support

TH-OR041

CTGF Influences Blood Pressure and Albumin Excretion in Mice Leighton R. James,[1,2] Catherine Le.[2,3] *[1]Medicine, University of Florida, Jacksonville, FL; [2]Medicine, UT Southwestern Medical Center, Dallas, TX; [3]Department of Cellular and Molecular Biology, Colorado State University, Fort Collins, CO.*

Background: CTGF (CCN2) is a pleiotropic growth factor belonging to the 30-40kDa CCN family of proteins that have been shown to exhibit a diverse array of cellular effects. We, and others, have shown that reduced ctgf expression attenuates the response to hyperglycemia. CTGF protein levels are increased in resistance and capacitance vessels in spontaneously hypertensive rats (SHR) and maneuvers that reduce blood pressure also lead to reduced CTGF expression. Given the role of CTGF in extracellular matrix production, it is proposed that ctgf expression will impact on glomerular physiology and function.

Methods: The potential effect(s) of CTGF on blood pressure, albumin excretion and kidney histopathology was tested in mice models. We studied CTGF knockout and gene-duplicated mice, both generated through gene targeting using standard gene-targeting methodologies.

Results: We observed a graded increase in CTGF gene expression with increasing gene copy number. There was no significant difference in expression between mice or mice embryonic fibroblasts (MEFs) with 3 and 4 copies of ctgf, suggesting a possible plateau effect in which expression does not increase above a specific number of copies of ctgf gene. Analysis of blood pressure by automated tailcuff method revealed an increasing mean systolic blood pressure with ctgf gene copy-number, suggesting that ctgf expression may influence blood pressure in this line of mice. There was attenuation of basal albumin excretion rate (AER) in CTGF heterozygotes and increased excretion in 3-copy animals compared to 2-copy (wt) mice. In 9 month mice, there was evidence of increased interstitial fibrosis in kidney sections from 3- and 4-copy animals compared with wildtype and heterozygous age-matched animals.

Conclusions: These observations suggest that CTGF expression may be a determinant of blood pressure and kidney function in mice.

Funding: Other NIH Support - NHLBI, NIDDK, Private Foundation Support

TH-OR042

The Role of Erythropoietin Receptors in Kidney Disease Progression Masayuki Takase,[1] Jin Nakamura,[1] Atsushi Fukatsu,[1] Masayuki Yamamoto,[2] Motoko Yanagita.[1] *[1]Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Graduate School of Medicine, Tohoku University, Sendai, Miyagi, Japan.*

Background: Erythropoietin (EPO) is a hormone indispensable for red blood cell production, and mainly produced in the fibroblast-like cells in the kidney after birth. In addition to the role in erythropoiesis, potential protective activity of EPO is reported in various tissues. EPO binds to receptor complex consisting of EPO receptor (EPOR) and ß common receptor (CD131) subunit for tissue protective effect, while it binds to homodimeric EPOR for maturation of erythrocytes. However molecular mechanism and the contribution of endogenous EPO in tissue protection remained elusive.

Methods: In the present study, we analyzed the renoprotective function of endogenous EPO utilizing EPOR knock out mice.

Results: First we analyzed the expression of EPO receptors during kidney injury. The expression of EPOR was decreased whereas the expression of CD131 was increased in mouse models of kidney disease. We further demonstrated that the administration of EPO significantly ameliorated renal dysfunction, and restored the expression of EPOR in cisplatin nephrotoxicity.

We further analyzed renal phenotypes of EPOR null mutant mice expressing EPOR in hematopoietic lineage (EpoR-/-:Tg mice). In the baseline analysis, kidney function, blood pressure and histological observation in the kidney of EpoR-/-:Tg mice was indistinguishable from those of EpoR+/+:Tg mice, while serum EPO concentration of EpoR-/-:Tg mice was higher than that of EpoR+/+:Tg mice. EPO expression in the kidney, liver, brain and testis was indistinguishable between both genotypes, suggesting that elevated serum EPO concentration in EpoR-/-:Tg mice might be due to lack of endocytosis via EPOR. We also induced cisplatin nephrotoxicity to both genetypes, and found that the expression of osteopontin and TGF-β in the kidney of EpoR-/-:Tg mice was higher than that of EpoR+/+:Tg mice

Conclusions: Taken together, endogenous EPO exerts tissue protective function during kidney injury.

Funding: Government Support - Non-U.S.

TH-OR043

Rap1b Ameliorates the DN Progression Via Modulating Mitochondrial Homeostasis Lin Sun,[1] Xuejing Zhu,[1] Li Xiao,[1] Guanghui Ling,[1] Yashpal S. Kanwar,[2] Fu-You Liu,[1] You-Ming Peng.[1] *[1]Dep. Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]Dep. Pathology, Northwestern University, Chicago, IL.*

Background: We have demonstrated that Rap1b modulates the progression of diabetic nephropathy (DN), and ameliorates glucose-induced mitochondrial dysfunction in renal tubular cells (KI 2001, JBC 2002, JASN 2008) , However, it is unclear whether or how Rap1b can protect the tubular cell from damage induced by hyperglycemia.

Methods: 12 DN patients were observed and 24 Rats with STZ-induced DN were used in this study. Rap1b gene was transferred into the kidney of rats with DN by an ultrasound-microbubble-mediated technique(UMT). Immuno-EM was used to evaluate the localization of Rap1b in mitochondria of HK2 cell. Gene and protein expression analyses were carried out by the QPCR, Western blotting and IF. Mitochondrial functional analysis were carried out by measuring ROS, TMRE, ATP and mitochondrial apotosis genes.

Results: Rap1 expression was decreased in renal proximal tubular cells of DN patients, which was related to the tubular atrophy and decreased tubular functions. Although overexpression of Rap1b in DN Rats by UMT had no effect on blood glucose levels, it significantly attenuated the development of microalbuminuria, inhibited mitochondria mediated renal tubular cells death and ameliorated mitochondrial dysfunctions in proximal tubular cells. *In vitro*, Rap1b expression was localized to mitochondrial cristae of HK2 cells. Compared to control, 30 mM D-gluocse (HG) induced mitochondrial dysfunction, altered mitochondrial morphology, transmembrane potential, ATP levels, increased expression of mitochondrial fission gene Drp1. While a decreased expression of mitochondrial fusion gene in HK2 cells was observed. In addition, HG enhanced the overproduction of mitochondrial superoxide, decreased antioxidant enzymes and mitochondrial biogenesis genes expression. Increased expression of apoptosis related gene and protein expression was observed, and these effects were partially reduced with the transfection of Rap1b in HK2 cells.

Conclusions: These data indicate that Rap1b is capable of dampening the DN progression via modulating mitochondrial homeostasis.

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

TH-OR044

Bif-1 Regulates Mitochondrial Dynamics during ATP Depletion-Induced Apoptosis Sunggyu Cho,[1] Zheng Dong.[1,2] *[1]Department of Cellular Biology and Anatomy, Georgia Health Science University, Augusta, GA; [2]Charlie Norwood VA Medical Center, Augusta, GA.*

Background: Originally identified as a Bax-interacting factor, Bif-1 has sequence homology to endophilins and is also called endophilin B1. Nevertheless, Bif-1 does not play an essential role in membrane trafficking or endocytosis. It has been suggested that Bif-1 may contribute to mitochondrial damage and the consequent release of apoptotic factors during apoptosis. However, it is largely unclear how Bif-1 regulates mitochondria under these conditions.

Methods: In this study, we have examined the role of Bif-1 in mitochondrial regulation during ATP-depletion-induced apoptosis.

Results: In renal proximal tubular cells, Bif-1 translocated to mitochondria during ATP-depletion. Knockdown of Bif-1 in these cells suppressed apoptosis. Consistently, Bif-1-knockout mouse embryonic fibroblasts (MEFs) were resistant to ATP-depletion-induced apoptosis. Interestingly, Bif-1 knockout did not affect Bax translocation to mitochondria during ATP-depletion, but it prevented cytochrome c release from mitochondria. Mitochondrial fragmentation induced by ATP-depletion was markedly suppressed in Bif-1-knockout cells, suggesting that Bif-1 may participate in changes of mitochondrial dynamics resulting in mitochondrial damage. Mechanistically, Bif-1 was shown to interact with prohibitin-2, which regulates OPA1, a key regulator of mitochondrial inner membrane fusion.

Conclusions: Together, the results reveal a novel pathway of mitochondrial regulation by Bif-1 during apoptosis.

Funding: NIDDK Support, Veterans Administration Support

TH-OR045

Cathepsin-Mediated Depletion of Sirtuin-1 (SIRT1) in Endothelial Progenitor Cells (EPC) Jun Chen,[1] Eliza Moskowitz-Kassai,[1] Robert Chen,[1] Connie Y. Lu,[1] Ales Spes,[2] Boris Turk,[2] Michael S. Goligorsky.[1] *[1]Medicine, New York Medical College, Valhalla, NY; [2]Biochemistry and Molecular and Structural Biology, Jozef Stefan Institute, Ljubljana, Slovenia.*

Background: Stress-induced premature senescence (SIPS) of endothelial cells has emerged as a notable contributor to global endothelial cell dysfunction (ECD) in diverse diseases. One of the critical cellular abnormalities mechanistically linked to SIPS is lysosomal dysfunction. In the present study, we attempted to integrate the previous findings on endothelial SIPS with the exponentially growing field of SIRT1 effects on aging processes as applied to senescence of EPC.

Methods: Specifically, we examined the impact of a range of relevant cardiovascular risk factors on the expression of SIRT1, SIPS and apoptosis and documented the role of SIRT1 in the changes of EPC viability.

Results: Furthermore, studies showed reciprocal relations between SIRT1 and p62/SQSTM-1 expression in stressed EPC, thus demonstrating an attendant abnormality of autophagy. The described effects of stressors could be partially mimicked by inducing lysosomal membrane permeabilization or inhibiting autophagy and effect of stressors could be reversed by a cell-permeable inhibitor of cathepsins. Here we provide evidence, for the first time, that SIRT1 is an important substrate of cysteine cathepsins B, S and L. An antioxidant/peroxynitrite scavenger, ebselen, shown in the previous studies to protect endothelial cells and EPC from SIPS, prevented stress-induced SIRT1 depletion and subversion of autophagy by mitigating lysosomal dysfunction.

Conclusions: 1) these data advance the concept of "stem cell aging" by establishing the critical role of lysosomal dysfunction in the development of SIPS through the cathepsin-induced depletion of SIRT1, a hitherto hidden mechanism linking cell stress with apoptosis or SIPS; 2) Ebselen potently protects lysosomal membrane integrity, preventing cathepsin-induced cleavage of SIRT 1 in murine EPC, as well as blunting SIPS and apoptotic cell death induced by relevant cardiovascular stressors; 3) The proposed mechanism of SIRT1 depletion in stress has all the attributes of being a paradigm of SIPS.

Funding: NIDDK Support

TH-OR046

Protein Domains of Kidney Injury Molecule 1 That Mediate Phagocytosis and Induction of Autophagy Craig R. Brooks, Joseph V. Bonventre. *Medicine/Renal, Brigham and Women's Hospital, Boston, MA.*

Background: Autophagy has been shown to protect against acute kidney injury *in vitro* and *in vivo*. We have found that Kidney injury molecule-1 (KIM-1) expression induces autophagy, which is enhanced following phagocytosis. Kim-1 is a type I transmembrane phosphatidylserine receptor which is highly upregulated by proximal tubule cells in the injured kidney. Upon recognition, Kim-1 induces the phagocytosis of apoptotic cells, ox-LDL and other ligands. To identify regions of KIM-1 important for its functions and for therapeutic targeting, we examined which domains of Kim-1 are important for phagocytosis and autophagy.

Methods: We utilized KIM-1 constructs which have mutations in the phosphatidylserine binding site or which produce truncated proteins lacking either the extracellular domain or the cytosolic domain. Phagocytosis was studied by the uptake of fluorescently labeled apoptotic cells and oxidized low density lipoprotein. Autophagy was measured by formation of LC3-GFP puntae and LC3-II bands by western blot analysis.

Results: Expression of wild-type KIM-1 induces autophagy in renal proximal tubule cells, which is enhanced following phagocytosis. Mutation of the phosphatidylserine binding domain of the protein (which recognizes apoptotic cells) inhibits phagocytosis and blocks autophagy induction. The KIM-1 extracellular domain together with the transmembrane domain of the protein was sufficient to induce both phagocytosis and autophagy. Thus the cytosolic domain of Kim-1 was not required for phagocytosis or autophagy. Indeed, when the cytosolic domain was expressed alone there was no phagocytosis or upregulation of autophagy. In addition, wild-type KIM-1 and KIM-1 ectodomain were found to localize to the autophagosome following phagocytosis, while KIM-1 cytodomain or phosphatidylserine binding site mutants were absent from the autophagosome.

Conclusions: KIM-1 induces autophagy through its phagocytotic function. Targeting of KIM-1 to the autophagosome is also dependent on the phagocytic function of the protein. Both KIM-1 phagocytosis and the role of KIM-1 in autophagy are independent of the intracellular domain of the molecule.

Funding: NIDDK Support

TH-OR040

Signaling Mechanisms for Targeted Inactivation of Epidermal Growth Factor Receptor-Elicited Inhibition of Renal Fibrogenesis Shougang Zhuang.[1,2] *[1]Department of Medicine, Rhode Island Hospital, Brown University School of Medicine, Providence, RI; [2]Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China.*

Background: Although development and progression of renal fibrosis is associated with enhanced activation of epidermal growth factor receptor (EGFR), the underlying mechanisms by which EGFR mediates renal fibrosis remain elusive.

Methods: We used genetic, pharmacologic, in vivo, and in vitro experiments to study the molecular basis of EGFR-mediated renal fibrogenesis.

Results: In a mouse model of obstructive nephropathy, a sustained EGFR phosphorylation was detected in the kidney of wild-type mice, and these mice developed more severe renal fibrosis than waved-2 mice that have reduced EGFR tyrosine kinase activity. Attenuation of renal fibrosis in waved-2 mice was associated with reduced numbers of renal tubular cells arrested at G2/M, inhibition of α-smooth muscle actin (α-SMA) expression, down-regulation of gene expression of multiple profibrogenic cytokines including transforming growth factor-β1 (TGF-β1), and dephosphorylation of Smad3, STAT3 and ERK1/2. This phenotype was fully recapitulated in injured kidney of wild-type mice given gefitinib, a specific EGFR inhibitor. Furthermore, inactivation of either EGFR or STAT3 reduced UUO-induced expression of lipocalin–2, a molecule associated with the pathogenesis of chronic kidney disease. In cultured renal interstitial fibroblasts, inhibition of EGFR also abrogated TGF-β1 or serum-induced phosphorylation of EGFR, STAT3, ERK1/2 and Smad3 as well as expression of α-SMA and extracelluar matrix proteins.

Conclusions: These data suggest that EGFR may mediate renal fibrogenesis via induction of transition of renal epithelial cells to a profibrotic phenotype, overproduction of inflammatory factors, and activation of renal interstitial fibroblasts. Inhibition of EGFR may hold a therapeutic potential for treatment of fibrotic kidney disease.

Funding: NIDDK Support

TH-OR048

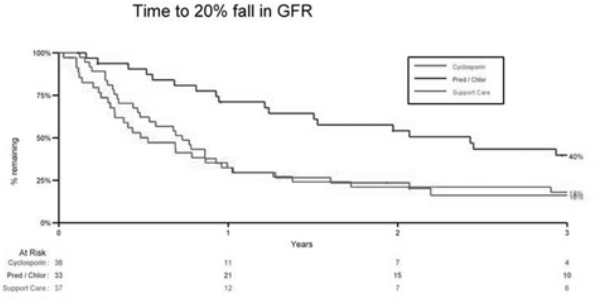
The UK Randomised Controlled Trial of Immunosuppression for Progressive Membranous Nephropathy Peter W. Mathieson. *Academic Renal Unit, University of Bristol, Bristol, United Kingdom.*

Background: Optimal treatment for idiopathic membranous nephropathy (IMN) remains controversial despite numerous controlled trials, not least because the condition has a variable natural history and even severely affected individuals can undergo spontaneous remission. Only a subset of patients (25-30% in most series) develops progressive loss of kidney function and since the available therapies have considerable adverse effects, a prominent school of thought is that aggressive therapy should be reserved for that subgroup.

Methods: In the mid 1990s we embarked on a multicentre randomised controlled trial to compare the three approaches that were popular at that time (and remain so today): six months of alternating cycles of prednisolone and chlorambucil (PC), twelve months of cyclosporine (Cy); or supportive therapy alone (ST) in patients with IMN who had shown a 20% deterioration in excretory renal function.

Results: 108 patients were recruited from centres across the UK (33 randomised to receive PC, 36 Cy, 37 ST, 2 proved ineligible). Recruitment was completed in 2008; analysis commenced when two year follow-up was complete for the last recruited subjects. Primary end-point was a further 20% decline in renal function; secondary end-points were proteinuria and adverse effects.

Regarding renal function, there was a highly statistically significant difference ($p<0.004$) in favour of PC.



Comparisons: Hazard Ratio (95% CI) and p-value
Cyclosporin vs Supportive care. HR 1.17 (0.7, 1.96), 2p=0.5
Prednisolone / Chlorambucil vs Supportive care. HR 0.43 (0.25, 0.76), 2p=0.004

This group also showed the greatest fall in proteinuria. Adverse events were common in all 3 groups, significantly higher in PC and Cy compared to ST but not significantly different between PC and Cy.

Conclusions: We conclude that six months' therapy with prednisolone and chlorambucil is superior to cyclosporine or supportive therapy alone in patients with IMN whose renal function is deteriorating. This effect is maintained to at least 3 years.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

TH-OR049

Oral Calcitriol for Reduction of Proteinuria in Patients with IgA Nephropathy: A Randomized, Controlled Trial

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Background: Vitamin D has shown efficacy in the reduction of proteinuria in patients with chronic kidney disease. This study aimed to determine the effect of calcitriol on urinary protein excretion in patients with IgA nephropathy.

Methods: In this open-labeled, blank-controlled study, 50 IgA nephropathy patients were enrolled. The main criterion for inclusion was urinary protein excretion greater than 0.8g/d following renin-angiotensin-system (RAS) inhibitor treatment for at least three months. Patients were randomly assigned (1:1) to receive two doses (0.5 μg) of calcitriol per week or no treatment, for 48 weeks. The primary endpoint of urinary protein excretion was measured from the baseline to 24 hours post-treatment. Concentrations of monocyte chemoattractant protein 1 (MCP-1) and transforming growth factor- β (TGF-β) in serum and urine were also measured.

Results: Measurement of the primary endpoint showed changes in the urinary protein excretion of +21% (from 1.29 to 1.58 g/24 h; 95% CI -9 to 52) in the control group and -19% (from 1.60 to 1.30 g/24 h; 95% CI -42 to 4) in the calcitriol-treated group (*p=0.03*). A 15% decrease in proteinuria was measured in controls (7 of 24, 29.2%) and the calcitriol-treated treatment group (17 of 26, 65.4%; *p=0.02*). No significant differences were observed in the decline in the estimated glomerular filtration rate (eGFR) and changes in blood pressure. Incidence of recorded adverse events was similar between the two groups.

Conclusions: Addition of calcitriol to RAS inhibitor resulted in a safe reduction of proteinuria in patients with IgA nephropathy.

Funding: Government Support - Non-U.S.

TH-OR050

The Effect on the Microcirculation of Ergocalciferol (Vitamin D2) Versus Placebo in Chronic Kidney Disease 3-4 and Vitamin D Deficiency: A Pilot, Double Blind, Randomised Controlled Trial

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Background: Observational studies have demonstrated reduced cardiovascular disease (CVD) in patients with kidney disease who receive vitamin D therapy. The exact mechanism leading to these observations is unclear and there are very few prospective studies of vitamin D therapy in kidney disease. We conducted a pilot randomised trial testing the hypothesis that vitamin D therapy improves microcirculatory function, a known surrogate marker for future cardiovascular dysfunction and disease.

Methods: 38 non-diabetic, non-transplant patients with CKD 3-4 and vitamin D deficiency (25 OH vitamin D < 40nmol/L) were enrolled. 20 received ergocalciferol as 50,000 iu weekly for 1 month followed by 50,000 iu monthly for 5 months. 18 patients received a matching placebo. The primary endpoint was microcirculatory function after 6 months therapy assessed by % change in skin vessel flux measured by laser doppler flowmetry after iontophoresis of acetylcholine (AcH).

Results: Treatment groups were similar at baseline with respect to age, sex, eGFR, blood pressure, proteinuria, medication and tobacco use, Hb, CRP and 25 OH vitamin D levels. After 6 months, 25 OH vitamin D levels were 91.4 nmol/L in the treatment vs 26.2 nmol/L in the placebo group (p<0.001). Percentage increase in flux after iontophoresis of AcH was similar at baseline in both groups (ergocalciferol - 826.0% +/- 170.0%, placebo - 785.9% +/- 121.3%, p=0.85) but was significantly higher in ergocalciferol treated patients at 6 months (ergocalciferol 1130% +/-182.3%, placebo 540.6% +/- 112.6%, p=0.012). eGFR, blood pressure, proteinuria, Hb and CRP did not change with treatment.

Conclusions: Improved microcirculatory function after treatment with vitamin D compounds may be contributing to the reduced burden of CVD in previous observational studies. Multi-centre, randomised trials which compare different vitamin D compounds and include measurement of both microcirculatory and hard cardiovascular endpoints are now required to optimise therapy in this patient group.

TH-OR051

Effect of Dual Blockade of Renin Angiotensin System on the Progression of Type 2 Diabetic Nephropathy

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Background: The blockade of the RAAS has been shown to reduce the rate of loss of glomerular filtration rate in proteinuric nephropathies, including diabetic nephropathy. It's possible the combination therapy of ACE inhibitors (ACEi) and ARBs may offer more complete blockade of the RAAS than monotherapy.

Methods: Clinical trial phase III, randomized (1:1:2), multicenter, open to compare the effect of lisinopril (40 mg), irbesartan (600 mg) or combination (lisinopril 20 mg + irbesartan 300 mg) on the progression of established diabetic nephropathy.

Primary composite outcome: ↑ 50% of the Cr or dialysis or renal transplantation. Secondary outcomes: ↓baseline proteinuria and cardiovascular events Inclusion criteria: Age 35-75 y, diabetes 2, CKD stage 2-3, ratio MAU/cr>300 mg/g All patients gave informed consent. (EUDRACT 2004-002470-31)

Results: We have included 131 patients in 23 units of Nephrology. The baseline characteristics of patients were: age 65 ± 8.3 y, BMI 34.8 kg/m2, SBP 155±19 mmHg, DBP 81±11 mmHg, Hemoglobin 13.4 ± 2.6 g / dl, LDL 104±36 mg / dl, GlicoHb 7±1.2%, SCr 1.5±0.5 mg /dl, Ks 4.5±0.6 mEq /l, eGFR MDRD4 45±24 mL/min/1.73m2, proto 2.6±1.8 g/24 h. The median follow up time was 49 months.

The frequency of the composite primary outcome was similar: lisinopril 7/35 (20%); irbesartan 6/28 (21.4%); lisinopril+irbesartan 16/70 (22.8%) (NS).

Loss Renal function was similar in the three groups: lisinopril 5, Irbesartan 3 .5, Lisinopril+irbesartan 3.5 mL/min/1.73 m2 year (NS) .

BP control was not optimal but there were no differences between groups. The lipid and glycemic control was optimal. There were no differences between groups. 4 patients discontinued the study because of hyperkalemia. ACEi (2) ARBs (1) combinated treatment (1)

Conclusions: In patients with established diabetic nephropathy, the monotherapy with ACEi and ARBs in high doses provides equivalent renoprotection combined treatment at equipotent doses.

Funding: Other NIH Support - Instituto de Salud Carlos III Spain

TH-OR052

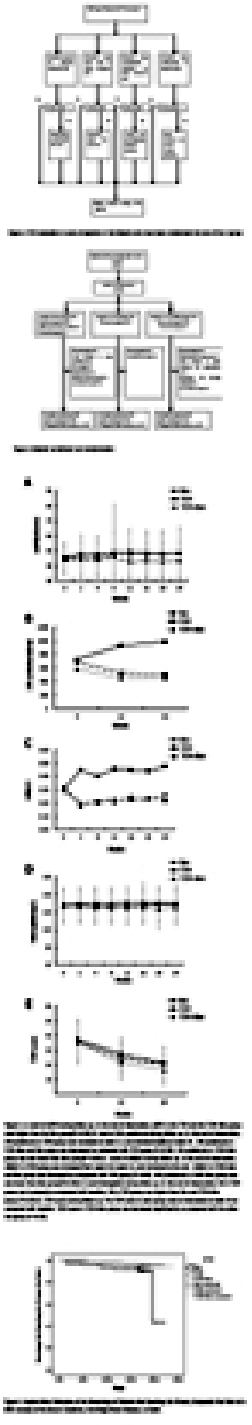
Optimized Project of Traditional Chinese Medicine in Treating Chronic Kidney Disease Stage 3: A Multicenter Randomized Double-Blinded Controlled Trials (RCT Registration Code: ChiCTR-TRC-00000204)

Yongjun Wang,[1] Wei Sun,[2] Li-Qun He.[3] *[1]Nephrology, Hangzhou Hospital of Traditional Chinese Medicine affiliated to Zhejiang University of Chinese Medicine, China; [2]Nephrology, Shanghai Shuguang Hospital, China; [3]Nephrology, Affliated Hospital, Jiangshu University of Chinese Medicine, China.*

Background: Stage 3 is the key phase of chronic kidney disease. Traditional Chinese Medicine has been used for the treatment of Chronic Kidney Disease. But a large sample multicenter randomized double-blinded controlled trial is desirable.

Methods: A total of 578 Chinese patients with primary glomerulonephritis in CKD stage 3 were randomly assigned to: Patients received TCM decoction granules(TCM group), benazepril (Ben group), TCM decoction granules combined with benazepril (TCM+Ben group) and followed up for 24 weeks. The primary endpoint was the time to the composite of 50% increased of serum creatinine, end stage renal disease or death.

Results: eGFR in the TCM and the TCM+Ben group were improved (week 24 vs. baseline, *P*<0.05) while eGFR in the Ben group was decreased (week 24 vs. baseline, *P*>0.05). 24h urinary protein excretion (UP) and urinary albumin/creatinine (UAlb/Cr) were decreased in the TCM+Ben (week 24 vs. baseline, *P*<0.05) and the Ben group (week 24 vs. baseline, *P*>0.05). UP and UAlb/Cr were increased in the TCM group to week 12, then was stable (week 24 vs. baseline, *P*<0.05). The hemoglobin in the TCM group was improved (week 24 vs. baseline, *P*<0.05). The accumulative survive rate in the TCM+Ben group was higher than that in the other 2 groups (P=0.14). The patients with dry cough in TCM group was decreased as compared with the other 2 groups(*P*<0.05)



Conclusions: For the patients with CKD stage 3, TCM can improve eGFR and hemoglobin. Chinese medicine integrated with benazepril can ameliorate renal function and decrease proteinuria synergistically.

Funding: Government Support - Non-U.S.

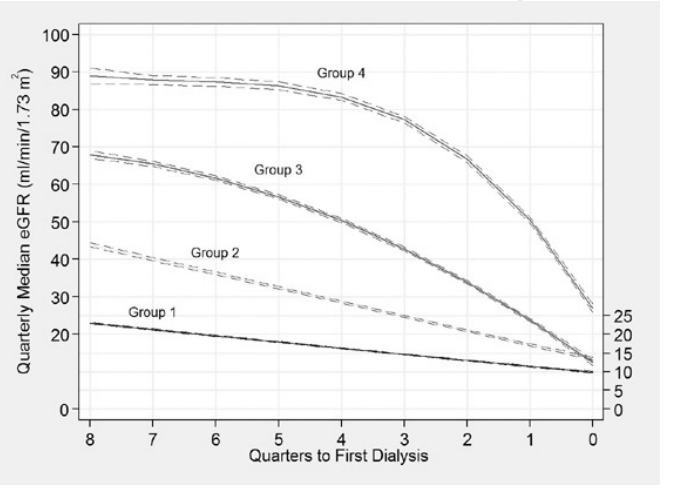
TH-OR053

Trajectories of Kidney Function before Initiation of Chronic Dialysis Ann M. O'Hare,[1] Adam J. Batten,[1] Meda E. Pavkov,[2] Nilka Rios Burrows,[2] Indra Gupta,[1] Jeffrey Todd-Stenberg,[1] Rudolph A. Rodriguez,[1] Fliss E. Murtagh,[4] Leslie L. Taylor,[1] Eric Larson,[3] Desmond Williams.[2] *[1]Medicine, University of Washington and VAPSHCS, Seattle, WA; [2]CDC, Atlanta, GA; [3]GHRI, Group Health, Seattle, WA; [4]Kings College, London, United Kingdom.*

Background: Little is known about patterns of kidney function decline leading up to initiation of chronic dialysis.

Methods: We used data from the VA, Medicare and USRDS to model pre-dialysis eGFR trajectories among 7,322 patients who initiated chronic dialysis in 2001-2003.

Results: We identified four distinct trajectories of eGFR during the two year period before dialysis initiation: 63% of patients experienced relatively slow loss of eGFR from levels that were already severely reduced two years before initiation (Group 1); 25% declined from moderately reduced levels of eGFR two years before initiation (Group 2); 9% had normal levels of eGFR until two years before initiation (Group 3), and 3% had normal levels of eGFR until six months or less before initiation (Group 4).



More rapid loss of eGFR was associated with a greater likelihood of hospitalization and inpatient acute kidney injury and a lower likelihood of outpatient nephrology care and vascular access placement during the two year period before initiation. Those with more rapid loss of eGFR were more likely to initiate dialysis in the hospital, at a higher level of eGFR and in the setting of an acute kidney injury. Median survival ranged from 1.0 year (25th to 75th percentile, 0.3 to 4.4 years) for Group 4, to 3.2 years (25th to 75th percentile, 1.3 to 6.2 years) for Group 1. Differences in survival persisted in analyses adjusted for patient characteristics and care practices.

Conclusions: Patterns of eGFR decline before initiation of chronic dialysis are heterogeneous and are strongly associated with pre-dialysis care and survival after initiation.

Funding: Other NIH Support - NIA, Other U.S. Government Support, Veterans Administration Support

TH-OR054

A Breath Test for Chronic Kidney Disease and Disease Progression Farid M. Nakhoul,[1] Ophir Marom,[2] Ulrike Tisch,[2] Ala Shiban,[2] Zaied Abassi,[3] Hossam Haick.[2] *[1]Nephrology Division, Poria Med Ctr, Tiberias; [2]Depart.of Chemical Engineering & Nanotechnology, Technion; [3]Research, Rambam, Haifa, Israel.*

Background: A novel approach that overcomes many of the conventional diagnostic techniques relies on patterns of volatile biomarkers in the exhaled breath. Some of the volatile organic compounds among the plasma chronic kidney dis.(CKD) biomarkers, or their metabolic products,are transmitted to the alveolar exhaled breath through exchange via the lung, at the very onset of the disease,Accurate determination of kidney function is essential in the treatment of CKD in order to identify patients with early renal impairment and to follow the course.we report a novel method to identify CKD and progression that is based on breath testing by using acustom-designed, nanoscale artificial NOSE(NA-NOSE).

Methods: Alveolar exhaled breath samples were collected from 62 volunteers and were analyzed using a custom-designed array of nanosensors that is based on organically functionalized gold nanoparticles, combined with support vector machine (SVM) analysis, to detect statistically significant differences between the sub-populations. Sensitivity and specificity with reference to CKD patient classification according to eGFR, were determined using cross-validation. Chemical composition of the breath samples was studied using gas chromatography linked with mass spectrometry (GC-MS).

Results: Excellent distinction was achieved with the nanosensor array between: (i) early stage CKD and healthy states, and (ii) stage-4 and stage-5 CKD states, with an accuracy of 79% and 85%, respectively. Several substances in the breath were identified and could be associated with CKD related biochemical processes or with the accumulation of toxins through kidney function loss.

Conclusions: This study focuses on testing the feasibility of a novel method in nanomedicine for identifying early stage of CKD and monitoring disease progression from exhaled breath of patients. The biomarker-based NA-NOSE breath test could form the basis of a future cost-effective, fast and early diagnostic test for CKD and progression. The nanosensor array could distinguish with high accuracy between the exhaled breath of (i) early &advanced CKD syages.

Funding: Technion Institute, Government Support - Non-U.S.

TH-OR055

A New Equation To Estimate GFR from Standardized Creatinine and Cystatin C Lesley Stevens Inker,[1] Christopher H. Schmid,[1] Hocine Tighiouart,[1] John H. Eckfeldt,[2] Harold I. Feldman,[3] Tom H. Greene,[4] Jane Manzi,[5] John W. Kusek,[6] Josef Coresh,[5] Andrew S. Levey.[1] *[1]Tufts Medical Center; [2]University of Minnesota; [3]University of Pennsylvania; [4]University of Utah; [5]John Hopkins University; [6]NIDDK; [7]Cleveland Clinic.*

Background: GFR estimates based on serum creatinine (cr) are routinely used, however, are imprecise due to variation among people in non-GFR determinants of creatinine. Cystatin C (cys) is a potential alternative filtration marker.

Methods: We developed estimating equations based on cys alone (eGFRcys) and in combination with cr (eGFRcr-cys) in a cross-sectional analysis using separate databases for equation development (13 studies of 5352 people) and validation (5 studies with 1119 people). GFR was measured (mGFR) using urinary clearance of iothalamate in the development dataset, and clearance of other markers in the validation dataset. Cr and cys assays were traceable to high-level reference materials.

Results: Mean mGFR (SD) was 68 (39) and 70 (41) ml/min/1.73m^2 in the development and validation dataset, respectively. eGFRcr-cys performed better than eGFRcys or eGFRcr, with similar bias, improved precision, and greater accuracy (table). Compared to eGFRcr, eGFRcr-cys improves classification of subjects with mGFR ≥ or < 60 ml/min/1.73m^2 [net reclassification improvement (NRI) (95% CI) 4.93% (2.19%-7.67%)]. In the subgroup of eGFRcr of 45-59 ml/min/1.73 m^2, NRI was 32.7% (15.4-50.1%).

Metric	Equation	Overall	Estimated GFR		
			<60	60-89	>90
Bias, Median Difference	Cr	3.7	1.8	6.6	11.5
	Cys	3.4	0.4	6.0	8.5
	Cr-cys	3.9	1.3	6.9	10.6
Precision, IQR of the Difference	Cr	15.4	10.0	19.6	25.0
	Cys	16.4	11.0	19.6	22.6
	Cr-cys	13.4	8.1	15.9	18.8
Accruacy,1-P30	Cr	12.8	16.6	10.2	7.8
	Cys	14.1	21.4	12.7	2.2
	Cr-cys	8.5	13.3	5.3	2.3

Difference refers to mGFR - eGFR. Interquartile range refers to the 25-75th percentile. Units are ml/min per 1.73 m 2. 1- P30 refers to the percent of GFR estimates that deviate by > 30% of mGFR.

Conclusions: The combined Cr-Cys equation is more accurate than equations with either marker alone, and can be used as a confirmatory test for people decreased eGFRcr.

Funding: NIDDK Support

TH-OR056

Assessing Improvement of eGFR Risk Classification in Meta-Analysis: An Example of CKD-EPI and MDRD Study Equations (for the CKD-PC Collaborators) Kunihiro Matsushita, Yingying Sang, Mark Woodward, B. Khan Mahmoodi, Brad C. Astor, Andrew S. Levey, Josef Coresh. *Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Background: Risk prediction models are assessed by calibration, discrimination, and reclassification. Net reclassification improvement (NRI) is a popular reclassification statistic in clinical epidemiology. However, methods to assess NRIs in a meta-analysis are not well described. Using an example of the CKD-EPI and the MDRD Study equations, we demonstrate a method to meta-analyze NRIs for eGFR category based on different equations.

Methods: In a sample of 16 of 46 cohorts joining the CKD Prognosis Consortium (CKD-PC), NRI was calculated for reclassification across the following six eGFR categories (≥90, 60-89, 45-59, 30-44, 15-29, <15 ml/min/1.73m^2) for CKD-EPI vs. MDRD Study equations. Standard errors of NRIs were calculated in analogy to McNemar's test for paired proportions as √{(proportion of reclassification to high risk [low eGFR] category in those who developed events + proportion of reclassification to low risk [high eGFR] category in those who developed)/(# of events) + (reclassification to low risk category in those who did not develop events + reclassification to high risk category in those who did not develop)/(# of no events)}. NRIs were meta-analyzed in a random effects model applying the *metan* command in STATA.

Results: There were a total of 85,245 participants and 16,864 deaths. NRIs for death based on eGFR category varied from 0.01 to 0.24 (p-values from <0.001 to 0.860) among cohorts. Standard errors also varied from 0.005 to 0.049. Meta-analyzed NRI was 0.10 (95% CI: 0.07-0.14, p<0.001), suggesting that the CKD-EPI equation classifies individuals more correctly than the MDRD Study equation. The I-squared was 95.8% (p<0.001), suggesting significant heterogeneity across studies.

Conclusions: We demonstrated how to meta-analyze NRIs, which will precisely estimate clinical impact of new eGFR equations or other prediction tools for CKD outcomes.

Funding: Private Foundation Support

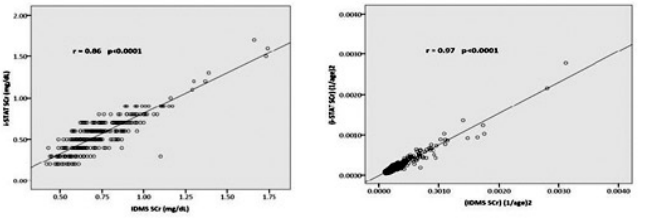
TH-OR057

Comparison of Serum Creatinine Measured with a Point-of-Care Testing Device (i-STAT) and with IDMS Methodology among Mexico's Kidney Early Evaluation Program (KEEP) Participants Gregorio T. Obrador,[1,2] Nadia Olvera,[2] Daniela Ortiz de la Peña,[2] Veronica Gutierrez,[2] Antonio Villa.[1] *[1]Universidad Panamericana School of Medicine; [2]Mexican Kidney Foundation.*

Background: Measurement of serum creatinine (SCr) with a method traceable to IDMS is optimum to estimate GFR (eGFR) in CKD screening programs. Unfortunately, most Mexican laboratories don´t use IDMS methods to measure SCr. KEEP Mexico is a CKD screening program directed to high risk individuals, including those with DM, HTN, or family history of DM, HTN, or CKD. Escalation of KEEP Mexico to other Mexican states as well as some Latin American countries has been challenged by variability in SCr measurements due to use of non-IDMS methods and to significant differences in quality control among laboratories. The aim of this study was to compare SCr measured with a point-of-care testing (POCT) device (i-STAT) and with IDMS methods in a representative sample of KEEP participants.

Methods: TThe i-STAT SCr method was assessed by analyzing capillary blood samples of 282 KEEP participants and comparing POCT results with those of an Olympus 5400 spectrophotometry instrument, which measures IDMS SCr in venous blood. Correlation between i-STAT and IDMS SCr was calculated.

Results: Mean age ± SD of the 282 participants was 53.4±15.1 and 76% were women. Mean SCr±SD by the i-STAT method was 0.56±0.23 mg/dl and by the IDMS method 0.73±0.20 mg/dl. Mean eGFR±SD estimated with the MDRD and CKD-EPI equations from the IDMS SCr were 102±25 and 97±19 ml/min/1.73m2, respectively. Correlation for i-STAT and IDMS SCr was high (r= 0.86, p<0.0001) and improved when a correction factor (1/age)2 was applied (r = 0.97, p<0.0001).



Conclusions: i-STAT SCr measured in capillary blood is as accurate as IDMS SCr when a correction factor is applied. Further linear regression analysis will be done to use i-STAT SCr for GFR estimation with equations.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-OR058

Rapid Kidney Function Decline in Young Adults without Chronic Kidney Disease: Data from the CARDIA (Coronary Artery Risk Development in Young Adults) Study Carmen A. Peralta,[1,2] Eric Vittinghoff,[1] Michael Shlipak,[1,2] David Siscovick,[3] David R. Jacobs,[7] Holly J. Kramer,[4] Michael Steffes,[5] Paul Muntner,[6] Kirsten Bibbins-Domingo.[1] *[1]UCSF, SF, CA; [2]SFVAMC, SF, CA; [3]U Washington, Seattle, WA; [4]Loyola, Maywood, IL; [5]U Minnesota, Minneapolis, MN; [6]U Alabama Birmingham, Birmingham, AL; [7]U Minnesota Public Health, Minneapolis, MN.*

Background: Blacks have been reported to have faster rates of kidney function decline in middle-aged or older adults with or without chronic kidney disease (CKD). Whether race differences in kidney function decline are detectable in young adults without CKD is not well studied.

Methods: CARDIA is a longitudinal cohort of young blacks and whites (age 18-30 at enrollment) with over 20 year follow-up. We included participants at years 10, 15 and 20 with at least two measures of cystatin C (N=3658). Rapid kidney function decline was defined as annual eGFRcys decline ≥3% per year. We evaluated race differences in rapid decline by study period (period 1: year 10-15 (age 28-40) and period 2: years 15-20 (age 33-45)) using Poisson regression. We adjusted for age, sex, diabetes, albuminuria, and time-averaged cumulative exposure to systolic blood pressure ≥120mmHg throughout follow up.

Results: At baseline, mean age was 35 ± 4, eGFRcys 112 ± 26ml/min/1.73m² for blacks and 105 ± 21ml/min/1.73m² for whites. Blacks were more likely to have rapid eGFRcys decline during both periods, but the magnitude of the differences varied by period. After age adjustment, during period 1, 14.3% of Blacks had rapid decline vs,1.8% of whites. During period 2, 26.4% of Blacks and 8.7% of whites had rapid decline. Multivariate adjustment did not attenuate race differences: blacks were nearly 8 times as likely to have rapid decline in kidney function as whites during the first period (prevalence ratio for blacks to whites=7.6 (5.1-11.3)) and nearly three times as likely during period 2 (prevalence ratios 2.7 (2.3-3.2)).

Conclusions: Among young adults without chronic kidney disease, Blacks were more likely to have rapid decline in eGFR than whites. Future studies are needed to elucidate the mechanisms predisposing young blacks to rapid kidney function decline.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support

TH-OR059

***APOL1* Variants Are Associated with Subclinical Albuminuria and Lower Glomerular Filtration Rate in the Black Young Adults: Findings from the Coronary Artery Risk Development in Young Adults (CARDIA) Study**

Kirsten Bibbins-Domingo,[1] Eric Vittinghoff,[1] Myriam Fornage,[2] George W. Nelson,[3] Cheryl Ann Winkler,[3] Jeffrey B. Kopp.[3] *[1]Department of Medicine and of Epidemiology and Biostatistics, University of California, San Francisco, CA; [2]University of Texas Health Science Center at Houston, Houston, TX; [3]National Institutes of Health, Bethesda, MD.*

Background: Genetic variants in *APOL1*, encoding apolipoprotein L1, are strongly associated with focal segmental glomerulosclerosis, HIV-associated nephropathy, and hypertensive kidney disease, but the association with subclinical kidney disease has not been examined.

Methods: The Coronary Risk Factors in Young Adults (CARDIA) is an on-going longitudinal study that enrolled 5115 participants age 18-30 years in 1985-6. We genotyped *APOL1* kidney risk mutations in all participants with samples at Year 10 and explored the association of 2 *APOL1* mutations with repeated measures of albuminuria (urine albumin/creatinine ratio >30 mg/g) and cystatin C-estimated glomerular filtration rate (eGFR) from Years 10, 15, and 20 among 1764 black participants. We also assessed effects of metabolic syndrome.

Results: The *APOL1* risk genotype (2 *APOL1* risk alleles as homozygotes or compound heterozygotes) was present in 12.6% of black participants. After adjustment for visit year, age, and sex, albuminuria (nearly all of which-86%- was microalbuminuria) was associated with the *APOL1* risk genotype (adjusted prevalence 17.2% vs 6.0%, p<0.0005). The *APOL1* risk genotype was associated with 5.5% lower eGFR at each exam year (p=0.003) and also predicted eGFR<60 ml/min (adjusted prevalence 3.3% vs 1.0%, p=0.02). Compared to normal controls, participants with both *APOL1* risk genotype and metabolic syndrome had 14.6% lower average eGFR (p=.003), while those with either metabolic syndrome (5.3% lower, p<0.0005) or the *APOL1* risk genotype (4.8% lower, p=.009) had intermediate levels. Annual rates of eGFR decline were similar across the four groups (p for heterogeneity = 0.36).

Conclusions: The increased prevalence of albuminuria and reduced eGFR may indicate subclinical kidney disease associated with *APOL1* risk alleles.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-OR060

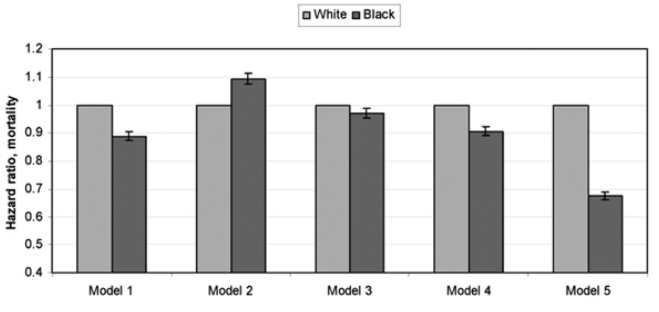
Black Patients Experience Lower Mortality Compared to White Patients in a Large Cohort of US Veterans with Non-Dialysis Dependent CKD

Csaba P. Kovesdy,[1,2] Evan H. Lott,[3] Jun Ling Lu,[4] Sandra M. Malakauskas,[1,2] Jennie Z. Ma,[2] Mark D. Okusa,[2] Kamyar Kalantar-Zadeh.[5] *[1]Salem VA Medical Center; [2]University of Virginia; [3]VA Informatics and Computing Infrastructure; [4]Salem Research Institute; [5]Harbor UCLA.*

Background: Blacks with end stage renal disease experience significantly lower mortality compared to whites. A similar paradoxical association in non-dialysis dependent CKD is unknown.

Methods: We compared 54,154 black and 468,233 white patients in a nationally representative cohort of US veterans with non-dialysis dependent CKD stages 1-5 in 2005-2006. Crude mortality rates (Model 1) were compared with the Kaplan Meier method and in Cox models. In order to explain observed differences in mortality, models were adjusted for age (Model 2), sociodemographics (Model 3), comorbidities (Model 4), and laboratory variables (Model 5).

Results: Blacks were younger, more likely to be unmarried and uninsured and to have diabetes and hypertension, but less likely to have coronary artery disease. Over a median follow-up of 4.7 years, 14,848 blacks (64.3 deaths/1000 patient-years (95% CI: 64.3, 63.3-64.5)) and 143,107 whites died (75.2 (71.8-72.5)). Black race was associated with lower crude mortality (hazard ratio, 95%CI: 0.89, 0.88-0.90), which reversed after adjustment for age (1.09, 1.07-1.11). Further adjustments resulted in black race being again associated with lower mortality (Figure; all p values <0.001). Similar trends were seen in all stages of CKD.



Conclusions: Blacks with non-dialysis dependent CKD experience lower mortality compared to white patients, which reverses after controlling for age. A survival advantage of blacks re-emerges upon controlling for differences in various characteristics. General population studies are needed to clarify the reasons for the selection of black patients with CKD possessing such different characteristics.

Funding: NIDDK Support, Veterans Administration Support

TH-OR061

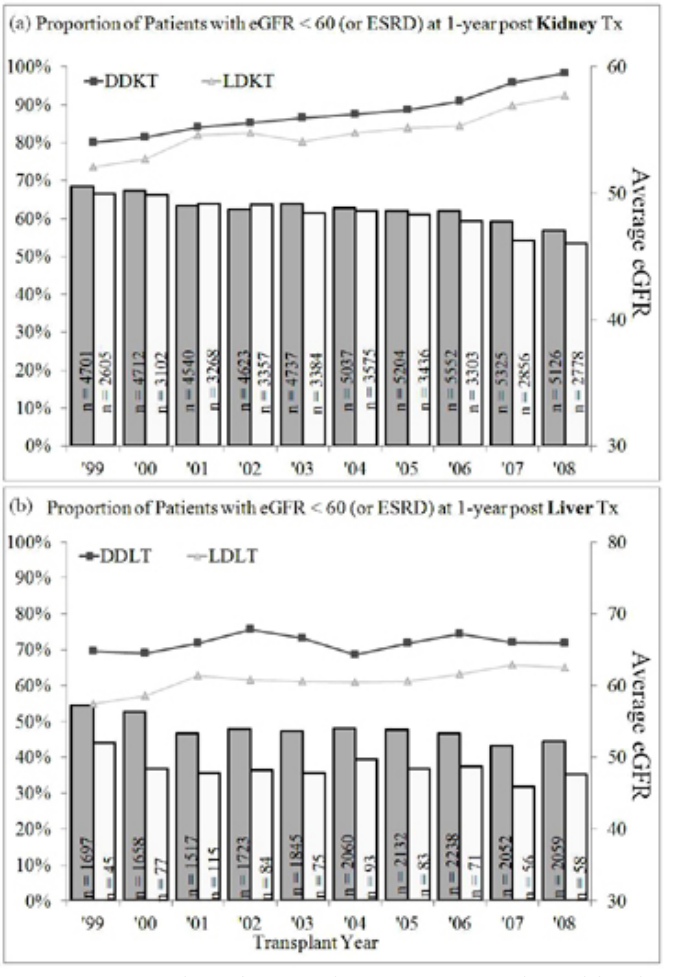
National Trends in Prevalence of Chronic Kidney Disease in Kidney & Liver Transplant Recipients

Anca Tilea,[1] Yihung Huang,[1] Vahakn B. Shahinian,[1] Vanessa Grubbs,[2] Neil R. Powe,[2] Nilka Rios Burrows,[3] Desmond Williams,[3] Rajiv Saran.[1] *[1]U of MI; [2]UCSF; [3]CDC.*

Background: High prevalence of CKD has been recognized as a major complication of solid organ transplantation. We postulated that prevalence of CKD in organ transplant recipients may be on the rise, as average age of both transplant donors and recipients has increased.

Methods: Adult solid organ (kidney (KT) and liver (LT)) transplant recipients (age>20) engrafted between 1999 and 2008 were identified in the US Scientific Registry of Transplant Recipients (SRTR). Patients with a death event or missing eGFR prior to 1-year post-transplant were excluded. GFR was estimated by the MDRD equation. Logistic regression was used to model the probability of eGFR<60 ml/min/1.73m^2 or ESRD at 1-year post-transplant, adjusted for transplant year, recipient and donor demographics by donor type (deceased (DD) or living (LD) donor).

Results: In adjusted models, the odds of CKD at 1-year post KT were significantly lower for both DDKT recipients and LDKT recipients (OR=0.96, and OR=0.93 respectively, Figure Panel [a], bars). Correspondingly, there was a significantly higher mean eGFR (lines) for both donor types (p<0.05). Similar results were seen in CKD prevalence for LT recipients (Panel [b]). In adjusted models, the odds of CKD at 1-year post LT were 3.4% lower per year (p=0.01) for DDLT recipients, but remained relatively unchanged for LDLT recipients (p=0.47). The average eGFR was significantly higher for DDLT recipients (β=0.53,p<0.05). There was no significant trend for the relatively small numbers of LDLT recipients.



Conclusions: Prevalence of post-transplant CKD among KT and LT recipients has steadily decreased in the US over the last decade, despite aging donors and recipients. Explanations for such overall trends may include changes in immunosuppression practices or other practices, and require further study.

Funding: Other U.S. Government Support

TH-OR062

Pregnancy in a Prospective Cohort of Women with CKD 3-5: Maternal Outcomes Sajeda Youssouf,[1] Matt Hall,[1] Liz Lightstone,[3] Nigel J. Brunskill,[1] Sue Carr.[1] *[1]John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom; [2]Renal Section, Dept of Medicine, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: CKD is associated with an increased risk of adverse fetal outcomes at all levels of renal dysfunction. For some women, pregnancy is associated with an accelerated decline in renal function. This retrospective analysis of data collected prospectively since 2003 sought to identify maternal outcomes in women with excretory renal dysfunction at conception.

Methods: We analysed prospective data from 3 specialist renal-obstetric services in the United Kingdom and identified those with excretory renal dysfunction (CKD 3 to 5) as women with a pre-conception estimated GFR <60ml/min or a serum creatinine >110µmol/l (>1.25mg/dl) prior to 12 weeks gestation.

Results: 42 pregnancies in 39 women with CKD stage 3 to 5 were identified, of whom sufficient data was available in 30 women. Mean maternal age at conception was 32±4.5 years. Mean eGFR prior to conception was 46 ± 10 ml/min, with 4 (12.5%) having eGFR<30ml/min. Eleven women (34%) were treated for hypertension prior to conception, 4 (12.5%) had a renal transplant and 5 (16%) had diabetes mellitus.

6 women (20%) required renal replacement therapy (RRT); 1 during pregnancy and 5 starting 469 to 1319 days postpartum. Preconception eGFR (39.3 vs 47.5ml/min, p=0.083) and serum creatinine (154 vs 115 µmol/l, p=0.059) identified more advanced renal dysfunction in those going on to require RRT.

Baseline renal function was worse in women with a 25% increase in serum creatinine in pregnancy (eGFR 30.0 vs 46.3 ml/min, p=0.022) and persistent 25% increase in serum creatinine at 6 months post-partum (eGFR 40.2 vs 51.9 ml/min, p=0.019). No association with blood pressure, proteinuria, medication or co-morbidity was identified.

The risk of a persistent 25% increase in serum creatinine at 6 months post-partum was 100% in women with eGFR ≤30ml/min and 33% in those with eGFR>30ml/min (p=0.385). The risk of requiring RRT was 50% in women with eGFR ≤30ml/min and 15% in those with eGFR>30ml/min (p=0.169).

Conclusions: Women with CKD 3 to 5 are at risk of loss of renal function as a result of pregnancy, particularly if eGFR ≤ 30ml/min.

Funding: Clinical Revenue Support

TH-OR063

Serum Uric Acid Predicts Incident Stage 3 Chronic Kidney Disease in a Middle Aged Population with Excess Obesity and Diabetes: The Strong Heart Family Study Jason G. Umans,[1,2] Hong Wang,[1] Nawar M. Shara.[1,2] *[1]MedStar Health Research Institute; [2]Georgetown-Howard Universities Center for Clinical and Translational Science.*

Background: Hyperuricemia has been associated with prevalent and incident CKD, with hypertension, and with the metabolic syndrome in observational studies of unselected and low risk populations. Studies in higher risk populations are limited by concerns that serum uric acid (UA) may more sensitively detect subtle decrements in GFR than creatinine-based methods. We sought to determine if elevated UA predicted incident stage 3 CKD (CKD3) in a population with high prevalence of obesity, diabetes and at high risk for CKD and CVD.

Methods: The genetic epidemiologic Strong Heart Family Study (SHFS) included 3665 American Indians (60% female) with median age 39(14-93)y. At the baseline examination (2001-03), 22.8% of participants had DM, 33.4% had HTN, 57.3% were obese with BMI ≥30, 18% (648) had albuminuria (UACR ≥ 30mg/g), and 6% (217) had CKD3-5 (MDRD eGFR ≤60ml/min/1.73m2 or ESRD). After excluding those with albuminuria or decreased eGFR at baseline, we assessed the association of serum UA and incident CKD3 in the remaining 2896 participants by conditional logistic regression, accounting for the extended family structures in the study population.

Results: Over a median 5.2y follow up, there were 170 incident cases of CKD3. The 1st, 2nd, 3rd, and 4th quartiles of serum UA spanned values from 0.5-4.0, 4.1-5.0, 5.1-6.0, and 6.1-11.1 mg/dl, respectively; mean serum UA in the 4th quartile was 7.04±0.84 mg/dl. The multivariate-adjusted (for age, sex, LDL-C, HDL-C, HTN, DM, and smoking) odds ratios for incident CKD3 (95% CI) across increasing quartiles of serum UA were: 1 (referent), 0.98(0.48-1.99), 1.46(0.71-2.99), and 2.65(1.17-6.00).

Conclusions: In this high risk group with an excess of obesity and DM, clinically-elevated values of serum UA, occurring only in the 4th quartile of our population, were significantly associated with incident CKD3. This extends observations from other populations with lesser burdens of disease

Funding: Other NIH Support - NHLBI, NCRR

TH-OR064

Association of Tenofovir Exposure with Kidney Disease Risk in HIV-Infection Rebecca Scherzer,[1,2] Michelle M. Estrella,[3] Yongmei Li,[1] Steven Deeks,[4] Carl Grunfeld,[1,2] Michael Shlipak.[1,2] *[1]Medicine, San Francisco VA Medical Center, SF, CA; [2]Medicine, University of California, San Francisco, CA; [3]Johns Hopkins School of Medicine; [4]Positive Health Program, San Francisco General Hospital, SF, CA.*

Background: Despite the widespread use of highly active antiretroviral (ARV) therapy, HIV disease remains associated with increased risk of kidney disease. Whether or not tenofovir use is associated with higher risk of kidney disease is controversial.

Methods: We designed a cohort of 10,841 HIV-infected patients who initiated antiretroviral therapy in the Veterans Health Administration from 1997-2007. Using proportional hazards survival regression and marginal structural models, we evaluated association of cumulative exposure to tenofovir with kidney outcomes: first occurrence of (1) proteinuria, (2) rapid decline in kidney function (≥3ml/min/1.73m² annual decline), and (3) estimated glomerular filtration rate (eGFR) <60ml/min/1.73m². Time-dependent models adjusted for antiretroviral drugs, demographics, baseline comorbid conditions, and current measurements.

Results: During follow-up, 3,400 proteinuria, 3,078 rapid decline, and 1,712 CKD events occurred. After multivariable adjustment, each year of exposure to tenofovir was associated with increased risk of all 3 endpoints. Other ARVs showed weaker or inconsistent associations with kidney disease risk. Among those who discontinued tenofovir use, the risk of kidney disease events did not appear to decrease during follow-up.

Conclusions: Tenofovir exposure was independently associated with increased risk for kidney disease events, and did not appear to be reversible. The balance between tenofovir's efficacy and these probable adverse effects requires further study.

Association of Cumulative Tenofovir Exposure with Risk of Kidney Disease Outcomes

Outcome	Hazard Ratio (95% CI) per year of Tenofovir	P Value
Proteinuria (n=3400 events)	1.34 (1.25-1.45)	<0.0001
Rapid decline (n=3078 events)	1.11 (1.03-1.18)	0.0033
CKD (n=1712 events)	1.23 (1.12-1.35)	<0.0001

Funding: NIDDK Support, Other NIH Support - 1R03AG034871 - NIA NIH HHS, K24AI069994 - NIAID NIH HHS, Veterans Administration Support

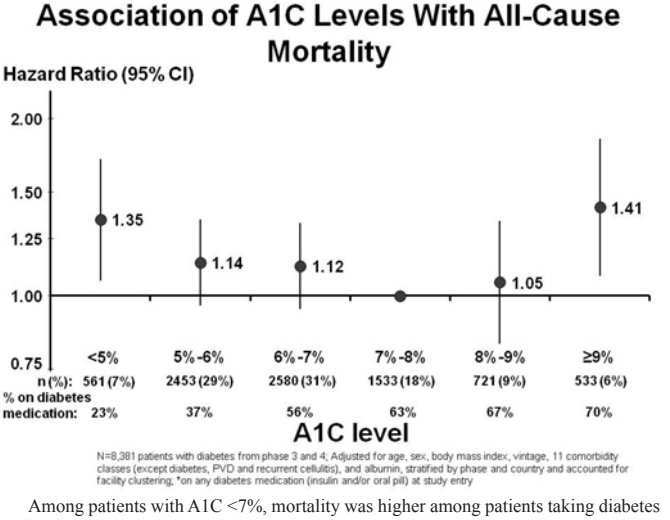
TH-OR065

Hemoglobin A1C Levels and Mortality in the ESRD Population: Findings from the DOPPS Sylvia Paz B. Ramirez,[1] Jyothi R. Thumma,[1] Francesca Tentori,[1] Brenda W. Gillespie,[2] Masaaki Inaba,[3] Robert G. Nelson,[4] Ronald L. Pisoni,[1] Bruce M. Robinson.[1,2] *[1]Arbor Research, MI; [2]Univ of MI, MI; [3]Osaka City Univ, Japan; [4]NIDDK, AZ.*

Background: Lowering hemoglobin A1C (A1C) levels to <7% can lower the risk of developing the microvascular complications of diabetes, but the value of maintaining this A1C goal in diabetic patients (pts) who have already progressed to kidney failure is less certain. In this study, we present analyses evaluating the relationship between glycemic control based on mean A1C levels and mortality in international DOPPS data.

Methods: 8,437 hemodialysis pts from 12 countries (DOPPS 3 & 4, 2006-2010) were identified who had diabetes at study entry. Associations between average A1C over 8 months (mo) after study entry and subsequent mortality was assessed using multivariable Cox regression models adjusting for age, sex, body mass index, vintage, comorbid conditions and serum albumin.

Results: Pts had 1 to 3 reported A1C values over 8 mo. 5th and 95th %ile A1C levels were 4.9 and 9.2%. The relationship between average A1C levels and mortality risk was U-shaped, with the lowest risk associated with A1C levels of 7-8%. All-cause mortality risk appears to be greatest at A1C levels ≥9% and <5%, however a trend toward higher mortality risk was seen below A1C levels of 7%.



Among patients with A1C <7%, mortality was higher among patients taking diabetes medications (p<0.01 for interaction).

Conclusions: These analyses support the importance of measuring A1C levels in the ESRD population. Target A1C levels may be higher among ESRD pts as compared to the general population since mortality risk appears to be lowest at A1C levels of between 7-8%. Use of diabetes medications is common among patients with A1C <7%; avoiding excessively low blood glucose levels by tapering these medications may be a readily modifiable practice to improve outcomes.

Funding: Pharmaceutical Company Support

TH-OR066

Intensive Glucose Lowering and End Stage Kidney Disease Vlado Perkovic,[1] Hiddo Jan Lambers Heerspink,[1,2] John P. Chalmers,[1] Mark Woodward,[1] Min Jun,[1] Alan Cass,[1] Mark E. Cooper,[4] Michel Marre,[5] Carl Erik Mogensen,[6] Sophia Zoungas.[1] *[1]George Institute for Global Health, University of Sydney, NSW, Australia; [2]Clinical Pharmacology, University Medical Centre Groningen, Netherlands; [3]Monash University, Melbourne, Victoria, Australia; [4]Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; [5]Hospital Bichat - Assistance Publique des Hôpitaux de Paris, Paris, France; [6]Aarhus Kommunehospital, Aarhus, Denmark.*

Background: Blood glucose levels have been linked to the risk of kidney disease, but the effects of intensive glucose control on major kidney outcomes among people with diabetes are not known.

Methods: This analysis from ADVANCE compared the effects of an intensive glucose lowering target (HbA1c < 6.5%) using a gliclazide MR based regimen to a standard target (below 7%) on major renal events. The outcomes assessed were end-stage kidney disease (ESKD, defined as maintenance dialysis or transplantation), renal death, confirmed doubling of creatinine to above 200 mcmol/L, and sustained doubling of creatinine to the same level (final recorded value consistent with the doubling criteria).

Results: 11,140 patients were randomised to intensive or standard glucose lowering. The mean HbA1c levels were 6.5% in the intensive and 7.3% in the standard arm. After 5 years average follow up, the risk of ESKD was significantly lower in the intensive glucose lowering arm: hazard ratio [HR] 0.35, 95% confidence interval [CI] 0.15-0.83, p= 0.02. There was a non-significant trend towards benefit for renal death (HR 0.85, CI 0.45-1.63, p=0.63) and the composite of ESKD and renal death (HR 0.64, CI 0.38-1.08, p=0.09). No clear effect on doubling of creatinine (HR 1.15, CI 0.82-1.63, p= 0.42) or sustained doubling of creatinine 0.83 (0.54- 1.27, p= 0.39) was observed.

Conclusions: An intensive blood glucose lowering regimen based on gliclazide MR reduced the risk of ESKD in the ADVANCE study, but the effects on other renal outcomes were less clear. The interpretation of doubling of creatinine as a component of renal endpoints requires further consideration. Additional studies of glucose lowering in people with diabetes at high risk of ESKD are needed.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

TH-OR067

Correlation of Plasminogen Activator Inhibitor-1 and Its Gene Polymorphism with Diabetic Nephropathy in Type 2 Diabetes Mellitus Liu Hui Lan, Xu Feng Bo. *Department of Nephrology, Fuxing Hospital, Capital Medical University, Beijing, China.*

Background: In this study, plasma plasminogen activator inhibitor-1(PAI-1) level of type 2 diabetes was measured and PAI-1 genotype was determined in order to elucidate the association of plasma PAI-1 level and its 4G/5G insertion/deletion polymorphism with type 2 diabetic nephropathy.

Methods: A total of 107 patients with type 2 diabetes were randomly recruited in the study. These patients were further divided into three groups according to their urinary albumin to creatine ratio. Group A(n=44) consised of type 2 diabetic patients without diabetic nephropathy(serum creatinine<106umol/L and normoalbuminuric). Group B consisted of patients (n=30) with microalbuminuria(A/C 20-200mg/g). Group C consisted of patients(n=33) with macroalbuminuria(A/C>200mg/g). At the same time 102 healthy controls were selected for the healthy controls. Plasma level of PAI-1 was measured by ELISA and PAI-1 polymorphism was measured DNA sequencers.

Results: (1)The plasma PAI-1 level in type 2 diabetes is higher than these in healthy controls. (2)Plasma PAI-1 level was correlated positively with glocuse,Triglyceride, High-density lipoprotein and uric acid. (3)The genotype 4G/4G distribution frequency in group C were 42.4%, which were significantly higher than those normal controls (28.7%). (4) The PAI-1 level are (68.33±15.95)ng/L, (64.57±19.39)ng/L and (58.00±18.34)ng/L in genotype 4G/4G, 4G/5G and 5G/5G. (5)Multiple logistic analysis showed that the plasma PAI-1 were the important risk factors of type 2 diabetic nephropathy.

Conclusions: (1) Plasma PAI-1 level goes up in 2 diabetes. High PAI-1 level is an important risk factor for type 2 diabetic. (2) Individuals with 4G/4G gene have higher plasma PAI-1 level. (3) PAI-1 4G/5G polymorphism is associated with the development and progression of predominant proteinuria diabetes nephropathy. 4G/4G gene probably is a susceptible gene of predominant proteinuria diabetes nephropathy.

TH-OR068

Low Plasma Adiponectin Levels Predict Increase of Urinary Albumin/ Creatinine Ratio in Type 2 Diabetes Patients Ina Maria Kacso,[1] Alina Lenghel,[1] Remus Aurel Orasan,[3] Rodica Rahaian,[2] Mirela Gherman.[1] *[1]University of Medicine and Pharmacy, Cluj Napoca, Romania; [2]Emergency County Hospital, Cluj Napoca, Romania; [3]Nefromed Dialysis Centers, Cluj Napoca, Romania.*

Background: Experimental studies have shown that adiponectin has antiproteinuric and nephroprotective effects. The purpose of the study was to assess the value of plasma adiponectin as a predictor for progressive diabetic kidney disease in type 2 diabetes (T2D) patients.

Methods: In a one-year prospective follow-up study we included microalbuminuric type 2 diabetes patients. Exclusion criteria were acute infection/inflammation, uncontrolled hypertension or history of coronary heart disease, stroke, atherosclerosis obliterans. The main outcome measure was difference in urinary albumin/creatinine ratio (UACR) between one year and baseline (Δ UACR).

Results: Fifty-six patients (66% males) completed the study. Mean UACR was in the microalbuminuric range (81.58±26.42 mg/g creatinine) and GFR close to normal (mean GFR=81,63±4,00ml/min). At baseline, simple regression disclosed significant correlations between UACR on one hand and plasma adiponectin (r=0.54, p=0.00002) and GFR (r=-0.29, p=0.03) on the other hand, findings confirmed by multiple regression analysis (p=0.0007). Baseline plasma adiponectin was significantly correlated to body mass index (r=-0.28, p=0.04), waist circumference (r=-0.27, p=0.05), HDL cholesterol (r=0.35, p=0.01), LDL cholesterol (r=0.27, p=0.04). Δ UACR correlated in simple regression significantly to baseline adiponectin values (r=-0.38, p=0.004); in multiple regression baseline plasma adiponectin remained the only predictor of Δ UACR (p=0.001). If patients are divided according to Δ UACR in nonprogressors (Δ UACR<0) and progressors (Δ UACR >0), logistic regression shows that baseline GFR (OR=1.04, CI95%: 1.00-1.09, p=0.048) and plasma adiponectin (OR=1.16, CI95%: 1.02-1.32, p= 0.02) are the only factors that predict weather the patient will be a progressor or not.

Conclusions: In microalbuminuric T2D patients lower plasma adiponectin levels seem to be predictive of increasing UACR.

Funding: Government Support - Non-U.S.

TH-OR069

The Majority of Type 2 Diabetic Patients with Renal Impairment Have Non-Albuminuric Renal Disease – The Swedish National Diabetes Register (NDR) Hanri Afghahi,[1] Mervete Miftaraj,[2] Ann-Marie Svensson,[2] Henrik Hadimeri,[1] Bjorn Eliasson,[3] Maria Svensson.[4] *[1]Nephrology, Kärnsjukhuset, Skövde, Västra Götaland, Sweden; [2]Center of Registers in Region Västra Götaland, Center of Registers in Region Västra Götaland, Gothenburg, Västra Götaland, Sweden; [3]Medicine, Sahlgrenska University Hospital, Gothenburg, Västra Götaland, Sweden; [4]Nephrology, Sahlgrenska University Hospital, Gothenburg, Västra Götaland, Sweden.*

Background: Albuminuria and renal impairment are two manifestations of renal disease but are not entirely linked in patients with type 2 diabetes (T2D). The aim of this cross-sectional study was to study prevalence and clinical characteristics associated with non-albuminuric renal impairment in T2D in a nation-wide population-based diabetes register.

Methods: 94446 patients with T2D and serum creatinine reported to the Swedish National Diabetes Register in 2009 were included. Renal impairment was defined as eGFR < 60 ml/min/1.73 m^2 (MDRD) and albuminuria as AER > 20 µg/min. A registry linkage was performed between the NDR and the Swedish Prescribed Drug Register to evaluate ongoing anti-diabetic, lipid-lowering, anti-hypertensive and aspirin medication.

Results: 17% of all patients had renal impairment and 62% of these were non-albuminuric. Patients with non-albuminuric renal impairment were more often women, non-smokers and more seldom had a history of CVD and heart failure and had lower HbA1c, triglycerides, BMI and systolic blood pressure compared to patients with albuminuric renal impairment. 27% of the patients with non-albuminuric renal impairment had no ongoing treatment with any RAAS-blocking agent. These patients had lower BMI and systolic blood pressure and were older, more often women and smokers and fewer patients had a history of CVD and heart failure compared to patients with non-albuminuric renal impairment and ongoing RAAS-blockade.

Conclusions: The majority of patients with type 2 diabetes and renal impairment were non-albuminuric. Non-albuminuric renal impairment can be explained by the use of RAAS-blockers but since only 75% of these patients were treated with RAAS-blockade this also supports the concept of different underlying pathophysiology mechanisms.

TH-OR070

Effect of Fenofibrate on Cardiovascular Events According to Changes in Plasma Creatinine Levels during the Pre-Randomization Period: The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study Jean-Claude Ansquer,[1] Karine Le Malicot,[1] Ru-Dee Ting,[2] Anthony C. Keech.[2] *[1]Laboratoires Fournier SA, Daix, France; [2]NHMRC-CTC University of Sydney, Australia.*

Background: Assessment of renal function (eGFR and albuminuria) is essential in risk stratification for cardiovascular disease (CVD) in subjects with type 2 diabetes mellitus (T2DM). FIELD was a double-blind, placebo-controlled, randomized study in

9795 T2DM subjects to assess the effects of 200mg/day micronized fenofibrate for an average 5 years on CVD events.

Methods: Prior to randomization, all the participants underwent sequentially 2 successive 6-week run-in periods with placebo and then fenofibrate 200 mg/day. This permitted an unbiased evaluation of the effect of treatment on CVD events (CVD death, non fatal MI or stroke, coronary or carotid revascularisation) according to tertiles of both baseline renal function and short term effect of fenofibrate. Analysis was intent to treat with time to first CVD event evaluated by log-rank and Cox proportional hazards model to adjust for covariates.

Results: The absolute risk reduction (ARR) in CVD events was 1.4% (relative reduction 11% p=0.035). Plasma creatinine changes in the upper tertile (>16%) were associated with the highest 5-year risk on placebo (17.7%) and the largest ARR with fenofibrate (3.5% p=0.005). ARR was 0.6% and 0.4% in the middle and lower tertile (≤8%), respectively. These results persist after adjustment for age, gender, prior CVD, use of ACE inhibitors, angiotensin receptor blockers and diuretics. Conversely, lower eGFR by tertiles or by chronic kidney disease stage is associated with higher risk of CVD.

Conclusions: The implications for CVD events of baseline renal function and the early changes in creatinine on fenofibrate treatment are different. Whereas eGFR using pre-treatment creatinine values predicted CVD events in the study, in contrast, relative changes in creatinine observed early with fenofibrate were associated with the largest reduction in CVD events.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

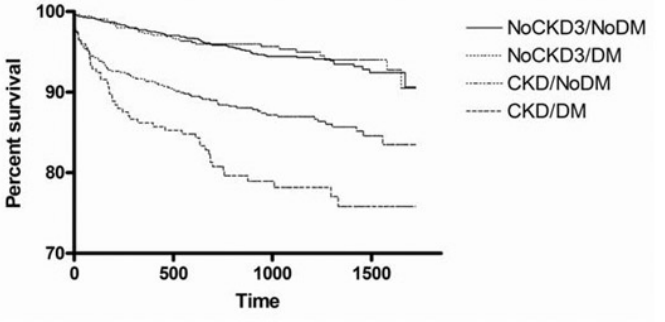
TH-OR071

Chronic Kidney Disease and Diabetes Mellitus: Can We Do More To Modify Cardiovascular Risk after Coronary Bypass Graft Surgery? Sean Gallagher, Matt Lovell, Dan A. Jones, Andrew Wragg, Akhil Kapur, Rakesh Uppal, Magdi Yaqoob. *Cardiac and Renal, Barts and the London NHS Trust, United Kingdom.*

Background: Chronic kidney disease stage 3 (CKD3) and diabetes mellitus (DM)in combination is anecdotally associated worse outcomes following coronary artery bypass graft surgery (CABG). Long term mortality data upon patients with both CKD3 and DM following CABG is currently lacking.

Methods: We analysed prospective data upon 2713 consecutive patients undergoing CABG at a tertiary cardiac centre between 2003 and 2007. Patients were divided into 4 groups for analysis: NoCKD/NoDM. NoCKD/DM. CKD/NoDM and CKD/DM. All-cause mortality was determined via Office of National Statistics data.

Results: There were 1286 No CKD/NoDM patients, 553 NoCKD/DM patients, 611 CKD/NoDM patients and 263 CKD/DM patients. In hospital mortality (0.78vs0.54%, p=0.373) and 5 year mortality (94.3vs94.8%, p=0.685)were not different between NoCKD/NoDM and NoCKD/DM groups despite more LV dysfunction (41.4vs 35.8%, p=0.037), and more previous MIs (54.0vs48.0%, p=0.0001) in the NoCKD/DM group. Comparing CKD/NoDM with CKD/DM; there was no difference in hospital mortality (2.45vs1.90%, p=0.806) but significant difference in 5 year mortality (86.2vs78.0%, p<0.0261).



CKD/DM patients had more previous MIs (53.8vs43.3%, p=0.003), more LV dysfunction (53.2vs41.9%, p<0.0001), more PVD (26.1vs16.1%, p=0.0014), and lower eGFR (45.6+/-11.9vs48.3+/-9.44, p=0.0005).

Conclusions: DM is not associated with increased mortality following CABG when eGFR is more than 60mls/min. The combination of CKD3 and DM, is associated with worse long term outcomes following CABG than with CKD3 alone. Excess late mortality is likely due to the augmentation of vascular risk factors and therapeutic nihilism. Currently, aggressive medical therapy is the mainstay of therapy but further research within this high risk group is urgently needed.

TH-OR072

Sevelamer Carbonate Improves Metabolism and Reduces Risk Factors for Progressive Nephropathy in Type 2 Diabetics with Stage 2-4 CKD by Sequestering Oral Advanced Glycation End Products Helen Vlassara,[1] Jaime Uribarri,[1] James B. Post, Fabrizio Grosjean,[3] Gary E. Striker.[1] *[1]Medicine and Geriatrics, Mount Sinai School of Medicine, New York, NY; [2]Epidemiology, University of Sydney, Australia; [3]Medicine, University of Pavia, Italy.*

Background: Increased inflammation and oxidative stress (Infl/OS) in stable diabetes mellitus (DM) are partly due to advanced glycation end products from food, and restricting AGE-intake significantly reduces these risk factors in DM. High levels of circulating advanced glycation end products (AGEs) and TNFR1/2 have been shown to be associated with progression in diabetic nephropathy. We hypothesized that sevelamer carbonate (SC), but not calcium carbonate (CC), would sequester AGEs in the GI tract and reduce Infl/OS, including circulating AGEs and TNFα, in T2DM with Stage 2-4 CKD. We conducted a proof-of-concept trial.

Methods: In a randomized two-month crossover study we compared stable diabetic patients with stage 2-4 CKD treated with either SC or CC for 2 months, a 1 week wash-out, and then the opposite drug for 2 months. There were no changes in medications and food intake.

Results: Urinary phosphate excretion was decreased by both SC and CC. Serum AGEs, lipids, HbA1c, FGF23, and 8-isoprostanes were significantly reduced by SC, but not by CC. In addition, serum chloride and potassium levels were decreased, and cystatin C showed a trend to decrease. Peripheral blood mononuclear cell AGE-receptor 1 and SIRT1 levels were decreased and TNFα were decreased by SC. These changes did not occur with CC. SC bound AGEs (AGE-BSA) (but not BSA) at pH 7.0, but not at pH 1.0 in vitro. BSA binding was <5% at either pH.

Conclusions: Sevelamer carbonate reduces circulating HbA1c, AGEs, FGF23, lipids, TNFα, other markers of inflammation and oxidative stress in stage 2-4 diabetic CKD. The proposed mechanism of action is sequestration of dietary AGEs in the GI tract and elimination in the stool. These changes were not seen with calcium carbonate. A larger and longer trial is indicated to confirm these results.

Funding: Pharmaceutical Company Support

TH-OR073

Advanced Diabetic Nephropathy with Nephrotic Range Proteinuria: Long-Term Efficacy of Subcutaneous Adrenocorticotrophic Hormone (ACTH) Therapy on Proteinuria and Urinary Vascular Endothelial Growth Factor (VEGF) Levels James A. Tumlin,[1] Claude Mabry Galphin,[3] Brad H. Rovin.[2] *[1]Internal Medicine/Nephrology, University of Tennessee College Medicine, Chattanooga, TN; [2]Renal Division, Ohio State University, Columbus, OH; [3]Clinical Research, Southeast Renal Research Institute, Chattanooga, TN.*

Background: Activation of melanocortin receptor-1 (MC1R) in podocytes and endothelium can lower proteinuria. We have shown that 6 months of ACTH gel reduces proteinuria in over 50% of patients with nephrotic diabetic nephropathy. Because ACTH increases expression of vascular endothelial growth factor (VEGF), we investigated whether the reduction in proteinuria with ACTH gel involves alteration of VEGF expression.

Methods: A total of 14 patients with diabetic nephropathy and 3.0 gm proteinuria/24 hrs on ACE inhibitor alone or 2.0 gm/24 hrs on combination ACE/ARB were enrolled. All patients had eGFR≥20 mls/min and HgBA1c ≤ 9%. Patients were randomized to ACTH gel (16U or 32U) SQ daily for 6 months. Using a Luminex or ELISA assay, urinary VEGF and monocyte chemotactic protein-1 (MCP-1) were measured at baseline and after 6 months of ACTH gel. All urinary samples were normalized to Cr.

Results: Table-1 Data

ACTH gel-16U	Baseline	6 Mth ACTH	6 Mth Post ACTH
Proteinuria	6395±735	2237±399*	1248±235*
Urinary VEGF	379±107	1539±403*	
Urinary MCP-1	569±254	1401±735	

*=P<0.05

ACTH gel (16U) reduced proteinuria from 6395 to 2237 mg/24 hrs (P=0.015). After drug withdrawal (6 mths), proteinuria further fell to 1248 mg/24 hrs (P=0.08). ACTH gel 16U increased urinary VEGF (379 to 1539 pg/mg Cr) (P=0.04). Patients responding to ACTH gel therapy (>50% reduction in proteinuria) had lower baseline urinary VEGF than non-responders (388 vs 689 pg/mg Cr)(P=0.022). Urinary MCP-1 tended to rise following treatment but did not reach statistical significance.

Conclusions: ACTH gel reduces urinary protein in diabetic nephropathy for up to 6 months after withdrawal of therapy. ACTH gel increased urinary VEGF 5-fold with a non-significant trend toward increased MCP-1. ACTH gel may represent a novel therapy for advanced diabetic nephropathy, and may act in part by restoring appropriate expression of VEGF.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-OR074

Assessment of Markers of Glycemic Control in Diabetic Patients with Chronic Kidney Disease Using Continuous Glucose Monitoring Frederiek E. Vos, John B.W. Schollum, Carolyn V. Coulter, Patrick Manning, Stephen Duffull, Robert J. Walker. *University of Otago, Dunedin, New Zealand.*

Background: Due to altered red blood cell survival and erythropoietin therapy glycated hemoglobin (HbA1c) may not accurately reflect long-term glycemic control in patients with diabetes and chronic kidney disease (CKD). Glycated albumin (GA) and fructosamine are potential alternative markers since their production is not affected by anemia or ESA. The exact relationship between glucose and the different indices of glycemia in advanced CKD have yet to be established. The aim of this study was to determine the correlation of indicators of diabetic control CKD to continuous glucose monitoring (CGM). to investigate the accuracy of HbA1c, GA and fructosamine as indicators of glycemic control using continuous glucose monitoring (CGM).

Methods: HbA1c, GA and fructosamine concentrations were measured in 25 diabetics with CKD stages 4 and 5 (eGFR < 30 mL/min/1.73m2) and 25 diabetics without nephropathy otherwise appropriately matched to the CKD subjects. Simultaneous real-time glucose concentrations were monitored by CGM over 48 hours.

Results: Mean glucose, GA and fructosamine concentrations were similar in both groups, HbA1c tended to be lower in CKD diabetics. GA correlated significantly to mean glucose concentrations in diabetics with and without CKD (r = 0.54 vs 0.49, p < 0.05). A

similar relationship was observed with fructosamine relative to glucose. A poor correlation between HbA1c and glucose was observed with CKD ($r = 0.38$, p=ns) but was significant in the non-CKD group ($r = 0.66$, $p < 0.001$). The GA/HbA1c ratio was significantly higher in diabetics with CKD compared to controls (2.5 ± 0.4 vs 2.2 ± 0.4, $p < 0.05$). HbA1c values were significantly lower in CKD diabetics, relative to non-CKD diabetics at comparable mean glucose concentrations.

Conclusions: HbA1c significantly underestimates glycemic control in CKD stage 4 & 5 diabetics. In severe CKD, GA more accurately reflects glycemic control compared to fructosamine and HbA1c in this patient cohort and should be the preferred marker of glycemic control.

Funding: Government Support - Non-U.S.

TH-OR075

Identification of Novel Genetic Factors for Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) Kirsten Y. Renkema,[1,2] Ernie M.H.F. Bongers,[2] Iris Van Rooij,[3] Loes F.M. Van der Zanden,[3] Nel Roeleveld,[3] Wout F. Feitz,[4] Helen McNeill,[5] Barbara Franke,[2] Nine V. Knoers.[1] *[1]Medical Genetics, University Medical Center Utrecht, Netherlands; [2]Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Epidemiology, Biostatistics, and HTA, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [4]Pediatric Urology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [5]Molecular Genetics, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, Canada.*

Background: CAKUT occur frequently in man and comprise the most common cause of end-stage renal disease in children. Structural anomalies belonging to the CAKUT spectrum include renal agenesis, multicystic dysplastic kidney, and duplex collecting system. Not much is known about the origin of CAKUT. Often, renal abnormalities are found in close relatives of CAKUT cases, showing a genetic contribution to CAKUT pathogenesis. In particular, alterations in genes expressed during nephrogenesis are considered to be important. The aim of this study is to identify new genetic factors involved in CAKUT aetiology.

Methods: Over 700 well-documented CAKUT case-parent triads and 45 families with multiple affected members were included in this study via the AGORA biobank project and European CAKUT consortium (EUCAKUT).

Results: Mutation analysis in a systematically selected and enriched set of CAKUT candidate genes was performed and revealed novel variants implicated in CAKUT pathogenesis. Moreover, linkage analysis in a multiplex family with 8 members dysplaying duplex collecting system identified suggestive linkage for loci on chromosome 3 and 4. Interestingly, the powerful combination of linkage analysis and next generation sequencing approaches brought disease-gene identification in close proximity.

Conclusions: The present identification and characterization of novel genetic factors for CAKUT contributes to the understanding of the pathogenesis and the design of genetic diagnostic screening tests, facilitating early detection and improving genetic counseling for CAKUT patients and their relatives.

Funding: Government Support - Non-U.S.

TH-OR076

Novel Amniotic Fluid Biomarkers of Congenital Anomalies of the Kidney and Urinary Tract Wendy Heywood, Darrell Wang, Kevin Mills, Paul J.D. Winyard. *Institute of Child Health, University College London, London, United Kingdom.*

Background: Congenital anomalies of the kidney and urinary tract (CAKUT) are often detected on pregnancy ultrasound, yet we lack good antenatal markers of prognosis. Amniotic fluid (AF) is 95% fetal urine and is collected during amniocentesis for karyotyping. We predict that changes in AF reflect the underlying severity of CAKUT. Hence, AF biomarkers may correlate with long-term prognosis.

Methods: After research ethical approval and maternal consent, we collected AF fluid from 10 controls, and 12 CAKUT cases comprising 6 isolated kidney malformations and 6 lower urinary tract obstruction. These were then analysed independently using three proteomic techniques: SELDI-TOF MS, 2D DiGE and label free quantitation (LC/MSE).

Results: In obstruction, SELDI-TOF revealed two down-regulated proteins of 11,789 and 26,301Da and two up-regulated proteins of 8,080 and 15,078Da; 2D DiGE identified significant down-regulation of alpha-1-microglubulin (AMBP) and gelsolin. Reduced AMBP was confirmed by LC/MSE, and this technique also revealed down-regulation of transthyretin, transforming growth factor-beta-induced protein ig-h3, SPARC, and plasma C1-inhibitor.

In renal malformations, SELDI-TOF detected a down-regulated protein of 40,890Da, whilst both 2D DiGE and LC/MSE identified down-regulation of collagen type-1 proteins and glycodelin; LC/MSE identified increased immunoglobulins, kininogen and IBP1.

Transthyretin and AMBP were reduced in both obstruction and kidney malformations by label free quantitation, but none of the other factors were consistently abnormal for both conditions using the same technique.

Conclusions: Many studies demonstrate common molecular pathology in CAKUT, usually with up-regulation of factors in kidney parenchyma. We identified markers that discriminate within the CAKUT spectrum, and most are down-regulated rather than increased. Key factors reduced included AMBP and glycodelin. Moreover, use of more than one proteomic technique identified a wider range of candidates. Further evaluation is needed to determine if these novel biomarkers are consistently deranged in CAKUT, with robust collection of outcome data for prognostic significance.

TH-OR077

Angiotensin (Ang) II Stimulates Papillogenesis during Late Metanephric Development Renfang Song, Graeme James Preston, Ihor V. Yosypiv. *Pediatrics, Tulane University, New Orleans, LA.*

Background: We tested the hypothesis that lack of Ang II production in angiotensinogen (*AGT*)-null mice impairs papillary collecting duct elongation thus contributing to the hypoplastic renal medulla phenotype observed in these mice.

Methods: Papillas were dissected from *AGT*+/+, +/- and -/- mouse metanephroi on postnatal day P3 and grown in 3-dimentional collagen matrix gels located on air-fluid interface in the presence of media (control, n=6/genotype) or Ang II (10^{-5}M, n=6/genotype) for 24 hours. Images were acquired at time of dissection ("0" hours) and after 24 hours of culture. Percent change in papillary length, determined by Slide book 4.0 software, at 24 hours relatively to time "0" was compared between the groups. We next examined the role of Ang II AT1R in papillary collecting duct growth, cell proliferation and apoptosis using P3 papillas from Hoxb7-GFP+ mouse grown ex vivo for 24 hours in the presence of the specific AT1R antagonist, candesartan (10^{-6}M, n=5) or media (control, n=5).

Results: Observed reduction in papillary length was attenuated in *AGT*+/+ compared with *AGT*-/- papillas (26±2 vs. 35±3%, p<0.01). Treatment with Ang II blunted the decrease in papilla length observed in respective media-treated controls (*AGT*+/+: 16±1.3 vs. 24±1.6%, p<0.01; *AGT*+/-: 16±1.1 vs. 23±0.9%, p<0.001; *AGT*-/-: 28±1.3 vs. 35±1.4%, p<0.01). Treatment with AT1R antagonist candesartan decreased papillary length after 24 hours of culture (87±4.2 vs. 100±0%, p<0.01). In contrast, papillary length in control group (media, n=5) did not differ from baseline (101±4.3 vs. 100±0%, p=0.6. The number of proliferating phospho-histone H3 (pH3)-positive collecting duct cells, visualized with anti-pancytokeratin antibody, was lower whereas the number of caspase 3 (Casp 3)-positive cells undergoing apoptosis was higher in candesartan- vs. media-treated papillas (pH3: 12±1.4 vs. 21±2.1, p<0.01; Casp 3: 3.8±0.5 vs. 1.7±0.2, p<0.01).

Conclusions: In summary, Ang II, acting via the AT1R, promotes papillary growth by stimulating collecting duct cell proliferation and survival. We conclude that defects in collecting duct elongation may be causally linked to medullary hypodysplasia observed in *AGT*- and *AT1R*-null mice.

Funding: NIDDK Support

TH-OR078

MRI Reveals Postnatal Functional Obstruction and Abnormal Peristalsis in Crim1KST264/KST264 Mice Lorine J. Wilkinson,[1] Nyoman Dana Kurniawan,[2] Yu Leng Phua,[1] Joan Li,[1] Melissa H. Little,[1] Richard J. Lang.[3] *[1]Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia; [2]Centre for Advanced Imagin, University of Queensland, Brisbane, Queensland, Australia; [3]Department of Physiology, Monash University, Melbourne, Victoria, Australia.*

Background: Crim1 hypomorphic (Crim1KST264/KST264) mice display renal disease characterised by podocyte effacement, excessive glomerular permeability, leaky peritubular vasculature, focal glomerular cysts and progressive interstitial fibrosis. A portion of Crim1KST264/KST264 mice develop hydronephrosis of unknown aetiology.

Methods: To investigate this phenomenon, the clearance of the contrast agent Magnavist® from the kidneys was analysed using dynamic contrast enhanced Magnetic Resonance Imaging (DCE-MRI). To examine the potential for a functional obstruction, ureteropelvic peristalsis was investigated using live imaging of whole kidney hemimounts.

Results: Using DCE-MRI, 95% of Magnavist was cleared from wild-type kidneys by 140 minutes after injection, however, contrast continued to accumulate in the kidneys of the Crim1KST264/KST264 mice over the imaging period of two hours. This result suggested obstructed flow of filtrate from these kidneys. Similarly, DEC-MRI was performed on a model of unilateral ureteral obstruction showed a similar accumulation of Magnavist in the obstructed kidneys. Immunofluorescence of kidney sections from Crim1KST264/KST264 mice after tail-vein injection of rhodamine dextran (10KDa) showed accumulation of dextran in the collecting ducts, particularly in the papilla. Peristalsis in the wild-type kidney originated at the kidney/pelvis junction and propagated distally along the pelvis and ureter. In contrast in the Crim1KST264/KST264 mouse peristalsis consisted of either small contractions that appeared near synchronously along the full length of the renal pelvis or appeared to originate in the distal pelvis, but only partially propagated in the proximal direction along the pelvic wall.

Conclusions: These results reveal a functional obstruction in the Crim1KST264/KST264 mouse resulting from abnormal ureteropelvic peristalsis and suggest a role for Crim1 in the development and/or maintenance of the peristaltic machinery.

Funding: Government Support - Non-U.S.

TH-OR079

Identification of microRNA and microRNA Targets in Human Podocyte Culture: A Deep Sequencing Approach Iddo Zeev Ben-Dov,[1] Moin Saleem,[2] Thomas Tuschl.[1] *[1]Rockefeller University, New York, NY; [2]Bristol Royal Hospital for Children, Bristol, United Kingdom.*

Background: Disruption of miRNA processing in mouse podocytes results in lethal glomerulopathy. Systematic identification of podocyte-specific miRNA and miRNA targets may thus facilitate further studies.

Methods: We profiled miRNA in cultured human podocytes using barcoded RNA sequencing. miRNA were grouped by sequence or genomic clustering. We also sequenced

argonaute-bound RNA by immunoprecipitation of in-vivo crosslinked miRNPs ('PAR-CLIP') to identify engaging miRNA and their targets.

Results: Podocyte miRNA profiles were typical of epithelial cells. Top miRNA sequence families were miR-21(1), let-7a-1(12), miR-27a(2), miR-30a(6), miR-29a(4) and miR-17(8) (parenthesis denote # of members). sf-miR-30a(6) ranked higher in mature cells, while sf-miR-17(8) were higher in immature. Higher expression of miR-29a(4), miR-10a(1) and several additional miRNA genomic clusters was found in podocytes compared to other epithelia (panel A).

PAR-CLIP on mature cells identified 860 high confidence mRNA clusters (showing a unique photo-crosslinking signature); a lower estimate of the true number of miRNA targets in podocytes due to the scale of the experiment. The clusters mapped to 634 genes (1 to 5 mRNA clusters per target gene). 89% of clusters matched exons. Of these, 70% mapped to 3'UTRs and 28% to CDS (28%). mRNA clusters were enriched with 7-mer seed complementary sequences to top miRNA, but the overall correlation was low (r=0.17, p<0.01), i.e., some abundant miRNA had only few matches and vice versa (see example in panel B)

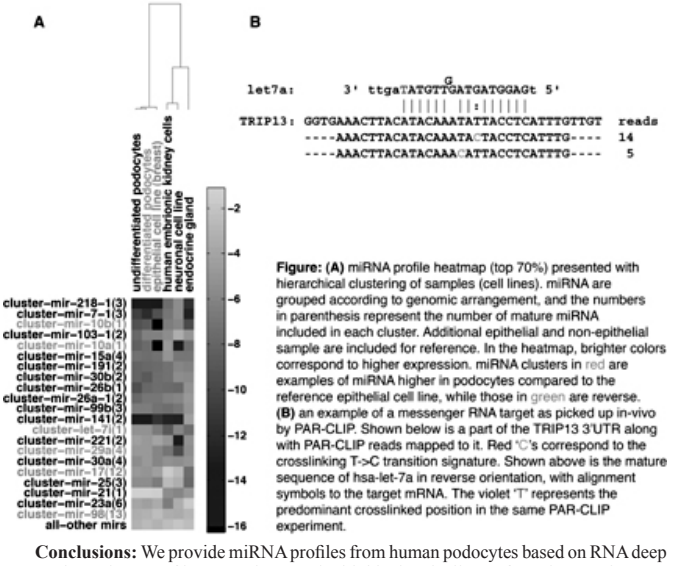


Figure: (A) miRNA profile heatmap (top 70%) presented with hierarchical clustering of samples (cell lines). miRNA are grouped according to genomic arrangement, and the numbers in parenthesis represent the number of mature miRNA included in each cluster. Additional epithelial and non-epithelial sample are included for reference. In the heatmap, brighter colors correspond to higher expression. miRNA clusters in red are examples of miRNA higher in podocytes compared to the reference epithelial cell line, while those in green are reverse. **(B)** an example of a messenger RNA target as picked up in-vivo by PAR-CLIP. Shown below is a part of the TRIP13 3'UTR along with PAR-CLIP reads mapped to it. Red 'C's correspond to the crosslinking T->C transition signature. Shown above is the mature sequence of hsa-let-7a in reverse orientation, with alignment symbols to the target mRNA. The violet 'T' represents the predominant crosslinked position in the same PAR-CLIP experiment.

Conclusions: We provide miRNA profiles from human podocytes based on RNA deep sequencing. These profiles, complemented with biochemically confirmed transcriptome-wide in-vivo mRNA target PAR-CLIP data, can serve to direct further research on miRNA involvement in podocyte development and disease.

Funding: Other NIH Support - The project described was supported by Grant Award Number UL1RR024143 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research

TH-OR080

High Resolution Genome Scans Combined with Principal Component Analysis Identifies Two Novel Nephropathy Loci

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Background: HIV associated nephropathy (HIVAN) is a secondary form of collapsing glomerulopathy and a major complication of HIV-1 infection. Previous analysis of an F2 intercross between HIV-transgenic FVB/NJ, the murine model for HIVAN, and C57BL/6J, identified two QTL on chr. 13 and 4 (HIVAN1 and HIVAN2 loci). We further investigated nephropathy QTLs in this cross, through increased mapping resolution, measurement of 12 new metabolic traits and principal component (PC) analysis.

Methods: 189 F2 mice were genotyped at 718 informative SNPs and 17 traits were measured; glomerulosclerosis (GS), tubular epithelial re-/degeneration (T-R), tubular casts/dilatation (T-C), podocyte hypertrophy (PH), proteinuria (Prot), blood urea nitrogen (BUN), calcium (Ca), phosphorus (P), uric acid (UA), cholesterol (Chol), amylase (Am), creatinine phosphokinase, albumin (Alb), alkaline phosphatase (AP), alanine transaminase (ALT), aspartate transaminase (AST), and glucose levels (Gl). Traits were transformed and standardized prior to non-parametric linkage analysis. To augment the power of linkage analysis, we also performed PC analysis of 7 nephropathy relevant traits (GS, T-C, T-R, PH, Prot, BUN & Alb).

Results: Using GS and sex as covariates, we found significant novel QTLs for PH on chr. 10 (LOD = 3.7, 40.7 cM), and for BUN on chr. 8 (LOD = 4.1, 63.9 cM). A highly significant QTL was found for Chol on chr. 1, (LOD = 8.6, 77.8 cM), co-localizing with Apoa2, a well-documented QTL for Chol. Suggestive QTL were identified for PH, T-C, Chol, BUN, Am, Al, Ca, UA, P, Gl, ALT, AST, & AP. We identified two PCs: PC1 captured 55% variance of the 7 nephropathy traits and mapped to HIVAN2 and HIVAN3. PC2 explained 17% of the variation and identified a novel QTL on chr. 8 (LOD = 4.6, 65.9 cM).

Conclusions: Increased mapping resolution and PC analysis identified two new nephropathy QTL, on chr. 10 and on chr. 8. These QTLs influence global components of disease as well as a specific histologic subcategory (podocyte hypertrophy), reflecting the genetic complexity of disease. These approaches may enable dissection of the different pathogenic mechanisms underlying HIVAN.

Funding: NIDDK Support

TH-OR081

An ENU-Induced Mutation Mapping to Chromosome 9 Is Associated with Diabetic Nephropathy in Mice

Elena E. Tchekneva,[1] Jennifer Kearney,[2] Agnes B. Fogo,[3] John N. Calley,[4] Jeffrey K. Cecil,[4] Matthew D. Breyer.[4] *[1]Medicine/Nephrology, Vanderbilt University, Nashville, TN; [2]Division of Genetic Medicine, Vanderbilt University, Nashville, TN; [3]Pathology, Vanderbilt University, Nashville, TN; [4]BioTechnology Discovery Research, Eli Lilly and Company, Indianapolis, IN.*

Background: Accumulating evidence suggests heritable factors predispose 25-40% of diabetic patients to diabetic nephropathy (DN). In mice, genetic background also appears to critically determine predisposition to DN. We mutagenized C57BL/6 mice, which are relatively resistant to DN, using ethyl-nitrosourea, and generated a dominant mutation designated Nphrp2 predisposing B6 mice to DN on a sensitizing type 1 diabetic background contributed by the dominant Akita mutation in insulin2 gene.

Methods: To map the genetic interval in which this mutation reside we performed a backcross of these C57Bl/6 mutant mice to Balb/c background. Genome scanning was performed using a panel of 478 SNPs. A reduction of interval of interest was performed using DNA microsatellite markers analysis.

Results: About 50% of the progeny of Nphrp2/Akita mutants, backcrossed B6 to Balb/C showed persistent albuminuria. N2 mutants exhibited renal failure within 19 months of age with an increase of BUN (90±15 mg/dl vs. 25±1 mg/dl in control Balb/cAkita mice, p<0.005) and by progressively increasing albuminuria. Renal hypertrophy,glomerular mesangial expansion and mesangial nodules, and pronounced tubulointerstitial fibrosis in Nphrp2 congenic mice demonstrated the persistence a dominant trait associated of the DN phenotype. Affected N2-N6 Nphrp2 mice were genotyped. Haplotype data showed retention of alleles from C57Bl/6 mutagenized strain with the only linkage being to distal Chr. 9 (LOD of 5.34). Association of the C57BL/6J chromosomal segments inherited in the backcross with the albuminuria in diabetic Balb/C mice allowed us to determine a candidate locus within which the ENU mutagenized genes reside at [101515262-104018155 bp].

Conclusions: We mapped the DN trait to 2.5 Mb locus in Chr.9. The identification of the gene involved in this mutation should facilitate the identification of new molecular pathways of DN.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-OR082

Systems Approach Identifies HIPK2 as a Critical Regulator of Kidney Tubulointerstitial Fibrosis

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Background: Tubulointerstitial fibrosis is a common process that leads to the progression of kidney disease. We describe an integrated computational/experimental approach to identify upstream protein kinases that regulate gene expression changes in kidneys of HIV-1 transgenic mice (Tg26), which exhibits significant tubulointerstitial injury. Using this approach, we identify the homeo-domain interacting protein kinase 2 (HIPK2), a previously unrecognized protein kinase for kidney disease, is critically involved in the regulation of the gene network in HIVAN kidney. We find that HIPK2 expresses mostly in renal tubular epithelial cells (RTEC) of HIVAN kidneys. We confirm that HIPK2 mediates HIV-induced apoptosis of renal tubular epithelial cells (RTEC). HIPK2 also mediates TGF-beta and HIV-induced expression of EMT markers. Here, we further determined the role of HIPK2 in vivo by crossing HIPK2-/- with Tg26.

Methods: HIPK2-/- mice on a mixed C57B6/129svj background were first backcrossed onto the FVB/N background for 6 generations and then cross with Tg26 to generate mice that are HIPK2 deficient and express the HIV-1 transgene. HIPK2-/-;Tg26 mice were compared to HIPK2+/+;Tg26 at 8 weeks of age. Renal function, proteinuria, and kidney histology were assessed in these mice. The activation of signaling pathways were also studied in kidneys of these mice.

Results: Compared to HIPK2+/+;Tg26 group, the HIPK2-/-;Tg26 mice had a significant decrease in serum urea nitrogen, urine protein/creatinine ratio, collagen deposition and tubulo-interstitial injury and fibrosis scores. Knockout of HIPK2 also significantly attenuated the activation of TGF-beta/smad, Wnt/beta-catenin, Notch, and p53 pathway in kidneys of Tg26 mice.

Conclusions: HIPK2 is a critical regulator of kidney fibrosis in the HIVAN model and a potential target for anti-fibrosis therapy.

Funding: NIDDK Support

TH-OR083

Geographic Differences in Genetic Susceptibility to IgA Nephropathy: GWAS Replication Study and Geospatial Risk Analysis Krzysztof Kiryluk,[1] Yifu Li,[1] Mersedeh Rohanizadegan,[1] Simone Sanna-Cherchi,[1] Murim Choi,[2] Francesco Scolari,[3] Loreto Gesualdo,[4] Silvana Savoldi,[5] Antonio Amoroso,[6] Bruce A. Julian,[7] Jan Novak,[7] Robert J. Wyatt,[8] Marie Metzger,[9] Benedicte Stengel,[9] Lise Thibaudin,[10] Francois C. Berthoux,[10] Frank Eitner,[11] Jurgen Floege,[11] Ulf Panzer,[12] Judit Nagy,[13] Richard P. Lifton,[2] Ali G. Gharavi.[1] *[1]Columbia U, US; [2]Yale U, Howard Hughes Med Inst, US; [3]U of Brescia, Italy; [4]U of Foggia, Italy; [5]Ciriè Hosp, Italy; [6]U of Torino, Italy; [7]U of Alabama at Birmingham, US; [8]U of Tennessee, US; [9]INSERM, France; [10]U N Hosp, St Etienne, France; [11]U of Aachen, Germany; [12]U Clin Hamburg-Eppendorf, Germany; [13]U of Pécs, Hungary.*

Background: In a recent GWAS, we localized 5 common susceptibility loci for IgAN. The goals of this study are to replicate these findings in independent cohorts and to model the genetic risk for IgAN in the worldwide populations.

Methods: We tested for association, assessed heterogeneity, and calculated a genetic risk score for IgAN in independent cohorts of 5 nationalities (French, Italian, German, Hungarian & AA; 1,120 cases & 1,665 controls) in addition to the original GWAS cohorts (Asians and Europeans; combined N=8,687). After validation, the risk model was applied to 1,042 HGDP individuals (57 populations); spatial interpolation of the risk trend surface was used to construct topographical IgAN risk maps across 6 continents.

Results: 4 of 5 loci demonstrate significant replication and no heterogeneity (OR=0.70-0.86, P=9x10-8-1x10-2). Heterogeneity is observed only for the MHC locus of TAP1/2-PSMB8/9 (I2=75%, p<0.01). This locus replicates in Italians & Germans (OR=0.60-0.67, P=5x10-3-2x10-2), but not in French, Hungarians, or AAs. In the combined analysis, all 5 loci remain highly significant with minor alleles conferring protection (OR=0.66-0.81, P=10-27-10-10). In the geospatial analysis, the genetic risk increases sharply with distance from Africa (P=2x10-16), and thus closely parallels the geographic distribution of IgAN.

Conclusions: These data suggest that the five IgAN risk loci contribute to the known geographic variation in disease prevalence.

Funding: NIDDK Support

TH-OR084

Mitochondrial Mistargeting Causes Autosomal Dominant Renal Fanconi Syndrome Markus Reichold,[1] Enriko Klootwijk,[2] Horia Stanescu,[2] Detlef Bockenhauer,[2] Carsten Broeker,[1] Dominika Peindl,[1] Kathrin Renner,[1] Karin Eberhart,[1] Joerg Reinders,[1] Katja Dettmer,[1] Robert Kleta,[2] Richard Warth.[1] *[1]University of Regensburg, Regensburg, Germany; [2]University College London, United Kingdom.*

Background: Renal Fanconi syndromes are characterized by a generalized renal proximal tubular dysfunction. For this study the causative genetic factor as well as the underlying molecular pathology in an extended family with autosomal dominantly inherited renal Fanconi syndrome without kidney failure was unraveled.

Methods: Whole genome multipoint parametric linkage analysis was performed resulting in a significant LOD score (> 3) for a single locus. All genes in the linked area were sequenced resulting in the identification of a heterozygous mutation in a gene, which we call Fanconin that leads to a de-novo formation of a mitochondrial targeting motif. To assess the functional impact a stable permanently transfected inducible renal proximal tubular cell model was generated. Immunohistochemical analysis showed appropriate intracellular localization for the wild type and mitochondrial mistargeting for mutant Fanconin.

Results: Since Fanconin is mistargeted to mitochondria the impact of this mutation on mitochondrial function was investigated. When mutated Fanconin was overexpressed, lactate levels in the medium were increased and cells consumed more glucose compared to overexpression of wild type protein pointing towards enhanced glycolysis. Respirometric measurements of these cells indeed showed a reduced response to ADP-triggered stimulation of the respiratory chain. According to our hypothesis, mutated Fanconin is interfering with oxidative phosphorylation leading to diminished ATP production in mitochondria.

Knockout mice for Fanconin, as expected, did not show a proximal tubular transport defect as assessed by aminoacid analysis using GC-MS.

Conclusions: We therefore hypothesize that the renal Fanconi phenotype in our family is not caused by haploinsufficiency rather than by a dominant negative effect of the Fanconin protein.

To our knowledge this is the first description of a genetic defect leading to intracellular mistargeting of a mutant protein resulting in mitochondrial pathology.

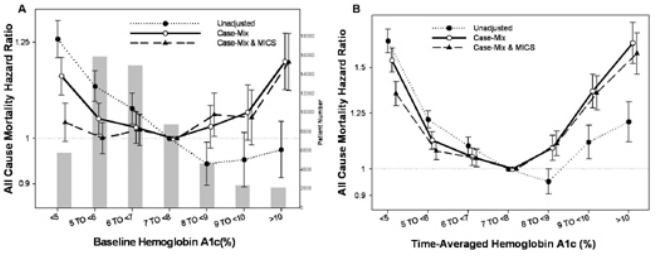
TH-OR085

Glycemic Control and Mortality in Hemodialysis Patients with Diabetes Mellitus: A Six Year Cohort Study Miklos Z. Molnar,[1] Joni L. Ricks,[1] Csaba P. Kovesdy,[2] Anuja P. Shah,[1] Allen R. Nissenson,[3] Mark E. Williams,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Torrance, CA; [2]Salem VA Medical Center, Salem, VA; [3]DaVita, Inc, Denver, CO; [4]Joslin Diabetes Center, Harvard Medical School, Boston, MA.*

Background: Observational studies examining the association of hemoglobin A1c (A1c) with outcomes in diabetic patients (pts) on maintenance hemodialysis (MHD) have used different methodologies & reached somewhat contrasting conclusions.

Methods: We examined mortality-predictability of A1c & random serum glucose over time in a cohort of diabetic MHD pts treated in DaVita dialysis clinics from July 2001 through June 2006 with follow-up through June 2007.

Results: We identified 54757 diabetic MHD pts with A1c data (age, 63±13 years, 51% men, 30% African Americans). Adjusted all-cause death hazard ratio (HR) and 95% confidence interval for baseline A1c increments of 8.0-8.9%, 9.0-9.9% and ≥10%, compared to 7.0-7.9% (reference), were 1.06(1.01-1.12), 1.05(0.99-1.12), & 1.19(1.12-1.28); and for time-averaged A1c were 1.11(1.05-1.16), 1.36(1.27-1.45), & 1.59(1.46-1.72), respectively. A consistent increase in mortality also occurred with time-averaged A1c levels in the low range 6.0-6.9% (1.05(1.01-1.08), 5.0-5.9% (1.08(1.04-1.11)) & ≤5% (1.35(1.29-1.42)) compared to 7.0-7.9% A1c in fully adjusted models.



Adjusted all-cause death HR for time-averaged blood glucose 175-199 mg/dl, 200-249 mg/dl, 250-299 mg/dl & ≥300 mg/dl, compared to 150-175 mg/dl (reference), were 1.03(0.99-1.07), 1.14(1.10-1.19), 1.30(1.23-1.37) and 1.66(1.56-1.76), respectively.

Conclusions: Poor glycemic control (A1c ≥ 8% or serum glucose ≥ 200mg/dl) appears associated with high death in MHD pts. Very low glycemic levels also add mortality risk. Clinical trials are needed to better define the target A1c levels in long-standing diabetic pts on MHD.

Funding: NIDDK Support

TH-OR086

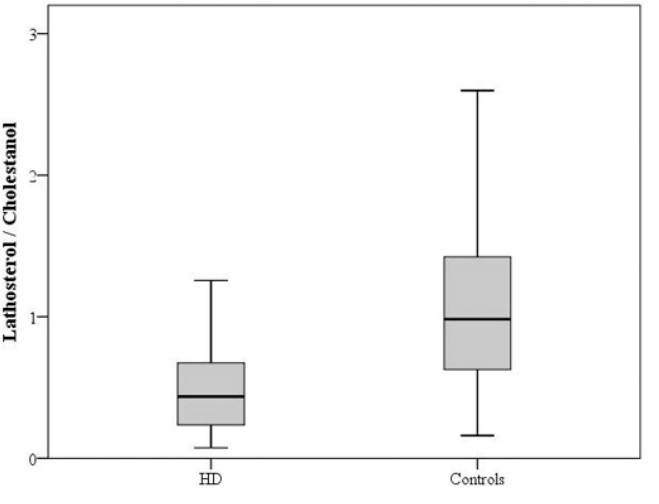
Analysis of Cholesterol Homeostasis Characterizes Dialysis Patients as "Cholesterol Absorbers" Kyrill S. Rogacev,[1] Tobias Pinsdorf,[2] Oliver Weingärtner,[4] Julius Popp,[3] Danilo Fliser,[1] Dieter Lutjohann,[2] Gunnar H. Heine.[1] *[1]Nephrology & Hypertension, Saarland University Hospital, Homburg, Germany; [2]Clinical Chemistry/Pharmacology, University of Bonn, Bonn, Germany; [3]Psychiatry, University of Bonn, Bonn, Germany; [4]Cardilogy, Angiology, Intensive Care, Saarland University Hospital, Homburg, Germany.*

Background: Recent clinical trials on cholesterol-lowering in chronic kidney disease patients yielded conflicting results, which might result from different treatment strategies used. Serum cholesterol levels are determined by both endogenous synthesis and intestinal absorption, which are differentially influenced by various classes of cholesterol-lowering agents. Assessment of cholesterol homeostasis has thus been proposed for guidance of lipid-lowering therapy. We analysed established surrogate markers of cholesterol homeostasis in patients with chronic kidney disease.

Methods: In 113 hemodialysis patients, we measured lathosterol and desmosterol as markers of cholesterol synthesis, and cholestanol, sitosterol and campesterol as markers of cholesterol absorption via gas chromatography. 229 healthy subjects served as controls. Overall survival in dialysis patients was recorded over a follow-up of 28 months.

Results: Hemodialysis patients displayed a striking shift towards cholesterol absorption compared to synthesis (p<0.001). High absorption markers and concomitantly low synthesis markers indicated poor outcome among dialysis patients in univariate Kaplan-Meier analysis, and in multivariate Cox regression analysis after adjustment for potential confounders.

Conclusions: Our analysis of cholesterol homeostasis characterises hemodialysis patients as "cholesterol absorbers". These findings supplement data from a recent randomised controlled trial on dual cholesterol-lowering therapy in chronic kidney disease.



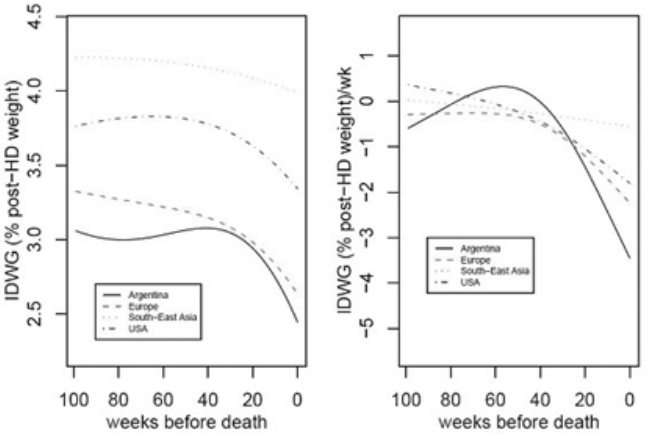
TH-OR087

Declining Interdialytic Weight Gain (IDWG) in Chronic Hemodialysis Patients (HD) – An Ominous Sign? Results of an International Study Adrian Marcos Guinsburg,[1] Cristina Marelli,[1] Adam Tashman,[2] Michael Etter,[3] Daniele Marcelli,[4] Gero D. von Gersdorff,[6] Mathias Schaller,[6] Yuedong Wang,[2] Nathan W. Levin,[5] Peter Kotanko,[5] Len A. Usvyat.[5] *[1]FMC Latin America, Buenos Aires, Argentina; [2]University of California - Santa Barbara, Santa Barbara, CA; [3]FMC Asia Pacific, Hong Kong, Hong Kong; [4]FMC Europe, Bad Homburg, Germany; [5]RRI, NY, NY; [6]University of Cologne Medical Center, Germany.*

Background: Chronic HD patients (pts) frequently present with high IDWG, presumably due to high fluid intake. It has been reported that in pts from US, contrary to widespread believe, declining IDWG precedes death (Kotanko 2009). We extend this observation to diverse HD populations from 3 continents.

Methods: HD databases from FMC clinics in Europe, Asia, Latin America, RRI clinics in US, and KfH in Germany were queried. IDWG was calculated as % of post-HD weight. IDWG dynamics were analyzed by estimating the mean IDWG level before death and its 1st derivative using quintic splines.

Results: Chronic HD pts from 23 countries were studied (Europe 17 [N=12333; age 71.7, 59% males]; South-East Asia 4 [N=1484; age 68; 53% males]; Argentina [N=10517; age 63.1; 58% males]; USA [N=3473; age 69.9; 56% males]). IDWG [mean (SD)] before death was 2.3 (2.6); 3.8 (3.2); 2.9 (3.1); 2.9 (3.1); same order as above. Irrespective of the region, IDWG dropped in males between 0.23% in South-East Asia and 0.68% in Europe in the 2 years preceding death (left).



The rate of IDWG change was identical in Europe, Argentina, and US, and less in South-East Asia (right). The results were materially identical in females and pts from KfH (not shown).

Conclusions: This international study corroborates that opposite to common believe, a decline in IDWG may be an ominous sign. Insights into pre-death biology may aid the development of alert systems to facilitate timely interventions.

TH-OR088

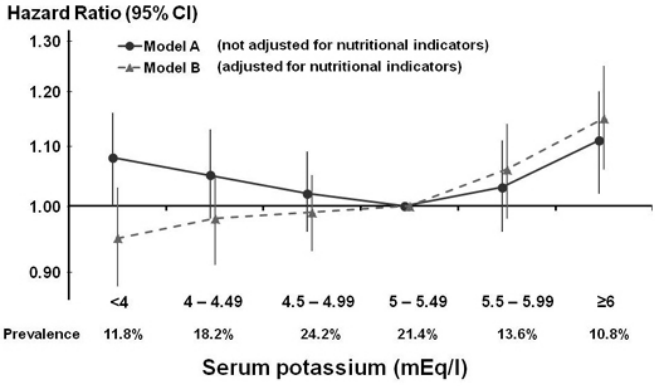
Associations of Serum Potassium with All-Cause Mortality: Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Rajiv Saran,[1] Jinyao Zhang,[2] Ananda Sen,[1] Hal Morgenstern,[1] Francesca Tentori,[2] Antonio Alberto Lopes,[3] David C. Mendelssohn,[4] Vittorio E. Andreucci,[5] Hideki Kawanishi,[6] Bruce M. Robinson.[1,2] *[1]University of Michigan; [2]Arbor Research Collaborative for Health; [3]Bahia da Universidade Federal da Bahia; [4]Humber River Regional Hospital; [5]Universita Federico II; [6]Tsuchiya General Hospital.*

Background: Severe hyperkalemia, defined here as serum potassium (SK) > 6 mEq/l, has been observed in approximately 10% of maintenance hemodialysis (HD) patients. We investigated the relation between SK and mortality in a large international HD cohort study.

Methods: We analyzed data on 37,967 in-center hemodialysis (HD) patients from 12 countries in DOPPS phases 1-3 (1996-2008). Patient demographics, laboratory values and dialysis treatment data were obtained at study enrollment. Cox regression was used to estimate the hazard ratio (HR and 95% CI) for the effect of SK on all-cause mortality, adjusting for several potential confounders, excluding and including 4 nutritional markers. SK was categorized and treated as 5 indicator variables in the model (<4, 4-4.49, 4.5-4.99, 5-5.49 [ref.], 5.5-5.99, 6+ mEq/l).

Results: When adjusting for patient demographics, 13 summary comorbid conditions, socioeconometric status, dialysis treatment and adherence indicators, but not nutritional markers, there was a U-shaped association between SK and mortality (Figure, Model A). When also adjusting for BMI, albumin, creatinine and normalized PCR, a positive monotonic association was observed (Model B).

Conclusions: The association between hypokalemia and mortality seems to be confounded by poor nutritional status. Therefore, SK < 4.0 mEq/l should alert physicians to the potential presence of poor nutritional status in HD patients. Levels approaching 6 mEq/l or higher, however, warrant appropriate therapeutic maneuvers.



N=37,967 patients; models stratified by country and phase, and accounted for facility level clustering
In Model A, the HR is adjusted for age, sex, race, vintage, 13 comorbidities, smoking, prior TX, catheter use, employment status, education level, living status, marriage status, skipped≥1 hemodialysis session in past 30 days, shortened≥1 hemodialysis session by ≥10 minutes in past 30 days, IDWG >5.7% of dry weight, PO4 >7.5 mg/dl, spKt/V, and hemoglobin. In Model B, the HR is adjusted for the Model A covariates plus BMI, albumin, creatinine and normalized PCR

Funding: Pharmaceutical Company Support

TH-OR089

Economic Evaluation of Frequent Home Nocturnal Hemodialysis Based on a Randomized Trial Scott Klarenbach,[1] Robert P. Pauly,[1] Michael Walsh,[3] Marcello Tonelli,[1] Helen Lee,[2] Brenda Hemmelgarn,[2] Braden J. Manns.[2] *[1]University of Alberta; [2]University of Calgary; [3]McMaster University.*

Background: There is interest in providing frequent home nocturnal hemodialysis (NHD) to patients on dialysis, and decision makers require information on its cost-effectiveness.

Methods: We did a cost-utility analysis of frequent home NHD compared with conventional hemodialysis (4 hours thrice weekly, including in-centre, satellite, and home HD) taking the perspective of the health payer over a lifetime horizon. Data on transition between health states, quality of life, costs, and other inputs were from a recent RCT. Costs, including training costs, were obtained using micro-costing, trial, and administrative data (CAN$). We determined the incremental cost per quality adjusted life year (QALY) gained, and robustness was assessed using scenario, sensitivity, and probabilistic sensitivity analyses.

Results: Compared with conventional HD (61% in-centre, 14% satellite, and 25% home as in the trial), frequent home NHD led to incremental costs of $290 and additional 0.38 QALYs, with a cost/QALY gained of $740. In sensitivity analyses, when the annual probability of failing home NHD and commencing conventional HD was assumed to be ≤ 6%, home NHD was dominant (greater benefit at lower costs), however if ≥19%, the cost/QALY was >$75k/QALY. The cost/QALY gained increased to $29k if average training time for NHD increased from 3.65 weeks to 6 weeks. Results were sensitive to quality of life estimates. In scenario analyses where the comparator modality was changed, frequent home NHD was dominant compared to in-centre HD, and was associated with a cost/QALY gained of $35k, $203k, and $481k when compared with satellite HD, home HD, and peritoneal dialysis respectively.

Conclusions: By current standards used to inform reimbursement decisions in publically funded health care systems, frequent home NHD appears attractive compared with conventional HD. However, the attractiveness varies based on the mix of comparator dialysis modalities. The modality mix provided by a health system and used in patients who would be offered frequent home NHD is a critical factor when considering establishing a program.

Funding: Government Support - Non-U.S.

TH-OR090

Anti-Inflammatory & Anti-Oxidative Nutrition in Hypoalbuminemic Dialysis Patients (AIONID) Double-Blind Randomized Placebo-Controlled Trial Kamyar Kalantar-Zadeh, Martin Lee, Ramanath B. Dukkipati, Jennie Jing, Youngmee Kim, Anne Coble Voss, Deborah A. Benner, Miklos Z. Molnar, Iain C. Macdougall, John Tayek, Keith C. Norris, Csaba P. Kovesdy, Joel D. Kopple. *LABioMed/Harbor-UCLA.*

Background: Low serum albumin (alb) is common & associated with wasting, inflammation & poor outcomes in hemodialysis (HD) patients (pts). We hypothesized that high protein oral nutritional supplements (ONS) tailored for HD pts combined with anti-oxidants & anti-inflammatory ingredients with/without anti-inflammatory appetite stimulator (pentoxifylline, PTX) is well tolerated & improves albumin level.

Methods: 84 adult hypoalbuminemic (<4.0 g/dL) HD pts in several DaVita clinics were double-blindly randomized (2x2 factorial) to receive 16 wks of placebo-controlled interventions including: (1) ONS consisting of high (19 g) protein supplement (Nepro™, 8 oz/d) combined with a concentrated anti-oxidant module (fish oil, borage oil, beta-carotene, vit C & E, zinc &selenium, 2 oz/d) vs. same volume placebo ONS without protein or anti-oxidants; &/or (2) PTX, 400 mg/d, vs. placebo pill.

Results: Out of 84 pts (age: 59±12 yrs, vintage: 33 mo), 74 completed the entire 16 wks.

Group (n)	A (n=19)	B (n=22)	C (n=22)	D (n=21)	ANOVA-p
Assignments	ONS+PTX	ONS+placebo	PTX+placebo	placebo+placebo	-
Age (yrs)	59.2±13.6	54.6±10.3	57.7±13.9	63.9±9.1	0.09
Gender (% men)	53	45	55	62	0.77
Pre-trial albumin (g/dl)	3.46±0.38	3.56±0.45	3.60±0.45	3.60±0.20	0.51
Change in albumin	+0.18±0.23	+0.21±0.30	+0.14±0.26	+0.03±0.24	-
Paired t-test p	0.001	0.004	0.008	0.587	-

Paired t-test showed significant alb increase of +0.14 to +0.21 g/dl in any intervention other than combined placebos. However, the intention-to-treat analysis of changes in alb using predetermined equation (*post-albumin = pre-albumin+A+B+C)* showed that only ONS was associated with a significant change: ONS, PTX or both were associated with change in alb of +0.17 (p=0.02), +0.11 (p=0.12) & -0.16 g/dL (p=0.10), respectively. No serious adverse events were observed.

Conclusions: Daily intake of CKD-specific high-protein ONS with anti-inflammatory/ anti-oxidative ingredients for 16 wks was well tolerated and associated with improved albumin level of about +0.17 mg/dL per ITT (p=0.02).

Funding: NIDDK Support

TH-OR091

More Efficient Removal of Serum Bilirubin by a Novel Artificial Liver Support System: A Pilot Study Dehua Gong, Dongdong Zhu, Daxi Ji, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China.*

Background: In this study, we established a novel artificial liver support (ALS) system based on fractionated plasma separation and adsorption system integrated with continuous veno-venous hemofiltration (FPSA-CVVH), and compared its efficacy with the traditional system of plasma separation and adsorption (PSA).

Methods: Sixteen patients with hyperbilirubinemia due to acute liver failure were included. For PSA, plasma was separated and perfused through an adsorber. For FPSA-CVVH, albumin-rich plasma was separated using a fraction plasma separator, followed by a concentration through ultrafiltration using a hemofilter; The concentrated plasma was then perfused through an adsorber and returned to the patients with a simultaneous infusion of the same amount of replacement fluid via a predilution route into blood. The duration for each session of ALS was 8 hours.

Results: The comparisons of total bilirubin clearance (TB, ml/min) by PSA versus FPSA-CVVH at the time points of 0.5, 2, 4, 6 and 8 hour were as follow: 22.3 ± 2.2 vs. 28.7 ± 13.1, 12.2 ± 4.4 vs. 21.9 ± 9.1 (P<0.05), 9.0 ± 2.8 vs. 16.1 ± 4.3 (P<0.01), 9.5±3.9 vs. 11.2±3.7, and 8.3±3.0 vs. 9.3±4.1, respectively. The average reduction rates (%) of TB, bile acid, urea and creatinine after a single session of PSA versus FPSA-CVVH were 46.1 ± 8.3 vs. 54.4 ± 5.2 (P<0.05), 36.5 ± 5.2 vs. 47.6±14.7, -14.9±16.8 vs. 31.4±10.6(P<0.01), and -17.1±14. 7 vs.36.3±9.2 (P<0.05), respectively. On the other hand, there was a significant decline of albumin level (11.8±5.9%, P<0.001), prolongation of prothrombin time (41.1±27.2%, P<0.01) and activated partial thromboplastin time (26.6±30.4%, P<0.05) observed after PSA but not FPSA-CVVH. The survival rates at the 30th day of ICU and hospital discharge were 69% and 56%, respectively.

Conclusions: In conclusion, compared with PSA system, the novel FPSA-CVVH system provides a more efficient removal of both albumin-bound toxins, like bilirubin, and water-soluble toxins, like urea and creatinine, with less deleterious effects on serum albumin level and blood coagulation.

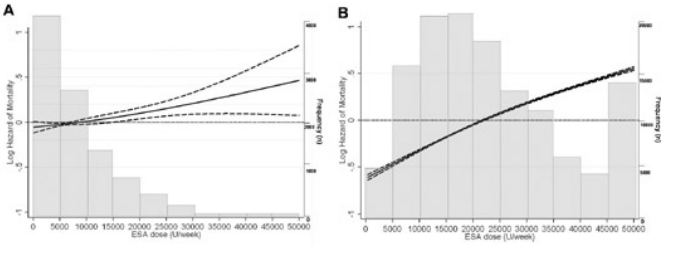
TH-OR092

Comparing Erythropoiesis Stimulating Agent Dose and Responsiveness between Peritoneal and Hemodialysis Patients Uyen Duong,[1] Kamyar Kalantar-Zadeh,[1,2] Miklos Z. Molnar,[1,3] Joshua Zaritsky,[2] Isaac Teitelbaum,[4] Csaba P. Kovesdy,[5] Rajnish Mehrotra.[1,2] *[1]Harold Simmons Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Semmelweis University, Hungary; [4]University of Colorado, Denver, CO; [5]Salem VA Medical Center, VA.*

Background: We hypothesized that peritoneal dialysis (PD) patients (pts) required lower erythropoiesis stimulating agent (ESA) dose for the same achieved hemoglobin compared to hemodialysis (HD) pts & that ESA dose-mortality associations were different between PD and HD patients.

Methods: We compared the prescribed doses of ESA between 139,103 HD & 10,527 PD pts treated from 7/2001 through 6/2006 using adjusted Poisson regression & examined mortality-predictability of prescribed ESA dose & ESA resistance index (ESA/hemoglobin) in PD & HD with follow-up through 6/2007 using Cox regression models.

Results: Poisson adjusted relative ratio of ESA dose of HD to PD was 3.6 (95% confidence interval: 3.5-3.7). In PD patients, adjusted all-cause death hazard ratios (HR) for ESA doses of 3,000-5,999, 6,000-8,999 & ≥9,000 U/week (reference<3,000 U/week) were 0.97(0.87-1.07), 0.85(0.76-0.95) & 1.08(0.98-1.18), respectively (Figure A); whereas in HD pts across ESA dose increments of 10,000-19,999, 20,000-29,999 and ≥30,000 U/ week (reference <10,000 U/week) were 1.14(1.11-1.17), 1.54(1.50-1.58) & 2.15(2.10-2.21), respectively (Figure B).



In PD and HD pts, the adjusted death HR of the 4th to 1st quartile of ESA resistance index were 1.14(1.04-1.26) & 2.37(2.31-2.43), respectively.

Conclusions: During the 2001-2006 time period most PD patients received substantially lower ESA dose for same achieved hemoglobin levels, consistent with the known selection bias for patients with less comorbidities benign on PD. ESA resistance was associated with much higher mortality in HD than PD patients.

Funding: NIDDK Support

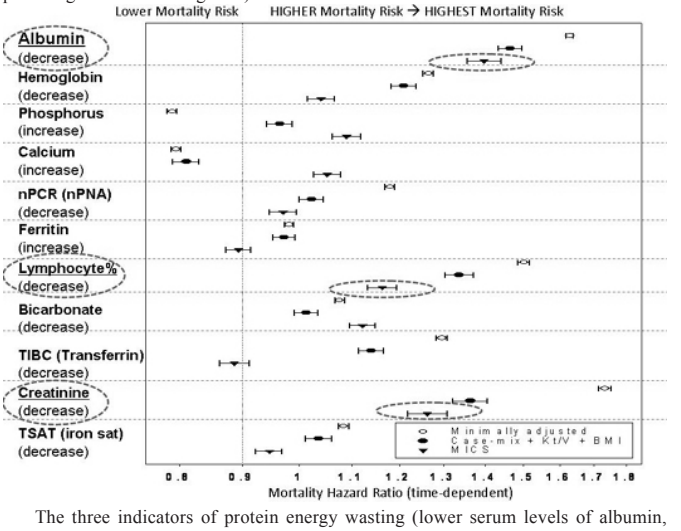
TH-OR093

Superiority of the Survival-Predictability of Laboratory Measures of the Nutritional Status in Hemodialysis Patients: A Comparative Effectiveness Study Jessica Miller,[1] Csaba P. Kovesdy,[2] Miklos Z. Molnar,[1] John J. Sim,[3] Ramanath B. Dukkipati,[4] Parta Hatamizadeh,[1] Deborah A. Benner,[5] Joel D. Kopple,[4] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center, Torrance, CA; [2]Salem VA Medical Center, Salem, VA; [3]Kaiser Permanente, Los Angeles, CA; [4]Harbor UCLA, Torrance, CA; [5]DaVita Inc., Denver, CO.*

Background: It is not clear which potentially modifiable laboratory measures are associated with greater survival in maintenance hemodialysis (MHD) patients (pts).

Methods: In a large cohort of 140146 MHD pts who underwent MHD treatment for at least 3 months in all legacy DaVita dialysis clinics between July 2001 & June 2006, changes in values across consecutive 20 calendar quarters for laboratory measures that may represent "malnutrition inflammation complex syndrome" (MICS) were calculated. Values were standardized with mean of 0 and standard deviation of 1, and hazard ratios for the increase or decrease (as commensurate) in 1 standard deviation were estimated.

Results: Pts were 61.5±15.5 years old & included 45% women, 32% African Americans, 14% Hispanics & 44% diabetics. Cox models were case-mix adjusted (sex, age, race/ethnicity, diabetes, dialysis vintage, Kt/V, BMI) & MICS adjusted (serum creatinine, albumin, calcium, nPCR, ferritin, bicarbonate, TIBC, TSAT, phosphorus, lymphocyte percentage & blood hemoglobin):



The three indicators of protein energy wasting (lower serum levels of albumin, creatinine & lymphocyte percentage) showed the strongest association with death risk, followed by lower serum bicarbonate & higher levels of serum phosphorus & calcium.

Conclusions: Measures of nutritional/inflammatory status are by far the strongest predictors of survival in MHD patients. Interventions to improve nutritional/inflammatory status may improve longevity. This hypothesis needs testing in randomized trials.

Funding: NIDDK Support

TH-OR094

Hepcidin Isoforms – Biomarkers for Optimal Managements of Renal Anemia in Hemodialysis Patients Satoshi Yamazaki,[1] Yusuke Sasaki,[2] Masahiro Hagiwara,[1] Shunichi Furuhata,[1] Minoru Murakami,[1] Yasushi Shimonaka,[2] Masaya Ikezoe.[1] *[1]Dept. of Nephrology, Saku Central Hospital, Minamisaku-Gun, Nagano, Japan; [2]Product Ressearch Dept., Chugai-pharmaceutical Co., Ltd., Kamakura-shi, Kanagawa, Japan.*

Background: It has been known that serum levels of hepcidin-25 (Hep-25) and its isoforms, hepcidin-20 and -22, are significantly higher in hemodialysis (HD) patients than in healthy subjects, however, the significance of these isoforms have not been well elucidated. In the present study, we analyzed the relationship between serum levels of hepcidin isoforms and clinical parameters in HD patients to clarify the possibility of hepcidin isoforms as biomarkers for anemia treatment.

Methods: We enrolled 100 HD patients, provided written informed consent in Saku Central Hospital. Patients with infection, severe inflammatory disorders or hematologic disorders were excluded. Anemia and other complications were treated according to national and K/DOQI guidelines. Erythropoiesis stimulating agents (ESA) was used to maintain a target hemoglobin (Hb) level of 10-11 g/dL. Clinical parameters including iron parameters and serum hepcidin isoforms were measured. Patients characteristics were analyzed in 4 groups divided by median of serum levels of Hep-25 (High, Low) and the ratio of active hepcidin (Hep-25/total hepcidin isoforms levels) (High, Low).

Results: Patients with high Hep-25 levels and low active hepcidin ratio, named HL group, showed the lowest Hb and highest mean cellular hemoglobin (MCH) than any other groups. HL group also exhibited high transferrin saturation (TSAT) and serum ferritin levels. Instead, patients with low Hep-25 levels and low active hepcidin ratio (LL group) showed the highest Hb and lowest MCV than any other groups. LL group exhibited low TSAT and serum ferritin levels. There was no difference in ESA dosage between groups.

Conclusions: Present results indicate that patients with high Hep-25 levels and low active hepcidin ratio characterized enough iron storage and supply but low hematopoietic activity. It is recommended that these patients should be treated ESA rather than iron supplementation. Moreover, iron status and hematopoietic activity might affect proteolysis of hepcidin isoforms.

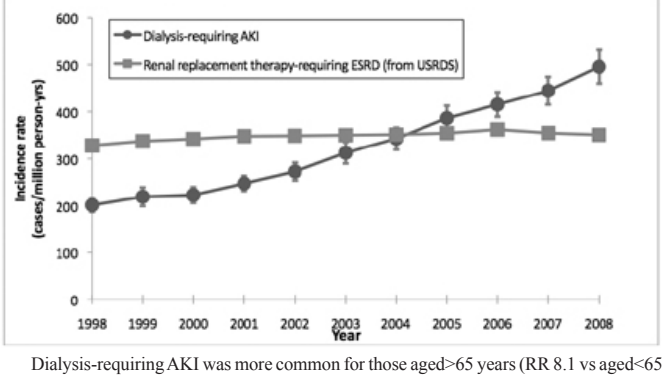
TH-OR095

Dialysis-Requiring AKI Is Now More Common Than Renal Replacement Therapy-Requiring ESRD Raymond K. Hsu, Charles E. McCulloch, Lowell J. Lo, R. Adams Dudley, Chi-Yuan Hsu. *UCSF.*

Background: There are limited data on the population epidemiology of acute kidney injury (AKI).

Methods: To determine the incidence rate of dialysis-requiring AKI from 1998-2008, we analyzed data from the US Census and the Nationwide Inpatient Sample (NIS), a nationally representative sample of hospitalizations, accommodating for NIS's sampling scheme. Cases were identified using validated ICD9 codes (Waikar 06). To explore reasons for temporal trends, we used multivariate logistic regression, with dialysis-requiring AKI as the primary outcome, year as main predictor, and additional covariates such as demographic variables and prespecified AKI risk factors.

Results: From 1998-2008, the incidence rate of dialysis-requiring AKI increased from 201 to 496 cases/million person-yrs, averaging 9% per yr (OR 1.09, 95% CI 1.08-1.10). Since 2005, the population incidence of dialysis-requiring AKI has exceeded that of renal replacement therapy (RRT)-requiring end-stage renal disease (ESRD).



Dialysis-requiring AKI was more common for those aged>65 years (RR 8.1 vs aged<65 years, 95% CI 7.9-8.3), blacks (RR 1.7 vs whites, 95% CI 1.6-1.8), and men (RR 1.3 vs women, 95% CI 1.2-1.4). Accounting for temporal changes in population distribution of age, race, gender, as well as trends of sepsis, acute heart failure, and receipt of cardiac catheterization and mechanical ventilation only slightly attenuated this trend to an adjusted rate of 8% per yr (OR 1.08, 95% CI 1.07-1.09).

Conclusions: The incidence of dialysis-requiring AKI has increased rapidly in the past decade and now exceeds the incidence of RRT-requiring ESRD. Factors such as changing demographics, increased incidence of sepsis and acute heart failure, or increased utilization of cardiac catheterization and mechanical ventilation do not appear to account for this strong secular trend. The public health burden of AKI is larger than previously appreciated.

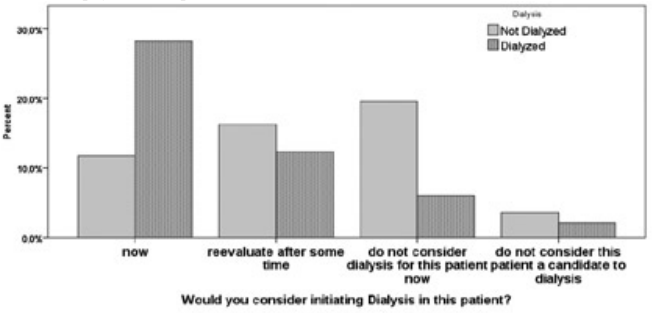
TH-OR096

Factors Associated with the Decision Making Process in Dialysis Initiation in AKI: Results of a Survey Etienne Macedo,[1] Josee Bouchard,[2] Jorge Cerda,[3] Ashita J. Tolwani,[4] Anjali Acharya,[5] Gary R. Cutter,[4] Ravindra L. Mehta.[6] *[1]University of Sao Paulo, Brazil; [2]Hôpital du Sacré-Coeur de Montréal, Canada; [3]Albany Medical College; [4]University of Alabama; [5]Jacobi Medical Center; [6]University of California San Diego.*

Background: In AKI, the benefits of dialysis must outweigh the associated risks. We hypothesized that physician decisions for initiating dialysis in AKI are affected by patient and processes of care factors.

Methods: We utilized a web-based survey to present 22 real case scenarios. For each case we asked whether physicians would initiate dialysis and the reason for their choice. The presentation included all pertinent clinical and laboratory data from 48 to 72 hours prior and up to the day of dialysis in actually dialyzed patients (n=12) or the day of peak creatinine in those patients that where not dialyzed (n=10).

Results: There were a total of 332 questionnaires representing 12-28 physician responses to each case. Physicians preferred to initiate dialysis immediately in 40%; reevaluate after some time in 29%; did not think the patient needed dialysis in 26%; and considered the procedure futile in 5% of the responses. There was substantial discordance between the physician responses and the actual events.



Process-of-care factors frequently influenced the decision: Nurse availability (27%); vascular access issues (25%); dialysis need at night time (17%) or holiday and weekends (17%). Physician's perception of futility correlated well with their choice to withhold dialysis, whereas prediction of renal recovery was associated with greater likelihood that the patient did not need dialysis.

Conclusions: Decisions to initiate dialysis are widely variable and uncertainty is common. Perception of patient prognosis and process-of care-factors influence the decisions.

Funding: NIDDK Support

TH-OR097

A Multi-Center Pilot Study To Assess the Safety and Efficacy of Selective Cytopheretic Device (SCD) Therapy in Patients with Acute Kidney Injury (AKI) James A. Tumlin,[1] Lakhmir S. Chawla,[2] Ashita J. Tolwani,[3] John J. Dillon,[4] Ravindra L. Mehta,[5] Kevin W. Finkel,[6] J. Ricardo Da Silva,[7] Alexander S. Yevzlin,[8] David Humes.[9] *[1]SERRI; [2]George Washington University; [3]University of Alabama; [4]Mayo Clinic; [5]UCSD; [6]University of Texas; [7]Cytopherx, Inc.; [8]University of Wisconsin; [9]University of Michigan.*

Background: Dialysis requiring AKI in critically ill patients is associated with hospital mortality rates between 50-60%. Currently, there are no proven therapies that can improve renal function or reduce overall mortality.

Methods: To address this problem, we conducted a multicenter, single arm, pilot study of the Selective Cytopheretic Device (SCD), a novel cartridge that is able to selectively bind and deactivate activated neutrophils from the circulation. Enrolled patients received up to 7 days of SCD therapy in conjunction with citrate-based continuous renal replacement therapy (CRRT). The primary endpoints of the study were safety of the SCD and mortality at 28 and 60 days.

Results: A total of 35 patients were enrolled by 6 centers in the United States between May 2010 and January 2011. The mean age of patients enrolled was 56.9 years with 60.0% (21/35) male. The mean Sequential Organ Failure Assessment (SOFA) score at baseline was 11.3 and 88.6% (31/35) were ventilated. The overall mortality rate was 28.6% (10/35) in hospital, 25.7% (9/35) at 28 days and 31.4% (11/35) at 60 days. The rate of renal recovery in survivors was 73.1% (19/26) by day 28 and 100% (24/24) by day 60. During the study, a total of 28 serious adverse events were reported of which 7.1% were determined to by investigators to be related to the SCD device. Elastase, IL-6, and sICAM, markers associated with inflammation, decreased substantially over the course of therapy.

Conclusions: This trial provides preliminary data of the safety and efficacy of the SCD treatment. The SCD compares favorably to the 50-60% mortality reported in previous AKI trials. The addition of the SCD to conventional citrate-based CRRT was well tolerated and led to a reduction in serum markers of activated neutrophils.

Funding: Pharmaceutical Company Support

TH-OR098

High Cut-Off Hemodialysis for the Management of Myeloma Kidney: An International Study Colin A. Hutchison,[2] Anne Bevins.[1] *[1]The Binding Site Group Ltd, Birmingham, United Kingdom; [2]Renal Unit, University Hospital Birmingham, United Kingdom.*

Background: The early reduction of serum free light chains (FLC) improves clinical outcomes for patients with acute kidney injury secondary to multiple myeloma. We studied an international cohort of patients treated FLC removal by high cut off (HCO) hemodialysis to determine treatment patterns and clinical outcomes associated with its use in this setting.

Methods: Data was collected from 54 patients, from 18 centers in 10 countries, using electronic case report forms. Rates of renal recovery were determined in relationship to baseline variables, treatment patterns and degree of FLC reduction achieved.

Results: Patients were predominately Caucasian, median age 65 years (range 43-81), with a median GFR of 8 (1-27). Baseline serum biochemistry was: creatinine 633.5µmol (168-2263); calcium 2.3mmol (0.91-3.83); albumin 34g/L (14-46) and β2M 9.45mg/L (0-55.7). Myeloma kidney was the primary diagnosis in 88.1% of the population who had a renal biopsy. Monoclonal κ and λ FLCs levels were: κ 5070mg/L (range 2250-20200) and λ 4200mg/L (range 300-13300), respectively at presentation. 68.75% of patients were treated with the Theralite dialyzer; another 31.25% used the HCO1100. 78% received bortezomib and 34% received thalidomide as part of their initial treatment. In total there were 626 HCO dialysis sessions, with a median of 13 (3-35) treatments per patient. HDF was used in 3 patients. Median FLC reduction was 72.96% (15.09-99.62%) by day 12 and 93.03% (40.23-99.96%) by the last dialysis treatment. There was no difference in the percentage FLC reduction achieved between bortezomib and thalidomide treatment groups (p=0.140). Dialysis independence occurred in 55.6% of patients, median time 32 days (10-249). Independence of dialysis was greater in patients who had a reduction in serum FLCs by day 12 (p=0.038). No significant adverse events related to the study device were reported.

Conclusions: Targeting early FLC reductions in patients with AKI secondary to multiple myeloma should become standard of care. This study adds further weight to the potential benefit of FLC removal by HCO-HD in this setting.

TH-OR099

Exposure to Potentially Toxic Hydro- and Halocarbons Released from Dialyzer and Tubing Sets during Hemodialysis Hyun Ji Julie Lee,[2] Madeleine V. Pahl,[1] Nosratola D. Vaziri,[1] Donald R. Blake.[2] *[1]Nephrology and Hypertension, University of California, Irvine, CA; [2]Chemistry, University of California, Irvine, CA.*

Background: While much is known about the effect of ESRD and dialysis on the composition of solutes in the plasma, little is known about their impact on composition of gaseous compounds in the exhaled breath. This study was designed to explore the effect of uremia and hemodialysis (HD) on the composition of exhaled breath.

Methods: Exhaled breath samples were collected from 10 ESRD patients immediately before, during and after dialysis. Ten age-matched healthy subjects served as controls. To determine the potential introduction of gaseous compounds from the components of dialysis, gasses were eluded from dialyzers, tubing sets, dialysate and water supplies. A five column/detector gas chromatography (GC) system was employed to measure different hydrocarbon, halocarbon, oxygenate, and alkyl nitrate sulfur containing compounds.

Results: The concentration of 11 hydrocarbons and 4 halocarbons in the patients' breath rose rapidly after the onset of the HD. All of the 15 compounds appearing in the breath (and 5 others not found in the breath) were eluded from the dialyzers and tubing sets, but not the water supplies or final dialysate solution.

Breath Composition. Average breath concentrations collected before, at the start (0 minutes) and 3 minutes after start of HD. Number of samples averaged were labeled as n.

Compounds	Average ± SE, pptv			p-value	
	pre (n = 60)	0 (n = 20)	3 (n = 20)	pre vs 0	0 vs 3
i-butane	1703 ± 271	1511 ± 245	1908 ± 297	0.5	0.3
n-butane	2144 ± 477	1873 ± 459	3523 ± 671	0.6	0.05
n-pentane	1639 ± 420	1364 ± 307	2492 ± 426	0.5	0.04
n-hexane	454 ± 68	388 ± 52	2059 ± 595	0.3	0.01
n-heptane	865 ± 234	925 ± 267	1963 ± 411	0.8	0.04
n-octane	161 ± 24	134 ± 15	961 ± 140	0.2	8 E-07
n-nonane	6870 ± 1444	6633 ± 1486	456170 ± 96808	0.9	4 E-05
n-decane	208 ± 65	198 ± 52	231 ± 45	0.9	0.6
3-methylpentane	343 ± 62	288 ± 51	1729 ± 639	0.4	0.03
3-methylhexane	380 ± 67	312 ± 51	677 ± 129	0.3	0.01
3-methylheptane	98 ± 25	54 ± 11	800 ± 133	0.1	2 E-06
CH_3CH_2Cl	37 ± 13	27 ± 10	78 ± 16	0.5	0.01
CH_2CHCl	1 ± 1	1 ± 0.4	22 ± 44	0.5	0.04
CH_2Cl_2	1453 ± 279	1371 ± 200	41873 ± 11822	0.8	0.001
$CHCl_3$	156 ± 28	143 ± 25	319 ± 101	0.7	0.1

Contrary to earlier reports which showed increased ethane levels during HD, exhaled breath ethane concentrations remained virtually unchanged during HD and mirrored the concentrations found in room air.

Conclusions: The present study documented release of several potentially toxic hydrocarbons and halocarbons to the circulation from the dialyzer and tubing sets during HD. The long-term exposure to these compounds may contribute to the morbidity and mortality in ESRD population and this issue should be considered in manufacturing of new generation of dialyzers and dialysis tubing sets.

Funding: Clinical Revenue Support

TH-OR100

^{23}Na-MRI Monitoring of Sodium Removal in Dialysis Patients Anke Dahlmann,[1] Peter Linz,[2] Florian Eicher,[1] Kathrin Dörfelt,[1] Matthias Hammon,[3] Christoph Kopp,[1] Alexander Cavallaro,[3] Dominik N. Muller,[5] Kai-Uwe Eckardt,[1] Friedrich C. Luft,[4] Michael Uder,[3] Jens Titze.[2] *[1]Nephrology & Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Interdisciplinary Centre for Clinical Research, University of Erlangen-Nuremberg, Erlangen, Germany; [3]Radiology, University of Erlangen-Nuremberg, Erlangen, Germany; [4]Experimental & Clinical Research Centre, Charité & MDC, Berlin, Germany; [5]Experimental Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Sodium and water are removed by hemodialysis. While water removal can easily be determined by ultrafiltration rates and weight loss, no valid method for the assessment of tissue Na^+ content is available. ^{23}Na magnetic resonance could offer spectroscopic and imaging (MRI) options.

Methods: We used ^{23}Na magnetic resonance spectroscopy and imaging at 3Tesla (T) to quantify Na^+ content in skeletal muscle of the left lower leg. 15 dialysis patients (9 men, 6 women) were measured before and after regular dialysis with ^{23}Na MRI. Blood pressure was measured before and after dialysis treatment. Ultrafiltration rate was adjusted to individual interdialytic weight gain.

Results: Initial sodium content of M. triceps surae in dialysis patients was 21.2±6.3 mmol/l and decreased significantly to 17.1±6.8 mmol/l after dialysis treatment, while serum Na^+ concentrations were unaffected by dialysis. Muscle Na^+ content before dialysis was higher in men than in women; however, age was the strongest predictor for muscle Na^+ content in patients with endstage renal disease (< 60 years, n=7: 17.8±2.7 mmol/l; > 60 years, n=8: 24.5±7.2 mmol/l). Na^+ removal from muscle tissue with dialysis treatment was inversely correlated with ultrafiltration rate (y=-0.00007x+0.90452, R^2 = 0.47), but not with blood pressure.

Conclusions: Non-invasive measurement of tissue Na^+ content with ^{23}Na MRI provides a novel clinical tool to assess Na^+ removal in patients with end-stage renal disease during hemodialysis treatment. The method could allow efficacy monitoring for Na^+ removal and permit a better understanding of the relationship between body-Na^+ accumulation, blood pressure, and cardiovascular disease in patients with end-stage renal disease.

Funding: Government Support - Non-U.S.

TH-OR101

Increasing Protein-Bound Solute Clearances Independent of Urea Clearance Tammy L. Sirich,[1] Frank Jiann-Gang Luo,[1] Thomas H. Hostetter,[2] Timothy W. Meyer.[1] *[1]Medicine, Stanford, Palo Alto, CA; [2]Medicine, AECOM, New York, NY.*

Background: The toxicity of bound solutes could be evaluated better if we could adjust the clearance of such solutes independent of unbound solutes. This study evaluated a means to increase the clearance of bound solutes independent of unbound solutes during nocturnal dialysis. It tested the hypothesis that bound solute clearances could be increased by raising the dialysate flow and dialyzer size above the low levels which are sufficient to achieve target Kt/V_{urea} values during the extended hours of nocturnal treatment.

Methods: Nine patients on thrice-weekly in-center nocturnal dialysis underwent two experimental sessions one week apart. The sessions were designed to provide the same urea clearance but widely different bound solute clearances. The *Low* K_oA-Q_d session employed a small dialyzer, dialysate flow of 300 ml/min, and blood flow of 350 ml/min. The *High* K_oA-Q_d session employed a larger dialyzer and dialysate flow of 800 ml/min while restricting blood flow to 270 ml/min. The bound solutes p-cresol sulfate (PCS) and indoxyl sulfate (IS) were measured by HPLC and the unbound solute urea was measured enzymatically.

Results: Results showed (mean±sd; [a], p<0.05 *High* K_oA-Q_d vs *Low* K_oA-Q_d; [b], p<0.05 PCS and IS clearance vs urea).

		Low K_oA-Q_d	*High* K_oA-Q_d
Urea	Clearance (ml/min)	193 ± 16	204 ± 20
	Kt/V_{urea}	1.8 ± 0.3	1.9 ± 0.4
	Removed in dialysate (g)	43 ± 6	41 ± 11
PCS	Clearance (ml/min)	14 ± 6 [b]	27 ± 9 [a, b]
	Removed in dialysate (mg)	207 ± 86	375 ± 200 [a]
IS	Clearance (ml/min)	14 ± 5 [b]	26 ± 8 [a, b]
	Removed in dialysate (mg)	153 ± 74	201 ± 137 [a]

Urea clearance and removal were similar during both treatments. As expected, the clearances of the bound solutes PCS and IS were much lower than the clearance of urea. But the *High* K_oA-Q_d session nearly doubled the clearances of PCS and IS without changing the urea clearance. Higher clearances during the *High* K_oA-Q_d session resulted in the removal of more PCS and IS in the dialysate.

Conclusions: Increasing dialyzer size and dialysate flow during nocturnal dialysis increases the clearance of bound solutes and could provide a means to further test the contribution of such solutes to morbidity in ESRD.

Funding: Other NIH Support - NIH Nephrology Research Training Grant T32 DK7357

TH-OR102

Associations of Dialysate Bicarbonate (DB) with Mortality: Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Francesca Tentori,[1] Jinyao Zhang,[1] Ananda Sen,[2] Hal Morgenstern,[2] Bruce M. Robinson,[1,2] Hugh C. Rayner,[3] Rachel B. Fissell,[4] Raymond C. Vanholder,[5] Tadashi Tomo,[6] Friedrich K. Port.[1,2] *[1]Arbor Research Collaborative for Health; [2]University of Michigan; [3]Birmingham Heartlands Hospital; [4]Cleveland Clinic Foundation; [5]University Hospital Gent; [6]Oita University.*

Background: Severe pre-dialysis metabolic alkalosis has been associated with elevated mortality risk in patients on maintenance hemodialysis (HD) [Bommer AJKD 2004]. We hypothesized that use of high dialysate bicarbonate (DB), which could result in severe post-dialysis metabolic alkalosis, may adversely affect clinical outcomes.

Methods: This study included 16,899 patients on thrice-weekly in-center HD using DB as the predominant dialysate buffer (20 to 45 mEq/L) from 12 countries in DOPPS 2 (2002-2004) and 3 (2005-2008). Cox regression was used to estimate the effect of DB on all-cause mortality, adjusting for potential confounders including predialysis serum bicarbonate. DB was analyzed as a continuous variable and as a categorical variable (<33, 33-36.9, ≥37 mEq/L).

Results: DB varied across facilities and countries. The overall median facility mean DB was 35 mEq/L (inter-quartile range=33.2, 36.5), with the lowest median level in Japan (30) and the highest level in the US (37). About 65% of facilities prescribed the same DB dose to more than 90% of its patients. DB was positively associated with all-cause mortality in the total sample and in facilities using a single DB for most patients (Table). The adjusted HR for DB did not vary appreciably by level of pre-dialysis serum bicarbonate, serum or dialysate potassium, serum or dialysate calcium.

Conclusions: High DB was associated with elevated mortality risk in the DOPPS. Changes in DB can easily be implemented in dialysis treatment and may have a beneficial impact on clinical outcomes.

Table: Adjusted HR (95% CI)* for all-cause mortality, by category of dialysate bicarbonate or continuous DB and patient sample

Dialysate bicarbonate (mEq/L)	All patients (N=16,899) %	HR	95% CI	P-value	Facilities with more than 90% patients on the same dialysate bicarbonate prescription (N=8,345 patients)** HR	95% CI	P-value
Per 10 mEq/L (continuous)	-	**1.21**	**(1.03,1.43)**	**0.02**	**1.28**	**(1.01,1.61)**	**0.04**
By DB category:							
<33	24%		Ref.			Ref.	
33-36.9	51%	1.09	(0.97,1.24)	0.15	1.19	(0.97,1.46)	0.10
≥37	25%	**1.21**	**(1.05,1.40)**	**0.01**	**1.37**	**(1.08,1.74)**	**0.01**

*Adjusted for serum bicarbonate, age, sex, BMI, vintage, 13 comorbidities, albumin, hemoglobin, SBP, residual kidney function, catheter use, and spKt/V, stratified by country and study phase, and accounted for facility level clustering
**Restricted to patients from the initial cross-section and facilities with at least 5 patients and ≥90% of patients prescribed the same dialysate bicarbonate

Funding: Pharmaceutical Company Support

TH-OR103

Resource Conservation in Hemodialysis: Two Simple, Practical, Eco-Sensitive, Cost Efficient Initiatives Alwie Tjipto, John W.M. Agar. *Renal Unit, Geelong Hospital, Melbourne, Victoria, Australia.*

Background: Conventional hemodialysis (HD) is a rapacious (ab)user of water and power, yet most HD services ignore its environmental impact. While HD session duration, frequency and equipment varies, our measured mean mains water use (4-5 hr x 3/wk HD) is ~450L/treatment using a low-efficiency reverse osmosis (RO) plant (dialysate flow rate 500ml/min: 66% reject rate). Further, the metered power-draw from our paired Fresenius 4008B + Aquauno RO home systems is ~1.3kWh/hr. If similar data are notionally applied to all 2.0 x 10^6 HD patients world-wide, the extrapolated annual world-wide water use would approximate 33.7 trillion liters and the power draw 1.62 trillion kWh.

Methods: Two parallel eco-sensitive programs now address resource utilization in our service. From 2004/5, a range of simple, inexpensive RO reject water (RW) systems recycle the biochemically and biologically WHO and EPA 'potable' RO RW generated at our in-centre, multiple satellite and home patient HD sites for use in: surgical instrument sterilization, janitor services, off-site sporting facility and garden maintenance. From June 2010, 18 solar panels (area = 23.41m^2: cost = A$16,219), have powered our 4 chair home HD facility (HTU) - the first known solar-assisted HD project in the world.

Results: The water reuse program reclaims up to 100,000L RO RW/week. The return on investment (RoI) for capital outlays are accrued within 36 months. In the 1st 11 months of operation, our solar project has created 107% of all the HD-process-related power needs of the HTU. Total solar power generated 3,496 kWh, total power drawn 3,262 kWh. Power use is currently billed at A$0.267/kWh. Power generation is grid-reimbursed at A$0.235/kWh, we are meeting 94.3% of the power cost for the HTU. The current modeled RoI for capital repayment = 19.7 years. The estimated panel life is 30 years. This should result in free power and a future income stream in the 3rd operational decade. With electricity costs projected to double over coming years, there is a potential that the modeled RoI will improve significantly.

Conclusions: Solar assisted power generation for dialysis is feasible and we recommend similar practices be considered by all dialysis services.

TH-OR104

The Long Interdialytic Interval and Mortality in Hemodialysis Patients Robert N. Foley,[1,2] David T. Gilbertson,[1] Thomas A. Murray,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: As end-stage renal disease limits the tolerance of metabolic and volume-related excursions from normality, and cardiovascular disease is highly prevalent, we set out to address the hypotheses that the long interdialytic interval is associated with adverse events in hemodialysis patients.

Methods: We studied 32,065 participants in the CMS End-Stage Renal Disease Clinical Performance Measures (CPM) Project, a nationally representative sample of US patients receiving hemodialysis three times weekly at the end of the calendar years 2004 through 2007. We compared rates of death and cardiovascular hospital admissions on the day following the long (two-day) interdialytic interval with rates on other days.

Results: Mean age of the cohort was 62.2 years; 24.2% had been receiving dialysis treatment for ≤ 1 year. Over a mean follow-up interval of 2.2 years, the following event rates were higher on the day after the long interval: all-cause mortality (22.1 versus 18.0 per 100 person-years, $P < 0.001$), cardiac mortality (10.2 versus 7.5, $P < 0.001$), infectious mortality (2.5 versus 2.1, $P = 0.007$), mortality from cardiac arrest (1.3 versus. 1.0, $P = 0.004$), mortality from myocardial infarction (6.3 versus 4.4, $P < 0.001$); admissions with myocardial infarction (6.3 versus 3.9, $P < 0.001$), congestive heart failure (29.9 versus 16.9, $P < 0.001$), stroke (4.7 versus 3.1, $P < 0.001$), dysrhythmia (20.9 versus 11.0, $P < 0.001$), and any cardiovascular event (44.2 versus 19.7, $P < 0.001$).

Conclusions: The long interdialytic interval is a time of heightened risk in hemodialysis patients.

Funding: NIDDK Support

TH-OR105

Overexpression of Mouse Angiotensinogen in Renal Proximal Tubule Causes Salt-Sensitive Hypertension in Mice Donald E. Kohan, Deborah Stuart, Jean-Marc Lalouel, Jian Ying. *University of Utah Health Sciences Center, Salt Lake City, UT.*

Background: The role of proximal tubule angiotensinogen (AGT) in modulating blood pressure has previously been examined using mice co-expressing proximal tubule human AGT and human renin. These animals are hypertensive, however the question remains whether alterations in "endogenous" mouse proximal tubule AGT, interacting with endogenous renin, affects blood pressure (BP).

Methods: Mouse AGT cDNA was knocked-in to the endogenous kidney androgen promoter (KAP) gene using an internal ribosomal entry site-based strategy.

Results: The KAP-mAGT animals showed kidney-specific KAP-AGT mRNA expression; renal immunostaining detected AGT only in proximal tubule. Urinary uncleaved AGT was markedly increased in KAP-mAGT mice (5.3 ± 2.1 nmole) vs. wild-type controls (0.1 ± 0.1 nmole, p<0.001, N=4). On a high Na diet, radiotelemetric BP showed a mean systolic BP elevation of 5 ± 2 mmHg during the day and a 10 + 2 mmHg elevation during night in KAP-mAGT animals comparing to litermater control mice; no significant difference in BP was observered when fed a normal salt diet. Plasma renin concentration was reduced to 80% of control animal values by proximal tubule mAGT overexpression in animals given a high Na diet, but was not different between mouse lines during normal Na intake (n=7). Plasma AGT concentration was not altered by overexpression of proximal tubule mAGT (n=7). Renal renin mRNA tended to decrease in targeted mice (79 ± 7% of controls, N=7, p=0.08), while renal NHE3 and ENaC-α mRNA tended to increase (134 ± 14% of controls, N=7, p=0.06 for NHE3; 151 ± 25% of controls, N=7, p=0.09 for ENaC). No changes were detected in renal mRNA levels for ACE, NKCC2, ENaC-β, or NCC.

Conclusions: In summary, proximal tubule overexpression of mAGT leads of hypertension and salt-sensitivity without recruitment of the systemic renin-angiotensin system. The mechanisms responsible for the hypertensive effects of mAGT overexpression are unresolved, but studies suggest that further attention should be focused on alterations in NHE3 and ENaC-α.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-OR106

Angiotensin II Type 1A Receptors in Vascular Smooth Muscle Cells Contribute to Blood Pressure Control, Salt Sensitivity and Hypertension Matthew A. Sparks, Johannes Stegbauer, Susan B. Gurley, Thomas M. Coffman. *Nephrology, Duke Hospital.*

Background: Ang II acting through AT1 receptors promotes hypertension. However,AT1 receptors are expressed in many tissues relevant to BP control. In previous studies, we found that AT1 receptors in proximal tubule play a key role in determining level of BP. Here,we examine the impact of vascular actions of AT1 receptors in chronic BP homeostasis.

Methods: We generated a mouse with a conditional allele of the Agtr1a gene encoding the major AT1 receptor isoform, AT1A. To eliminate AT1A from VSMCs, we used transgenic mice with Cre driven by SM22 promoters. In a previous study, we found that a heterologous SM22-Cre transgene induced deletion of AT1A from large conduit arteries, but not resistance vessels. In these mice, BP and hypertensive responses were unaffected. Therefore, we now utilize a transgenic line with Cre knocked-in to the SM22 locus(KISM22-Cre).

Results: KISM22-Cre mice were crossed with the mTmG reporter to map Cre expression, documenting Cre in SM throughout the vasculature including small arterioles. We then generated mice homozygous for the conditional Agtr1a allele also bearing KISM22-

Cre(SMKOs). Compared to controls, acute constrictor responses to ang II were decreased by 25% in SMKOs(P<0.05), consistent with partial deletion of AT1A from resistance arteries and arterioles. Resting BP measured by telemetry was reduced in SMKOs(105.6 2.3 mmHg vs. 114.3 1.8 mmHg; P=0.01). While BPs in the control mice were not affected by varying sodium, MAP fell by 5mmHg in SMKO(P=0.01) mice during low salt and increased in the SMKO mice by 7 mmHg(P=0.01) on high salt. Similarly, hypertension induced by chronic infusion of ang II was attenuated in SMKOs(MAP:121.7 2.8 mmHg vs. 145 3.5 mmHg; P=0.004) and this was associated with diminished aortic media thickening(63 3.8 vs. 82 2.8 m; P<0.006) and reduced cardiac hypertrophy(5.5 0.2 vs. 7.4 0.6 heart/body,mg/g; P=0.01)in SMKOs.

Conclusions: Thus, a mutation causing partial attenuation of Ang II-induced vasoconstriction reduces BP, enhances sodium sensitivity and confers substantial resistance to hypertension indicating a major role for vascular AT1A receptors in BP control and hypertension pathogenesis.

Funding: NIDDK Support, Veterans Administration Support

TH-OR107

Responses of the Renal Afferent Arterioles to Increased Perfusion Pressure and Angiotensin II: Role of $P47^{phox}$

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Background: The tone of the renal afferent arteriole regulates renal vascular resistance (RVR) and transmission of arterial pressures into the kidneys. We reported that mouse isolated perfused afferent arterioles challenged with either angiotensin II (Ang II; 10^{-6} M) or an increasing perfusion pressure generated reactive oxygen species (ROS: from PEG-SOD inhibitable ethidium: dihydroethidium fluorescence, E:DHE) and that the contractile responses to both were blunted by 10^{-4}M tempol. We detected the mRNA for $p47^{phox}$, Nox-2 and -4 in a gene array from individual microdissected afferent arterioles. Since Ang II infusion downregulated Nox-4 and only Nox-2 requires $p47^{phox}$, we tested the hypothesis that Nox-2/$p47^{phox}$ is the source of the ROS that enhanced afferent arteriolar tone and thereby RVR with Ang II infusion. Ang II increased MAP and RVR in $p47^{phox}$ +/+ mice (77±2 to 91±3 mmHg; P<0.05 and 7.5±0.4 to 10.5±0.8 mmHg/mL/min/100 g; P<0.05) but did not change these variables in -/- mice (74+/-3 to 76+/-1 mmHg; NS and 8.9±0.5 to 9.7±0.7 mmHg/mL/min/100 g; NS). Afferent arterioles dissected from mouse kidneys and perfused at 40 mmHg had similar basal diameter in $p47^{phox}$ +/+ and -/- mice (8.8±0.6 vs 9.1±0.5 μm; NS). Compared to isolated perfused afferent arterioles from $p47^{phox}$ +/+ mice, those from -/- mice had a lesser myogenic response (MR; increase in active wall tension with perfusion pressure from 40 to 135 mmHg: 3.1±0.4 vs 1.4±0.2 dynes/cm/mmHg; p<0.02) and a lesser change in luminal diameter with 10^{-6}M Ang II (-4.6±0.4 vs -2.9±0.3 μm; p<0.03). These were accompanied by lesser increases in E:DHE. Two weeks Ang II infusion (200 ng/kg/min) enhanced the reduction in luminar diameter with 10^{-6}M Ang II in afferent arterioles from $p47^{phox}$ +/+ mice (-5.9±0.4 μm; P=0.05) but not from -/- mice (-2.5±0.5; NS). We concluded that ROS derived from Nox-2/$p47^{phox}$ enhanced Ang II hypertension by augmenting the increase in renal vascular resistance and the afferent arteriolar myogenic and Ang II contractile responses.

Funding: NIDDK Support, Other NIH Support - P01HL-068686, R01-DK049870

TH-OR108

Intrarenal Dopamine Counteracts Angiotensin II-Mediated Progressive Renal Injury

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Background: Angiotensin II (Ang II) is a mediator of progressive renal injury. Previous studies by us and others have indicated that dopamine may modulate actions of Ang II in the kidney. The current studies examined the role of altering intrarenal dopamine on Ang II-mediated renal fibrosis.

Methods: Mice were subjected to chronic Ang II infusion by osmotic minipumps. We utilized a model of increased intrarenal dopamine, COMT KO mice, which have increased kidney dopamine levels due to deletion of a major intrarenal dopamine metabolizing enzyme. We also used a model of intrarenal dopamine deficiency due to selective proximal tubule AADC deletion (AADC KO), which inhibits dopamine synthesis.

Results: In wild type mice, Ang II infusion increased expression of both of the major dopamine metabolizing enzymes, COMT and MAO. After 8 weeks of Ang II infusion, there were no significant differences in blood pressure between either wild type and COMT KO or AADC KO mice. Compared to wild type, AADC mice had increased, and COMT mice had decreased albuminuria and tubulointerstitial injury. In response to Ang II, there was increased expression of both glomerular and tubulointerstitial injury markers (fibronectin, CTGF, FSP1, KIM-1, collagen I podocyte VEGF) in AADC KO mice and decreased expression in COMT KO mice. There was also differential macrophage infiltration in the two models. We have recently reported that Ang II-mediated tubulointerstitial fibrosis is mediated by src-dependent EGFR activation. In AADC KO mice, Ang II infusion further increased expression of p-src and pTyr845-EGFR, while their expression was markedly attenuated in COMT KO mice.

Conclusions: These results demonstrate a role for intrarenal dopamine to buffer the detrimental effects of angiotensin II upon the kidney.

Funding: NIDDK Support, Veterans Administration Support

TH-OR109

Impaired Pressure Natriuresis Is Associated with Interstitial Inflammation in Salt Sensitive Hypertension

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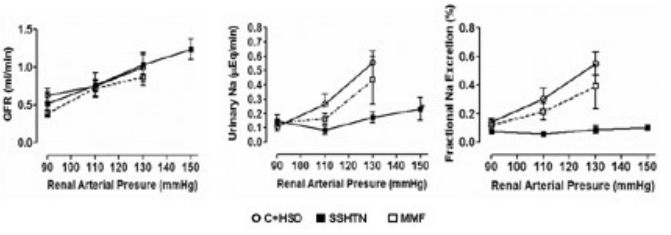
Background: Impairment of pressure natriuresis is a physiopathological characteristic of salt-sensitive hypertension. The shift to the right of the pressure natriuresis is likely a consequence of changes present in tubulointerstitial areas of the kidney, among them, accumulation of immunocompetent cells and increase in renal angiotensin II (AII) activity.

Methods: We studied the pressure natriuresis relationship in relation to severity of interstitial inflammation in salt sensitive hypertension (SSHTN). SSHTN was induced in 250-270g male Wistar rats by administration of L-NAME for 3 weeks; after a 2 week washout period a high (4%) salt diet was given (SSHTN group, n=6). One group received mofetil mycophenolate (MMF) (20mg/kg/day) during the L-NAME administration for reduction of tubulointerstitial inflammation and amelioration of salt-induced hypertension (MMF group, n=8) (AJP2001;281:F38-F47). Controls were untreated rats under high (C-HSD, n=5) and normal (0.4%) salt diet (C-NSD, n=9). Pressure natriuresis was evaluated after 6 weeks using an aortic clamp to modify renal artery pressure (RAP) with measurements at 90, 110, 130 and 150 (hypertensive rats) mmHg of RAP.

Results:

	SSHTN	MMF	C-HSD	C-NSD
CD5+CD68	84.4±5.6°	50.2±2.3°	21.6±1.2	17.6±1.1
AII+cells	31.4±9.0*	19.5±6.9	10.6±1.0	8.8±0.8

*p<0.05 vs. MMF; °p<0.001 vs. the rest



Conclusions: Impairment of pressure natriuresis in salt sensitive hypertension is associated with tubulointerstitial infiltration of immune cells and AII expressing cells and corrected by suppression of tubulointerstitial inflammation.

Funding: Government Support - Non-U.S.

TH-OR110

Intra-Renal Delivery of Autologous Endothelial Progenitor Cells (EPC) Improves Renal Function after Revascularization in Swine Renal Artery Stenosis (RAS)

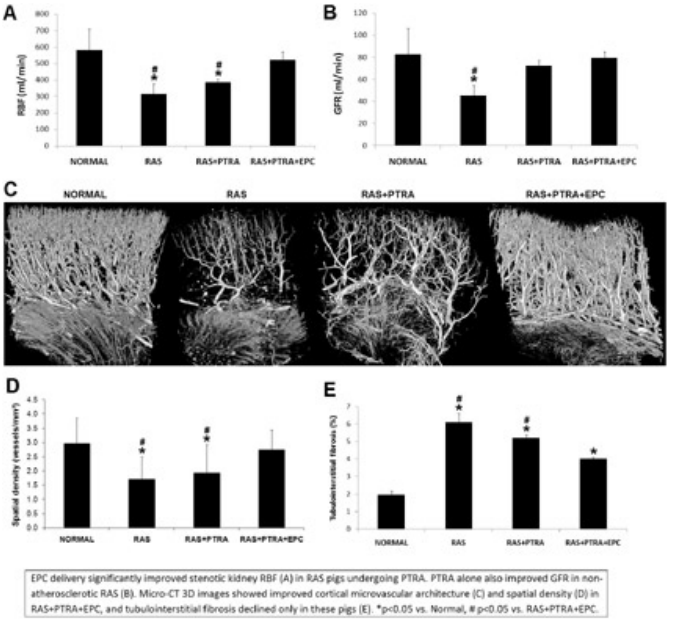
Xiang-Yang Zhu,[1] Alfonso Eirin,[1] Behzad Ebrahimi,[1] Zilun Li,[1] Amir Lerman,[2] Stephen C. Textor,[1] Lilach O. Lerman.[1] *[1]Divisions of Nephrology and Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

Background: Percutaneous transluminal renal angioplasty (PTRA) alone fails to restore the renal function and microvascular network in a swine model of RAS. This study tested if replenishment with EPC in conjunction to PTRA would improve renal function in swine RAS.

Methods: Pigs with 10 weeks of RAS were studied 4 weeks after PTRA (+stenting) or sham, with or without adjunct intrarenal delivery of autologous EPC expanded from peripheral blood, and controls (n=7 each). Stenotic kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were evaluated in vivo with multidetector computed tomography, and microvascular remodeling and fibrosis ex vivo with micro–CT and trichrome staining respectively.

Results: PTRA normalized blood pressure and GFR in all revascularized pigs (p<0.05 vs. RAS, p>0.05 vs. Normal). EPC were retained (~12%) and engrafted in injected kidneys. Stenotic kidney RBF, tubulo-interstitial fibrosis, and microvascular rarefaction were normalized only in PTRA+EPC-treated pigs (Figure).

Conclusions: A single intrarenal infusion of EPC following PTRA preserved microvascular architecture and function and decreased fibrosis in the stenotic kidney, suggesting a unique and novel therapeutic potential for adjunctive EPC delivery in improving PTRA outcomes in chronic experimental renovascular disease.



EPC delivery significantly improved stenotic kidney RBF (A) in RAS pigs undergoing PTRA. PTRA alone also improved GFR in non-atherosclerotic RAS (B). Micro-CT 3D images showed improved cortical microvascular architecture (C) and spatial density (D) in RAS+PTRA+EPC, and tubulointerstitial fibrosis declined only in these pigs (E). *p<0.05 vs. Normal, # p<0.05 vs. RAS+PTRA+EPC.

Funding: Other NIH Support - DK73608, DK77013, HL77131, HL085307, and RR018898.

TH-OR111

Increased Expression and Activity of the Sodium Chloride Cotrasnporter Mediates Salt Sensitivity in Mice Xiaoyan Wang, Laureano D. Asico, Ines Armando, Pedro A. Jose. *Center for Molecular Physiology Research, Chilren's National Medical Center, Washington, DC.*

Background: We have reported that C57BL6 and SJL mice from Jackson Laboratory have different responses to a chronic salt load: blood pressure increases in C57BL6 but not in SJL mice. Our purpose for the current study is to determine if the salt sensitivity of C57BL6 mice is related to increased expression of sodium transporters in renal distal convoluted tubule.

Methods: We examined the protein abundance of the sodium chloride cotransporter (NCC) which is predominantly expressed in the distal tubule by semiquantitative immunoblotting and measured blood pressures, urinary sodium excretion and aldosterone levels in the two mouse strains (10 weeks old, male) on normal (0.8%, NS) and high NaCl (6%, HS) diets (n=5/group).

Results: The blood pressures (telemetry) were similar at baseline and increased in C57BL6 but not in SJL mice after 1 and 3 weeks of HS. The urinary sodium excretions were also similar at baseline (2.3±0.3 vs 2.3±0.2,mEq/mg Cr, respectively) and increased to a lesser extent in C57BL6 than in SJL mice (1 wk: 4.5±1 vs 8.0±0.6, P<0.05; 3 wks: 3.5±0.4 vs 5.0±0.9, P=0.16, respectively). On NS, the renal protein expression (immunoblotting) of NCC was greater in C57BL6 (167±24, % of SJL mice) than in SJL mice (100±16, %, P<0.05). Urinary aldosterone excretion and renal aENaC expression were decreased by HS to a similar extent in the two mouse strains. In contrast, HS decreased NCC in SJL mice (58±4, % of SJL-NS) but not in C57BL6 mice (146±20, %, P<0.05). The NCC inhibitor, hydrochlorothiazide (30 mg/kg, IP), increased sodium excretion to a greater extent in C57BL6 (NS=0.054±0.01, HS=0.975±0.106, mEq/6hrs) than in SJL (NS=0.023±0.01, HS=0.56±0.147, P<0.01) mice on both diets indicating increased transporter activity. No differences were found in food and water intakes between strains.

Conclusions: We conclude that increased renal NCC expression and activity, which is independent of aldosterone levels, may be responsible for the salt sensitivity in C57BL6 mice.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-OR112

The Glutathione S-Transferase µ-1 Null Allele GSTM1(0) Is Associated with an Accelerated Progression of Hypertensive Kidney Disease in the African American Study of Kidney Disease (AASK) Jamison W. Chang,[1] Jennie Z. Ma,[1] Qing Zeng,[1] Michael S. Lipkowitz,[2] Thu H. Le.[1] *[1]University of Virginia; [2]Georgetown University.*

Background: We previously identified *Gstm1* as a candidate gene influencing susceptibility to renal vascular injury in a mouse model with lesions that closely resemble renal vascular pathology in human hypertensive nephrosclerosis (HN). There is also evidence that humans who are homozygous for the null allele of *GSMT1 (0)* have increased risks of vascular disease. We evaluated whether the null allele of *GSMT1* modifies the clinical course of African Americans with CKD due to hypertension in the African American Study of Kidney Disease (AASK).

Methods: 722 AASK trial participants with DNA samples were genotyped and classified into three groups based on the number of null alleles: homozygous null (0/0), heterozygous (0/1), and homozygous active (1/1). Differences in the time to 50% or 25ml/min/1.73m^2 decline in glomerular filtration rate (GFR), time to dialysis, or the composite events of time to GFR event or dialysis or the time to GFR event, or dialysis or death were compared between genotype groups. The effect of the *GSTM1* genotype was explored using Cox regression.

Results: The genotype groups differed significantly in the time to a GFR event or dialysis (p=0.04) and in time to a GFR event, dialysis, or death (p=0.02, figure 1). The hazard ratios for the time to GFR event or dialysis in those with 2 (0/0) or 1 (0/1) null alleles relative to those with none (1/1) were 1.96 (95% CI, 1.12 to 3.44, p=0.01) and 1.72 (95% CI, 1.02 to 2.89, p=0.04), respectively. For the time to GFR event, dialysis, or death the hazard ratios (in same order) were 2.15 (95% CI, 1.26 to 3.69, p=0.005) and 1.73 (95% CI, 1.05 to 2.87 and p=0.03).

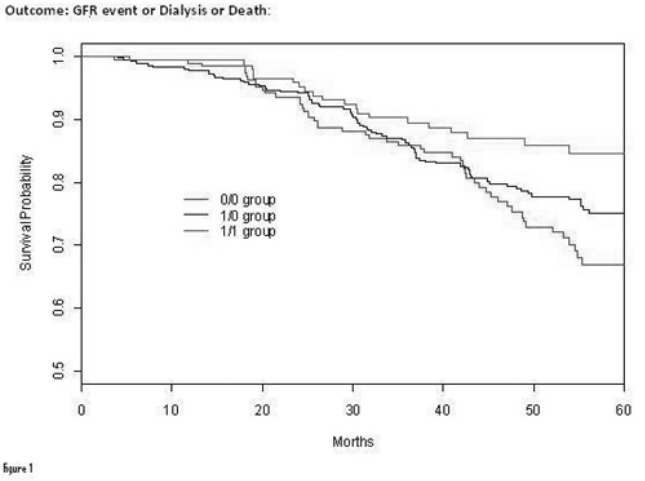


figure 1

Conclusions: The *GSTM1* null allele is associated with a more rapid progression to important clinical outcomes in the AASK trial participants.

Funding: NIDDK Support, Private Foundation Support

TH-OR113

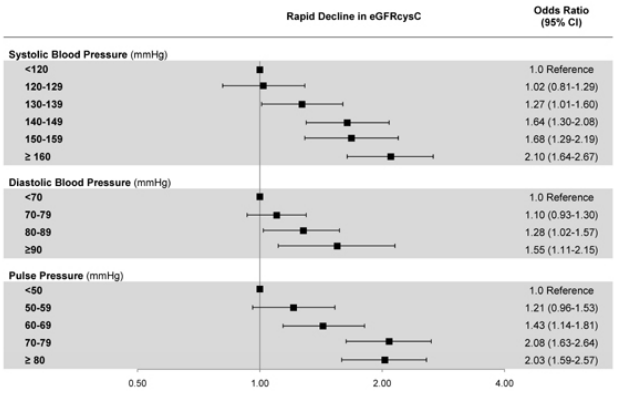
Blood Pressure Components and Decline in Kidney Function in Older Adults Dena E. Rifkin,[1] Ronit Katz,[2] Michel B. Chonchol,[3] Michael Shlipak,[4] Mark J. Sarnak,[5] Linda F. Fried,[6] Anne B. Newman,[6] David Siscovick,[2] Carmen A. Peralta.[4] *[1]UCSD; [2]Univ. of Washington; [3]Univ. of Colorado; [4]UCSF; [5]Tufts Univ.; [6]Univ. of Pittsburgh.*

Background: Progressive decline in kidney function(KF) is associated with mortality in older adults. Although hypertension is known to contribute to KF decline in the general population, the relative contributions of elevated systolic(SBP), diastolic(DBP), and pulse pressure(PP) to KF decline in community dwelling older adults are not known.

Methods: We used linear and logistic regression to examine the separate and combined associations of SBP, DBP, and PP at baseline with KF decline among 4365 older adults in the Cardiovascular Health Study. We used cystatin C to estimate glomerular filtration rate(eGFRcys) at three occasions over seven years of follow-up. Rapid decline in KF was defined as loss of ≥3ml/min/year.

Results: Average age was 72.2 and mean(SD) SBP, DBP and PP were 135(21), 71(11) and 65(18) mm Hg, respectively. SBP and PP were significantly associated with KF decline. In adjusted linear models, each 10 mm Hg increment in SBP was associated with an added decline of 0.13 ml/min/year (-0.19, -0.08, p < 0.001); each 10 mm Hg increment in PP associated with an added decline of 0.15 ml/min/yr (-0.21, -0.09, p < 0.001). Each 10 mm Hg increment in DBP was associated with a more modest decline of 0.10 ml/min/yr (-0.20, 0.01, p < 0.051). In adjusted logistic models, SBP had the strongest associations with rapid decline, with each 10 mm Hg increase conferring a 14% increased hazard of rapid decline (95% CI 10%, 17%, p < 0.01). In models combining BP components, only SBP was independently associated with rapid decline. Findings were similar regardless of BP medication use.

Conclusions: Our findings suggest that elevated BP, particularly SBP, contributes substantially to declining KF in older adults.



Funding: Other NIH Support - NHLBI, Veterans Administration Support

TH-OR114

Improvement of Vascular Function and Cardiac Autonomic Control Following Renal Transplantation: A Prospective Longitudinal Study

Roberto S. Kalil,[1] Kimberly Ferrante,[2] Harald M. Stauss,[2] William G. Haynes.[1] *[1]Dept. of Internal Medicine, University of Iowa, Iowa City, IA; [2]Health and Human Physiology, University of Iowa, Iowa City, IA.*

Background: Uremic state is strongly associated with autonomic nervous system dysfunction and impaired endothelial vascular function. Furthermore, decreased heart rate variability (HRV) in patients with ESRD is associated with higher rates of cardiovascular events and predicts progression of chronic kidney disease.

Methods: Therefore, we studied endothelial vascular function and cardiac autonomic control in patients with ESRD before and 3 months, 12 months, and 36 months following renal transplantation (RT). Vascular function was assessed by flow mediated dilatation (FMD) and cardiac autonomic control was assessed by time- and frequency-domain HRV analysis.

Results: FMD increased by 50% at one year after RT, indicating improved endothelial vascular function. During the first year following RT, time- and frequency-domain HRV parameters tended to increase (e.g., RMSSD 20.7±1.9 ms before RT vs. 25.3±5.4 ms one year after RT), indicating improved autonomic cardiac control and suggesting reduced cardiovascular risk. Compared to one year following RT, HRV parameters tended to be slightly lower at 3 years following RT (e.g., RMSSD 21.7±2.3 ms), but were still greater than before RT. Importantly, HRV (SDNN) correlated significantly (R=0.4, P<0.05) with FMD.

Conclusions: We conclude that renal transplantation in ESRD patients chronically improves endothelial vascular function and cardiac autonomic control for at least 3 years. Because of the significant correlation between the recovery of FMD and HRV, we speculate that improved endothelial vascular function contributes to reduced cardiovascular events following RT in ESRD patients.

Funding: NHLBI, NKF

TH-OR115

Women with a History of Hypertensive Pregnancy Disorders Are at Increased Risk for Future Cardiovascular and Renal Disease: A Population-Based Cohort Study

Vesna D. Garovic,[1] Catherine M. Brown,[1] Slavica Katusic,[2] Cynthia L. Leibson,[2] Jeanine Ransom,[2] Stephen T. Turner,[1] Veronique L. Roger.[2] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Health Sciences Research, Mayo Clinic, Rochester, MN.*

Background: Using the unique population-based longitudinal resources of the Rochester Epidemiology Project (REP), we tested the hypothesis that hypertensive pregnancy disorders (HPD) are a risk factor for future renal and cardiovascular disease (CVD) among women in the community.

Methods: Using REP resources we retrospectively identified female residents of Rochester, MN and the surrounding townships in Olmsted County who delivered in 1976-1982. We used the REP diagnostic index and International Classification of Diseases codes to identify women with (cases)and without (controls) diagnostic codes consistent with HPD and followed them after age 40 years for diagnostic codes consistent with adverse CVD and renal outcomes.

Results: We identified 6051 mothers who delivered between 1976-1982, of whom 607 women (mean age 26 years) had diagnostic codes consistent with HPD and 5444 women (mean age 27 years) had no such codes. Follow up after age 40 years was available for 465 of the 607 (77%) cases and 3898 of the 5444 (72%) controls. The percentage assigned renal and CVD diagnoses was significantly greater among cases than controls for hypertension diagnosed after age 40 (p<0.0001), renal disease (p<0.01), and stroke (p<0.001).

Table 1 Results of outcome events cases vs. controls

Follow up at ≥40 years of age	Cases n=465	Controls n=3898	P value
Outcome event	288(62%)	1839(47%)	p<0.0001
Median age in years (range) *	44 (40-67)	50 (40-72)	p<0.0001
Hypertension	236(51%)	1222(31%)	p<0.0001
Coronary heart disease	110(24%)	823(21%)	p=0.38
Renal (proteinuria, CKD or ESRD)	63(14%)	374(10%)	p<0.01
Stroke	35(8%)	160(4%)	p<0.001
Venous thromboembolism	13(3%)	75(2%)	p=0.21

* Age at first outcome event.

Conclusions: A history of hypertensive pregnancy disorders among women in the community is associated with significantly increased risks of future hypertension, renal and stroke events compared to women who have had normotensive pregnancies.

TH-OR116

Factors Contributing to the Development and Control of Elevated Blood Pressure in Pediatric CKD Patients

Amy Kogon,[1,2] Christopher B. Pierce,[2] Tammy M. Brady,[2] Mark Mitsnefes,[2] Bradley A. Warady,[2] Susan L. Furth,[2] Joseph T. Flynn.[1,2] *[1]Seattle Childrens's Hospital; [2]CKiD Study Group.*

Background: Few data exist regarding longitudinal blood pressure(BP) status changes in pediatric chronic kidney disease(CKD). Using Chronic Kidney Disease in Children(CKiD) cohort study data, we assessed factors associated with the rates at which normotensive patients develop elevated BP(eBP) and patients with eBP develop controlled BP.

Methods: *Development of eBP*: Children with no history of eBP, measured BP<90th %ile and no BP medication use at baseline, were followed prospectively with annual visits for the outcome of a measured BP>90th %ile. Analysis was performed using Cox proportional hazards models. *Control of eBP*: Children with measured BP>90th %ile were followed prospectively for the outcome of measured BP<90th %ile at two consecutive visits. To account for the competing events of progression to replacement therapy(RT) or death, cause-specific relative hazards are reported.

Results: Of 124 children(21% of CKiD cohort) with normal baseline BP and median follow-up of 1.5 years(IQR:0.9, 3.0), 50% were estimated to develop eBP within 2.5 years, regardless of glomerular filtration rate(GFR), race and gender. 205 children(35% of cohort) had baseline eBP; 85 developed controlled BP and 43 progressed to RT with median follow-up of 1.4 years(IQR: 0.6,3.0). African American(AA) race and urine protein:creatinine(uP:C)>2 were associated with persistently eBP. Older age, glomerular CKD and GFR<45 showed an increased risk of RT or death.

Results of Multivariate Cause-specific Relative Hazards Models, N=205

Factor	HR for BP Control, Estimate (95% CI)*	HR for RT/death, Estimate (95%CI)*
Age, per year	0.96(0.92,1.02)	1.14(1.03,1.26)
Male	1.31(0.81,2.11)	2.25(0.95,5.31)
AA race	0.54(0.29, 0.98)	0.99(0.47, 2.10)
Glomerular CKD	0.9 (0.47, 1.97)	2.10(1.00, 4.39)
GFR<45	0.79(0.50, 1.27)	7.09(2.70, 18.62)
uP/C		
<0.2	-	-
0.2-2.0	0.72(0.45, 1.16)	1.26(0.26, 6.12)
>2.0	0.17(0.05, 0.61)	4.54(0.95, 21.61)

* HR=Hazard ratio; adjusted for variables show plus BP med use.

Conclusions: In pediatric CKD, eBP develops irregardless of GFR and demographics. In those with eBP, achieving controlled BP is more difficult in AA patients and those with uP:C>2.

Funding: Other NIH Support - National Institute of Neurological Disorders and Stroke (UO1-DK-66143), National Institute of Child Health and Human Development (UO1-DK-66174), National Heart, Lung, and Blood Institute (UO1-DK-66116)

TH-OR117

Renal Inflammation and Injury Signals in Patients with Atherosclerotic Renal Artery Stenosis (ARAS)

Alfonso Eirin,[1] Monika L. Gloviczki,[1] Hui Tang,[1] Mario Gossl,[2] Kyra L. Jordan,[1] John R. Woollard,[1] Amir Lerman,[2] Joseph P. Grande,[3] Stephen C. Textor,[1] Lilach O. Lerman.[1] *[1]Divisions of Nephrology and Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic; [3]Pathology, Mayo Clinic, Rochester, MN.*

Background: The mechanisms underlying irreversible renal injury in ARAS patients have not been elucidated. This study tested the hypothesis that the injured ARAS kidney releases inflammatory mediators and retains circulating endothelial progenitor cells (EPC) to promote regeneration.

Methods: We prospectively measured renal vein (RV) and inferior vena cava (IVC) levels of inflammatory biomarkers and CD34+/KDR+EPC in essential hypertensive (EH) and ARAS patients (n=24 each) studied under constant sodium intake and anti-hypertensive regimens. Cytokine gradient (RV-IVC) and net renal release (gradient x RBF) were estimated for each product and compared to systemic levels in age-matched normotensive subjects (n=24).

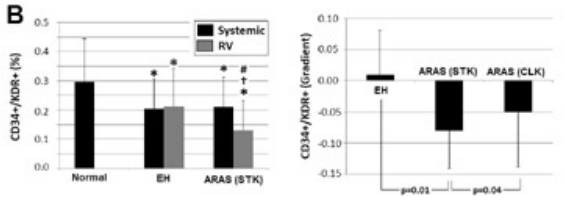
Results: Blood pressure was similar in ARAS and EH, but glomerular filtration rate was lower in ARAS. RV levels of inflammatory cytokines (Table) and EPC homing factors (SCF and SDF-1) were higher in the stenotic ARAS kidney vs. normal and EH RV (p<0.05), and their net release increased. Conversely, RV levels and net release of anti-inflammatory IL-10 were decreased in the stenotic ARAS kidney. Moreover, there was a negative gradient of CD34+/KDR+ EPC across the ARAS kidney, suggesting EPC retention (Figure).

Conclusions: These studies demonstrate for the first time that the post-stenotic human ARAS kidney releases inflammatory biomarkers and retain circulating EPC. These cytokine likely accelerate kidney injury and provide signals to EPC that may participate in reparative processes within the affected kidneys. These observations identify novel therapeutic targets to attenuate tissue injury and promote repair in the stenotic kidney.

A

	Normal	EH		ARAS		
	Systemic	*Systemic*	*RV*	*Systemic*	*CLK RV*	*STK RV*
sE-Selectin ng/ml	16.1±8.7	20.4±12.9	15.7±11.7	20.7±15.0	21.1±11.2	26.3±16.3*†
sVCAM-1 (ng/ml)	706.2±386.2	897.2±261.7	674.6±258.5	843.2±268.0	866.3±378.0	1052.4±413.3*†#
MCP-1 (pg/ml)	119.9±27.7	141.2±39.1	121.0±41.7	134.5±57.5	142.1±84.5	175.2±72.4*†#
IF-γ (pg/ml)	4.7±2.7	7.9±5.0	7.3±5.4	7.3±3.5	7.0±4.4	10.0±4.6*†#
TNF-α (pg/ml)	3.7±1.6	3.9±1.9	4.1±1.9	5.7±3.0	5.9±2.3	6.7±2.3*†#
sTNFR-1 (pg/ml)	964.9±261.0	1102.7±483.2	1066.5±446.9	1441.6±377.3	1485.9±813.6	1454.7±816.4*†
IL-6 (pg/ml)	3.2±2.9	4.5±7.8	4.3±7.8	3.8±2.9	4.5±4.4	5.6±3.6*#
IL-10 (pg/ml)	5.6±7.3	3.7±4.2	3.6±3.7	4.4±3.0	3.1±3.0	2.7±2.6*#
SDF-1 (pg/ml)	1622.8±1148.1	1713.7±1320.6	1555.5±1215.5	2341.1±1488.0	2326.9±1405.2	2426.5±1458.4*†
SCF (pg/ml)	9.3±10.1	11.4±17.6	9.2±11.8	15.6±14.6	13.1±13.9	19.7±14.9*†

B



A: Systemic and renal vein (RV, mean±SD) cytokine levels measured with ELISA in normal volunteers, essential hypertensive (EH), and atherosclerotic renal artery stenosis (ARAS) patients (n=24 each).
B: Systemic and renal vein (RV) levels (left) and gradients (right) of CD34+/KDR+ EPC in normotensive volunteers, EH, stenotic (STK) and contralateral (CLK) kidney of ARAS patients. *p≤0.05 vs. Normal, † p≤0.05 vs. EH (RV), # p≤0.05 vs. ARAS (Systemic), °p≤0.05 vs. ARAS (CLK).

Funding: Other NIH Support - HL085307, DK73608, DK77013, HL77131 and UL1-RR024150, and by Mayo Clinic Center for Individualized Medicine.

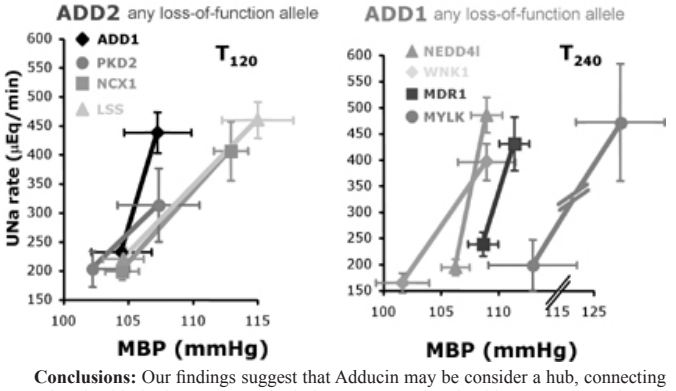
TH-OR118

Network of Salt-Senstive Hypertension: Time Effect of a Genetic Hub Marco Simonini, Guido Gatti, Simona Delli Carpini, Simona Pozzoli, Lorena Citterio, Chiara Lanzani, Elena Brioni, Stefano Tentori, Paolo Manunta. *Chair of Nephrology, San Raffaele Scientific Institute, Milan, Italy.*

Background: The emerging tools of network of hypertension offer a platform to explore systematically not only the molecular complexity of a particular disease, leading to the identification of disease modules and pathways, but also the molecular relationships among apparently distinct phenotypes. The effects of ADD1and ADD2 mutated allele and how they interact with other genes affecting the Pressure Natriuresis relationship (PNat) have been tested.

Methods: We performed a Na load test (iv. infusion of 2L of 0.9% NaCl in 2h) in 513 new diagnosed, never treated essential hypertensive patients. We tested relationship between BP change and urinary sodium excretion during the test (samples were collected every 120 min).

Results: We studied the slope of PNat at the end of infusion time (T_{120}) and after recovery (T_{240}). No differences are identifiable under the ADD1 and ADD2 wild-type allele background. A time-depending effect of ADDs genes was detected. We observed an epistatic ($p<0.001$) effect on PNat of ADD2 with the recessive alleles of ADD1, PKD2, SLC8A1 (NCX1), and LSS all related to vascular component at T_{120}. However, after recovery (T_{240}) we found an epistatic interaction on PNat ($p<0.01$) between ADD1 and genes related to tubular transporters (WNK1, MDR1, NEDD4L) and MYLK gene, encoding myosin light chain kinase which is a calcium/calmodulin dependent enzyme.



Conclusions: Our findings suggest that Adducin may be consider a hub, connecting genes involved in both vascular and tubular transporter involved in pressures natriuresis control.

Funding: Government Support - Non-U.S.

TH-OR119

Vascular Endothelial Growth Factor C Levels Are Modulated by Dietary Salt Intake in Humans Arjan J. Kwakernaak,[1] Maartje C.J. Slagman,[1] Saleh Yazdani,[1] Gozewijn Dirk Laverman,[1] Jacob Van den Born,[1] Jens Titze,[2] Gerjan Navis.[1] *[1]Department of Nephrology, University Medical Centre Groningen, Netherlands; [2]Department of Nephrology and Hypertension, Friedrich-Alexander University, Erlangen-Nürnberg, Germany.*

Background: Recent experimental findings demonstrate vascular endothelial growth factor C (VEGF-C) mediated water-free storage of salt in the interstitium, which prevents a salt-sensitive blood pressure state. It is unknown whether this mechanism plays a role in salt homeostasis and regulation of blood pressure in humans as well. Therefore, we investigated circulating VEGF-C levels and blood pressure during different well-controlled salt intakes in healthy subjects and in chronic kidney disease (CKD) patiens.

Methods: In two cross-over studies, non-diabetic proteinuric CKD patients (n=32), and healthy subjects (n=31) were treated with consecutively a high sodium diet (HS, aim 200 mmol Na^+/d) and a low sodium diet (LS, aim 50 mmol Na^+/d) in random order, during two 6-week (CKD) and two 1-week (healthy subjects) periods.

Results: We found that VEGF-C levels are higher during HS than during LS in CKD patients (median (IQR) 1228 (1024-1471) and 1004 (857-1177) pg/mL, resp; p=0.034) as well as in healthy subjects (881 (758-1023) and 773 (748-921) pg/mL, resp; p=0.070). In CKD patients HS was associated with higher NT-proBNP levels (HS: median (IQR) 91 (60-137) and LS: 62 (41-93) pg/mL, resp; p=0.005) and body weight (HS: mean (SD) 91 (3) and LS: 89 (3) kg, resp; p=0.013), consistent with ECV expansion, and with higher mean arterial pressure (HS: mean (SD) 105 (15) and LS: 101 (11) mmHg, resp; p<0.001), indicating salt-sensitivity. In healthy subjects blood pressure was not affected by dietary salt (HS: 87 (7) and LS: 86 (8) mmHg, resp; p=0.251), despite a rise in ECV (HS: mean (SD) 20.8 (0.5) and LS: 19.8 (0.5) L, resp; p=0.023).

Conclusions: Our findings support a role for VEGF-C mediated salt homeostasis in humans. Considering the salt-sensitivity of blood pressure, this buffering mechanism appears to be insufficient in proteinuric CKD patients. Future studies should investigate the clinical and therapeutic relevance of this VEGF-C regulatory mechanism in humans.

TH-OR120

Cardiovascular Disease in Fabry Patients Is Related to Dysfunctional Circulating Angiogenic Cells Johan M. Lorenzen,[1] Hermann G. Haller,[1] Christoph Wanner,[2] Thomas Thum.[1] *[1]Nephrology, Hanover Medical School, Hannover, Germany; [2]Nephrology, University Hospital Würzburg, Würzburg, Germany.*

Background: Fabry disease is an X-chromosomal recessive deficiency of the lysosomal hydrolase alpha-galactosidase A (alpha-Gal A), which catalyzes the hydrolytic cleavage of the terminal galactose from globotriaosylceramide (GL-3). This results in an accumulation of GL-3 in a variety of cells such as endothelial cells, smooth muscle cells and cardiomyocytes impairing their physiological function. Enzyme replacement therapy can prevent or attenuate onset of cardiovascular complications. We investigated the impact of this disease on the biology of circulating angiogenic cells (CACs) and endothelial function in patients with Fabry disease and healthy controls.

Methods: 26 patients with untreated Fabry disease, 16 patients after enzyme replacement therapy (ERT) and 26 healthy controls were investigated. Endothelial function was assessed by the EndoPat device, left ventricular hypertrophy by echocardiography. Circulating angiogenic cells were analyzed by fluorescence associated cell sorting (FACS) analysis (CD34+/CD133+/KDR+). The migratory capacity of CACs was assessed by a modified Boyden chamber. GL-3 was visualized by immunofluorescence and electron microscopy. Alpha-Gal A was knocked out in CACs by a specific siRNA.

Results: Fabry patients showed a hyperactive, but impaired endothelial function and signs of cardiac hypertrophy, which normalized after enzyme replacement therapy. Fabry patients displayed increased numbers, but impaired function of CACs. We performed immunofluorescence and electron microscopy of CACs to further investigate causes of this functional impairment and identified an excessive accumulation of GL-3 in Fabry disease CACs. Enzyme replacement therapy attenuated CAC dysfunction in patients via a reduction in GL-3 accumulation in vitro and in vivo. SiRNA-mediated knockdown of alpha-Gal A in healthy CACs also led to an impairment of migratory capacity.

Conclusions: Cardiovascular disease in Fabry patients may be in part related to CAC dysfunction. Enzyme replacement therapy improves CAC function and may attenuate development of cardiovascular disease in the long-term.

Funding: Government Support - Non-U.S.

TH-OR121

Gitelman Syndrome: Hypertension in Adulthood Is a Common Sequela, and Female Patients Have Higher Potassium Requirements Miriam Rose Berry,[1] Caroline M. Robinson,[2] Fiona E. Karet.[1, 2] *[1]Renal Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Medical Genetics, University of Cambridge, United Kingdom.*

Background: Gitelman Syndrome (GS) is a rare inherited disorder caused by mutations in *SLC12A3*, which encodes the thiazide-sensitive NCC transporter in the distal tubule. GS is characterised by potassium (K) and magnesium (Mg) wasting, relative hypotension and usually, hypocalciuria; however there is wide phenotypic variability.

Methods: We performed a retrospective study of all patients with genetically proven Gitelman syndrome known to our specialist adult nephrology service. Differences between group medians were tested for significance by Mann-Whitney U-testing.

Results: 37 patients (21 male and 16 female) with median age 39 ± 14 y were studied. All were hyper-reninemic at diagnosis. 35% (n=13) patients were hypertensive (defined as BP > 130/80 despite amiloride or anti-hypertensive agents). Of these 13, 11 were male (p=0.017) with median age 53 y, *vs* two female, 45 y (p=0.55). One patient was already hypertensive by age 21 y. Overall, currently normotensive patients were significantly younger: median 37.5 y (p=0.03), and also younger at the time of genetic diagnosis: 35.5 *vs* 47 y (p=0.04).

Females required significantly higher daily doses of K compared to males: median 124 *vs* 72 mmol (p=0.007). There was also a trend towards higher Mg requirements: 30 *vs* 15 mmol (p=0.15). 5 patients were in the top quartiles of both K and Mg requirements, 4 female and 1 male (p=0.14), median age 36 y. Patients with mutations in exon 26 had significantly higher K requirements than others: 120 mmol *vs* 80 (p=0.018).

Conclusions: Despite obligate salt-wasting in GS, secondary hypertension has developed in more than one third of our cohort, and may be an expected feature of the aging GS population. It appears to be more prevalent in male patients. It may be related to chronic hyperreninism and/or hyperfiltration. The higher female requirement for potassium supplementation we observed may be related to the effects of oestrogens on expression or function of the NCCT. Mutations in exon 26 (C-terminal) may affect targeting of NCCT and hence explain the apparently more severe phenotype observed in these patients.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-OR122

An ADPKD Cyst Epithelial Cell Secreted Factor Activates STAT3 and Promotes M2-Like Polarization in Macrophages Katherine Swenson-Fields, Jacqueline D. Peda, Sally M. Salah, Carolyn J. Vivian, Darren P. Wallace, Timothy A. Fields. *Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

Background: Alternatively-activated macrophages (M2 MΦ) are known to play a pro-fibrotic role in chronic kidney disease (CKD). Since MΦ have been demonstrated within the stroma of autosomal dominant polycystic kidney disease (ADPKD), we assessed whether M2 MΦ are present and whether ADPKD cells affect their polarization.

Methods: Immunohistochemical characterization of macrophages in human ADPKD tissue was performed using HAM56 and anti-CD163 antibodies. Primary human ADPKD cyst cells were co-cultured with murine bone-marrow derived macrophages (BMDM) and a murine macrophage-like cell line, RAW264.7 (RAW). Signaling was assessed using phospho-specific Western analysis; gene expression was analyzed using qRT-PCR.

Results: HAM56 identified MΦ within ADPKD stroma, especially adjacent to basement membranes underlying cysts. Many of these MΦ also stained for the M2 marker CD163. Since murine proximal tubule epithelial cells have been shown to influence MΦ polarization in vitro, we tested whether ADPKD epithelial cells could effect polarization of BMDM. Strikingly, ADPKD cell co-culture caused a >200 fold induction of the M2 MΦ marker Arg1 in both BMDM and RAW cells, while the inflammatory MΦ marker Nos2 was not induced. ADPKD cell conditioned media (ADPKD CM) also induced Arg1 expression (~75 fold), indicating the presence of an ADPKD cell-derived, soluble, M2-polarizing factor. STAT6 activation, which is essential for Arg1 induction and M2 polarization induced by the cytokines IL4 and IL13, was not detected in RAW cells after ADPKD CM treatment. However, ADPKD CM induced a rapid activation of STAT3 in RAW cells, and the presence of a STAT3 inhibitor completely blocked the ADPKD CM Arg1 induction.

Conclusions: ADPKD cyst cells produce a soluble factor that activates STAT3 and induces Arg1 expression and M2-like macrophage polarization. M2 MΦ in the ADPKD environment are likely to promote disease progression. Thus, understanding the mechanisms by which polarization occurs could allow the development of targeted therapies to diminish M2 polarization and disease progression in ADPKD.

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TH-OR123

Polycystin-1 Binds Par3-aPKC To Control Polarized Cell Migration and Renal Tubular Morphogenesis Maddalena Castelli,[1,2] Manila Boca,[1,2] Marco Chiaravalli,[1,2] Alessandra Boletta.[1,2] *[1]Dulbecco Telethon Institute - DTI, Milan, Italy; [2]Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy.*

Background: Loss of function mutations in the PKD1 gene, encoding Polycystin-1 (PC-1) result in Autosomal Dominant Polycystic Kidney Disease (ADPKD), characterized by renal cyst formation. PC-1 is a large receptor localized in cilia and at sites of cell adhesion. Its precise function remains largely elusive. It was recently proposed that renal cystogenesis results from defective planar cell polarity. Here we link PC-1 to a highly conserved polarity pathway, showing that PC-1 interacts with a complex composed of aPKC and Par3, both in vitro and in vivo.

Results: In previous studies we have demonstrated that PC-1 regulates cell migration. Furthermore, we found that Pkd1-/- Mouse Embryonic Fibroblasts (MEFs) in wound-healing assays do not migrate in a directional manner and are not able to establish front-rear polarity, as compared to controls. This phenotype depends on the dynamic interplay between the actin and the microtubular cytoskeleton.

We next tested a potential role of the Pars/aPKC polarity complex. Using a novel system carrying endogenous tagged PC-1, we found that PC-1 interacts with Par3/aPKC, but not with Par6 in vitro. Moreover, PC-1 favors the association of aPKC with Par3 at the expense of Par6 to achieve polarized migration. Interestingly, this effect appears to be non cell-autonomous.

Furthermore we found that PC-1 associates with Par3/aPKC in the developing kidneys in vivo and that PC-1 and Par3 appear to undergo a similar regulation of expression during renal development. In line with this, developing kidneys of Pkd1 mutants show defective convergent extension and tube morphogenesis accompanied by defective association of aPKC/Par3.

Conclusions: We propose that Par3 and Par6 compete with each other for binding aPKC and that PC-1 associates with and favors aPKC/Par3 complex to control polarized migration, convergent extension and tube morphogenesis, thus preventing cystogenesis.

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TH-OR124

PDE1 and AKAP79 Interact with B-Raf To Mediate cAMP-Induced ERK Activation and Proliferation of Human ADPKD Cells Cibele S. Pinto, Gail Reif, Emily Nivens, Corey White, Darren P. Wallace. *Internal Medicine, University of Kansas Medical Center, Kansas City, KS.*

Background: cAMP plays a central role in the pathogenesis of ADPKD by activating the B-Raf/MEK/ERK signaling pathway and promoting cyst epithelial cell proliferation. By contrast, cAMP fails to stimulate B-Raf and ERK-mediated proliferation of normal human kidney (NHK) cells. The molecular mechanism for this difference in cAMP-dependent B-Raf signaling is still unclear. Scaffolding proteins, such as A-kinase anchoring proteins (AKAPs), assemble multi-enzyme signaling complexes with cAMP effector molecules (i.e phosphodiesterases (PDEs) and B-Raf) to promote spatial and temporal regulation of the cAMP signal. PDEs, enzymes that degrade cAMP, also function as scaffolding proteins to compartmentalize cAMP signal. In melanoma cells, PDE4 has been shown to interact with Raf-1, and switch the Raf isoform involved in growth factor signaling; thereby causing hyperactivation of the MEK/ERK pathway. Previously, we showed that PDE1, a Ca^{2+}-regulated isoform, is involved in the phenotypic switch in the mitogenic response of ADPKD cells to cAMP. We hypothesize that cAMP induces the formation of macromolecular complexes involving AKAPs and PDEs that facilitate the cAMP-dependent B-Raf activation and coordinate the cross-talk between cAMP and ERK pathways in ADPKD cells.

Methods: To determine if PDE1 and AKAP79 interact with B-Raf, ADPKD and NHK cells were treated with 100 μM cAMP or 10^{-9} M AVP for 15 min, and PDE1 and AKAP79 were immunoprecipitated and immunoblots were probed for B-Raf.

Methods: Here, we show that AKAP79, an isoform expressed in ADPKD and NHK cells, directly interacts with B-Raf. Surprisingly, cAMP caused a 40% increase in the AKAP79 and B-RAF interaction in ADPKD cells, while causing a small decrease in this interaction in NHK cells. PDE1 was found to also be part of the AKAP79/B-Raf complex. Treatment with cAMP or AVP caused a 70% increase in the interaction between PDE1 and B-Raf in ADPKD cells, while having no effect in NHK cells.

Conclusions: These data indicate that the assembly of a multi-protein signaling complex involving AKAP79, PDE1 and B-Raf may be differentially regulated by cAMP in ADPKD cells.

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TH-OR125

Ire1-Xbp1 Unfolded Protein Response (UPR) Pathway Is a Genetic and Molecular Regulator of Cystogenesis through a Polycystin-1 (PC1) Dependent Mechanism Sorin V. Fedeles,[1] Ann-Hwee Lee,[2] Seung H. Lee,[1] Ming Ma,[1] Rachel Gallagher,[1] Laurie H. Glimcher,[2] Stefan Somlo.[1] *[1]Internal Medicine/Nephrology, Yale School of Medicine, New Haven, CT; [2]Immunology/ Infectious Diseases and Medicine, Harvard Medical School, Boston, MA.*

Background: This work examined the relationship between up-regulation of the unfolded protein response (UPR, a housekeeping pathway involved in counteracting ER stress) and cyst formation due to the absence of the Autosomal Dominant Polycystic Liver Disease (ADPLD) gene orthologs, *Prkcsh* and *Sec63*, both of which are involved in ER protein biogenesis.

Methods: We used mouse models of *Prkcsh*, *Sec63*, and *Xbp1* based on conditional inactivation in the kidney by *Ksp-Cre*, as well as kidney tubule cell lines isolated from these models.

Results: Inactivation of *Prkcsh* did not result in up-regulation of any of the three branches of the UPR pathway. Inactivation of *Sec63*, on the other hand, resulted in selective activation of the Ire1-Xbp1 pathway, as evidenced by *Xbp1* splicing, protein expression and up-regulation of Xbp1 transcriptional targets. The other two branches of the UPR pathway, Atf6 and Perk, remained at basal levels. Double knockout (DKO) animals with conditional inactivation of *Sec63* and *Xbp1* in distal nephron segments (*Sec63$^{flox/flox}$;Xbp1$^{flox/flox}$;Ksp-Cre*) showed a marked exacerbation of the cystic phenotype compared to single knockout (SKO; S*ec63$^{flox/flox}$;Ksp-Cre*) mice; *Xbp1$^{flox/flox}$;Ksp-Cre* mice had no cysts. DKO mice had a ~2.5 fold increase in kidney weight (KW), kidney-to-body weight ratio (KW/BW), cystic index (CI) and BUN compared to SKO animals. Introduction of a 3-copy *Pkd1BAC* trangene in DKO mice resulted in a significant reduction in the KW, KW/BW ratio, CI and BUN compared to the DKO animals alone. Furthermore, expression of the Xbp1 transcriptional target ER chaperone BiP was decreased in the cystic epithelia of DKO vs. SKO animals. This correlated with a decrease in the expression of PC1 and PC2 in the DKO vs. SKO backgrounds.

Conclusions: Taken together our data demonstrate that the most conserved branch of the UPR pathway, Ire1-Xbp1, can act as a modifier of cyst formation by impacting the biogenesis of PC1/PC2.

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TH-OR126

Pyrimethamine: A Potential Novel Therapeutic Intervention for ADPKD
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Background: Autosomal dominant polycystic kidney disease (ADPKD) is a commonly inherited disorder mostly caused by mutations in PKD1. The disease is characterized by development and growth of epithelium-lined cyst in kidney. There is no specific treatment for ADPKD. The signal transducer and activator of transcription (STAT) pathway is critical for developmental regulation and growth control in organs.

Methods: We measured STAT3 activation in kidneys from Col2Cre+Pkd1flox/flox conditional knockout, Mx1Cre+Pkd1flox/flox inducible knockout and their littermate wild-type mice by western blot and immunostaining. We performed cell based functional assay of FDA-approved compounds to identify small molecule inhibitors of STAT3 transcriptional activity. Two PKD mouse models were used for the treatment: 1) an adult-onset PKD mouse model (adult inactivation of Pkd1 followed by renal injury), and 2) a neonatal PKD mouse model (Pkd1 inactivation at P7). The kidney weight, the kidney body weight ratio, serum creatinine, cyst volume were measured. Proliferation index was assessed using Ki67 and BrdU incorporation.

Results: A sustained activation of the transcription factor STAT3 was observed in ischemic injured and uninjured mouse polycystic kidneys and in human ADPKD kidneys. Furthermore, Jak2 inhibitors reduced STAT3 activation in human ADPKD cells in a dose-dependent manner, indicating increased Jak2 activity in ADPKD cells. Through a chemical library screen, we identified the anti-parasitic compound pyrimethamine as an inhibitor of STAT3 function. Treatment with pyrimethamine decreased STAT3 activation and cell proliferation preferably in human ADPKD cells and reduced renal cyst formation in an adult and a neonatal PKD mouse models. Moreover, we demonstrated that another STAT3 inhibitor, S3I-201, reduced cyst formation and growth in a neonatal PKD mouse model.

Conclusions: Blocking STAT3 signaling with pyrimethamine or similar drugs may be an attractive therapy for human ADPKD.

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TH-OR127

Smac-Mimetic Prevents Renal Cyst Formation by Inducing TNFa-Dependent Cystic Epithelial Cell Death
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Background: Past efforts to pharmacologically interfere with the development and growth of cystic renal lesions have been designed to "normalize" the activity of a specific signaling molecule. Recent efforts in our laboratory have focused on developing a novel strategy by targeting pathways that can induce only cystic renal epithelia apoptosis, leading to remove the dead cells from kidney tissues, to prevent cyst formation.

Results: In this study, we present for the first time evidence that second mitochondria-derived activator of caspase (Smac) or a Smac mimetic induces cell death in a TNFα-dependent manner, in Pkd1 mutant (*Pkd1*$^{-/-}$) renal epithelial cells without any effect on Pkd1 wild type (*Pkd1*$^{+/+}$) or Pkd1 heterozygous (*Pkd1*$^{+/-}$) renal epithelia. Following activation by TNF-α, the C-tail death domain of TNF-α receptor 1 (TNFR1) recruits a multimeric protein complex that includes receptor associated protein kinase 1 (RIPK1), and the cellular inhibitor of apoptosis proteins 1 and 2 (cIAP1 and cIAP2), to form a pro-survival signaling complex. In Pkd1 mutant (*Pkd1*$^{-/-}$) renal cells, both TNFR1 and cIAP1 but not cIAP2 are upregulated compared to levels in Pkd1 wild type and Pkd1 heterozygous renal epithelia. A Smac-mimetic (GT13072) induced the degradation of cIAP1 in (*Pkd1*$^{-/-}$) renal epithelia but had no effect on cIAP1 in Pkd1 wild type or heterozygous epithelia. Degradation of cIAP1 releases RIPK1 from the TNFR1 complex, and leads to the formation and activation of RIPK1-dependent caspase-8, pro-death complex. This complex specifically induces cell death in mutant Pkd1 renal epithelia only. In addition, we found that Smac-mimetic alone or in combination with TNF-α, stimulated further expression of TNF-α only in cystic renal epithelia. Furthermore, we found that treatment with the Smac-mimetic only prevented edema and cyst formation in Pkd1 knockout embryos and kidneys.

Conclusions: These data suggest the use of Smac-mimetics alone provide a novel and specific therapeutic approach to prevent cyst formation in ADPKD patients.

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TH-OR128

Hepatocyte Nuclear Factor 1 beta (HNF1b/MODY5) Controls Nephron Morphogenesis
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Background: The deficiency for Hepatocyte Nuclear Factor 1 Beta (HNF1B), a gene encoding for a homeobox-containing transcription factor, is a major cause for developmental renal malformation characterized by the occurrence of cysts and hypo-dysplasia. The current knowledge about its molecular and cellular mechanisms is still incomplete.

Methods: A homozygous germ-line deletion of Hnf1b in mouse leads to embryonic lethality shortly after implantation (E6.5) due to defective differentiation of extraembryonic tissues. To circumvent this early lethality we inactivated this gene specifically in the embryo proper and not in the extraembryonic compartment with a set of Cre recombinases (Mox2Cre, RARbCre and Six2-GFP::Cre).

Results: Similarly to what was already shown (Lokmane et al,2010), we found that a total embryonic (Mox2-Cre driven) Hnf1b inactivation led to a drastic defect of renal morphogenesis. Branching of the Ureteric Bud (UB) was distorted and the induction of renal vesicles was impaired.

Our results also demonstrated that Hnf1b-deficient embryos displayed Wolffian duct malformations, characterized by the emergence of multiple bulges (ectopic UBs).

To further identify the potential role played by Hnf1b in developing nephrons, we used a Cre recombinase specifically expressed in Metanephric Mesenchyme (MM) and not in the UB (Six2-GFP::Cre). Contrary to the Mox2-Cre driven deletion, this inactivation did not prevent the induction of renal vesicles. However, nephron precursors gave rise to aberrant globular structures and did not produce renal tubules.

Interestingly, with a highly chimeric inactivation of Hnf1b in the MM (RARb-Cre), we were able to identify a further crucial role of Hnf1b in proximal tubular morphogenesis. In fact, partial loss of Hnf1b led to defective lengthening of this segment, and mutant animals suffered from defective reabsorption of glucose.

Conclusions: Our results demonstrate that HNF1 beta plays essential roles in the first morphogenetic events that shape nephrons and, subsequently, in proximal tubular elongation.

These results provide a novel perspective on the function of a gene that is frequently mutated in children or fetuses with cystic/dysplastic kidneys.

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TH-OR129

Hedgehog Signaling Is Increased in Glis2 Knockout Kidneys Resulting in the Persistence of Nephron Developmental Programs
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Background: Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, is the most frequent genetic cause of chronic renal insufficiency in the first three decades of life (1). Glis2/NPHP7 loss of function causes NPHP type 7 in humans and mice (2). Kidney tubular cells in Glis2 knockouts acquire mesenchymal phenotype, but the cellular mechanisms of this transition are unknown.The high sequence homology between Glis2 and Gli proteins, key effectors in the Hedgehog (Hh) pathway and the localization to the primary cilia, suggest that Glis2 is a functional component of Hh signaling, a pathway activated during embryonic kidney development (3).

Results: To test this hypothesis we crossed Glis2 knockout with the Gli1+/lacZ transgenic mouse (4), which were used as an in vivo reporter of Hh activity; β-gal activity in Glis2+/-;Gli1+/lacZ kidneys was significantly increased, indicating that Glis2 is a repressor of Hh. Glis2 suppression in cultured IMCD3 cells by shRNA interference resulted in the acquisition of mesenchymal hallmarks that was partially rescued by treatment with the specific Gli inhibitor GANT58. We found, by chromatin immunoprecipitation using an anti-Glis2 antibody, that Glis2 binds regulatory elements in the promoters and inhibits the transcription of the epithelial-to-mesenchymal regulator Snai1 and of the developmental gene Wnt4 through a competition with Gli1.

Conclusions: According to our results, Glis2 is a repressor of the Hh pathway, which is unexpectedly latent in postnatal kidneys, and sustains the aberrant persistence of developmental programs. This is the first description of a molecular mechanism that links the hedgehog signaling pathway to cystic kidney diseases and can open new avenues for treatment of diverse ciliopathies.

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TH-OR130

Identification of 3 Novel NPHP Genes by Whole Exome Capture (WEC) Implicates DNA Damage Response (DDR) Signaling in the Pathogenesis of Nephrono-Phthisis-Related Ciliopathies
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Background: Nephronophthisis-related ciliopathies (NPHP-RC) are recessive disease of kidney, retina, and cerebellum. Identification of 10 disease-causing genes revealed that their products are located at primary cilia and centrosomes, but disease mechanisms remain poorly understood.

Methods: We identify by whole exome capture (WEC), mutations of the centrosomal proteins FAN1, ZNF423, and CEP164, as novel causes of NPHP-RC. Surprisingly, these genes serve functions within the DNA damage response (DDR) pathway.

Results: Specifically, we identify a homozygous truncating mutation (W707X) of *FAN1* in 2 siblings who exhibit NPHP with karyomegaly. FAN1 is essential for the DNA interstrand crosslink (ICL) repair pathway of DDR signaling. Second, we detect in 2 siblings with NPHP and cerebellar hypoplasia a homozygous missense mutation in *ZNF423*. Interestingly, ZNF423 interacts with the DNA ds-break sensor PARP1, ATM

to sites of DNA damage. Third, we show that ATM is activated in turn by the 'TIP60 complex'. And we demonstrate colocalization to TIP60-positive nuclear foci for other products of NPHP-RC genes. In 4 different families with NPHP-RC we identify mutations of *CEP164* as a novel cause of NPHP-RC. CEP164 acts in the ATR-Chk1-related arm of DDR, where it is necessary for ATR-dependent Chk1 activation upon induced replication stress (Sivasubramaniam 2008).

Conclusions: We suggest a new pathogenic working hypothesis for NPHP-RC proposing the following cascade of events: i) defects of DDR - lack of Chk1 (Chk2) activation - inadequate G_2/M cell cycle arrest.

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TH-OR131

Disrupted Retrograde Ciliary Transport Causes Sensenbrenner Syndrome with Nephronophthisis Heleen H. Arts,[1,2] Cecilie Bredrup,[3,4] Machteld Oud,[1] Torunn Fiskerstrand,[3] Alexander Hoischen,[1] Damien Brackman,[5] Sabine Leh,[6] Marit Midtbø,[7] Christian Gilissen,[1] Olav H. Haugen,[3,8] Helge Boman,[4] Eyvind Rødahl,[3,8] Joris A. Veltman,[1] Per Knappskog,[4,8] Nine V. Knoers,[2] Ronald Roepman.[1] *[1]Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Department of Medical Genetics, University Medical Center Utrecht, Netherlands; [3]Department of Opthalmology, Haukeland University Hospital, Bergen, Norway; [4]Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway; [5]Department of Pediatrics, Haukeland University Hospital, Bergen, Norway; [6]Department of Pathology, Haukeland University Hospital, Bergen, Norway; [7]Department of Clinical Dentistry, University of Bergen, Bergen, Netherlands; [8]Institute of Clinical Medicine, University of Bergen, Bergen, Netherlands.*

Background: Intraflagellar transport (IFT) occurs along microtubules in the cilium, an antenna-like organelle present on the apical surface of almost any vertebrate cell (including renal cells). The IFT-B and IFT-A protein complexes regulate upward- and downward ciliary transport, respectively, in association with motor proteins such as kinesins and dynein. The IFT-A complex consists of 6 proteins of which 3 (IFT122, IFT121 and IFT43) are associated with Sensenbrenner syndrome, an autosomal recessive disorder characterized by skeletal- and ectodermal defects.

Methods: Exome-sequencing; SNP array analysis; Sanger sequencing; Immunocytochemistry.

Results: We used exome-sequencing to identify the genetic defect in a Norwegian family with Sensenbrenner syndrome accompanied by nephronophthisis-like nephropathy. We identified multiple genomic variants, however, only the compound heterozygous mutations in *WDR19* encoding IFT144 (an IFT-A protein) segregated with disease in the family. IFT144 is normally present in the base and the tip of the cilium. However,IFT144 was absent in cilia from fibroblasts from one of our patients.

Conclusions: We conclude that *WDR19* is a novel Sensenbrenner gene and that defective tip-to-base (retrograde) ciliary transport causes Sensenbrenner syndrome.

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TH-OR132

Proteomic Analysis of Class IV Lupus Nephritis: Global Versus Segmental Mamdouh N. Albaqumi, Dania Alkhafaji, Lutfi Alkorbi. *KFSHRC, Saudi Arabia.*

Background: There have been several attempts to standardize definitions and increase reproducibility in classifying lupus nephritis. The last classification added subcategories global (IVG) and segmental (IVS). Whether this subdivision has an impact on clinical practice or patient's outcome is controversial.

Methods: A retrospective reviewe with 2DE gel electrophoresis and MALDI-TOF/MS analysis of renal biopsies

Results: Renal biopsies from patients with IVG, IVS, ANCA, normal were used to extract proteins. Proteins were then separated by 2D gel electrophoresis and the expression levels of the protein spots were determined. Principal Component Analysis (PCA) Plot was applied for the four groups using the dataset of statistically differentially expressed protein spots. Global and segmental groups cross over each other and clearly separate away from both normal biopsies and those with ANCA indicating that both IVG and IVS may represent a similar entity .42 protein spots corresponding to 28 protein species were successfully identified by MALDI-TOF/MS. We then compared IVG vs. IVS using the quantitative expression levels of the 28 identified differentially expressed proteins. IVG and IVS were similar in their expression levels of these proteins; supporting the notion that they are similar entity. When IVG and IVS are classified as one group and compared to ANCA and normal tissue, the differences were significant across nine proteins. Using a quantitative expression of these nine proteins, there is no statistical difference between IVG and IVS, but when compared to ANCA or normal tissue, there are several differences among the expression level of these proteins.

To examine whether these data can be correlated with clinical outcome, a total of 78 patients were re-classified. 50 patients had class IVG LN while 28 patients had class IVS LN. Serum creatinine showed no statistical difference at baseline between the two groups. .

There was no statistical difference in therapy between the two groups. The probability of renal survival and patient survival were not statistically different between the two groups as.

Conclusions: There is no strong evidence to support a different outcome or etiology between the two subcategories of class IV LN

TH-OR133

C3 Glomerulonephritis Is a Disease of the Alternative Pathway of Complement Sanjeev Sethi,[1] Fernando C. Fervenza,[1] Yuzhou Zhang,[2] Samih H. Nasr,[1] Richard J. Smith.[2] *[1]Mayo Clinic, Rochester, MN; [2]University of Iowa, Iowa City, IA.*

Background: C3 Glomerulonephritis (C3GN) is a recently described glomerulonephritis (GN) with extensive C3 deposits. The purpose of this study was to describe the clinicopathologic findings and study the alternate pathway of complement (AP) in cases of C3GN.

Methods: The diagnosis of C3GN was based on a proliferative GN on light microscopy (LM) with extensive C3 deposits and absence of immunoglobulins (Ig's), kappa and lambda light chains on immunofluorescence microscopy (IF).

Results: Ten cases of C3GN were studied. There were 6 male and 4 female patients. The age ranged from 4 to 73 yrs (mean 44.4 yrs). The serum creatinine ranged from 0.5 to 3.1 mg/dL (mean 1.52 mg/dL). Urinary protein ranged from 0.3 to 9 gms (mean 2.7 gms/day). UA showed hematuria and proteinuria in all cases. All cases had low C3 and normal C4 levels. LM showed a membranoproliferative GN with extensive C3 deposition in the mesangium and capillary walls on IF. IF was negative for Ig's, kappa and lambda light chains. EM often showed large confluent hazy electron dense deposits in the mesangium, with subendothelial, intramembranous and occasional subepithelial deposits. Based on the presence of subepithelial deposits, 4 of the 10 cases were previously diagnosed as post-infectious GN. Three cases were previously labeled MPGN type I. Evidence of AP activation was demonstrable in all cases. Specific abnormalities of the AP were as follows: Autoantibodies to C3 convertase (2 cases), antibodies to factor H (1 case), homozygous risk alleles for p.His 402 (1 case), c.C2867T p.T956M factor H missense mutation (2 cases), heterozygous risk alleles for factor H p.Val62 and p.His402 (1 case), mutation in factor I gene and polymorphisms in factors B and H (1 case), frameshift c.2171delC, p.Thr724fsSTOP725 variant mutation in factor H resulting a truncated factor H (1 case), and deletion of CFHR3-CFHR1 (1 case).

Conclusions: This study show that C3GN can result from a diverse set of abnormalities affecting the AP. Treatment needs to be tailored based on the underlying etiology of AP dysfunction. Finally, C3GN should be considered in differential diagnosis of post-infectious GN.

Funding: NIDDK Support

TH-OR134

Association of Anti-PLA2R with Disease Activity and Outcome in Idiopathic Membranous Nephropathy Durga A.K. Kanigicherla,[1] Paul E. Brenchley,[1] Michael Venning,[1] Kay V. Poulton,[1] Edward A. McKenzie,[2] Jennet O. Gummadova,[2] Colin D. Short.[1] *[1]Renal Medicine, Manchester Royal Infirmary, United Kingdom; [2]FLS, Manchester University, United Kingdom.*

Background: Antibodies to PLA2R have been identified in 70% cases of idiopathic membranous nephropathy (IMN), which has a strong association with HLA DQA1. The interaction between anti-PLA2R levels, HLA genes and PLA2R polymorphism is unknown. We measured anti-PLA2R antibodies in 88 IMN patients typed for DQA1: DQB1 and PLA2R 292 SNP and describe the results in the context of disease activity and outcome.

Methods: Biopsy proven IMN patients (mean age 55.6 yrs; 63 M, 25 F; mean follow up 65±11 months) were selected for a retrospective cross sectional study of clinical outcomes with samples obtained from our BioBank. 40 patients had active disease, 22 were in partial and 26 in complete remission. DQ high resolution typing used LABType ®RSSO (One Lambda Inc). The PLA2R gene M292V SNP, was typed by TaqMan PCR. PLA2R was cloned and expressed in HEK cells and protein purified for use in an ELISA. 38 normal plasma samples defined the normal range (mean +2SD =32 units). Clinical status, pre and post plasma sample, (earliest sample available) was recorded.

Results: High anti-PLA2R levels were significantly associated with active disease (73% positivity; median=171 units) compared to remission (15% positivity; median=24units; $p< 0.001$; ANOVA). Antibody level was associated with concurrent proteinuria ($p<0.001$) but not with gender or ethnicity. High antibody level was associated with DQA1 05:01 (p adj<0.001) and DQB1 02:01 (p adj=0.05) and PLA2R 292 M allele (ptrend=0.04). Of the 29 patients positive for anti-PLA2R with active disease, 9/17 with high anti-PLA2R compared to 1/12 with low antibody reached CKD5 or doubling of serum creatinine within 10 years.

Conclusions: High anti-PLA2R levels are associated with active disease, possession of DQA1 05:01; DQB1 02:01 tissue type and poor long term outcome, suggesting that anti-PLA2R may be pathogenic.

Monitoring antibody levels over time in response to treatment will help to define anti-PLA2R as a surrogate marker of the immunopathological mechanism.

Funding: Private Foundation Support

TH-OR135

Response of Anti-PLA2R to Adrenocorticotropic Hormone (ACTH) Gel in Membranous Nephropathy Laurence H. Beck,[1] Fernando C. Fervenza,[2] Andrew S. Bomback,[3] Rivka Ayalon,[1] Maria V. Irazabal,[2] Alfonso Eirin,[2] Daniel C. Cattran,[4] Gerald B. Appel,[3] David J. Salant.[1] *[1]Boston University; [2]Mayo Clinic; [3]Columbia University; [4]Toronto General Hospital.*

Background: Autoantibodies to the phospholipase A2 receptor (PLA2R) define most cases of primary membranous nephropathy (MN); their presence correlates with clinical disease activity. We analyzed anti-PLA2R in patients treated with long-acting ACTH gel

(H.P. Acthar® Gel, repository corticotropin; Questcor) to better understand its potential mechanism of action.

Methods: Samples were collected from 14 patients in 2 pilot studies (Mayo Clinic, n=9; Columbia, n=5) that treated MN patients with ACTH gel for 6 mo. Longer term follow-up samples were available in 11 cases. Serum was assayed for anti-PLA2R by immunoblotting and subsequent densitometry.

Results: 86% of patients were anti-PLA2R positive at baseline (Mayo Clinic, n=8; Columbia, n=4). Two patients with undetectable baseline anti-PLA2R rapidly achieved clinical remission and may have already entered immunologic remission prior to treatment. All 12 patients experienced a reduction in anti-PLA2R (17-100%) by 6 mo with disappearance of anti-PLA2R in 5 cases. There were 5 partial remissions at 6 mo: 3 in patients who cleared their anti-PLA2R and 2 in those lacking baseline anti-PLA2R. Another patient later achieved a 96% reduction in anti-PLA2R without further treatment while 2 others, after receiving rituximab for perceived failure of ACTH gel, fully cleared anti-PLA2R. With long term (12-18 mo) follow-up of those with undetectable anti-PLA2R at baseline or after ACTH gel ± rituximab, there were 4 complete and 2 partial remissions, one non-responder, and a final patient who achieved partial remission but relapsed coincident with a return of anti-PLA2R. Two other patients also showed an increase in anti-PLA2R after discontinuing treatment with ACTH gel.

Conclusions: Measurement of anti-PLA2R provides useful information relating immunological and clinical disease activity in MN patients treated with new agents such as ACTH gel. This study suggests that ACTH gel may work in part by suppressing autoantibody production; the duration and degree of this response need further study.

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TH-OR136

Clinical Pathologic Correlations in 190 Multiple Myeloma Patients with Kidney Biopsy Samih H. Nasr,[1] Anthony M. Valeri,[2] Sanjeev Sethi,[1] Mary E. Fidler,[1] Lynn D. Cornell,[1] Nelson Leung.[3] *[1]Pathology, Mayo Clinic, Rochester, MN; [2]Nephrology, Columbia University, New York, NY; [3]Nephrology, Mayo Clinic, Rochester, MN.*

Background: Renal involvement is common in multiple myeloma (MM). In this largest study to date, we examined kidney biopsy (KBx) findings in MM patients (pt) and correlated them with clinical data at KBx and outcome.

Methods: The characteristics of 190 Mayo Clinic pts with MM who underwent KBx between 1997-2011 are provided. MM was diagnosed before or at time of KBx.

Results: On KBx, paraprotein-related lesions (PRL) were seen in 73% of pts, non-PRL in 25%, and no pathology in 2%. PRL were myeloma cast nephropathy (MCN)(33% of pts), monoclonal immunoglobulin deposition disease (MIDD)(22%), amyloid (21%), fibrillary GN (FGN)(1%), immunotactoid GN (0.5%) and light chain proximal tubulopathy (0.5%). Two forms of PRL were seen in 6% (MCN and MIDD in 5 pts, MCN and amyloid in 4, MIDD and amyloid in 2, and MCN and FGN in 1). Direct MM infiltration was seen in 1%. Non-PRL included ATN (9%), HTN arteriosclerosis (6%), diabetic nephropathy (5%), FSGS (3%), APIGN (3%), and interstitial nephritis (2%). 1/3 of MIDD pts were <50 yrs vs. 9% of MCN and 9% of amyloid pts (p=0.035). Serum complete Ig was more common in MCN than amyloid or MIDD. The size of urine paraprotein and % of plasma cells in bone marrow were higher in MCN than amyloid or MIDD. Markedly abnormal FLC ratio was more common in MCN than amyloid. 37% of MCN pts required dialysis at KBx vs. 22% of MIDD and 9% of amyloid pts. >50% albuminuria on UPEP was highest in amyloid>MIDD>MCN. After a mean follow-up of 30 mos, 52% died. Median pt survival was 45 mos for MCN, 61 for amyloid and 84 for MIDD (p=0.193). Independent predictors of death were reaching ESRD, no treatment with stem cell transplant, and hypercalcemia. Median renal survival was 36 mos for MCN vs. 113 for amyloid vs. 74 for MIDD (p=0.003). The only independent predictor of progression to ESRD was dialysis at KBx.

Conclusions: The current spectrum of renal lesions in MM pts is more heterogenous than previously reported. KBx is essential to establish the individual diagnoses and provide important prognostic and therapeutic information.

TH-OR137

Natural History of Serum Free Light Chains Prior to the Clinical Presentation of AL Amyloidosis Joseph D. Hebreo,[1] Kevin C. Abbott,[1] Brendan M. Weiss,[2] Stephen W. Olson.[1] *[1]Department of Medicine, Nephrology Service, Walter Reed Army Medical Center, Washington, DC; [2]Department of Medicine, Hematology/Oncology Service, Walter Reed Army Medical Center, Washington, DC.*

Background: AL amyloidosis is a rare plasma cell dyscrasia with significant morbidity (including acute kidney injury) and mortality, in the absence of timely diagnosis and treatment. Abnormal serum free light chain (SFLC) ratios are associated with AL amyloidosis at time of diagnosis but no prior studies have evaluated the natural history of SFLC prior to diagnosis.

Methods: Using serum from the Department of Defense Serum Repository (DoDSR), we assessed SFLC levels in up to three longitudinal serum samples per patient for 20 AL amyloidosis cases and 20 healthy controls matched for age, sex, race, and age of serum.

Results: A greater percent of cases versus controls had a single abnormal serum free light chain ratio (0.26-1.65 mg/L) any time prior to diagnosis (90% vs. 5%, p<0.001), <4 years prior to diagnosis (92% vs. 0%, p<0.001), 4 to 11 years prior to diagnosis (67% vs. 7%, p<0.001), and >11 years prior to diagnosis (42% vs. 0%, p=0.015). In the 75% of cases with an elevated lambda light chain (LLC) level, a rise in LLC of greater than 1mg/L per year was 100% sensitive and specific for future disease (100% vs. 0%, p<0.001).

Conclusions: An abnormal SFLC ratio and a LLC rate of rise greater than 1 mg/L per year were associated with the subsequent diagnosis of AL amyloidosis. Our data could allow for an earlier diagnosis in those with clinical concern or asymptomatic elevations in SFLC prior to extensive end organ damage that prohibits a definitive stem cell transplant.

Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of the Army, the Department of the Navy, the Department of Defense, or the United States government.

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TH-OR138

Novel Noninvasive Biomarkers for Disease Activity of IgA Nephropathy (IgAN) Yusuke Suzuki,[Inst#1] Keiichi Matsuzaki,[Inst#1] Hitoshi Suzuki,[Inst#1] Keiko Okazaki,[Inst#1] Hiroyuki Yanagawa,[Inst#1] Norio Ieiri,[Inst#2] Mitsuhiro Sato,[Inst#2] Toshinobu Sato,[Inst#2] Yoshio Taguma,[Inst#2] Jan Novak,[Inst#3] Osamu Hotta,[Inst#2] Yasuhiko Tomino.[Inst#1] *[1]Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Sendai Shakaihoken Hospital, Sendai, Japan; [3]University of Alabama at Birmingham.*

Background: The primary abnormal manifestation in IgAN is recurring bouts of hematuria with or without proteinuria. Although immunohistochemical analysis remain the gold standard not only for diagnosis but also for evaluating the activity of IgAN, new sensitive and reasonably specific tests are emerging to guide therapeutic strategy applicable to all stages of IgAN. Present study examined serum levels of galactose-deficient IgA1 (GdIgA1) and IgA-IgG immune complex (IgA-IgGIC) as noninvasive markers for the disease activity.

Methods: We enrolled patients (n=48) with IgAN who showed complete or partial clinical remission after tonsillectomy and steroid-pulse therapy (TSP) and were followed up for 3-5 years. Baseline clinical data were evaluated just before TSP; serum levels of GdIgA1 and IgA-IgGIC and seminquantitative hematuria and proteinuria were assessed just before TSP, 1 year, and 3-5 years after TSP. GdIgA1 and IgA-IgGIC were measured by ELISA.

Results: Cross-sectional analysis revealed that the degree of hematuria and proteinuria were significantly associated with levels of GdIgA1 (p for trend=0.004), and IgA-IgG IC (p for trend<0.001), respectively. Longitudinal analysis showed that from the group of 91.7% patients with heavy hematuria (≥3+) just before TSP, 64.6% patients showed complete disappearance of hematuria (group A), but the remaining patients did not (group B), during the 3-5-year follow up. Although the levels of GdIgA1 and IgA-IgG IC in the two groups just before TSP were similar (A vs. B; 122.2 vs 107.7 units p=0.36, and 0.77 vs 0.85 OD p=0.43), decrease of GdIgA1 levels in group A was significantly higher than that in group B (27.4 vs 13.6%, p=0.02).

Conclusions: Disease activity of IgAN assessed by hematuria and proteinuria correlated with changes of serum levels of GdIgA1 and IgA-IgG IC, respectively. These new noninvasive disease activity markers can be used to guide the therapeutic approaches.

TH-OR139

APOL1 Is Expressed in Human Kidney with Dynamic Cellular Localization Patterns in Health and Disease Sethu M. Madhavan, John F. O'Toole, Martha Konieczkowski, Leslie A. Bruggeman, John R. Sedor. *MetroHealth System, Case Western Reserve University, Cleveland, OH.*

Background: Genetic variants in *APOL1* , which encodes apolipoprotein L1 (APOL1), associate with the non-diabetic kidney diseases in patients of African ancestry. APOL1 is a component of HDL_3 particles and mediates trypanolytic activity. Renal disease-associated APOL1 variants have been positively selected in African populations by conferring resistance to trypanosomiasis. Mechanisms by which *APOL1* variants promote nephropathy are unknown.

Methods: APOL1 localization was examined using immunohistology in normal human kidney sections and in FSGS, HIVAN, diabetic and hypertensive-associated nephropathy and minimal change disease (MCD) biopsies. Biopsies were genotyped for *APOL1* variants. APOL1 expression was also examined in human podocytes, endothelial and proximal tubular cells.

Results: APOL1 was only present in podocytes in normal glomeruli. Podocyte staining of APOL1 was decreased in diseased glomeruli, although podocyte markers, GLEPP1 and synaptopodin, were maintained. Only in MCD glomeruli, APOL1 expression was induced in endothelia concomitant with the loss of podocyte APOL1. APOL1 localized to proximal tubular epithelia in a vesicular pattern and was detected in the arteriolar endothelium of normal and diseased kidney sections. Unexpectedly, in biopsies but not normal kidney, medium artery and arterioles contained a subset of α-smooth muscle actin-positive cells that stained for APOL1. RT-PCR demonstrated *APOL1* transcripts in podocytes and normal glomeruli. By immunoblotting, podocyte, proximal tubular and vascular endothelial cell lines expressed APOL1. Stimulation of human endothelial cells with TNF or LPS induced expression of APOL1.

Conclusions: APOL1 is present in normal kidney, suggesting that a functional role in normal kidney. The cell compartments in which it localizes change with disease, and the patterns do not appear to correlate with kidney disease-associated APOL1 variants, suggesting that these variants do not cause disease by altering APOL1 trafficking or protein levels. Rather the risk variants of *APOL1* may dysregulate its function within the kidney cells where it is localized.

Funding: NIDDK Support

TH-OR140

Chronic West Nile Nephropathy: A New Clinical Entity Amber S. Podoll,[1] Nashila Abdul Rahim,[1] Kevin W. Finkel,[1] Kristy Murray.[2] *[1]Division of Renal Diseases and Hypertension, University of Texas Health Science Center, Houston, TX; [2]School of Public Health-Center for Infectious Diseases, University of Texas Health Science Center, Houston, TX.*

Background: Infection with West Nile Virus (WNV), an RNA flavivirus, occurs in 25,000 people worldwide annually and results in more than 1,000 deaths. Symptoms of infection range from a nonspecific febrile illness with fatigue, lymphadenopathy and malaise, to more serious consequences of meningitis and encephalitis. Although previously thought to be a self-limited disease, we have shown that some patients have persistent infection with WNV up to 9 years based on elevated serum WNV IgM and viral shedding in the urine. Hamsters infected with WNV develop chronic infection of distal renal tubule epithelium and shed virus in the urine. Immunohistochemical staining of hamster kidney tissue shows evidence of WNV RNA. This data raises the possibility that persistent viral infection of the kidney occurs in humans.

Methods: From a cohort of 131 patients with a history of WNV infection, 45 were found to have persistent shedding of WNV RNA in the urine. Full medical assesments were performed. Four patients were found to have impaired renal function or abnormal urinalysis and underwent a percutaneous renal biopsy.

Results: Examination of the renal tissue revealed a prominant mononuclear infiltrate with signficant chronic tubulointerstitial changes. The degree of chronic changes correlated with diminished renal function. The renal tubules showed reactive epithelial changes with focal hyaline proteinaceous casts. Abnormal inclusion vescicles, presumed viral particles, were identified within renal epithelial cells by electron microscopy. No glomerular abnormalities or immune complexes were noted. PCR of renal tissue verified the presence of WNV-specific RNA.

Conclusions: These results demonstrate persistent renal infection by WNV in humans that results in chronic tubulointerstitial nephritis associated with varying degrees of CKD. Whether treatment of the infection will alter the course of CKD requires further study. This is an important question given the high incidence of infection worldwide and may reveal a new occult pathologic process.

TH-OR141

A Comparative Histomorphological, Immunohistochemical and Ultrastuctural Study on Pre and Post Calcineurin Inhibitor Therapy Renal Biopsy of Childhood Steroid Resistant Nephrotic Syndrome: An Insight for Pathogenesis of Its toxicity Amit K. Dinda, Arvind Bagga, Lavleen Singh. *All India Institute of Medical Sciences, New Delhi, Delhi, India.*

Background: Long term calcineurin inhibitor (CNI) therapy is a common practice in childhood steroid resistant nephritic syndrome (SRNS). Current study has been undertaken to study the early renal injury with treatment.

Methods: 24 children of SRNS with a mean age of 4.2±2.8 years (M:F = 4.8:1) were included with paired renal biopsies, before initiation and 1 year after CNI therapy with standard dosage. Semi-quantitative grading was done on light microscopic (LM) features for assessing CNI toxicity. Ultrastructural study was done with special emphasis on mitochondrial morphology in the tubular epithelial and endothelial cells. The mitochondrial alterations were segregated (*Walker et al)* into four semi quantitative grades. Immunohistochemical (IHC)staining for nitrotyrosine, eNOS and TGFβ1 was performed and graded.

Results: Out of 24, 15 cases were diagnosed as minimal change disease, and 9 cases focal segmental glomerulosclerosis. With CNI therapy remission was obtained in 75% cases. Serum creatinine and CNI level were with in normal limits in all cases. Post-treatment biopsies showed significant increase in glomerulosclerosis, JGA hyperplasia, tubular atrophy, interstitial fibrosis, arteriolar hyalinosis and smooth muscle vacuolization ($p<0.05$ to <0.001). Grade 2/3 alterations were seen in endothelial mitochondria in 79.1% biopsies with CNI therapy, while such change was not seen in pre-treatment group. Significantly higher expression of eNOS was noted (91.6%) with increased nitrotyrosine (75%) and TGFβ1 (79.1%) after treatment.

Conclusions: Ultrastructural alterations in endothelial mitochondria may be related to endothelial injury which are early and constant features associated with CNI therapy.

Free radical (peroxynitrite) mediated endothelial injury with higher eNOS expression may be an important event associated with development of CNI toxicity.

The increased TGFβ1 expression supports that with prolonged CNI therapy there may be initiation of events leading to chronic CNI toxicity with normal renal parameter and drug level in cases of SRNS.

TH-OR142

There Is an Increased Risk of Peritonitis in the 21 Days Prior to Death in Peritoneal Dialysis Patients: A Case-Crossover Study Neil Boudville,[1 2] Anna Kemp,[2] Philip A. Clayton,[1] Wai Hon Lim,[1] Carmel M. Hawley,[1] Sunil V. Badve,[1] Fiona Brown,[1] Brian E.R. Livingston,[1] Stephen P. McDonald,[1] Kym M. Bannister,[1] Kathryn J. Wiggins,[1] David W. Johnson.[1] *[1]Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, Adelaide, SA, Australia; [2]University of Western Australia, Perth, WA, Australia.*

Background: Peritoneal dialysis(PD)-related peritonitis may be associated with increased risks of both infectious and non-infectious death as a result of inflammation, declining health and other factors. However, the evidence for this is primarily descriptive. Our aim was to examine the relationship between mortality and peritonitis in PD patients, utilising a novel method of analysis, where patients serve as their own controls.

Methods: All patients on the ANZDATA Registry receiving PD for ≥7 months between 1/5/2004 and 31/12/2009 and who died on PD or within 30days of transferring to HD were included. We used a case-crossover design so patients had to be on PD for ≥7months to allow a comparator time period. Conditional logistic regression was used to compare the risk of peritonitis in the 'case' window 21days immediately before death, and a 'control' window of 21days 6months before death. This method eliminated the influence of patient-level confounders.

Results: 1316 PD patient were included with a mean age at death of 70.5±11.7years, 44.2% female, and a mean time on dialysis of 3.2±2.2years. 1446 peritonitis episodes were documented, with 43.1% experiencing ≥1 episode. 5.9% of deaths were documented as being due to peritonitis, with 207 (15.7%) experiencing an episode in the 21days before death. There was a significant 8.0 fold increased odds ratio (95%CI=5.3-12.1) of an episode of peritonitis in the 21days before death compared to a 21day window 6months prior. Sensitivity analysis showed no significant difference in the odds of having peritonitis in a 21day window 60days prior to death compared to 120, 190 and 250days.

Conclusions: We have established that there is a significantly increased risk of peritonitis in the 21days prior to death. An examination of the definition of peritonitis-related mortality is required.

TH-OR143

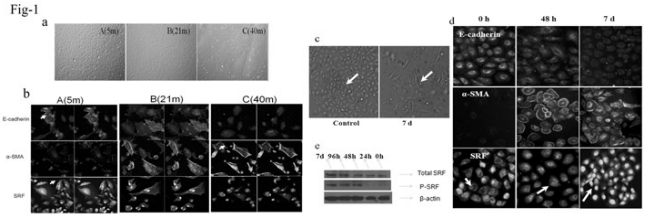
Translocation of Nuclear Transcription Factor Serum Response Factor Expedites Epithelial-to-Mesenchymal Transition in Human Peritoneal Mesothelial Cells Lijie He,[1] Nan Zhang,[2] Hanmin Wang,[3] Shiren Sun.[4] *[1]Department of Nephrology, Xijing Hospital, the Fourth Military Medical University, Xi'an, Shaan Xi, China; [2]Department of Nephrology, Xijing Hospital, the Fourth Military Medical University, Xi'an, Shaan Xi, China; [3]Department of Nephrology, Xijing Hospital, the Fourth Military Medical University, Xi'an, Shaan Xi, China; [4]Department of Nephrology, Xijing Hospital, the Fourth Military Medical University, Xi'an, Shaan Xi, China.*

Background: Epithelial-to-mesenchymal transition (EMT) causes denudation of mesothelial cells and peritoneal fibrosis, so the molecular is thus of significance. Serum response factor (SRF) could regulate tumor cell migration by expediting EMT,but the mechanism is unclear.

Methods: We isolated mesothelial cells from effluents in dialysis fluid from 30 patients, undergoing continuous ambulatory peritoneal dialysis for less than 6 months or more than 20 months. All these cells were characterized by phenotype markers. The location of SRF were tested by confocal immunofluorescence. We also tested location of SRF and EMT markers in HPMCs in vitro.

Results: With the dialysis time of 5 m (A), 21m (B) to 40 m (C), human peritoneal mesothelial cells underwent a transition from an epithelial phenotype to a mesenchymal phenotype with a progressive loss of epithelial morphology [figure1a], a decrease in the expression of E-cadherin, an increase in the expression of SMA and the translocation of SRF from cytoplasm into nucleus [figure1b]. In vitro analysis, during the inducing time, high glucose-induced HPMCs were changed into fibroblast-like cells [figure1c], downregulation of E-cadherin, upregulation of SMA and translocation into nucleus of SRF [figure1d]. The p-SRF was also upregulated after being induced for 48 h [figure1e].

Fig-1



Conclusions: The findings suggest SRF might be a potential targets and marker for the design and monitor of new dialysis solutions of patients.

Funding: Government Support - Non-U.S.

TH-OR144

Distinctive Role of Smad2 and Smad3 in Peritoneal Dialysis-Related Peritoneal Fibrosis and Mesothelial-Myofibroblast Transition *In Vivo* and *In Vitro* Wenjuan Duan,[1,2] Xueqing Yu,[1] Xiao Ru Huang,[2] Xiaoming Meng,[2] Hui Y. Lan.[2] *[1]Department of Nephrology, First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; [2]Department of Medicine and Therapeutics, and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China.*

Background: It is well accepted that TGF-β signaling plays an essential role in peritoneal fibrosis including the process of mesothelial-myofibroblast transition (EMT). However, the functional role and the underlying mechanisms by which TGF-β1 signals through to mediate peritoneal fibrosis under peritoneal dialysis (PD) conditions remain largely unclear.

Methods: We first examined TGF-β/Smad signaling in peritoneal tissues from patients with PD. Then, the role of Smad2 and Smad3 in peritoneal fibrosis including EMT was determined in a mouse model of PD induced in Smad3 KO and conditional Smad2 KO

mice. Mechanisms of Smad2 and Smad3 in PD-associated peritoneal fibrosis was studied in primary culture of peritoneal mesothelial cells (PMCs) that lack Smad3 or have conditional KO for Smad2.

Results: We found that TGF-β/Smad2/3 was markedly activated in PD patients with severe peritoneal fibrosis and EMT. In a mouse model of PD, mice lacking Smad3 were protected against peritoneal PET dysfunctions and peritoneal fibrosis including EMT such as increased collagen matrix and α-SMA$^+$ myofibroblast accumulation(Col1 86.6%↓α-SMA44.9↓ all P<0.05), but diseased E-cadherin expression. Surprisingly, in contrast to Smad3 KO mice, mice with conditional Smad2 KO from the peritoneal tissues substantially enhanced peritoneal fibrosis(Col1 79.9%↑P<0.01) and EMT. Similarly, deletion of Smad3 from PMCs prevented, but disruption of Smad2 enhanced TGF-β1-induced collagen matrix expression and EMT *in vitro*. Further study revealed that increased peritoneal fibrosis in conditional Smad2 KO mice and PMCs was associated with enhanced Smad3 signaling *in vivo* and *in vitro*.

Conclusions: The present study demonstrates that Smad3, but not Smad2, plays an essential role in PD-related peritoneal fibrosis and EMT. Enhanced Smad3 signaling may be a mechanism by which disrupted Smad2 promotes peritoneal fibrosis and EMT *in vivo* and *in vitro*.

Funding: Government Support - Non-U.S.

TH-OR145

Effect of Treatment Time Per Session and Dialyzer Phosphate Clearance on Predialysis Serum Phosphorus Concentration during Short Daily Hemodialysis

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Background: Short daily hemodialysis (SDHD) has the potential to reduce predialysis serum phosphorus (P) concentrations; however, not all previous SDHD studies have demonstrated this clinical benefit. In this study we use P kinetic modeling to determine the effect of treatment time per session (T) and dialyzer phosphate clearance (K) on predialysis serum P concentration during SDHD.

Methods: We recently demonstrated that a pseudo-one compartment kinetic model, including P mobilization from other body compartments into extracellular fluids, can be used to describe P kinetics during conventional (4 hr) and short (2 hr) HD treatments (Hemodial Int 2011, in press). This kinetic model is advantageous since it characterizes individual patient differences in P removal by a single parameter, the P mobilization clearance (KM, determined range: 45-208 ml/min). We used this model, combined with a P mass balance relationship, to perform computer simulations of predialysis serum P concentrations during 6-times per week SDHD.

Results: Results from representative simulations are tabulated for patients at steady state with a P central distribution volume of 10 L (estimate of extracellular fluid volume); it was assumed that net P intake was increased by 20% during SDHD. K values were varied over the range reported in previous clinical studies.

Simulated Predialysis Serum P Concentrations (mg/dL)

	KM = 50 ml/min (Low)			KM = 150 ml/min (High)		
	CHD	SDHD	SDHD	CHD	SDHD	SDHD
T (min)	240	120	180	240	120	180
K = 80 ml/min	8.34	8.53	6.38	6.98	7.85	5.52
K = 110 ml/min	7.11	6.97	5.36	5.62	6.22	4.42
K = 140 ml/min	6.43	6.10	4.80	4.86	5.29	3.80

Doubling HD treatment frequency without a change in total weekly treatment time (CHD vs SDHD at T=120 min) may result in either an increase or a decrease in predialysis serum P concentration. Further, SDHD with low K does not result in reductions in predialysis serum P concentration unless T is increased substantially.

Conclusions: Both increasing T and maintaining a high K during SDHD are important to provide significant reductions in predialysis serum P concentrations.

Funding: Pharmaceutical Company Support

TH-OR146

Histone Acetyltransferase Activity Is Involved in the Pathogenesis of Experimental Peritoneal Fibrosis

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Background: Peritoneal fibrosis is a serious complication in long-term peritoneal dialysis. However, the precise pathogenic mechanisms of peritoneal fibrosis remain to be determined. Circulating mesenchymal progenitor cells 'CD45/COLI dual positive cells', originally named as fibrocytes, have been reported to participate in tissue fibrosis. In addition, histone acetyltransferase (HAT) has also been demonstrated to be involved in the pathogenesis of fibrotic conditions. Therefore, we examined the participation of CD45/COLI dual positive cells dependent on HAT activity in experimental mouse model of peritoneal fibrosis.

Methods: To address this, peritoneal fibrosis was induced by the injection of 0.1% chlorhexidine gluconate (CG) into the abdominal cavity in mice.

Results: A considerable number of CD45/COLI dual positive cells infiltrated in the peritoneum accompanied with the progression of peritoneal fibrosis. CG injection also increased number of cells immunoreactive for acetylated histone H3. The treatment with curcumine, a potent inhibitor of HAT, reduced the extent of peritoneal fibrosis, peritoneal transcripts of transforming growth fatcor-β1 as well as the numbers of acetyl-histone H3 positive cells and infiltraled CD45/COLI dual positive cells. In isolated CD45/COLI dual positive cells from human peripheral blood, pre-treatment with curcumin inhibited transforming growth fatcor-β1-induced expression of pro-collagen type Iα.

Conclusions: These results suggest that HAT activity contributes to peritoneal fibrosis via regulation of CD45/COLI dual positive cells.

Funding: Pharmaceutical Company Support

TH-OR147

LPA-LPA1 Signaling Drives Peritoneal Fibrosis by Inducing Fibroblast Accumulation and MRTF-SRF-Dependent Profibrotic Gene Expression

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Background: Peritoneal fibrosis (PF) is a potentially fatal complication of peritoneal dialysis, but the mechanisms driving it remain to be fully identified. We have previously implicated the lipid mediator lysophosphatidic acid (LPA) and one of its receptors, LPA_1, in lung and skin fibrosis. Additionally, LPA induces actin polymerization, which has recently been shown to induce profibrotic gene expression through a myocardin-related transcription factor (MRTF)-serum response factor (SRF) axis.

Methods: We therefore examined the role of LPA-LPA_1 signaling in PF induced by intraperitoneal injection of chlorhexidine gluconate (CG).

Results: CG-induced increases in peritoneal thickness and hydroxyproline content were significantly attenuated in LPA-deficient mice (LPA_1 KO) compared with wild-types (WT). Peritoneal accumulation of myofibroblasts and connective tissue growth factor (CTGF) expression induced by CG were also significantly reduced in LPA_1 KOs. To determine whether LPA and LPA_1 mediated the accumulation of peritoneal fibroblasts as a source of myofibroblasts, we determined the effect of the LPA_1-selective antagonist AM095 on CG challenge of mice whose fibroblasts could be identified by fibroblast-specific expression of EGFP. AM095 dramatically attenuated CG-induced increases of EGFP-positive fibroblasts. We also found that LPA directly induced CTGF gene expression by mouse primary peritoneal mesothelial cells. To determine the molecular pathway through which LPA induced CTGF expression, we performed a series of experiments genetically deleting, knocking down or chemically inhibiting potential components of this pathway. We found that this pathway involves LPA_1 signaling, RhoA activation, actin polymerization, and the transcription factors MRTF-A, MRTF-B and SRF.

Conclusions: Our results suggest that LPA and LPA_1 drive PF by inducing fibroblast accumulation and MRTF-SRF-dependent profibrotic gene expression.

Funding: Other NIH Support - NIH/NHLBI, Government Support - Non-U.S.

TH-OR148

Association of Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers on Peritoneal Dialysis Technique Survival

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Background: Recent studies suggest that angiotensin converting enzyme inhibitors (ACE) and angiotensin receptor blockers (ARB) exert a protective effect on peritoneal membrane transport status, thereby preserving membrane function in peritoneal dialysis (PD) patients. We therefore sought to examine the association of ACE and ARB use on technique survival in our PD patients.

Methods: We identified patients in our database who initiated PD at our institution between January 1, 2001 and December 31, 2010. We used our pharmacy database to identify patients who filled prescriptions for ACEs/ARBs at least 60% of the time during the second year of therapy (ACE/ARB access). We excluded patients who remained on PD less than one year. Patients who received a renal transplant were censored from the analysis. Results were adjusted for age, sex and Charlson co-morbidity index (CCI).

Results: Baseline patient demographics are presented in the table below by ACE/ARB access status. The unadjusted hazard ratio (HR) for technique survival in patients with ACE/ARB access was 0.72 (p=0.010) and the adjusted HR was 0.68 (p=0.003).

Patient Demographics

	ACE/ARB access (n=318)	<60% ACE/ARB access (n=637)	p value
Median age in years	57.6 (49.5-66.9)*	56.5 (46-64.9)*	0.049
Number of females (%)	144 (45.3)	303 (47.6)	0.536
Number of diabetics (%)	222 (69.8%)	339 (53.2)	<0.001
CCI	6 (5-7)*	5 (3-7)*	0.004
PD first modality	237 (74.5)	390 (61.2)	<0.001

* 95% confidence interval

Conclusions: We found that PD patients who had access to ACE/ARBs at least 60% of the time during the second year of PD were approximately 30% less likely to transition off PD for any reason (other than transplant) compared to patients who had no or less access to ACE/ARB. Our data suggest that ACE/ARB therapy may be associated with longer time on therapy for PD patients, though the exact mechanism for this has not been determined.

Funding: Pharmaceutical Company Support

TH-OR149

Abstract Withdrawn

TH-OR150

Effect of BMP-7 and Tamoxifen in Animal Model of Peritoneal Fibrosis Developed in Uremic Rats Filipe Miranda Silva,[1] Dayana G. Viloslada,[1] Humberto Dellê,[1] Erik Halcsik,[2] Mari C. Sogayar,[2] Irene L. Noronha.[1] *[1]Nephrology, Univ. Sao Paulo, Brazil; [2]Chemistry Institute, Univ. Sao Paulo, Brazil.*

Background: Progressive increase in the thickness of peritoneal membrane and peritoneal sclerosis are considered serious complications of long-term peritoneal dialysis. There is still no recognized treatment to reduce these fibrotic changes. In this context, the use of drugs with anti-fibrotic properties such as bone morphogenic protein-7 (BMP-7) and tamoxifen (TAM) may be of relevance. The aim of this study was to analyze the effect of BMP-7 and TAM in an experimental model of peritoneal fibrosis induced by chlorhexidine gluconate (CG), developed in uremic rats.

Methods: End stage chronic kidney disease (CKD) was induced in male Wistar rats using diet containing 0.75% adenine for 30 days. At day 15, rats with CKD, characterized by hypertension (168±13mm/Hg), high BUN (64±25mg/dL) and high serum creatinine (0.70±0.4mg/dL) levels, were subjected to intraperitoneal injections of 0.1% CG daily for 15 days to induce peritoneal fibrosis (PF). The animals were divided into 4 groups (n=5 per group): **CKD,** CKD rats receiving only vehicle; **CKD+PF**, peritoneal fibrosis induced in CKD rats; **CKD+PF+BMP7**, CKD rats with PF treated with BMP-7 (30ng/Kg every 3 days intraperitoneally), and **CKD+PF+TAM**, CKD rats with PF treated with TAM (10mg/Kg/day) by gavage.

Results: Treatment with BMP-7 and TAM was effective in reducing the thickness of the peritoneal membrane and the number of macrophages and T-lymphocytes. Reduction of TNF-α and collagen III mRNA levels was also observed.

	CKD	CKD+PF	CKD+PF+BMP7	CKD+PF+TAM
Thickness of peritoneal membrane (μm)	30±24	123±32*	41±10#	44±4#
Macrophages (cells/mm²)	311±115	1380±295*	685±185#	453±65#
T-lymphocytes (cells/mm²)	14±12	119±71*	89±20#	31±17#
TNF-α (mRNA level)	1.0±0.8	8.0±0.3*	1.4±0.9#	2.9±0.5#
Collagen III (mRNA level)	1.0±0.8	18.9±2.4*	2.6±1.5#	2.6±1.5#

*p<0.05 vs. CKD, #p<0.05 vs. CKD+PF

Conclusions: BMP-7 and TAM were effective in reducing the thickness of peritoneal membrane in the experimental model of peritoneal fibrosis induced by CG developed in uremic rats, possible due to their anti-fibrotic effects.

Funding: Government Support - Non-U.S.

TH-OR151

Intensive Home Hemodialysis and Patient Survival: A Multinational Cohort Study Gihad E. Nesrallah,[1] Robert M. Lindsay,[1] Meaghan S. Cuerden,[1] Amit X. Garg,[1] Friedrich K. Port,[2] Peter Austin,[3] Louise M. Moist,[1] Rita Suri.[1] *[1]IQDR Investigators; [2]DOPPS, Arbor Research; [3]ICES, Canada.*

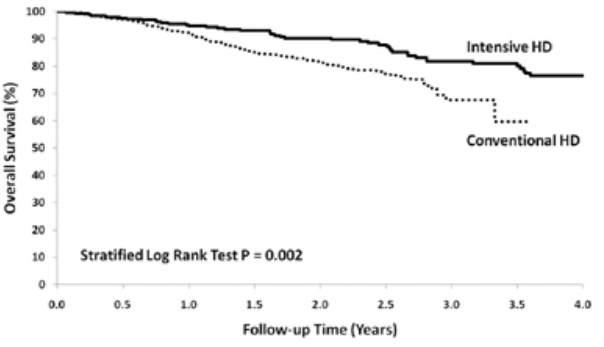
Background: Patients undergoing hemodialysis (HD) typically receive 3 sessions per week, each lasting 2.5-5.5 hours (conventional HD). Recently, the use of more intensive HD (>5.5 hours, 3-7 times per week) has increased, but the effects of these regimens on survival is uncertain.

Methods: We conducted a retrospective cohort study to examine whether intensive HD is associated with better survival than conventional HD. We identified 420 patients in the International Quotidian Dialysis Registry (IQDR) who received intensive home HD in France, the US, and Canada between January 2000 and August 2010. We matched 338 of these patients by country, duration of end-stage renal disease before study enrollment, and propensity score, to 1388 patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) who received in-center conventional HD during the same time period. We compared mortality rates using Cox regression.

Results: The intensive HD group received 4.8±1.1 sessions/week, with a mean treatment time of 7.4±0.87 hours/session, while the conventional HD group received 3 sessions/week, with a mean treatment time of 3.9±0.32 hours/session. During 3008 patient-years of follow-up, 45/338 patients receiving intensive home HD died, compared with 293/1388 patients receiving conventional in-center HD (6.1 vs. 10.5 deaths per 100 person-years, HR=0.55 (95% CI 0.34-0.87; p=0·01). This effect estimate was robust across all pre-specified subgroups and sensitivity analyses.

Conclusions: We demonstrate a strong association between intensive home HD and improved survival. Since we cannot exclude residual confounding in this observational study, confirmation in a large clinical trial is desirable, but may not be feasible.

Figure 1. Kaplan-Meier plot for intensive and conventional hemodialysis. Two-sided p = 0.002 by log rank test, stratified by matched set and country.



TH-OR152

Development of Circulating Anti-HLA Antibodies Is Associated with Acute Rejection after Conversion: Interim Report of CTOT-02 Sacha A. De Serres,[1] Indira Guleria,[1] Nader Najafian,[1] David N. Ikle,[3] Flavio G. Vincenti,[2] William E. Harmon,[1] Mohamed H. Sayegh,[1] Anil K. Chandraker.[1] *[1]Brigham and Women's Hospital and Children's Hospital Boston, Boston, MA; [2]UCSF, San Francisco, CA; [3]Rho Federal Systems Division, Chapel Hill, NC.*

Background: The clinical characteristics and the impact of development of anti-HLA alloantibodies (Abs) in renal transplant recipients is not well defined. This report looks at possible associations between Ab development, clinical characteristics, allograft histology at time of Ab development, and acute rejection following Ab conversion.

Methods: Over 750 subjects have been enrolled in the screening phase of the NIH CTOT-02/CCTPT-02 study, a multi-center prospective trial where unsensitized kidney transplant recipients are screened for development of de novo anti-HLA Abs up to 48 months post transplant. Subjects were divided into those who developed anti-HLA antibodies (Ab+) and those who did not (Ab-) as detected by Luminex. Ab+ subjects were offered treatment with anti CD20 therapy.

Results: 92 (15%) subjects developed Abs, at a fairly constant rate throughout the study period. 26, 51 and 15 subjects developed class I, II or both I & II Abs respectively. Mean time of Abs development was 18±10 months post transplant. Compared to Ab- subjects, Ab+ subjects were younger (36±18 vs. 43±17; p<0.01) and had lower serum creatinine (SCr) (1.3±0.5 vs. 1.4±0.5; p=0.03) at enrollment. There was no evidence of an association between Ab development and gender, donor type or DGF status. SCr at time of Ab conversion in Ab+ subjects was similar to the last SCr at follow-up in Ab- subjects (1.5±1.8 vs. 1.4±0.6; p=0.65). The proportion of subjects who developed acute rejection (AR) was higher in the Ab+ group (14 vs. 3%; p<0.01); most of the AR (77%) were noted after Ab development, at a mean time of 4.8±4.8mo post conversion. Moreover, biopsies in 7/18 Ab+ subjects prior to treatment showed evidence of acute rejection.

Conclusions: This interim analysis of CTOT-02 reveals unexpected differences in the baseline characteristics and a high proportion of subclinical and clinical rejection associated with anti-HLA Abs.

Funding: Other NIH Support - This work was supported by the Cooperative Clinical Trials in Organ Transplantation (CTOT)

TH-OR153

The Joint Economic Impact of Acute Rejection and Glomerular Filtration Rate in Contemporary Kidney Transplantation Adrian Gheorghian,[1] Mark Schnitzler,[1] Gilbert Litalien,[2] Anupama Kalsekar,[2] Krista L. Lentine.[1] *[1]Saint Louis University; [2]Bristol-Myers Squibb.*

Background: The economic implications of acute rejection (AR) in contemporary kidney transplant are not defined. We assessed the combined impact of 1st year AR and estimated glomerular filtration rate (eGFR) on 2nd and 3rd yr Medicare costs.

Methods: Data for Medicare-insured kidney transplant recipients in 2000-2007 (n=32,520) who survived with graft function to12 mo were drawn from the United States Renal Data System. AR events were ascertained from OPTN reports. AR was classified as Antibody-Treated AR (Ab-AR) or other management (non-Ab-AR). The primary cost measure was payments for all healthcare services made by Medicare during intervals of the 2nd and 3rd yrs post-transplant. Multivariate linear regression was used to quantify: 1) the marginal cost impact of first-year AR and eGFR on subsequent costs, and 2) total predicted costs. Covariates included recipient, donor and transplant factors in the UNOS Kidney Allocation Review Committee survival model.

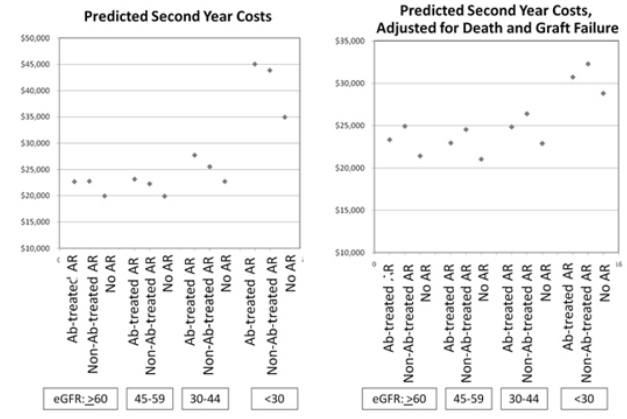
Results: Ab-AR and non-Ab-AR within the 1st yr were associated with significant increases in 2nd yr costs of $5,755 and $5,019, respectively, after adjustment including eGFR and baseline factors (**Table**). AR was also significantly associated with 3rd yr costs.

Total 2nd & 3rd costs were higher in those with AR compared to no AR. However, markedly stronger variation in costs was seen across eGFR levels **(Figure).** For example, among those with non-Ab-AR, adjusted total 2nd yr costs were $22,747 with eGFR >60 but $43,881 with eGFR ≤30 ml/min/m². Cost impacts of AR and eGFR were similar but of lower magnitude after additional adjustment for death and graft failure.

Table. Associations of AR within the 1st Yr and 12-month eGFR with Costs in Years 2 and 3 after Transplant by Multivariate Regression.

P-values: † P 0.0001–0.01; ‡ P < 0.0001	2nd Year Costs*	2nd Year Costs,* Adjusted for death and graft failure	3rd Year Costs*	3rd Year Costs,* Adjusted for death and graft failure
	$ per yr	$ per yr	$ per yr	$ per yr
Ab-treated in Yr1	5,755.40‡	1,923.02	7,962‡	7,838‡
Non-Ab-treated in Yr1	5,019.69‡	3,483.01‡	4,502‡	3,059‡
eGFR30 ml/min/m²				
≥60	*Reference*	*Reference*	*Reference*	*Reference*
45–59	-43.03‡	-387.40	-284	-450
30–44	2,854.24‡	1,477.56†	2,562‡	1,403†
<30	16,349.00‡	7,372.62‡	15,077‡	10,410‡

Figure. Predicted Costs in Yr 2 after Transplant according to 1st Yr AR and eGFR level



Conclusions: AR is a significant predictor of post-transplant costs. However, the cost impact of AR is markedly higher among affected patients with reduced compared to preserved eGFR.

Funding: Pharmaceutical Company Support

TH-OR154

Efficacy and Safety of Early Cyclosporine Conversion to Sirolimus with Continued MMF: 5-Year Results of the Post-CONCEPT Study Yvon Lebranchu,[1] Matthias Buchler,[1] Eric Thervet,[2] Isabelle Etienne,[3] Pierre-Francois Westeel,[4] Jean-Philippe Rerolle,[5] Sandrine Girardot-Seguin,[6] Bruno Moulin.[7] *[1]CH Bretonneau; [2]CHU Necker; [3]CH Bois Guillaume; [4]CH Sud; [5]Civil Dupuytren; [6]Roche, Neuilly-sur-Seine; [7]Civil Hospital.*

Background: CNI induces long-term nephrotoxicity and is associated with moderate renal dysfunction.

The *de novo* introduction of sirolimus (SRL) has demonstrated a positive impact on renal function. However, the adverse effects of early SRL introduction could limit this approach. In the CONCEPT study, the delayed conversion to SRL demonstrated an improved renal function with a similar tolerance, at one year. 60 months follow up study investigates the long term impact of this strategy.

Methods: In this prospective trial, renal function by simplified MDRD formula and safety were assessed from 12 to 60 months post transplantation in the two groups (group A: SRL+MMF, group B: CsA+MMF).

Results: Among the 162 patients (89.5%) entered in the follow up at M12, 139 patients were evaluated at M60; 67 in group A, 72 in group B. eGRF was significantly higher in group A, 58.31 vs 49.89 ml/mn/1.73m², respectively (p=0.0012). This difference was observed particularly in population "on treatment" (group A, n= 39) 65.05 ml/mn/1.73m² vs (group B, n= 67) 50.31 ml/mn/1.73m² (p<.0001).

On ITT analysis, 7 deaths (3 in SRL group and 4 in CsA group) not related to immunosuppressive treatment, 2 graft losses in group A and 4 BPAR (2 in each group) were observed between M12 and M60.

Concerning the lipid profile and proteinuria, the two groups were not different at M60.

The occurrence of cancers was lower in patients treated by MMF+SRL (n= 4) than MMF+CNI (n=12).

Conclusions: The significant improvement in renal function observed at 12 months in patients receiving MMF + SRL was maintained at 60 months compared to MMF + CSA and the occurrence of cancers was lower in patients treated by MMF + SRL

Funding: Pharmaceutical Company Support

TH-OR155

A Multicentre RCT of Early Switch to Everolimus Plus Steroids or Everolimus Plus CsA Versus CsA, MPA and Steroids in *De Novo* Kidney Transplant Recipients: 12 Month Analysis Steven J. Chadban,[1] Graeme Russ,[2] John Kanellis,[3] Helen L. Pilmore,[4] Yu Seun Kim,[5] Si-Yen Tan,[6] Nicol Kurstjens,[7] Josette M. Eris.[1] *[1]Royal Prince Alfred Hospital; [2]Royal Adelaide Hospital; [3]Monash Medical Centre; [4]Auckland City Hospital; [5]Severance Hospital; [6]University Malaya Medical Centre; [7]Novartis Australia.*

Background: To determine whether addition of everolimus(EVR) to cyclosporine(CsA), mycophenolic acid(MPA) and steroids at 2 weeks post-kidney transplantation can enable elimination of either CsA and MPA or steroids and MPA without compromising efficacy and safety.

Methods: SOCRATES is a 36-month(M), prospective, multi-centre, randomised, open-label study. Kidney transplant recipients(KTR) received CsA, MPA, prednisone and basiliximab and were randomized commencing week2 to: add EVR and withdraw CsA&MPA(CsA-WD); add EVR and withdraw steroids & MPA(steroid-WD); or remain on CsA, MPA & steroid(control). Primary outcome was non-inferiority at M12 eGFR(Nankivell formula). Key secondary endpoints were a composite of biopsy proven acute rejection(BPAR), graft loss, death, loss to follow-up and each of these individually.

Results: 126 KTR were randomized to CsA-WD n=49, steroid-WD n=30, or control n=47. The steroid-WD arm was prematurely terminated due to a higher treatment discontinuation rate attributed to acute rejection, unsatisfactory effect, or other reasons. At M12, CsA-WD was non-inferior to control for eGFR 72.3 vs 71.3 mL/min/1.73m²(p=0.006). Change in mean eGFR from week2 to M12 was numerically greater for CsA-WD vs control(+17.5 vs +5.7mL/min/1.73m²).

Key M12 efficacy parameters (ITT population)

	EVR with CsA-WD n=49 (%)	Control n=47 (%)	EVR with Steroid-WD n=30 (%)	P-value*
Composite efficacy failure by Month 12	16 (32.7)	8 (17.0)	11 (36.7)	0.1001
BPAR	15 (30.6)	6 (12.8)	5 (16.7)	0.0479
Graft loss	0 (0.0)	2 (4.3)	0 (0.0)	0.2371
Death	0 (0.0)	1 (2.1)	0 (0.0)	0.4896
Loss to follow-up	1 (2.0)	0 (0.0)	6 (20.0)	1.0000

*The p-value is from Fisher's exact test comparing CsA-WD versus control.

Conclusions: Early switch to everolimus enabled withdrawal of CsA and MPA without compromising M12 eGFR. This regimen was associated with improved eGFR from week2 to M12 but a higher rate of acute rejection. Continuation of MPA may be preferred when switching from a CNI to everolimus strategy.

Funding: Pharmaceutical Company Support

TH-OR156

Endothelial Dysfunction in Renal Transplant Recipients with Functioning Arteriovenous Fistula Yasar Caliskan,[1] Mehmet Besiroglu,[1] Halil Yazici,[1] Ibrahim Altun,[2] Ahmet Gurdal,[2] Tevfik Ecder,[1] Aydin Turkmen,[1] Mehmet Sever.[1] *[1]Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [2]Department of Cardiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.*

Background: Endothelial dysfunction is a common precursor and denominator of atherosclerosis and atherosclerotic cardiovascular diseases (CVD) are still the major cause of death among renal transplant recipients. We investigated the effects of functioning arteriovenous fistula (AVF) on endothelial functions in renal transplant recipients.

Methods: A total of 82 renal transplant recipients who were undertaken hemodialysis or peritoneal dialysis before transplantation enrolled in this study. Patients were divided into three subgroups [patients with functioning AVF (n=46), patients whose fistulas were spontaneous stopped or closed (n=25) and peritoneal dialysis patients who had never been constructed AVF]. Serum creatinine, uric acid, albumin, calcium, phosphorus, albumin, PTH, triglyceride, total, LDL- and HDL- cholesterol levels were measured. Endothelial function was measured non-invasively with high resolution ultrasound equipment as the percentage of post-ischemic flow-mediated dilation (FMD) of the brachial artery of the non-dominant or non- fistula arm**.**

Results: There were no significant differences regarding age, gender, smoking status, time on dialysis, immunosuppressive treatment, blood pressures, serum creatinine, uric acid, albumin, hs-CRP, calcium, phosphorus, albumin, intact PTH, triglyceride, total, LDL- and HDL- cholesterol levels among the study groups. Pulmonary artery pressure and ejection fraction were also similar among the three groups. FMD value of patients with functioning AVF (7.9±6.1%) were significantly lower then patients with non-functioning AVF (12.2±5.8%) and patients never been constructed AVF (10.4±5.8%) (p=0.017). In correlation analysis, FMD was not significantly correlated with any laboratory parameters.

Conclusions: Endothelial dysfunction which is a common precursor of cardiovascular events is more prominent in renal transplant recipients with functioning AVF.

Funding: Government Support - Non-U.S.

TH-OR157

Short Course, High Dose Erythropoietin in Deceased Cardiac Death Kidney Transplant Recipients Zeynep Aydin, Marko J. Mallat, Ton J. Rabelink, Johan W. De Fijter. *Nephrology, Leiden University Medical Center, Leiden, Netherlands.*

Background: Ischemia-reperfusion injury is a problem in kidneys derived from DCD donors. In preclinical models with ischemic insults, exogenously administered Erythropoietin proved to be renoprotective.

Methods: All consecutive DCD transplant recipients were included in this single center prospective, randomized, double blind, placebo-controlled study. Erythropoietin-was administered to the recipient as an intravenous bolus of 3.3 x 10^4 IU, 3-4 hours before the transplantation, as well as 24 and 48 hours after reperfusion.

Primary end point was the composite of delayed graft function/primary non function. Secondary end points included duration of DGF, acute rejection, measured creatinine clearance (mGFR) and survival at 1 year after transplantation.

For safety purposes any arterial or venous thrombosis was recorded.

Results: A total of 92 patients were included in the study. DGF occurred in 39/45 recipients in the EPO treated group (86,7%) as compared with 41/47 patients in the placebo group (87,2%) (P=1.00).

The incidence of PNF was 6.7% in the EPO group (3 patients) and 2.1% in the placebo group (1 patient).

If DGF developed, the median duration was 10 days in the EPO group and 9 days in the placebo group (NS).

Acute rejections occurred in 20.5% in the EPO group and in 26.1 % in the placebo group (P=0.62).

The measured creatinine clearance showed no difference at 6 weeks (44±19 ml/min and 46±18 ml/min in EPO and placebo group respectively), but was significantly better in the EPO group 1 year after transplantation (68±23 vs 57±25 ml/min) (P<0.05).

One-year patient and graft survival were respectively 96/93% and 96/96% in the EPO and placebo group.

Thromboembolic events occurred in 14 patients in the total group, the majority consisted of thrombosis of the vascular access (4 in EPO group, 1 in placebo group).

Conclusions: High-dose Erythropoietin did not reduce the incidence or duration of DGF. However, treatment with EPO resulted in a significantly better recovery of mGFR, but also more thrombotic events.

TH-OR158

Replacement of Vitamin D by Cholecalciferol or Cholecalciferol Plus Doxercalciferol Lowers Parathyroid Hormone Levels without Raising FGF23 or Altering Kidney Function in Kidney Transplant Recipients Mariana S. Markell, Sima Terebelo. *Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Hypovitaminosis D (HYPOD) and hyperparathyroidism (HPTH) occur commonly in kidney transplant recipients (KTR's). Optimal vitamin D replacement has not been established because of concerns regarding hypercalcemia, immune stimulation and potential increase in FGF23 secondary to increased 1,25 Vit D levels.

Methods: 39 stable KTR's with HPTH and HYPOD were enrolled. Pts were randomized to receive cholecalciferol 1000U daily plus placebo (CHOLE) or cholecalciferol plus 0.5 mcg doxercalciferol (DOXE) for 6 months. PTH values were measured at 4 weeks and doxercalciferol (or placebo) dose was titrated up by 0.5mcg if PTH values had not fallen. Full-length FGF-23 levels were determined using an ELISA assay.

Results: 33 pts completed the study, 3 pts dropped out from each group. No pt dropped out because of hypercalcemia. No pt had rejection. One CHOLE pt developed hypophosphatemia. 4 pts had constipation (3 DOXER/1 CHOLE) but none had hypercalcemia. The population included 16 men (37%), 29 Blacks (68%). Mean age was 49.2±13.5; 43.6±6.5 months since last transplant, creat 1.5±0.7 (GFR 54.8±3.1 by MDRD). By ANOVA, there was no difference in response to CHOLE vs DOXER as regards effect on PTH so the groups were combined for evaluation of treatment effect. By paired sample t-test there were no differences for creat, GFR by MDRD, prot/creat ratio, calcium or phosphorous after 6 month treatment. PTH fell from 189.7±11.8 to 134.7±10.8 (p<0.0001), 25-OH Vit D rose from 16.4±0.7 to 30.8±1.4 (p<0.001), while 1,25 Vit D remained the same (53.5±3.4 vs 56.1±3.5) as did FGF23 (97.9±8.9 vs 102.9±12.4).

Conclusions: 1. Cholecalciferol 1000U with or without doxercalciferol up to 1 mcg has a low side effect profile in stable long-term KTR's with HPTH. 2. Repletion of vitamin D can be accomplished within 6 months and results in an average decrease in PTH of 30%. 3. Repletion of Vitamin D with cholecalciferol or active vitamin D (doxercalciferol) does not cause an increase in 1,25 Vit D or FGF23 or adverse effects on kidney function 4. These regimens should be studied in KTR's with advanced CKD as a therapy for HPTH.

Funding: Pharmaceutical Company Support

FR-OR159

Macrophages Play an Essential Role in Renal Repair Following Apoptotic Tubule Injury Bing Yao,[1] Shilin Yang,[1] Ming-Zhi Zhang,[1] Raymond C. Harris.[1] *[1]Medicine, Vanderbilt University, Nashville, TN; [2]Vanderbilt University, Nashville, TN; [3]Nashville, TN.*

Background: There is increasing evidence that macrophages may contribute to both injury and repair in different experimental models of acute kidney injury.

Methods: To study the role of macrophages in recovery from acute kidney injury characterized predominantly by epithelial apoptosis, we utilized a transgenic mouse expressing the human diphtheria toxin receptor (DTR) selectively in kidney proximal tubule.

Results: DT administration induced acute kidney dysfunction in transgenic (TG) mice but not wild-type (WT) mice following DT administration, with increased creatinine and BUN and renal proximal tubule dilation, cell sloughing and cast formation. Marked renal proximal tubular cell apoptosis was detected by TUNEL-staining. In the TG mice, macrophage/monocyte depletion by clodronate either prior to, or after induction of injury by DT administration led to significantly greater functional and histologic injury, increased and prolonged KIM-1 expression, increased apoptosis and delayed cell proliferation. Similar results were seen with macrophage deletion in γ-GT/CD11c-DTR double transgenic mice. Following ischemia-reperfusion, there was an early increase in macrophages with "M1" (inflammatory) markers. In contrast, in injury induced by DT, there were no increases in M1 macrophages but significant increases in M2 (wound healing) macrophages; expression of these M2 markers was markedly inhibited by clodronate or in the double transgenic mice. Of interest, prior splenectomy did not alter the recovery in this model. Furthermore, unlike ischemia/reperfusion injury, there was no increase in renal accumulation of labeled monocytes in DT-induced injury above that seen in kidneys from untreated animals, but there was an increase in cells double labeled with F4/80 and Ki67, a marker of proliferation.

Conclusions: In this model of AKI characterized by renal tubular apoptosis, M2 macrophages increase in response to injury and serve as a crucial component mediating epithelial regeneration. There appears to be an important role for local macrophage proliferation in the reparative process.

Funding: NIDDK Support, Veterans Administration Support

FR-OR160

Oxygen-Sensing Prolylhydroxylases (PHDs) in Acute Kidney Injury Alexander Badulak, Douglas Ridyard, Eunyoung Tak, Holger Eltzschig, Almut Grenz. *Dept. of Anesthesiology, UC Denver, Denver, CO.*

Background: During renal ischemia, shifts in the metabolic supply to demand ratio – particularly for oxygen – result in severe tissue hypoxia. Cellular responses to hypoxia are regulated by enzymes that sense cellular oxygen levels and coordinate transcriptional responses to hypoxia or ischemia. Central among these enzymes are three oxygen-sensing prolyl hydroxylases (PHD1-3). Limited oxygen availability results in inhibition of PHDs with subsequent stabilization of hypoxia-inducible factors (HIFs). Activation of HIF results in a transcriptionally regulated response that re-programs cellular metabolism towards hypoxia adaptation. *Thus, we hypothesize that genetic deletion or pharmacologic inhibition of PHDs mediates kidney protection from ischemia.*

Methods: Gene targeted mice for PHD1, 2 and 3 ($PHD1^{-/-}$, $PHD2^{+/-}$, $PHD3^{-/-}$) were studied in an ischemic model of acute kidney inury (AKI) by utilizing a hanging weight system. Renal function was determined by FITC-labeled inulin clearance, serum creatinine, renal cytokine levels, renal myeloperoxidase (MPO ELISA), histology and TUNEL staining.

Results: To pursue our hypothesis, we first treated wild type mice with a PHD inhibitor (DMOG, dimethyloxallyl glycine) before renal ischemia. Mice with DMOG treatment showed attenuated kidney injury following renal ischemia compared to vehicle treated mice. The decrease in GFR following ischemia was attenuated and the increase in serum creatinine reduced. Furthermore, inflammatory cytokines (TNF-alpha, IL-6) and neutrophil infiltration (MPO assay) were attenuated in DMOG treated mice compared to vehicle treated mice. Further studies in gene-targeted mice for PHD1, 2 or 3 showed a selective phenotype in $Phd1^{-/-}$ mice with remarkable protection from ischemic AKI. The GFR was tremendously improved, serum creatinine significant lower and histological damage attenuated.

Conclusions: In summary, we could show a selective phenotype in Phd1-/- mice with improved renal function following AKI due to ischemia. Thus, PHD inhibitors could prove to be a novel therapeutic agent in the treatment of AKI in the near future.

FR-OR161

Chitinase 3-Like 1 Regulates the Renal Response to Ischemic Injury and Predicts Delayed Allograft Function Insa Marie Schmidt,[1] Isaac E. Hall,[1] Gilbert W. Moeckel,[2] Chirag R. Parikh,[1] Lloyd G. Cantley.[1] *[1]Department of Internal Medicine, Section of Nephrology, Yale University School of Medicine, New Haven, CT; [2]Department of Pathology, Yale University School of Medicine, New Haven, CT.*

Background: Acute kidney injury can trigger a series of responses that promote tubule repair. Defining those pathways may provide novel targets for therapeutic intervention to promote the repair process.

Methods: Using proteomic analysis, western analysis and quantitative PCR, we found that Chitinase 3-like 1 (Chi3l1,named Brp-39 in mouse,YKL-40 in humans), is highly upregulated in the kidney and urine during the repair phase after ischemia/reperfusion (I/R) injury. The functional importance of this pathway was analyzed using Brp-39 null mice, cultured epithelial cells and urine from patients after kidney transplant.

Results: Mice lacking Chi3l1 demonstrate significantly worse outcomes following AKI compared to control animals, with more severe tubular injury, increased apoptosis, decreased reparative tubular proliferation, a persistent reduction of kidney function and decreased survival. Stimulation of mouse tubular epithelial cells with Brp-39 induces activation of Akt and Erk, with a reduction of H2O2-induced apoptosis by 50%. Inhibition of Brp-39 stimulated PI3-K/Akt activation prevented the anti-apoptotic effect, suggesting that this pathway is critical for the protective effects of Brp-39. In recipients of deceased-donor kidney transplants who exhibited delayed graft function (DGF, indicative of severe ischemic injury,n=26), urinary YKL-40 levels at 0 (immediately post-op) and 1 day after

transplant were markedly higher as compared to patients without DGF (indicative of less severe ischemic injury, n=51). Receiver-operating characteristic analysis revealed urinary YKL-40 had an area under the curve for the development of DGF of 0.84.06 immediately post-op and 0.88.05 1 day after transplant.

Conclusions: These studies demonstrate that Chi3l1 is expressed in a graded fashion in response to ischemic kidney injury where it acts to limit tubular apoptosis and promote kidney repair. This newly identified pathway may thus serve as both a potential therapeutic target and biomarker for AKI.

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FR-OR162

PTEN Loss Defines the Tubule Phenotype of Failed Differentiation Associated with Kidney Fibrosis in Rodent Models and Human Disease

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Background: High TGFβ after acute kidney injury (AKI) leads to failed differentiation of regenerating epithelium, defective tubule repair, and fibrosis.

Methods: We studied how the "failed differentiation" tubule phenotype develops in cultured cells, mice with tubule specific TGFβ induction, rat models of ischemic and maleate AKI, and human chronic kidney disease.

Results: Tubule specific induction of TGFβ in vivo led to loss of tumor suppressor PTEN and increased Jun N-terminal kinase (JNK) signaling in proximal tubules (PT), and to fibrosis. In cultured PT cells, high TGFβ depleted PTEN, raised JNK signaling and inhibited differentiation. Conversely, TGFβ antagonism increased PTEN, decreased JNK signaling and promoted differentiation. Selective Cre-Lox PTEN deletion suppressed PT differentiation despite TGFβ status, triggered PI3K and JNK signaling, and induced growth arrest. PTEN declined in a subpopulation of PT during repair of AKI in vivo. These cells lacking PTEN were growth arrested, but showed increased PI3K and JNK signaling, failed to re-differentiate, expressed vimentin and keratin, produced PDGF-B and CTGF, and were surrounded by fibrosis; whereas PTEN recovery was associated with PT re-differentiation and normal repair. Pharmacologic TGFβ antagonism promoted PTEN recovery, PT differentiation, and normal repair. Similar improvement of low PTEN and tubulo-interstitial pathology were induced by contralateral nephrectomy done 2 weeks after unilateral ischemia, showing reversibility of this dysfunctional phenotype. Vimentin and keratin expressing tubules with low PTEN and increased phospho-c-Jun were associated with fibrosis also in human chronic kidney disease of diverse etiology.

Conclusions: The low PTEN, vimentin-keratin positive tubule phenotype that expresses phlogistic JNK-Jun signaling and fibrogenic peptides is associated with fibrosis in diverse animal models and human disease. We propose that it plays a central pathogenic role in triggering fibrotic events in the renal interstitium, and is potentially treatable.

Funding: NIDDK Support

FR-OR163

Exosome-horizontal Transfer of IGF-1 Receptor to Cisplatin-Damaged Tubular Cells Potentiates the Reparative Effect of Mesenchymal Stem Cells (MSC)-Derived IGF-1

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Background: Bone marrow MSC ameliorate renal dysfunction and repair tubular damage of acute kidney injury through their ability to locally release growth factors including IGF-1. Beside soluble factors, cell-cell communication occurs through vesicles named exosomes containing mRNA that can be delivered and translated into the recipient cell. Finding few MSC at the site of injury led us to investigate a possible gene-based communication mechanism between MSC and tubular cells amplifying the renoprotective effect of IGF-1.

Methods: Transmission electron microscopy allowed identification of microvesicles (MV) and exosomes by size and expression of antigen surface proteins CD63 and CD9 by immunogold. Expression profile of genes involved in the insulin-signalling pathway was assessed by PCR arrays in MV/exosomes and donor MSC.

Results: MV (110-150 nm) and exosomes (45-90 nm) released from either serum-free cultured mouse and human MSC did not contain DNA but were enriched in mRNA (10-60 ng and 20-300 ng/1x10^6 cells for mouse and human MSC). Gene profile analysis revealed the presence of selected pattern of transcripts in MV/exosomes versus donor cells. MV/exosomes expressed IGF-1 receptor (IGF-1R) but not IGF-1 mRNAs, while MSC contained both mRNAs. Human tubular cells exposed to mouse MSC-derived exosomes acquired mouse IGF-1R transcript demonstrating genetic exchange between MV/exosomes and tubular cells. mRNA shuttled by exosome was functional as addition of human MV/exosomes to cisplatin-damaged mouse tubular cells (MTC) significantly increased MTC proliferation. Gene silencing of IGF-1R in MSC significantly reduced the proliferative effect induced by exosome on damaged MTC suggesting that MTC proliferation was exosome-IGF-1R dependent.

Conclusions: Horizontal transfer of mRNA for the IGF-1R to tubular cells through MV/exosomes potentiates tubular cell sensitivity to locally produced IGF-1 providing a new mechanism underlying the powerful renoprotection of MSC.

Funding: Pharmaceutical Company Support

FR-OR164

Exploring the Origin of the Cells Responsible for Regeneration, and the Mechanism of Repair in the Kidneys

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Background: Kidneys repair after acute kidney injury (AKI) with a rapid proliferative response, which leads to the restoration of nephron structure and function. However the origin of the proliferating cells after AKI remains controversial. We hypothesized that the mature proximal tubule cells proliferate and repair kidney after injury.

Methods: To test the hypothesis, we generated proximal tubule-specific inducible Cre mice (NDRG1CreERT2/+ mice), which could achieve almost 100% recombination in proximal tubules without any leakage, only after the administration of tamoxifen. To explore the origin of the proliferating cells after AKI, we performed ischemia-reperfusion injury (I/R injury) to NDRG1CreERT2/+:R26R mice, and administered BrdU.

Results: Many LacZ/BrdU double positive cells were observed within injured tubules, indicating that mature proximal tubule cells proliferated. Furthermore, no dilution of genetic label was observed in the proximal tubules of the repaired kidney at day45, despite of extensive proliferation after I/R injury.

We also analyzed whether other cell source is employed after repeated injury. Even after three times I/R injuries, however, no dilution of the genetic label was observed in the repaired proximal tubules, indicating that proximal tubule cells are the only cell source to repair proximal tubules even after repeated injuries.

Furthermore, the size of the injured kidney decreased after repeated injuries, even after the repair of tubules was completed. We further demonstrated that the complexity of proximal tubules decreased significantly after repeated injuries. Because the volume of proximal tubules is about 80 % of whole kidney, the shortening of proximal tubules might account for the size reduction of kidneys after repeated injuries.

Conclusions: Proximal tubule is a unique segment of nephron, which is capable of self-proliferation and self-repair, although the repair capacity is not sufficient after repeated injuries.

Funding: Government Support - Non-U.S.

FR-OR165

IQGAP1 Is Critical for Recovery from AKI by Enhancing Tubular Cytoskeleton Regeneration

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Background: IQ-domain GTPase-activating protein 1 (IQGAP1) is a scaffold protein that plays a critical role in regulating actin cytoskeleton by interacting with actin, via Rac1/Cdc42, Arp2/3 and N-WASP signaling. Since actin cytoskeleton remodeling plays an import role in the repair of acute kidney injury (AKI), we investigated the role of IQGAP1 in tubular regeneration after endotoxin induced AKI.

Methods: IQGAP1 knock out (KO) mice and wild type control (WT) received lipopolysaccharide (LPS, 10 mg/kg, i.p.) or vehicle and blood and kidneys were collected 24 h after for BUN, histology and immunofluorescence staining (IF).

Results: KO had normal baseline renal function (BUN: 21±3). After LPS treatment for 24 h, BUN and acute tubular necrosis (ATN) score were significantly higher in KO vs. WT (BUN: 151±23 vs. 37±8; ATN: 4.2±0.3 vs. 1.6±0.2, both $p<0.05$). Interaction between IQGAP1 and F-actin was analyzed using IF and confocal microscope. In WT proximal tubule, IQGAP1 and F-actin co-localized in the apical microvillar. In distal convoluted tubules, IQGAP1 was diffusely expressed and co-localized with F-actin at lateral cell-cell junctions. In collecting ducts, F-actin co-localized with IQGAP1 in both intercalated and principal cells. After LPS treatment, the cytoskeleton was disrupted with the reduction of both IQGAP1 and F-actin in the damaged renal tubules. The reappearance of IQGAP1 along with F-actin was observed in the damaged renal tubules after 24 h of LPS treatment, suggesting the regeneration of actin cytoskeleton. Uninjured KO showed normal distribution of F-actin, however, there was no reappearance of F-actin in the damaged renal tubules of KO at 24 h after LPS.

Conclusions: The co-localization of IQGAP1 with F-actin suggests that IQGAP1 is important in actin cytoskeleton organization. During the early phase of AKI, IQGAP1 is removed from the apical membrane along with the loss of F-actin and regenerated along with F-actin during the recovery phase, suggesting that IQGAP1 is critical for tubular regeneration after AKI.

Funding: NIDDK Support, Private Foundation Support, Government Support - Non-U.S.

FR-OR166

Understanding Endogenous Repair Following Acute Kidney Injury Using Macrophage Profiling and Phenotypic Analysis

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Background: Ischemia-reperfusion (IR) injury is characterised by the production of pro-inflammatory cytokines, such as IL-6, TNF and MCP-1, that drive M1 macrophage polarisation resulting in tissue damage. We have reported that colony stimulating factor (CSF)-1 stimulates macrophage differentiation towards a reparative phenotype leading to accelerated kidney repair following IR (Alikhan et al. Am J Pathol 2011). Identifying

further cytokines and growth factors involved in macrophage polarisation may lead to the development of therapeutics aiding in the rapid reversal of the inflammatory response and promotion of tissue remodeling.

Methods: Flow cytometry was used to assess macrophage phenotype during the inflammatory and tissue remodeling phases of IR injury (n=5/group) with/without the administration of CSF-1. The production of IFN-γ, TNF, IL-6, IL-10, IL-12p70 and MCP-1 was assessed using cytokine bead arrays. In conjunction, macrophages (CD45+CD11b+CD11c-) from damaged kidneys were FACS sorted and subjected to microarray gene expression profiling using IlluminaTM Mouse gene expression arrays. Candidate therapeutic target genes were identified using the Ingenuity Pathway Analysis software.

Results: Total kidney cellularity decreased over the time course of injury but with a concomitant rise in CD45+ cells. Ly6Chigh inflammatory monocytes comprised the majority of the infiltrate at 24hrs. This was followed by the rapid maturation of macrophages, identified by the upregulation of MHC class II and F4/80. Gene profiling arrays showed the increased expression of the M2 associated glucocorticoid receptor, IL-10 and IGF-1 genes at day 5 and repair associated PDGF, GM-CSF and apoptosis signaling pathways by day 7 in CSF-1 treated mice.

Conclusions: Macrophages play a central role in the endogenous repair process following IR injury as evident by the phenotypic switch from a pro-inflammatory to an anti-inflammatory cell type. Further gene profiling of the cells involved in CSF-1 enhanced repair has highlighted potential therapeutic targets that may prevent or retard the progression of renal disease.

FR-OR167

Hedgehog Signaling Pathway Is Activated during Kidney Repair Massimo Attanasio,[1,2] Alysha Rauhauser,[1] Binghua Li.[1] *[1]Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX; [2]McDermott Center for Growth and Development, UT Southwestern Medical Center, Dallas, TX.*

Background: Acute kidney injury (AKI) is a frequent cause of long term renal failure or death (1). Unilateral ureteral obstruction (UUO) in mice has been widely used as a model of severe AKI. Hedgehog (Hh) is a core signaling pathway implicated in fundamental processes during embryonic kidney development (2) but its role in the adult kidney has not been explored.

Experiments performed in our laboratory show that persistence of hedgehog signaling in the adult kidney leads to acquisition of mesenchymal hallmarks by tubular epithelial cells, similarly to what is observed after AKI. This similarity prompted us to test the participation of Hh pathway in kidney repair.

Results: Using Gli1lacZ transgenic mice (3) as an in vivo reporter of Hh activity, we found that Hh signaling is latent in adult fully developed kidneys and is significantly upregulated after UUO. Increased Gli1 activity was more evident at the cortico-medullary junction, where it is mostly concentrated in interstitial cell populations, but was also sparsely detected in cells of various tubular segments.

To test if pharmacological modulation of Hh activity would affect kidney repair, we inhibited Hh signaling at different time points after injury using the specific Smoothened inhibitor, cyclopamine. Hh inhibition resulted in net improvement of kidney morphology, significant reduction of collagen deposition and diminished apoptosis, indicating a central role of Hh signaling in kidney repair. Interestingly, the favorable effects of Hh inhibition on the injured kidney were dependent on the time of the drug administration, suggesting that Hh might have different functions and signal to different cell populations in different phases of the repair process.

Conclusions: We conclude that activation of the Hh signaling pathway has a key role in kidney repair and its modulation might be useful for future therapy of AKI.

(1) Lo, L. J. et al. Kidney Int. 76:893–899, 2009
(2) Hu MC et al. Development 133:569-78, 2006
(3) Bai et al. Development 129:4753-4761, 2002

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FR-OR168

Microparticles from Kidney Derived-Mesenchymal Stem Cells Act as Carriers of Proangiogenic Signals and Contribute to Renoprotection in Acute Kidney Injury Hyeong Cheon Park, Hoon Young Choi, Geum-Ock Kim, Jin Seol An, Sun Hee Ahn, Sung-Kyu Ha. *Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.*

Background: Microparticles (MP) shed from bone marrow mesenchymal stem cells (MSC) conferred protective effects against acute tubular injury via transfer of messenger RNA and microRNA. We recently demonstrated that in vitro expanded kidney derived-MSC (KMSC, Kidney International 2008; 74:879-889) protected peritubular capillary endothelial cells in acute ischemic reperfusion injury (IRI).

Methods: KMSC were cultured in hypoxic chamber in serum deprived MEM with hydrogen peroxide (200 μM) for 24 hours. MP were isolated from supernatants by differential ultrafiltration (2,000x g, 10 min, 100,000x g, 1hr) for electron microscopy and flowcytometric characteriaztion and MP RNA was extracted using ExoMir kit (BIOO Scientific). Isolated MP were co-cultured with human umbilical vein endothelial cells (HUVEC) on growth factor reduced Matrigel to assess their effect on endothelial tube formation. Mice subjected to bilateral IRI were injected with PKH26 stained MP ($4x10^6$/mice) to assess its renoprotective effects.

Results: Presence of MP was confirmed by electron microscopy. Flow cytometric analysis of MP demonstrated presence of several adhesion molecules shown to be expressed on KMSC membrane such as CD29, CD44, CD73, alpha4- and alpha 6 integrins. Quantitative real time PCR confirmed the presence of 3 splicing variants of VEGF-A (120, 164, 188) and IGF-1 in isolated MP. MP labeled with PKH26 red fluorescence dye were incorporated by cultured HUVEC via surface molecules such as CD44 and CD29. MP dose dependently improved in vitro HUVEC viability and promoted endothelial tube formation. Furthermore, injection of MP after IRI significantly improved renal function in mice subjected to 32 minutes of bilateral IRI.

Conclusions: Our results support the hypothesis that KMSC-derived MP may act as a source of proangiogenic signals and confer renoprotective effects.

Funding: Government Support - Non-U.S.

FR-OR169

Therapeutic Targeting of Vascular Klotho Inhibits Calcification and Unmasks FGF23 Vasculo-Protective Effects in CKD Kenneth Lim,[1,2] Tzong-Shi Lu,[1] Guerman Molostvov,[2] Daniel Zehnder,[2] Li-Li Hsiao.[1] *[1]Brigham and Women's Hospital, Harvard Medical School; [2]Warwick Medical School, United Kingdom.*

Background: Klotho-FGFR1 functions as a receptor for the phosphatonin, FGF23 at the kidney. FGF23 levels rise in CKD despite progressive vascular calcification (VC). We postulate functional vascular Klotho expression as a therapeutic target for VC in CKD.

Methods: In vitro model: Human aortic smooth muscle cells (HA-SMCs) +/- Klotho siRNA. Calcification: Alizarin red and Arsenazo III detection.

Results: We show for the first time Klotho expression in human arteries and HA-SMCs. Analysis of human arteries from CKD and healthy controls revealed CKD as a state of vascular Klotho and FGFR1/3 deficiency. Klotho and FGFR1/3 deficiency was driven by uremic serum, calcification medium (CM: 2.7mM CaCl2 +/- 2mM β-glycerophosphate) and TNF-α (20ng/ml), in vitro. Klotho knockdown potentiated development of accelerated calcification. These cells exhibited osteogenic transformation (increased Cbfa1 and ALP), and concomitant loss of myocardin-SRF regulated contractile phenotype. In addition, in vitro studies revealed that vascular cells are a Klotho-dependent target for FGF23. FGF23 (5ng/ml) induced cellular activation of ERK, AKT and proliferative effects, which were abrogated by Klotho knockdown. We next showed that calcitriol or calcidiol reversed loss of Klotho and FGFR1/3 in HA-SMCs. Human arterial organ cultures from CKD patients confirmed these findings, with upregulation of Klotho and FGFR1 mRNA after calcitriol and paricalcitol treatment. Furthermore, calcitriol or FGF23 pre-treatment alone followed by combined treatment with CM did not modulate development of HA-SMC calcification. However, pre-treatment with calcitriol and FGF23 together, significantly inhibited the development of calcification. These effects mitigated by Klotho knockdown.

Conclusions: Chronic metabolic stress factors found in CKD promote vascular Klotho deficiency. Functional studies reveal a bifunctional role for vascular Klotho, first as an endogenous inhibitor of VC, and second as a co-factor for FGF23 signaling. Furthermore, VDR activators can restore Klotho expression and unmask FGF23 vasculo-protective effects.

Funding: Private Foundation Support

FR-OR170

FGF23 Inhibits Innate Immune Responses to Vitamin D in Human Monocytes Justine Bacchetta, Thomas S. Lisse, Jessica L. Sea, Rene Chun, Katherine Wesseling-Perry, Isidro B. Salusky, Martin Hewison. *David Geffen School at UCLA, Los Angeles, CA.*

Background: Vitamin D is a potent stimulator of innate immunity. This facet of vitamin D physiology is highly dependent on efficient synthesis of 1,25-dihydroxyvitamin D (1,25D) by monocytes, the 1-alpha hydroxylase CYP27B1 being induced by immune activators. FGF23 suppresses renal CYP27B1 but its role in non-classical actions of vitamin D is less clear. We hypothesized that FGF23 inhibits vitamin D-induced antibacterial activity by targeting monocyte CYP27B1 similar to its actions in the kidney.

Methods: We performed rt-PCR, Western blots and immunohistochemistry in human monocytes obtained from healthy donor peripheral blood mononuclear cells (PBMCm) and from peritoneal dialysate effluent (PDm).

Results: In untreated PBMCm, initial rtPCR studies confirmed the presence of receptors for FGF23, with klotho and FGFR1 being more strongly expressed than FGFR2, 3 or 4; there was a positive relationship between FGFR1 and klotho expression, as well as between FGFR-1 and CYP27B1. Immunohistochemistry showed that klotho and FGFR1 proteins colocalize, this effect being enhanced following treatment with FGF23. Western blot analyses showed that treatment with FGF23 activated both Erk1-2 and Akt pathways, known to be downstream of FGFRs. Treatment of PBMCm with FGF23 (100 ng/ml) demonstrated a downregulation of Klotho, FGFR1 and CYP27B1 expression, as determined by rtPCR. This effect was also observed in PBMCm pre-treated with IL-15 to stimulate CYP27B1 expression. As a consequence of decreased CYP27B1 expression, FGF23-treated PBMCm showed lower levels of antibacterial LL37, and other 1,25D targets such as CYP24A1. Similar observations were also made in PDm, which showed stronger expression and colocalization of klotho and FGFR1 at baseline.

Conclusions: These data show for the first time that FGF23 can inhibit extra-renal metabolism and function of vitamin D. We therefore propose that elevated expression of FGF23 may play a crucial role in defining immune responses to vitamin D and this, in turn, may be a key determinant of infection in patients with CKD.

Funding: NIDDK Support, Private Foundation Support

FR-OR171

Chronic Phosphate Restriction Fails To Prevent Fibroblast Growth Factor 23 (FGF23) Elevation in Chronic Kidney Disease Mouse Model Jason R. Stubbs, Shiqin Zhang, Ryan Gillihan. *Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

Background: FGF23 is a phosphaturic and vitamin D regulatory hormone that is markedly elevated in end-stage renal disease (ESRD). While elevations in FGF23 are associated with morbidity and mortality in ESRD, the mechanism responsible for this elevation is unclear.

Methods: We tested the hypothesis that phosphate retention in chronic kidney disease (CKD) is the primary stimulus for FGF23 elevations by studying the effects of dietary phosphate restriction on FGF23 levels in Col4a3 null mice, a proteinuric mouse model with a mineral metabolism profile that mimics that found in humans with CKD.

Results: We observed a significant increase in serum FGF23 in Col4a3 null mice at 8 weeks-of-age (1160.8 ± 401.1 vs 183.5 ± 11.3 pg/ml for WT controls; $p<0.05$), with a marked elevation by 12 weeks (5407.0 ± 1269.0 vs 236.3 ±23.2 pg/ml for WT controls; $p<0.001$), when mice were uremic. Severe phosphate restriction (0.02%) initiated at weaning failed to prevent the rise in FGF23, with Col4a3 null mice on phosphate restriction demonstrating FGF23 levels similar to those of null mice on the control diet at 8 (1304.0 ± 151.0 pg/ml; p=NS) and 12 weeks (8228.3 ± 2314.0 pg/ml; p=NS). Col4a3 null mice also exhibited a decline in serum $1,25(OH)_2D$ levels by 8 weeks (142.0 ± 16.5 vs 215.5 ± 39.7 pmol/l for WT controls; p=0.06), with severe deficiency present by 12 weeks (39.0 ± 10.5 vs 175.7 ± 31.9 pmol/l for WT controls; $p<0.01$). At 8 weeks, a 3-fold increase in renal Cyp24 gene expression was present in Col4a3 null mice ($p<0.05$ vs WT), suggesting the activation of vitamin D degradation pathways. Phosphate restriction improved $1,25(OH)_2D$ levels in these mice (192.0 ± 71.7 pmol/l at 12 weeks; $p<0.05$ compared to null mice on control diet) and normalized the renal Cyp24 gene expression, despite persistent elevation of FGF23 levels.

Conclusions: Our data suggest that phosphate retention may not be the primary cause for the elevated FGF23 levels in CKD. However, phosphate restriction can improve $1,25(OH)_2D$ levels in advanced CKD despite persistent FGF23 elevations, suggesting a retained capacity for renal $1,25(OH)_2D$ generation in ESRD.

Funding: NIDDK Support, Pharmaceutical Company Support

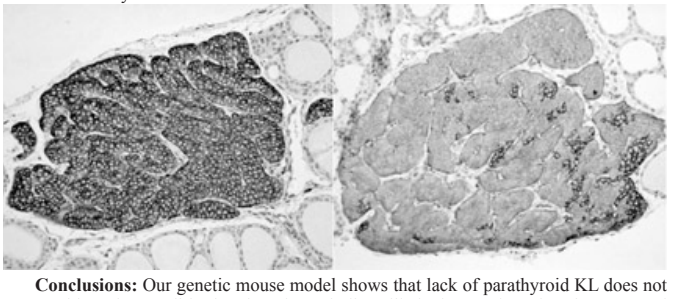
FR-OR172

Role of Parathyroid α-Klotho in Maintenance of Mineral Metabolism Hannes Olauson,[1] Karolina Lindberg,[1] Göran Andersson,[2] Tobias Larsson.[1] *[1]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden; [2]Department of Pathology, Karolinska Institutet, Stockholm, Sweden.*

Background: Parathyroid type 1 membrane-bound α-Klotho (KL) has been reported to mediate PTH secretion at low extra-cellular calcium concentrations. KL also functions as a co-receptor for FGF23, mediating its suppressive action on PTH release. Thus, the overall physiological role of parathyroid KL remains unclear. Further, KL expression is reduced in CKD patients, possibly leading to secondary hyperparathyroidism (sHPT) through loss of FGF23 inhibition.

Methods: We generated mice with a targeted deletion of parathyroid KL (PTG-KL-/-) by employing Cre-Lox recombination using transgenic PTH-Cre mice. Immunohistochemical detection showed efficient deletion of KL in the parathyroid glands of PTG-KL-/- mice compared to wild-type controls.

Results: No gross phenotypic changes were observed in adult PTG-KL-/- mice, although female PTG-KL-/- mice were smaller than its wild-type counterparts. In 3 and 8 week-old mice, no gross histological- or serum abnormalities were found. When challenged with a low calcium diet, PTG-KL-/- mice had a trend towards lower PTH levels than controls although statistically non-significant (p=0.07), however calcium, phosphate and FGF23 remained normal. Immunohistological analysis revealed reduced expression of CaSR and VDR in PTG-KL-/-. There were no changes in gland size or cell proliferation as determined by Ki67 index.



Conclusions: Our genetic mouse model shows that lack of parathyroid KL does not cause evident abnormalities in mineral metabolism, likely due to adaptations in CaSR and VDR expression. Reduced KL, as found in CKD patients, is therefore unlikely to play a primary pathogenic role in development of sHPT.

FR-OR173

Possible Involvement of PTH in the Secreted Frizzled Related Proteins (sFRPs) Regulation and Wnt Signalling Pathway Natalia Carrillo-Lopez, Maria Arias, Pablo Roman-Garcia, Sara Panizo, Manuel Naves, Jorge B. Cannata-Andia. *Bone and Mineral Research Unit, Hospital Universitario Central de Asturias. Instituto Reina Sofía de Investigación. REDinREN del ISCIII. Universidad de Oviedo, Oviedo, Asturias, Spain.*

Background: The aim of this study was to evaluate in vivo and in vitro the effect of different degrees of secondary hyperparathyroidism and different PTH concentrations on bone turnover-related and Wnt pathway signaling-related gene expression.

Methods: After inducing chronic renal failure (CRF) by 7/8 nephrectomy in 36 rats, one group was fed normal phosphorus (P) diet (NPD) (0.6%P) and other was fed high P diet (HPD) (0.9%P). Rats were sacrificed at 8, 16 and 20 weeks. Blood samples were collected and the left tibia was removed to assess gene expression. In the in vitro study, UMR106 cells were exposed to vehicle or different concentrations of PTH (1-34). After 24 hours, cells were collected to analyze gene expression.

Results: After 20 weeks, CRF rats fed HPD diet showed a significant increase in serum PTH and P levels, together with a significant decrease in serum calcium. Moreover, the bone gene expression of bone turnover markers together with Wnt inhibitors, such as sFRP1, sFRP2, sFRP4 and DKK1, was significantly increased. In vitro, cells exposed to PTH were able to significantly increase FGF23, osteocalcin, OPG, Cbfa1 and cathepsin K gene expression. Like in the in vivo experiments, PTH were also able to significantly increase sFRP1, 2 and 4 gene expression in a concentration dependent manner.

Conclusions:

Bone gene expression of bone turnover markers and Wnt-related gene expression measured by qRT-PCR in the *in vivo* and *in vitro* studies. R.U.: Relative Units referred to Reference group (rats with normal renal function and NPD diet) (*in vivo* study) and to Vehicle group (*in vitro* study). *In vivo* study: $^{\#}p<0.05$ compared to time-matched NPD group and $^{*}p<0.05$ compared to Reference group. *In vitro* study: $^{*}p<0.05$ compared to Vehicle group.

IN VIVO STUDY	Osteocalcin (R.U.)	OPG (R.U.)	Cbfa1 (R.U.)	Cathepsin K (R.U.)	Lrp5 (R.U.)	sFRP1 (R.U.)
8 weeks NPD	1.20±0.34	0.87±0.36	0.82±0.36	2.41±0.80	1.56±0.84	1.63±0.45
16 weeks NPD	0.73±0,.59	0.83±0.28	0.81±0.18	2.57±1.36	0.95±0.39	2.73±0.93
20 weeks NPD	1.06±0.15	0.89±0.06	0.96±0.22	2.33±0.45	0.97±0.09	1.34±0.30
8 weeks HPD	2.27±0.98*,#	4.33±1.50*,#	2.81±0.88*,#	3.34±0.69*	1.33±0.41	5.89±1.62*,#
16 weeks HPD	8.84±1.43*,#	6.50±2.40*,#	3.73±1.70*,#	5.99±2.80*	1.75±0.96	8.51±2.69*,#
20 weeks HPD	13.06±3.75*,#	9.33±4.39*,#	5.96±1.50*,#	27.03±6.69*,#	1.20±0.36	17.87±2.99*,#
Reference group	1.00±0.50	1.00±0.31	1.00±0.15	1.00±0.36	1.00±0.26	1.00±0.21

IN VIVO STUDY	sFRP2 (R.U.)	sFRP4 (R.U.)	DKK1 (R.U.)
8 weeks NPD	1.90±0.91	0.96±0.22	1.75±0.54
16 weeks NPD	1.17±0.38	1.25±0.29	1.30±0.36
20 weeks NPD	1.47±0.40	1.07±0.09	0.98±0.04
8 weeks HPD	2.12±0.38*	2.87±1.39*,#	5.52±2.14*,#
16 weeks HPD	19.13±10.30*,#	13.99±5.35*,#	3.05±1.62
20 weeks HPD	8.59±2.59*,#	30.17±6.61*,#	4.57±1.57*,#
Reference group	1.00±0.20	1.00±0.30	1.00±0.24

IN VITRO STUDY	FGF23 (R.U.)	Osteocalcin (R.U.)	OPG (R.U.)	Cbfa1 (R.U.)	Cathepsin K (R.U.)	Lrp5 (R.U.)
Vehicle	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
10-8M PTH	1.32±0.32	1.17±0.15	2.83±0.88*	1.45±0.06*	1.49±0.12*	0.89±0.17
10-7M PTH	1.72±0.25*	1.71±0.47	9.87±2.16	2.05±0.53*	2.38±0.41*	1.17±0.18
10-6M PTH	4.96±1.44*	2.66±0.85*	5.34±1.44*	1.60±0.19*	2.12±0.24*	1.00±0.16

IN VITRO STUDY	DKK1 (R.U.)	sFRP1 (R.U.)	sFRP2 (R.U.)	sFRP4 (R.U.)
Vehicle	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
10-8M PTH	1.10±0.15	1.52±0.54	1.56±0.43	1.93±0.37
10-7M PTH	0.99±0.2	1.97±0.51*	5.90±1.68*	4.43±0.55*
10-6M PTH	1.07±0.36	2.05±0.36*	11.01±0.86*	6.72±1.11*

In vivo, the PTH increments were associated with a significant increase in the expression of genes involved in bone turnover and Wnt pathway inhibition. The in vitro study partly confirmed the in vivo results, demonstrating for the first time that PTH directly increase sFRPs, suggesting that PTH is involved in the sFRPs and Wnt signaling pathway regulation.

Funding: Government Support - Non-U.S.

FR-OR174

Conditional Knockout of Dicer in the Parathyroid Shows That miRNAs Are Necessary for the Response of the Parathyroid to Short and Long-Term Hypocalcemia Vitali Shilo, Chofit Chai, Justin Silver, Tally Naveh-Many. *Nephrology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.*

Background: MicroRNAs (miRNAs) are short non-coding RNA molecules that affect protein levels by sequence-specific repression of translation and mRNA degradation. The final step in miRNA maturation is mediated by Dicer, a RNase III-like enzyme expressed in all cell types that is essential for life.

Methods: We have disrupted miRNA maturation specifically in the parathyroid using parathyroid specific *dicer* knock-out (PT-*Dicer*$^{-/-}$) mice to study the role of miRNAs in parathyroid physiology and the development of SHPT.

Results: The PT-*Dicer*$^{-/-}$ mice develop normally and are fertile with no marked differences in serum PTH and calcium levels compared to control littermates. However, a short-term decrease in serum Ca^{2+} by EGTA administration at 40 min did not increase serum PTH in the PT-*Dicer*$^{-/-}$ mice but led to the expected 3-fold increase in serum PTH in the control mice. To induce secondary hyperparathyroidism (SHPT) mice were fed a calcium depleted diet for 3 weeks. PT-*Dicer*$^{-/-}$ mice showed only a moderate 2-fold increase in serum PTH compared to the 10-fold increase in control mice. Moreover, chronic hypocalcemia also failed to increase PTH mRNA levels in the PT-*Dicer*$^{-/-}$ mice.

Conclusions: These results show that parathyroid miRNAs are necessary for the response of the parathyroid to both acute and chronic hypocalcemia. The impaired response of the PT-*Dicer*$^{-/-}$ mice to the challenge of hypocalcemia may be due to an effect of miRNAs on calcium sensing, PTH secretion, gene expression and/or parathyroid cell proliferation.

FR-OR175

Transgenic Approach Reveals Strong Protective Effects of Vitamin D Signaling on Podocytes Youli Wang, Dilip K. Deb, Yan Chun Li. *Department of Medicine, University of Chicago, Chicago, IL.*

Background: Podocytes play a key role in maintaining the integrity of the glomerular filtration barrier, and podocyte injury is a major cause for renal dysfunction in diabetic nephropathy. Clinical and animal studies have demonstrated potent anti-proteinuric activity for vitamin D and its analogs, suggesting podocytes as important reno-protective target of vitamin D signaling.

Methods: To test this hypothesis, we targeted Flag-tagged human (h) VDR to podocytes in DBA/2J mice using the 2.5 kb podocin gene promoter. The transgenic (Tg) mice were analyzed using the model of streptozotocin (STZ)-induced diabetic nephropathy.

Results: Podocyte-specific expression of hVDR was confirmed by Western blotting and immunostaining in hVDR-Tg mice. Diabetic hVDR-Tg mice exhibited less albuminuria compared to wild-type (WT) counterparts. While treatment with a low dose vitamin D analog doxercalciferol (Dox, 30 ng/kg bw, i.p. 3x/wk) had little effects on the progression of diabetic nephropathy in WT mice, this treatment almost completely prevented albuminuria and markedly reduced glomerular fibrosis in diabetic hVDR-Tg mice. WT1 and synaptopodin staining demonstrated decreased podocyte injury, and TUNEL assays showed attenuation of podocyte apoptosis in the hVDR-Tg mice. Dox treatment also prevented the elevation of renal renin and fibronectin and preserved the expression of nephrin in the hVDR-Tg mice. Moreover, when the hVDR transgene was bred into VDR-null mice to generated VDR knockout (KO) mice that express hVDR only in podocytes, the hVDR transgene was able to partially rescue the severe renal damages seen in VDR KO mice in the STZ-diabetic model, manifested by marked reduction in albuminuria and podocyte loss. In podocyte culture exposure to high glucose (30 mM) induced apoptosis with up-regulation of p53, Bad, Bak and p-Erk, and vitamin D treatment blocked this pro-apoptotic pathway and podocyte apoptosis.

Conclusions: Taken together, these data provide strong evidence that vitamin D signaling plays a critical role in the protection of podocytes from diabetic injury.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-OR176

Cardiac Remodeling and Vitamin D Status in Chronic Kidney Disease: A CRIC Study Bonnie Ky,[2] Justine Shults,[2] Martin Keane,[2] Myles S. Wolf,[3] Harold I. Feldman,[2] Mary B. Leonard.[1] *[1]Childrens Hospital of Philadelphia; [2]University of Pennsylvania; [3]University of Miami.*

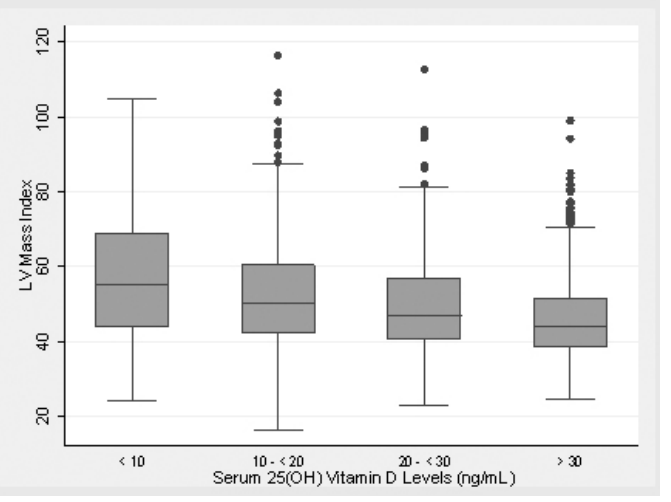
Background: Animal studies link abnormal vitamin D metabolism and left ventricular (LV) hypertrophy. The objective was to determine associations between vitamin D levels and LV remodeling in CKD.

Methods: We evaluated 1283 CRIC study participants with vitamin D levels and echocardiography at Year 1 [52% male, 53% Caucasian, 40% African American; median (interquartile range, IQR) age 62 (54-67) yrs, and eGFR 45 (35-55) ml/min/1.73m^2]. Serum 25(OH)D and 1,25$(OH)_2$D were measured by tandem mass-spectrometry and I^{125} RIA, respectively. LV mass index (LVMI) was calculated by the area-length method and volumes by Simpson's method. Linear and logistic regression analyses were used to determine the relations between 25(OH)D and 1,25$(OH)_2$D and echo. LV hypertrophy (LVH) was defined as LVMI >50 in males and >47g/m$^{2.7}$ in females.

Results: The median (IQR) 25(OH)D levels were 32 (23-41) in Caucasians and 18 (11-27) ng/ml in African Americans. The median LVMI was 48.4 in males and 46.1g/m$^{2.7}$ in females. LVH was present in 46% of participants. Lower 25(OH)D levels were significantly associated with greater LVMI in univariate models ($p<0.001$). This remained significant ($p = 0.04$) in multivariable models adjusted for age, sex, race, BMI, eGFR, cardiac disease history, smoking, diabetes, and anti-hypertensive therapy. Compared to a 25(OH)D level ≥20 ng/mL, a level <20 ng/mL was associated with an odds ratio of LVH of 1.36 (95% CI 1.01, 1.84, $p<0.05$) in adjusted models. Lower 1,25$(OH)_2$D levels were associated with greater LVMI in both unadjusted ($p<0.001$) and adjusted analyses ($p=0.03$). Vitamin D levels were not associated with volumes.

Conclusions: These data demonstrate a significant association between lower 25(OH)D and 1,25$(OH)_2$D levels and higher LVMI in CKD.

Figure: LVMI According to 25(OH)D Levels



Funding: NIDDK Support

FR-OR177

Calcitriol Promotes Vascular Calcification in Renal Failure through Systemic Rather Than Local Activation of Vitamin D Receptors Koba A. Lomashvili, Faten Hasounah, W. Charles O'Neill. *Renal Division, Emory University, Atlanta, GA.*

Background: Calcitriol promotes vascular calcification in experimental renal failure but it is not known whether this is due to local or systemic activation of vitamin D receptors. This was examined by transplanting aortas from mice lacking the vitamin D receptor (VDR-/-) into wild-type mice.

Methods: Sections of abdominal aorta (5-8 mm) were transplanted from 4 month old VDR-/- mice into 5 wild type (WT) littermates using end-to-end anastomosis. A second group of 8 wild-type animals received abdominal aortic grafts from wild-type littermates and was used as a control. Uremia was induced by feeding powdered chow with 2% phosphorus and 0.6 % adenine, and calcitriol (1 microgram/kg) was given by subcutaneous injection three times per week. After two months, aortas were removed and the calcium contents of the allograft and adjacent native aorta were compared (excluding the suture lines).

Results: Plasma calcium, phosphorus, and urea were 1.9 ± 0.1mM, 2.8 ± 0.3mM, and 68.7 ± 9.1 mg/dl respectively, with no difference between the two groups. Staining of calcium with alizarin red showed no difference between transplanted aortas and native aortas. The ratio of the calcium content in transplanted aortas to that in adjacent native aortas was 2.1 ± 0.74 (mean ± SE) for VDR-/- transplants and 1.5 ± 0.58 for the WT transplants, with no significant difference between the two groups.

Conclusions: Vascular calcification was the same in VDR-/- and wild-type aortas. This indicates that calcitriol promotes vascular calcification through stimulation of systemic rather than local vitamin D receptors.

Funding: NIDDK Support

FR-OR178

Mutations in CYP24A1 Encoding Vitamin D-24-hydroxylase Cause Idiopathic Infantile Hypercalcemia Karl P. Schlingmann,[1] Martin Kaufmann,[2] Stefanie Weber,[3] Guenter Klaus,[4] Henry Fehrenbach,[5] Ulrike John,[6] Joachim Misselwitz,[6] Tulay Guran,[7] Joost G. Hoenderop,[8] Rene J. Bindels,[8] David E. Prosser,[2] Glenville Jones,[2] Martin Konrad.[1] *[1]Pediatrics, Wilhelms University, Münster, Germany; [2]Biochemistry, Queen's University, Kingston, Canada; [3]Pediatrics, University of Duisburg-Essen, Essen, Netherlands; [4]KfH Pediatric Kidney Center, Marburg, Germany; [5]Children's Hospital, Memmingen, Germany; [6]Pediatrics, Schiller University, Jena, Germany; [7]Pediatrics, Marmara University, Istanbul, Turkey; [8]Physiology, Radboud University, Nijmegen, Netherlands.*

Background: Vitamin D supplementation during infancy to prevent rickets is one of the most effective prophylactic measures in medicine. Though the margin between prophylactic and toxic doses is broad, an increased incidence of Idiopathic Infantile Hypercalcemia (IIH) was observed in the 1950s during a period of high vitamin D content in fortified milk products in Great Britain. IIH is characterized by severe hypercalcemia, vomiting, dehydration, and nephrocalcinosis. Laboratory evaluation reveals a suppressed PTH and high normal 1,25(OH)2-VitD3 levels.

Methods: In a cohort of familial cases with IIH and suspected autosomal recessive inheritance, we performed an extended candidate gene approach to identify the causative genetic defect.

Results: We detected homozygous or compound-heterozygous mutations in CYP24A1 encoding Vitamin D-24-hydroxylase responsible for the inactivation of 1,25(OH)2-VitD3. CYP24A1 mutations were not only identified in IIH patients given regular 500 IU VitD daily, but also in a second cohort of patients from German Democratic Republic who presented during infancy with suspected VitD intoxication after receiving a bolus prophylaxis of 600.000 IU VitD2 in the late 1980s. The functional analysis of mutant CYP24A1 in a mammalian overexpression system revealed a lack of 24-hydroxylated VitD metabolites after incubation with radioactively labeled 1,25(OH)2-VitD3 indicating a complete loss-of-function.

Conclusions: In conclusion, we not only highlight the role of CYP24A1 mutations as causative for IIH but identify a genetic risk factor for the development of a serious adverse effect of generally advocated VitD prophylaxis.

FR-OR179

Risks of ESRD and Death Following Coronary Revascularization in Individuals with CKD

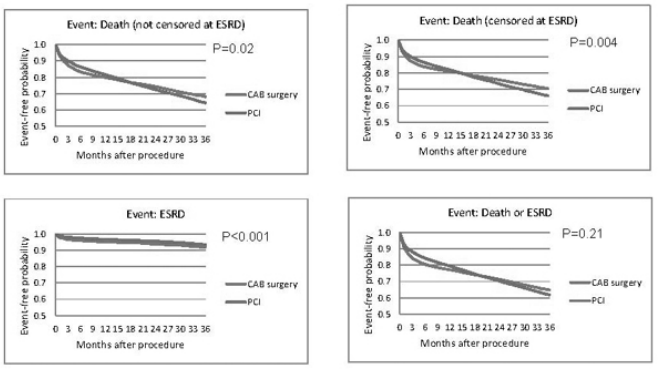
David M. Charytan,[1] Shuling Li,[2] Charles A. Herzog.[2] *[1]Medicine, Brigham and Women's Hospital, Boston, MA; [2]CVSSC, USRDS, Minneapolis, MN.*

Background: Surgical (CABG) and percutaneous (PCI) intervention revascularization are frequently deferred in individuals with CKD out of concern that they will precipitate ESRD, but reliable estimates of the absolute and relative risks of death and ESRD following PCI and CABG are unavailable.

Methods: Individuals with CKD undergoing PCI (8,620) or CABG (4,547) were identified using the 5% Medicare sample. Long-term outcomes were tracked, and ESRD was identified via linkage to the USRDS. Multivariable Cox regression was used to asses the relative hazards (HR) of death and ESRD.

Results: PCI patients were older, less likely to have heart failure, arrhythmias, anemia, COPD, diabetes, or cerebrovascular disease, and more likely to have a myocardial infarction (P<0.001 for all comparisons). The overall HR for death was lower with CABG than PCI (HR 0.89, P<0.001). Early mortality was higher after CABG, but the HR increasingly favored CABG over time [figure 1]. Results were similar when death was censored for ESRD. Conversely, the risk of ESRD was higher throughout follow-up following CABG (HR 1.18, P=0.04). The absolute incidence of ESRD at 3 years was markedly lower (PCI-3.0%, CABG-3.6%) than the incidence of death (PCI-20.1%, CABG-15.6%). As result, the adjusted HR of the combined outcome of ESRD or death was higher during the first 3 months (HR 1.30, P<0.01) and lower from 6-36 months (HR 0.66, P<0.001.

Conclusions: Among individuals with CKD undergoing coronary revascularization, the incidence of death is much higher than ESRD. The risk of ESRD is lower throughout follow-up with PCI, but the long-term risk of death or combined death and ESRD is lower with CABG. Our data suggest that overall clinical outcomes are better with CABG than PCI in patients with CKD.



FR-OR180

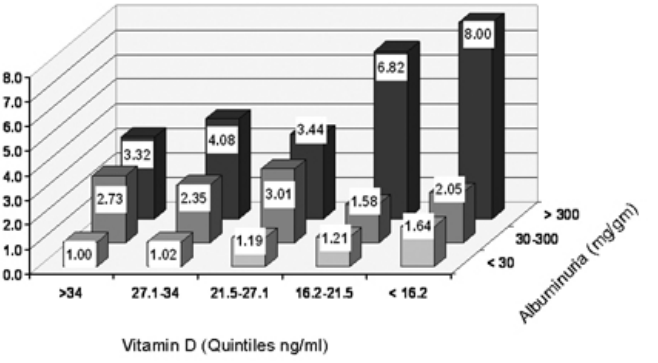
The Impact of Low Vitamin D Levels on Mortality Is Influenced by Urinary Albumin Excretion Rates in Chronic Kidney Disease

Austin G. Stack,[1, 2] Arif I.F. Mutwali,[1] Rajiv Saran.[2] *[1]Regional Kidney Centre, Letterkenny General Hospital, Letterkenny, Donegal, Ireland; [2]Kidney Epidemiology & Cost Center, University of Michigan, Ann Arbor, MI.*

Background: Reduced 25-(OH)-Vitamin D (Vit D) levels and the presence of albuminuria are independent predictors of mortality in chronic kidney disease. The combined impact of these two potentially modifiable risk factors on mortality has to our knowledge not been explored in a population-based cohort.

Methods: We tested this hypothesis in cohort of 13,455 subjects age >= 20 without diabetes or pre-diabetes, and representative of the U.S. population from the Third National Health and Nutrition Examination Survey (1988-1994). Vital status through to 2006 was obtained from linkage with the National Death Index. The interaction of Vit D and albumin/creatinine ratio with mortality was tested. Vit D levels in quintiles [Q1< 16.2, Q2 16.2-21.5, Q3 21.5-27.1, Q4 27.1-34, Q5 > 34 ng/ml] and urinary albumin/creatinine ratio categorized as < 30, 30-300 and > 300 mg/gm was modeled with all-cause and cardiovascular mortality using weighted Cox regression with Q5 Vit D and albumin < 30 as a single referent. Adjustment was made for demographic factors, cardiovascular conditions, hypertension, body mass index, physical inactivity, smoking, CRP, and eGFR

Results: The p-value for interaction was 0.02. The relative mortality risks were significantly greater for subjects in the lowest quintile of Vit D, and for subjects with the highest albumin excretion rates. Subjects who fell into both categories experienced the greatest mortality risks. The patterns of association were similar for CV mortality (data not shown).



Conclusions: Vit D deficiency and albuminuria were synergistically associated with higher mortality risk. Combined strategies to correct these related abnormalities could therefore yield greater benefit than therapy focused on either alone.

FR-OR181

Mortality in Individuals with Acute Coronary Syndrome and CKD: An Analysis of the Myocardial Infarct National Audit Project (MINAP) Database

Catriona Shaw,[1] Sapna Shah,[1] Cornelia Junghans,[1] Donal O'Donoghue,[2] Claire C. Sharpe.[1] *[1]Renal Medicine, King's College London, London, United Kingdom; [2]Salford Royal Foundation Trust, United Kingdom.*

Background: CKD is a recognised risk factor for cardiovascular mortality. Our aim was to assess the mortality risk following acute coronary syndrome (ACS) across all stages of CKD using the UK's MINAP database.

Methods: Data from January 2007 to April 2011 on 94,165 individuals with NSTEMI and 54,824 individuals with STEMI were included in multivariate Cox regression models to investigate the association of eGFR and intervention (CABG/PCI) with mortality and in multivariate logistic regression models to study the association between intervention and eGFR.

Results: eGFR is strongly associated with mortality after adjustment for confounders following ACS (p<0.0001). After NSTEMI, an increase in hazard ratio (HR) for mortality from 1.23 (1.11,1.36) to 3.38 (2.97,3.84) was demonstrated in those that did not receive an intervention as eGFR declined from CKD3a to CKD5 compared to those with CKD1. In those who received an intervention, an increase in HR from 1.68 (1.29, 2.18) to 7.38 (4.50,11.03) was seen across the same eGFR categories.

After STEMI, an increase in HR for mortality from 1.17 (0.99,1.38) to 4.01 (3.21,5.02) in the non-intervention group and an increase in HR from 1.22 (0.99,1.49) to 4.87 (3.24,7.30) in the intervention group was demonstrated as eGFR declined.

Intervention in all stages of CKD demonstrated a survival benefit compared with no intervention (p<0.001). After an NSTEMI, the odds ratio (OR) of receiving an intervention decreased from 0.87 (0.82,0.94) to 0.34 (0.28,0.42) as eGFR declined from CKD3a to CKD5 and from 0.66 (0.61,0.72) to 0.26 (0.20,0.34) in the STEMI population.

Conclusions: eGFR is strongly associated with increased mortality after ACS. The greater increment in risk in individuals with NSTEMI who underwent intervention, likely reflects the increased severity of their cardiac disease. In all stages of CKD, intervention demonstrated a survival benefit. However individuals with increasingly severe renal dysfunction were less likely to receive an intervention compared to those with normal kidney function.

FR-OR182

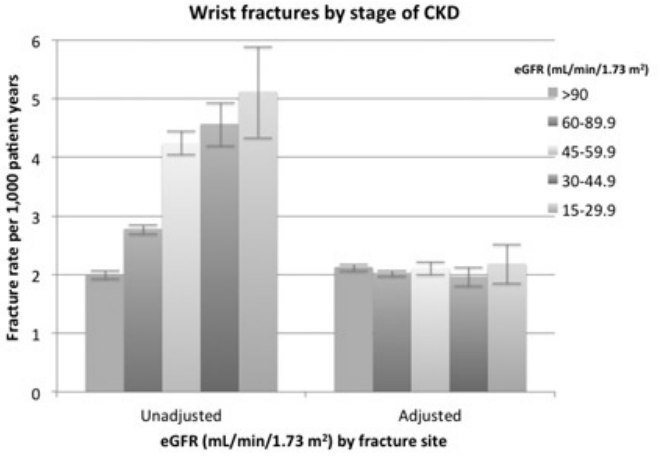
Fracture Risk Is Not Increased in Patients with Chronic Kidney Disease

Meghan J. Elliott,[1] Marcello Tonelli,[2] Braden J. Manns,[1] Gregory Kline,[1] Matthew T. James,[1] Andrea Soo,[1] Brenda Hemmelgarn.[1] *[1]University of Calgary, Calgary, AB, Canada; [2]University of Alberta, Edmonton, AB, Canada.*

Background: In patients with end-stage kidney disease, fractures occur four-times more frequently than in the general population. The incidence of fractures in those with chronic kidney disease (CKD) not receiving renal replacement treatment has not been well-studied.

Methods: We did a population-based longitudinal study with participants aged ≥18 years identified from a province-wide laboratory database from Alberta, Canada between 2002 and 2008. 1,815,957 patients had at least one outpatient serum creatinine measurement and did not require renal replacement therapy at baseline. The eGFR was estimated using the CKD-EPI equation, and categorized as ≥90, 60-89, 45-59, 30-44, 15-29 mL/min/1.73 m². The cohort was linked to administrative data to define demographics, comorbidities and outcomes. Outcomes of non-traumatic incident hip, wrist or clinical vertebral fractures were identified during the follow-up period to March 31, 2009. Poisson regression was used to determine unadjusted and adjusted rates of each fracture outcome by level of eGFR.

Results: The mean (SD) age of patients was 47.7 (17.2) years, and 7.1% had eGFR <60 mL/min/1.73 m². Unadjusted rates of wrist fractures increased at lower levels of eGFR, however, after adjusting for age, sex, diabetes and hypertension, the wrist fracture rate was similar across all eGFR levels. Similar trends in the unadjusted and adjusted results were observed for hip and clinical vertebral fractures.



Conclusions: Lower levels of kidney function are associated with an increased risk of non-traumatic fractures, an association that is primarily related to confounding by age. Future studies of fracture risk should include adjustment for potential confounders, and in particular patient age.

FR-OR183

Effect of IL-1β Inhibition with Canakinumab Compared to Triamcinolone Acetonide on Pain Intensity and New Flares in Gouty Arthritis Patients with Chronic Kidney Disease Stage 2-5 P. Sunkureddi,[1] A. So,[2] T. Bardin,[3] R. Alten,[4] M. Bloch,[5] T. Kiechle,[6] G. Krammer,[6] A. Shpilsky,[7] N. Schlesinger.[8] *[1]Clear Lake Rheumatology Center; [2]University of Lausanne, Switzerland; [3]Hôpital Lariboisière, France; [4]Charité Teaching Hospital, Germany; [5]Holdsworth House General Practice, Australia; [6]Novartis Pharma AG, Switzerland; [7]Novartis Pharmaceuticals Corporation; [8]UMDNJ-RWJMS.*

Background: Many patients with gouty arthritis (GA) have pre-existing comorbidities. Chronic kidney disease (CKD) is one of the most common comorbidities and significantly limits treatment options with standard therapy. Canakinumab, a fully human monoclonal anti-IL-1β antibody, is a potential new therapy for treating GA pain and delaying new flares. We present 24-week data for a subgroup of patients with renal insufficiency.

Methods: In 2 multicenter, double-blind, active controlled studies (β-RELIEVED, N=228; β-RELIEVED-II, N=226), patients, ≥18-≤85 yrs meeting ACR 1977 criteria for acute GA and contraindicated, intolerant or unresponsive to NSAIDs and/or colchicine received canakinumab 150 mg sc or triamcinolone acetonide (TA) 40 mg im. The co-primary endpoints were pain intensity at 72h post dose on a 0-100 mm visual analog scale (VAS) in the most affected joint and time to first new GA flare.

Results: A total of 380 (83.7%) patients had renal impairment (baseline eGFR <90mL/min/1.73m²; corresponding to CKD stages 2-5): 188 (83.6%) canakinumab group and 192 (83.8%) TA group. Mean VAS scores for canakinumab and TA were 73.9mm vs 73.8mm at baseline and 24.4 mm vs 35.3 mm at 72h (Diff: -10.9 mm; 95% CI: -16.1, -5.8, p<0.0001), respectively. The significant difference was maintained up to 7 days. At 24 weeks, significantly fewer canakinumab patients experienced new flares vs TA (25.5% vs 47.4%, OR 0.38, 95% CI 0.25-0.59, p<0.0001). 66.5% of patients had adverse events (AEs) with canakinumab vs 52.6% with TA. Serious AEs (canakinumab: n=15, 8%; TA: n=6, 3.1%) were not considered to be related to treatment by the investigator.

Conclusions: In GA patients with CKD stage 2-5 canakinumab was superior to the TA in reducing pain and the risk of new flares.

FR-OR184

Coronary Artery Calcification and Risk of Cardiovascular Disease among Patients with Chronic Kidney Disease: A Prospective Analysis from the CRIC Study Jing Chen, Matthew Jay Budoff, Muredach Reilly, Wei Yang, Sylvia E. Rosas, Jackson T. Wright, Mahboob Rahman, Eva Lustigova, Lisa C. Nessel, Xiaoming Zhang, Virginia Ford, Dominic S. Raj, Anna C. Porter, Elsayed Z. Soliman, Myles S. Wolf, Jiang He. *Tulane University.*

Background: Vascular calcification is associated with increased cardiovascular disease (CVD) risk and mortality in dialysis patients. However, the predictive value of coronary artery calcification (CAC) on clinical CVD has not been well studied in chronic kidney disease (CKD) patients.

Methods: We examined the prospective association of CAC and risk of CVD events among 1,704 participants who had electron beam computed tomography (EBCT)/ multidetector CT measures in the Chronic Renal Insufficiency Cohort (CRIC) study. Patients aged 21 to 74 years old with an estimated glomerular filtration rate (eGFR) of 20-70 mL/ min/1.73 m² were recruited from 7 clinical centers in the US. CAC was classified as none (0), moderate (>0-100) or severe (>100) according to Agatston scores.

Results: Compared to those without CAC, the patients with moderate and severe CAC had higher event rates (1000 person-years) for myocardial infarction (MI): 1.3, 3.9 and 13.2; congestive heart failure (CHF): 7.8, 13.5 and 30; total CVD: 11.1, 25.8, and 50.2; and all-cause mortality: 3.2, 8.6, and 18.0. After adjusting for age, gender, race, and clinical sites, the hazard ratio (95% confidence interval) associated with moderate and severe CAC was 3.1 (0.6-16.5) and 11.3 (2.4-52.5) for MI; 1.8 (0.8-3.8) and 4.4 (2.2-8.9) for CHF; 2.3 (1.2-4.1) and 4.5 (2.5- 8.1) for total CVD; and 2.2 (0.7- 6.5) and 4.1 (1.5-11.6) for all-cause mortality. After further adjustment for established CVD risk factors including eGFR and history of CVD, severe CAC was only significantly related to MI with a hazard ratio of 6.0 (1.2 -29.5).

Conclusions: These data indicate that CAC provides additional predictive value for the risk of MI in CKD patients.

Funding: NIDDK Support

FR-OR185

A Multi-Centre, Double-Blind, Randomised, Placebo-Controlled, Multiple Fixed-Dose Study of Colestilan (MCI-196) Versus Placebo in Chronic Kidney Disease Stage 5 Subjects on Dialysis (CKD 5D) with Hyperphosphatemia and Dyslipidemia (DL): Efficacy Results Francesco Locatelli,[1] Nada Dimkovic,[2] Goce Spasovski.[3] *[1]Nephrology, Ospedale Manzoni, Lecco, Italy; [2]Zvezdara University Hospital, Belgrade, Serbia; [3]University of Skopje, Macedonia, The Former Yugoslav Republic of.*

Background: Hyperphosphatemia in CKD 5D is associated with increased mortality. Colestilan (COL) is a new non-metallic, non-calcium anionic resin that binds both phosphate (P) and bile acids.

Methods: In this global, 642 patient (100 site) study, after washout of lipid-lowering medication (8 weeks) and P binders (1-4 weeks) subjects received fixed doses of COL (3, 6, 9, 12 or 15 g/day) for 12 weeks or 9, 12 or 15 tablets/day placebo. 99-104 pts were randomised to each dose COL and 132 total to placebo. Primary objectives were to demonstrate efficacy of COL compared to placebo in control of both serum P and serum LDL cholesterol (LDL-C) in subjects with CKD 5D. Secondary objectives were to study the efficacy of COL in control of other lipid parameters, iPTH, serum calcium and safety.

Results: The overall withdrawal rate was 36.1%. After 12 weeks treatment or Last Observation Carried Forward, serum P was reduced from baseline by 0.18 (pooled placebo) and 0.28, 0.72, 0.87 and 1.06 mg/dl in the 3, 6, 9 and the pooled 12+15g/day groups respectively, p<0.05 versus placebo for 6, 9 and 12+15g groups. LDL-cholesterol was raised 4.1% by pooled placebo and reduced by 15.9, 23.6, 27.6 and 27.6% in 3, 6, 9 and pooled 12+ 15g/day groups respectively (p<0.001 versus placebo in all cases). Similarly oxidised LDL-C was significantly reduced in all treated groups. HDL-C, iPTH and serum calcium were not significantly altered. COL produced limited reduction in overall HbA1c but in patients with high baseline HbA1c values (>7%), HbA1c was substantially reduced by up to 1.2%. Uric acid was also reduced significantly by up to 40.9 mcmol/L.

Conclusions: This study suggests that COL is effective as a combined treatment for reducing P and LDL-C in CKD 5D. Significant reductions in high HbA1c and uric acid could offer additional benefits. The safety profile suggested COL to be safe and well-tolerated.

Funding: Pharmaceutical Company Support

FR-OR186

AKB-6548, a Novel Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor Reduces Hepcidin and Ferritin While It Increases Reticulocyte Production and Total Iron Binding Capacity in Healthy Adults Robert Shalwitz,[1] Charlotte Hartman,[1] Cindy Flinn,[1] Isaiah Shalwitz,[1] Douglas K. Logan.[2] *[1]Akebia Therapeutics, Inc., Cincinnati, OH; [2]Medpace, Inc., Cincinnati, OH.*

Background: Current treatment of anemia associated with chronic kidney disease with erythropoiesis-stimulating agents can lead to unusually sustained, high levels of circulating erythropoietin (EPO). Therefore, a drug that more closely mimics physiologic conditions by replicating more intermittent daily rises in EPO may be a very effective and well-tolerated treatment. AKB-6548, a new short-acting hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitor, was selected to induce daily rises in EPO at a level that closely simulates physiologic responses changes in altitude. This physiologically similar mechanism, which is characterized by stabilization of HIF, is hypothesized to also improve iron mobilization.

Methods: In a double-blind, placebo-controlled Phase 1b study, 33 healthy males were randomized to three treatment groups of 500, 700, or 900mg AKB-6548. In each dosing group, 8 volunteers received AKB-6548 and 3 received placebo once daily for 10 days. Pharmacodynamic response was evaluated on Day 11.

Results: Dosing was generally well tolerated, and the results show a dose-related increase in maximum EPO, reticulocytes, and total iron binding capacity (TIBC), as well as dose-related reductions in hepcidin and ferritin.

Conclusions: From this study, we conclude that AKB-6548 enhances erythropoiesis by inducing controlled increases in EPO production in concert with enhanced iron mobilization and could represent a safer and more effective approach to treating anemia than currently available agents.

	Dosing Groups			
	Placebo	**500 mg**	**700 mg**	**900 mg**
Average, Max EPO (mIU/mL)	15.50±2.87	18.01±5.15 p=0.8951	28.03±9.20 p=0.0207	32.44±15.29 p=0.0023
Abs Reticulocyte on Day 11 (thousands/μL)	61.59±18.31	84.41±7.91 p=0.1009	96.85±16.23 p=0.0067	110.21±37.15 p=0.0004
Mean TIBC % Change from BL	1.78±2.80	9.17±3.99 p=0.0176	8.94±5.37 p=0.0219	15.72±7.87 p=0.0001
Mean Hepcidin % Change from BL	22.12±124.49	-31.57±30.40 p=0.3215	-50.81±45.86 p=0.1226	-59.94±23.32 p=0.0862
Mean Ferritin % Change from BL	-7.16±12.55	-2.49±21.87 p=0.9381	-20.21±29.95 p=0.4338	-36.00±11.36 p=0.0246

BL:Baseline

Funding: Pharmaceutical Company Support

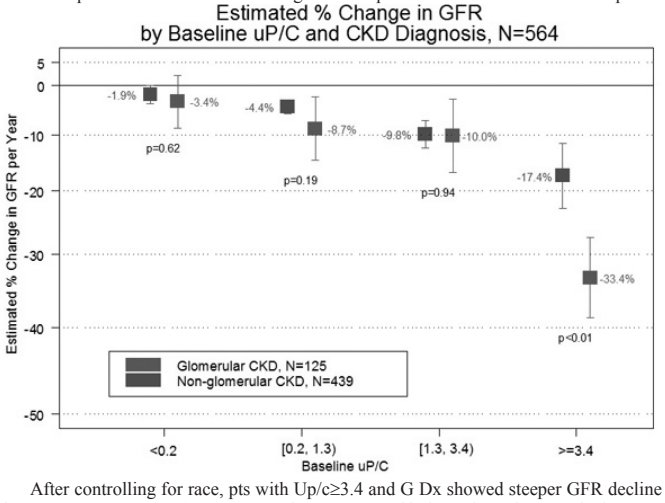
FR-OR187

Proteinuria Predicts a Decline in Glomerular Filtration Rate Particularly in African-Americans and Glomerular Chronic Kidney Disease Sahar A. Fathallah-Shaykh,[1] Christopher B. Pierce,[2] Tom D. Blydt-Hansen,[2] Alison G. Abraham,[2] Susan F. Massengill,[2] Bradley A. Warady,[2] Susan L. Furth,[2] Craig S. Wong.[2] *[1]Pediatrics, University of Alabama at Birmingham, AL; [2]CKiD Study.*

Background: Proteinuria is associated with progressive kidney disease. CKiD cohort cross-sectional data revealed a log-linear relationship between proteinuria and glomerular filtration rate (GFR). Here we evaluate a relationship between urine protein-to-creatinine ratio (Up/c) level and prospective changes in GFR

Methods: GFR was measured in the first two visits and then annually by Iohexol blood-disappearance or estimated using the CKiD equation; proteinuria was assayed by first morning baseline Up/c. We used a linear mixed model with random intercept and slope to account for individual variability in baseline GFR and % decline.

Results: 564 CKiD subjects with median age 11 yrs, GFR 44 ml/min/1.73 m^2, and baseline Up/c 0.47 were studied: 62% males, 24% African Americans (AA), 22% glomerular (G) cause CKD, 54% on ACE/ARB. For both non-G and G CKD, GFR % decline increased with rising baseline Up/c; linearly in non-G pts and biphasic in G CKD pts. Those with baseline Up/c≥ 3.4 had GFR decline larger than expected under a linear relationship.



After controlling for race, pts with Up/c≥3.4 and G Dx showed steeper GFR decline than non-G CKD (p<0.01). As compared to non-AA and independent of Dx, AA had steeper decline in GFR beginning at Up/c = 0.2; this disparity increased with higher levels of Up/c. Baseline use of ACE/ARB did not affect GFR.

Conclusions: Up/c level predicts magnitude of future decline in GFR. In pts with G CKD, baseline Up/c is associated with a biphasic rate of decline in GFR, which is accelerated when Up/c≥ 3.4. The effects of Up/c are more pronounced in AA.

Funding: NIDDK Support

FR-OR188

Determinants of Progression of Microalbuminuria in Non-Diabetic Persons from the General Population Marit D. Solbu,[1] Bjorn Odvar Eriksen,[1,2] Trond G. Jenssen.[2] *[1]University Hospital of North Norway, Tromsø, Norway; [2]University of Tromsø, Norway.*

Background: Low-grade albuminuria is a risk factor for chronic kidney disease and cardiovascular disease. Knowledge about predictors and consequences of change in albuminuria over time in non-diabetic populations is incomplete. Our aim was to assess predictors for increase in urinary albumin-creatinine ratio (ACR) in a non-diabetic population.

Methods: From the population based Tromsø study, non-diabetic persons with morning ACR in the upper tertile at least two out of three consecutive days at baseline (1994/1995), and who were attending follow-up (2006/2007), were divided into two groups; upper tertile ("increasers") vs. the two lower tertiles ("non-increasers") of ACR change. Logistic regression was used to assess predictors of being an "increaser".

Results: Among the 6528 non-diabetic baseline participants, 2001 had ACR in the upper tertile in at least two specimens. Between baseline and follow-up, 715 participants died. At follow-up 662 persons participated and were divided into 221 "increasers" (ACR change ≥0.45 mg/mmol) and 441 "non-increasers" (ACR change <0.45 mg/mmol). Baseline predictors of being an "increaser", were age (OR per 5 years 1.26; 95% CI 1.10 – 1.46; P=0.001), gender (OR for men 1.92; 95% CI 1.23 – 2.99; P=0.004), waist circumference (OR per 5 cm 1.12; 95% CI 1.01 – 1.24; P=0.03) and cystatin C (OR per 0.1 mg/L 1.15; 95% CI 1.04 – 1.28; P=0.006). Increase in systolic blood pressure (OR per 10 mm Hg 1.17; 95% CI 1.08 – 1.27; P<0.001), increase in HbA1c (OR per % 1.38; 95% CI 1.04 – 1.84), and increase in cystatin C (OR per 0.1 mg/L 1.16; 1.07 – 1.26; P<0.001) also were significant predictors. Smoking cessation, initiation of antihypertensive medication or physical activity were not significant predictors.

Conclusions: In non-diabetic persons with elevated ACR, gender, age, obesity, increasing glucose and blood pressure predicted further increase in ACR. However, most of the increase was not explained by cardiovascular risk factors. Increasing albuminuria seemed to parallel changes in renal function more closely than changes in cardiovascular risk factors.

Funding: Government Support - Non-U.S.

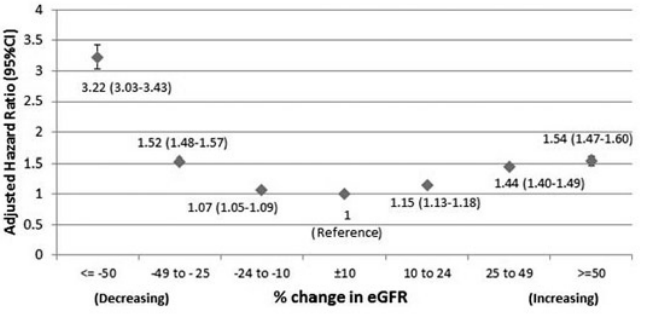
FR-OR189

Association between Change in eGFR and Mortality Tanvir Chowdhury Turin,[1] Josef Coresh,[2] Marcello Tonelli,[3] Paul E. Stevens,[4] Brenda Hemmelgarn.[1] *[1]University of Calgary, Calgary, AB, Canada; [2]Johns Hopkins University, Baltimore, MD; [3]University of Alberta, Edmonton, AB, Canada; [4]East Kent Hospitals University Foundation NHS Trust, Canterbury, Kent, United Kingdom.*

Background: The effect of change in kidney function, in particular the degree of change, and risk of death is not well understood. We aimed to investigate the association between change in eGFR over a one year period and risk of mortality in a community based population.

Methods: We included 843,417 adults who had at least 2 outpatient eGFR measurements in a one-year period, separated by at least two weeks, from a laboratory registry from Alberta, Canada. The percent change in eGFR was calculated using the first and last eGFR within the period. Change in eGFR was categorized as: ≤ -50%; -49 to -25%; -24% to -10%; ±10%; 10 to 24%; 25 to 49%; and ≥50% to reflect both decline and increase in eGFR. Cox hazard models, adjusting for baseline covariates, kidney function and proteinuria, were used to estimate the HR of all-cause mortality (follow-up to March 31 2009) associated with categories of percent change in eGFR. Stable eGFR (change ±10%) was the reference.

Results: Among the participants (mean age 55.6 years, 42% male), 61.2% had stable, 19.9% had an increase and 18.9% had a decline in eGFR. Compared to participants with stable eGFR, those with the largest decline in eGFR (change ≤-50%) had a greater than three-fold increased risk of death.



Participants with moderate declines also exhibited a higher mortality risk, as did participants with the greatest increase in eGFR

Conclusions: Our results demonstrate an independent and graded association between change in kidney function and mortality, with both a decline and increase in kidney function associated with increased risk. These results suggest that overall variation in eGFR over time, rather than decline alone, may be an important prognostic marker.

FR-OR196

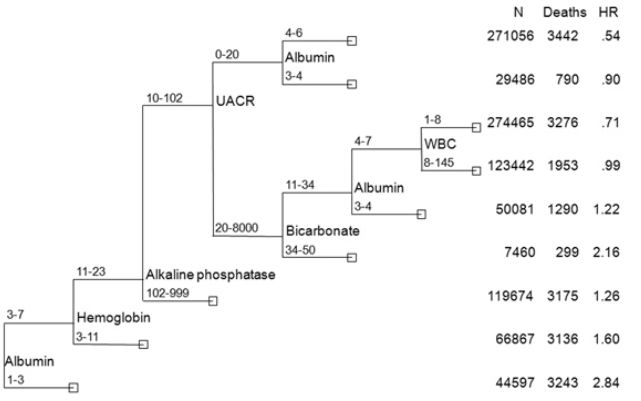
Comparative Effectiveness of Modifiable Risk Factors To Predict Mortality in a Large Cohort of US Veterans with Non-Dialysis Dependent CKD Csaba P. Kovesdy,[1,2] Evan H. Lott,[3] Jun Ling Lu,[4] Sandra M. Malakauskas,[1,2] Jennie Z. Ma,[2] Mark D. Okusa,[2] Kamyar Kalantar-Zadeh.[5] *[1]Salem VA Medical Center; [2]University of Virginia; [3]VA Informatics and Computing Infrastructure; [4]Salem Research Institute; [5]Harbor-UCLA.*

Background: CKD results in the development of multiple metabolic disorders which individually are associated with increased mortality. The head-to-head relative importance of these abnormalities remains unclear.

Methods: We determined the hierarchy of multiple modifiable risk factors (blood pressure, urine albumin/creatinine ratio (UACR), serum albumin, bicarbonate, calcium, alkaline phosphatase, hemoglobin, white blood cell count (WBC) and cholesterol) in predicting all-cause mortality in a nationally representative cohort of 657,614 US veterans with non-dialysis dependent CKD stage 1-5 in 2005-06. The comparative effectiveness of the modifiable risk factors was examined by constructing classification and regression

trees (CART) from time-dependent Cox models adjusted for non-modifiable risk factors of mortality (age, gender, race, marital and insurance status, geographic location and comorbidities).

Results: Patients were 73.9±9.8 years old, 97% were males and 71% were white. 189,565 patients died over a median follow-up of 4.7 years. Of the modifiable risk factors included in the CART model low serum albumin, low hemoglobin, high alkaline phosphatase, high UACR, high bicarbonate and high WBC predicted mortality in decreasing order of significance (Figure).



Conclusions: In a comparative effectiveness model in CKD patients markers of protein-energy wasting, anemia and increased bone turnover appeared to be the most important predictors of mortality, followed by albuminuria. Since these risk factors are readily modifiable, therapeutic interventions targeting them may hold the largest promise of significant benefit towards lowering mortality in CKD.

Funding: NIDDK Support, Veterans Administration Support

FR-OR191

Comparative Effectiveness of Incident Oral Antidiabetic Drugs on Kidney Function: A National Veterans Cohort Study

Adriana Hung,[1,2] Christianne Roumie,[1,2] Robert Greevy,[2,4] Xulei Liu,[2,4] Carlos Grijalva,[2,3] Harvey J. Murff,[1,2] Talat Alp Ikizler,[1,2] Marie Griffin.[1,2,3] *[1]Medicine, Vanderbilt University, Nashville, TN; [2]Veterans Administration TVHS, Nashville, TN; [3]Department of Preventive Medicine, Vanderbilt University, Nashville, TN; [4]Department of Biostatistics, Vanderbilt University, Nashville, TN.*

Background: Diabetes is a major cause of chronic kidney disease (CKD). Oral antidiabetic drugs (OADs) are the mainstay of therapy for most patients with type 2 diabetes; however, data comparing their effect on kidney function decline are sparse.

Methods: We used national Veterans Administration (VA) databases to assemble a retrospective cohort of 93577 diabetic veterans who filled an incident OAD prescription between 10/1/2001 and 9/30/2008, and had an estimated glomerular filtration rate (eGFR) ≥60 ml/min. Exposure groups were incident users of metformin (n=61104), sulfonylurea (n=30550), or rosiglitazone (n=1923). The primary composite outcome was persistent decline in eGFR from baseline of ≥25% (GFR event) or diagnosis of end-stage renal disease (ESRD). The secondary outcome was an eGFR event, ESRD or death. Sensitivity analyses included 1) using a more stringent definition of eGFR event which required reaching an eGFR<60ml/min in addition to a persistent 25% decline in GFR, 2) controlling for baseline proteinuria, restricted to those with this measurement (n=15065); and 3) not requiring persistent exposure to the initial OAD.

Results: Mean age was 62 (23-97), 78% were whites and 96% were males. Compared with metformin users, sulfonylurea use was associated with an increased risk for both the primary outcome, adjusted hazard ratio (*aHR*) 1.20 (95% confidence interval1.13, 1.28) and the secondary outcome *aHR* 1.20 (95% CI 1.13, 1.28). This association was observed across all planned sensitivity analyses. There was no difference in risk between rosiglitazone and metformin for either the primary *aHR* 0.92 (0.71, 1.18) or the secondary outcome: *aHR* 0.89 (0.69, 1.12).

Conclusions: Compared with metformin, initiation of sulfonylureas is associated with increased the risk of eGFR decline, ESRD or death.

Funding: Other NIH Support - AHRQ

FR-OR192

Association of Kidney Function with Abnormal Cardiac Structure and Function by Echocardiography in the Chronic Renal Insufficiency Cohort (CRIC) Study

Meyeon Park,[1] Chi-Yuan Hsu,[1] Yongmei Li,[2] Rakesh Mishra,[2] Martin Keane,[3] Sylvia E. Rosas,[4] Alan S. Go,[5,6] Michael Shlipak.[6] *[1]Nephrology, University of California, San Francisco, CA; [2]Medicine, SF VA Medical Center, San Francisco, CA; [3]Cardiovascular Division, University of Pennslyvania, School of Medicine, Philadelphia, PA; [4]Renal Division, University of Pennsylvania, School of Medicine, Philadelphia, PA; [5]Kaiser Permanente of Northern California, Oakland, CA; [6]Departments of Epidemiology, Biostatistics and Medicine, University of California, San Francisco, CA.*

Background: Heart failure (HF) is a common consequence of chronic kidney disease (CKD) and portends high risk for mortality. The prevalence of abnormalities in cardiac structure and function preceding clinical HF in a community-based population with CKD is not known.

Methods: We evaluated cross-sectionally the association of kidney function with echocardiographically determined stages of cardiac structural and functional abnormalities among 3496 participants in CRIC without a diagnosis of HF. Kidney function was defined by glomerular filtration rate estimated from cystatin C (eGFRcys) (≥ 60 ml/min/1.73m^2, 45-59, 30-44, or <30).

Results: The prevalence of left ventricular hypertrophy (LVH) was 27%, 39%, 44%, and 54% for eGFRcys categories ≥ 60, 45-59, 30-44, and <30 respectively. In fully adjusted multivariate analyses, subjects with eGFRcys levels of <30 ml/min/1.73m^2 had nearly 3-fold odds of LVH (2.8; 1.9-4.1) relative to subjects with eGFR ≥ 60. This reduction in kidney function was also significantly associated with abnormal LV geometry (2.1; 1.6-2.9) and diastolic dysfunction (1.4; 1.1-1.9), but not systolic dysfunction (1.0, 0.6-1.7); an eGFR of 30-44 was also significantly associated with LVH and abnormal LV geometry compared with eGFR≥60.

Conclusions: In this large CKD cohort, reduced kidney function appears to have substantial associations with abnormal cardiac structure, weaker associations with diastolic dysfunction, and no independent association with systolic dysfunction.

Funding: NIDDK Support

FR-OR193

Chronic Kidney Disease (CKD) Progression and Death among Hispanics and Non-Hispanics in the Chronic Renal Insufficiency Cohort (CRIC) Study

Michael J. Fischer,[1] Ana C. Ricardo,[1] Wei Yang,[3] Claudia M. Lora,[1] Amanda Hyre Anderson,[3] Lydia Bazzano,[3] Magdalena M. Cuevas,[3] Chi-Yuan Hsu,[3] John W. Kusek,[2] Amada Lopez,[3] Akinlolu O. Ojo,[3] Dominic S. Raj,[3] Sylvia E. Rosas,[3] Leigh Rosen,[3] Valerie L. Teal,[3] Kristine Yaffe,[3] James P. Lash.[1] *[1]Jesse Brown VA/U.Illinois; [2]NIH/NIDDK; [3]CRIC Study Group.*

Background: Despite the high prevalence of end-stage kidney disease (ESKD) in Hispanics, little is known about the earlier stages of chronic kidney disease (CKD) in this group. We compared CKD progression and death in Hispanics and non-Hispanics.

Methods: We conducted a prospective longitudinal analysis of CRIC participants. Race/ethnicity was self-reported and classified in three exclusive categories: Hispanic, non-Hispanic White (NHW), and non-Hispanic Black (NHB). Cox proportional hazards models were used to determine the association between race/ethnicity, 50% eGFR loss or incident dialysis/transplantation (renal outcome), and death.

Results: Among 3785 participants, 13% were Hispanics, 43% NHW, and 44% NHB. Over 3 years of follow up, Hispanics had significantly higher rates of the renal outcome compared with both NHW and NHB (p<0.05) but similar death rates.

	Hispanic (H)*	White (NHW)*	Black (NHB)*	Model 1 HR (H v. NHW)	Model 2 HR (H v. NHW)	Model 1 HR (H v. NHB)	Model 2 HR (H v. NHB)
50% eGFR loss or incident dialysis/ transplantation	10.7	2.7	6.2	2.49**	0.94	1.12	0.63**
death	2.3	1.8	2.3	0.84	0.66	0.68	0.60

*rates per 100 person-years, **p<0.05

In regression models adjusted for sex and gender (Model 1), Hispanic ethnicity was associated with a higher risk of the renal outcome compared with NHW and a similar risk compared with NHB. However, in regression models adjusted for demographics, comorbidities, eGFR, and proteinuria (Model 2), Hispanic ethnicity was associated with a similar risk for the renal outcome compared with NHW and a lower risk compared with NHB.

Conclusions: Although Hispanics have higher crude rates of CKD progression, Hispanic ethnicity is independently associated with similar or lower rates of this outcome relative to non-Hispanic race/ethnicity in CRIC. Disparities in renal outcomes for Hispanics appear to be explained by factors other than race/ethnicity.

Funding: NIDDK Support

FR-OR194

Chronic Kidney Disease and End Stage Renal Disease Predict Higher Mortality in Primary Upper Gastrointestinal Bleed Patients

Puneet Sood,[1] Gagan Kumar,[1] Ankit Sakhuja,[1] Edward A. Ross.[2] *[1]Medical College of Wisconsin; [2]University of Florida.*

Background: The outcome from upper GI bleeding (UGIB) in CKD and ESRD patients is difficult to discern from the older literature of small patient numbers prior to advanced endoscopy, proton pump inhibitors or H. pylori treatment. We sought a large national database to quantify the role of CKD and ESRD as independent predictors of mortality for patients hospitalized with a principal diagnosis of primary UGIB.

Methods: We used the Healthcare Cost and Utilization Project's Nationwide Inpatient Sample (NIS) 2007, the largest US all-payer database publicly available: an administrative dataset for approx. 8 million admissions in 1,000 hospitals approximating a 20% stratified sample of all US hospitals. Patients over 18 years, discharged with primary diagnoses of UGIB and CKD or ESRD were identified by ICD-9-CM codes. The outcome variables included frequency and in-hospital mortality in CKD and ESRD patients as compared to non-CKD patients. We also analyzed the effect of other risk factors and co-morbid conditions on outcomes of primary UGIB and all-cause mortality using multiple regression modeling.

Results: In the NIS dataset, out of a total of 398,213 admissions with a diagnosis of primary UGIB, 35,985 were for CKD patients, 14,983 for ESRD patients and the remaining 347,245 for patients without renal problems. Primary UGIB hospitalizations in CKD and ESRD patients were 30% and 84% higher respectively, compared to no-renal disease group, after controlling for potentially confounding covariates and adjusting for interaction between the independent variables (OR 1.30, 95% CI 1.17-1.46, P< 0.001 and OR 1.84, 95% CI 1.61-2.09, P<0.001). The CKD patients had 47% higher adjusted all-cause in-hospital mortality when compared to the no-renal disease group (OR 1.47, 95% CI 1.21-1.78, P< 0.001). The ESRD patients with UGIB three times higher adjusted all cause mortality as compared to the no-renal disease group (OR 3.02, 95% CI 2.23-4.1, P<0.001).

Conclusions: Patients with CKD or ESRD admitted with primary UGIB have up to three times higher all-cause in-hospital mortality. The clinicians need to be exceptionally vigilant in the care of these renal patients.

FR-OR195

A Varying Patient Safety Profile across Racial Groups with Chronic Kidney Disease

Clarissa Jonas Diamantidis,[1] Stephen L. Seliger,[1] Loreen D. Walker,[3] Van Doren Hsu,[3] Jeffrey C. Fink,[1] Min Zhan,[2] Gail B. Rattinger.[3] *[1]Nephrology, University of Maryland School of Medicine, Baltimore, MD; [2]Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD; [3]Pharmaceutical Research Computing, University of Maryland School of Pharmacy, Baltimore, MD.*

Background: Chronic kidney disease (CKD) is a high risk condition for a variety of adverse safety events yet little is known about differential rates of safety events across racial groups with CKD. We sought to examine the incidence of an array of disease-specific adverse safety events in black versus non-black patients with CKD.

Methods: A retrospective observational study of a national VA cohort of veterans with CKD and race/ethnic designation, and at least 1 hospitalization during federal fiscal year 2005 (n= 70,154). Primary measures included hospital discharge coding for Agency for Healthcare Research and Quality Patient Safety Indicators (AHRQ PSI), laboratory records for detection of hyperkalemia and hypoglycemia, and pharmacy records to determine dosing of four selected medications with potential safety hazards in CKD (atenolol, glyburide, allopurinol, digoxin).

Results: The majority of participants had at least one adverse safety event during the study period (57%, n=40,003). Black veterans (16% of study cohort) were more likely than non-black veterans to experience 1 safety event (33% versus 32%, respectively), and multiple safety events (32% versus 23%, respectively, both < 0.001). After adjustment, black veterans were 21% and 50% more likely to have at least one episode of hyperkalemia or hypoglycemia respectively, than non-black veterans, but were 22% less likely to experience a medication error (all p < 0.001). There was no association between the occurrence of AHRQ PSI and race after adjustment.

Conclusions: Black veterans with CKD are more likely to experience a broad array of safety events than non-blacks with CKD with a preponderance of electrolyte disturbances rather than medication errors or AHRQ PSI. The differential safety phenotype in blacks versus non-blacks may have implications for preventive strategies to improve patient safety in an integrated health system.

FR-OR190

Antibiotics in Patients with Severe Chronic Kidney Disease: An Epidemic of Dosing Errors

Alexandra Farag,[1] Amit X. Garg,[1,2,3] Lihua Li,[1] Arsh Jain.[1] *[1]Division of Nephrology, University of Western Ontario, London, ON, Canada; [2]Department of Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada; [3]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada.*

Background: Antibiotics are frequently misprescribed in patients with chronic kidney disease (CKD) and are implicated in over one third of preventable adverse drug events in these patients. To improve the safety of CKD patients, in January 2006, outpatient laboratories in Ontario, Canada began reporting estimated glomerular filtration rate (eGFR). We sought to describe the rate of ambulatory antibiotic dosing errors in Southwestern Ontario and examine the impact of eGFR reporting on these errors.

Methods: We linked health administrative data for ambulatory residents in Southwestern Ontario from January 2003 to April 2010. Patients had severe CKD (eGFR < 30 ml/min/1.73m^2) and were 66 years of age or older. We conducted a population-based intervention analysis with time-series modeling on the monthly rate of dosing errors.

Results: Of the total 1464 prescriptions filled for study antibiotics throughout the study period, 970 (66%) were dosed in excess of guidelines. Nitrofurantoin, which is contraindicated in patients with creatinine clearance < 60 ml/min, was prescribed 169 times. The initiation of eGFR reporting was not associated with a decline in the rate of antibiotic dosing errors (p =0.85, Figure 1). Prior to eGFR reporting the average rate was 636 per 1000 antibiotic prescriptions; after eGFR reporting, the rate was 680 per 1000 antibiotic prescriptions.

Conclusions: Our findings demonstrate that ambulatory antibiotic dosing errors are exceedingly common among severe CKD patients. Moreover, eGFR reporting has not impacted the rate of these errors at the population level. Further interventions to reduce medication errors in CKD patients may therefore be warranted.

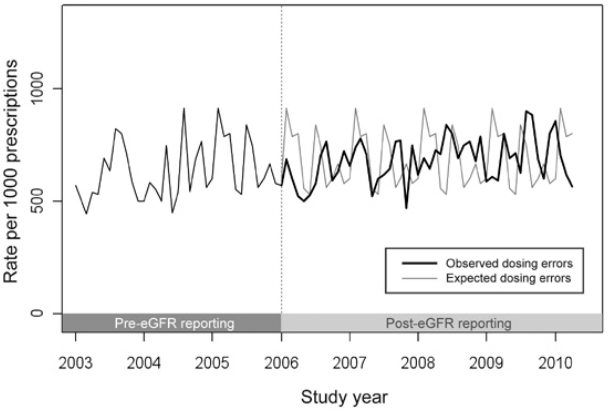


Figure 1. Antibiotic dosing errors among severe CKD patients of Southwestern Ontario.

Funding: Private Foundation Support

FR-OR197

Beyond Measured GFR: Prognostic Importance of Novel and Traditional Filtration Markers

Navdeep Tangri,[1] Lesley Stevens Inker,[1] Hocine Tighiouart,[1] Eric P. Sorensen,[1] Vandana Menon,[1] Gerald J. Beck,[2] Michael Shlipak,[3] Josef Coresh,[4] Andrew S. Levey,[1] Mark J. Sarnak.[1] *[1]Tufts Medical Center, Boston, MA; [2]Cleveland Clinic, Cleveland, OH; [3]UCSF, San Francisco, CA; [4]Johns Hopkins, Baltimore, MD.*

Background: Decreased GFR is a risk factor for kidney failure, cardiovascular disease (CVD), and all cause mortality. GFR can be estimated from serum creatinine, cystatin C or beta trace protein (BTP). The prognosis related to these filtration markers beyond GFR has not been determined.

Methods: Serum creatinine, cystatin C and BTP were assayed at baseline in 816 participants from the Modification of Diet in Renal Disease Study. GFR was measured using 125-I iothalamate. All markers were transformed to inverse variables and associations were examined per 1 SD to allow standardized comparisons with measured GFR. We examined the association of each filtration marker with kidney failure (dialysis or transplantation), all cause mortality and CVD mortality, in Cox proportional hazards models with and without adjustment for GFR.

Results: The mean (SD) GFR was 33 (12) ml/min/1.73 m2. Median follow up was 16.6 years. In univariate analysis, each filtration marker was associated with a higher risk for all outcomes. After adding GFR to the model, the associations differed among filtration markers. A 1 SD lower 1/Screat was independently associated with a higher risk of kidney failure (HR 1.79, 95% CI 1.54 - 2.08), but a lower risk of all-cause mortality (HR 0.82, 95% CI 0.69 - 0.96). A 1 SD lower 1/Scyc was associated with higher risk of kidney failure (HR 1.62, 95% 1.37 - 1.91), all-cause mortality (HR 1.72, 95% CI1.38 - 2.15) and CVD mortality (HR 1.90, 95% CI 1.37 - 2.63). A 1 SD lower 1/SBTP was associated with higher risk of kidney failure (HR 1.73, 95% CI 1.48 - 2.01), all-cause mortality (HR 1.33, 95% CI 1.10 - 1.61) and CVD mortality (HR 1.24, 95% CI 0.94-1.63).

Conclusions: The risks related to creatinine, cystatin C and BTP are similar for kidney failure, but differ for mortality when GFR is included in the multivariate model. These risks may be due to non-GFR associations of filtration markers or residual confounding due to error in measurement of GFR.

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

FR-OR198

Biochemical Data and Growth in Children with Chronic Kidney Disease: A Report from the Chronic Kidney Disease in Children (CKiD) Cohort Study
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Background: Growth failure is common among children with chronic kidney disease (CKD) and is associated with declining glomerular filtration rate (GFR). However, its relationship to a variety of biochemical parameters is poorly characterized.

Methods: We examined the cross-sectional association of baseline growth parameters (height [Ht] and weight [Wt] age-gender specific standard deviation scores [SDS]) with hemoglobin (Hg), C-reactive protein (CRP), and serum levels of CO_2, phosphate and albumin while adjusting for GFR, CKD cause, recombinant human growth hormone usage, and gender among 430 children (median age: 11 yo; median GFR via plasma disappearance of iohexol: 45 ml/min/1.73m^2) from the CKiD cohort study using two multivariate linear regression models.

Results: The median SDS for Ht and Wt were -0.70 (IQR: -1.42, 0.13) and -0.08 (IQR: -0.94, 0.84), respectively. When compared to children with serum CO2 ≥ 22mEq/L, children with serum CO2 <18mEq/L had heights that were on average 0.56 SDS lower (95% CI: -1.01, -0.10); Wt SDS were similar. Children with serum albumin < 3g/dL had weights that were on average 2.46 SDS lower (95% CI: -4.28, -0.65) than children with albumin ≥ 3 g/dL; Ht SDS were similar. Children with CRP > 10mg/L had SDS for Ht and Wt that were lower than children with CRP between 0.03 and 10mg/L, but the difference was not significant. Higher Hg and elevated phosphate levels (age-based) were associated with non-significantly higher Ht and Wt SDS.

Conclusions: Moderate to severe metabolic acidosis was associated with deficits in height, whereas low serum albumin was associated with severe deficits of weight. Longitudinal analysis of biochemical data in the CKiD cohort will allow further assessment of the risk factors associated with growth failure and targets for intervention in children with CKD.

Funding: NIDDK Support, Other NIH Support - NICHD

FR-OR199

High NaCl-Induced Nuclear Accumulation of the Osmoprotective Transcription Factor NFAT5 Depends on Binding to Osmotic Response Elements (OREs)
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Background: Nuclear Factor of Activated T-Cells 5 (NFAT5), also called TonEBP/OREBP, induces transcription of osmoprotective genes in response to hyper tonicity. High NaCl causes translocation of NFAT5 from cytoplasm to nucleus. Threonine 298 is a known DNA contact site in NFAT5.

Methods: The effects of mutation of threonine 298 on binding of NFAT5 to its cognate DNA element and its nuclear localization were examined. We measured binding of NFAT5-V5 to OREs by Electrophoretic Mobility Shift Assay (EMSA), using an IR-800 labeled probe and whole cell extracts from HEK293 cells. We measured nuclear localization of NFAT5-V5 by quantitative Western analysis of nuclear and cytoplasmic proteins. We also used confocal microscopy to confirm effects on nuclear localization with NFAT5-GFP in HeLa cells.

Results: Mutating NFAT5-T298 to aspartate (T298D), which introduces a negative charge, inhibited binding to OREs. Specificity of binding of wild type NFAT5-V5 was confirmed with a non labeled probe. High NaCl increases the nuclear to cytoplasmic ratio of wild type NFAT5-V5, but not of NFAT5-T298D-V5. That result was confirmed by confocal microscopy of HeLa cells in which high NaCl increases nuclear localization of wild type NFAT5-GFP, but not of NFAT5-T298D-GFP.

Conclusions: Substitution of a negatively charged amino acid for NFAT5-T298 inhibits both its specific DNA binding and its high NaCl-induced nuclear localization. We suggest that binding to OREs may contribute directly to high NaCl-induced nuclear localization of NFAT5. We are currently investigating possible phosphorylation of NFAT5 at threonine 298.

Funding: Other NIH Support - The Intramural Research Program of NHLBI

FR-OR200

Vasopressin Receptor Type 2 Membrane Trafficking and Protein Interaction at the Plasma Membrane
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Background: Cell membrane water permeability increases due to vasopressin-induced accumulation of the water channel, AQP2, at the plasma membrane of kidney principal cells. Activation of the vasopressin type 2 receptor (V2R) by VP is terminated by the downregulation of V2R. In contrast to the V2R downregulation mechanism, events taking place at the cell surface following ligand binding are still elusive.

Methods: We used total internal reflection fluorescence (TIRF) microscopy to study V2R trafficking at the cell surface prior to endocytosis.

Results: Under basal conditions, green fluorescent protein tagged V2R (V2R-GFP) is very mobile, moving randomly in all directions with erratic velocity, but with an average speed of movement of 0.2 μm/sec in human embryonic kidney cells. In the presence of our newly developed fluorescent VP ligand (VP-TMR), the average speed of V2R-GFP movement was reduced to an average of 0.1 μm/sec. During its time at the cell surface, TIRF and subsequent fractionation and western blot analysis revealed that the V2R moves rapidly through lipid raft domains. Next, the VP-TMR-receptor complex is immobilized in specific structures that recruit beta arrestin-GFP and then clathrin-GFP, confirming the role of clathrin-coated pits in V2R internalization. Cell fractionation and western blot analysis showed that internalized VP-V2R moves from light vesicles to heavier vesicles along with β-arrestin. We also observed a similar pattern of distribution of the Gs alpha subunit (Gs) protein, which despite being in the same membrane domains as the V2R, did not seem to interact directly with the receptor by immunoprecipitation. In contrast, immunoprecipitated c-myc tagged V2R expressed in HEK cells co-IP'd with β-arrestin .

Conclusions: This result suggests that cell surface mobility of the V2R is reduced by ligand binding, and that V2R is then immobilized in specific membrane domains along with proteins that may or may not interact directly with the receptor, but that are involved in its trafficking and/or signaling after VP binding and subsequent internalization.

Funding: NIDDK Support

FR-OR201

Genetic Ablation of Aquaporin-2 in the Mouse Connecting Tubules Results in a Mild Urinary Concentrating Defect
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Background: Body water balance is regulated in the kidney via the vasopressin (AVP) regulated water channel aquaporin-2 (AQP2) expressed in the connecting tubule (CNT) and the collecting duct (CD). Although crucial for the urinary concentrating mechanism, the relative role of AQP2 in the CNT and CD are not fully understood.

Methods: To study the role of AQP2 in the CNT we generated and characterized a mouse model with a CNT-specific knock-out of AQP2.

Results: Confocal immunofluorescence microscopy of kidney sections demonstrated an absence of AQP2 immunolabeling in the CNT of the knock-out animals. 24 hour urine output was significantly increased (KO: 3.0 ± 0.3 mL/20 g BW versus WT: 1.9 ± 0.3 mL/20 g BW) and urine osmolality decreased (KO: 1179 ±107 mOsm/kg versus WT: 1790 ±146 mOsm/kg) in knock-out mice compared to controls. After a 24 hour water restriction, urine volume was decreased in both groups, while urine osmolality was increased. Urine osmolality was still significantly lower in knock-out mice (KO 2087 ±169 mOsm/kg versus WT: 2678 ±144 mOsm/kg), although 24 hour urine output was not significantly different between groups. A significant difference in urine osmolality before intraperitoneal injection of dDAVP (KO: 873 ± 129 versus WT 1387 ± 163 mOsm/kg) was no longer observed 2 hours after injection, where urine osmolality had increased significantly from baseline in both groups (KO: 2944 ±41; WT 3133 ±66 mOsm/kg). Immunoblotting demonstrated a significant decrease in AQP2 abundance in cortical kidney fractions with no compensatory changes in the AVP-regulated transporters NKCC2, AQP3 and AQP4.

Conclusions: In conclusion, we have demonstrated that deletion of AQP2 from the CNT results in a mild urinary concentrating defect, without diminished maximal urinary concentrating ability. Our studies suggest that the CNT plays a minor role in regulation of whole body water balance, or that dysfunction of the CNT can be compensated for by increased water reabsorption in the collecting duct.

Funding: Government Support - Non-U.S.

FR-OR202

Inhibition of GSK3α Is Critical for Lithium-Induced NDI
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Background: Glycogen synthase kinase 3 (GSK3) is a protein kinase, which occurs in two isoforms, 3α and 3β. Lithium (Li), a potent inhibitor of GSK3 and a treatment for bipolar disorders, causes nephrogenic diabetes insipidus (NDI) in up to 40% of patients. In previous studies using renal collecting duct (CD) specific GSK3β knockout mice (3β-CDnull), we showed that GSK3β deletion reduced CD's response to vasopressin. However, these mice did not become polyuric when treated with Li. Hence, to compare the isoform specific roles of GSK3α and GSK3β in renal urine concentration and Li induced NDI, we used wild type (WT), 3β-CDnull, GSK3α global knockout (3α-/-) and 3α+/-/3β -CDnull mice.

Results: Our attempts to make a CD specific total knockout of both α and β isoforms resulted in total renal agenesis. The 3β–CDnull, 3α-/- and GSK3α+/-/3β-CDnull mice showed normal development. Baseline urine output in 3α-/- and 3α+/-/3β-CDnull mice was 2-fold higher than WT, accompanied by reduced urine osmolality and aquaporin 2 levels. Though 3β–CDnull mice showed 48% increase in urine output compared to WT, their urine osmolality and AQP2 levels were not different. To examine if renal GSK3 is the target for Li, mice were treated with lithium chloride (4mmol/Kg, daily IP injection). By day 9 of Li treatment, urine output increased by 7 fold and osmolality decreased by 3-fold in WT mice compared to their baseline levels. 3β-CDnull mice also showed a 3-fold increase in urine output and a 22% decrease in urine osmolality. In comparison, the 3α-/- and 3α+/-/3β-CDnull mice did not show any significant change in urine volumes or osmolality.

Conclusions: These studies show for the first time that GSK3 is critical for renal development and that 3α and 3β isoforms compensate for each other. Also, GSK3α may play a more important role in renal urine concentration than GSK3β because: 1) at basal levels GSK3α deletion caused higher urinary concentrating defect and polyuria compared to GSK3β; and 2) GSK3α, rather than GSK3β, is a critical target for Li.

Funding: NIDDK Support

FR-OR203

Pharmacological Blockade of $P2Y_{12}$ Receptor Ameliorates Lithium-Induced Polyuria in Rats

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Background: Previously, we showed the potential involvement of ATP/UTP/UDP-activated $P2Y_2$ or $P2Y_4$ receptors in a rat model of lithium (Li)-induced polyuria, and observed that genetic deletion of $P2Y_2$ receptor results in significant resistance to Li-induced polyuria. In contrast, $P2Y_{12}$ receptor ($P2Y_{12}$-R) is an ADP receptor expressed predominantly in blood platelets, and in the brain (microglia and astrocytes). Stimulation of $P2Y_{12}$-R also inhibits adenylyl cyclase. We discovered that $P2Y_{12}$-R mRNA and protein are expressed in rat kidney, and pharmacological blockade of $P2Y_{12}$-R with clopidogrel bisulfate (Plavix®) results in increased urinary concentration in rats, independent of $P2Y_2$ receptor (companion abstract). Here we document that pharmacological blockade of $P2Y_{12}$-R with Plavix® significantly ameliorates Li-induced polyuria in rats.

Methods: Groups of rats were fed normal (ND) or Li-added (LD; 40 mmol/kg food) diets with free access to drinking water and with/without added Plavix® (20 mg/kg/day in water) for 14 days, and then euthanized. Water intake and urine output were monitored and kidney tissue was analyzed.

Results: Co-administration of Plavix® resulted in significant improvement in: 1) Li-induced polyuria ($P < 0.01$) and polydipsia ($P < 0.001$), and decreased urinary concentration ($P < 0.05$); and 2) effectively reversed Li-induced increased solute-free water excretion ($P < 0.01$). These changes in whole body water metabolism were matched with a significant improvement in Li-induced decrease in APQ2 water channel in the inner medulla (2-fold difference; $P < 0.04$). Co-administration of Plavix® also significantly augmented Li-induced urinary AVP levels (1.7-fold vs. Li alone group; $P < 0.05$). The observed effects of Plavix® were not due to a reduction in blood Li levels or kidney medullary accumulation of Li. Finally, co-administration of Plavix® significantly improved Li-induced polyuria in mice also.

Conclusions: Taken together, these data unravel the potential therapeutic utility of drugs that target $P2Y_{12}$-R to ameliorate Li-induced polyuria and possibly, other forms of acquired nephrogenic diabetes insipidus.

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FR-OR204

Vasopressin V1a Receptor Is Required for Nucleocytoplasmic Transport of Mineralocorticoid Receptor

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Background: Type 4 renal tunbular acidosis (RTA) is caused by insufficient aldosterone action. We reported that the dificiency of vasopressin V1a receptor resulted in type 4 RTA by decreasing the expressions of H,K-ATPase and Rhcg and by increasing the expression of H-ATPase in the intercalated cells of the collecting ducts. We investigated the mechanisms of regulation of aldosterone action by vasopressin in the intercalated cells.

Methods: Mice lacking V1a receptor and the cell line of intercalated cells (IN-IC cells) from SV40 Tg rat were used in this study. V1a receptors in the IN-IC cells were knocked down by siRNA and the effects of aldosterone and vasopressin on the nuclear translocatoin of mineralocorticoid receptor (MR) was examined by Western blot. Immunohistochemistry of MR expression in IN-IC cells was also examined.

Results: Fludrocortisone stimulated MR and 11βHSD2 expression in wild type imce and those effects were largely depressed in the V1aR dificient mice. Aldosterone dose-dependently increased MR expression in whole IN-IC cells. Aldosterone decreased cytoplasmic and increased nuclear expression of MR after 30 min and 24 hr. Immunohistochemistry revealed nuclear expression of MR after 30 min and 24 hr stimulation by aldosterone. Vasopressin mimicked the effects of aldosterone on the nuclear expression of MR, and knockdown of V1a receptor abolished the effects of aldosterone. Although vasopressin stimulated PKC α and β1 expressions, aldosterone stimulated not PKC α and β1 but PKC ζ expressions.

Conclusions: Our data show that vasopressin regulates nucleocytoplasmic transport of MR and aldosterone requires vasopressin V1a receptor for nucleocytoplasmic transport of MR.

Funding: Government Support - Non-U.S.

FR-OR205

Microtubule Remodeling by Hyperosmotic Stress

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Background: Acute increase of extracellular osmolyte concentration is a common challenge that induces cell shrinkage and increases intracellular ionic strength, Cell survival depends on intracellular trafficking events that help restore cell volume and eliminate protein damage. Microtubules (MT), together with actin filaments, play an outstanding role in membrane trafficking. While effects of hyperosmotic stress on actin structure is well described its effect on MTs is unknown. The aim of this study was to examine whether hyperosmotic stress affects MT structure and behavior and the consequences that such changes may impart on actin structure and vesicle motility.

Methods: We examined MT and actin remodeling in porcine proximal LLC-PK1 cells before and after hyperosmotic challenge (NaCl or urea 500 mOsmol/kg) by tubulin immunostaining, phalloidin microscopy, Western blot analysis of free and polymerized tubulin and actin and live cell imaging of actin, tubulin and end-binding protein 1, a MT plus-end binding factor. Motility of endosomes loaded with FITC-dextran was examined by live cell imaging and analyzed using ImageJ software.

Results: Data shows that MTs are immediately immobilized by hyperosmotic stress and that this is accompanied by their dramatic depolymerization and subsequent repolymerization. A comparison between events induced by hyperosmotic stress and chemicals that interfere with MT and actin polymerization revealed that while MT remodeling participates in triggering actin polymerization MT depolymerization, more specifically, reduces spatial movement patterns of F-actin networks. MT remodeling and altered actin behavior both contribute to endosome paralysis during early phases of challenge. Endosome motility recovers as cytoskeletal remodeling matures, during and well after cell volume is restored.

Conclusions: These data link changes of cell volume by hyperosmotic stress to cytoskeletal remodeling and demonstrate ensuing consequences on endosome motility.

Funding: Government Support - Non-U.S.

FR-OR206

miR-148b Upregulation Modulating Core 1, β1,3-Galactosyltransferase 1 Expression Explains the Abnormal Glycosylation Process of IgA1 in IgA Nephropathy

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Background: IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide characterized by aberrant O-glycosylation in the hinge region of IgA1 involving the enzyme core 1, β1,3-galactosyltransferase 1 (C1GALT1). To date, the role of microRNAs (miRNAs) in the IgAN pathogenesis has not yet been reported.

Methods: Global miRNA profile of peripheral blood mononuclear cells (PBMCs) from IgAN patients and healthy subjects (HS) were identified using miRNA microarrays V2 Agilent. To study the molecular mechanisms in which the miRNAs were involved, we performed a bioinformatic analysis to predict target genes of the modulated miRNAs. To validate biologically miRNA targets, we performed transient transfection experiments ex vivo using PBMCs from an independent group of IgAN patients and HS.

Results: We identified 37 miRNAs differentially expressed in IgAN patients. Among them, upregulated miR-148b targeted C1GALT1, involved in abnormal glycosylation processes. C1GALT1 expression levels in IgAN patients were reduced and negatively correlated with the miR-148b expression. We demonstrated that miR-148b decreased the C1GALT1 levels in IgAN patients and that the loss of function of miR-148b in PBMCs led to an increase of C1GALT1 mRNA and protein levels similar to that observed in HS. Moreover, we showed that the upregulation of miR-148b directly correlated with the galactose-deficient (Gal-deficient) IgA1 levels supporting our data on C1GALT1 regulation by miR-148b and explaining the abnormal increase of Gal-deficient IgA1 consequent to higher miR-148b levels in IgAN patients. Finally, we validated the upregulated miR-148b in a population of 50 IgAN patients and 50 HS.

Conclusions: All together our data support the unreported role of miRNAs in the pathogenesis of IgAN by discovering the deregulation of miR-148b, which could explain the aberrant glycosylation of IgA1 and provide a potential pharmacological target for new therapeutic approaches in IgAN.

FR-OR207

Genetic Basis for Disease Severity in a Mouse Model of MPO ANCA Glomerulonephritis

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Background: Differences in disease severity among ANCA vasculitis patients may arise from genetic variability. Using a mouse model of MPO ANCA disease we tested the hypothesis that ANCA disease severity is genetically determined.

Methods: After i.v. injection of mouse anti-MPO IgG, glomerular crescents were measured in 4 inbred mouse strains: C57BL/6J, 129S6, 129S1/SvImJ, and LP/J. Genetic variation among the inbred strains was determined with the Mouse Diversity Array high-density genotypes. Genomic clustering of identical genotypes was performed to identify regions that are identical by descent (IBD). Glomerular crescents were also measured in a cohort of 100 mice from a C57BL/6Jx129S6 F2 population.

Results: The average percent glomerular crescents in each of the inbred strains ranged from 9% in C57BL/6 to 64% in 129S6, with 129S1/SvImJ and LP/J having 21% and 20% glomerular crescents, respectively. In the F2 mice the % glomerular crescents spanned the range observed in C57BL/6 and 129S6 mice. The C57BL/6J strain is IBD with each of the other three strains over a modest 28% of the genome and the LP/J strain is 72-74% IBD with the 129 substrains. Surprisingly, the 129 substrains, which have striking phenotypic differences, are IBD over 96.7% of their genome.

Conclusions: Differences in percent glomerular crescents among the 4 inbred strains of mice indicate that ANCA disease severity is genetically determined, and the distribution among F2 mice indicates the trait is polygenic. The IBD analysis between the 129 substrains reduced the fraction of the genome that contains candidate loci for ANCA disease severity to 90.8 Mb or 3.3% of the genome distributed among 15 regions. Mapping quantitative trait loci in the F2 population may corroborate one of the 15 candidate regions or identify additional modifier loci.

Funding: NIDDK Support

FR-OR208

The Role of C5a Receptors (C5aR and C5L2) and C6 in the Pathogenesis of Glomerulonephritis Induced by Myeloperoxidase (MPO) Specific Antineutrophil Cytoplasmic Autoantibodies (ANCA) Hong Xiao,[1] Bao Lu,[2] Peiqi Hu,[1] Ronald J. Falk,[1,3] Craig Gerard,[2] J. Charles Jennette.[1,3] *[1]Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; [2]Pediatrics, Childrens Hospital, Boston, MA; [3]Kidney Center, University of North Carolina, Chapel Hill, NC.*

Background: Anti-mouse MPO IgG causes GN in mice that mimics human ANCA GN. Alternative pathway complement activation is required for GN induction. C5a appears to mediate GN by chemotactic recruitment and priming of neutrophils for activation by ANCA. Engagement of C5a receptor (C5aR) is an important pathogenic event. The role of C5b and full MAC formation in anti-MPO GN has not been determined. We investigated the pathogenic roles of C6, C5aR and C5L2 (an alternative receptor for C5a).

Methods: Induction of GN was assessed after injection of 50mg/kg anti-MPO IgG into control B6 (n=7) or C3H/HeJ (n=5) wild type (WT), C3H C6-/- (n=5), B6 C5-/- (n=6), B6 C5aR-/- (n=6), or B6 C5L2-/- (n=7) mice. Mice were sacrificed at day 6 .

Results: C6-/- mice that received anti-MPO developed GN with % glomeruli with crescents (4.2%) similar to WT mice (5.2%, p=0.63). C5-/- mice that received anti-MPO developed no lesions. Only 1 of 5 C5aR-/- mice developed 1% crescents while the other 4 had no crescents. C5L2-/- mice developed more severe crescents (18.0%) compared to WT mice (8.14%, p=0.0035).

Conclusions: Absence of C6 has no effect on induction of anti-MPO GN, thus C5bC6 and MAC formation are not required. Absence of C5 and of C5aR abrogate induction of GN whereas absence of C5L2 enhances induction of GN by anti-MPO. Thus, engagement of C5aR has a pro-inflammatory effect whereas engagement of C5L2 has an anti-inflammatory effect. More severe disease in C5L2-/- mice is in line with observation that C5L2-/- mice have neutrophils with increased response to C5a (J Biol Chem 2005;280:39677). C5L2 may function as a decoy receptor reducing availability of C5a or by dominant-negative signaling that counteracts pro-inflammatory signaling from C5aR. Antagonists of C5, C5a or C5aR, but not of C6 or C5L2, may have therapeutic benefits in ANCA disease.

Funding: NIDDK Support

FR-OR209

Renal IL-17 Expression in Human ANCA-Associated Glomerulonephritis Joachim Velden,[1] Hans-Joachim Paust,[2] Elion Hoxha,[2] Jan-Eric Turner,[2] Saskia Schröder,[2] Ursula Kneissler,[1] Oliver M. Steinmetz,[2] Erik M. Disteldorf,[2] Hans-Willi Mittrücker,[3] Rolf A. Stahl,[2] Udo Helmchen,[1] Ulf Panzer.[2] *[1]Inst. f. Pathologie, Nierenregister, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]III. Med. Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3]Inst. f. Immunologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Background: Interleukin-17A (IL-17) promotes inflammatory renal tissue damage in mouse models of crescentic glomerulonephritis (GN) including murine experimental autoimmune anti-MPO GN, which most likely depends on IL-17 producing Th17 cells. In human ANCA-associated GN (ANCA-GN), however, the cellular sources of IL-17 remain to be elucidated.

Methods: We studied IL-17 expression in human kidney biopsies of acute necrotizing and crescentic ANCA-GN (n=20) by immunohistochemistry and immunofluorescence co-localization analysis. Further, we measured IL-17 levels in lysates and supernatants from purified human blood leukocytes by ELISA.

Results: Numerous IL-17 expressing (IL-17+) cells were detected in acute ANCA-GN biopsies, specifically in glomeruli with capillary necroses or cellular crescents and in the periglomerular as well as peritubular interstitium. Most of these IL-17+ cells (>80%) were MPO+ polymorphonuclear neutrophilic granulocytes. The small remaining fractions of IL-17+ mononuclear cells were tryptase+ mast cells and unexpectedly low numbers of CD3+ T cells (Th17). IL-17 was not detectable in other infiltrating or resident kidney cells. In vitro, purified human neutrophils were capable to release significant amounts of IL-17 upon stimulation.

Conclusions: We have shown that neutrophils are the numerically most abundant IL-17+ cell type in human kidneys affected by acute ANCA-GN, by far outnumbering IL-17+ mast cells and T cells, and that neutrophils can release IL-17 in vitro. Together, our findings suggest that neutrophils may contribute significant amounts of IL-17 to the sites of glomerular and tubulointerstitial inflammation in human ANCA-GN.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR210

Etanercept Delays the Progression of Alport Glomerulosclerosis by Preventing Tumor Necrosis Factor-Driven Loss of Podocytes Hans J. Anders,[1] Mi Ryu,[1] Shrikant Ramesh Mulay,[1] Oliver Gross.[2] *[1]Department of Nephrology, Universitiy of Munich, Munich, Germany; [2]Department of Nephrology and Rheumatology, Georg August University Göttingen, Göttingen, Germany.*

Background: Chronic renal failure involves the progressive loss of renal parenchymal cells. For example, Alport nephropathy develops from mutated type IV collagen which fosters the digestion of the GBM and podocyte loss, followed by progressive glomerulosclerosis. We found that Alport nephropathy in Col4a3-deficient mice is associated with an increased intrarenal expression of the proapoptotic cytokine tumor necrosis factor-alpha (TNF-α) in glomerular cells, particularly in podocytes as well as in infiltrating leukocytes. We therefore hypothesized that TNF-α contributes to Alport glomerulosclerosis by inducing podocyte apoptosis.

Methods: We treated 4 week old Col4a3-deficient mice on a 129/SvJ genetic background either with vehicle or the TNF-α antagonist etanercept, or an equivalent dose of human IgG as a control intraperitoneally for a period of 5 weeks. Lifespan was monitored and renal pathologic evaluations were performed (e.g. immunohistochemistry, TUNEL, FACS, electron microscopy, real time-RT-PCR, western blot, and functional assessment).

Results: Etanercept treatment prolonged mean survival compared to vehicle-treated Col4a3-deficient mice (p=0.0016). The beneficial effect of etanercept on survival was associated with a significant improvement of the glomerulosclerosis score, renal function (proteinuria, plasma creatinine, and BUN) and the glomerular filtration rate (GFR) at 9 weeks of age. Etanercept treatment specifically improved the numbers of glomerular podocytes (WT-1 and nephrin co-staining) and significantly increased the renal mRNA expression of nephrin and podocin without affecting markers of renal inflammation. The increased number of podocytes was consistent with less TUNEL-positive podocytes that undergo apoptosis.

Conclusions: Together, we concluded TNF-α-induced podocyte loss via apoptosis is a previously unrecognized pathomechanism of Alport nephropathy; hence, TNF-α blockade might be a therapeutical option to delay the progression of Alport nephropathy and potentially of other forms of glomerulosclerosis.

FR-OR211

Podocyte-Specific Inhibition of Signal Transducer and Activator of Transcription (STAT) 3 Attenuates Nephrotoxic Serum-Induced Glomerulonephritis Yan Dai,[1,2] Michael J. Ross,[1] John C. He,[1] Peter Y. Chuang.[1] *[1]Dept of Medicine, Div of Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Medicine, Div of Nephrology, Shanghai First Affiliated Hopsital, Shanghai, China.*

Background: STAT3 activation correlates with cell proliferation in Bowman's space and renal injury in glomerulonephritis (GN). Podocytes are involved in the formation of cellular crescents in GN. We hypothesize that STAT3 activation in podocytes causes the development of GN.

Methods: To test our hypothesis mice with podocyte-specific Cre recombinase-mediated excision of exons encoding for the SH2 domain of the STAT3 allelle (Cre F/F), which is required for STAT3 activation, were generated. Cre F/F and control littermates (Cre +/+) were generated by crossing Cre F/+ mice. GN was induced by tail vein injection of a sheep anti-mouse glomerular basement membrane antibody (NTS). Mortality was monitored. Urine, serum, total kidney, and isolated glomeruli were collected.

Results: Cre F/F lack renal phenotype and are born with the expected Mendelian frequency. Podocyte-specific deletion of the SH2 domain of STAT3 was confirmed by PCR using primers flanking the excised exons and DNA extracted from isolated glomeruli. Suppression of STAT3 signaling in Cre F/F mice was confirmed by a reduction of STAT3 phosphorylation at Y705. NTS induced a significant increase in albuminuria in Cre +/+, but not in Cre F/F (1.07+/-0.20 vs 0.20+/-0.02 g albumin/g creatinine, 8d post injection). Proteinuria correlated with glomerular deposition of mouse and sheep IgG. BUN of NTS-injected Cre +/+ mice was higher than NTS-injected Cre F/F mice (68.95+/-9.91 vs 21.90+/-4.30 mg/dl, 14d post injection). NTS failed to activate STAT3 in Cre F/F mice as demonstrated by reduced phospho-STAT3 (Y705) immunostaining. NTS-injected Cre F/F have preserved nephrin and synaptopodin immunostaining. NTS increased the glomerular expression of STAT3 target genes (ICAMI and MCP1) in Cre +/+, but not Cre F/F mice.

Conclusions: STAT3 activation in podocytes is required for the development of renal injury in NTS-induced GN. Additional studies to examine the underlying mechanism of STAT3 are needed. Inhibition of STAT3 activation is a potential therapeutic option for GN.

Funding: NIDDK Support

FR-OR212

Podocyte Injury Specific Microangiopathy in Collapsing FSGS; the Role of PAI-1 and the Beneficial Effect of Its Inhibitor TM5484 Namiko Kobayashi,[1] Toshiharu Ueno,[1] Toshio Miyata,[2] Taiji Matsusaka,[3] Michio Nagata.[1] *[1]Department of Pathology, University of Tsukuba, Ibaraki, Japan; [2]United Centers, Tohoku University School of Medicine, Sendai, Miyagi, Japan; [3]Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

Background: We previously established transgenic model of podocyte-specific injury resulting collapsing FSGS (NEP mice with LMB2).This model showed distinct microangiopathy at the site of glomerular epithelial hyperplasia suggesting podocyte injury provokes microangiopathy. This microangiopathy is associated with podocyte reduction, thrombosis and glomerulosclerosis. In addition, reduction of VEGF and eNOS, and increase of PAI-1 was observed. This suggests that podocyte injury causes dysregulation in podocyte-endothelial cells(ECs) homeostasis resulting site specific microangiopathy. To test whether microangiopathy promotes collapsing FSGS, present study administered novel PAI-1 inhibitor (TM5484) in this model.

Methods: Two groups of NEP mice, with (TM group, n=9) and without (controls, n=5) PAI-1 inhibitor (TM5484) were induced collapsing FSGS by LMB2. Immediately after injection of LMB2, TM5484 was administered orally for 12 days.

Results: Proteinuria was significantly reduced in TM 5484 group compared to controls on day12 ($p<0.05$). Of note, WT1 positive cells were not reduced in TM5484 group, whereas significant podocyte reduction was noted in controls on day8 (20.2 ± 2.39 vs. 9.23 ± 1.98 $p<0.001$) and day12(15.1 ± 2.42 vs. 4.44 ± 0.95 $p<0.001$). TI is significant lower in TM5484 on day8 (0.03 ± 0.05 vs. 0.12 ± 0.10, $p<0.05$) and day12 (0.05 ± 0.04 vs. 0.24 ± 0.10 $p<0.05$). SI was lower in TM5484 (0.05 ± 0.04 vs. 0.24 ± 0.1, $p<0.05$). Compared to controls, TM5484 group revealed significant reduction in mRNA levels of PAI-1 on day12, conversely VEGF on day8 and 12, eNOS on day8 and 12, u-PA on day8, and c-met on day12 was significantly increased in TM 5484 group.

Conclusions: Podocyte-specific injury mice model of collapsing FSGS showed corresponding microangiopathy due to increase of PAI-1. TM5484 significantly ameliorated progression of collapsing FSGS *via* protection of podocyte injury and epithelial hyperplasia.

FR-OR213

Intrauterine Growth Restriction Leads to Dysregulation of WT 1 and to Early Podocyte Alterations Carlos Menendez-Castro,[1] Fabian Fahlbusch,[1] Karl F. Hilgers,[2] Kerstin U. Amann,[3] Andrea Hartner,[1] Wolfgang Rascher.[1] *[1]Department of Pediatrics, University Hospital; [2]Department of Nephrology, University Hospital; [3]Department of Nephropathology, University Hospital, Erlangen, Germany.*

Background: Intrauterine growth restriction (IUGR) leads to a reduced nephron number and a higher incidence of renal disease. We tested the hypothesis that IUGR interferes with nephrogenesis by altered expression patterns of Wilms' tumor suppressor gene 1 (WT 1), a key player of renal development, and mediator of podocyte integrity in the mature kidney. We further hypothesized that early dysregulation of WT 1 results in later podocyte damage.

Methods: IUGR was induced in rats by maternal protein restriction (8.4% vs 17.2% casein diet) during pregnancy. Kidneys were harvested from offspring at day 1 and 70 of life. qRT-PCR, immunohistology and electron microscopy (EM) was performed in renal tissue. Albuminuria was assessed by ELISA.

Results: In neonatal kidneys of IUGR rats a significant reduction of the nephrogenic zone was observed. Furthermore, IUGR led to an increased expression (1.78-fold; $p<0.001$) of WT 1 and an imbalance of its splice variants +KTS and –KTS, persisting until adulthood. At day 70 of life IUGR rats showed increased glomerular immunoreactivity ($9.36\pm1.03\%$ positive glomerular cells vs $3.35\pm0.34\%$, $p<0.01$) and expression (1.93-fold, $p<0.05$) of desmin, a marker of podocyte injury. EM revealed overt alterations of podocyte ultrastructure in IUGR rats with podocyte enlargement and foot process effacement. Albuminuria was detected in IUGR rats at day 70 of life ($201.7\pm56.48\mu g/24h/100g$ vs 63.35 ± 19.29, $p<0.05$).

Conclusions: IUGR results in early podocyte damage possibly due to a persistent dysregulation of WT 1 and its isoforms. –KTS plays the major role during embryonal renal development. We suggest that early imbalance of WT 1 isoforms to the disadvantage of -KTS lead to the reduced nephron number in neonates. Since developmental pathways are also important for regeneration/repair, we assume that persistent dysregulation of WT 1 results in a reduced ability to compensate for renal damage, rendering IUGR rats more susceptible for renal disease.

Funding: Government Support - Non-U.S.

FR-OR214

Proximal Tubule Autophagic Changes Are Associated with the Development of Microalbuminuria in the PEXTKO Mouse Delecia R. Lafrance,[1] George Rhodes,[2] Bruce A. Molitoris,[2] Deborah J. McCarthy,[1] Kevin J. McCarthy.[1] *[1]Pathology, LSU Health Sciences Center, Shreveport, LA; [2]Medicine, Division of Nephrology, Indiana University Purdue University at Indianpolis, Indianapolis, IN.*

Background: PEXTKO mouse podocytes (Kidney Int. 74: 289-299, 2008) cannot assemble heparan sulfate glycosaminoglycans (HS) thus the anionic charge of the GBM is substantially depleted. The animals do not develop overt proteinuria over their lifetime, but gradual microalbuminuria does develop (>2yrs). Development of vacuolar changes in the proximal tubule cells (PTC) was coincident with the development of microalbuminuria. The purpose of this study was to investigate the functional status of the vacuolated PTE.

Methods: Control and PEXTKO mice (3,6, and 9 months old) were used in the study. Kidneys were removed and processed for frozen and paraffin sections and for electron microscopy (TEM) studies. Sections were stained for TUNEL and for autophagy (LC-3). For multiphoton microscopy studies, animals were injected with fluorescently labeled albumin 30 minutes prior to imaging. Intravital Z-stack images were collected during imaging. Post-imaging, the kidneys were processed for paraffin embedding, and sections re-imaged using widefield fluorescence microscopy.

Results: TEM of PEXTKO tubules showed PTE with large, lamellar vacuoles resembling lysosomes. PTE vacuolization were seen by 3 months of age and increased in size in PTE of older animals. No difference in the pattern or degree of TUNEL staining was seen between control and PEXTKO tissue sections but the vacuolated PTE of PEXTKO animals were LC-3 positive. Confirmation of autophagy was done by western blot for the processed form of LC-3. Intravital microscopy showed that the vacuolated PTE were incapable of resorbing albumin whereas the non-vacuolated PTE were capable of resorbing albumin. Widefield microscopy studies of sections from the same kidneys confirmed multiphoton observations.

Conclusions: The vacuolated PTE cells of PEXTKO tubules are dysfunctional, incapable of resorbing protein. The development of microalbuminuria in the PEXTKO mouse may be due to PTE autophagic change as shown by the TEM and validated by LC-3 staining.

Funding: NIDDK Support

FR-OR215

Light Chain-Associated Renal Fanconi Syndrome Triggers Oxidative Stress, Dedifferentiation and Defective Receptor-Mediated Endocytosis in Proximal Tubule Cells Sara Terryn,[1] Claudia Raggi,[1] Christophe Sirac,[2] Pierre Aucouturier,[3] Pierre M. Ronco,[3] Frank Bridoux,[4] Olivier Devuyst.[1,5] *[1]UCL Nephrology, Brussels, Belgium; [2]CNRS, Limoges, France; [3]INSERM, Paris, France; [4]CHU Jean Bernard, Poitiers, France; [5]University of Zurich, Switzerland.*

Background: Renal involvement often complicates multiple myeloma, and storage of monoclonal immunoglobulin light chain (LC) within the lysosomes of proximal tubule cells (PTC) may lead to LC-associated renal Fanconi syndrome (RFS). The pathogenic mechanisms linking accumulation of LC in lysosomes and dysfunction of PTC remain unknown.

Methods: To investigate the cellular mechanisms involved in PTC dysfunction after LC-accumulation, we used a transgenic mouse model overexpressing a pathogenic human monoclonal κLC (CHEB mouse) and primary cultures of PTC.

Results: The CHEB mice showed low-molecular weight (LMW) proteinuria, glucosuria and phosphaturia, indicating PTC dysfunction and RFS. Immunostaining in CHEB kidneys revealed a decreased expression of the endocytic receptors megalin and cubilin in segments accumulating the κLC, associated with oxidative stress and proliferation markers. Decreased expression of megalin/cubilin, with defective endocytosis of LMW ligands and oxidative stress was also observed in primary cultures of PTC from CHEB kidneys. The changes were specifically related to κLC purified from patients with κLC-RFS, as they were not observed with κLC from patients with cast nephropathy (CN). The link between lysosomal accumulation of LC and defective endocytosis was substantiated by exposing PTC to RFS-associated or CN-associated κLCs. Exposure to RFS κLC, but not to CN κLC, triggered the expression of ZONAB, a transcription factor which induces cell proliferation, dedifferentiation and repression of megalin/cubilin. The increased expression of ZONAB in the PTC resulted in loss of polarization as evidenced by the loss of apical megalin expression and disorganization of the tight junctions.

Conclusions: These results suggest that the lysosomal accumulation of specific κLC within PTC triggers a transcriptional mechanism of cellular dedifferentiation which leads to defective endocytosis and RFS.

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FR-OR216

Nicotine and Chronic Kidney Disease: Role of the Alpha 7 Nicotinic ACh Receptor Gabriel Rezonzew,[1] Phillip H. Chumley,[1] Gene P. Siegal,[1] Wenguang Feng,[1] Ping Hua,[1] Edgar A. Jaimes.[1,2] *[1]U of Alabama at Birmingham; [2]VA Medical Center, Birmingham, AL.*

Background: Cigarette smoking is an important risk factor in the progression of Chronic Kidney Disease (CKD). We have previously demonstrated that mesangial cells are endowed with nicotine receptors (nAChR) and that nicotine increases mesangial cell fibronectin via the α7-nAChR (AJP' 07). Herein, we tested the hypothesis that nicotine worsens the severity of renal injury in 5/6 nephrectomized rats (5/6Nx) and that these effects are mediated via the α7-nAChR.

Methods: SD rats were divided in the following groups: Sham, 5/6Nx, 5/6Nx + Nicotine (NIC, 0.1 gm/L, DW), 5/6Nx + NIC + α7-nAChR blocker methyllicaconitine (MLA,3 mg/Kg/day, SQ), Sham + NIC. Urine was collected for proteinuria and blood pressure (SBP) was measured by tail cuff. Rats were euthanized after 12 weeks, kidneys saved for histology and western blot (WB) and serum saved for creatinine (Cr).

Results: Rats with 5/6Nx developed progressive proteinuria associated with increases in Cr, glomerular injury score (GIS) and fibronectin (WB). The administration of nicotine to 5/6Nx rats did not modify SBP and resulted in worse renal injury as assessed by proteinuria, GIS, Cr and fibronectin (table). The expression of fibronectin was predominantly interstitial and perivascular (IF). The administration of MLA ameliorated the effects of

NIC suggesting they were mediated via the α7-nAChR. Sham + NIC rats had increased proteinuria, fibronectin and Cr but had no significant increase in GIS.

Table

	Sham (n=3)	5/6Nx (n=9)	5/6Nx + NIC (n=7)	5/6Nx + NIC + MLA (n=7)	Sham + NIC (n=7)
Proteinuria (mg/mg Cr)	21.5 ± 0.8	94 ± 8.3 *	153 ± 9.1 *#	84± 11.1 *	120 ±5.8 *
GIS	0.27 ± 0.1	0.85 ± 0.3 *	1.60 ± 0.3 *#	0.9 ± 0.4 *	0.2± 0.1 #
Serum Creatinine (mg/dL)	1.6 ± 0.1	2.5± 0.6 *	2.6 ± 0.3 *	1.7 ± 0.1 #	2.9± 0.2 *
SBP (mmHg)	140 ± 19	162 ± 8.4	159 ± 7.8	143 ± 6.5	149 ± 8.5
Fibronectin (fold increase)	1	5.6 ± 0.6 *	7.6 ± 1.3 *#	1.4 ± 0.3 *#	3.3 ± 0.7 *#

* p<0.05 vs Sham, # p<0.05 vs 5/6Nx.

Conclusions: The administration of nicotine worsens the severity of renal injury in a rat model of CKD via activation of the α7-nAChR suggesting a critical role for this receptor in the accelerated progression of CKD in smokers.

Funding: Other NIH Support - NIEHS, Veterans Administration Support, Private Foundation Support

FR-OR217

Diffusion of Laminin-521 in the Glomerular Basement Membrane Jung Hee Suh, Jeffrey H. Miner. *Renal Division, Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO.*

Background: Laminin-521 (α5β2γ1) is the major laminin isoform in the mature glomerular basement membrane (GBM). Lamβ2 defects cause congenital nephrotic syndrome in humans and mice. Laminin-521 trimers are secreted by both podocytes and glomerular endothelial cells and self-polymerize into a supramolecular network essential for GBM formation. Although laminin secretion and polymerization are relatively well understood processes, laminin dynamics within basement membranes is an unexplored area with relevance to glomerular disease.

Methods: To investigate the hypothesis that laminin diffusion in the GBM occurs, we produced chimeric mice by aggregating pairs of genetically distinct 4-celled mouse embryos, one expressing rat Lamβ2 in podocytes, and one expressing lacZ in all cells.

Results: Chimerism was confirmed by Xgal staining, which revealed a mosaic pattern. Using confocal microscopy we observed distinct segmental rat Lamβ2 deposition in the GBM in some glomeruli, but many glomeruli showed rat Lamβ2 deposition throughout the GBM, along with the presence of Xgal-positive (rat Lamβ2-negative) podocytes. The presence of segmental rat Lamβ2 deposition in the GBM implies that the diffusion of laminin-521 trimers is limited. However, because we observed many continuously rat Lamβ2-positive GBMs in glomeruli containing Xgal-positive podocytes, we conclude that secreted laminin-521 trimers can diffuse within the GBM to regions covered by rat Lamβ2-negative podocytes.

Conclusions: Laminin-521 trimers can diffuse in the GBM. These results contrast with the conventional view of type IV collagen α3α4α5 trimers, which are thought not to diffuse freely within the GBM, perhaps due to extensive cross-linking that fixes their location. These results have implications for Alport syndrome and other glomerular diseases with deposition of ectopic laminins that may contribute to filtration barrier defects.

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FR-OR218

Inhibiting Discoidin Domain Receptor 1 Expression Protects Mice Against the Development of Crescentic Glomerulonephritis Monique Kerroch, Dominique Guerrot, Sophie Vandermeersch, Laurent Mesnard, Eric Rondeau, Pierre M. Ronco, Christos Chatziantoniou, Jean-Claude Dussaule. *INSERM UMR 702, Tenon Hospital, Paris, France.*

Background: The present study investigated the role of discoidin domain receptor 1 (DDR1), a non-integrin collagen receptor displaying tyrosine-kinase activity and mediating vascular inflammation and fibrosis, in the development of crescentic glomerulonephritis

Methods: Experimental glomerulonephrits was induced by administration of nephrotoxic serum (anti-glomerular basement membrane) in wild type mice, mice treated daily either with antisens oligonucleotids against DDR1 or with 'scrambled' oligonucleotids, and mice lacking expression of DDR1 (KO).

Results: Anti-GBM administration resulted in a progressive glomerulonephritis in the wild type and scrambled groups as evidenced by the increasing levels of proteinuria and uremia, the presence of cellular infiltrate (mainly macrophages), and the formation of glomerular crescents, fibrin deposits and interstitial fibrosis. These pathological features were concomitant to a several- fold upregulation of mRNA and protein expressions of DDR1 within glomeruli (mainly podocytes). In sharp contrast, mice treated with DDR1 antisens or DDR1 KO mice displayed a significantly lower proteinuria ($p<0.001$) and uremia ($p<0.01$), a blunted infiltration of inflammatory cells ($p<0.05$), a decrease in the formation of glomerular crescents and fibrin deposits and a preserved renal structure ($p<0.01$). This protection was associated to decreased expressions of pro-inflammatory (ICAM, VCAM, MCP1, IL-1b) and pro-fibrotic (TGFb, Col I and III) factors. In addition, nephrin and podocin expressions were preserved in glomeruli of the antisens and KO groups. In separate experiments, we tested DDR1 presence in human biopsies of rapidly progressive glomerulonephritis (Goodpastures's syndrome and lupus nephritis). Interestingly, DDR1 was expressed in glomeruli, especially in crescents when they were visible whereas it was absent in glomeruli of control kidneys

Conclusions: DDR1 is a major mediator of renal inflammation and fibrosis; blocking DDR1 expression can be a novel, promising treatment against the progression of glomerulonephritis.

Funding: Government Support - Non-U.S.

FR-OR219

Sonic Hedgehog Signaling Promotes Myofibroblast Activation and Renal Interstitial Fibrosis Hong Ding, Sha Hao, Weichun He, Youhua Liu. *Department of Pathology, University of Pittsburgh, PA.*

Background: Sonic hedgehog (Shh) signaling is a developmental signal cascade that plays an essential role in regulating embryogenesis and tissue homeostasis. Hedgehog transmits its signaling through binding to the plasma membrane receptor, Patched 1 (Ptch1), leading to the de-repression of Smoothened (Smo). Activated Smo then moves from an intracellular vesicle to the cell membrane, where it activates the Gli family of transcription factors. Here we investigated the potential role of Shh signaling in renal interstitial fibrogenesis. Both Shh and Gli1 were induced in the fibrotic kidneys in obstructive nephropathy. Using the $Gli1^{lacZ}$ 'knock-in' mutant mice, we found that Shh/Gli1 signaling specifically targeted on renal interstitial myofibroblasts. *In vitro*, recombinant Shh did not promote renal fibroblast proliferation, but induced Gli1, Snail1, α-SMA, fibronectin and collagen I expression, suggesting a role of this signaling in myofibroblast activation and matrix production. *In vivo*, disruption of *Gli1* gene in mice protected kidneys against development of interstitial fibrosis, as evidenced by a decreased expression of α-SMA, fibronectin and collagen I after obstructive injury. Blockade of hedgehog signaling with cyclopamine abolished the Shh-mediated Gli1, Snail1, α-SMA, fibronectin, and collagen I induction in renal fibroblasts. Cyclopamine did not affect renal Shh expression but inhibited Gli1 and Snail1 induction in obstructive nephropathy. Accordingly, cyclopamine also inhibited renal α-SMA and matrix expression and mitigated fibrotic lesions. These results indicate that Shh signaling plays a critical role in the pathogenesis of myofibroblast activation, matrix production and renal interstitial fibrosis.

Funding: NIDDK Support

FR-OR220

Pericyte Derived TIMP3 and ADAMTS1 Regulate Vascular Stability in the Kidney after Injury Claudia Schrimpf,[2] Benjamin D. Humphreys,[2] Jeremy S. Duffield.[1] *[1]Medicine, University of Washington, Seattle, WA; [2]Medicine, Brigham & Womens' Hospital, Boston, MA.*

Background: Recently we identified mural cells of peritubular capillaries called kidney pericytes (kPC) as progenitors of scar forming myofibroblasts but regulatory mechanisms by which kPC differentiate into myofibroblasts are unknown & molecular mechanisms by which kPC detachment from endothelial cells leads to microvascular instability is poorly understood.

Methods: Microarray analysis and vascular stabilization assay

Results: Using microarray analysis, we identified more than 1000 genes that are regulated in kPC during detachment in response to kidney injury. Among the enriched functional categories were genes involved in migration, chemotaxis, extracellular matrix & angiogenesis. Among these genes we identified the metalloproteinase ADAMTS1 as highly up-regulated early during kPC detachment & its endogenous inhibitor TIMP3 as down-regulated during detachment. The specificity of these observations was confirmed by ISH, immunostaining, quantitative assays & by cell purification. In order to study the role of these genes in kPC function we generated & characterized primary kPC cultures, & developed a 3D capillary tube stabilization assay. Primary kPC strongly expressed TIMP3 which was down regulated in primary myofibroblasts. ADAMTS1 was not expressed by kPCs but was highly expressed by myofibroblasts. In a functional 3D regression assay primary kPC attached to capillary tubes with characteristic processes & stabilized the microvasculature with similar capacity to brain PC, while fibroblast cultures did not show these stabilizing effects. In the absence of kPCs, recombinant TIMP3 stabilized 3D capillary tubes, whereas ADAMTS1 accelerated destabilization. Lower levels of TIMP3 stimulated & ADAMTS1 inhibited kPC function in stabilizing capillaries in this assay. Consistent with these in vitro findings, mice deficient in TIMP3 had a spontaneous microvascular phenotype, impaired angiogenesis & accelerated fibrosis in response to kidney injury.

Conclusions: kPC-derived TIMP3 and ADAMTS1 are important regulators of vascular integrity and fibrosis after renal injury.

Funding: NIDDK Support

FR-OR221

Inhibition of Mitochondrial Fission by the DRP1-Inhibitor Mdivi Blocks High-Glucose and TGF-beta Induced PAI-1 and Fibronectin Expression in Renal Cells Jens Gaedeke, Hans-Hellmut Neumayer. *Nephrology, Charité, Campus Mitte, Berlin, Germany.*

Background: Mitochondria are dynamic networks that constantly change morphology through fusion and fragmentation (fission).

A known stimulus for fragmentation is exposure to high glucose. In a previous study, we showed that mild inhibition of mitochondrial respiration blocked TGF-beta effects in renal cells. Recently, an inhibitor of the mitochondrial fission protein DRP-1 (Mdivi) has been described (Dev Cell 2008:14(2)).

Here we analysed if inhibition of mitochondrial fission by Mdivi has effects on glucose and TGF-beta mediated fibrotic effects in renal cells.

Methods: Rat renal fibroblasts (NRK-49F) were kept in DMEM containing 1g/l Glucose and stimulated with 4.5 g/l glucose or TGF-beta (0-5 ng/ml).

Mdivi (Calbiochem) was used at 0-25μM. PAI-1 and fibronectin (FN) was measured by western blot. Signaling pathways were analysed by western blot using phospho-specific antibodies for SMAD3, ERK-, p-p38-MAPK and AMPK.

Results: Treatment of fibroblasts for 48hrs in high glucose led to a strong increase in PAI-1 and FN production. A single dose of Mdivi (25μM) reduced high-glucose induced PAI-1 (-72%) and FN (-88%) significantly.

Similarly, the increase in PAI-1 and FN induced by TGF-beta was blocked by pre-treatment with Mdivi at 12.5 and 25 μM (PAI-1: -64% at 12,5μM, 88% at 25 μM; FN: -55 % at 12,5μM, -76% at 25μM).

Pre-treatment with Mdivi failed to block TGF-beta induced activation of p-p38-MAPK and SMAD3, and Mdivi failed to induce the inhibitory SMAD7 (mRNA analysed by real-time PCR).

Treatment with Mdivi alone led to a time and dose-dependent activation of ERK-MAPK and AMP-activated kinase (AMPK) starting at doses of 12.5 μM, but not at lower doses.

Conclusions: The data show that inhibition of the mitochondrial fission protein DRP-1 blocks expression of high glucose and TGF-beta induced markers of fibrosis. This was associated with an activation of ERK-MAPK and AMPK.

We did not observe any direct effect on the TGF-bta signaling pathway, indicating that Mdivi blocks the nuclear effects of activated SMAD proteins.

Our data raise the possibility that changes in mitochondrial morphology are involved in cellular responses to fibrotic stimuli.

FR-OR222

Regulation of Renal Fibroblast Function by Potassium Channels Michael Kacik, Joachim Hoyer, Brajesh Pratap Kaistha. *Philipps-University-Medical Center, Nephrology, Marburg, Hessen, Germany.*

Background: Potassium channels are important regulators of cellular function during cell cycle and proliferation. In recent studies proliferation of renal fibroblasts has been shown to depend on Ca-activated potassium channels.In the present study we identified a new type of potassium channels and characterized its role in resting and proliferating fibroblasts.

Methods: Potassium channel function was characterized in murine renal fibroblasts with whole cell and single channel patch-clamp experiments and cell potential recordings. Expression of potassium channels was determined with quantitative RT-PCR. Channel localisation was examined by immunofluorescence microscopy. Fibroblast proliferation was activated by TGF-β or bFGF and quantified with a colorimetric assay(MTT).

Results: In murine renal fibroblasts we newly identified the THIK-1 potassium channel, a member of two-pore-domain potassium channel (K2P) family.In resting fibroblasts next to the Kv potassium channel THIK-1 was the predominant potassium channel and responsible for maintaining the hyperpolarized cell membrane potential after activation of resting fibroblast. THIK-1 current was Ca-independent and typically activated by arachidonic acid (AA) at a EC50 of 13 ± 2 μmol/L leading to a mean current of 45,9 pA/pF at 0mV. THIK-1 was blocked by typical K2P blockers chlorpromazine and flupentixol.

After treatment (24h) with profibrotic factors like bFGF or TGF-β the proliferating fibroblasts THIK-1 current was down regulated by about 70%. Instead potassium current was carried by the Ca-activated potassium channel KCa3.1. Tyrosine kinases are important pathways by profibrotic agents. The incubation (24h) with the TK inhibitor genistein led to a significant reduction of the channel activity of THIK-1.

Conclusions: During transition from resting to proliferation state fibroblasts show a distinct switch in potassium channel expression and function. A newly identified Ca-independent THIK-1 is predominant in resting fibroblasts and enables a hyperpolarized membrane potential. During proliferation the channel is downregulated and substituted by the Ca-activated KCa3.1 channel which then regulates Ca2+ ion fluxes in the proliferation state.

FR-OR223

CXCR6 Mediates Recruitment of Bone Marrow-Derived Fibroblast Precursors in Renal Fibrosis Song-Chang Lin, Jiyuan Chen, William E. Mitch, Yanlin Wang. *Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Renal fibrosis is a prominent pathological feature of chronic kidney disease. Although activated fibroblasts are responsible for the production and deposition of the extracellular matrix, the origin of activated fibroblasts mediating renal fibrosis has been controversial. Recent evidence indicates that circulating fibroblast precursors termed fibrocytes contribute to the pathogenesis of renal fibrosis. However, the mechanisms accounting for the recruitment of bone marrow-derived fibroblast precursors into the kidney are not fully understood. We have found that circulating fibroblast precursors express CXCR6. In the present study, we examined the role of CXCR6 in the recruitment of bone marrow-derived fibroblast precursors and renal fibrosis.

Methods: Chimeric mice that express GFP driven by collagen α1(I) promoter were used to demonstrate the bone marrow origin of fibroblast precursors. Unilateral ureteral obstruction was performed to induce renal fibrosis in wild-type (WT) and CXCR6-deficient (CXCR6-KO) mice.

Results: We found that bone marrow-derived GFP positive cells were present in kidneys 5 days post obstructive injury while not in normal kidneys. Furthermore, most of the GFP positive cells stained positively for α-smooth muscle actin. Immunohistochemical studies revealed that a significant number of bone marrow-derived fibroblast precursors dual positive for CD45 and vimentin accumulated in obstructed kidneys of WT mice. In contrast, these infiltrating bone marrow-derived fibroblast precursors were significantly reduced in obstructed kidneys of CXCR6-KO mice. Using immunohistochemistry and Western blot analysis, we found the expression of collagen type I, fibronectin, and α-SMA was upregulated in obstructed kidneys of WT mice. These responses were significantly reduced in obstructed kidneys of CXCR6-KO mice.

Conclusions: These data indicate that bone marrow-derived fibroblast precursors are recruited into the kidney and capable of differentiating into myofibroblasts during the pathogenesis of renal fibrosis and CXCR6 plays a pivotal role in the development of renal fibrosis by recruiting bone marrow-derived fibroblast precursors.

Funding: Other NIH Support - NHLBI, AHA

FR-OR224

Basement Membrane Proteoglycans Mediate Leukocyte Influx in Renal Ischemia/Reperfusion Jacob Van den Born,[1] Gerjan Navis,[1] Azadeh Zaferani,[1] Johanna W.A.M. Celie,[2] Raija Soininen,[3] Ritva Heljasvaara.[3] *[1]Nephrology, University Medical Center, Groningen, Netherlands; [2]Molecular Cell Biology and Immunology, Vrije Universiteit, Amsterdam, Netherlands; [3]Medical Biochemistry and Molecular Biology, University of Oulu, Finland.*

Background: Basement membranes (BM) have important roles in trafficking, signaling, differentiation, and regeneration of cells. Perlecan, collagen XVIII and collagen XV are three major proteoglycans of BM. Renal ischemia/reperfusion (I/R) is characterized by early renal injury and inflammation, followed by regeneration and resolution phase.

Methods: To investigate the role of perlecan, collagen XVIII and collagen XV in renal I/R, we used mice mutated for perlecan (Hspg2Δ3/Δ3), collagen XVIII (Col18a1-/-), collagen XV (Col15a1-/-), and compound mutants (Hspg2 Δ3/Δ3 x Col18a1-/- and Col18a1-/- x Col15a1-/-). Serum urea and creatinine, tubular injury, neutrophil and macrophage influx were determined at day 1, 5 and 10 after 25 minutes of bilateral renal arteries clamping. Wild type (WT) male adult C57BL/6 mice were used as controls. To mimic migration over BM, transwell migration experiments were done with freshly isolated human monocytes.

Results: One day after I/R, Hspg2 Δ3/Δ3 mice showed a transient, but 3-fold rise in serum urea and creatinine compared to WT, (both $p< 0.05$). Five days after I/R, Col15a1-/- and especially Col15a1-/- x Col18a1-/- compound mutant mice showed a reduction of serum urea and creatinine compared to WT mice ($p<0.05$ and $p<0.01$ respectively). Histology showed significantly less tubular damage and reduced leukocytes influx in (Col18a1-/- x) Col15a1-/- mutants at day 1 and 5 after I/R (p-values between $p<0.05$ and $p<0.01$). In a transwell system, the migration of monocytes towards MCP-1 increased over 2-fold ($p<0.05$) when heparin-albumin was immobilized on the filter, confirming involvement of proteoglycans in leukocytes migration.

Conclusions: BM proteoglycans modulate inflammation and tubular damage after renal I/R, probably via the formation of stable chemokine gradients and/or interaction with leukocyte receptors. Thus proteoglycan- leukocyte interaction might be a potential intervention target for heparinoids in order to reduce inflammation.

FR-OR225

Mast Cell Degranulation Promotes Renal Fibrosis in Murine Unilateral Ureteric Obstruction Shaun A. Summers,[1] Poh-Yi Gan,[2] David J. Nikolic-Paterson,[1] A. Richard Kitching,[1] Stephen R. Holdsworth.[1] *[1]Department and Medicine and Nephrology, MMC and Monash University; [2]Departmnet of Medicine, Monash University.*

Background: Mast cells (MCs) are pluripotent innate immune cells. After degranulation MCs release cytokines, chemokines and pro-fibrotic factors including transforming growth factorβ (TGFβ)and matrix metalloproteinases (MMPs). In human and experimental studies MCs are linked with progressive fibrosis. We explored the role of MCs in renal fibrosis induced by unilateral ureteric obstruction (UUO).

Methods: To define a role for MCs in renal fibrosis, we performed UUO on C57BL/6 wild type (WT) and mast cell deficient, KitWsh/Wsh mice. Experiments ended 7 days later. We measured collagen deposition (%), intrarenal mRNA expression of pro-fibrotic factors, TGFβ, MMP and smooth muscle actin (αSMA)relative to a house-keeping gene and interstitial leukocyte recruitment. To confirm these results were exclusively due to MC deficiency we measured renal fibrosis in KitWsh/Wsh mice reconstituted with MCs from WT mice. Finally we assessed renal fibrosis after administration of sodium chromoglycate, a mast cell stabiliser.

Results: Compared to WT mice, renal fibrosis was decreased in KitWsh/Wsh mice 7 days after UUO. Histological assessment demonstrated decreased collagen deposition (12.7±1.2 vs. 9.9±0.9%, $P<0.05$). Intrarenal TGFβ (1.0±0.1 vs.0.7±0.1, $P<0.05$), αSMA (1.0±0.1 vs. 0.7±0.1, $p<0.05$) and MMP12 mRNA expression was decreased in KitWsh/Wsh mice. Fewer interstitial macrophages (score 2.4±0.1 vs. 1.6±0.1 $P<0.001$) and CD4+T cells (7.1±0.4 vs. 4.1±0.6cells/ high power field, $P<0.001$) were seen in KitWsh/Wsh mice. Collagen deposition (17.0±2.5 vs.9.1±1.2%, $P<0.05$) and mRNA expression of TGFβ and αSMA was decreased in (unreconstituted) KitWsh/Wsh mice compared to KitWsh/Wsh mice reconstituted with MCs from WT mice. Pre-emptive administration of sodium chromoglycate to WT mice resulted in decreased collagen deposition (16.3±1.3 vs. 10.5±0.7%, $P<0.01$) and decreased intrarenal mRNA expression of TGFβ (1.0±0.1 vs.0.7±0.1, $P<0.01$) and αSMA (1.0±0.2 vs. 0.6±0.1, $P<0.05$).±

Conclusions: MCs mediate renal fibrosisi after UUO. MC stabilisation is a potential novel therapy for treating renal fibrosis.

Funding: Government Support - Non-U.S.

FR-OR226

Ciliary Neurtrophic Factor Deficiency Protects Against Angiotensin II-Dependent Hypertension Sebastian Alexander Potthoff, Henning Hoch, Paul Probst, Eva Koenigshausen, Magdalena Woznowski, Oliver Vonend, Lorenz Sellin, Lars C. Rump, Johannes Stegbauer, Ivo Quack. *Department of Nephrology, University of Duesseldorf, Germany.*

Background: JAK2/STAT3 signaling cascade modulates AngII dependent hypertension. Ciliary neutrophic factor (CNTF) is an interleukin-6-like cytokine which plays a distinct role in survival and differentiation of neuronal cells by activating the JAK2 / STAT3 signaling cascade. However, CNTF function in other tissues is poorly understood. This study focuses on the role of CNTF in AngII-dependent hypertension model.

Methods: One week after uninephrectomy, Ang II osmotic minipumps (1000 ng/min/kgBW) were implanted in CNTF KO and age-matched C57Bl/6J male mice (WT). Blood pressures (BP) were measured for 3 weeks, starting one week before implantation. Histological and mRNA analysis were performed at the end of the observation period. Renal vascular function was evaluated in the isolated perfused kidney.

Results: Baseline systolic BPs were similar in CNTF KO and WT mice (119±2 vs.124±1 mmHg). Chronic AngII infusion caused a significantly attenuated increase in BP in CNTF-KO mice compared to WT mice (week 1: 139±3 vs. 153±3 mmHg; week 2: 151±5 vs. 168±4 mmHg; n=19; $P<0.01$). Heart hypertrophy was significantly less in the CNTF KO compared to the WT-group (6.5±0.4 vs. 8.2±0.6 mg/g BW; $P<0.01$). Histological and mRNA analysis revealed significantly attenuated renal vascular hypertrophy, tubulo-interstitial damage and CD4/CD8 positive cell infiltration as well as reduced NGAL expression (3.2±0.8 vs. 13.9±2.6 arbitrary units; $P<0.01$) in kidneys of AngII treated CNTF KO mice compared to WT mice. In the isolated perfused kidney, pressor response to AngII was significantly attenuated in CNTF KO mice. AG-490 (5 mM), a JAK2/STAT3 inhibitor, reduced the pressor response to AngII in kidneys of WT mice but had no effect in CNTF KO mice.

Conclusions: CNTF has a major impact on blood pressure regulation in Ang II-dependent hypertension. CNTF seems to modulate the Ang II induced renal pressor response via a JAK2/STAT3 dependent mechanism. Thus, CNTF could be an important regulatory cytokine in the pathogenesis of AngII-dependent hypertension.

Funding: Government Support - Non-U.S.

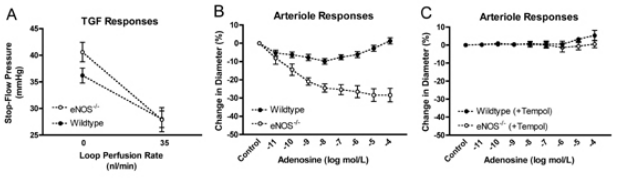
FR-OR227

Adenosine-Mediated Tubuloglomerular Feedback Responses Are Modulated by eNOS in the Afferent Arteriole Mattias Carlstrom, Enyin Lai, Zaiming Luo, Christopher S. Wilcox, William J. Welch. *Dept. of Medicine, Georgetown University, Washington, DC.*

Background: Tubuloglomerular feedback (TGF) is mediated via activation of A1 receptors on the afferent arteriole, and A2 receptors attenuate the response. Whereas NO derived from nNOS in macula densa regulates TGF, the role of eNOS is not clear. We tested the hypothesis that eNOS in the afferent arteriole modulates adenosine-mediated TGF responses by reducing oxidative stress.

Methods: TGF was measured in eNOS-knockout (eNOS-/-) and wildtype mice, as changes in proximal stop-flow pressure (ΔPSF) in response to increased loop perfusion (0 to 35 nl/min). Isolated and perfused afferent arterioles, the effector of TGF, were used to study adenosine responses (10-11 to 10-4 M) with or without simultaneous application of superoxide scavenger (Tempol; 10-4 M).

Results: TGF responses (ΔPSF) were significantly stronger in eNOS-/- mice (12.7±0.7 mmHg) than in wildtypes (8.2±0.5 mmHg) (A). Adenosine caused a biphasic response with contraction in the lower concentration range (-10±2% at 10-8 M) and dilatation in the higher concentration range (1±1% at 10-4 M). In eNOS-/- mice the contractile response to adenosine was significantly stronger (-25±2 % at 10-8 M), with absence of dilatation at higher concentrations (-28±4 % at 10-4 M) (B). Simultaneous application with Tempol prevented adenosine-mediated contraction in both eNOS-/- and wildtype mice, but did not significantly influence dilatation at higher concentrations (C). Western Blot analysis of renal cortex revealed no differences between genotypes in adenosine A1, A2A or A2B receptors.



Conclusions: eNOS in the afferent arteriole importantly regulates TGF by modulating the contractile response to adenosine. Mechanistically, adenosine-mediated contraction is linked to increased superoxide production in the arteriole, and the contractile response is attenuated by eNOS-derived NO.

Funding: NIDDK Support

FR-OR228

Activation of Adenosine A3 Receptor in the Afferent Arteriole (Af-Art) Blunts the Effect of A1 Receptor and AngII Stimulation Yan Lu,[1] Ying Ge,[1] Luis A. Juncos,[2] Ruisheng Liu.[1] *[1]Physiology & Biophysics, University of Mississippi Medical Center, Jackson, MS; [2]Medicine, University of Mississippi Medical Center, Jackson, MS.*

Background: Adenosine plays an important role in tubuloglomerular feedback and renal microcirculation.There are 4 known receptors for adenosine, A1,A2a,A2b and A3(A3R).Activation of the A1 receptor constricts,whereas activation of the A2 receptor dilates the Af-Art.Whether the A3R exists in the Af-Art and its function are not clear.

Methods: A superficial Af-Art was microdissected from a mouse kidney, and mRNA of the A3R was measured with RT-PCR and real-time PCR. We found that the A3R is expressed in the Af-Art. Then, a superficial Af-Art with its attached glomerulus was microdissected from a mouse kidney and perfused at 60 mm Hg.

Results: We measured the diameter of the Af-Art when stimulated with selective A3 receptor agonist IB-MECA at 10-4 M and found no effect. Next, we used a selective A1receptor agonist CHA at concentrations from 10-6 M to 10-4 M. CHA induced a dose-responsive vasoconstriction of the Af-Art, and diameters were 10.9±0.2 μm at control,9.0±0.4 μm at 10-6 M,8.5± 0.5 μm at 10-5 M,8.0± 0.4 μm at 10-4 M. When we repeated the CHA dose response experiment in the presence of the A3R agonist IB-MECA, we found that activation of A3 receptor blocked the constrictive effect of A1 receptor on the Af-Art.We measured intracellular calcium concentration with fura-2.In the presence of IB-MECA, the CHA-induced calcium increase was blocked. Next, we used AngII at concentration from 10-9 M to 10-8 M. AngII induces a dose-responsive vasoconstriction of the Af-Art, and diameters were 10.7±0.4 μm at control,9.3±0.6 μm at 10-9 M, 9.0±1.1 μm at 10-8 M. When we repeated the AngII dose response experiment in the presence of the A3 receptor agonist IB-MECA, we found that activation of A3 receptor blocked the constrictive effect of AngII on the Af-Art.

Conclusions: We conclude that A3 receptor is expressed in the Af-Art and activation of A3 blunts the vasoconstrictive effect of A1 receptor and AngII stimulation. Therefore, the adenosine A3 receptor may play an important role in modulating the tubuloglomerular feedback response and renal hemodynamics.

FR-OR229

Increased Na-Cl Cotransporter Phosphorylation in Hyperinsulinemic db/db Mice Is Regulated by Insulin/PI3K Pathway Hidenori Nishida,[1] Eisei Sohara,[1] Dario Alessi,[2] Naohiro Nomura,[1] Tatemitsu Rai,[1] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan; [2]MRC Protein Phosphorylation Unit, University of Dundee, United Kingdom.*

Background: The metabolic syndrome causes hyperinsulinemia, as a result of insulin resistance, and hyperinsulinemia causes an aberrant increase in sodium reabsorption by the kidney. However, the mechanism responsible for this greater salt-sensitivity in hyperinsulinemic patients is still unknown. We recently discovered the WNK kinase-OSR1/SPAK kinases-NCC transporter phosphorylation cascade, whose abnormal activation results in salt-sensitive hypertension in pseudohypoaldosteronism type II.

Methods: In this study, we investigated whether and how WNK-OSR1/SPAK-NCC cascade is activated in kidney of the db/db mouse, a model of hyperinsulinemic metabolic syndrome.

Results: Thiazide sensitivity in terms of sodium excretion was increased in the db/db mice compared to wild-type mice, suggesting greater activity of NCC in the db/db mice. In fact, increased phosphorylation of OSR1/SPAK and NCC was observed in the db/db mice kidney. This increase in NCC phosphorylation was decreased when 1) the db/db mice were mated with the SPAK(T243A/+) and OSR1(T185A/+) knockin mice which carry the mutations disrupting the signal from WNK kinases, and 2) the db/db mice were treated with a PI3K inhibitor, NVP-BEZ235. Furthermore, increased blood pressure in the db/db mice disappeared when they were mated with the SPAK/OSR1 knockin mice.

Conclusions: We conclude that WNK-OSR1/SPAK kinase cascade is activated in the db/db mouse kidney, and that PI3K is an upstream regulator of this cascade. This mechanism may be one of the pathogenesis of salt sensitive hypertension in hyperinsulinemic conditions, such as metabolic syndrome.

Funding: Government Support - Non-U.S.

FR-OR230

Nitric Oxide Decreases Transforming Growth Factor Beta Type I Receptor Surface Expression through a Dynamin 2-Mediated Process Michael B. Hovater,[1] Wei-Zhong Ying,[1,2] Paul W. Sanders.[1,2] *[1]Department of Medicine, Division of Nephrology, University of Alabama at Birmingham, AL; [2]Medicine, Veterans Affairs Medical Center, Birmingham, AL.*

Background: Transforming growth factor-β (TGF-β) is widely accepted as a profibrotic growth factor, and signal propagation is responsible for deposition of extracellular matrix proteins and promotion of vascular fibrosis. Previous studies from our lab demonstrated that nitric oxide (NO) mitigates the deleterious effects of TGF-β. Studies in bladder endothelial cells and human embryonic kidney (HEK) cell lines indicated that NO activates dynamin 2. We hypothesized that NO increases endocytosis of the TGF-β type I receptor (TBRI) in vascular smooth muscle cells (VSMC) through activation of dynamin 2, potentially shielding the cells from the effects circulating TGF-β.

Methods: Primary cultures of VSMC from Sprague-Dawley rats were treated with dynamin 2 siRNA or control 48 hours prior to treatment with NOR3, a NO chemical donor, ODQ, an inhibitor of soluble guanylyl cyclase, or vehicle. Dynamin 2 was detected using a commercially available rabbit polyclonal IgG antibody, 1:1000 dilution. Surface expression of TBRI was detected with fluorescence-activated cell sorting (FACS).

Results: NO stimulated a time- and dose-dependent endocytosis of TBR1. This effect was independent of guanylyl cyclase since ODQ did not inhibit the NO-mediated decrease in surface expression of TBRI. Physiological levels of NO also stimulated in a dose-dependent fashion dynamin 2 multimerization indicating activation of dynamin 2. Cells pretreated with dynamin 2 siRNA did not demonstrate a NO-stimulated decrease in surface expression of TBRI.

Conclusions: NO decreased TBRI surface expression in VSMC through a dynamin 2-mediated process. This may help explain an important way in which NO protects the vasculature by decreasing TGF-β surface expression and, therefore, decreasing the cellular response to this profibrotic growth factor.

Funding: NIDDK Support, Veterans Administration Support

FR-OR231

Intravital Reactivity to Angiotensin II and In Vitro Preglomerular Myogenic Constriction Predict Renal Damage after 5/6 Nephrectomy Peter Vavrinec, Robert H. Henning, Maaike Goris, Hendrik Buikema, Richard P. Van Dokkum. *Dep of Clinical Pharmacology, UMCG, Groningen, Netherlands.*

Background: Susceptibility to renal injury varies among individuals. Previously, it was shown that individuals with healthy baseline endothelial dilatory ability in isolated renal arterioles developed less renal damage after 5/6 Nx. Whether in vivo pre-existing vascular integrity also predicts subsequent renal damage to 5/6 Nx is subject of the current study using intravital microscopy. In addition, we explored whether in vitro myogenic constriction of small renal arteries extirpated at 5/6 Nx surgery also predicts subsequent development of renal damage in the animal.

Methods: Anaesthetized rats underwent intravital microscopy to visualize glomerular afferent and efferent arterioles, with continuous measurement of arterial blood pressure, heart rate and renal blood flow. After stabilization on saline infusion, Ang II (30 ng/kg/min) was infused for 10 min. Thereafter, 5/6 Nx was performed and blood pressure and proteinuria were assessed weekly for 12 weeks. Images of glomeruli were recorded for measurements of vascular diameter of afferent and efferent glomerular arterioles. Small arteries were isolated from the extirpated kidney at 5/6 Nx and myogenic constriction was assessed in a perfused vessels setup.

Results: Infusion of Ang II induced significant contraction of both afferent and efferent glomerular arterioles ($p<0.001$ compared to saline). The contraction in afferent and efferent glomerular arteriolar diameter upon Ang II infusion correlated strongly with proteinuria 12 weeks after 5/6 Nx ($r = 0.73$; $p=0.01$ and $r = 0.90$; $p=0.01$, respectively). Additionally, in vitro measured myogenic constriction of small renal arteries correlated negatively with proteinuria 12 weeks after 5/6 Nx ($r = - 0.71$, $p = 0.02$).

Conclusions: Both in vivo afferent and efferent responses to Ang II and in vitro myogenic constriction of small renal arteries in the healthy rat predicts the severity of renal damage induced by 5/6 Nx. Further research into this relationship may lead to novel preventive renoprotective therapies.

FR-OR232

Sympathetic Nerves Are in Close Proximity to Vasa Recta Pericytes, and the Co-Transmitters Noradrenaline and ATP Evoke Pericyte-Mediated Regulation of Vasa Recta Diameter Carol Crawford,[1] Teresa M. Kennedy-Lydon,[1] Liam Sawbridge,[1] Jessica Munday,[1] Robert J. Unwin,[2] Scott S.P. Wildman,[1] Claire M. Peppiatt-Wildman.[1] *[1]Royal Veterinary College, London, United Kingdom; [2]University College London, United Kingdom.*

Background: Pericytes reside at regular intervals along vasa recta capillaries and have been shown to regulate *in situ* vasa recta diameter, in response to a number of vasoactive agents, including the co-transmitters noradrenaline (NA) and ATP (1, 2). Here we show vasa recta pericytes exist in close apposition to sympathetic nerves, a potential endogenous source of NA and ATP, and determine the mean distance between sympathetic nerves and pericytes in the inner and outer medulla.

Methods: Live kidney slices, obtained from adult male Sprague Dawley rats, were secured in an open bath chamber set on the stage of an upright microscope and continually superfused with oxygenated physiological saline solution. Video-imaging techniques were used to capture pericyte-mediated changes in vasa recta diameter following exposure of live slices to NA and ATP. Pericytes and sympathetic nerves were labeled in fixed kidney slices using anti-NG2 and anti-TH antibodies and appropriate fluorescently-conjugated secondary antibodies. Fluorescent images of sympathetic nerves and pericytes in the inner and outer medulla were taken with a Zeiss LSM 510 confocal microscope and the distance between sympathetic nerves and the nearest pericyte measured using LSM image browser software. Data are mean±SEM.

Results: NA (10 nM) and ATP (100 μM) evoked a significantly greater constriction of vasa recta at pericyte sites (20.8±5.1% and 19.4±2.8%, respectively) compared with non-pericyte sites (7.6±2.0% and 3.3±2.9%, respectively). Sympathetic nerves were identified in both the inner and outer medulla, and varicosities were found to be 3.5±0.5 μm from the nearest pericyte.

Conclusions: Given the close proximity of sympathetic nerves to pericytes in the medulla, they are a likely endogenous source of the co-transmitters NA and ATP, which we demonstrate evoke pericyte-mediated constriction of vasa recta.

(1) Peppiatt-Wildman CM, et al 2009 *FASEB J* **23** 969.4
(2) Crawford C, et al 2011 *Acta Physiologica* **202**

FR-OR233

Adenosine Receptors Regulate Fluid Uptake in the Proximal Tubule Carolina Panico, Zaiming Luo, William J. Welch. *Medicine, Division Nephrology, Georgetown University, Washington, DC.*

Background: Adenosine enhances fluid uptake in the proximal tubule (PT) via adenosine type 1 receptors (A1-AR). The effect of adenosine type 2 receptors (A2-AR) on PT function is unknown. We showed that fluid uptake (Jv) in the S2 segment of the PT was lower in adult spontaneously hypertensive rats (SHR) compared to normotensive rats, therefore we hypothesized that Jv in this model is reduced by changes in adenosine receptor activity.

Methods: We measured Jv by microperfusion and recollection of accessible S2 segments of the PT in WKY and SHR.

Results: An A1-AR antagonist reduced Jv in normotensive rats (WKY: 2.7±0.3 vs A1-AR Ant: 1.3±0.3 nl/min/mm, $p<0.001$), but had no effect on the lower Jv in SHR (SHR: 1.1±0.2 vs A1-AR Ant 1.3±0.3 nl/min/mm, ns), suggesting that A1-AR activity is impaired in the SHR. However, microperfusion of an A2-AR antagonist (ZM241385 10-7 M) increased Jv in WKY (WKY: 2.6±0.3 vs ZM: 3.1±0.2 nl/min/mm, $p<0.01$), and completely restored Jv in SHR (SHR: 1.2±0.3 vs SHR + ZM: 2.9±0.3 nl/min/mm, $p<0.0001$).

Conclusions: This suggests that A2-AR inhibits Jv in the PT and that A2-AR activity is increased in SHR. Protein expression of A1-AR in microdissected PTs was not different between strains, however A2-AR expression was 2.4 fold higher in PT from SHR. This suggests that activation of A2-ARs normally inhibits Jv and therefore offsets the action of A1-ARs in the PT and that PT dysfunction in SHR is related to overexpression of A2-AR.

Funding: NIDDK Support

FR-OR234

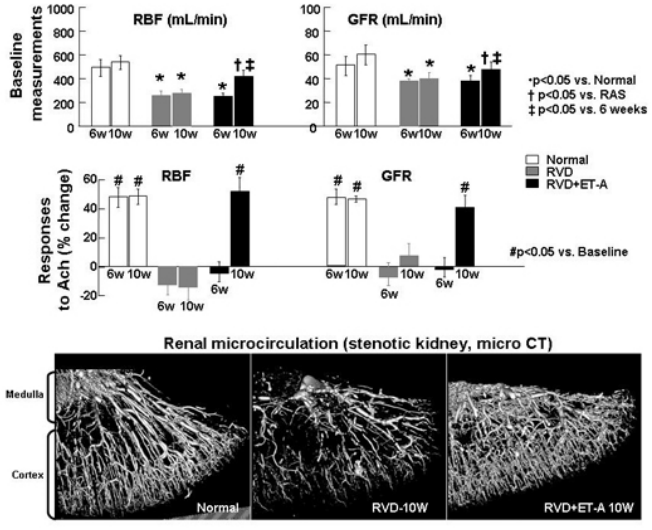
Endothelin-A Receptor Blockade in Chronic Renovascular Disease: A Novel Therapeutic Application Alejandro Chade. *Physiology and Biophysics, Medicine, University of Mississippi, Jackson, MS.*

Background: Circulating and renal endothelin (ET)-1 is increased in patients with chronic renovascular disease (RVD). We have recently shown that chronic blockade of the ET-A receptor prevents functional and structural damage in the stenotic kidney of experimental RVD. This study was designed to extend those observations and determine whether chronic ET-A blockade can halt the progression and reverse renal injury in RVD.

Methods: Unilateral RVD was induced in 8 pigs. After 6 weeks, single-kidney blood flow (RBF) and glomerular filtration rate (GFR) was quantified in the RVD kidney using multi-detector CT (MDCT), before and after endothelium-dependent challenge (acetylcholine, Ach). Then, RVD pigs were randomized in either untreated (RVD, n=4) or treated with chronic-oral ET-A blocker (RVD+ET-A, 0.75 mg/kg/day, n=4) and observed for 4 additional weeks. At 10 weeks, *in vivo* MDCT studies were repeated, and renal microvascular (MV) density quantified *in situ* using 3D micro-CT.

Results: Stenotic kidney RBF, GFR, and MV density and function were similarly blunted after 6 weeks of RVD in all pigs. Untreated RVD showed no changes after 10 weeks, while RVD+ET-A showed improved RBF, GFR and MV endothelial responses to Ach, accompanied by a significant expansion of the renal microcirculation (Figure). Renal artery stenosis and hypertension were similar in RVD and RVD+ET-A at 6 and 10 weeks (P=NS)

Conclusions: This study shows renoprotective effects of chronic ET-A blockade in the stenotic kidney and underscores the importance of renal MV integrity as a determinant of the progression of renal injury. Furthermore, it supports the potential of a novel therapeutic approach to protect the kidney in chronic RVD.



Funding: Other NIH Support - NIH-NHLBI HL095638

FR-OR235

Increased Protein Abundance of the Mutant WNK4 May Be a Cause of the Increased WNK4 Kinase Activity in the Mouse Model of Pseudohypoaldosteronism Type II (PHA II) Mai Wakabayashi, Shotaro Naito, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Mutations in two WNK kinase genes, WNK1 and WNK4, lead to PHA II characterized by hypertension, hyperkalemia and acidosis. We previously generated *Wnk4* $^{D561/+}$ knock-in mice, carrying a heterozygous D561A mutation, and found that phosphorylation of NaCl cotransporter (NCC) was increased by the activation of WNK-OSR1/SPAK kinase cascade. However, mechanism(s) how the mutant WNK4 activated the kinase cascade remains to be determined. In this study, we investigated biochemical characteristics of the mutant WNK4 (D561A) protein in vivo and in vitro.

Methods: To perform in vivo analyses, we used the *Wnk4* $^{D561A/D561A}$ homozygous knock-in mice since they only have the mutant WNK4 protein.

Results: We first confirmed that the homozygous mice showed PHA II phenotypes, indicating the same pathogenic mechanism(s) are working in the homozygous mice as in the heterozygous mice. We also confirmed the increased phosphorylation of OSR1/SPAK and NCC. Using a new anti-WNK4 antibody, we could detect increased WNK4 protein expression (2.2 fold) in the homozygous knock-in mice. Although intrarenal and intracellular localization of the mutant WNK4 was not different from those in wild-type mice, the increased signal was also evident under immunofluorescence. Total WNK4 kinase activity in kidney measured on immunoprecipitated WNK4 was 2.8 times higher in the homozygous mice. However, the kinase activity of the mutant WNK4 per molecule was only slightly (1.3 times) increased after correction by the increased WNK4 protein abundance in the homozygous mice. Since WNK4 mRNA expression in kidney was not increased in the homozygous mice, we measured protein stability of the mutant WNK4 in MDCK cells. Pulse-chase experiment revealed that the stability of mutant WNK4 was significantly increased.

Conclusions: These results suggested that the increasing WNK4 kinase activity in the PHAII model mice could result mainly from the increased WNK4 protein abundance probably due to its increased protein stability.

Funding: Government Support - Non-U.S.

FR-OR236

Improvement of Mild and Moderate Hyponatremia Is Associated with Enhanced Cognitive Function Rick P. Vaghasiya, Maria V. DeVita, Michael F. Michelis. *Division of Nephrology, Lenox Hill Hospital, New York, NY.*

Background: Hyponatremia affects as many as 15-30% of hospitalized patients. Mild hyponatremia has generally been considered asymptomatic. However, a recent study showed that patients with mild to moderate chronic hyponatremia had an increased risk of falls, as well as impairments in gait and attention. This study was designed to assess cognition levels using the Mini-Mental Status Exam (MMSE) in hospitalized patients with varying degrees of hyponatremia pre and post serum sodium (SNa) improvement.

Methods: Thirty patients with SNa values ≤134 mEq/L were included. The MMSE was administered to these patients and scores recorded to a maximum of 30. SNa improved appreciably in 24 of these patients (improved group) after management with 0.9% saline, fluid restriction, vasopressin receptor antagonists, withholding medications, or 3% saline as clinically indicated. The remaining 6 patients (control group) had no change in SNa levels (±1 mEq/L). The MMSE was readministered to all 30 patients after at least 72 hrs. MMSE scores were compared between the improved group and control group.

Results: The initial SNa levels of the improved group ranged from 117 to 134 mEq/L with a mean of 124.3 mEq/L (SD±4.4) and post-improvement SNa ranged from 127 to 143 mEq/L with a mean of 133.7 mEq/L (SD±4.1, p=0.016). The control group had a mean SNa level of 128.9 mEq/L (range 127-131 mEq/L). Twenty-one patients of the improved group (88%) had an increase in MMSE score: 9 patients had a 4-10% increase, 8 patients had an 11-20% increase, 3 patients had a 21-35% increase and 1 patient had a 100% increase on retesting. Of note, 7 improved patients had mild hyponatremia with pre SNa ≥ 127 meq/L. In the control group, 67% had no change, 1 patient had a 3.5% increase and 1 patient had a 7.4% increase on MMSE retesting.

Conclusions: Improving hyponatremia at all levels, mild, moderate and severe, was associated with higher cognitive function on MMSE when compared to patients without correction. Correcting hyponatremia to improve cognitive function should be considered.

FR-OR237

Prognostic Impact of Severe Hyponatremia Following Liver Transplantation Jeonghwan Lee,[1] Jung Pyo Lee,[2] Dong Ki Kim,[1] Yon Su Kim,[1] Curie Ahn,[1] Jin Suk Han,[1] Kwon Wook Joo.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Internal Medicine, Seoul National University Boramae Hospital, Seoul, Korea.*

Background: Hyponatremia is known as a risk factor of reduced survival in patients with end stage liver disease. But, the result on the outcomes after orthotopic liver transplantation (OLT) according to the degree of hyponatremia is controversial.

Methods: We conducted a retrospective analysis of 517 adult patients who underwent OLT at Seoul National University Hospital between 1 January 2005 and 31 December 2010. Patients were divided into three groups according to serum sodium values; normal (more than 135 mEq/L), mild hyponatremia (125-134mEq/L), and severe hyponatremia (<125 mEq/L). In-hospital mortality, duration of admission on ICU and ward, delirium, neurologic complications, acute kidney injury (AKI) and infections were analyzed by ANOVA, Chi-square and logistic regression method.

Results: Out of 517 patients, mild hyponatremia was present in 235 (45.5%) and severe hyponatremia in 75 (14.5%). Hyponatremia had no impact on in-hospital mortality (odds ratio=1.48, p=0.38). Compared with patients of normal serum sodium, patients with severe hyponatremia did not differ in the duration (days) of admission either on ICU (9.6±7.5 vs. 8.1±11.1, p=0.24) or general ward (28.8±16.7 vs. 24.9±32.0, p=0.51). Patients with severe hyponatremia had higher rates of delirium (56.0%/29.5%; OR=1.44, p=0.018), neurologic complications (24.0%/7.2%; OR=1.538, p=0.037), and AKI (57.3%/35.3%; OR=1.35, p=0.045). In 75 patients with severe hyponatremia, patients with rapid correction of hyponatremia over 12 mEq/L/24hr showed higher mortality than others (16.2%/5.3%, p=0.002). Rapid correction of hyponatremia was associated with higher mortality in univariate logistic regression analysis (OR=4.90, p=0.042) but not in multivariate analysis (OR=3.53, p=0.149).

Conclusions: Pre-OLT hyponatremia does not affect on the outcomes of in-hospital mortality or the duration of admission. Hyponatremia is independently associated with post-OLT delirium, neurologic complications and AKI. Rapid correction of serum sodium is associated with higher mortality after OLT, but statistically not significant.

FR-OR238

3% Saline and DDAVP: A Simple Strategy for Safe Correction of Severe Hyponatremia Lonika Sood,[1] John Kevin Hix,[1,2] Richard H. Sterns.[1,2] *[1]Dept. of Medicine, Rochester General Hospital, Rochester, NY; [2]Nephrology Division, University of Rochester School of Medicine and Dentistry, NY.*

Background: Prompt but limited correction of severe hyponatremia is important to avoid morbidity and mortality. An unanticipated water diuresis can destabilize the pace of therapy causing an unintended overcorrection (>10 mEq/L/24 hrs and/or >18 mEq/L/48 hrs)(Clin J Am Soc Nephrol. 2007;2:1110-7). After success treating a patient with a serum sodium(sNa) of 96 mEq/L with 3% saline and DDAVP (AJKD 2010;56:774-9), we adopted this strategy more routinely in managing severe hyponatremia.

Methods: Case records of all 12 patients with sNa <120 mEq/L who received 3% saline and DDAVP concurrently between 11/01/2008 and 01/31/2011 were reviewed retrospectively to determine effectiveness in achieving treatment goals, predictability of response to 3% saline, and optimum dosage of DDAVP.

Results: Eight patients (sNa 106-118 mEq/L) were treated with 2 mcg DDAVP every 8 hours and 3% saline (initially 0.2 to 0.7 ml/kg/hr, usually for less than 24 hours): all met treatment goals at 24 hrs (5.4 ± 1.4 mEq/L); the observed increase in sNa was close to that predicted by the Adrogue-Madias formula (4 ± 2.7 mEq/L), with deviations from predicted ranging from -2 to +5.3 mEq/L. Four patients (sNa 111-113 mEq/L) were treated with 1 mcg DDAVP and 3% saline (0.3 to 0.8 ml/kg/hr): two exceeded treatment goals at 24 hours with increases of 10 and 11 mEq/L, 5.5 and 7.5 mEq/L higher than predicted, in one case because of 3.3 liters urine output despite DDAVP. All 12 patients treated concurrently with DDAVP and 3% saline met the 48 hour treatment goal (9.8 ± 3.0 mEq/L). Except for one patient with preexisting heart disease who developed rapid atrial fibrillation and mild CHF, 3% saline and DDAVP were well tolerated without neurological complications.

Conclusions: Concurrent infusion of 3% saline along with 2 mcg DDAVP every 8 hours is an effective strategy to achieve controlled and predictable correction of severe hyponatremia. Lower doses of DDAVP have little advantage and do not always prevent an unwanted water diuresis.

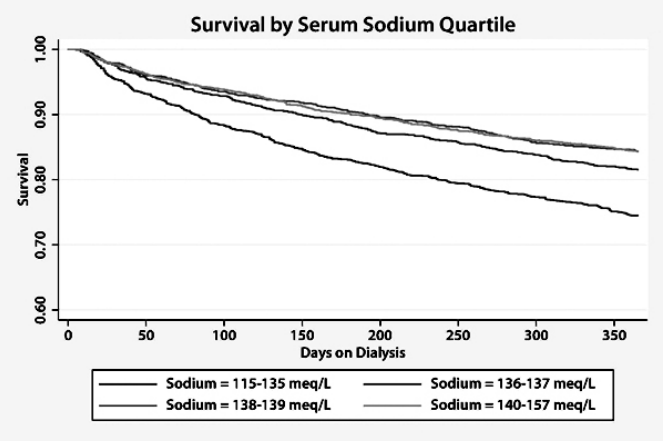
FR-OR239

Low Serum Sodium, Bone Mineral Disease and Mortality in Incident Chronic Hemodialysis Patients Sagar U. Nigwekar, Julia Beth Wenger, Ravi I. Thadhani, Ishir Bhan. *Massachusetts General Hospital.*

Background: In the general population, hyponatremia has been associated with mortality. In addition, recent data suggest that hyponatremia may contribute to a reduction in bone mass. It is unclear whether these associations exist in the hemodialysis (HD) population. We tested the hypothesis that low serum sodium predicts mortality and correlates with markers of bone disease in hemodialysis patients.

Methods: We studied 7,972 patients in the Accelerated Mortality in Renal Replacement (ArMORR) cohort of incident HD patients. Baseline serum sodium, calcium, phosphorous, alkaline phosphatase (AP), and parathyroid hormone (PTH) levels were obtained from blood samples taken at the time of HD initiation. Baseline sodium levels were analyzed by quartile. Hazard of all cause and cardiovascular mortality were calculated using Cox proportional hazard models. Multiple linear regression models were used to predict change in serum calcium, phosphorus, AP, and PTH across the 1-year period.

Results: Lower sodium levels were associated with older age (p=0.007), Hispanic ethnicity (p<0.001), congestive heart failure (p=0.004), diabetic nephropathy (p<0.001), lower BMI (0.008), and catheter access (p<0.001). In multivariate Cox models adjusted for demographic, clinical and laboratory factors, lower sodium levels were independently associated with an increased risk for 1-year all cause mortality (quartile 1 vs quartile 4, HR 1.75, p<0.001) and cardiovascular mortality (HR 1.72, p<0.001).



Lower sodium was independently associated with a progressive decrease in serum calcium, phosphorus, and PTH (p<0.05); however, no association was seen with AP levels.

Conclusions: In a cohort of incident HD patients, low serum sodium is independently associated with all cause and cardiovascular mortality. Additionally, low sodium produces an effect on mineral metabolism consistent with PTH suppression.

FR-OR240

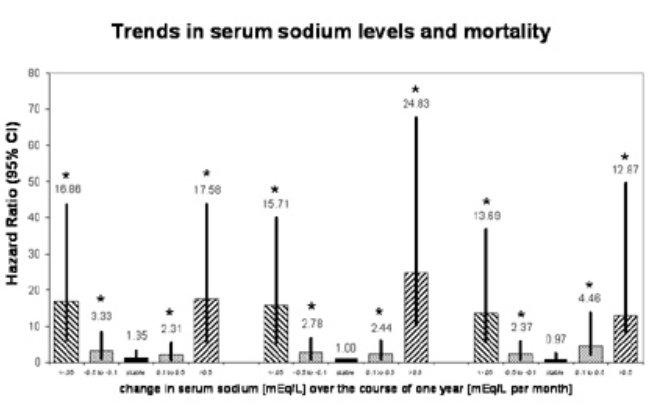
Trends in Serum Sodium Levels (SNa^+) Relate to Mortality in Incident Hemodialysis (HD) Patients Jochen G. Raimann,[1,2] Len A. Usvyat,[1] Jeroen Kooman,[3] Frank Van der Sande,[3] Stephan Thijssen,[1,2] Peter Kotanko,[1,2] Nathan W. Levin.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center; [3]University of Maastricht.*

Background: Low pre-HD SNa^+ is related to increased mortality in HD patients (pts; Waikar 2011). It is not yet clear if this is the reflection of disease or a causal determinant of mortality. This analysis aims to investigate the relation of mortality and temporal evolution of SNa^+ in incident patients during the first two years of HD.

Methods: Pts who started HD between 1/1/2001 and 7/30/2008, with at least 3 SNa^+ during the first 3 months were included and observed over 2 years. SNa^+ changes were quantified as slopes [mEq/L/month], computed by linear regression of SNa^+. Pts were stratified in groups of SNa^+ (average over the first three months): (1) <138, (2) 138 to 141, (3) > 141 mEq/L; and by the slope of SNa^+ change over time: (1) decreasing (<-0.1 mEq/L/month), stable (-0.1 to -0.5) and increasing (>0.1 mEq/L/month). Cox Regression was used to compute hazard ratios (HR) adjusted for age, race, gender, diabetes (DM), ultrafiltration volume, albumin, and pre HD systolic blood pressure and pre HD weight.

Results: Out of 4012 eligible pts (56% males, 43% black, 48% white, 50% DM, age 61.5±15.3 yrs) 607 pts (15%) died in the first 2 years. In the presence of stable SNa^+ over time, low SNa^+ levels during the first three months were not associated with increased mortality. Both in unadjusted and adjusted analysis rising or falling SNa^+ levels over time were significant predictors of outcomes (**Figure 1**).

Conclusions: In incident HD pts changes of SNa^+ over time appear to be a novel and significant predictor of mortality, outweighing the effects of absolute SNa^+ levels. Within the limitations of an observational study these findings suggest that low SNa^+ levels are rather a reflection of yet to be defined underlying illnesses than a direct cause of death.



FR-OR241

Is the Hyponatremia Related to Mortality in Peritoneal Dialysis Patients? A Single-Centre Retrospective Observational Study Seokhui Kang, Jun-Young Do, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. *Division of Nephrology, Department of Internal Medicine, Yeungnam University Hospital, Daegu, Korea.*

Background: Hyponatremia is one of the most common electrolyte abnormality. There are few reports on the incidence, etiology and mortality of the peritoneal dialysis (PD) patients with hyponatremia.

Methods: We reviewed the medical records and identified all the adults who received PD between May 2001 and March 2010. Hyponatremia was defined as a serum sodium concentration less than 135 mmol/L. We enrolled 99 patients who did not show hyponatremic episodes and 297 who showed hyponatremia during follow-up. For evaluation of volume status, all patients had undergone bioelectrical impedance analysis every 6 months. Water gain as a cause of hyponatremia was defined as more than 5% increase of mean total body water (TBW) during normonatremia in the patient with hyponatremic episodes.

Results: The incidence of hyponatremia increased as the grade of the Davies risk index increased. The most common cause of hyponatremia was sodium chloride deficit (39.5%).

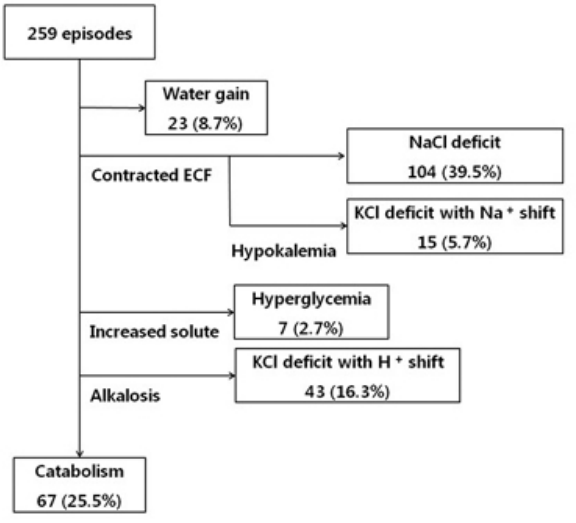


Figure 1. Summary of a diagnostic approach for hyponatremia in peritoneal dialysis patients. Abbreviations: ECF, extracellular fluid; NaCl, sodium chloride; KCl, potassium chloride; Na^+, sodium ion; K^+, potassium ion; H^+, hydrogen ion.

On the univariate analysis, old age (≥65-years-of-age), presence of hyponatremia during follow-up, hypoalbuminemia (<35g/L), residual renal function (RRF) (<2ml/min) and high comorbidity conditions are associated with mortality in the PD patients. On the multivariate analysis, old age, hypoalbuminemia, low RRF and high comorbidity conditions are associated with mortality in the PD patients.

Conclusions: The risk for hyponatremia was associated with higher comorbidity in the PD patients. However, the comorbidity conditions are more important than hyponatremia per se in predicting the mortality. For prevention or management of hyponatremia in the PD patients, we should pay more attention to correct fluid-electrolyte abnormalities but also the underlying comorbidity.

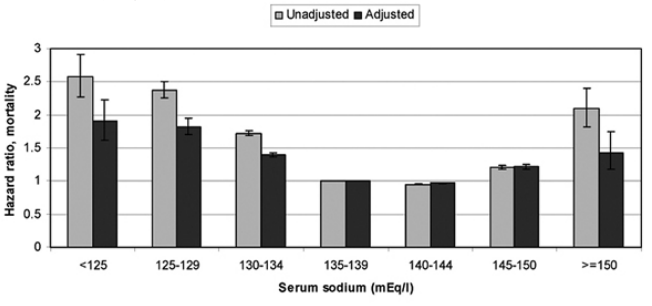
FR-OR242

Association of Serum Sodium (SeNa) Concentration with Mortality in a Large Cohort of US Veterans with CKD Csaba P. Kovesdy,[1,2] Evan H. Lott,[3] Jun Ling Lu,[4] Sandra M. Malakauskas,[1,2] Jennie Z. Ma,[2] Mark D. Okusa,[2] Kamyar Kalantar-Zadeh.[5] *[1]Salem VA Medical Center; [2]University of Virginia; [3]VA Informatics and Computing Infrastructure; [4]Salem Research Institute; [5]Harbor-UCLA.*

Background: The association of serum sodium concentration with mortality in patients with various stages of CKD is not well characterized.

Methods: We examined the association of SeNa with all-cause mortality in a nationally representative cohort of 655,493 US veterans with non-dialysis dependent CKD stages 1-5. Associations were examined in time-dependent Cox models. Non-linear associations were examined by categorizing SeNa in increments of 5 mEq/l. Models were adjusted for sociodemographics, comorbidities (including congestive heart failure (CHF) and liver disease), blood pressure and laboratory variables. Analyses were repeated in patients without CHF or liver disease.

Results: Patients were 73.9±9.8 years old, 97% were males and 71% were white. During a median follow-up of 5.5 years 193,956 patients died (mortality rate, 95%CI: 62.5/1000 patient-years, 62.2-62.8). The association of SeNa with mortality was U-shaped: compared to patients with SeNa 135-139 mEq/l, patients with SeNa <125, 125-129, 130-134, 140-144, 145-149 and ≥150 had adjusted mortality hazard ratios (95%CI) of 1.90 (1.62-2.26), 1.82 (1.71-1.95), 1.40 (1.36-1.43), 0.97 (0.98-0.99), 1.22 (1.18-1.26) and 1.43 (1.18-1.74)(Figure).



Associations were similar when restricting the study cohort to patients without CHF or liver disease.

Conclusions: Both lower and higher SeNa are associated with higher mortality in patients with CKD. These associations are present even in patients without significant comorbid conditions known to affect SeNa, suggesting that abnormal SeNa may not be merely a surrogate of more sever comorbid states. Therapeutic interventions targeting abnormal SeNa will need to be tested in clinical trials.

Funding: NIDDK Support, Veterans Administration Support

FR-OR243

Nephrocystins Regulate the Wnt Pathways and Are Required for Ciliogenesis and Epithelial Morphogenesis Helori-Mael Gaudé,[1] Celine Burckle,[1,2] Rodrick Montjean,[1] Flora Silbermann,[1] Emilie Montenont,[1] Thierry Blisnick,[3] Anthony Henneveu,[4] Cecile Jeanpierre,[1] Alexandre Benmerah,[4] Philippe Bastin,[3] Corinne Antignac,[1] Sylvie Schneider-Maunoury,[2] Sophie Saunier.[1] *[1]INSERM U983, Hopital Necker Enfants Malades, Paris, France; [2]CNRS UMR 7622, UPMC, Paris, France; [3]CNRS URA 2581, Institut Pasteur, Paris, France; [4]INSERM U1016, Institut Cochin, Paris, France.*

Background: Nephronophthisis is a hereditary nephropathy characterized by interstitial fibrosis and cyst formation that is caused by mutations in NPHP genes encoding the nephrocystin family of ciliary proteins.

Methods: We have previously shown that nephrocystin-1 and -4 associate with tight junction protein complexes and that depletion of NPHP1 or NPHP4 by shRNA-mediated knockdown in MDCK cells led to a delay in tight junction formation and disorganized structures in 3D culture.

Results: Interestingly, knockdown of either gene in MDCK and IMCD also increased cell migration and enhanced spreading on collagen. This was accompanied by over-activation of proteins regulating focal adhesions (p130cas and Pyk2), as well as of RhoGTPases, disorganisation of the actin cytoskeleton, enhancement of microtubule polymerization and alteration of ciliogenesis. In vivo experiments performed in zebrafish demonstrate that nephrocystin-4 depletion results in abnormal ciliogenesis and planar cell polarity (PCP) defects, leading to convergent extension defects and pronephric cysts associated with alterations in early cloacal morphogenesis. Depletion of either inversin, nphp2 gene product, or of Wnt-PCP pathway components increases cyst formation in nphp4-depleted embryos. In agreement with these observations, we demonstrate that nephrocystin-4 interacts with inversin and dishevelled and regulates dishevelled stability and subcellular localization in mammalian kidney cells, thus repressing the Wnt-beta-catenin pathway.

Conclusions: Altogether, our data point to crucial functions of nephrocystins in epithelial cell organization and kidney morphogenesis, tight regulation of cytoskeleton organization, cell adhesion and the PCP pathway, through their functional interaction with dishevelled and other polarity determinants such as focal adhesion complexes.

Funding: Government Support - Non-U.S.

FR-OR244

Toward Understanding Pkd1 Cystogenetic Mechanism Marie Trudel, Almira Kurbegovic. *Molecular Genetics and Development, Institut de Recherches Cliniques de Montreal, Montreal, QB, Canada.*

Background: Autosomal dominant polycystic kidney disease(ADPKD) is a genetic disease that displays renal and extrarenal phenotypes. The PKD1 gene responsible for most cases of ADPKD, was shown to have developmentally regulated expression pattern. The pathogenetic mechanism underlying ADPKD induced by mutations in the PKD1 gene is an enigma. While evidence of Pkd1 loss-of-heterozygosity and hypomorph alleles can cause renal cyst in mice, paradoxically high levels of human PKD1 are detected in ADPKD kidneys/tissues.

Methods: To determine whether enhanced Pkd1 expression could be a pathogenetic mechanism, 3 transgenic mouse lines were generated with a tagged Pkd1-BAC.

Results: These mice overexpressed wild type Pkd1 transgene with proper temporal regulation in renal and extrarenal tissues from ~2- to 15-fold over Pkd1 endogenous levels. All mice reproducibly developed tubular and glomerular cysts leading to renal insufficiency that correlated with level of Pkd1 imbalance. $Pkd1_{TAG}$ mouse model also displayed hepatic cyst, brain aneurysm, cardiovascular defect and provide evidence that gain-of-function can be a pathogenetic mechanism. To verify that the Pkd1-BAC transgene produced a functional protein, we questioned whether low and high transgene expressor could rescue the lethality of Pkd1 null newborn. The low Pkd1 expressor in Pkd1 null newborn did not prevent renal cystic or pancreatic phenotypes. These results contrast with those of Pkd1 hypomorphic alleles at 15-20% Pkd1 with less severe renal cysts than null Pkd1 mice. It suggests that either targeted hypomorphic Pkd1 produce abnormal transcripts that protect from cyst formation or the $Pkd1_{TAG}$ transgene lack regulatory elements for proper expression. However, the high Pkd1 transgene expressor (>endogenous) on a null Pkd1 background are healthy with no evidence of renal or extrarenal PKD phenotype and displayed complete rescue, supporting proper transgene regulation. Only older adult mice showed renal cystic anomalies, much milder than in the high transgene expressors.

Conclusions: Together our data support a Pkd1 gene dosage pathogenetic mechanism for ADPKD and highlight our $Pkd1_{TAG}$ mouse as the model most closely mimicking ADPKD phenotype and progression.

Funding: Government Support - Non-U.S.

FR-OR245

Copeptin, a Surrogate Marker for Vasopressin, Is Associated with Disease Progression in the CRISP Cohort of ADPKD Patients Wendy E. Boertien,[1] Esther Meijer,[1] Li Jie,[2] James E. Bost,[2] Joachim Struck,[3] Michael F. Flessner,[4] Ron T. Gansevoort,[1] Vicente E. Torres.[5] *[1]On behalf of the CRISP Consortium; Nephrology, UMC, Groningen, Netherlands; [2]Biostatistics, University of Pittsburgh; [3]BRAHMS Biomarkers, ThermoFisher Scientific, Hennigsdorf, Germany; [4]NIH/NIDDK, Bethesda; [5]Nephrology, Mayo Clinic, Rochester.*

Background: Experimental studies suggest a detrimental role for vasopressin in the pathogenesis of ADPKD. The significance of vasopressin in human ADPKD, however, is yet unclear. We therefore investigated whether vasopressin is associated with disease progression in a cohort of ADPKD patients.

Methods: Baseline plasma copeptin, a reliable surrogate for vasopressin, was measured in 241 ADPKD patients who participated in the CRISP study (a longitudinal, observational study). Patients were followed for 3 years. Every year total kidney volume (MRI) and renal function (iothalamate clearance) were measured.

Results: In these 241 patients (age 32.4 ± 8.9 years, 40% male, GFR 97.8 ± 24.7 ml/min/1.73m^2), median copeptin level was 2.9 (IQR 1.8 – 5.0) pmol/L. Copeptin concentration was higher in males than in females. Remarkably, baseline copeptin concentration was not associated with plasma osmolality ($p = 0.29$), urine osmolality ($p = 0.16$) and 24 hour urine volume ($p = 0.17$). In contrast, baseline copeptin concentration was significantly associated with change in total kidney volume during follow-up (std. Beta = 0.24, $p < 0.01$). This association remained significant after adjusting for gender, age, cardiovascular risk factors and baseline TKV (std. B = 0.14, $p = 0.03$). Baseline copeptin concentration was also significantly associated with change in GFR after adjusting for gender, age cardiovascular risk factors and baseline GFR (std. B = -0.15, $p = 0.03$).

Conclusions: These data show that in ADPKD patients, copeptin levels, as a marker for vasopressin, are not correlated with normal physiologic parameters as plasma nor urinary osmolality or urine volume. Most importantly, high copeptin levels are independently associated with disease progression in ADPKD patients, confirming experimental studies suggesting a detrimental role for vasopressin.

Funding: NIDDK Support

FR-OR246

Expression of the Cytoplasmic Tail of Fibrocystin Causes Cystogenesis through PI3K/Akt/mTOR Pathway Shixuan Wang,[1] Gang Yao,[1] Maoqing Wu,[1] Jingjing Zhang,[2] Jing Zhou.[1] *[1]Medicine, Brigham and Women's Hospital, Boston, MA; [2]Medicine, Mount Auburn Hospital, Cambridge, MA.*

Background: FPC (fibrocystin or polyductin) is a single transmembrane receptor-like protein, responsible for the human autosomal recessive polycystic kidney disease (ARPKD). It was recently proposed that FPC could undergo a Notch-like cleavage and subsequently releases the cleaved carboxy(C)-terminal fragment that enters the nucleus of the cell. However, the functions of the cleaved C-tail are unknown.

Methods: We have used a series of morphological, biochemical, immunological, and cell biology approaches to explore the roles of the isolated FPC C-tail.

Results: By 3D tubulogenesis assay and confocal microscopy, we found that in contrast to tubule-like structures formed by vector-transfected control cells (100%), these hICD-expressing cells (hICD) form cyst-like structures (100%). By western blotting and immunostaining, we observed altered expression of integrins (alpha2, 3, beta1), translocation of E-Cadherin from adherent junctions to the cytoplasm, reduced cell growth rate, and increased apoptosis/autophagy in hICD cells. Flow cytometry analysis showed an increased accumulation of hICD cells in S-phase after 48-h serum starvation (G0). Furthermore, the Akt/mTOR pathway, demonstrated by increased phosphorylation of Akt at serine 473 and S6 kinase 1 at threonine 389, are constitutively activated. More importantly, rapamycin of mTOR inhibitor or wortmannin and LY294002 of phosphoinositide 3-kinase (PI3K) inhibitors can inhibit the activation of mTOR and partially rescue the cyst-forming phenotype in hICD cells. Interestingly, hICD cells display cell attachment defects upon FBS deprivation and high osmolality, which can also be partially rescued by mTOR and PI3K inhibitors.

Conclusions: FPC C-tail overexpression leads to activation of mTOR pathway, reduced cell growth rate, increased apoptosis and autophagy, altered cell cycle profile and cyst formation in 3D cultures in mIMCD3 cells. We propose that FPC C-tail modulates tubulogenesis through PI3K/Akt/mTOR pathway.

Funding: NIDDK Support, Private Foundation Support

FR-OR247

The Role of the Exocyst in Renal Cilia Assembly and Function Ben Fogelgren,[1,3] Shin-Yi Lin,[2] Xiaofeng Zuo,[3] Kimberly Jaffe,[2] Sarah McKenna,[3] Ryan J. Reichert,[4] Kwon Moo Park,[5] P. Darwin Bell,[4] Rebecca D. Burdine,[2] Joshua H. Lipschutz.[1, 6] *[1]University of Hawaii; [2]Princeton University; [3]University of Pennsylvania; [4]Medical University of South Carolina; [5]Kyungpook National University, Korea; [6]Philadelphia VAMC.*

Background: The pathogenesis of polycystic kidney disease (PKD) is dependent on disruptions in primary cilia function in renal epithelial cells. Despite intense study, it remains poorly understood how proteins are targeted and delivered to cilia.

Methods: We established in vitro and in vivo models to analyze the role of the exocyst complex and one of its regulating GTPases (Cdc42) in cilia assembly and function. shRNA was used to knockdown exocyst Sec10 and Cdc42 expression in MDCK cells, and antisense morpholinos were used to disrupt Sec10 during zebrafish development.

Results: In Sec10-knockdown MDCK cells, ciliogenesis was severely disrupted and the cellular phenotype recapitulated that seen in ADPKD cells: loss of flow-generated calcium increases; hyperproliferation; and overactivation of the MAPK pathway. The exocyst bound to the Par complex, which co-localizes with the exocyst at the primary cilium. Dominant negative Cdc42 expression, shRNA-mediated knockdown of Cdc42, and shRNA-mediated knockdown of Tuba, a Cdc42 GEF, all prevented ciliogenesis in MDCK cells. In vivo, Sec10 knockdown in zebrafish phenocopied many aspects of polycystin-2 knockdown, including: curly tail up; left-right patterning defects; glomerular expansion; and MAPK activation. Sec10 morphants also had disorganized cilia. Importantly, there was a synergistic genetic interaction between zebrafish sec10 and pkd2, in that co-injection of small amounts of sec10 and pkd2 morpholinos, which individually had no effect, together resulted in a severe phenotype. Supporting this observation, the Sec10 protein pulled down and co-immunoprecipitated with polycystin-2 and intraflagellar transport proteins IFT20 and IFT88.

Conclusions: Taken together, these data support a model of exocyst ciliary trafficking whereby Cdc42 localizes the exocyst to primary cilia, the exocyst binds to the Par complex, and the exocyst then docks Rab8-positive vesicles carrying ciliary proteins.

Funding: NIDDK Support, Other NIH Support - NICHD, Veterans Administration Support, Private Foundation Support

FR-OR248

Induction of Ran GTP Drives Ciliogenesis Shuling Fan,[1] Eileen L. Whiteman,[1] Toby W. Hurd,[3] Benjamin L. Margolis.[1,2] *[1]Department of Internal Medicine; [2]Department of Biological Chemistry; [3]Department of Pediatrics and Communicable Disease, University of Michigan Medical School, Ann Arbor, MI.*

Background: The small GTPase Ran and its binding partners, the Importins, regulate transport from the cytoplasm into the nucleus. New evidence suggests that Ran GTP and the Importins are also involved in regulating protein movement into the cilia. Our previous studies found that importin beta2 collaborates with Ran to promote cilia targeting of Retinitis Pigmentosa 2 and the Kif17 motor protein.

Methods: We used Retinal Pigment Epithelial (RPE), Human Primary Airway Epithelial or MDCK cells to study ciliogenesis. Ciliogenesis was induced in RPE cells by serum starvation and in MDCK and Human Primary Airway Epithelia by growing the cells to confluence. Ran GTP levels were measured with Ran GTP specific antibodies or by pull down with GST Importin beta1 followed by immunoblotting with Ran antibodies.

Results: We found that Ran GTP can be localized to centrosomes/basal bodies as well as the tips of cilia. Strikingly, we find that Ran GTP accumulation at basal bodies is coordinated with the initiation of ciliogenesis. The Ran Binding Protein 1 (RanBP1), which accelerates hydrolysis of Ran GTP to Ran GDP also localizes to basal bodies and cilia. To confirm the crucial link between Ran GTP and ciliogenesis, we manipulated the levels of RanBP1 and determined the effects on Ran GTP and primary cilia formation. We discovered that RanBP1 knockdown in RPE cells results in an increased concentration of Ran GTP at centrosomes leading to ciliogenesis. In contrast, overexpression of RanBP1 in RPE cells antagonizes primary cilia formation and attenuates Ran GTP accumulation at centrosomes. Furthermore, we demonstrate that RanBP1 knockdown disrupts the proper localization of Kif17 at the distal tips of primary cilia in MDCK cells.

Conclusions: Our studies illuminate a new function for Ran GTP in stimulating cilia formation and reinforces the notion that Ran GTP and the Importins play key roles in ciliogenesis and ciliary protein transport.

Funding: NIDDK Support

FR-OR249

The Cilia-Associated Protein NPHP4 Translocates Canonical Wnt-Regulator Jade-1 to the Nucleus Lori Borgal,[1] Sandra Habbig,[1,2] Max C. Liebau,[1,2] Claudia Dafinger,[1] Roman-Ulrich Mueller,[1] Thomas Benzing,[1] Bernhard Schermer.[1] *[1]Renal Division, Department of Medicine and Center for Molecular Medicine Cologne, University of Cologne, Germany; [2]Department of Pediatrics, University of Cologne, Germany.*

Background: Nephronophthisis is the most common genetic cause of endstage renal disease in children, with no causative treatment currently available. The disease is characterized histologically by a hallmark triad of tubular basement membrane disruption, interstitial inflammation and fibrosis, and corticomedullary cyst development. Pathogenesis involves the loss of function of one or more nephrocystin proteins (NPHPs), leading to the disease classification of a "ciliopathy" because all NPHPs localize to primary cilia or centrosomes. Cystogenesis is thought to involve over-activation of canonical Wnt signaling, but the mechanism remains unclear.

Jade-1 has recently been identified as a novel ubiquitin ligase targeting beta-catenin for proteasomal degradation. Jade-1 was further shown to be regulated by pVHL. Here, we demonstrate that Jade-1 localizes to the ciliary base, and interacts with NPHP4. NPHP4 stabilizes protein levels of Jade-1 and is involved in the translocation of Jade-1 to the nucleus. Finally, NPHP4 and Jade-1 were demonstrated to additively inhibit Wnt signaling, and this interaction was shown to exist genetically in zebrafish.

The stabilization and nuclear translocation of Jade-1 by NPHP4 enhances the ability of Jade-1 to negatively regulate canonical Wnt signaling. Mutation of NPHP4 in Nephronophthisis could disrupt this mechanism, leading to increased Wnt activation and contributing to cyst formation.

Funding: Government Support - Non-U.S.

FR-OR250

Generation of a Mouse Knockout Model To Functionally Characterize the New Ciliopathy Gene Sdccag8 Rannar Airik,[1] Friedhelm Hildebrandt.[1,2] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, Chevy Chase, MD.*

Background: Nephronopthisis (NPHP) is a heterogenetic autosomal recessive renal ciliopathy that represents the most frequent cause of kidney failure in the first 30 years of life. It is frequently associated with retinal degeneration, liver fibrosis, mental retardation or malformations of brain, heart and bone. We have previously shown that recessive truncating mutations in the human gene SDCCAG8/NPHP10 gene cause NPHP with retinitis pigmentosa (Otto et al., Nat Genet 42:840, 2010) and primary cilia dyskinesia (Dollfus H, unpublished communication). We demonstrated that SDCCAG8 localizes to several different subcellular structures, including centrioles and cell-cell junctions. However, the pathogenic mechanism of SDCCAG8 mutation remains unknown.

Methods: We therefore generated an Sdccag8 knock-out mouse model to study the function of Sdccag8 in tissues affected by its ablation.

Results: Our first data indicate that Sdccag8 is strongly expressed in nephron segments of the cortico-medullary zone of the developing mouse kidney and in bronchioles of the developing mouse lung. Morphologically, Sdccag8$^{-/-}$ mice display hindlimb polydactyly with triphalangeal thumbs. In addition, we demonstrate that Sdccag8 is also localized to nuclear speckles – foci of mRNA synthesis.

Conclusions: The nuclear localization indicates that Sdccag8 may be involved in regulating processes so far not ascribed to NPHP-causing genes. Further understanding of Sdccag8 subcellular and signalling functions will provide valuable insight into the pathogenetic mechanisms of NPHP.

Otto EA, Hurd TW, Airik R, et al. Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. Nat Genet 42:840-850, 2010

Funding: NIDDK Support

FR-OR251

Renal Phenotype of a Mouse Model of HANAC Syndrome Revealing a New Role for Col4a1 in Glomerulogenesis Emmanuelle M. Plaisier,[1] Zhiyong Chen,[1] Migeon Tiffany,[1] Mohamad Zaidan,[1] Dontscho Kerjaschki,[2] Pierre M. Ronco.[1] *[1]INSERM UMRS 702, Tenon Hospital, Paris, France; [2]Medical university of Vienna, Austria.*

Background: We have described a multisystemic dominant syndrome called HANAC (Hereditary Angiopathy, Nephropathy, Aneurysms, and Cramps) related to *COL4A1* mutations. Renal abnormalities include hematuria and renal cysts. To get insight into the pathogenesis of HANAC, we have generated *Col4a1*^{G495V} mutant mice.

Methods: The *Col4a1*^{G495V} mouse strain was generated by homologous recombination, using a targeting construct containing a 16kb *Col4a1* genomic region, in which the c.1706G>T mutation was introduced in exon 25.

Results: Despite intracerebral bleeding and growth retardation at birth, homozygous mice lived through adulthood. At birth, albuminuria and hematuria were detected in homozygous and heterozygous animals. Homozygous neonates showed morphological glomerular defects with ultrastructural abnormalities of podocytes and glomerular

basement membrane (GBM). Focal proximal tubular dilation and cell vacuolization were present. Basement membrane expression of the α1α1α2(IV) molecule persisted at a low level in homozygous mutants, demonstrating that the mutation did not abolish the trimer formation and secretion. Abnormal expression of nephrin was detected in mutants as well as accumulation of the ER stress protein Bip in podocytes. Albuminuria progressively disappeared during the first week and neonate morphological changes resolved. However, at 3 months, renal defects developed in both heterozygous and homozygous animals, that associated bilateral glomerular cysts and large periglomerular and vascular inflammatory infiltrates.

Conclusions: Renal phenotype of the Col4a1^{G495V} mice reveals a potential contribution of the α1α1α2(IV) collagen network to glomerulogenesis. Because HANAC mutations affect an integrin-binding site domain of the α1(IV) chain, and given that glomerular defects observed in Col4a1^{G495V} neonates are similar to the ones observed in α3-integrin deficient mice, we speculate that the α1α1α2(IV) molecule expressed in embryonic GBM, contribute to a developmental pathway through the interaction with the α3β1 integrin expressed in podocytes.

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FR-OR252

Dicer$^{flox/flox}$-Pax8$^{Cre/+}$ Mice a Model for Studying the Role of miRNAs in Renal Cysts Development Anna Iervolino,[1] Francesco Trepiccione,[1] Daniela Frezzetti,[2] Marzia Scarfò,[2] Mario De Felice,[2] Giovambattista Capasso.[1] *[1]Chair of Nephrology, Second University of Naples, Naples, Italy; [2]Institute of Genetic Research, Biogem, Ariano Irpino, Italy.*

Background: MicroRNAs (miRNAs) are small, non-coding, regulatory RNAs that control gene expression at the post-transcriptional level. Dicer1 is a RNAse III protein essential for the biogenesis of active miRNAs. Here we want to assess the role of miRNA in renal cysts development in mice cko for Dicer1.

Methods: We studied mice cko for Dicer1 in Pax8 expressing organ, namely thyroid gland and kidney. We matched Pax8-Cre mice with mice carrying a Dicer floxed allele. The loxP sequences were intercalated around exons 22 and 23 of the Dicer gene, encoding for catalytic RNAseIII domains. Mice carrying both Pax8-Cre and floxed-Dicer alleles had an impairment in miRNA generation.

Results: Dicer cko mice have morphological and functional signs of hypothyroidism. They have a smaller size and a higher mortality rate. Kidneys were smaller than age-matched control mice, but they do not show any difference in KW/BW ratio. At 1 month, histological examination does not demonstrate macroscopic changes as a further evidence of lack of renal organogenesis impairment. At 2 months cko mice have multicystic kidneys. Renal cysts are mainly located in the cortex, but also in outer and inner medulla as well. Some cysts appear as an enlargement of the Bowman'space, containing the glomeruli, others look like more tubular dilatations. Only few cysts present a AQP2 positive staining. Dicer cko mice have a higher cellular turn-over as shown by increased cellular proliferation (ki-67) and apoptosis (cleaved caspase-3) markers. At 2 months cko mice are characterized by increased urine volume associated with reduced urine osmolality. This is coupled with a lower expression of inner medullar AQP2 protein. Dicer cko mice at 2 months are also affected by severe proteinuria.

Conclusions: In conclusion Dicer cko mice have no renal morphological alteration until 1 month from the birth but then, they progressively develop a multicystic kidney phenotype at 2 months. It is therefore evident that our Dicer cko mice are a good model to investigate the miRNA function in cystogenesis.

FR-OR253

Robust Assays Confirm the High Prevalence of Antibodies to LAMP-2 in European Patients with Pauci-Immune FNGN (piFNGN) Andrew J. Rees, Renate Kain. *Clinical Department of Pathology, Medical University of Vienna, Vienna, Austria.*

Background: We reported that antibodies to LAMP-2 were detected in 93% of a cohort of patients presenting with piFNGN but not found once remission had been induced (Nature Med. 14:1088. 2008) but these findings have been challenged. The present study had two purposes: to test the robustness of three independent assays for anti-LAMP-2 antibodies and the concordance between them; and to re-asses the prevalence of antibodies to LAMP-2 in piFNGN in three new groups of European patients from Austria, UK and the Netherlands.

Methods: The patient groups were: newly presenting and relapsing patients from Vienna (n=11) together with healthy and disease controls; cohorts of newly presenting patients with piFNGN (n=55) from Cambridge, UK (kindly provided by Professor K. Smith); and newly presenting and sequentially followed patients from Groningen, Netherlands (n=48) (kindly provided by Professor C Kallenberg and C. Stegeman). Antibodies to LAMP-2 were assayed using three independent tests; an ELISA using unglycosylated recombinant human LAMP-2 extracellular domain expressed in E.coli; Western blots using the same substrate; and indirect immunofluorescence of ldlD cells expressing human LAMP-2 with a mutation in the cytoplasmic lysosmal retrieval signal that targeted it exclusively to the cell surface.

Results: Inter- and intra-assay variation was less than 10% for each assay and all three assays gave the same result in 121 of 144 patient sera. This gives a concordance rate of 84% proving the assays measure authentic antibodies to LAMP-2. We detected antibodies to LAMP-2 in piFNGN in 9/11 (82%) Vienna patients; 38/55 (69%) of the Cambridge cohort; and 40/48 (82%) of the Groningen cohort. Sera taken from the Groningen cohort 1, 2, 3, 6 and 12 months after starting treatment showed rapid resolution of the antibodies which were uniformly negative at 1 month. Subsequently anti-LAMP-2 antibodies became detectable again in 5 patients.

Conclusions: These results confirm a uniformly high prevalence of anti-LAMP-2 antibodies in European cohorts with piFNGN and show the antibody concentrations fall rapidly after the start of treatment.

Funding: Government Support - Non-U.S.

FR-OR254

Human Anti-PR3 ANCA Recapitulate Systemic Vasculitis in Mice with a Humanised Immune System Jeremy S. Duffield,[1] Mark Little,[3] Bahjat Al-Ani,[2] Charles E. Alpers,[1] Caroline O.S. Savage.[2] *[1]Medicine, University of Washington, Seattle, WA; [2]Medicine, Birmingham University, Birmingham, United Kingdom; [3]Medicine, UCL, London, United Kingdom.*

Background: Evidence is lacking for a direct pathogenic effect of anti-proteinase 3 (PR3) antibodies. Progress has been hampered by different PR3 expression patterns in the mouse, the neutrophils of which do not express PR3 on the cell surface, and by the presence of different Fc receptors across the two species. Therefore, we sought to test whether human anti-PR3 ANCA were capable of inducing acute vasculitis in mice with a human immune system.

Methods: We generated chimeric mice by injecting mobilised human haematopoetic stem cells into NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice, which lack functional B, T and NK cells. We confirmed myeloid chimerism Mean achieved human CD45+ cell chimerism was 18.5% of circulating cells (range 6.3-38.2). Both human monocytes & granulocytes were detectable by FACS using human CD11b, CD15 & CD66b antibodies. Human neutrophils were detectable in the bone marrow, with typical c-ANCA and p-ANCA staining demonstrated using human anti-PR3 & anti-MPO antibodies.

Results: We injected IV 4mg of protein G purified human IgG from patients with Renal $ Lung vasculitis (anti-PR3, n=18 mice from 3 donors), patients with non-vasculitic renal disease (disease controls, n=5) and healthy controls (n=3). To maximise myeloid cell recruitment mice were pre-treated with LPS 1500EU/g. By sacrifice on day 6, seven of anti-PR3 treated mice (39%) had haematuria, whereas none of the control animals did. There was punctate haemorrhage on the surface of the lungs of anti-PR3 treated animals, with histological evidence of acute vasculitis and haemorrhage. Anti-PR3 treated mice had mild pauci-immune proliferative glomerulonephritis, with infiltration of human & mouse leukocytes. In 3 mice (17%) more severe glomerular injury was present. There were no glomerular changes in controls. There were no glomerular changes in control mice.

Conclusions: Human anti-PR3 autoantibodies are pathogenic. This model of anti-PR3 associated vasculitis may be useful in dissecting mechanisms of vascular injury.

Funding: NIDDK Support, Private Foundation Support, Government Support - Non-U.S.

FR-OR255

Mast Cells Attenuate Autoimmune Anti-Myeloperoxidase Glomerulonephritis by Mast Cell IL-10 Directed Enhanced T Regulatory Cell Immunosuppression Poh-Yi Gan, Shaun A. Summers, A. Richard Kitching, Stephen R. Holdsworth. *Medicine, Monash Medical Centre, Monash University, Clayton, Victoria, Australia.*

Background: Mast cells (MCs) are pleiotrophic and can release cytokines that mediate effector or regulatory immune responses. We previously showed that MCs are protective in anti-myeloperoxidase (MPO) GN. This study explores the mechanism of this protection.

Methods: Autoimmunity to MPO and anti-MPO GN was examined among MC deficient (Wsh) and Wsh mice reconstituted with WT (WTMC→Wsh) or IL-10-/-MC (IL-10-/-MC→Wsh). MPO immunization with Freunds adjuvant was used to induce autoimmunity and GN was triggered using low dose of anti-glomerular basement membrane antibody.

Results: WTMC→Wsh mice developed significantly less intense anti-MPO autoimmunity and renal injury than Wsh mice confirming that protection is MC dependent (proteinuria; 10.1±1.3 vs 4.8±0.8mg/24hr and glomerular CD4 cells; 0.8±0.1 vs 0.5±0.1c/gcs). MPO autoimmunity measured by dermal DTH (1.1±0.1 vs 0.7±0.1Δmm), CD4 anti-MPO proliferation responses (19.2±2.2 vs 13.3±1.8 x10^{3}counts/min) and IL-17A (15.8±1.3 vs 9.5±1.4ng/ml). The proportion of LN Tregs was decreased in Wsh mice (12.2±1.2 vs 13.4±0.4 %CD4+Foxp3+) all P<0.05.

Reconstitution with IL-10-/-MC offered no protection compared with WTMC→Wsh mice, confirming that protection is MC IL-10 dependent. Proteinuria (13.0±2.2 vs 6.8±0.4) and glomerular CD4 cells (0.8.0±0.1 vs 0.6±0.01). Dermal MPO DTH (0.8±0.1 vs 0.4±0.1), MPO recall proliferation (12.8±1.5 vs 8.1±1.3 x10^3) and IL-17A (7.0±1.8 vs 2.5±0.9).

Tregs (CD4+Foxp3+) and Teffectors (CD4+Foxp3-) were isolated from LNs of MPO immunized Foxp3-GFP mice. The capacity of Tregs to suppress MPO recall responses of Teffs was assessed in the absence and presence of either WT or IL-10-/- MCs. WT MCs induced Treg production of IL-10, TGFβ and enhanced Treg suppression of Teff responses (17.1±1.0 vs 10.6±1.7 x10^{2}counts/min). Whereas IL-10-/- MCs induced no increase in production of IL-10, TGFβ and had significantly less capacity to enhance Treg suppression of Teffectors (15.7±1.3 vs 14.2±17.3 x10^2).

Conclusions: MCs play a protective role in autoimmune anti-MPO GN by enhancing T regulatory cell suppression mediated by MC production of IL-10.

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FR-OR256

Neutrophil Serine Proteases Mediate Anti-Myeloperoxidase Antibody Induced Crescentic Glomerulonephritis Adrian Schreiber,[1,2] Christine T.N. Pham,[3] Friedrich C. Luft,[1] Ralph Kettritz.[1,2] *[1]Experimental Research Center (ECRC), Charite Berlin-Buch, Berlin, Germany; [2]Medizinischen Klinik mit Schwerpunkt Nephrologie und internistische Intensivmedizin am Campus Virchow-Klinikum der Charité, Charite Berlin, Berlin, Germany; [3]Division of Rheumatology, Washington University School of Medicine, St. Louis, MO.*

Background: Anti-neutrophil cytoplasmic antibodies (ANCA) are associated with necrotizing crescentic glomerulonephritis (NCGN) and their pathogenicity has been firmly established in several animal models. Dipeptidyl peptidase I (DPPI) is a cysteine protease required for the activation of neutrophil serine proteases (NSP) cathepsin G (CG), neutrophil elastase (NE), and proteinase 3 (PR3), enzymes that are thought to play an important role in inflammation. We tested the hypothesis that active NSP are essential to anti MPO Ab-induced NCGN.

Methods: An experimental model of anti-MPO induced NCGN was used in which MPO-deficient animals were immunized with murine MPO followed by irradiation and bone marrow (BM) transplantation with MPO-positive bone marrow cells.

Results: Mice transplanted with wild type (WT) BM developed NCGN whereas mice transplanted with DPPI-/- BM were protected (37.4±8.2% crescents in WT vs. 1.3±0.7% crescents in DPPI-/-). This protective effect correlated with inactivation of NSP as mice reconstituted with NE-/-xPR3-/- BM were equally protected against NCGN induced by anti-MPO Ab. Protection was accompanied by a significant reduction in in vivo IL-1β generation by DPPI-/- BM-reconstituted animals (103.0 ± 29.6 pg/ml in WT kidneys vs. 5.9±1.1 pg/ml in DPPI-/-) and in vitro production of IL-1β by anti-MPO Ab-activated protease-deficient monocytes. Lastly, the specific IL-1 receptor antagonist Anakinra protected animals against anti-MPO Ab-induced NCGN (16.7±6.0% crescents in untreated mice vs. 2.4±1.7% crescents in Anakinra-treated mice), suggesting that IL-1β is a critical inflammatory mediator in this model.

Conclusions: Our data strongly suggest that NSP-dependent IL-1β generation is required for the development of anti-MPO Ab-induced NCGN and together NSP and IL-1β may provide novel therapeutic targets for ANCA-mediated diseases in humans.

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FR-OR257

Membranous Nephropathy-Associated Anti-Phospholipase A2 Receptor IgG4 Autoantibodies Activate the Lectin Complement Pathway Hong Ma, Laurence H. Beck, David J. Salant. *Medicine, Boston University Medical Center, Boston, MA.*

Background: IgG4 is the major IgG subclass in the glomerular immune deposits of patients with primary membranous nephropathy (MN). IgG4 antibodies to the phospholipase A2 receptor (PLA2R) also predominate in the serum of most cases of MN, and IgG4 anti-PLA2R can be eluted from their glomerular deposits. Whereas IgG4 does not activate complement via the classical pathway, C3 and C5b-9 are typically present in MN glomerular deposits in the absence of C1q, which suggests that another complement pathway might be involved. Given that mannan-binding lectin (MBL) has been found in MN glomeruli, we hypothesized that IgG4 might bind MBL and activate complement via the lectin pathway.

Methods: IgG4 from normal and MN sera was isolated and IgG4 anti-PLA2R was further purified from the total MN IgG4 by affinity chromatography on an immunoreactive fragment of PLA2R. MBL binding to IgG4 anti-PLA2R was assessed by Far-Western blot analysis and by ELISA on IgG4 anti-PLA2R-coated plates. The ability of IgG4 anti-PLA2R to activate complement via MBL was assessed by generation of C4b in the presence of high salt buffer to inactivate the classical pathway.

Results: For the Far-Western assay, normal human IgG4, IgG4 anti-PLA2R, and MN IgG4 that had been preabsorbed with PLA2R was resolved by SDS-PAGE, transferred to nitrocellulose membranes and incubated with purified MBL. MBL bound strongly to the anti-PLA2R IgG4 band and only weakly to normal IgG4 and MN IgG4 that had been depleted of anti-PLA2R. Under reducing conditions, MBL bound exclusively to the H chain of anti-PLA2R IgG4. ELISA assay further demonstrated that MBL binding to IgG4 anti-PLA2R was significantly greater than to normal control IgG4 (0.317±0.148 vs -0.007±0.007, N=3, P<0.05). Moreover, IgG4 anti-PLA2R significantly activated C4 to C4b in the presence of MBL as compared to control IgG4 (0.193±0.084 vs -0.012±0.012, N=3, P<0.05).

Conclusions: MN-associated IgG4 anti-PLA2R autoantibodies are able to bind MBL directly and activate complement in situ. These findings suggest that the Fc region of anti-PLA2R IgG4 may possess unique properties and explain how complement is activated in the glomeruli of patients with MN.

Funding: NIDDK Support, Other NIH Support - NIAID

FR-OR258

Exosomes Released from Dendritic Cells Are Taken up by Kim-1-Expressing Proximal Tubule Cells and Increase Cellular MHC-II Protein Levels Dhruti D. Patel,[1,2] Suetonia Palmer,[1] Craig R. Brooks,[1] Takaharu Ichimura,[1] Joseph V. Bonventre.[1] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]School of Medicine, University of North Carolina, Chapel Hill, NC.*

Background: Chronic kidney disease is often marked by an infiltration of inflammatory cells. The mechanisms underlying the facilitation of this inflammatory response and the role of antigen presentation by the proximal tubule remains incompletely defined. Kidney Injury Molecule 1 (Kim-1) recognizes phosphatidylserine and converts proximal tubule cells to phagocytes. We evaluated whether Kim-1 mediates uptake of exosomes released from denditic cells and whether this process results in enhanced proximal tubule presentation of antigen by upregulation of major histocompatibility complex class II (MHC-II).

Methods: The relationship between Kim-1 and MHC-II expression was determined by immunocytochemical techniques in a novel transgenic animal model of Kim-1 expression which results in chronic kidney disease. Isolated exosomes from cultured mouse dendritic cells were presented to proximal tubule cells. MHC-II and Kim-1 co-expression was evaluated by flow-cytometry and MHC-II RNA expression levels through real time PCR.

Results: In the animal model there was a marked increase of MHC-II expression in tubular cells expressing Kim-1. After treatment of proximal epithelial cells in culture with exosomes derived from DC, MHC-II expression was increased in the tubular cells, specifically in cells expressing higher levels of Kim-1. There was no significant difference in MHC-II mRNA expression levels, indicating the protein is transferred from exosomes, and not newly synthesised in the Kim-1 expressing epithelial cells.

Conclusions: Dendritic cell-derived exosomes confer antigen-presentation capabilities to proximal tubule cells. This process is facilitated by Kim-1 mediated uptake of exosomes from dendritic cells, resulting in the transfer of MHC-II to tubular cells. Increasing the antigen presentation capability of renal tubule cells through transfer of exosomes after injury may lead to propagation of inflammatory changes resulting in fibrosis and eventual renal failure.

Funding: NIDDK Support

FR-OR259

Autocrine Adenosine 2A Receptor Signaling Promotes PD-1 Expression and Innate Regulatory T Cell Function during Ischemic Kidney Injury Gilbert R. Kinsey, Liping Huang, Konstantine Khutsishvili, David Becker, Hong Ye, Peter I. Lobo, Mark D. Okusa. *Department of Medicine and Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia.*

Background: Regulatory T cells (Tregs) suppress innate inflammation and injury associated with kidney ischemia-reperfusion injury (IRI). Tregs express CD73, which is the final enzyme in production of extracellular adenosine and previous studies have demonstrated that adenosine 2A receptor ($A_{2A}R$) activation on immune cells inhibits inflammation and preserves kidney function after IRI. We hypothesized that the production of adenosine by Tregs is required to block innate immune responses in kidney IRI and that the Treg-generated adenosine would signal through $A_{2A}Rs$ in an autocrine manner on Tregs.

Methods: Freshly-isolated $CD4^+CD25^+$ Tregs from WT, $A_{2A}RKO$ or CD73KO mouse spleen were adoptively-transferred to naive C57/Bl6 (WT) mice 18 hr prior to bilateral renal ischemia or sham surgery. Kidney function was estimated by plasma creatinine measurement and kidney inflammation was assessed by flow cytometry and immunohistochemistry. The $A_{2A}R$ specific agonist ATL1222 (0.1 to 30 nM) and $A_{2A}R$ specific antagonist ZM241385 (100nM) were used *in vitro* to investigate the role of $A_{2A}R$ signaling in Tregs.

Results: Adoptively-transferred WT Tregs protected WT mice from kidney IRI, but in the absence of adenosine generation (CD73KO Tregs) or $A_{2A}Rs$ ($A_{2A}RKO$ Tregs), Treg function was completely inhibited. *In vitro* activation of $A_{2A}Rs$ on CD73KO Tregs, prior to adoptive transfer, restored their protective ability, and augmented the ability of WT Tregs to suppress kidney IRI (an effect blocked by pretreatment with ZM241385). ATL1222 dose-dependently enhanced surface expression of the negative co-stimulatory molecule PD-1 on Tregs. PD-1 blocking antibody treatment of Tregs, prior to adoptive transfer, reversed their protective effects *in vivo,* even if they had been pre-treated with ATL1222.

Conclusions: Taken together these findings demonstrate that the simultaneous ability to generate and respond to adenosine is required for Tregs to suppress innate immune responses in ischemia-reperfusion injury through a PD-1-dependent mechanism.

Funding: NIDDK Support

FR-OR260

Critical Role of the Chemokine Receptor CXCR6 for Renal NKT Cell Localization and Function in Murine Crescentic Glomerulonephritis Jan-Hendrik Riedel,[1] Hans-Joachim Paust,[1] Jan-Eric Turner,[1] Erik M. Disteldorf,[1] Joachim Velden,[2] Hans-Willi Mittrücker,[3] Rolf A. Stahl,[1] Oliver M. Steinmetz,[1] Ulf Panzer.[1] *[1]Universitätsklinikum Hamburg Eppendorf, III. Medizinische Klinik, Hamburg, Germany; [2]Universitätsklinikum Hamburg Eppendorf, Institut für Pathologie, Hamburg, Germany; [3]Universitätsklinikum Hamburg Eppendorf, Institut für Immunologie, Hamburg, Germany.*

Background: The chemokine receptor CXCR6 is expressed on T cells and Natural Killer T (NKT) cells and might play a role in trafficking or activation of these cells via its unique chemokine ligand CXCL16.

Methods: To investigate the role of CXCR6 in renal inflammation we induced a T cell-dependent model of glomerulonephritis (nephrotoxic nephritis) in BL6/CXCR6$^{-/-}$ and BL6 WT mice.

Results: Induction of nephritis resulted in upregulation of renal CXCR6 and CXCL16 mRNA expression. CXCL16 expression peaked between days 7-10 and was mostly detectable in periglomerular and tubulointerstial areas. Unexpectedly CXCR6$^{-/-}$ mice developed an aggravated course of nephritis (day 8) in terms of T cell recruitment, tissue injury, serum creatinine and BUN level compared to WT mice. In the inflamed kidney CXCR6 was highly expressed (> 90%) on invariant NKT (iNKT) cells and to a much lesser degree on CD4 and CD8 T cells. In contrast to the enhanced numbers of T cells, the infiltration of iNKT cells into the kidney was markedly reduced in nephritic CXCR6$^{-/-}$ mice. RT-PCR and FACS analyses revealed the production of anti-inflammatory IL-4 and TGFβ but no proinflammatory IL-17 by renal iNKT cells, supporting their protective role. To assess the function of CXCR6^{+} NKT cells, FACS-sorted TCRb^{+}NK1.1^{+} NKT cells from livers of WT mice (purity >95%) were transferred into WT and CXCR6$^{-/-}$ mice (10^6 NKT cells/animal) followed by nephritis induction. The transfer of CXCR6^{+} NKT cells resulted in an almost identical course of nephritis (day 8) in WT and CXCR6$^{-/-}$ mice (assessed by morphological and functional parameters) demonstrating the protective role of CXCR6^{+} NKT cells in glomerulonephritis.

Conclusions: Nephritic CXCR6$^{-/-}$ mice had a defect in the recruitment of protective NKT cells into the kidney, resulting in an accelerated course of nephritis.

Funding: Government Support - Non-U.S.

FR-OR261

Complement C3a Induces IL-1beta Production in Human Monocytes Which Leads to Th17 Lineage Decisions Elham Asgari, Steven H. Sacks, Esperanza Perucha, Claudia Kemper. *MRC Centre for Transplantation, King's College London, London, United Kingdom.*

Background: IL-1beta is among the most potent pro-inflammatory cytokines and mediates important immune functions such as promoting Th17 linage commitment. Monocytes/macrophages are the major IL-1beta sources. IL-1beta secretion by these cells requires TLR (LPS) and P2X7-receptor (ATP) signals, which in turn activate the inflammasome. However, how exactly LPS signals and ATP availability are regulated during monocyte activation is unclear and the requirement for a second danger signal has long been proposed. Considering the importance of anaphylatoxins C3a and C5a in innate immunity, we hypothesised that they participate in IL-1beta production.

Methods: Freshly isolated monocytes have been stimulated with C3a or C5a with and without LPS at different concentrations and time points and IL-1beta measured as an indication of inflammasome activation. Relationship of inflammasome activation with pannexin 1 channel has been investigated by inhibition of the channel with carbenoxolone.

For assessing Th17 induction, activated monocyte supernatants were used in T cell cultures and IL-17 production was measured.

Results: Both LPS and C3a were absolutely required for IL-1beta production in human macrophages while in monocytes, C3a increased LPS-induced IL-1beta dramatically. Neither C3adesArg, nor C5a showed any effect on IL-1beta production. We suggest that C3a drives IL-1beta production by controlling the release of intracellular ATP into the extracellular space via regulating the function of the ATP-releasing channel pannexin 1.

Importantly, we found that C3a/LPS-stimulated monocytes induce strong Th17 cell induction in in vitro cultures.

Conclusions: Our data indicate that C3aR-mediated signalling events are important components of the IL-1beta/Th17 axis in humans. This has significant implications in IL-17-driven disease states such as rheumatoid arthritis or asthma as well as kidney transplantation where Th17 cells have been shown to participate in allograft rejection and causing resistance to tolerance induction.

Funding: Private Foundation Support

FR-OR262

Role of a Novel Rat-Specific Fc Receptor in Macrophage Activation Associated with Crescentic Glomerulonephritis Jacques Behmoaras,[3] Theresa H. Page,[1] Zelpha D'souza,[2] Charles D. Pusey,[3] Timothy J. Aitman,[2] H. Terence Cook.[3] *[1]Kennedy Institute of Rheumatology; [2]MRC Clinical Sciences Centre; [3]Renal Medicine, Imperial College London, United Kingdom.*

Background: Crescentic glomerulonephritis (Crgn) is a complex disease where the initial insult is often the glomerular deposition of antibodies against intrinsic or deposited antigens in the glomerulus. The role of Fc receptors in the induction and progression of Crgn is increasingly recognised and our previous studies have shown that copy number variation in *Fcgr3* partially explains the genetic susceptibility of the Wistar-Kyoto (WKY) rat to nephrotoxic nephritis (NTN), a rat model of Crgn. The Fcgr3-related sequence (*Fcgr3-rs*) is a novel rat-specific Fc receptor with a cytoplasmic domain 6 amino acids longer than its paralogue, *Fcgr3*. The Fcgr3-rs gene is deleted from the NTN-susceptible WKY rat genome and this deletion is associated with enhanced macrophage activity in this strain. Here, we have investigated the mechanism by which the deletion of *Fcgr3-rs* in the WKY strain leads to increased macrophage activation.

Methods: By lentivirus-mediated gene delivery, we have generated stably transduced human U937 cells expressing either *Fcgr3-rs* or *Fcgr3*.

Results: In these cells, which lack endogenous Fcgr3 receptors, we show that Fcgr3-rs interacts with the common Fc-gamma chain but that Fc receptor-mediated phagocytosis and signalling are defective. Furthermore, in primary macrophages, expression of *Fcgr3-rs* inhibits Fc-receptor mediated functions, as WKY bone marrow derived macrophages (BMDMs) transduced with *Fcgr3-rs* had significantly reduced phagocytic activity. This inhibitory effect on phagocytosis was mediated by the novel cytoplasmic domain of *Fcgr3-rs*.

Conclusions: In conclusion, these results suggest that the rat specific *Fcgr3-rs* may act to inhibit *Fcgr3*-mediated signalling and phagocytosis and could be considered as a novel mechanism in the modulation of Fc receptor mediated cell activation in autoimmune diseases.

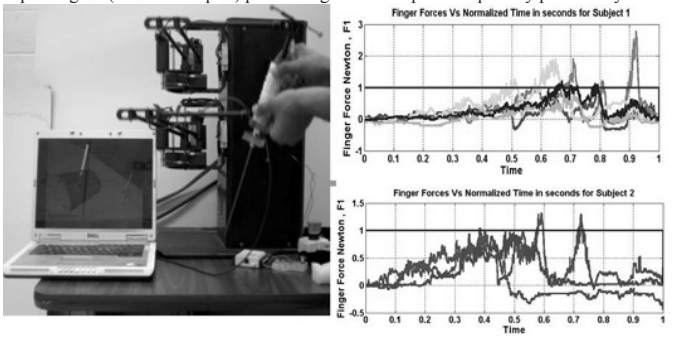
FR-OR263

Quantitative Skill Evaluation for Kidney Biopsies Sudha Garimella,[1] Madusudanan Sathia Narayanan,[2] Xiaobo Zhou,[2] Seung-Kook Jun,[2] Wayne R. Waz,[1] Frank C. Mendel,[3] Venkat N. Krovi.[2] *[1]Pediatric Nephrology, State University of New York at Buffalo, Buffalo, NY; [2]Mechanical and Aerospace Engineering, State University of New York at Buffalo, Buffalo, NY; [3]Pathological and Anatomical Sciences, State University of New York at Buffalo, Buffalo, NY.*

Background: Certification in kidney biopsy procedures requires both cognitive and sensorimotor skill acquisition which has been linked to duration, realism and diversity of training exposure. There is a critical need for a comprehensive and realistic training system which also facilitates monitoring and evaluation of procedural skills.

Methods: We created SIMBiopsies, a visual and kinesthetic environment that mimics the look and feel of a kidney biopsy, to allow trainees to improve their skills while performing simulated biopsies. A mock-biopsy needle mounted on a haptic device produces the appropriate 'feel' as the trainee inserts a needle into the virtual model of a Blue Phantom®, a block of tissue-like gel with inclusions of denser gel (to mimic different tissues). This virtual model was created by coupling material-testing data with a 3D visual model of the Blue Phantom.

Results: A series of needle insertion studies were carried out by two nephrologists and sample characteristic haptigrams (force-motion profiles) were recorded. We are currently working on comparing, cataloging and validating captured haptigrams of a range of nephrologists (novice to expert) performing virtual biopsies to quantify proficiency.



Conclusions: The SIMBiopsies virtual immersive environment offers a convenient interactive means to develop, practice and quantitatively assess biopsy skills. By overcoming various economic, logistical and safety issues that hinder extensive conventional training on real patients, we anticipate it will also provide a standardized means for certification in this critical skill.

Funding: Clinical Revenue Support

FR-OR264

Education Intervention Increases Patient Knowledge about Chronic Kidney Disease Julie A. Wright, Jane H. Greene, Kenneth A. Wallston, Tom A. Elasy, Svetlana Eden, Ayumi Shintani, Russell Rothman, Talat Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt University Medical Center, Nashville, TN.*

Background: We evaluated the impact of a 2-3 minute, physician delivered, literacy sensitive education worksheet on patient knowledge about chronic kidney disease (CKD).

Methods: Adult patients with CKD Stages 1-5, seen in nephrology clinic, were enrolled into an education intervention pilot (April-October 2010) and compared to a historical cohort (April-October 2009). A validated survey assessing CKD awareness and knowledge was given to patients in both the pilot intervention group and historical cohort. Areas of low knowledge from the historical cohort informed development of a patient CKD education worksheet (adapted from: National Kidney Disease Education Program). This worksheet was reviewed by nephrologists with patients during appointments for the pilot intervention. The proportion of patients correctly answering survey questions was compared between groups.

Results: 401 patients were in the historical cohort, and 155 received the intervention. The mean (SD) age of the combined population (N=556) was 57 (16) years. 53% were male, 81% White, and 78% had CKD Stage 3-5. There were no significant differences in patient demographics by intervention status. Unadjusted analysis showed a higher proportion of patients receiving the intervention were aware of CKD diagnosis (78% vs. 69%; p=0.04), and correctly defined "GFR" (85% vs. 68%; p<0.001), their kidney function (68% vs. 49%; p<0.001), and their stage of CKD (65% vs. 36%; p<0.001). Compared to controls, patients receiving the intervention had higher odds of knowing they had CKD [OR 2.20, CI 1.16-4.17; p=0.016], of knowing their kidney function [OR 2.25, CI 1.27-3.97; p=0.005], and of knowing their stage of CKD [OR 3.22, CI 1.49-6.92; p=0.003] in

analyses adjusted for age, sex, race, health literacy, income, number of provider visits, eGFR, and knowing someone with CKD. 80% of patients deemed the worksheet helpful, and 98% recommended it for future use.

Conclusions: A physician delivered education intervention taking less than 3 minutes, increased patient knowledge on topics important to understanding CKD diagnosis.

Funding: NIDDK Support, Other NIH Support - T32 DK007569, Private Foundation Support

FR-OR265

International Society of Nephrology Sister Renal Center Program between Gold Coast Hospital and Adam Malik Hospital, North Sumatra, Indonesia

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Background: Gold Coast Hospital (GCH) renal department has provided education and experience to hospitals in the Tsunami devastated North Sumatra. This partnership was formalized in 2011 by ISN SRC program and a team comprising Nephrologist and Advanced trainee spent 1 week in Medan and Banda Ache, assessing the needs of the department and educating clinicians in areas of interest and concern in order to build strong partnership and advance the practice of Nephrology in North Sumatra.

Methods: GCH team toured both major public hospitals in Medan. We made ward and dialysis rounds with the renal doctors to get a thorough understanding of the renal service and the medical system. During these, diagnostic and treatment protocols present in Medan public hospitals were reviewed directly with the local clinicians. The GCH team compared these protocols or the lack thereof with those established in GCH. An assessment was presented to the department and a plan to improve the service and practice of nephrology was constructed. Regular teaching and meetings between the two centers via videoconference should commence within 3 months.

Results: We identified that renal biopsy service was virtually non-existent in North Sumatra. After deliberation with the renal and pathology departments, renal biopsy process was reviewed and encouraged. Ultrasound guided biopsy technique was taught and a post biopsy observation protocol was implemented. Also, prevalence of AV fistulas and access surveillance was low. Use of access blood flow is now being implemented.

Conclusions: Appropriate diagnosis and directed treatment of renal conditions was found to be lacking in North Sumatra and was strongly related to the poor renal biopsy service. This service is being developed while collaborating with Gold Coast Hospital.

FR-OR266

Patient's Perception of Life on Hemodialysis Scale: Instrument Development and Psychometric Evaluation

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Background: Using a grounded theory approach the overall psychosocial and physiological experience of patients with treated with in-center hemodialysis was examined. Three theoretical constructs emerged from the research: Physical Health, Quality of Supports, and Psychosocial Health. Researchers developed a 64 item rating instrument titled the Patient's Perception of Hemodialysis Scale (PPHS) to measure these concepts.

Methods: Using a cross-sectional design, data collection was completed in the hemodialysis units in two Canadian provinces (N =236). The purpose of the research was to examine data quality, internal consistency, reliability and validity of the instrument.

Results: Limited missing data and good ceiling/floor statistics for each question were observed. Following correlation of items within each scale the number of questions was reduced by 28. Five scales were identified: Physical Health, Emotional Well-being, Psychosocial Distress, Nurse Support, and Physician Support. The Cronbach's alpha for the new scales was 0.70-0.89, suggesting good internal consistency.

Construct validity was examined using factor analysis, with a principal component approach and varimax rotation. The total factor structure explained 51% of the variance, and eigenvalues implied no further item reduction was necessary. Convergent validity was confirmed for Physical Health, Emotional Well-being and Psychosocial Distress by comparison with SF-36, and divergent validity by the poor correlation of Nurse and Physician support scales with SF-36. Test-retest analysis demonstrated that the instrument was stable over time and intraclass correlation ranged from 0.72 to 0.94.

Conclusions: The PPHS is a valuable instrument for measuring disease specific concerns as patients experience hemodialysis.

Funding: Kidney Foundation of Canada

FR-OR267

US Non-Renal Internal Medicine Subspecialty Fellow Survey on Nephrology

Seyyar A. Khan,[1] Matthew A. Sparks,[3] Arun Chawla,[1] Emily L. Petersen,[2] Hitesh H. Shah,[1] Mark G. Parker,[4] Donald E. Kohan,[2] Kenar D. Jhaveri.[1] *[1]Nephrology, Hofstra North Shore-LIJ School of Medicine; [2]University of Utah; [3]Duke University; [4]Maine Medical Center.*

Background: Nephrology is facing a workforce shortage in the U.S, particularly among U.S. medical graduates. We designed a survey to explore the reasons why fellows from other internal medical subspecialties did not choose nephrology.

Methods: The anonymous survey was created online using SurveyMonkey in May 2011 and was distributed to all non-renal fellowship program directors in the US. The survey contained 11 questions regarding nephrology and choices of fellowship.

Results: Preliminary results have a 10% response rate with 650 subspecialty fellows responding. Over 75% of fellows did not know that a shortage of nephrologists is estimated to develop by 2020. When asked if nephrology was the most difficult physiology course in their medical school, 68.4% said "no" . 26.5% had considered nephrology as a career at some point and only 11% indicated that nephrology was their second choice. 40.9% of endocrinology fellows had considered nephrology at one point ($P<0.0072$) and GI fellows (17.7%) were least likely to consider nephrology($P<0.05$). For those who had considered nephrology, reasons for not choosing included: 86.8% "fell in love with another field", 16.2% were concerned about work hours and 16.2% felt that compensation was not adequate. Interestingly, 23.9% would have considered nephrology if it were taught better and 23% would have considered nephrology if nephrologists had higher incomes. When asked what they did not like about nephrology, 37.3% said that "dialysis and transplant patients are too complicated to take care of" and 29.1% said "No role model or mentor to guide me towards nephrology."

Conclusions: The majority of non-renal fellows never considered nephrology as a career choice. Among those who considered a nephrology career, a significant proportion was dissuaded by several factors, including lifestyle, income, patient complexity and lack of role models. Each of these factors can be addressed, but will require the concerted efforts of nephrologists throughout our training community.

FR-OR268

Pediatric Nephrology as a Specialty: Survey of USA Non-Renal Pediatric Fellows

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Background: The U.S. pediatric nephrology workforce is aging with an insufficient number of graduating fellows. The purpose of this survey is to assess why non-renal fellows choose areas of concentration other than nephrology.

Methods: A web-based self-administered survey was sent to all accredited ACGME pediatric fellowship programs. The survey focused on the perceived difficulty of nephrology as a discipline and factors involved in fellowship choice.

Results: Of the 313 responses, 294 (94%) were from pediatric programs; 194 (62%) females; and 236 (75%) graduated from USA schools. When asked about how difficult nephrology was, 194 (59%) respondents said it was difficult or very difficult. For all respondents, the odds of reporting nephrology as difficult among females was 1.3 times higher than among males and 2.3 higher for USA graduates. The odds of reporting nephrology as difficult among fellows in a non-acute specialty is 8% higher than among those in an acute specialty. Acute kidney injury and hypertension were rated with the lowest levels of difficulty whereas renal tubular acidosis, dialysis and transplantation had the highest.

At one point, 15% of respondents considered nephrology. Of those who did not consider nephrology, 80% stated that they were not attracted to the field because they did not like the work hours, patients/families, monetary benefits or pediatric nephrologists (10% each). When asked what would have made them consider nephrology, the most common responses were: more appealing teaching sessions (30%); higher income (16%); if they had not matched into their current specialty (11%).

Conclusions: Overall, pediatric fellows in non-renal specialties rated nephrology as a difficult specialty. This perception is stronger among females. Efforts to make pediatric nephrology more appealing to medical students and residents are necessary to address the perception of this specialty and deter gender disparities in the future workforce.

FR-OR269

Creative Nephrology Elective during Medical Residency: A Single Center Experience

Kenar D. Jhaveri, Peter B. Schrier, Hitesh H. Shah. *Nephrology, Hofstra North Shore- LIJ School of Medicine, NY.*

Background: Traditionally, most institutions offer a "pure" inpatient consult rotation as a standard elective for internal medicine residents and medical students (MS). Embarking on a combined inpatient (IP) and outpatient (OP) form of elective might spark more interest and give a better " flavor of nephrology" to the trainees.

Methods: From 2009-present, we randomly assigned medical residents and students taking the renal elective at our institution to either a "pure" inpatient form of elective group (Group A) or a combined elective group (Group B). The combined elective started with 2 weeks of IP consult rotation followed by 2 weeks of OP rotation. The OP rotation included: HD & PD unit rounding, several renal(glomerular, ckd, stones) and kidney transplant clinic sessions, and a research session with a faculty member. The trainees also completed a small assignment using games or concept maps and shared in a small group setting. After the elective,all trainees were asked to complete an anonymous survey of their nephrology rotation experience.

Results: 26 trainees completed the survey, 38.5% PGY1, 34.6% PGY3, 19.2% PGY2 and 7.6% were MS. 68% did a "pure" inpatient elective (Group A). In this group, 47.5% felt that a combined elective would have been more beneficial and 63% felt that they would have learned more renal medicine if they had done a combined elective. 50% of group A would consider a career in nephrology. 75% of group A had exposure to 2 faculty mentors. 32% of the trainees did a combined elective (Group B). 80% of group B thought that they had learned more renal medicine as a result of the combined experience and all 100% of them

would not have chosen to do a "pure" inpatient elective. 80% would consider a career in nephrology in group B. 60% of them worked with more than 3 faculty mentors. All trainees felt the need for combined electives and more mentorship by nephrologists.

Conclusions: Combined inpatient and outpatient nephrology elective during medical residency training with increase exposure to several nephrology faculty members seems to create a more positive and enriching experience perhaps leading to a more likelihood of choosing nephrology as a career choice.

FR-OR270

Career Choice Selection and Satisfaction among US Nephrology Fellows

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Background: Interest in obtaining training in nephrology continues to decline. We wanted to find out why current fellows selected nephrology and if they are satisfied with this choice. We conducted an online survey with current US nephrology fellows.

Methods: The anonymous survey was created online using SurveyMonkey in May 2011 and was subsequently distributed to US nephrology training program directors.

Results: On initial evaluation, 137 respondents to our survey (10% of total nephrology fellows in the US), of which 55.1% were graduating from their training program. 41.4% were US medical school graduates. 65.8% chose nephrology during either their 1st or 2nd year of residency; 24.3% made this decision during medical school. The most common reasons for choosing nephrology included: interesting subject matter and excellent mentoring in nephrology. Interesting, only 55.7% of respondents were either extremely or very satisfied with their decision to enter nephrology fellowship. 32.8% were somewhat or slightly satisfied and 11.4% were not satisfied at all with their decision. Statistically, no differences were seen between graduating and non-graduating fellows or US and non-US medical school graduates. The top reasons respondents cited for being extremely or very satisfied with nephrology included: excellent teaching and mentoring by faculty, "variety of stimulating cases" and association between general medicine and nephrology. In contrast, top reasons that respondents were somewhat, slightly or not satisfied with nephrology were: poor income and job potential, and long work hours.

Conclusions: Majority of the current US nephrology fellows selected nephrology as their career choice during medical residency training. Strong mentorship and teaching was highly associated with both the decision to enter nephrology as well as satisfaction during nephrology fellowship. Reasons for less or no satisfaction with nephrology as a career choice included poor job opportunities, poor income potential, long work hours and poor teaching and mentoring by faculty. The nephrology training community should take measures to ensure higher level of satisfaction among US nephrology fellows.

FR-OR271

Using Community Kidney Disease Detection (CKDD) as a Community-Based Platform for Student Leadership Cultivation in Nephrology

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Background: Chronic Kidney Disease (CKD) is a major public health problem. Patient awareness of CKD is very low especially among the racial minorities, who often have poorer access to health care and are particularly vulnerable. However, despite the growing significance of CKD, there exists a disproportionate shortage of nephrologists and a diminishing interest in the field of nephrology that raise concerns about adequate renal health care provisioning in the future.

Methods: Here, we present Community Kidney Disease Detection (CKDD), a community outreach program for undergraduate students, as a viable solution for promoting prevention of CKD whilst cultivating student leadership and exposure to nephrology. CKDD holds monthly renal health screenings in underserved areas in Greater Boston to facilitate awareness and early detection of CKD. Such health screenings are entirely run by student volunteers, and hence offer undergraduates an unparalleled hands-on clinical experience and exposure to nephrology. CKDD also provides extensive pre-medical training by hosting workshops attended by nephrologists who teach and certify students for knowledge and command of a range of medical techniques, which include practicing universal precaution, obtaining pertinent medical history, measuring blood pressure, operating glucometers, and conducting urinalysis. At each health screening, students have the opportunity to undertake leadership roles as the clinic manager or as station leaders, through which they can develop leadership skills important for the medical career.

Results: Since its establishment in 2007, CKDD has recruited a stable volunteer base of more than 200 undergraduates, of which 78 students have been successfully trained and certified. Our preliminary data showed that the majority of participants found CKDD to be valuable and graded 4.5 out of 5 on CKDD's services.

Conclusions: Collectively, CKDD serves as an effective platform for undergraduate student's community outreach for CKD prevention, promoting student leadership, recruitment of interest, and exposure to nephrology.

Funding: Other NIH Support - sundry fund

FR-OR272

Examining the Role of Education of Nephrology Fellows in Utilization of Peritoneal Dialysis in the United States

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Background: The 2009 US Renal Data System annual report revealed that peritoneal dialysis (PD) was used by only 7.2% of end stage renal disease (ESRD) patients (vs. use of hemodialysis (HD) by >90% of patients), a decline from a 1984 peak of 15%. We examined whether education of nephrology fellows contributed to the underutilization of PD in the US.

Methods: Self-report questionnaires were administered via Survey Monkey to nephrology fellowship training program directors during October 2010-March 2011. Names/addresses of all US training program directors were obtained from the American Society of Nephrology list-serve. Project protocol was approved by the Stony Brook University IRB. Statistical analyses included: descriptive statistics (frequencies and proportions) and Wilcoxon signed-rank tests for comparisons.

Results: 55% (78) of program directors responded. Median number of training faculty and patients/fellow were significantly lower for PD training vs. HD training (0.5 vs. 1.2, $p<0.001$ and 5 vs. 37.5, $p<0.001$; respectively). Hours of didactic teaching for fellows over their 2 year training period was also lower for PD vs. HD (6 vs. 10 hours, $p<0.001$). Most nephrology programs provide training in PD and HD; however, PD training was only 20% of total training vs. 80% for HD. Among program directors, 76% indicated that >5% of PD training and 81% indicated that >5 PD patients/fellow were adequate for PD training over a 2 year training period. 87% believed that PD training for fellows was inadequate because of lack of trained faculty in PD and insufficient PD patient population. 67% reported that fellows did not participate in ESRD/chronic kidney disease education to patients.

Conclusions: Current US nephrology fellowship training in PD is inadequate and contributes significantly to the underutilzation of PD. The adequacy of PD training resources needs vigorous discussion and evaluation by the nephrology community.

Funding: Private Foundation Support

FR-OR273

Association of Serum Glycated Albumin and Fructosamine with Outcomes in Incident Hemodialysis Patients

Tariq Shafi,[1] Stephen M. Sozio,[1] Bernard G. Jaar,[1] Rulan S. Parekh,[2] Laura Plantinga,[3] Neil R. Powe,[3] Josef Coresh,[1] Elizabeth Selvin.[1] *[1]Johns Hopkins University; [2]University of Toronto; [3]University of California, San Francisco.*

Background: Serum total glycated proteins (fructosamine) and the more specific glycated albumin may be better indicators of glycemia in dialysis patients compared with hemoglobin A1C as they are not affected by red cell turnover. However, the association of glycated albumin and fructosamine with long-term outcomes in dialysis patients is not well-described.

Methods: We measured baseline levels of glycated albumin and fructosamine in 503 incident hemodialysis (HD) participants of the CHOICE Study, a national prospective cohort study. The association of these glycemic markers with mortality and infection-related hospitalizations were determined using Cox proportional hazards and Poisson models, adjusting for potential confounders.

Results: Mean age was 58 years; 64% white; 54% male; and 57% had diabetes. Higher baseline levels of glycated albumin and fructosamine were independently associated with higher risk of death in patients with and without diabetes over a median follow-up of 3.1 years. There was a non-significant trend towards higher risk of infection-related hospitalizations.

Association of Glycemic Markers and Outcomes in 503 Incident HD Patients

	Diabetes (n=287)		No Diabetes (n=213)	
	Glycated Albumin %	Fructosamine	Glycated Albumin %	Fructosamine
Mean±SD	24±9%	360±94 umol/L	14±2%	271±46 umol/L
All-Cause Mortality*	**1.22(1.02-1.45)**	**1.24(1.05-1.46)**	**1.29(1.03-1.61)**	1.28(0.99-1.67)
Cardiovascular Mortality*	1.15(0.90-1.47)	1.15(0.90-1.47)	1.35(0.97-1.86)	**1.86(1.13-3.06)**
Infection-Related Hospitalizations†	1.12(0.98-1.28)	1.09(0.96-1.24	0.85(0.66-1.08)	1.01(0.84-1.20)

*HR per SD increase adjusted for age, sex, race, smoking, BMI, pulse pressure, comorbidities, albumin, hemoglobin and CRP. †Incidence rate ratio per SD increase adjusted for age, sex, race and comorbidities.

Conclusions: In this national cohort, glycemic markers glycated albumin and fructosamine were associated with adverse outcomes in HD patients with and without diabetes. Measurements of these serum glycemic markers may be useful for management of diabetes in dialysis patients.

Funding: NIDDK Support, Private Foundation Support

FR-OR274

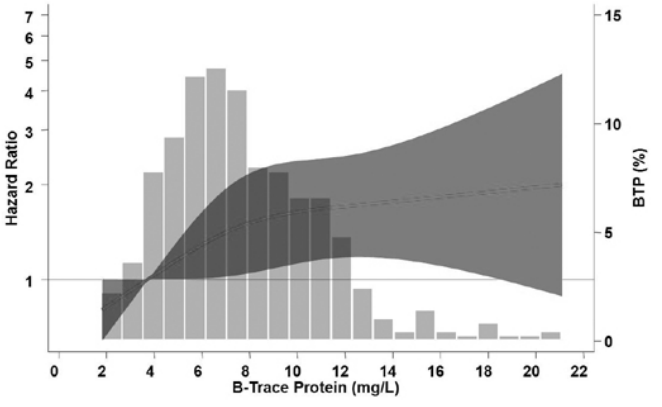
Serum Beta-Trace Protein Marks Low Residual Kidney Function and Risk of Mortality in Incident Hemodialysis Patients Tariq Shafi,[1] Rulan S. Parekh,[2] Bernard G. Jaar,[1] Laura Plantinga,[3] Pooja C. Oberai,[1] John H. Eckfeldt,[4] Andrew S. Levey,[5] Neil R. Powe,[3] Josef Coresh.[1] *[1]Johns Hopkins University; [2]University of Toronto; [3]University of California, San Francisco; [4]University of Minnesota; [5]Tufts Medical Center.*

Background: Residual kidney function (RKF) in dialysis patients is associated with improved survival, but there are no simple methods for assessing RKF. Beta-trace protein (BTP) is a novel endogenous filtration marker of kidney function that is not removed during hemodialysis and may serve as a marker for RKF, similar to serum creatinine in patients not on dialysis.

Methods: We measured serum BTP in 503 participants of the CHOICE Study, a national prospective cohort of incident dialysis patients. Outcomes analyzed were all-cause and cardiovascular disease (CVD) mortality using Cox proportional hazards regression adjusted for demographic (age, race, gender, education, marital status and employment) and clinical and treatment factors (smoking, pulse pressure, body mass index, cause of kidney failure, Index of Coexistent Disease (ICED) score (0-3), cardiovascular disease, congestive heart failure, left ventricular hypertrophy, diabetes, serum albumin).

Results: Serum BTP levels were higher in individuals with no self-reported urine output at baseline (p<0.001) or year 1 (p=0.001).There were 321 deaths (159 from CVD) over 1,814 person-years of follow-up. Higher BTP levels were independently associated with higher risk of all-cause and CVD mortality.

Figure 1: Adjusted Relative Hazard of All-cause Mortality with Serum Beta Trace Protein (BTP) in 503 Incident Hemodialysis Participants of the CHOICE Study.



Bars, distribution of serum BTP; **Line**, association of serum BTP with mortality, modeled as a cubic spline.

Compared with the lowest tertile, adjusted HR and 95% CI for all-cause mortality in the middle and highest tertile were: 0.95 (0.69-1.32) and 1.72 (1.25-2.37); p-value for linear trend 0.001. Similar results were noted for CVD mortality.

Conclusions: The serum level of BTP, a novel marker of residual kidney function, is an independent predictor of death and CVD mortality in hemodialysis patients.

Funding: NIDDK Support

FR-OR275

Effects of Six Versus Three Times Per Week Hemodialysis on Physical Performance, Health and Functioning: Frequent Hemodialysis Network (FHN) Trials Yoshio N. Hall,[1] Brett Larive,[2] Patricia Lynn Painter,[3] George A. Kaysen,[4] Robert M. Lindsay,[5] Allen R. Nissenson,[6] Mark L. Unruh,[7] Michael V. Rocco,[8] Glenn M. Chertow,[9] The FHN Trial Group.[10] *[1]University of Washington; [2]Cleveland Clinic; [3]University of Minnesota; [4]UC Davis; [5]University of Western Ontario; [6]DaVita Inc.; [7]University of Pittsburgh; [8]Wake Forest University; [9]Stanford University; [10]NIDDK, Bethesda, MD.*

Background: Relatively little is known about whether and to what extent the frequency of hemodialysis may influence the substantial disability and functional dependence of patients with end-stage renal disease.

Methods: We examined changes in physical performance, health and functioning among subjects randomized to frequent (six times per week) as compared with conventional (three times per week) hemodialysis in both the Frequent Hemodialysis Network Daily (n=245) and Nocturnal (n=87) Trials. The primary outcomes were adjusted change in scores over 12 months on the short physical performance battery (SPPB), RAND 36-item self-reported physical health composite (PHC) and physical functioning subscale (PF).

Results: In the Daily Trial, subjects randomized to frequent as compared with conventional in-center hemodialysis experienced no significant change in SPPB (adjusted mean change of -0.14 ± 0.19 vs. -0.39 ± 0.21, P=0.38), but experienced clinically significant improvements in PHC (3.4 ± 0.8 vs. 0.2 ± 0.8, P=0.004) and in PF (4.8 ± 2.1 vs. -0.3 ± 2.2, P=0.081). The effects of frequent in-center hemodialysis did not appreciably differ according to sex, race or diabetic status. In the Nocturnal Trial, there were no significant differences among subjects randomized to frequent as compared with conventional hemodialysis in SPPB (adjusted mean change of -0.89 ± 0.44 vs. -0.44 ± 0.43, P=0.45), PHC (2.8 ± 1.5 vs. 1.8 ± 1.5, P=0.64), or PF (-3.1 ± 3.5 vs. 1.0 ± 3.5, P=0.41).

Conclusions: Frequent in-center hemodialysis, as compared with conventional in-center hemodialysis, improved self-reported physical health and functioning but had no significant effect on objective physical performance.

Funding: NIDDK Support, Other NIH Support - NIH Research Foundation, Other U.S. Government Support, Pharmaceutical Company Support, Private Foundation Support

FR-OR276

Both Excessive Weight Loss and Gain over Time Are Associated with Increased Mortality in Incident Dialysis Patients Sunil V. Badve, Carmel M. Hawley, Stephen P. McDonald, Kym M. Bannister, Neil Boudville, Fiona Brown, Philip A. Clayton, Brian E.R. Livingston, Kevan R. Polkinghorne, Kathryn J. Wiggins, David W. Johnson. *Australia and New Zealand Dialysis and Transplant Registry, Australia.*

Background: The prevalence of obesity is increasing in incident dialysis patients. There is limited information on the association between longitudinal changes in body mass index (BMI) and mortality in incident dialysis patients.

Methods: The study included all adult patients from the ANZDATA Registry who started hemodialysis (HD) or peritoneal dialysis (PD) between 2001 and 2008 with ≥ 2 measurements of dry weight and ≥ 6-mo follow up. Annualized change in BMI was calculated using a least-squares regression slope. Patients were divided into quintiles of slope of annual BMI change (Q1 to Q5). Dialysis modality was classified according to modality in use at 90 d. Survival analysis was done using Cox proportional hazards model.

Results: A total of 16,869 incident dialysis patients were included in the study (mean age 60 yrs, male 60%, obese 29%, HD 64%). The median[interquartile range] slopes of annual BMI change in Q1, Q2, Q3, Q4 and Q5 were -2.3[-3.5, -1.6] kg/m²/yr, -0.7[-0.9, -0.5] kg/m²/yr, -0.2[-0.3, -0.5] kg/m²/yr, 0.3[0.2, 0.4] kg/m²/yr and 1.2[0.9, 1.9] kg/m²/yr, respectively. There was a significant interaction between the dialysis modality and the slope of annual BMI change. Over a mean follow up of 2.8 yrs, 5896 patients died. Compared to Q3, the adjusted hazard ratio (95%CI) for mortality in Q1, Q2, Q4 and Q5 were 3.97(3.43 – 4.58), 1.52(1.35 – 1.72), 1.09(0.95 – 1.26) and 2.41(2.13 – 2.72), respectively.

Conclusions: A U-shaped association was present between the slope of annual BMI change and mortality, suggesting both excessive weight loss and gain over time may be associated with increased mortality in incident dialysis patients.

FR-OR277

Limitations in Activities of Daily Living Independently Influences One-Year Survival of Hemodialysis Patients Eiichiro Kanda,[1] Jenna O. Krisher,[2] William M. McClellan.[3] *[1]Tokyo Kyosai Hospital, Japan; [2]ESRD Network 6; [3]Emory University.*

Background: We examined the independent contribution of activities of daily living (ADL) limitations with one-year mortality among incident hemodialysis patients.

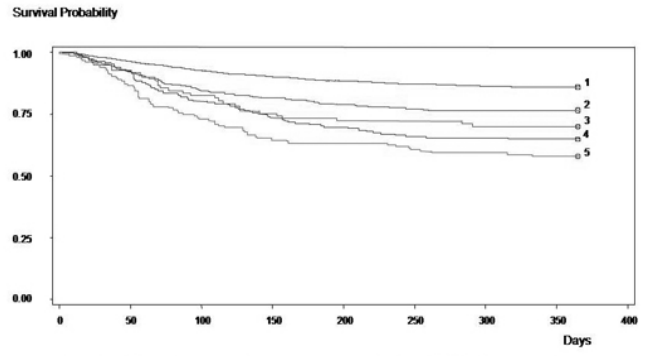
Methods: We enrolled 6217 incident hemodialysis patients and followed up these individuals for up to one year. ADL limitations were assessed using data from the Centers for Medicare & Medicaid Services (CMS) 2728 Form including inability to ambulate, inability to transfer from bed to chair, and the need for assistance with daily activities. The patients were categorized into five groups according to their ADL limitations.

ADL Groups

ADL Group	Inability to ambulate	Inability to transfer	Needs assistance with daily activities
Group 1	No	No	No
Group 2	No	No	Yes
Group 3	Yes	No	No
Group 4	Yes	No	Yes
Group 5	Yes	Yes	Yes

Mortality was evaluated using a fully adjusted Cox proportional hazard model.

Results: The mean age was 61.3 years; 47.7% were female; 46.3% had diabetes. Group 1 consisted of 5414 patients (87.1% of sample); Group 2, 363 (5.8%); Group 3, 97 (1.6%); Group 4, 188 (3.0%); Group 5, 150 (2.4%). ADL limitations correlated with mortality: no ambulation, hazard ratio (HR) 2.12 (95% confidence interval 1.77-2.53); unable to transfer 2.13 (1.64-2.76); need for assistance with daily activities, 1.58 (1.34-1.85). Patients in Group 5 had the worst outcomes.



Unadjusted Kaplan-Meier survival curve showing survival probability by strata of ADLs

Log rank test P<0.0001, Wilcoxon test P<0.0001

HRs (CIs) for Groups 2 to 5 compared with Group 1 (no disability) were as follows: Group 2, 1.29 (1.02-1.63); Group 3, 1.87 (1.28-2.71); Group 4, 2.07 (1.60-2.68); Group 5, 2.38 (1.82-3.07).

Conclusions: Inability to ambulate and transfer and need for assistance with daily activities strongly associated with a higher risk of one-year mortality among hemodialysis patients. More attention should be given to the evaluation of ADL limitations and their improvement.

FR-OR278

Influence of Dialysis Unit Affiliation on Involuntary Discharges (IVD) Brian T. Chu,[1] Abey K. Thomas,[2] Senthil P. Ramaiyah,[2] Carol Lyden,[3] Chaim Charytan,[2] George N. Coritsidis.[1] *[1]Nephrology, Elmhurst Hospital Center, Elmhurst, NY; [2]Nephrology, New York Hospital Medical Center of Queens, Flushing, NY; [3]ESRD Network of New York, IPRO, Lake Success, NY.*

Background: Dialysis facilities (DF) may need to involuntarily discharge patients under certain circumstances set forth in Medicare's conditions of coverage. Minimal literature has been published on IVD. We wanted to analyze IVD rates by the different types of DF and by insurance status of patients as this may have significant implications in the bundling payment era.

Methods: We collected IVD data reported to ESRD Network 2 (EN2) between July 2006 and March 2011. We used the annual average EN2 population in that period to calculate IVD incidence rate for various types of DF. We looked at facility characteristics like profit status, large dialysis organization (LDO) affiliation and hospital/nonhospital based DF. We also looked at the initial insurance status of IVDs. Data was analyzed by chi square test to look for statistical significance.

Results:

	IVDs	Anualized average IVDs	Anualized average EN2 population	Annual IVD Incidence	P-value
Profit Status					
Profit	55	10.48	14427	0.073	
Non-Profit	17	3.24	10307	0.031	0.002
Unit Type					
Non-Hospital Based	69	13.14	24806	0.053	
Hospital Based	3	0.57	4070	0.014	0.004
Affiliation					
LDO	44	8.38	9285	0.09	
Other	28	5.33	15449	0.035	<0.0001
LDO					
LDO 1	28	5.33	5051	0.106	
LDO 2	12	2.29	3459	0.066	*0.04
LDO 3	4	0.76	1030	0.074	

*comparing LDO 1 to LDO 2

Reasons for IVD were the following: behavioral 76.4%, nonpayment 16.7%, noncompliance 2.8%, and unknown 4.2%. Initial insurance coverage was the following: Medicaid 34.7%, Medicare 20.8%, other 12.5%, none 4.2%, and unknown 27.8%. Initial insurance status was similar in IVD patients regardless of the unit affiliations.

Conclusions: Our analysis of EN2 data shows IVD rates are significantly higher in LDOs, non hospital based units and for-profit DFs. The rates also vary significantly among the different LDOs. Medicaid is the most common primary insurance among IVD patients. Interestingly most IVDs are reported to be due to behavioral reasons with financial concerns cited in only 17% of cases. These intriguing findings need to be compared to national trends and further monitored during the new bundled era.

FR-OR279

The End-Stage Renal Disease (ESRD) Prospective Payment System (PPS): Who Gains and Who Loses? Fredric O. Finkelstein,[2] Alan S. Kliger,[1] Peter Juergensen,[1] John J. Kochevar,[3] John Mark Stephens.[3] *[1]Medicine, Hospital of St. Raphael, New Haven, CT; [2]Medicine, Yale University School of Medicine, New Haven, CT; [3]Kochevar Associates, Boston, MA.*

Background: The ESRD PPS was designed to reduce dialysis facility Medicare payment (pymt) by 2% without introducing disparities. There was no demonstration project and the potential impact of the PPS on independent and small chain dialysis organizations (SDOs) has raised concerns. The study objective was to determine which patient (pt) and facility characteristics would be associated with income gains and losses under the PPS.

Methods: We recruited SDOs stratified by region, size, urbanicity and minority zip code. Facilities entered 2009 pt demographics, comorbidities and Medicare pymts into a HIPAA compliant database. We calculated pt pymt gains/losses under the PPS compared to 2009 prior pymts, updated for inflation, systematically evaluated their association with pt demographics, comorbidities, outcomes, Erythropoiesis-Stimulating Agent (ESA) use, and facility characteristics.

Results: A total of 41 facilities (SDOs) entered data on 3039 pts. Overall,the PPS led to a 5.1% reduction in Medicare income. The top 20% of pts (outliers) lost $6.1 Million (about - $102 per treatment, - $10,000 per pt), 72% of all losses. They were more likely to have died, be Black and live in rural areas. About 60% of their losses were from ESA use. Most clinicians did not know if they could reduce outlier pt ESA use by 25% and still meet treatment guidelines. Main gains were among pts new to dialysis and with low ESA use.

Average PPS/Tx Gains/Losses, Patient disposition, Race and Rural Location

	Losses/Gains by Quintile				
N	607	608	608	608	608
Ave $ Loss/Gain Tx	- $101.63	-$30.08	-$181	$21.04	$54.46
% Died 2009	19.5%	14.9%	13.8%	10.5%	11.3%
% Black	38.2%	30.9%	27.2%	26.6%	24.3%
% Rural	14.5%	10.2%	8.2%	8.2%	11.0%
% New to Dialysis	9.2%	8.4%	10.4%	17.4%	45.9%

Conclusions: Pymt reductions in this sample of SDOs were more than twice those mandated and due largely to high cost pt outliers that were not compensated by PPS case mix adjustors. Losses may produce adverse treatment incentives and increase disparities.

Funding: Pharmaceutical Company Support

FR-OR280

Higher Weekend Mortality in Teaching Hospitals in End Stage Renal Disease Patients Ankit Sakhuja, Gagan Kumar, Aaron T. Dall, Rahul S. Nanchal, Puneet Sood. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Background: We have previously demonstrated that patients with End Stage Renal Disease (ESRD) admitted over weekends have worse outcomes than those admitted over weekdays. In this study we analyzed if the outcomes of patients admitted to teaching hospitals on weekends were different than those admitted to non-teaching hospitals.

Methods: We analyzed the data from years 2004-2008 using the Nationwide Inpatient Sample (NIS) database. Adult patients (age ≥18 years) with ESRD were included. The primary outcome measured was all cause in-hospital mortality. Chi square test was used to compare the variables for univariate analysis and logistic regression was used to obtain adjusted Mortality odds ratios (OR) in teaching and non-teaching hospitals. We then compared the two Mortality ORs by using Cochran-Mantel-Haenszel (CMH) test. Alpha was set at 0.05. The teaching hospital status was defined as hospitals with either an American Medical Association approved residency program, or those that are members of the Council of Teaching Hospitals, or those that have fulltime equivalent interns and residents to patient ratio of 0.25 or higher.

Results: Of total 756,987 admissions in ESRD patients, 19.5% (147,730) were admitted over a weekend. A total of 49.9% (73,684) of weekend admissions were admitted to teaching hospitals. The unadjusted all cause mortality was 6.1% on weekends in comparison to 5.4% on weekdays ($p < 0.001$). On adjusting for patient and hospital characteristics the overall Mortality OR for weekend admissions was 1.15 (95% CI: 1.12-1.18). On further stratifying the overall weekend mortality into teaching and non-teaching hospitals, Mortality OR in teaching hospitals was 1.22 (95% CI: 1.17-1.26) in comparison to 1.09 (95% CI: 1.06-1.13) in non-teaching hospitals. The difference between the two Mortality ORs was statistically significant (CMH $p<0.001$).

Conclusions: ESRD patients admitted over weekends have a higher mortality. This weekend mortality is significantly higher in teaching hospitals as compared to non-teaching hospitals. Further research and effort is needed to elucidate the reasons for this difference.

FR-OR281

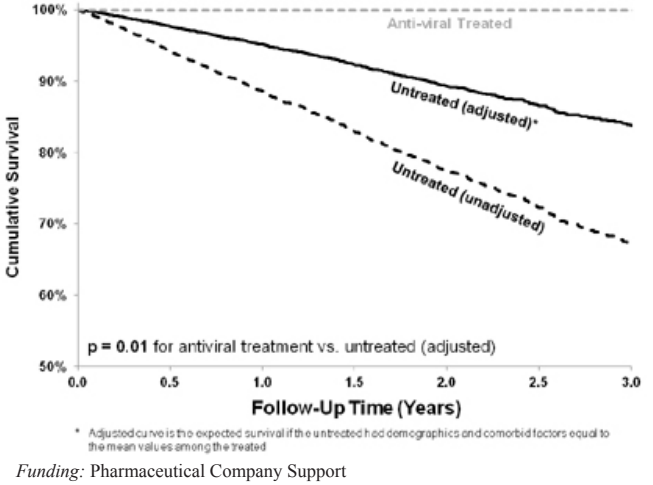
Treatment of Hepatitis C in Hemodialysis Patients Is Associated with Markedly Decreased Mortality, but Is Rarely Prescribed David A. Goodkin,[1] Brian Bieber,[1] Bruce M. Robinson,[1, 2] Michel Y. Jadoul.[3] *[1]Arbor Research Collaborative for Health; [2]U. of Michigan; [3]Univ. Cathol. de Louvain.*

Background: Hepatitis C infection is common among HD patients internationally, with a prevalence exceeding 9%. Data regarding antiviral treatment of HCV among HD patients are very limited.

Methods: The DOPPS is a cohort study of HD patients in Australia, Belgium, Canada, France, Germany, Italy, Japan, New Zealand, Spain, Sweden, the UK, and the US . We examined data from 43,828 patients, collected between 1996 and 2010. Patients were defined as HCV+ if they had the diagnosis in their medical record or if they tested positive for HCV antibody. Patients with prescriptions for an interferon or ribavirin were classified as "treated" for HCV. Cox regression was used to compare survival between treated HCV+ patients, and untreated HCV+ patients, stratified by country and adjusted for case-mix (age, sex, vintage, US black race, and 14 comorbid conditions) to match the treated patients.

Results: Out of a total of 4,494 HCV+ patients, only 35 (0.8%) were treated with antiviral medication. Median duration of follow-up was 1.4 years. The treated patients were younger, less often male, and had lower prevalence of CAD, cerebrovascular disease, CHF, DM, HT, and PVD. None of the treated patients died. 861 of the 4,459 untreated HCV+ patients (19%) died. Mortality risk was significantly worse for untreated patients, even after adjustment for case-mix ($P = 0.01$).

Conclusions: HCV infection among HD patients is very rarely treated internationally. Antiviral therapy is associated with significantly lower mortality risk. This first study of mortality among treated versus untreated HCV+ HD patients suggests that HD patient survival may be increased if HCV+ patients are treated with antiviral therapy.



Funding: Pharmaceutical Company Support

FR-OR282

Higher Serum Phosphorous Is Associated with Increased Risk for End Stage Renal Disease across All Stages of Chronic Kidney Disease Simran K. Bhandari,[1] John J. Sim,[1] Antoine C. Abcar,[1] Ning Smith,[2] Joanie Chung,[2] Dean A. Kujubu,[1] Scott A. Rasgon,[1] Kamyar Kalantar-Zadeh.[3] *[1]Nephrology and Hypertension, KPSC LAMC, Los Angeles, CA; [2]Research and Evaluations, KPSC, Pasadena, CA; [3]Nephrology, Harbor UCLA, Torrance, CA.*

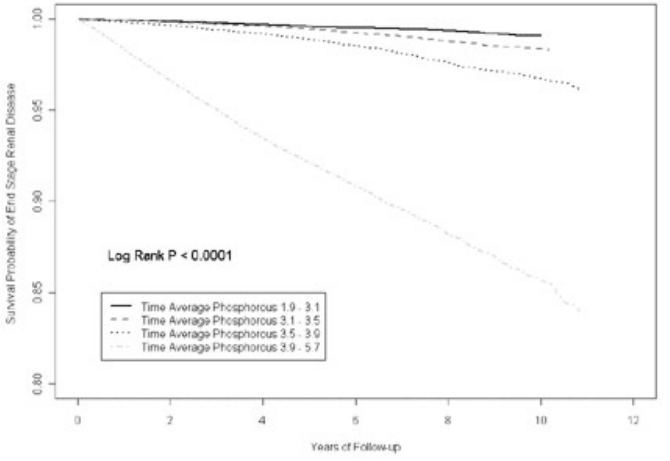
Background: We sought to determine whether higher levels of serum phosphorus (Phos) increased risk for end stage renal disease (ESRD) in a large ethnically diverse population of primarily non-CKD subjects.

Methods: Retrospective cohort study of persons age ≥ 18 yrs during 1/1/1999-12/31/2008 with ≥ 1 Phos and 1 yr min follow up. Subjects categorized into population-based quartiles by time dependent average Phos: 1.9-3.1, 3.1-3.5, 3.5-3.9, 3.9-5.7. The association between Phos quartiles and incident ESRD defined as need for dialysis, transplant, or estimated glomerular filtrate rate [eGFR (mL/min/1.73 m^2)] < 15 using Kaplan Meier survival curve and Cox proportional hazard modeling to calculate hazard ratios (HR) after adjusting for age, gender, race, HTN, DM, eGFR, and Charlson comorbidity index.

Results: 209,865 subjects were evaluated. Mean eGFR was 82; 24% eGFR > 90, 21% eGFR 60-89, and 13% eGFR < 60. Compared to the lowest Phos quartile adjusted HR for ESRD was greater across the 3 higher quartiles.

Phos Quartile	HR for ESRD	95%CI
1.9-3.0	-	-
3.1-3.4	1.47	1.20-1.79
3.5-3.8	2.89	2.41-3.46
3.9-5.7	13.58	11.53-16.01

Linear analysis for ESRD found HR of 1.7 for every 0.5mg/dL increase in Phos. Survival curve also demonstrated ESRD free survival with lower serum Phos.



ESRD rates were evaluated across eGFR (<30, 30-59, 60-89, ≥ 90) and the highest quartile of Phos demonstrated significantly greater HR for ESRD within each eGFR class.

Conclusions: In a large ethnically diverse population primarily without CKD, higher Phos was associated with greater rates of incident ESRD.

FR-OR283

Effects of Rituximab in 100 Consecutive Patients with Idiopathic Membranous Nephropathy (IMN) and Nephrotic Syndrome Despite RAS Inhibition Piero Ruggenenti, Paolo Cravedi, Maddalena Marasa', Barbara Ruggiero, Antonietta Chianca, Giuseppe Remuzzi. *Mario Negri Institute for Pharmacological Research, Bergamo, Italy.*

Background: B-cell depleting therapy has been suggested to reduce proteinuria in patients with IMN and nephrotic syndrome.

Methods: We prospectively studied over a median (range) follow-up of 30.5 (1-115) months, 100 consecutive patients with biopsy-proven IMN and persistent proteinuria >3.5g/24h despite ≥6 months RAS inhibition therapy, who received rituximab therapy at our center.

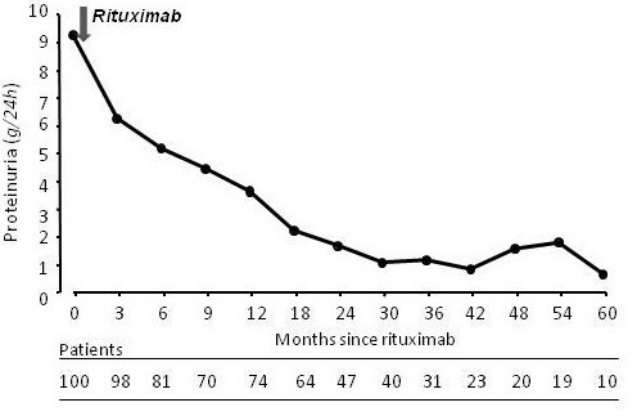
Baseline patients' characteristics

Age (y)	51.3±16.0
Male gender (n)	69 (69%)
Serum creatinine (mg/dL)	1.4±0.7
Serum albumin (g/dL)	2.2±0.6
Total Cholesterol (mg/dL)	274.1±72.3
Proteinuria (g/24h)	10.6±6.4

Proteinuria was the primary outcome.

Results: Mean(± SD) proteinuria significantly decreased from 10.6±6.4, to 4.7±0.5, 3.0±0.5, and 2.0±0.4 g/24h at 1, 2, and 3 years after rituximab, respectively (P <0.05 for all comparisons with Bonferroni adjustment, in parallel with normalization of edema, hypoalbuminemia and hypercolesterolemia.

Figure. Changes in 24-h proteinuria after rituximab therapy



At 3 years, 94.4% of patients reached complete (47.2%) or partial (47.2%) remission defined as proteinuria <0.3g/24h, or <3.5 g (with ≥50% reduction vs. baseline) in 2 consecutive evaluations, respectively. Six patients with baseline creatinine 1.9±0.6 mg/dL reached ESRD over 35 (7-68) months. In the remaining 94 patients serum creatinine was stable from basal (1.4±0.7 mg/dL) to final visit (1.6±1.4 mg/dL). Circulating B cells were fully depleted shortly after rituximab and recovered to basal in 9-12 months. Treatment was well tolerated.

Conclusions: Rituximab safely induced sustained remission in a large cohort of IMN patients with severe nephrotic syndrome. The long-term risk/benefit profile of this selective therapy seems much more favorable than that of commonly used immunosuppressive regimens.

Funding: Private Foundation Support

FR-OR284

Phospholipase A_2 Receptor Autoantibodies (PLA_2R-AB) and Increased PLA_2R Expression in Glomeruli Discriminate Primary from Secondary Membranous Nephropathy (MN) Elion Hoxha,[1] Gunther Zahner,[1] Joachim Velden,[2] Kai Fechner,[3] Gesa Stege,[1] Sigrid Harendza,[1] Rolf A. Stahl,[1] Udo Helmchen.[2] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Nierenregister Hamburg, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3]Institute of Experimental Immunology, Euroimmun AG, Lübeck, Germany.*

Background: M-Type PLA_2R is a target antigen in MN. Autoantibodies against PLA_2R are detected in up to 80% of patients with MN. Since PLA_2R-AB in the serum can become or be negative, PLA_2R-AB detection does probably not allow to differentiate in every single patient between primary and secondary MN.

Methods: In order to achieve higher specificity in the diagnosis of patients with MN, we performed a prospective study. Between November 2009 and April 2011, in 81 consecutive patients with the histologic diagnosis MN, PLA_2R-AB were measured in the serum with an indirect immunofluorescence test (Hoxha et. al. NDT in press). In 64 of these patients, in addition to standard histology, kidney biopsies were immunohistochemically stained for PLA_2R (Atlas Antibodies) and IgG4 (Binding Site).

Results: The mean age of the patients, (60 males, 21 females) was 55.9 ± 15.3 years. In 53 patients (65%) serum was positive for PLA_2R-AB: All these patients were positive for IgG4-PLA_2R. Immunohistochemistry for PLA_2R was only faintly positive in normal renal biopsies and biopsies from patients with minimal change disease (n=11). In 47 patients PLA_2R expression was strongly increased in glomeruli, 45 (96%) of those patients were positive for PLA_2R-AB in serum, 44 (94%) stained positive for IgG4. In all 13 PLA_2R-AB negative patients (which had a secondary diagnosis of MN such as SLE, Hep. B, malignancies, NSAID-related MN) PLA_2R and IgG4 staining in renal biopsies were negative.

Number of patients (%)	PLA2R-AB in Serum	PLA2R staining	IgG4 staining
42 (66%)	+	+	+
3 (5%)	+	+	-
13 (20%)	-	-	-
4 (6%)	-	-	+
2 (3%)	-	+	+
64 (100%)			

Conclusions: Measurement of PLA_2R-AB in the serum, in combination with PLA_2R and IgG4 staining of renal biopsies can discriminate between primary and secondary MN and may become a standard approach in the diagnosis of MN.

Funding: Government Support - Non-U.S.

FR-OR285

Abstract Withdrawn

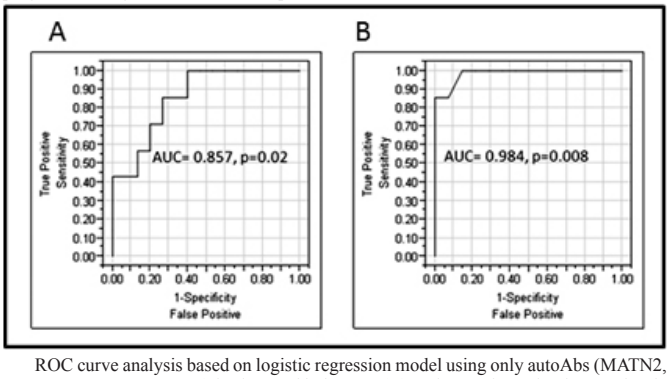
FR-OR286

Integrative Antibiomics Identifies Potential Novel Autoantibody Biomarkers for IgA Nephropathy Tara Sigdel,[1] Sang Hoon Woo,[2] Purvesh Khatri,[1] Li Li,[1] Minnie Sarwal,[1] Richard A. Lafayette.[2] *[1]Department of Pediatrics-Nephrology, Stanford University, Stanford, CA; [2]Department of Medicine, Stanford University, Stanford, CA.*

Background: Immunoglobulin G (IgG) often deposits with Immunoglobulin A in the glomerular mesangium of patients with IgA nephropathy (IgAN) but its clinical relevance is unclear. Autoantibody (autoAb) biomarkers to detect and track progression of IgAN is an unmet need.

Methods: Protein microarrays were used to evaluate IgG autoAbs in the serum of IgAN patients (n=22) and controls (n=10). Clinical parameters were collected on all patients over 5 years. Bioinformatics analysis was performed to choose the targets for further validation by immunohistochemistry (IHC). Significance of autoAbs in IgAN was determined by regression analysis using clinical parameters.

Results: A total of 117 (1.4%) unique antibodies were found to be increased in IgAN. IgAN specific autoAb (~50%) were mounted against proteins predominantly expressed in glomeruli and tubules. The presence of selected antigens in kidney tissue was verified by IHC. ROC analysis demonstrated that IgG autoAbs levels (MATN2, UBE2W, DDX17, PRKD1) might be used in combination with 24 hr proteinuria to improve prediction of the progression of IgAN (AUC = 0.857, p=0.02).



ROC curve analysis based on logistic regression model using only autoAbs (MATN2, UBE2W, DDX17, PRKD1) is showen if Figure 1 (A) and autoAbs and 24hr proteinuria (AUC=0.984; p=0.0008) is shown in Figure 1 (B).

Conclusions: This first analysis of the repertoire of autoAbs in IgAN identified novel, immunogenic protein targets, expressed in the kidney glomerulus and tubules that may be relevant in the pathogenesis and progression of IgAN. Our data suggests that using IgG autoAbs in addition to 24hr proteinuria could improve the ability to predict the progression of disease.

FR-OR287

IgA Nephropathy with Minimal Change like Lesion:Clinicopathologic Features and Response to the Therapy of Steroid Xiran Zhang, Qingwen Wang, Hui-Ping Chen, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China.*

Background: Immunoglobulin A nephropathy (IgAN) is the most common glomerular nephritis in the world. Some patients of IgAN manifest minimal change like lesions which have not been well characterized. The purpose of this study was to investigate the clinicopathologic features and response to prednisone treatment of the patients of IgAN with minimal change like lesion.

Methods: 61 patients [46 males and 15 females with an average age of 23.92±8.65] of biopsy-proven IgAN with minimal change like lesions were enrolled in this retrospective study.

Results: All patients had edema, and infection is the major cause in 15 patients (24.6%). Nephrotic syndrome was the primary clinical manifestation. 14 patients (23.0%) had AKI, 5 patients (8.2%) had hypertension and 14 patients (23.0%) had hematuria. The elevation of urine n-acety glucosaminidase (NAG) and urine retinol binding protein (RBP) were observed in the majority of the patients (78.7% and 75.4% respectively). Glomerular changes were minimal with deposition of immunoglobulin A alone (14.3%) or together with immunoglobulin M (61.9%) in mesangial region. 80.3% of the patients manifested acute tubular injury, while 65.6% had interstitial injury. During the follow-up of 46.00±13.93 months (24~70 months), 91.8% of the patients responded to the treatment of prednisone, but 76.8% of which relapsed. Compared with the responders, the non-responders had a higher incidence of hematuria and manifested more severe ($P<0.01$), and their hypoproteinemia was not as evident as the former ones ($P<0.05$). Besides, tubular atrophy, interstitial fibrosis and hyaline degeneration of arteriole were seen more often in non-responders ($P<0.01$). Until the last follow-up, 59 patients (96.7%) still had normal renal function as evidenced by normal serum creatinine**.**

Conclusions: In conclusion**,** IgAN with minimal change like lesion was similar to minimal change disease both in clinical manifestation and pathologic features. Most patients of the patients were remitted with steroid treatment, and had good prognosis.

FR-OR288

Tacrolimus Is an Effective Steroid Sparing Treatment for Lupus Nephritis in Pregnancy Alexander Paul Wardle,[1] Kate Bramham,[2] Catherine Nelson-Piercy,[3] Liz Lightstone.[1] *[1]Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom; [2]Maternal and Fetal Research Unit, King's College London, London, United Kingdom; [3]Obstetric Medicine, Guy's & St Thomas' Hospitals NHS Foundation Trust, London, United Kingdom.*

Background: Treatment options for pregnant women with lupus nephritis (LN) were previously limited to steroids +/- azathioprine (aza). Steroids in pregnancy are linked with increased pre-term delivery, infection & gestational diabetes. In non-pregnant women we have successfully treated LN with tacrolimus (Tac) in steroid-sparing regimens. Safety of Tac in pregnancy has been established in transplant patients. We report our use of Tac both as maintenance & as an alternative treatment for LN flare in pregnancy, where steroid avoidance was desirable.

Methods: 5 pregnant women taking Tac or Tac added for control of LN. Tac dosed to trough levels of 5-8ng/ml. Remission- Complete (CR): urine PCR <50mg/mmol; Partial (PR): PCR non-nephrotic + >50% fall

Results:

Renal & pregnancy outcomes

Case	Class LN	Immunosuppression	Gestation at flare	Urine PCR	Pre preg CKD stage	Gestation Delivery (wks)	Birth Weight (g)
1	V	Started Tac/MPx2/Aza	10/40	10/40 600, PR 23/40, CR 3/12 post	1	38	3320
2	IV-G (A/C)	Tac maintained	No flare	Pre 106; CR by 23/40	2	37	2500
3	IV-G (A/C)	Tac maintained	No flare	Pre 149, 35/40 129, 37/40 663, CR 1yr post	2	37	2780
4	IV	Aza, Tac started	8/40	Pre 24, 8/40 302, 30/40 777, CR 3/12 post	3	30	1100
5	IV-G (C)	Pred/Aza increased, Tac started	8/40	Pre 52, 8/40 222, 35/40 563, PR 4/12 post	3	35	2910

Delivery: all spontaneous labour; 1 emergency C-section; all live births. In both cases with CKD stage 3, creatinine rose during pregnancy; by 3/12 post partum case 5 eGFR returned to baseline but case 4 had persistent reduction.

Conclusions: To our knowledge, this is the first case series reporting Tac enabling induction of disease remission with steroid sparing in LN in pregnancy. Tac was well tolerated, avoided steroid associated complications and resulted in a reduction or stabilisation of proteinuria, even in women with CKD 3. We propose that it may be considered as an adjuvant or alternative therapy to steroids for pregnant women with LN for maintenance or treatment of disease flare.

FR-OR289

Tacrolimus as an Induction Agent for Proliferative Lupus Nephritis Is as Effective as Cyclophosphamide Sanjay Gupta,[1] Sheel Bhadra Jain,[1] Uma Kumar,[2] Amit K. Dinda.[1] *[1]Nephrology, AIIMS, India; [2]Medicine, AIIMS, India.*

Background: Conventional therapy for lupus nephritis has limitations, cyclophosphamide (cyclo) carries the risk of severe infections, ovarian failure and malignancies mycophenolate has risk of infections. Less toxic treatment with at least equal efficacy is necessary. Tacrolimus (tac) target B cells indirectly by interfering with T cell help by inhibiting IL-6, IL-10 production. The aim of study was to assess the efficacy and safety of Tacrolimus as induction therapy in class III and IV lupus nephritis and compare it with cyclo.

Methods: In an open label non randomized control trial involving 40 female patients with biopsy proven class III and class IV lupus nephritis, we compared the Tac in combination with steroid as an induction therapy with cyclo monthly pulses (NIH protocol). Twenty patients were treated with Tacrolimus, starting at 0.1 mg/kg/day in divided doses maintaining trough levels 5-8 ng/ml and prednisolone at 0.6 mg/kg/day for 6 weeks and then tapered. Response rate and adverse effects were compared with 20 consecutive patients treated with IV cyclo.

Results: The tac group and cyclo group had similar baseline characteristics. Histopathology was similar, in tac group class III in 10, IV in 9 & III+V in 1 and in cyclo group class III in 6, IV in 13 & IV+V in 1. After 6 months in Tac group, complete remission was achieved in 11(55%), partial response in 4 (20%) and no response in 5 (25%) while it was 8(40%), 9 (45%) and 3 (15%) in Cyclo respectively (p=0.23). Proteinuria decreased from 2.2±0.8 to 0.85±1.1 gm/day in tac group & 2.8±2 to0.78±1.2gm/day in Cyclo group(p=0.8) & S albumin increased from 3.1±0.7 to 4.1±0.5 g/dl in tac group & from 2.8±0.8 to 4±0.7 in cyclo group(p=.46). In both groups there was significant improvement in C3 level and decline in dsDNA (IU/ml) titre from baseline. There were more infections in Cyclo group, total 6 episodes (including 2 tubercular) versus 3 episodes in tac group (p=.45). There was no case of hyperglycemia or nephrotoxicity in both groups.

Conclusions: Induction with Tac is as effective as cyclo for proliferative lupus nephritis. It is an option for females desirous of preserving ovarian function or not tolerating Cyclo.

Funding: Government Support - Non-U.S.

FR-OR290

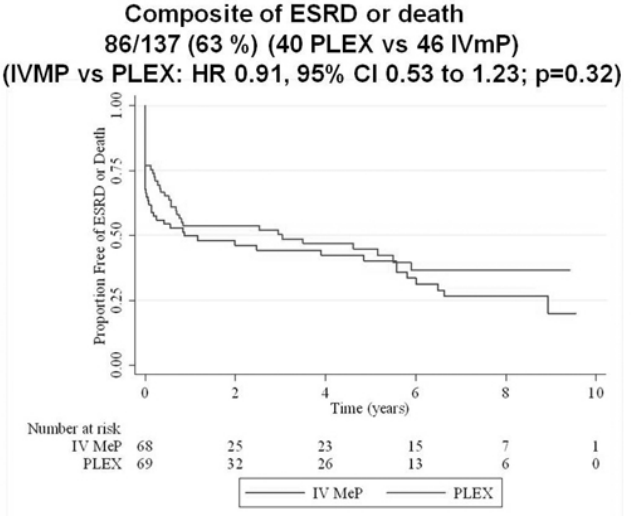
A Long-Term Analysis of the MEPEX Trial: Plasma Exchange for Severe Renal ANCA Vasculitis Alina L. Casian,[1] Michael Walsh,[2] David R.W. Jayne.[1] *[1]Vasculitis, Cambridge University NHS Foundation Trust, United Kingdom; [2]Epidemiology and Biostatistics, McMaster University, Hamilton, Canada.*

Background: Whether plasma exchange (PLEX) reduces end-stage renal disease (ESRD) and mortality in patients with severe ANCA vasculitis (AAV) is unclear. We examined the effect of PLEX on patient outcomes in the long term follow-up of the MEPEX trial.

Purpose of study: To determine whether PLEX compared to IV methylprednisolone (IVMeP) reduces the composite endpoint of ESRD or death or the endpoint of relapse in severe AAV.

Methods: Patients in MEPEX had severe AAV with a serum creatinine >500 µmol/L (5.8 mg/dl). They were randomized to receive either 7 treatments of PLEX or 3 pulses of IVMeP as adjunctive therapy to oral cyclophosphamide and prednisolone. Differences in ESRD/death between the groups were assessed using Cox proportional hazards models and differences in relapse were assessed using competing risk time to event models.

Results: 137 patients were included with a median follow-up time of 4 years. 70 (51%) developed ESRD, 56 (41%) died, and 26 (19%) experienced at least one relapse. The hazard ratio (HR) for the primary composite endpoint of ESRD and death was 0.91 (95% CI 0.53 to 1.23; p=0.32) for PLEX vs IVMeP.



For the endpoint of ESRD alone, the HR was 0.64 (95% CI 0.4 to 1.05, p=0.08) and for the endpoint of death alone the HR was 1.08 (95% CI 0.67 to 1.73). 37 out of 137 patients were alive and not ESRD at 5 years, with a median creatinine of 176 µmol/L in the PLEX group vs. 158 µmol/L in the IVMeP group (p=0.43). When considering the competing risk of death, the HR for relapse was 0.56 (95% CI 0.26 to 1.21, p=0.14) in patients treated with PLEX compared to IVMeP.

Conclusions: We were unable to demonstrate a long-term benefit of PLEX over IVMeP. Further trials are needed to determine if PLEX reduces the occurrence of clinically important outcomes in patients with AAV

FR-OR291

Aberrations in Genes Encoding CFHR5 and Terminal Complement Pathway Components in Patients with Atypical Hemolytic Uremic Syndrome Dineke Westra,[1] Katherine Anne Vernon,[2] Elena Volokhina,[1] Matthew C. Pickering,[2] Cees van Kooten,[3] Nicole Van De Kar,[1] Lambertus V. Heuvel.[1,4] *[1]Radboud University Nijmegen Medical Centre, Netherlands; [2]Imperial College, United Kingdom; [3]Leiden University Medical Centre, Netherlands; [4]University Hospital Leuven, Belgium.*

Background: Atypical HUS (aHUS) is a rare and severe renal disorder thought to be caused by predisposing aberrations in complement proteins. Previously, complement deficiencies in *CFH*, *CFI*, *MCP*, and *CFB*, or the presence of αFH were identified in 37% of our cohort of Dutch and Belgian patients. More than 4% of the patients carried an alteration in more than one gene. In this study, we identify potentially pathogenic aberrations in the regulator CFHR5 (*CFHR5*) and the terminal complement complex (TCC) components C8α (*C8A*), C8β (*C8B*), and C9 (*C9*).

Methods: Mutational screening was performed in *CFHR5*, *C8A*, *C8B*, and *C9* in 65 aHUS patients. Potential pathogenicity of genetic alterations was checked in literature, evolutionary conservation, and *in silico* mutation prediction programs. Influence of mutations in *C8A* on protein structure was analyzed with respect to available structural data. Hemolytic assays were performed for functional analysis of aberrations in TCC proteins. CFHR5 was detected in available serum by means of western blotting analysis and ELISA.

Results: In eleven patients (16.9%) we identified a potentially pathogenic sequence variation in a TCC gene or in *CFHR5*. The p.Arg444His aberration in C8α, located in the proximity of the interface with C8γ and in the binding site for CD59, results in increased hemolytic activity. Serum levels of CFHR5 were not altered. In three patients, a genetic defect was also found in one of the previously screened genes (*CFH*, *CFHR5*, and *C8A*: 2x; *CFHR5* and *CFH*: 1x).

Conclusions: A potentially pathogenic genetic abnormality in one of the components of the membrane attack complex or in *CFHR5* was observed in 16.9% of the patients. In total, in 51% of the patients at least one potentially pathogenic defect was identified in one of the complement genes screened in this cohort. The combined genetic defects identified in 9.2% might partly explain the incomplete penetrance described in the disease.

Funding: Private Foundation Support

FR-OR292

Podocyturia Is an Early Marker That Distinguishes among Normotensive Pregnancy, Gestational Hypertension, and Preeclampsia Iasmina Craici,[1] Steven Wagner,[1] Juan C. Calle,[1] Christina Wood-Wentz,[3] Kent R. Bailey,[3] Stephen T. Turner,[1] Joseph P. Grande,[2] Vesna D. Garovic.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [3]Biostatistics, Mayo Clinic, Rochester, MN.*

Background: Preeclampsia (PE) is a disorder of hypertension and proteinuria that affects 3% to 5% of pregnancies. We have previously shown that podocyturia, the shedding of live podocytes, is present at delivery in patients with PE. We aim to test whether podocyturia is predictive of later development of PE, and whether it can differentiate among normotensive pregnancies, gestational hypertension (GHTN), and PE.

Methods: From a prospective cohort of 315 patients, 15 developed PE and 15 developed GHTN. Forty four normal controls were selected. Blood pressure and protein/creatinine ratios were checked at mid-gestation prior to the onset of PE or GHTN. Urine sediments collected in mid pregnancy, prior to 210 days gestation, were cultured for 24 hours to select for viable cells. Podocytes were then identified on the basis of podocin staining.

Results: Age was not different between groups. At mid-pregnancy, the patients who later developed PE or GHTN had higher mean arterial pressure (MAP). There was an insignificant trend toward a higher protein:creatinine ratio in those who later developed PE. Of the 15 patients who later went on to develop PE, all had positive podocyturia early in pregnancy. None of the 44 normal pregnancies or 15 pregnancies complicated by gestational hypertension had podocyturia before 210 days gestation (Table 1).

Table 1

	Normotensive	GHTN	PE	p (anova)
Maternal age (years)	28.8 ± 4.4	29.9 ± 3.5	29.3 ± 6.4	0.72
Mid gestation MAP (mmHg)	77.6 ±7.1	84.0 ± 7.4	84.1 ± 8.4	0.002
Protein:creatinine ratio	0.06 ± 0.12	0.04 ± 0.02	0.25 ± 0.76	0.17
Podocyturia N (%)	0/44 (0%)	0/15 (0%)	15/15 (100%)	

Conclusions: Mid pregnancy, podocyturia is a highly accurate test for prediction of later development of PE. Podocyturia can also differentiate between later development of GHTN and PE. We postulate that the high accuracy of this test further supports the role of podocyte loss in the mechanism of proteinuria in preeclampsia.

FR-OR293

Mechanism of Selective Proteinuria in Minimal Change Nephrotic Syndrome Satoshi Kinugasa,[1] Akihiro Tojo,[1] Tatsuo Sakai,[2] Toshiro Fujita.[1] *[1]Department of Internal Medicine, University of Tokyo, Japan; [2]Department of Anatomy and Life Structure, Juntendo University School of Medicine, Tokyo, Japan.*

Background: As a mechanism of selective proteinuria in minimal change nephrotic syndrome, we have previously demonstrated albumin transport through the podocyte cytoplasm. In this study we investigated the time course of podocyte albumin transport by confocal laser-scanning microscopy, utilizing GFP-transgenic rats in which a minimal change nephrotic syndrome model was induced, and examined the role of FcRn as albumin receptor in podocytes.

Methods: A minimal change nephrotic syndrome model was induced in GFP-transgenic rats by puromycin aminonucleoside (PAN). After injection of Evans blue (EB)-labeled human serum albumin, kidneys were observed in vivo by real-time confocal microscopy. Subsequently, we observed the localization of EB-labeled human serum albumin in vibratome sections. We treated PAN nephrotic rats with anti FcRn antibody, and observed its effect on proteinuria and podocyte uptake of EB-labeled albumin.

Results: PAN nephrotic rats presented with selective proteinuria, which was confirmed by urine SDS-PAGE, and the urine IgG/Albumin ratio, an index of selectivity, was comparable with controls. In PAN nephrotic rats, fluorescence of EB was absent in the proximal tubular lumen until 5 minutes after injection, and increased at 15 minutes in PAN nephrotic rats, whereas it was almost negative in controls, indicating that a large proportion of albumin is transported slowly through podocytes. The cytoplasm of some podocytes appeared yellow in PAN nephrotic rats, due to merging of the green fluorescent signals of GFP expressed in podocytes and the red fluorescence of EB-labeled albumin, which was not observed in controls. Immunoprecipitation analysis showed an increase in Evans blue-labeled human serum albumin bound to FcRn. PAN nephrotic rats treated with antibody against rat FcRn showed significantly decreased proteinuria, compared to untreated animals (40.5±6.3 vs. 22.1±4.2 mg/mgCr, $p<0.05$), and podocyte uptake of EB-labeled albumin.

Conclusions: Podocyte albumin transport by FcRn may contribute to the selective albuminuria in minimal change nephrotic syndrome.

FR-OR294

Dynamin Oligomerization Cycle Directly Reglates the Actin Cytoskeleton and Alleviates Proteinuria Changkyu Gu, Sanja Sever. *Nephrology, Massachusetts General Hospital, Charlestown, MA.*

Background: Dynamin oligomerization into rings or spirals/helices has been linked to membrane tubulation and fission of clathrin coated vesicles at the plasma membrane. Recently, we implicated dynamin oligomerization in the regulation of the actin cytoskeleton. We now show that dynamin oligomerization in live cells is dependent on dynamin-actin interactions and GTP-binding. To elucidate the role of dynamin oligomerization in cells we turned to a small molecule that alters the dynamin oligomerization cycle by a novel mechanism. We identify Bis-T-23</A> as an archetypical small molecule "ring stabilizer" that promotes dynamin oligomerization into rings *in vitro* and in cells, promoting an activated conformation and reducing disassembly rates. In podocytes, Bis-T-23 promoted formation of focal adhesions and stress fibers independently of the RhoA pathway. Thus dynamin rings directly stimulate actin assembly by a novel mechanism. In contrast, dynole 34-2, a dynamin inhibitor lacking ring stabilizer activity, exhibited opposing cellular effects, ruling out endocytosis as an underlying mechanism for actin dynamics. Bis-T-23 protected against cathepsin L-driven proteolysis of dynamin *in vitro* and in podocytes. This in turn restored wild type organization of the actin cytoskeleton and protected podocytes from lipopolysaccharide (LPS)-induced apoptosis. Administration of Bis-T-23 in two different rodent models of proteinuric kidney disease significantly reduced proteinuria. Our study reveals that oligomerised dynamin directly regulates the actin cytoskeleton and identifies dynamin ring stabilizers as a potential novel therapeutic approach for proteinuria.

FR-OR295

Hyposialylation of Glycoproteins in the UDP-N-Acetylglucosamine-2-Epimerase/N-Acetylmannosamine Kinase (GNE) Point Mutant Mice Gives Rise to a Renal Impairment That Is Prevented by Sialic Acid Administration Mitutoshi Ito,[1] Tomoya Asaka,[2] Kazushi Sugihara,[1] Toru Yoshihara,[1,3] Takashi Wada,[4] Masahide Asano.[1] *[1]Advanced Science Research Center, Kanazawa University, Kanazawa, Japan; [2]Nanao National Hospital, Nanao, Japan; [3]Research Center for Child Mental Development, Kanazawa University, Kanazawa, Japan; [4]Department of Laboratory Medicine, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.*

Background: Sialic acid is one of acidic carbohydrates and well known to modify non-reducing terminal carbohydrates on glycoproteins and glycolipids. It is reported that mutations in the key enzyme of sialic acid biosynthesis, GNE, result in distal myopathy with rimmed vacuole (DMRV) in human. In this study, we generated mice with a point mutation (V572L) in its kinase domain.

Methods: Kidneys of GNE homozygous mutant mice (mt-mice) were investigated using biochemical, histopathological, and glycobiological analysis. N-acetylneuraminic acid (Neu5AC) treatment was carried out as follows. Before birth, pregnant heterozygous mutant mice were administrated with Neu5AC (1 g/kg/day) in drinking water. After birth, babies as well as mothers were administrated with Neu5AC (0.2 g/kg/day) in drinking water.

Results: Unexpectedly apparent myopathic features were not observed in mt-mice. However, they showed renal impairment with proteinuria and high level of serum cystatin C. Furthermore, abnormal structure of podocyte foot processes, hypoplastic glomeruli and cystic degeneration were observed in the mt-mice. Glycan analysis using several lectins showed that hyposialylation of renal tubules and renal corpuscles were observed, particularly, hyposialylation of a major podocyte sialoprotein, podocalyxin, was observed in the mt-mice. Administration of Neu5AC to the mt-mice from embryonic stage significantly supressed proteinuria and improved renal pathology with recovered sialylation of renal corpuscles.

Conclusions: These findings suggest that the proteinuric glomerular disease in the mt-mice was caused by impaired glomerular filtration due to hyposialylation of podocyte glycoproteins. Moreover, the renal disease in the mt-mice was prevented by the administration of Neu5AC from embryonic stage.

Funding: Government Support - Non-U.S.

FR-OR296

Single Injection of Stem Cells Derived from Amniotic Fluid Slows down the Progression of Kidney Failure in a Mouse Model of Alport Syndrome Laura Perin,[1] Sargis Sedrakyan,[1] Stefano Da Sacco,[1] Astgik Petrosyan,[1] Liron Shiri,[1] Kevin V. Lemley,[2] Roger E. De Filippo.[1] *[1]Urology, Children's Hospital of Los Angeles, Los Angeles, CA; [2]Nephrology, Children's Hospital of Los Angeles, Los Angeles, CA.*

Background: Alport Syndrome (AS) is a useful model for studying chronic kidney disease (CKD). This hereditary form of glomerulonephritis leads to interstitial fibrosis and eventual loss of renal function. Stem-cell based therapies may provide alternative therapeutic opportunities to treat CKD. Amniotic fluid stem cells (AFSC) present pluripotential capabilities and are reno-protective in mouse models of acute kidney injury. Herein, we investigate the potential of AFSC as a new approach to current therapies for CKD.

Methods: In this study we have administered AFSC in a murine model of AS (Col4a5-/-) before the onset of proteinuria. Mice were sacrificed at 5 days, 1 and 2 months post treatment and kidneys were harvested for molecular and histological analysis. Kidney function was assessed with serum creatinine measurements.

Results: AFSC infusion resulted in delayed renal fibrosis and prolonged animal survival, slower progression of glomerulosclerosis and ameliorated kidney function. Furthermore, injected animals exhibited decreased recruitment of macrophages within the kidney and an apparent preference towards M2 type macrophages favoring tissue remodeling. Our investigation supports a mechanism of renal protection through modulation of expression of genes promoting fibrosis such as Twist1, Snai2, Wnt11, Zeb1, Zeb2, SPARC and two potent BMP antagonists, Chordin and Noggin. Important changes in genes stimulating new extracellular matrix, such as Coll1A1, Fn1, Vcan, Itgb5, Coll3A1 and Coll5A2 appear to also be involved. Furthermore, injected AFSC are capable of downregulating MMP2 and MMP9 enzyme activity, critical modulators in GBM remodeling during disease progression.

Conclusions: Although injection of AFSC does not entirely reverse kidney disease, taken together our finding suggest that a single AFSC treatment delays progression of CKD and significantly improves survival in treated AS mice.

Funding: Private Foundation Support

FR-OR297

Role of Endothelin-1 in Podocyte-to-Endothelial Cross-Talk in Podocytopathies Ilse S. Daehn, Taoran Zhang, Gabriella Casalena, Franz Fenninger, Erwin P. Bottinger. *Medicine, Nephrology, Mount Sinai School of Medicine.*

Background: Podocyte-initiated glomerular diseases (podocytopathies) can progress to glomerulosclerosis with mesangial expansion. The molecular signaling mechanisms between glomerular cell types during initiation/progression of primary podocytopathies remain poorly understood. The purpose of this study was to examine cellular culprits and consequences of glomerular mitochondrial dysfunction (MD)and oxidative stress responses in-situ.

Methods: We assessed glomerular MD and oxidative stress in glomeruli of a Doxycycline (DOX)-inducible expression of a ligand-independent, constitutively active TGFβ receptor type 1 (Tgfbr1) mutant selective to podocytes; NPHS2-rtTA_tet-O-Tgfbr1(AAD) double transgenic mice (PodTbr1(AAD)), which develops progressive glomerulosclerosis characterized by mesangial expansion, podocyte apoptosis and depletion [Zhang et al, ASN 2010 free communication].

Results: After 2 days of DOX chow, markers of oxidative stress (3NT) and oxidative DNA damage (8-oxoguanine) were detected in mtDNA of endocapillary cells, but not in podocytes. 8-oxoG exclusively colocalized with endothelial-specific marker CD31. Because endothelin-1 receptor A (ETA) expression was increased already after 1day of DOX in glomerular endothelial cells, we applied inhibitor of ETA, BQ-123, by subcutaneous osmotic pump together with DOX chow in double transgenic mice. Inhibition of ETA signaling ameliorated endothelial oxidative mtDNA damage and MD, and prevented subsequent podocyte apoptosis, depletion, proteinuria and glomerulosclerosis. Conditioned media from DOX-treated podocyte cultures established from PodTbr1(AAD) mice induced mtDNA damage, loss of mitochondrial membrane potential and apoptosis of glomerular endothelial cell cultures, that were prevented in the presence of BQ-123 in conditioned media.

Conclusions: Our in-vivo and in-vitro studies of podocyte-restricted TGFβ signaling identify endothelin-1/ETA-mediated podocyte-to-endothelial crosstalk and suggest that TGFβ activation in podocytes induces endothelin-1 as essential mediator of endothelial mitochondrial dysfunction, oxidative stress and mtDNA damage, and that ETA inhibition prevents subsequent podocyte injury.

Funding: NIDDK Support

FR-OR298

Changes in Proximal Tubule Cell Phenotype Induced by Glomerular Proteinuria Sara Terryn,[1] Vanessa Guy-Viterbo,[1] Geraldine Mollet,[2] Marie-Claire Gubler,[2] Corinne Antignac,[2] Olivier Devuyst.[1,3] *[1]UCL Nephrology, Brussels, Belgium; [2]Inserm U983, Paris, France; [3]University of Zurich, Switzerland.*

Background: Glomerular proteinuria is a strong predictor of renal function decline in chronic kidney diseases, and the interaction between proteins filtered in excess and proximal tubule (PT) cells participates in the interstitial damage. The timing, nature and mechanisms of phenotype changes in PT cells remain poorly documented.

Methods: We analyzed the phenotype of PT cells exposed to a strictly time-controlled glomerular proteinuria, using the Nphs2flox/-Cre+ mice in which conditional inactivation of podocin induces a massive proteinuria followed by tubulo-interstitial lesions.

Results: Histological analysis revealed changes in PT morphology starting 5 days after podocin inactivation, as shown by dilated tubules with brush border disappearance progressing from the early to the straight proximal tubule, resulting in atrophic, dedifferentiated tubules at day 12. These changes in PT morphology coincided with the onset of low-molecular weight (LMW) proteinuria, glucosuria and phosphaturia. Immunostaining revealed a decreased expression of the multi-ligand receptor megalin starting 5 days after podocin inactivation, with further decline mirroring LMW proteinuria. The pathways involved were investigated in primary cultured PT cells exposed to albumin. Exposure of PT cells to high albumin concentration (10mg/ml) decreased the expression of the megalin and cubilin receptors and induced oxidative stress and fibrosis. These changes were associated with an increased expression of ZONAB, a transcription factor promoting epithelial cell dedifferentiation and a direct repressor of megalin/cubilin, paralleled by increased cell proliferation (PCNA) and a loss of polarization as evidenced by the disappearance of tight junctions (ZO-1).

Conclusions: These results show that exposure of PT cells to an excess of albumin in vivo or in vitro triggers a transcriptional mechanism that leads to cellular dedifferentiation and generalized dysfunction. Glomerular proteinuria is thus causing a rapid and specific toxicity on PT cells, involving their endocytic machinery.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR299

Urinary Cystatin C Excretion Is Controlled by Megalin Mediated Proximal Tubule Endocytosis Danny Jensen, Rikke Nielsen, Henrik Birn. *Dept. of Anatomy, Aarhus University Faculty of Health, Aarhus, Denmark.*

Background: Cystatin C is a protease inhibitor produced in all nucleated cells. Urinary Cystatin C is an established marker of kidney injury. Cystatin C is filtered freely in the glomeruli and in the normal kidney this is followed by complete reabsorption by the proximal tubule. Megalin and cubilin are endocytic receptors involved in the proximal tubule reabsorption of filtered proteins and the current study was undertaken to establish the role of these receptors for the urinary excretion and tubular uptake of endogenous Cystatin C.

Methods: Binding of recombinant Cystatin C to purified megalin and cubilin was analyzed by surface plasmon resonance analysis. Urinary excretion and proximal tubule uptake of endogenous Cystatin C was studied by immunoblotting and immunohistochemistry in wildtype and in conditional megalin and cubilin knockout (KO) mice as well as in rats treated with L-lysine known to inhibit proximal tubule protein uptake.

Results: Surface plasmon resonance analysis revealed a high affinity binding of Cystatin C to megalin (Kd ~78 nM) and to cubilin (Kd ~50 nM). Receptor associated protein, a known inhibitor of ligand binding to megalin, inhibited Cystatin C binding to megalin with 76%. Urinary excretion of Cystatin C was identified in megalin KO, but not in cubilin KO or wildtype mice. Immunocytochemistry showed vesicular labeling for Cystatin C in wildtype proximal tubular cells consistent with endocytic uptake of endogenous Cystatin C. Similar labeling was observed in cubilin deficient cells, however, no uptake in was identified in proximal tubule cells not expressing megalin. Finally, no uptake of endogenous Cystatin C was observed in proximal tubule cells from L-lysine treated rats.

Conclusions: The endocytic receptors megalin and cubilin bind Cystatin C with high affinity. Inactivation of megalin, but not cubilin, is associated with increased urinary excretion and inhibition of proximal tubule uptake of Cystatin C. These observations identifies megalin as essential for the normal, tubular recovery of endogenous Cystatin C. Increased urinary excretion of Cystatin C, as observed in kidney injury, is a marker of proximal tubule endocytic dysfunction.

Funding: Government Support - Non-U.S.

FR-OR300

Resveratrol, an Activator of Sirt1, Ameliorates Renal Damage in 5/6 Nephrectomized Rats Via the Smad Pathway Xinzhong Huang, Donghai Wen, Chuan-Ming Hao. *Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China.*

Background: Fibrosis plays an important role in the pathogenesis and progression of CKD. Resveratrol(RSV) is a polyphenol with antifibrotic properties. It has also been shown to activate Sirt1 which has health beneficial effect. In the present study, we investigated the effect of RSV on a 5/6 nephrectomized CKD rat model.

Methods: Three groups of rats were studied: sham operated, 5/6Nx+vehicle, and 5/6Nx+RSV 20mg/kg. RSV or vehicle was administered one week after 5/6 nephrectomy surgery.

Results: Subtotal nephrectomy significantly increased proteinuria (152.14±30.48mg/day vs 25.34±7.54 mg/day), blood urea nitrogen (16.75±3.99mmol/L vs 8.03±1.2mmol/L) and serum creatintine(111.6±21.5μmol/L vs 53.9±11.6μmol/L) at 12 weeks. RSV treatment significantly reduced urinary protein excretion (79.87±34.27mg/day), blood urea nitrogen (11.95±1.88mmol/L) and serum creatinine levels(83±14.69μmol/L). The glomerular sclerosis index (1.56±0.34 vs 0.35±0.08) and tubulointerstitial fibrosis (1.47±0.29 vs 0.18±0.04) were increased in nephrectomized rats, which were significantly reduced by RSV treatment. Further study shows that treatment of nephrectomized rats with RSV significantly reduced acetylation levels of Smad3. Immunoprecipitation studies revealed a binding of acetylated Smad3 with silent information regulator 1(Sirt1). In cultured murine mesangial cells, down-regulation of Sirt1 increased acetylation levels of Smad3, consistent with deacetylation effect of Sirt1. RSV significantly down-regulated TGF-β-induced fibronectin and type IV collagen expression in a dose- and time-dependent manner by immunoblot in cultured cells. Knocking-down Sirt1 using a RNAi markedly attenuated these effects of resveratrol, supporting that Sirt1 mediates the effect of RSV. In contrast, forced up-regulation of Sirt1 in cultured mesangial cells significantly reduced fibronectin and type IV collagen expression.

Conclusions: RSV attenuates renal damage in 5/6Nx rats. The renal protective effect is associated with reduced Smad acetylation and TGFβ signaling. These findings indicate that Sirt1 may be a potential therapeutic target for the treatment of CKD.

Funding: Government Support - Non-U.S.

FR-OR301

Mesangial Cell Susceptibility to IgA1 Is Important for Disease Development Kerstin Ebefors,[1] Ying Sun,[2] Johannes Elvin,[3] Borje Haraldsson,[3] Jenny C. Nystrom.[1] *[1]Physiology, Neurosience and Physiology, Gothenburg, Sweden; [2]AstraZenca, Sodertalje, Sweden; [3]Molecular and Clinical Medicine, Medicine, Gothenburg, Sweden.*

Background: IgA nephropathy (IgAN) is the most common type of glomerular nephropathy, with IgA depositions containing under-galactosylated IgA1 in the mesangial matrix as one of the key findings. However, these depositions are sometimes also found in kidneys of asymptomatic individuals. We therefore hypothesize that there must be both mesangial cell susceptibility to IgA, and under-galactosylated IgA1 in order for IgAN to develop.

Methods: To test the hypothesis, two protocols were used. Firstly, we collected and microdissected biopsies from patients with IgAN (n=15) and healthy kidney donors (n=23). Global gene expression was investigated with focus on matrix-associated genes. Secondly, we developed a unique method for culturing mesangial cells from patients with IgAN and healthy controls. These cells were treated with IgA1 purified from patients with IgAN (n=3) or healthy controls (n=3) and the expression of extracellular matrix (ECM) genes were studied.

Results: Patients with IgAN have increased gene expression of several ECM genes and a KEGG pathway analysis showed that the most up-regulated pathway was the ECM-receptor interaction pathway. Mesangial cells cultured from patients with IgAN had changed basal gene expression of several ECM genes including: COL1A1, DCN and SDC1 compared to control. Treating cells with purified IgA1 increased the changes in expression further. These changes in matrix gene expression may be necessary for the IgA depositions to be pathogenic.

Conclusions: To conclude, these experiments show that the gene expression of matrix genes is affected in IgAN, both on a systemic and cell-specific level. This strengthens the hypothesis that the mesangial cell response to IgA1 is important for the disease to develop and may differ between individuals.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR302

A Small-Molecule Inhibitor of Stat3 Prevents Muscle Wasting in Chronic Kidney Disease Liping Zhang, Yanlan Dong, William E. Mitch, David J. Tweardy. *Medicine, Baylor College of Medicine, Houston, TX.*

Background: Chronic kidney disease (CKD) is characterized by increased circulating IL-6, insulin/IGF-1 resistance, and abnormal protein metabolism causing body and muscel mass loss; administration of IL-6 alone to mice causes cachexia. There are no approved treatments for CKD-induced cachexia. Activation of signal transducer and activator of transcription (Stat) 3 is a key component of IL-6 signaling. We developed a small-molecule probe (C188-9) that targets the Src-homology 2 domain of Stat3 thereby blocking two steps in IL-6-mediated Stat3 activation—recruitment to the IL-6-activated receptor and Stat3 dimerization. We examined if C188-9 treatment improves muscle metabolism in mice with CKD.

Methods: Subtotal nephrectomy and a high protein diet were used to create mice with BUNs>80 mg/dL. The mice were paired by BUN and body weight and injected daily for 14 days with either C188-9 (6.25 mg/kg) in D5W or D5W alone.

Results: CKD mice developed weight loss and reduced muscle mass accompanied by increased muscle Stat3 activation. C188-9 treatment increased body weight (9.8% ±0.31, mean ± S.E.) and muscle weights (13.2±1.2% & 7.9±0.8 for gastrocnemius and tibialis anterior (TA) muscles, respectively) compared to D5W treatment ($p<0.05$ for all). C188-9 treatment also increased myofiber cross sectional areas in TA muscles by 41% (avg., 2400 mm^2 vs 1700 mm^2; control), increased muscle protein synthesis, and decreased protein degradation; the latter coincided with decreased expression of the E3 ligase Atrogin-1 (39.8%, $p<0.01$), indicating suppression of CKD-induced activation of ubiquitin-proteasome system. C188-9 treament reduced muscle Stat3 phosphorylation and decreased expression of the Stat3 gene target, SOCS3, while increasing Akt phosphorylation. These effects most likely were direct effects of C188-9 because C188-9 treatment of C2C12 muscle cells blocked the catabolic effects of IL-6 while improving insulin/IGF-1 intracellular signaling.

Conclusions: Thus, C188-9 treatment blocked the pathogenesis of CKD-induced cachexia in mice. Since C188-9 was well-tolerated and is water soluble, it, or a derivative, shows promise as an oral therapy for patients with CKD-induced cachexia.

Funding: NIDDK Support, Private Foundation Support

FR-OR303

Hypertension in African American Live Kidney Donors Mona D. Doshi,[1] Mariella Goggins,[2] Amit X. Garg.[3] *[1]Internal Medicine, Wayne State University, Detroit, MI; [2]Internal Medicine, Henry Ford Transplant Institute, Detroit, MI; [3]Nephrology, London Health Science Centre, London, ON, Canada.*

Background: Compared to those of Caucasian race, African Americans (AA) are at higher risk of developing hypertension (HTN). It is unclear if live kidney donation further increases this risk. We conducted a retrospective cohort study of donors and non-donors to examine this issue.

Methods: We enrolled 138 AA who underwent donor nephrectomy at Harper Hospital or Henry Ford Transplant Institute in Detroit between 1993-2007. We also enrolled 48 AA non-donors who were suitable candidates for live kidney donation but did not donate due to non-medical reasons. None of the donors were hypertensive or diabetic prior to donation. In follow-up, HTN was defined as either systolic BP > 140 or diastolic BP > 90 or use of antihypertensive agent(s). We used multivariable logistic regression models to account for differences in baseline characteristics between the two groups.

Results: The baseline characteristics of donors and non-donors at time of follow up are are shown in the table.

Comparison of characteristics between donors and non-donors at time of follow-up

	Donors (n=138)	Non-Donors (n=48)	p-value
Age (years)	45±10	42±11	0.05
% Women	63%	67%	0.60
Body Mass Index (kg/m^2)	32±5	28±6	0.39
Serum creatinine (mg/dL)	1.2±0.3	0.9±0.3	0.01

Mean ± standard deviation

Non-donors were younger than the donors by 3 years. At median follow up of 7 years (range 4-18 years), 50% of donors exhibited HTN compared to 27% of non-donors (p<0.01). The odds ratio for exhibiting HTN in donors was two and a half fold higher compared to non-donors (unadjusted odds ratio 2.7 (95% CI: 1.3-5.5; p<0.01), adjusted odds ratio 2.4 (95% CI: 1.1-5.3; p=0.01)). Since donation, 52% of donors were not seeing a physician regularly, and 49% of 69 donors with HTN were without treatment.

Conclusions: Live kidney donation increases the risk of hypertension in AA. In the US, many donors lack appropriate medical follow-up and care to maintain good long-term cardiovascular and renal health.

Funding: Private Foundation Support

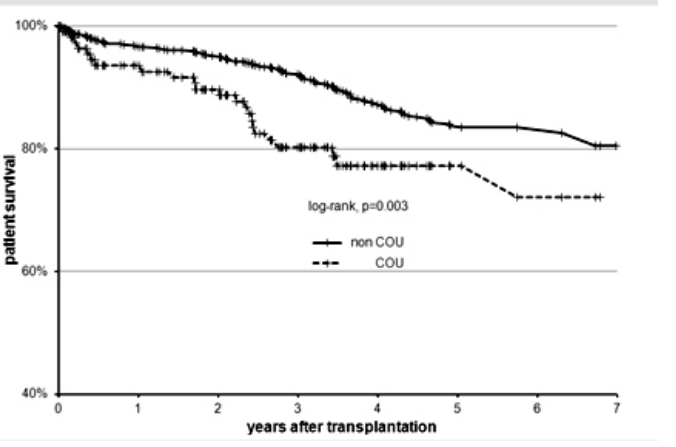
FR-OR304

History of Chronic Opioid Usage and Clinical Outcomes Post Kidney Transplantation Fidel Barrantes,[1] Fu L. Luan,[1] Mallika Kommareddi,[1] Kareem Alazem,[1] Alan B. Leichtman, Tareq Yaqub,[1] Diane M. Cibrik,[1] Randy S. Roth,[2] Silas Norman,[1] Randall Sung,[3] Timothy A. Horwedel,[4] Milagros D. Samaniego-Picota.[1] *[1]Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI; [3]General Surgery, University of Michigan, Ann Arbor, MI; [4]Pharmacy, University of Michigan, Ann Arbor, MI.*

Background: Chronic use of opioid analgesics for pain management is common among patients with end stage renal disease. We hypothesized that chronic opioid usage (COU) prior to kidney transplantation can negatively affect post-transplant outcomes.

Methods: We examined retrospectively the risk of graft loss and death in adult kidney transplant recipients according to their history COU prior to transplantation. Kaplan-Meier-survival and multivariate Cox-regression analysis were performed to compare patient and graft survival with and without COU.

Results: Among 1064 adult kidney transplant patients, 108 patients (10.2%) reported a history of COU. The cumulative mortality rate at one, three and five years was 6.5% vs. 3.2%, 18.5% vs. 7.5% and 20.4% vs. 12.7% for patients with and without a positive history of COU, respectively. A positive history of COU prior to transplantation was associated with a 65% increase in the risk of death (AHR 1.65, 95% CI 1.04, 2.60, p=0.033).



Furthermore, COU was associated with increased risk of graft loss within the first year (5.6% vs. 1.6%, p=0.005, AHR 2.90, 95% CI 1.10, 7.64, p=0.031), which did not persist during the subsequent follow up.

Conclusions: A positive history of COU prior to transplant is associated with inferior clinical outcomes after kidney transplantation.

FR-OR305

Recipient *klotho* genotypes Affect Outcome after Human Kidney Transplantation Leandro Cunha Baia, Gerjan Navis, Martin H. De Borst. *On behalf of the REGaTTA (REnal GeneTics TrAnsplantation) Groningen group, University Medical Center Groningen, Dept of Nephrology, Netherlands.*

Background: In experimental studies genetic or environmental loss of Klotho contributes to premature aging, renal damage and vascular calcification. As these factors may influence graft function and surivival, we studied the association between klotho gene variants and outcome after human kidney transplantation.

Methods: Thirteen tag SNPs covering all known variants in the human Klotho gene were genotyped in 1271 donor-recipient pairs transplanted between 1993 and 2008. Only SNPs in Hardy Weinberg equilibrium (HWE) were considered. With minor allele frequencies (MAF) <5%, dominant models were used. Donor and recipient genotypes were analyzed for associations with mortality, death-censored graft loss and clinical data (serum creatinine, PTH, phosphate) obtained 60 (range 58-64) months post-transplantation.

Results: During followup of median 5.5 [IQR 2.9-8.9] years, 175 (16.0%) recipients developed graft loss and 191 (17.4%) died with a functioning graft. On multivariate Cox regression analysis, recipient rs577912 major allele homozygote genotype (CC) was associated with mortality, also when adjusted for donor and recipient age, ischemia times, % of panel reactive antibodies and HLA mismatches (RR 1.606 (1.164-2.215), p=0.004).

The minor recipient rs553791 allele (TT, MAF 0.07) was associated with death-censored graft loss as compared to TG/GG genotypes (RR 1.903 (1.116-3.245), p=0.018). Oppositely, the GG genotype protected against graft loss (multivariate: RR 0.526 (0.308-0.896), p=0.018). In TT (n=57) as compared to age-matched GG/GT genotypes (n=135), serum creatinine (3.7±0.6 vs 2.3±0.2 mg/dl, p=0.002), PTH (505±171 vs 162±29 pg/ml, p<0.0001), and phosphorus (3.7±0.3 vs 3.1±0.3 mg/dl, p=0.02) were elevated 5 yrs post-transplantation.

Conclusions: Recipient but not donor klotho variants are associated with mortality (rs577912), in line with prior data in hemodialysis patients, and with death-censored graft loss, serum creatinine, PTH and phosphate 5 years after transplantation (rs553791). These findings link extrarenal Klotho to outcome after human kidney transplantation.

Funding: Government Support - Non-U.S.

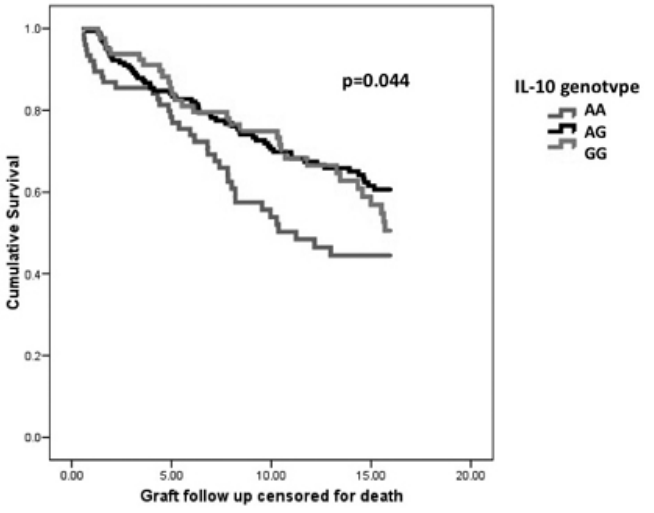
FR-OR306

The Impact of Interleukin-10 Genetic Polymorphism on Long-Term Graft Survival in Kidney Transplantation Patrick Barry Mark,[1] Jamie P. Traynor,[2] Kathryn K. Stevens,[1] Rajan Kantilal Patel,[1] Alan G. Jardine.[1] *[1]BHF Cardiovascular Research Centre, University of Glasgow, Scotland, United Kingdom; [2]Renal Unit, Monklands Hospital, Airdire, Scotland, United Kingdom.*

Background: Immunologically mediated damage contributes to chronic allograft nephropathy. Interleukin-10 (IL-10) has anti-inflammatory effects. Low IL-10 production is associated with the A allele of the IL-10-1082 G/A single nucleotide polymorphism (SNP). Transforming growth factorβ1 (TGFβ1) has pro-inflammatory, fibrotic and immunosuppressive actions. The implications of genetic polymorphisms encoding these cytokines on graft survival are unclear.

Methods: We studied 422 first renal transplant patients (median age 42.6 years; 56.6% male; 10% live donors). Patients were surveyed in 1994 and followed up after 16 years. Patients were genotyped for SNPs encoding IL-10 (G/A at position - 1082) and TGFβ1(C/T, C/G at codons 10 and 25).

Results: There were 179 (42.2%) deaths and 154 (36.5%) death-censored graft failures. No significant differences existed in patient survival between the polymorphisms studied. Age (HR 1.07, p<0.001) and diabetes (HR 2.54, p<0.01) were independent predictors of mortality on multivariate analysis. Graft survival was reduced in patients with IL-10 genotype AA but other polymorphisms did not influence graft survival.



Higher serum creatinine at one year (p<0.001), deceased donor transplantation (p<0.05), younger age (p<0.01) and IL-10 genotype AA (p<0.05) were associated with reduced graft survival on univariate analysis. Creatinine at 1 year (p<0.001) and IL-10 genotype (p<0.05) were independent predictors of long-term graft failure on multivariate analysis.

Conclusions: In addition to conventional risk factors for graft loss, polymorphism of SNPs encoding IL-10 may predict long-term graft failure.

FR-OR307

Disparities in Kidney Transplant Education for Patients with End-Stage Renal Disease Lauren M. Kucirka,[1] Morgan E. Grams,[2] Dorry L. Segev.[1] *[1]Surgery, JHU; [2]Medicine, JHU.*

Background: Inequitable transplant education might contribute to disparities in access to transplant (ATT). CMS recently began asking providers on Form 2728 whether they educated patients about transplant, and if not, to select a reason. The goals of our study were to describe national transplant education practices and to analyze associations between these practices and ATT.

Methods: We studied 236,079 incident ESRD patients captured in USRDS from 2005-2007. Multinomial logistic regression was used to examine factors associated with not being educated about KT, and modified Poisson regression was used to examine associations between not being educated and ATT. All models were adjusted for demographic factors and comorbidities.

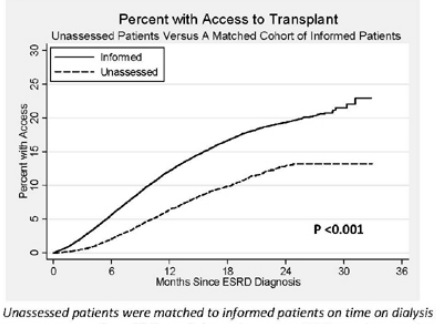
Results: Patients under nephrology care prior to ESRD and those with private insurance were more likely to be educated about transplant. However, 30.1% of patients were not educated about transplant, for reasons reported by the provider as follows: 43.1% unassessed, 32.3% medically unfit, 25.5% unsuitable due to age, 3.1% psychologically unfit, and 1.5% declined counseling. Patients at for-profit centers, older, obese, uninsured, or those who had Medicaid were more likely to be unassessed. Women were 23% more likely to be reported as unsuitable due to age, 7% more likely to be reported as medically unfit, and 25% more likely to be reported as having declined counseling. Patients not educated about transplant had a 53% lower rate of ATT (RR 0.41, 95% CI: 0.39-0.42), a disparity that persisted even in the subgroup who were simply unassessed at the time of Form 2728 reporting.

Table 1: Multivariate Multinomial Logistic Regression Accounting for Dialysis Center Clustering to Estimate the Relative Rate of Reasons for Not Informing Patients about Transplant, Including: (1) Patient Unassessed, (2) Unsuitable Due to Age, (3) Medically Unfit, (4) Declined Information, or (5) Psychologically Unfit, Compared to Being Informed about Transplant

	Informed	Unassessed RR (95% CI)	Unsuitable Due to Age RR (95% CI)	Medically Unfit RR (95% CI)	Declined Counsel RR (95% CI)	Psychologically Unfit RR (95% CI)
For Profit	Reference	**1.19 (1.01-1.41)**	1.03 (0.87-1.22)	**0.62 (0.54-0.72)**	0.96 (0.75-1.22)	**0.65 (0.55-0.77)**
Volume	Reference	1.03 (0.95-1.11)	1.05 (0.95-1.15)	0.98 (0.91-1.06)	1.01 (0.89-1.15)	0.95 (0.87-1.02)
Age *	Reference	**1.09 (1.07-1.11)**	**4.47 (4.31-4.64)**	**1.54 (1.50-1.57)**	**1.56 (1.46-1.67)**	**1.32 (1.26-1.38)**
Female	Reference	1.01 (0.99-1.04)	**1.23 (1.17-1.28)**	**1.07 (1.03-1.11)**	**1.25 (1.09-1.42)**	**1.07 (0.98-1.18)**
Afr. American	Reference	0.98 (0.90-1.07)	0.98 (0.89-1.07)	0.98 (0.91-1.06)	0.88 (0.74-1.06)	**1.26 (1.11-1.42)**
BMI > 35	Reference	**1.10 (1.06-1.15)**	**0.85 (0.78-0.92)**	1.14 (1.08-1.19)	**0.89 (0.74-1.07)**	**0.72 (0.62-0.83)**
Prior Care**	Reference	**0.68 (0.64-0.72)**	**0.91 (0.84-0.98)**	**0.73 (0.69-0.76)**	**0.81 (0.70-0.95)**	**0.67 (0.55-0.77)**
Insurance						
Medicare	Reference	Reference	Reference	Reference	Reference	Reference
Private	Reference	**0.81 (0.76-0.87)**	0.97 (0.88-1.06)	**0.87 (0.82-0.94)**	**0.78 (0.63-0.97)**	**0.57 (0.47-0.68)**
Medicaid	Reference	**1.11 (1.04-1.18)**	1.04 (0.96-1.13)	**1.19 (1.12-1.26)**	1.16 (0.98-1.39)	**2.12 (1.86-2.42)**
None	Reference	**1.14 (1.05-1.24)**	**0.57 (0.45-0.72)**	0.90 (0.75-1.08)	**0.80 (0.54-1.18)**	1.01 (0.79-1.30)

**Bold indicates statistical significance, defined as a p-value < 0.05*
Model also adjusted for other insurance, time on dialysis when 2728 form was filled out and all major comorbidities captured on 2728 form

Figure 1



Unassessed patients were matched to informed patients on time on dialysis when 2728 form was filled out, age, cause of renal failure, diabetes, insurance, COPD, cerebrovascular disease, congestive heart failure, artherosclerotic heart disease, peripheral vascular disease, and malignant neoplasm/cancer.

Conclusions: Disparities in ATT may be partially explained by disparities in the provision of transplant education; dialysis centers should ensure that this critical intervention is offered to all patients in an equitable and timely manner.

Funding: NIDDK Support

FR-OR308

Demonstrating Geographic Equity in Kidney Organ Allocation – Satisfying the Final Rule at Last Ashley E. Davis, Daniela Ladner, John J. Friedewald, Sanjay Mehrotra, Mark S. Daskin, Anton I. Skaro, Michael Abecassis. *Transplant Outcomes Research Collaborative (NUTORC), Comprehensive Transplant Center, Northwestern University, Chicago, IL.*

Background: The US Department of Health and Human Services' Final Rule dictated that organ allocation policies should not be biased towards a transplant candidate's place of residence or listing. Since this mandate, little has been done to lessen geographic disparities in kidney transplant rates. This study analyzes current cadaveric kidney allocation and compares present kidney transplant rates to those of an alternative strategy focused on optimally improving geographic equity.

Methods: Distribution of 2000-2009 kidney transplant candidates, recipients, and standard criteria donors (SCD) were obtained for each Donor Service Area (DSA) from United Network for Organ Sharing (UNOS) data. The proposed alternative kidney allocation sharing strategy allocates SCD kidney organs between DSAs but maintains current levels of local allocation. The DSA sharing levels minimizing the overall variability in transplant rates over a ten year period are determined through a mixed-integer, multi-period, linear optimization model, KSHARE.

Results: From 2000-2009, approximately 77% of procured SCD kidney organs were allocated within the UNOS Region of procurement, and in 2009, DSA SCD transplant rates ranged by 27%. With 600 mile optimized KSHARE SCD kidney organsharing, the geographic range in DSA SCD kidney transplant ratesfalls to 7.5%. Further, KSHARE allocates over 87% of procured SCD kidney organs within the UNOS Region of procurement. Extending the feasible sharing radius to a national kidney organ sharing strategy only incrementally improves the variability.

Conclusions: Enhancing the practice of sharing SCD kidney organs can increase geographic equity in DSA kidney transplant rates over time. The 600 mile sharing of SCD kidney organs employs a multi-period optimization model, KSHARE, to increase geographic equity in SCD kidney allocation while not affecting current local allocation.

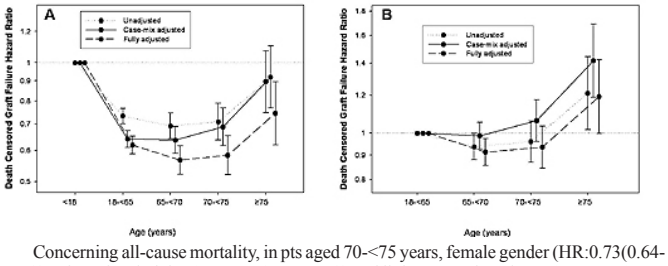
FR-OR309

Predictors of Kidney Transplantation Outcomes in the Elderly Miklos Z. Molnar,[1,2] Elani Streja,[1] Csaba P. Kovesdy,[3] Anuja P. Shah,[1] Edmund Huang,[4] Suphamai Bunnapradist,[4] Mahesh Krishnan,[5] Joel D. Kopple,[4] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]Salem VA Medical Center, Salem, VA; [4]David Geffen School of Medicine at UCLA, Los Angeles, CA; [5]DaVita, Inc, Denver, CO.*

Background: Recent studies show a survival advantage with kidney transplantation amongst elderly patients (pts) compared to those on dialysis.

Methods: We examine the predictors of outcome of kidney transplantation in different ages of elderly recipients. Using the Scientific Registry of Transplant Recipients, we identified 155005 kidney transplanted pts. Mortality&death censored graft failure risks were estimated by Cox proportional regression (hazard ratio(HR) and 95% of confidence interval) analysis over a median follow-up period of 4 years.

Results: Pts were 45±16(mean±SD) years old and included 40% women and 19% diabetics. Figure shows an association between age groups % death censored graft failure using different reference groups. The association between age groups&death censored graft failure was U-shaped.



Concerning all-cause mortality, in pts aged 70-<75 years, female gender (HR:0.73(0.64-0.85)), Hispanic ethnicity (HR:0.75(0.58-0.95)) and living transplant donor (HR:0.70(0.57-0.86)) were protective predictors and diabetes mellitus (HR:1.21(1.05-1.39)) and Black race (HR:1.22(1.03-1.45)) were risk factors. In pts ≥75 years old, only a living donor kidney (HR:0.64(0.44-0.93)) was an important predictor of lower all-cause mortality.

Conclusions: The strong association between living donor kidneys & all cause mortality suggest that, pts ≥70 years old should receive living donor kidney transplants. Having an expanded criteria donor kidney was not a predictor of either mortality or graft failure; consequently these kidneys may be used in elderly transplantation.

Funding: NIDDK Support

FR-OR310

Differential Survival Rates of Combined Liver Kidney Transplantation (CLKT) vs. Liver Alone Transplantation (LAT) vs. Kidney Alone Transplantation (KAT) David J. Leehey, Susan H. Hou, Holly J. Kramer. *Loyola University Medical Center, Maywood, IL.*

Background: Combined liver kidney transplantation (CLKT) is indicated in patients with combined end-stage liver disease (ESLD) and end-stage kidney disease (ESKD). Controversy exists as to allocation of donor kidneys to patients with ESLD with hepatorenal syndrome (HRS), because some of these patients will recover renal function after liver alone transplantation (LAT).

Methods: UNOS data during the period 1/1/2000 through 9/3/2010 were analyzed to determine one-year kidney allograft survival (defined as allograft failure or patient death) and one-year patient survival.

Results: Among 54,676 liver transplants performed, 8978 recipients had CKD defined as receiving dialysis or serum creatinine > 2.5 mg/dl at wait listing. 3893 recipients with CKD underwent CLKT and 12.8% had a reported diagnosis of HRS. Unadjusted one-year kidney allograft survival: CLKT (all) 82.7%; CLKT receiving dialysis 83.3%; CLKT with no dialysis 82.0%; CLKT with reported HRS 88.8%. One-year patient survival: CLKT (all) 85.4%; CLKT receiving dialysis 83.6%; CLKT with no dialysis 87.7%; CLKT with reported HRS 88.1%. One-year patient survival: LAT with CKD (with and without dialysis) 84.7%; LAT with dialysis 81.6%; LAT with CKD without dialysis 87.5%. A total of 92,771 dialysis-dependent and 7,701 non-dialysis-dependent individuals with no liver disease received a deceased donor KAT during this time period. One-year allograft survival among KAT patients who were or were not receiving dialysis was 93.2% and 95.1%, respectively, and one-year patient survival was 96.0% and 98.4%, respectively.

Conclusions: In summary, one-year patient survival among patients with ESLD receiving dialysis was approximately 2.0% higher with CLKT than with LAT, but there was only a 0.2% difference in patients not receiving dialysis. One-year kidney allograft failure and mortality are approximately 2.5-fold and 4-fold higher, respectively, in patients with ESLD undergoing CLKT as opposed to patients without liver disease undergoing KAT. These data support a reappraisal of the practice of preferential allocation of donor kidneys to patients with ESLD rather than to patients with ESKD.

FR-OR311

Sensitization Rates after 1st Graft Failure in Pediatric Renal Transplant Recipients Are Impacted by a De-Emphasis of HLA Matching in Organ Allocation and by Donor Type Alexander C. Wiseman, Jane Gralla, Suhong Tong. *University of Colorado.*

Background: Despite improvements in immunosuppression, sensitization after 1st renal graft failure remains a barrier to re-transplantation. US allocation policies currently place less emphasis on HLA matching in pediatric renal transplant (tx) candidates to minimize dialysis time, with undetermined long-term consequences.

Methods: Using the SRTR database, we examined HLA sensitization after graft loss and re-graft survival of all pediatric 1st renal tx recipients aged 0-17 yrs transplanted 1990-2008, by peak panel reactive antibody (%PRA), type of 1st graft donor (deceased, DD vs. living, LD) and HLA mismatch (MM) of 1st tx.

Results: Of 13,227 pediatric 1st renal tx recipients, 1,943 received a re-tx (60% of DD and 74% of LD) following 1st graft failure and 948 recipients were relisted but did not receive a re-tx (re-tx WL) by June 2009. Mean PRA increased from 7% prior to 1st tx to 53% after 1st graft failure, with a greater increase in the re-tx WL group for DD and LD (Table 1). The risk of sensitization significantly increased with number of 1st tx HLA MM (DD: p=0.001, LD: p=0.027). Re-graft survival at 5 years was significantly worse with more MM at 1st tx, even for re-tx in 2000-2008 (DD p=0.046, LD p=0.012).

	1st tx DD (N=1388)		1st tx LD (N=1503)	
RR for becoming sensitized, 4-6 MM vs. 0-3 MM at 1st tx	1.16 (95% CI 1.06-1.27), p=0.001		1.12 (95% CI 1.01-1.23), p=0.027	
% Re-tx of those relisted	60% Re-tx (N=838) (24% within 1yr)	40% No re-tx (N=550)	74% Re-tx (N=1105) (32% within 1yr)	26% No re-tx (N=398)
Mean waiting time	22 mo	58 mo	17 mo	56 mo
Mean %PRA increase from 1st tx	8% to 49%	10% to 77%	5% to 41%	5% to 71%

Conclusions: HLA sensitization in pediatric recipients increases with increasing number of MM and is associated with a decreased likelihood of re-tx, for deceased and living donors. Re-graft survival is lower after more HLA MM at 1st tx. The risks of sensitization vs. the benefits of earlier transplantation with a de-emphasis of HLA matching require further study, but these preliminary data suggest that pediatric transplant programs may benefit from evaluating local waiting times and donor type when considering HLA mismatched kidneys for transplant.

FR-OR312

Autoantibodies Specific for the Phospholipase A2 Receptor in Recurrent and De Novo Membranous Nephropathy: Are They Predictive of Recurrence? Hanna Debiec,[1] Laurent Martin,[2] Chantal Jouanneau,[1] Laurent Mesnard,[1] Eric Rondeau,[1] Christiane I. Mousson,[3] Pierre M. Ronco.[1] *[1]INSERM/UPMC UMR_S702, Tenon Hospital, Paris, France; [2]Plateau Technique de Biologie, Dijon, France; [3]University Hospital, Dijon, France.*

Background: Recent findings in idiopathic membranous nephropathy (MN) suggest that in most patients, the disease is due to anti-PLA2R1 autoantibodies. Our aim was to analyze the prevalence of anti-PLA2R1 antibodies in recurrent and *de novo* MN, and to determine whether these antibodies may identify a potential risk of recurrence of disease in the transplant.

Methods: Fifteen patients with recurrent MN, 15 patients with *de novo* MN and 6 patients with an idiopathic MN that did not histologically recur on the graft were included in the study. Diagnosis of recurrent and *de novo* MN was established histologically. Circulating PLA2R1 autoantibodies were assessed by a direct immunofluorescence assay based on human embryonic kidney cells transfected with PLA2R1 cDNA; The presence of PLA2R1 antigen and C4d in immune deposits was detected by confocal microscopy in paraffin-embedded native and graft biopsies with specific anti-rabbit PLA_2R1 or C4d antibody

Results: We showed that PLA2R1 was involved in 6 of 15 patients with recurrent MN, but in none of the 15 patients with *de novo* MN. C4d was detected along glomerular capillary walls in both recurrent and *de novo* MN as a consequence of complement activation by subepithelial immune complexes irrespective of deposited antigen. We also showed a marked heterogeneity in the kinetics and titers of anti-PLA2R1 antibodies which may relate to their different pathogenic potential. We found that 3 patients with PLA2R1-related idiopathic MN and anti-PLA2R1 antibodies at the time of transplantation did not develop histological recurrence.

Conclusions: Our findings indicate that PLA2R1 antibody is specific for recurrent vs de novo MN although 9/15 recurrent diseases were most likely not PLA2R1 related. Furthermore PLA2R1 autoantibody at transplantation was not always associated with recurrence; thus its predictive value should be carefully analyzed in prospective studies.

Funding: Government Support - Non-U.S.

FR-OR313

Differential Effects on Foxp3+ Tregs of Targeting Histone/Protein Deacetylases 6, 9 and Sirtuin-1 Ulf H. Beier,[1,2] Liqing Wang,[1] Tatiana Akimova,[1] Wayne W. Hancock.[1,2] *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania, Philadelphia, PA.*

Background: Histone/protein deacetylase (HDAC) targeting augments suppressive functions of human and murine Foxp3+ Tregs, but it is unclear if this involves a single mechanism, such that combined targeting would have little benefit compared to optimal targeting of one or other HDAC.

Methods: We compared the suppressive functions of Tregs of wild-type (WT) C57BL/6 mice or mice with global (HDAC6-/-, HDAC9-/-, dual HDAC6/9-/-) or conditional deletion (CD4-Cre or Foxp3-Cre and floxed Sirt1) alone, or after treatment with pan- or isoform-selective HDAC inhibitors (HDACi).

Results: Tregs deficient in expression of Sirt1, HDAC6 or HDAC9 had increased suppressive function compared to WT Tregs, but treatment with a pan-HDACi (Trichostatin) in each case further increased their suppressive function, indicating the benefit of combined vs. single HDAC targeting. This was reinforced by increased suppressive function of Tregs from HDAC6/9-/- mice vs. HDAC6-/- or HDAC9-/- Tregs. HDAC targeting increased Foxp3 gene expression 2-5 fold over control Tregs and involved different transcription factors. Sirt1 deletion stabilized p65 acetylation and promoted its nuclear translocation. Loss of HDAC6 or HDAC9 increased SMAD3 or STAT5 phosphorylation, respectively, consistent with data that HDAC6 promotes proteasomal degradation of SMAD3 and the STAT5-P dimer is stabilized by acetylation. Deletion of each HDAC increased Foxp3 acetylation, which protects Foxp3 from ubiquitination and proteasomal degradation. Tregs with HDAC6 or HDAC9 deletion had increased transcription of heat shock response (HSR) genes. HSP70 deletion reduced, but did not abolish, the therapeutic effects of HDAC6 inhibition, whereas Sirt1 deletion decreased HSR gene expression but increased Treg resistance to stress and HSP70 protein levels, in conjunction with increased acetylation of HSP70.

Conclusions: Targeting different HDAC can increase Treg function by multiple and additive mechanisms, indicating the therapeutic potential for combinations of HDACi in the therapy of autoimmunity and transplantation.

Funding: Other NIH Support - K08AI095353, AI073489

FR-OR314

Strategies To Promote Tolerance through Augmented Treg Homeostasis

Ying Wang, David M. Rothstein. *University of Pittsburgh, PA.*

Background: CD4+Foxp3+ Treg play a key role in transplant tolerance. Treg undergo higher homeostatic proliferation (HP) than Tconv cells. α-CD45RB induces tolerance through a 2X increase in Treg, induced by converting Foxp3- to Foxp3+ cells and by a specific increase in Treg HP. Thus, Treg HP can be specifically regulated to expand Treg in vivo. We asked whether IL2, which also supports Treg HP, synergizes with α-CD45RB. CFSE-stained congenic CD4 cells were transferred into naive wt mice, which were untreated, or received IL2 (1.5ugX9d), α-CD45RB (100ug d-1,0,5), or both. Transferred Foxp3+ and Foxp3- CD4 cells (spleen and LN) were assessed (d10). Both IL2 and α-CD45RB increase Treg HP resulting in a 1.5-3X increase in Treg. Combined, these agents exhibit dramatic synergy. >90% of Treg undergo HP such that 40% of transferred CD4 cells are now Foxp3+ (increase: 8X in % and 5X in #). While α-CD45RB induces >100d graft survival (GS) in 50% of B6 recipients of BALB/c islets, IL2 did not (MST 15d). Despite a marked increase in Treg, IL2+α-CD45RB shortened GS (MST 60d). The failure of regimens with IL2 to prolong GS may be due to a 2X expansion of CD8 effectors and NK cells vs. untreated controls. In this regard, Boyman reported that α-IL2 mAb (JES6-1) allows IL2 to bind the αβγ IL2R on Treg, but not the βγ IL2R on CTLs. They also found JES6-1+IL2 induced long-term islet GS. However, using the same treatments /strains, we found all mice rejected by d18. Moreover, JES6-1+IL2 did not inhibit CD8 effectors, allowing a 2X increase in INF-γ+ CD8 cells, and an ~2X increase in NK, PMNs, DCs and B cells. However, when α-CD45RB was added to IL2+JES6-1, it markedly improved long-term GS (80% >100d) vs. all other regimens. Together, JES6-1+IL2+α-CD45 promoted dramatic Treg expansion, but completely blocked expansion of non-Treg cell types (above) and reduced (70%) IFN-γ+ CD8 cells vs. JES6-1+IL2. Thus: a) Dramatic expansion of Treg is not sufficient to promote tolerance if certain effector, and innate populations also expand. b) α-CD45RB specifically augments the Treg promoting effects of JES6-1+IL2, while inhibiting the expansion of other cell types, resulting in robust and reproducible tolerance.

Funding: NIDDK Support

FR-OR315

CD4 Memory T Cells Persist in the Absence of T Cell Receptor Signals

Jonathan S. Maltzman, Elizabeth Staub, Karla Wiehagen, Evann Corbo. *Department of Medicine and Institute for Immunology, University of Pennsylvania.*

Background: Memory T cells are generated in response to transplant, pathogen exposure, and lymphopenia and are a barrier to tolerance induction. The importance of T cell receptor (TCR)- versus cytokine receptor- generated signals in the differentiation and persistence of CD4+ memory T cell populations has not been well defined. SLP-76 is an adaptor protein critical in mediating proximal TCR signals. We previously showed that continuous expression of SLP-76 is required for activation of naive T cells and for persistence of CD4 memory T cells generated by in vitro stimulation followed by adoptive transfer. The role of TCR signals in pathogen-generated CD4+ memory T cells remains unclear.

Methods: We developed a system combining in vivo temporally-mediated conditional deletion of SLP-76 with viral infection. Lymphocytic choriomeningitis virus (LCMV) Armstrong is used as a model pathogen for acute infection. Following viral clearance and T cell differentiation into memory, SLP-76 is deleted in vivo using a tamoxifen regulated Cre recombinase. Memory cells are identified by I-Ab:GP61 MHC:peptide tetramer staining. Absolute counts of CD4+ memory T cells in the spleen and bone marrow were determined at multiple time points. Homeostatic cell turnover was determined using in vivo BRDU administration. Cytokine production was determined using single cell FACS based assays.

Results: SLP-76 is required for effector function of CD4+ memory T cells. SLP-76 deficient memory T cells fail to produce effector cytokines in response to TCR crosslinking in vitro. Secondary immune responses to LCMV-GP61 are similarly impaired. In contrast to the requirements for effector function and in vitro generated CD4 memory, LCMV-GP61 specific CD4+ T cells persist at wild-type levels until 200 days post deletion regardless of SLP-76 expression.

Conclusions: CD4+ memory T cells have differential requirements for TCR-generated signals depending on the method of their generation. Potential therapeutic approaches that target memory T cell populations in the setting of transplantation and autoimmune disease must account for the diversity of cells in this population.

Funding: Other NIH Support - NIAID

FR-OR316

Recognition of Allogeneic Non-Self by Monocytes Leads to Their Differentiation into Mature Dendritic Cells in a Mouse Heart Transplantation Model

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Background: How the innate immune system recognizes an allograft is not known. Here we tested the hypothesis that, akin to sensing microbial non-self, the innate immune system distinguishes between self and allogeneic non-self, leading to APC maturation and activation of the adaptive alloimmune response.

Methods: T, B and NK cell-deficient B6 Rag-/-γc-/-CX3CR1-GFP+/- reporter mice in which monocyte-lineage cells express GFP received either syngeneic (B6) or allogeneic (Balb/c) vascularized heart grafts. Graft-infiltrating cells were analyzed on days 1, 3, 5, 10, 21 and 42 after Tx and the function of monocyte-derived dendritic cells (mono-DC) was tested in vivo and ex vivo.

Results: We found that the vast majority (> 90%) of DC present in allografts within 1 day after Tx was derived from host monocytes. Mono-DC (lineage-Ly-6G-CD45+GFP+CD11b+CD11c+F4/80lo) were present in significantly greater numbers in allogeneic than syngeneic grafts, had a mature phenotype (MHCII+CD80+), expressed IL-12p40, and stimulated allogeneic T cells in an ex vivo MLR both early (days 1–10) and late (days 21–42) after transplantation. In contrast, maturation of monocytes to mono-DC was self-limited in syngeneic grafts (disappearing between days 10 and 21) and no IL-12p40 expression was detected. Recipient monocyte depletion with clodronate abrogated the proliferation of adoptively transferred 1H3.1 CD4+ T cells that recognize BALB/c allopeptide in the context of H-2b (indirect alloreactivity). Except for higher numbers in allografts on day 1, there were no differences in the number of infiltrating total GFP+ cells, monocytes, macrophages, or GFP+ conventional DC between syngeneic and allogeneic grafts. Neutrophils transiently infiltrated allografts and syngeneic grafts in equal numbers.

Conclusions: Our data indicate that monocytes distinguish between self and allogeneic non-self. Non-self recognition causes their differentiation to mature DC long after inflammation (I/R injury) has subsided. The persistence of activated mono-DC in transplanted organs could perpetuate the immune response, eventually leading to allograft failure.

Funding: Other NIH Support - PO1AI064343, Private Foundation Support

FR-OR317

First MicroRNA Transcriptome of Human Renal Allograft Interstitial Fibrosis and Tubular Atrophy

Thangamani Muthukumar,[1] Iddo Zeev Ben-Dov,[2] Fabien Campagne,[1] Ruchuang Ding,[1] Thomas Tuschl,[2] Manikkam Suthanthiran.[1] *[1]Cornell University; [2]Rockefeller University.*

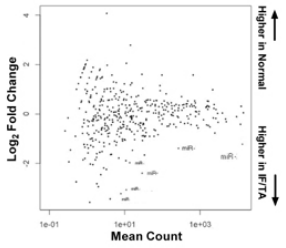
Background: Massively parallel sequencing (Next Generation Sequencing) has revolutionized sequencing. We developed & applied barcoded RNA sequencing to characterize the microRNA (miRNA) transcriptome of human kidney allograft biopsies with interstitial fibrosis/tubular atrophy (IFTA).

Methods: RNA from human renal allograft biopsies (3 IF/TA & 4 Normal, Discovery) were sequenced. Unique ('barcode') 3'-adapters were ligated to each sample & 32 cycle deep sequencing performed on pooled RNA. Using in-house computer pipeline, we extracted barcodes, aligned to genome & assigned read annotations. We determined miRNA abundance by the sum of all reads with up to 1 genome/2 annotation mismatches. We tested for differential expression by DESeq. We used EIMMo target prediction server to identify targets of differentially expressed miRNAs.

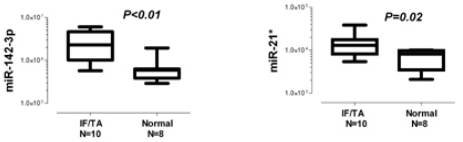
We used RT-PCR to validate selected miRNAs in an independent cohort of 18 kidney recipients; 10 IFTA & 8 Normal (Validation).

Results: The pooled library yielded 1.7 million sequences with overall 8x coverage. Major classes of small RNA annotations were miRNA (64%), synthetic calibrator (26%) and rRNA (4%). Predicted targets for top 3 differentially expressed miRNAs were overrepresented by genes involved in DNA-dependent transcription regulation, transmembrane receptor protein-kinase signaling pathway, protein import into nucleus, & RNA pol II transcription. 6 miRNAs were different between IF/TA & Normal (Top Panel).

Validation of the 2 differentially expressed miRNAs is shown (Bottom Panel).



Scatter plot of log_2 ratio (fold change) versus mean expression. Red data points mark miRNA detected as differentailly expressed at 5% false discovery rate (Benjamini-Hochberg multiple testing adjustment)



Box plots of miR-142-3p (left) & miR-21* (right). miRNA copies (ug total RNA) were normalized to U6 snRNA copies (fg total RNA). miRNA & siRNA were measured by RT-PCR & quantified by standard curve method

Conclusions: To the best of our knowledge, this is the first characterization of the miRNA transcriptome of human renal allograft IFTA using bar coded massively parallel sequencing. Our approach, discovery using deep sequencing and validation with PCR, has identified miRNAs associated with IFTA.

Funding: NIDDK Support

FR-OR318

Extracorporeal Photopheresis (ECP) as an Anti-Rejection Prophylaxis in Kidney Transplant Recipients: Preliminary Results Marian Klinger,[1] Mariusz Kusztal,[1] Katarzyna Koscielska-Kasprzak,[1] Wieslawa Gdowska,[1] Marcelina Zabinska,[1] Marta Myszka,[1] Renata Klak,[1] Magdalena Krajewska,[1] Maria Boratynska,[1] Przemyslaw Szyber,[2] Pawel Chudoba,[2] Dariusz Patrzalek.[2] *[1]Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland; [2]Vascular, General and Transplantation Surgery, Wroclaw Medical University, Wroclaw, Poland.*

Background: ECP is considered a promising immunomodulatory therapy of acute allograft rejection in organ transplantation and GVHD. We report on early (6 months) results of cadaver donor KTx in 20 paired recipients, all of them on standard immunosuppression (MPA+CNI+prednisone), and 10 of them additionally treated with 12-16 ECP procedures in the first 3 months following KTx.

Methods: There was no significant differences between controls and ECP group in terms of age (47±11 vs 43,4±13y), gender (3vs4 F), cause of kidney disease, cold ischemia time, diabetes, CsA/TAC and MPA doses, BMI, mean HLA mismatch and sensitization. ECP procedures were conducted using UVAR XTS (Therakos, Exton, PA), an automated system for leukocyte separation and photoactivation (with Methoxsalen). All recipients were followed by means of eGFR and peripheral T,B,NK, Treg and dendritic cells (DCs) counts and phenotype.

Results: Acute rejection appeared in one recipient from the control group. In ECP group a positive tendency to higher GFR at month 3 (53±11 vs 47,1±9;p=0.17) and 6 (67,5±10 vs 53,6±3;p=0.03 in Wilcoxon test) was observed. An increase in percent of Treg (CD3+CD4+CD25+) in total CD3 cell count (4,9±1 to 9,4±15%) as well as inducible Treg(CD3+CD8+CD28-) among CD3 cells (3,3±3 to 11,8±8%, p=0.025) was observed within 3 months of ECP treatment. A significant difference in percent of Treg in total CD3 cell count was found at month 3 (completed ECP) between ECP group and controls (9,4±15 vs 3±1%;p=0.01). No change was noticed in DCs count. In ECP group more myeloid DCs (BDCA1,3) were immature then in controls after 6 month (89% vs 69% and 88% vs 73%,p=0.08; BDCA1 and 3 respectively).

Conclusions: An addition of ECP to standard immunosuppression was associated with the significant higher GFR at 6 month and with the significant increase of natural Treg among CD3 cells.

Funding: Government Support - Non-U.S.

FR-OR319

A New Recombinant MnSoD Restores the Kidney Functions during Chronic Treatment with Cyclosporine A Sara Damiano,[170120] Roberto Ciarcia,[170153] Roberto Scanni,[170120] Leonida Manco,[170153] Salvatore Florio,[170153] Aldo Mancini,[170160] Giovambattista Capasso.[170120] *[1]Chair of Nephrology, Second University of Naples, Naples, Italy; [2]Department of Structures, Functions and Biological Technologies, School of Veterinary, University of Naples Federico II, Naples, Italy.*

Background: Cyclosporine A (CsA) is used for preventing graft rejections and autoimmune disease. Unfortunately, its use is hampered by its nephrotoxic effects and there is evidence suggesting that these effects are associated with free radical involvement. In this work we have tested if a new recombinant superoxide dismutases (rMnSOD) was able to prevent the renal damage induced by CsA.

Methods: Rats were injected i.p. for 7 days as follows: group1: control solution; group 2: CsA (25 mg/Kg/day); group 3: rMnSOD (10 ug/Kg/day); group 4: rMnSOD (10 ug/Kg/day) and CsA (25 mg/Kg/day) administered simultaneously. At the end of the treatment we measured: GFR by inuline clearance, ROS levels by 2'7' dichloroflurescin diacetate assay and morphology.

Results: GFR was significantly decreased in CsA rats as compared to the controls (0,37±0,1ml/min and 0,92±0,05ml/min respectively) while it was completely restored by rMnSOD administration (0,77±0,08 ml/min). ROS levels were larger in CsA rats than the control (3970±300 and 1459±500 IF/gr of tissue/ug of proteins respectively). In group 4 ROS were similar to control (1322±600). On renal histology, CsA treatment showed cellular vacuolization, tubular swelling, dilatation and necrosis. rMnSOD completely restored the renal damage (fig1).

Conclusions: We have showed that rMnSOD administration restored GFR, ROS levels and renal damage of CsA treated rats. This robust effect of rMnSOD is, most probably, related to the presence of leader peptide allowing rMnSOD to enter the cells and thus preventing its degradation by circulating proteases.

Funding: Private Foundation Support

FR-OR320

Abstract Withdrawn

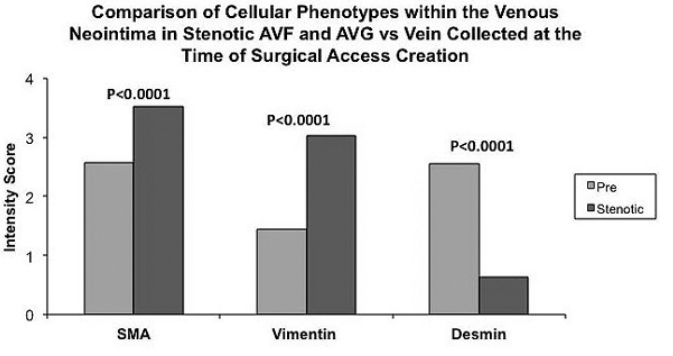
FR-OR321

Comparative Analysis of Cellular Phenotypes between Pre-Existing Venous Neointimal Hyperplasia Prior to Access Surgery and Venous Neointimal Hyperplasia from Stenotic Arteriovenous Dialysis Accesses Timmy C. Lee,[1] Prabir Roy-Chaudhury.[1] *[1]Internal Medicine, University of Cincinnati, OH; [2]Pathology, Johns Hopkins University, Baltimore, MD.*

Background: The histology of vascular access stenosis has been well characterized as aggressive venous neointimal hyperplasia (VNH) in both AV grafts (AVG) and AV fistulas (AVF). Recently we reported that severe VNH occurs prior to vascular access placement. The objective of this study was to perform a comparison of cellular phenotypes between venous tissue samples collected at the time of vascular access creation and stenotic vein samples from patients with failed AVGs and AVFs.

Methods: 55 vein samples,collected at the time of vascular access surgery,and 43 stenotic vein segments from failed AVGs and AVFs were examined. Sections from both groups were evaluated for expression of alpha-smooth muscle actin (SMA), desmin, and vimentin. A semiquantitative scoring system from 0-4+ was used to quantify positive cells for the specific marker compared to total cells (0 indicated 0-10% positive;1+=11-25%;2+=26-50%;3+=51-75% and 4+=76-100%).

Results: Within the neointima, the median semiquantitative scores for SMA, desmin, and vimentin between vein tissue collected at the time of surgery vs. vein tissue obtained from stenotic AVFs and AVGs were 2.57 vs. 3.53, 2.55 vs. 0.63, and 1.45 vs 3.03, respectively.



Conclusions: Our results demonstrate that the predominate cellular phenotype in pre-existing VNH are SMA +ve, desmin +ve, vimentin -ve contractile smooth muscle cells, while in the venous neointima of stenotic fistulas and grafts, SMA+ve, desmin -ve, vimentin +ve myofibroblasts dominate. These phenotypic differences could indicate the need for divergent therapeutic approaches because of differing mechanistic pathways for preexisting VNH compared to the VNH responsible for AVG and AVG stenosis.

Funding: NIDDK Support

FR-OR322

Factor V Leiden (F5) Gene Polymorphism Is Associated with Arteriovenous Graft Failure Michael Allon,[1] Li Zhang,[2] Ivan D. Maya,[1] Molly S. Bray,[3] Jose R. Fernandez.[4] *[1]Division of Nephrology, Univ of Alabama at Birmingham, Birmingham, AL; [2]Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH; [3]Department of Epidemiology, Univ of Alabama at Birmingham, Birmingham, AL; [4]Department of Nutrition Sciences, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Dialysis grafts fail due to recurrent stenosis and thrombosis. Vasoactive and pro-thrombotic substances affecting intimal hyperplasia or thrombosis may modify graft outcomes. We evaluated the association between polymorphisms of several candidate genes and graft patency.

Methods: The Dialysis Access Consortium (DAC) Study randomized patients receiving a new graft to treatment with aspirin+dipyridamole vs placebo, and evaluated graft outcomes. DNA samples from 354 subjects were used to measure genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR), heme-oxygenase 1 (HO-1), Factor V Leiden (F5), transforming growth factor beta-1 (TGF-β1), Klotho, and angiotensin converting enzyme (ACE). We evaluated the association between these polymorphisms and primary graft patency (time to first angioplasty or thrombosis), after adjusting for clinical factors and estimates of genetic admixture for the control of population stratification.

Results: On multivariate analysis, primary graft patency was associated with active drug treatment (HR 0.76; 95% CI 0.60 to 0.96; p=0.02) and with graft placement after starting dialysis (HR 1.38; 95% CI 1.05 to 1.82; p=0.02), but not with patient age, sex, diabetes, cardiovascular disease, graft location, baseline aspirin use, or BMI. After adjusting for the clinical factors and genetic admixture, F5 gene polymorphisms were significantly associated with graft survival in a dominant model (HR 1.70, 95% CI 1.32 to 2.19, p<0.0001 for G/C and G/G genotypes vs C/C genotypes). There was no significant association between primary graft patency and polymorphisms of MTHFR, HO-1, TGF-β1, Klotho, or ACE.

Conclusions: Factor V Leiden (F5), the most common inherited thrombophilia, is associated with an increased risk of graft failure. Anticoagulation may reduce graft failure in patients with the G/C or G/G genotypes (47% of our study).

Funding: NIDDK Support

FR-OR323

Wall Shear Stress (WSS) and Oscillatory Shear Index (OSI) in a Porcine Arteriovenous Graft (AVG) Model Yan-Ting E. Shiu,[1,2] Daniel B. Pike,[2] Christi M. Terry,[1] Huan Li,[1] Alfred K. Cheung.[1,3] *[1]Medicine, Univ. of Utah, SLC, UT; [2]Bioengineering, Univ. of Utah, SLC, UT; [3]Medical Service, VASLCHCS, SLC, UT.*

Background: AVG suffer from high rates of stenosis due to NH at the venous anastomosis (VA). Aberrant anastomotic hemodynamics likely play a role in the focal nature of NH formation, but this causal relationship is not well understood. Chronicling in vivo hemodynamics after AVG implantation will help illuminate its role in NH formation and develop therapies for preventing stenosis.

Methods: Four pigs received an AVG between the common carotid artery and the external jugular vein; the un-operated contralateral vessels served as a control. 3D reconstructions of the vessel and graft lumen geometry were created using Amira software from 3D black-blood MRI scans obtained weekly post-implantation. Blood flow rate was simultaneously obtained using cine phase-contrast MRI. CFD simulations of pulsatile blood flow were performed using FLUENT. CFD results were used to calculate WSS (the axial frictional force exerted by flowing blood on the luminal vessel wall) and OSI (a measure of the change in direction and magnitude of WSS, which is calculated from integrals of WSS at one location over the cardiac cycle).

Results: Compared to control vein, mean WSS, averaged over a cardiac cycle at the VA and downstream vein (DV) areas, was increased by 5.0- and 3.8-fold respectively, at day 5. At day 14, WSS at the VA remained 4.5-fold increased, while WSS at the DV almost returned to control vein levels. Similarly, mean OSI averaged at the VA and DV areas increased by 4.8- and 2.6-fold respectively, at day 5, as compared to control vein. At day 14, OSI at the VA remained 4.5-fold increased, while OSI at the DV also returned to near the control vein level.

Conclusions: We have previously shown in our porcine model of AVG stenosis that significant NH develops at the VA by week 6, but not at the DV. Here we found sustained increased WSS and OSI at the VA, the region most susceptible to NH, but not at DV, the region relatively free from NH. These results suggest a role for increased WSS and OSI at the VA in promoting venous NH.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support, Private Foundation Support

FR-OR324

Early Adventitial Activation and Proliferation in a Mouse Model of Arteriovenous Stenosis Begoña Campos, Yang Wang, Meenakshi J. Mistry, Benjamin K. Woodle, Virgilius Cornea, Timmy C. Lee, Prabir Roy-Chaudhury. *Dialysis Vascular Access Research Group, Division of Nephrology and Hypertension, University of Cincinnati, OH.*

Background: Early arteriovenous fistula (AVF) failure remains an important cause of hemodialysis vascular access dysfunction and a major impediment to the Fistula First initiative. Despite the magnitude of the clinical problem, there are currently no effective therapeutic interventions for early AVF failure. In order to better understand the cellular and molecular mechanisms involved in early AVF failure we have previously developed a mouse model which is characterized by significant stenosis at the AV anastomosis 14d post surgery. The aim of this study was to describe the pattern of cellular proliferation and macrophage infiltration at different time points in this model.

Methods: AVFs were created using an end to side anastomosis between the jugular vein and carotid artery in 13 mice. Animals were sacrificed at 2d (n=4), 7d (n=5) and 14d (n=4). A standard immunohistochemical analysis was performed to assess cellular proliferation (Ki-67) and macrophage infiltration (Mac-2), using a semiquantitative scoring scale (0=<10% of total cells positive; 1+=11-25%; 2+=26-50%; 3+=51-75%; 4+=>75%).

Results: Our results (Table shows Adventitial data only) demonstrate an early proliferation within the adventitia (ADV) which peaks at 7d and which is followed by a later endothelial (ENDO) proliferation which peaks at 14d (p<0.05 for ADV proliferation vs ENDO and intimal [INT] proliferation at 2d). This is accompanied by an early macrophage infiltration which peaks at 7d (p<0.05 for ADV macrophage infiltration vs INT and ENDO macrophage infiltration).

Conclusions: Our results suggest that the adventitia could be a key player in the pathogenesis of early AVF failure. In addition it is possible that early peri-adventitial therapies targeting cellular proliferation and macrophage infiltration might be effective in reducing early AVF failure.

Cellular Proliferation and Macrophage Infiltration in the Adventitia

	2 Days	7 Days	14 Days
Cellular Proliferation	1.75+/- 1.03	2.8+/-0.44	2.125+/0.64
Macrophage Infiltration	0.75 +/-0.88	1.6+/-0.69	0.875+/-0.64

Funding: Private Foundation Support

FR-OR325

Sunitinib Inhibits Venous Neointimal Hyperplasia (NH) Formation in a Perfused Organ Culture System Sun Hyung Kwon,[1] Li Li,[2] Yan-Ting E. Shiu,[2] Seung-Jung Kim,[3] Huan Li,[2] Donald Blumenthal,[1] Alfred K. Cheung.[4,2] *[1]Pharmacology & Toxicology, Univ of UT, SLC; [2]Medicine, Univ of UT, SLC; [3]Nephrology, Ewha Womans Univ; [4]Medicine, VASLHCS, SLC.*

Background: Stenosis associated with arteriovenous (AV) vascular access occurs frequently and predominantly as a result of NH formation at the venous anastomosis. Effective treatment for NH in this setting is lacking. The highly localized nature of NH at the venous anastomosis suggests that aberrant blood flow profiles may play a major role in NH development. We have established a perfused organ culture model that enables us to investigate the effects of various experimental conditions on NH development in intact explanted vessels, and to evaluate the effectiveness of anti-hyperplasia drug candidates.

Methods: In porcine vein segments exposed to non-physiologically low wall shear stress (WSS), we observed significant NH as assessed by intima-to-media (I/M) area ratio, while veins cultured under standard static culture conditions exhibited little or no NH. To evaluate the effect of sunitinib, a multi-receptor tyrosine kinase inhibitor, vein segments were exposed to non-physiological WSS (< 1 or >10 dyne/cm^2) for 12 days with or without 100 nM of sunitinib. Formalin-fixed vein sections were stained with van Gieson's stain to determine I/M area ratio and probed with antibodies for markers of proliferating cells (Ki-67), smooth muscle cells or transformed myofibroblasts (alpha-actin), and endothelial cells (von Willebrand factor).

Results: Sunitinib significantly inhibited the magnitude of NH formation, with the I/M area ratio decreasing from 0.43±0.26 to 0.15±0.04. Cells in the hyperplastic lesions were primarily positive for Ki-67 and alpha-actin, suggesting that the NH resulted from proliferation and migration of smooth muscle cells and/or myofibroblasts.

Conclusions: In summary, this perfused organ culture system allows for the dissection of the effects of various conditions, such as non-physiological WSS, on intact vein segments that could not be achieved using standard cell cultures or whole animal models. It also provides a more expeditious model than the whole animal for the study of anti-hyperplasia therapies.

Funding: Other NIH Support - NIH RO1 HL067646-04A1, Veterans Administration Support, Private Foundation Support

FR-OR326

Without Integrin β3, Endothelial Cell Regeneration Is Delayed and Contributes to AV Fistula Failure Jizhong Cheng, Yun Wang, Anlin Liang, Jie Du, William E. Mitch. *Nephrology Division, Baylor College of Medicine, Houston, TX.*

Background: The Achilles heel of dialysis patients is a functioning arteriovenous fistula (AVF). Failure of an AVF is generally due to proliferation of vascular smooth muscle cells forming a neointima. Whether endothelial cell (EC) damage contributes to this process is unclear. In AVF created in mice, there was an initial denudation of ECs followed by endothelial regeneration. Since integrin β3 is involved in angiogenesis and is essential for normal EC function, we studied how integrin β3 influences the course of endothelial regeneration in AVFs created in WT and integrin β3 KO mice.

Methods: regeneration and differentiation of endothelial progenitor cells (EPC) in AVF were studied *in vitro* and *in vivo* in WT and integrin b3 KO mice.

Results: AVF failed in 80% of integrin β3 KO mice but in only 5% of WT mice. Cultured bone marrow-derived EPCs (Sca-1$^+$) from integrin β3 KO mice showed suppressed attachment to extracellular matrix and differentiation into mature ECs vs WT BM EPCs. These changes were reflected *in vivo:* AV fistulas created in integrin β3 KO mice had fewer Sca I$^+$ EPCs in the denuded vessel wall compared to AVFs created in WT mice. Likewise, endothelial regeneration occurred at 5 days in AVFs in WT mice but was delayed to 10 days in AVFs of integrin β3 KO mice. The defects in AVFs of β3 integrin KO mice were

rescued when the mice received a bone marrow (BM) transplant from WT mice. AVFs in integrin β3 KO mice transplanted with WT BM cells and BM cells from integrin β3 KO mice indicated that WT BM cells were the principal contributor to new endothelium in AVFs of integrin β3 KO mice. Cell proliferation in neointima in the same AVFs was reduced by 40% and was due to impaired SGK-1 phosphorylation and decreased PI3K activity as found in our report about mechanisms of smooth muscle cell proliferation.

Conclusions: Integrin β3 is required for endothelial regeneration in newly created AV fistulas. The mechanism includes an improvement in the attachment and differentiation of EPCs in the AV fistula.

Funding: NIDDK Support

FR-OR327

Quaking as a Critical Determinant of Neoimtima Formation and Vascular Smooth Muscle Cell Homeostasis

Eric P. Van der Veer,[1,2] Ruben G. De Bruin,[1,2] ChunYu Wong,[1,3] Margreet De Vries,[2,3] Ton J. Rabelink,[1] Joris I. Rotmans,[1] J. W. Jukema,[4] Paul Quax,[2,3] Anton Jan Van Zonneveld.[1,2] *[1]Nephrology, LUMC, Leiden, Netherlands; [2]Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, Netherlands; [3]Surgery, LUMC, Leiden, Netherlands; [4]Cardiology, LUMC, Leiden, Netherlands.*

Background: Patients undergoing hemodialysis require a patent, vascular access point that can withstand high-flow rates. For this, an arteriovenous fistula (AVF) is commonly surgically created in the arm. However, low patency rates due to insufficient remodeling and excessive neointima formation are frequently observed in the clinic. Here, we present the mRNA-binding protein Quaking (QKI) as a critical regulator vascular smooth muscle cell (VSMC) homeostasis through modulation of actin cytoskeletal dynamics, and neointima formation following injury to the vessel wall.

Methods: Quaking viable mouse mutants (Qk^v), which as a result of a megabase deletion in the *qki* promoter region express reduced levels of QKI, were used to study neointima formation upon non-constrictive cuff placement around the femoral artery. Furthermore, we specifically investigated the consequences of abrogated QKI expression on VSMC behaviour in both murine VSMCs derived from C57Bl6 (WT) and Qk^v mice as well as in WT human VSMCs treated with either a non-coding shRNA (shRNA-non) and shRNA targeting QKI (shRNA-qki).

Results: Non-constrictive cuff placement in Qk^v mice resulted in a significant decrease in neointima formation and luminal stenosis as compared to control mice. Furthermore, attenuated expression of QKI in VSMCs (derived from both Qk^v mice or following shRNA-mediated knockdown in human VSMCs) resulted in significantly reduced cellular proliferation, migration and elaboration of extracellular matrix. The targeted disruption of QKI impaired both the capacity to remodel the actin cytoskeleton and perturbed VSMC contractile function.

Conclusions: Collectively, we have uncovered a novel role for QKI in regulating VSMC homeostasis, and propose that intervention in QKI activity could be an effective modality in the prevention of AVF stenosis, and potentially shunt failure.

FR-OR328

Matrix-Embedded Endothelial Cells Are Protected from the Uremic Milieu

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Background: Endothelial cells (ECs) embedded in 3D matrices (MEECs) of denatured collagen implanted around vascular access anastomoses preserve luminal patency. MEEC implant efficacy depends on embedded EC health. As the uremic milieu inhibits proliferation and induces apoptosis of ECs, we examined whether uremia might impact MEECs.

Methods: ECs grown on 2D gelatin-coated polystyrene culture plates (gTCPS) or in MEEC were treated with sera pooled from 20 control or ESRD patients. EC viability was examined using MTT, cell counting and Trypan blue. Media conditioned (CM) with 2 and 3D-supported ECs were examined for inhibition vascular smooth muscle cell (vSMC) proliferation using 3[H] thymidine and cyclin D1. ECs grown on gTCPS were treated with uremic serum filtered through matrices to examine if matrices retain uremic toxins or whether EC effects were cell-mediated.

Results: Uremic serum significantly reduced viability and number of live, and increased dead ECs on gTCPS, but not in MEECs. EC survival correlated with vSMC inhibition. While CM from ECs grown in gTCPS with uremic serum inhibited vSMC proliferation no better than uremic serum alone (22% vs 27%), MEEC CM inhibited vSMC proliferation by 47% ($p = 0.0004$). Cyclin D1 expression tracked with vSMC proliferation. There was no significant difference in EC viability between EC treated with matrix-filtered or unfiltered uremic serum.

Conclusions: The viability, number and efficacy of MEECs were preserved in uremic serum compared to that of ECs on gTCPS. MEECs are protected from uremic toxicity, not from retention of uremic toxins by matrices, but likely from intrinsic changes in EC sensitivity to uremia. MEECs implanted at vascular access should inhibit neointimal hyperplasia in uremia. This study underscores the robustness of matrix-embedding as cell-protectant, especially in hostile environment like uremia.

Funding: NIDDK Support, Private Foundation Support

FR-OR329

Venous Segment Calcification in a Mouse Model of Arteriovenous Stenosis

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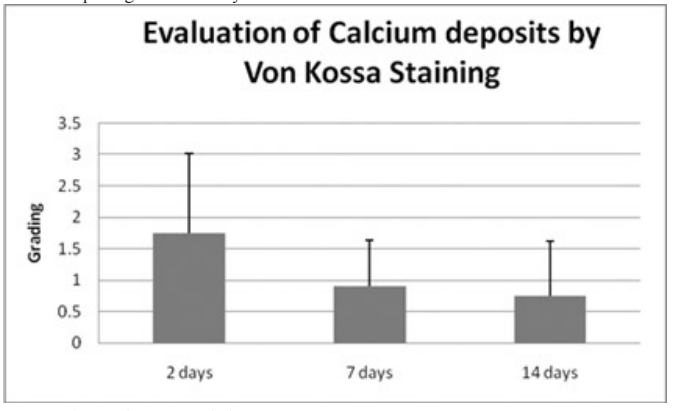
Background: Early arteriovenous fistula (AVF) failure remains an important cause of hemodialysis vascular access dysfunction, although the exact biological mechanisms involved remain unclear. Vascular calcification is known to be an important clinical problem in the setting of uremia but the exact role of calcification at different time points following creation of an AVF is unknown. The aim of this study was to describe the extent of venous segment calcification at different time points in a mouse model of AVF stenosis.

Methods: AVFs were created between the jugular vein and the carotid artery with an end to side anastomosis in 13 mice (this model results in significant stenosis at the AV anastomosis 14 days after surgery). Animals were sacrificed at the 2d (n=4), 7d (n=5) and 14d (n=4) time points and examined for calcification within the venous segment of the AVF (including the anastomosis) with a Von Kossa stain. Sections were scored using a semiquantitative scoring scale (1=1-10% calcification; 2=11-25%; 3=26-50%; 4=>51%).

Results: Maximal venous segment calcification occurred at 2d post surgery, often in the setting of a swollen venous wall. Although the magnitude of venous segment calcification appeared to decrease over time this was not statistically significant (see figure).

Conclusions: Our results clearly document the presence of venous segment calcification in a mouse model of AVF stenosis. It is unclear at present as to whether these calcium deposits are secondary to early hemodynamic injury or whether they could play a primary role in the pathogenesis of early AVF failure.



Funding: Private Foundation Support

SA-OR330

Generation and Examination of Proximal Tubule Specific Na^+/H^+ Exchanger NHE3 Knockout Mouse

Hong C. Li,[1] Zhaopeng Du,[2] Sharon L. Barone,[1] Hassane Amlal,[1] Alicia A. McDonough,[3] Tong Wang,[2] Manoocher Soleimani.[1] *[1]Medicine, University of Cincinnati; [2]Yale University; [3]University of Southern California.*

Background: The conventional NHE3 knockout mouse has a significant intestinal phenotype manifested with electrolyte malabsorption, diarrhea, and volume depletion. As a result the mutant mouse can not tolerate oral salt or acid load, making it unsuitable for the examination of the role of kidney NHE3 *in vivo*.

Methods: To overcome this problem, mice with tissue specific deletion of NHE3 were generated. NHE3 floxed mice were crossed with either the Sglt2 (sodium-glucose transporter 2) cre mice to generate proximal convoluted tubule specific NHE3 knockout (NHE3-PT KO) or with the villin cre mice to generate combined intestine and proximal tubule NHE3 knockout mice.

Results: The intestine and kidney specific NHE3 ko mice are smaller and have loose stool and caecal dilatation, consistent with an important role for NHE3 in salt absorption in the intestine. The NHE3-PT KO mice have ~ 90% ablation of NHE3 as determined by immunofluorescence labeling and western blotting. NHE3-PT KO animals show mild metabolic acidosis (21 mEq/l in ko Vs. 23 in wt, $p<0.05$). In situ microperfusion studies in proximal tubules demonstrate a ~36% reduction in bicarbonate reabsorption (J_{HCO3} = 83 pmol/min/mm in wt Vs. 54 in ko) and a ~27% reduction in volume reabsorption (Jv = 0.92 nl/min/mm in wt Vs. 0.67 in ko) in mutant mice. The NHE3-PT KO mice tolerated NH_4Cl acid load well and showed comparable NH_4 excretion rates Vs. wild type mice at 2 days and 5 days after acid loading.

Conclusions: We conclude that NHE3 plays an important role in bicarbonate reabsorption in the proximal convoluted tubule but does not play a major role in NH_4^+ secretion, at least in the first 5 days of acid loading. Whether the Na^+/H^+ exchanger NHE3 in the S3 segment of the proximal tubule plays a more important role in NH_4^+ secretion than the convoluted segment needs the generation and examination of appropriate mutant animals. The mild metabolic acidosis, despite a significant reduction in net bicarbonate reabsorption in the proximal tubule of NHE3 KO mice, suggests the presence of compensatory mechanisms in other nephron segments.

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SA-OR331

ErbB1-ErbB2 Heterodimer Activation in Rabbit Renal Proximal Tubules Exposed to Acute Respiratory Acidosis Lara A. Skelton, Walter F. Boron. *Dept. Physiology and Biophysics, CASE Western Reserve University, Cleveland, OH.*

Background: In a previous abstract, we reported that acute exposure of rabbit proximal tubule suspensions (PTs) to respiratory acidosis (RAc) increases both ErbB1 and ErbB2 tyrosine phosphorylation. Zhou et al have shown that single perfused PTs exposed to the ErbB-family inhibitor PD168393 cannot respond to changes in basolateral [CO_2] or [HCO_3]. Here we examine if heterodimers of ErbB1 and ErbB2 are involved in the CO_2/HCO_3 signaling cascade.

Methods: We exposed purified PTs for 5 min or 20 min to: *HEPES* (i.e., HEPES/pH 7.40); *Butyrate* (HEPES/pH 7.10 + 20 mM butyrate); *EGF* (*'HEPES'* + 10 nM EGF); *Physiological CO_2/HCO_3* (5% CO_2/22 mM HCO_3/pH 7.40); *RAc* (10% CO_2/22 mM HCO_3/pH 7.10); *Compensated RAc* (10% CO_2/44mM HCO_3/pH 7.40); or *Metabolic alkalosis* (*MAlk*; 5% CO_2/44 mM HCO_3/pH 7.66). Lysates were probed with phosphotyrosine (pY)-specific antibodies to ErbB1 or ErbB2, or with antibodies to total ErbB1 and ErbB2.

Results: PTs exposed to *HEPES* or *Butyrate* exhibit a low, basal level of pY for both ErbB1 and ErbB2. Exposure to *EGF, Physiological CO_2/HCO_3,* or *RAc* induces ErbB1-pY845, pY1173, and pY1068, as well as ErbB2-pY1221/pY1222 as early as 5 min, a response that is sustained at 20 min. PD168393 inhibited ErbB1 and ErbB2 activation (i.e., tyrosine phosphorylation at the above residues) under all conditions. To detect heterodimers, ErbB1 IPs were probed for ErbB2-pY1221/1222 and ErbB2 IPs were probed for ErbB1-pY1173. Activation of ErbB1-ErbB2 heterodimers occurred after 20-min treatment with *EGF, Physiological CO_2/HCO_3,* or *RAc*. In contrast, a 20-min exposure to *MAlk* versus *Physiological CO_2/HCO_3*, on average, resulted in reduced activation of ErbB1/ErbB2 heterodimers. Exposure to *RAc* appears to induce slightly greater heterodimer activation than exposure to *Compensated RAc*.

Conclusions: The results indicate that exposure of PTs to CO_2/HCO_3, especially during respiratory acidosis, induces rapid ErbB1-ErbB2 activation and heterodimerization.

Funding: Other NIH Support - RO1-DK081567 Regulation of Proximal Tubule bicarbonate transport

SA-OR332

Vacuolar H^+-ATPase Regulation by AMPK in the Kidney Proximal Tubule Mohammad M. Al-Bataineh,[1] Nuria M. Pastor-Soler.[1,2] *[1]Renal-Electrolyte Division, Department of Medicine, U. of Pittsburgh; [2]Department of Cell Biology and Physiology, U. of Pittsburgh, PA.*

Background: The Vacuolar H^+-ATPase (V-ATPase) is present at the apical membrane of proton secreting cells, such as kidney proximal tubule cells. We previously showed that the V-ATPase at the apical membrane of distal nephron intercalated cells is regulated by the metabolic sensor AMP-activated protein kinase (AMPK). In proximal tubule, the V-ATPase participates in luminal bicarbonate recovery. Defects in V-ATPase and in proximal tubule function can cause renal tubular acidosis with systemic metabolic acidosis. Studying the V-ATPase regulation in the proximal tubule is important as it may differ from regulation in other nephron segments. Here, we examined the role of AMPK, which can become active in settings of ischemia and metabolic stress, in the regulation of V-ATPase subcellular localization and activity in the proximal tubule.

Methods: We examined the effects of an AMPK activator in the S3 segment of rat kidney tissue slices ex vivo and in an immortalized S3 proximal tubule cell line (S3 cells) on subcellular V-ATPase immunolocalization by confocal microscopy and by functional acidification assays.

Results: The V-ATPase had an apical distribution in S3 proximal tubules of kidney slices incubated for 75 min in Ringer buffer alone and in S3 cell polarized monolayers, while the AMPK activator AICAR induced a decrease in V-ATPase apical accumulation in both systems. Moreover, AICAR treatment activated AMPK in S3 cells and decreased the rate of V-ATPase-dependent (bafilomycin-sensitive) extracellular acidification relative to untreated cells.

Conclusions: Taken together, our results suggest that in proximal tubule S3 cells the V-ATPase is inhibited by activation of the metabolic sensor AMPK. This pathway that may be very relevant in the conservation of cellular energy during kidney ischemia.

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SA-OR333

The Role of Ile-Pro-Met Tri-Peptide in the C-Terminus of Kidney NBC1 (NBCe1/SLC4A4) in Its Targeting to the Plasma Membrane Hong C. Li,[1, 2] Kamyar A. Zahedi,[1, 2] Manoocher Soleimani.[1, 2] *[1]Medicine, University of Cincinnati, OH; [2]Research Services, Veterans Administration Hospital, Cincinnati, OH.*

Background: The human kidney NBC1 mutant (NBC1Δ65) codes for a C-terminal truncated protein (total length 982 aa Vs. 1035 for wt) that is retained in the cytoplasm. Individuals homozygous for this mutation have proximal renal tubular acidosis (pRTA) and familial migraine (PNAS 2010).

Methods: Here we describe a novel motif in the C-terminus of NBC1 corresponding to the deleted region in NBC1Δ65 that contributes to its accurate targeting to the plasma membrane. Toward this goal, a number of GFP tagged kNBC1 C-terminal subdomain deletion expression constructs were developed and used to transiently transfect polarized MDCK cells. Functional studies were performed in Oocytes.

Results: Confocal microscopy indicates that kNBC1 Δ991-1005 was retained in the cytoplasm, while the wild type, Δ961-975, Δ976-990 and Δ1006-1009 kNBC1 proteins were appropriately targeted to the basolateral membrane. Functional studies in Oocytes indicated that while the electrogenic potential of kNBC1 Δ961-975, Δ976-990 and Δ1006-1009 mutants were not significantly different than that of wild type kNBC1, the kNBC1 Δ991-1005 mutant exhibited a significantly reduced electrogenic potential (*p<0.01). In order to more precisely identify the amino acid residues involved in the targeting of kNBC1, we generated a series of kNBC1 C-terminal subdomain triple-peptide deletions spanning the amino acid residues 991 to 1005. These mutant proteins were then expressed and their localization was examined. Our results indicate that kNBC1 Δ1003-1005 mutant was retained in the cytoplasm while the wild type, Δ991-993, Δ994-996, Δ997-999 and Δ1000-1002 GFP-kNBC1proteins were correctly targeted to the basolateral membrane. Functional studies revealed that only the kNBC1 D1003-1005 mutant was significantly less electrogenic than kNBC1 wild type (Δ1003-1005: -13±5.7mV vs kNBC1 wild type: -54.8±7.4 *p<0.01).

Conclusions: Our data indicate that the tri-peptide Ile 1003-Pro 1004-Met 1005 in the C-terminus of NBC1 is involved in its accurate targeting. These results provide novel insight into the mechanism through which kNBC1 Δ65bp mutation leads to pRTA.

Funding: NIDDK Support, Veterans Administration Support

SA-OR334

Lipopolysaccharide (LPS) Inhibits NHE3 and HCO_3^- Absorption in Medullary Thick Ascending Limb (MTAL) through a TLR4-ERK Pathway Upregulated by Sepsis Bruns A. Watts, Thampi George, Edward R. Sherwood, David W. Good. *Medicine and Anesthesiology, Univ TX Med Branch, Galveston, TX.*

Background: Recently we demonstrated that bacterial molecules can act directly through Toll-like receptors (TLR) to impair the transport function of renal tubules. In particular, basolateral LPS inhibits HCO_3^- absorption in perfused rat and mouse MTALs through TLR4-mediated activation of ERK. Here we examined the transport mechanism responsible for this inhibition and whether the LPS-induced pathway is influenced by sepsis.

Results: Activation of ERK can inhibit HCO_3^- absorption in the MTAL through primary inhibition of either basolateral NHE1 or apical NHE3. The inhibition of HCO_3^- absorption by bath LPS was unaffected by maneuvers that inhibit basolateral Na^+/H^+ exchange, indicating that NHE1 is not involved in mediating the inhibition by LPS. Bath LPS decreased apical NHE3 activity due to a decrease in V_{max}. The inhibition of NHE3 by LPS was eliminated by MEK/ERK inhibitors. The effects of sepsis were examined 18 h after surgery using a mouse cecal ligation and puncture (CLP) model. Bath LPS decreased HCO_3^- absorption by 45% in MTALs from CLP mice compared with a decrease of only 26% in tubules from sham-operated controls (P<0.05). MEK/ERK inhibitors eliminated the inhibition of HCO_3^- absorption by LPS in both groups. Exposure to LPS for 15 min increased ERK phosphorylation in sham and CLP MTALs but the level of phosphorylated ERK was higher in MTALs from CLP mice. The increased ability of LPS to inhibit HCO_3^- absorption through ERK in CLP MTALs was associated with increased expression of TLR4 in the basolateral membrane domain.

Conclusions: We conclude: 1) basolateral LPS inhibits HCO_3^- absorption in the MTAL through TLR4-ERK-dependent inhibition of NHE3; 2) this pathway is upregulated during sepsis. These results identify NHE3 as a target of TLR4 signaling in the MTAL and suggest that bacterial molecules can impair absorptive functions of renal tubules important for acid-base and volume homeostasis through inhibition of this exchanger. The ERK pathway links TLR4 to downstream modulation of ion transport proteins and represents a potential target for treatment of sepsis-induced renal tubule dysfunction.

Funding: NIDDK Support

SA-OR335

Double-Knockout of Pendrin and NaCl Cotransporter Causes Salt Wasting, Profound Dehydration and Nephrogenic Diabetes Insipidus Manoocher Soleimani,[1, 2] Hassane Amlal,[2] Sharon L. Barone,[1, 2] Jie Xu,[1, 2] Faraaz Siddiqui.[2] *[1]Research Services, Veterans Administration Hospital, Cincinnati, OH; [2]Medicine, University of Cincinnati, OH.*

Background: The Cl^-/HCO_3^- exchanger pendrin (SLC26A4) and the thiazide-sensitive NaCl cotransporter NCC are expressed in the distal nephron and mediate salt absorption. Single deletion of pendrin or NCC does not cause salt wasting or excessive diuresis under basal conditions, suggesting they might be predominantly active during salt depletion.

Methods: Alternatively, we hypothesized that each transporter may compensate for loss of the other under basal conditions, thereby masking the role that each plays in salt absorption.To test our hypothesis, we generated pendrin/NCC double knockout (dKO) mice.

Results: NCC/pendrin double KOs displayed profound polyuria (24 hr urine volume in ml: double KO, 5.3; pendrin KO, 1.5; NCC KO, 1.0; WT, 1.5; p<0.0001 vs single KO and WT) and polydipsia (24 hr water intake in ml: double KO, 8.0; pendrin KO, 4.0; NCC KO, 4.4; WT, 3.5; p<0.001 vs single KO and WT). Urine osmolarity was 790 mosm/kg H2O in double KO vs. 2500 or higher in other genotypes (p<0.001 Vs. single KO and WT). Urine chloride excretion was significantly increased in dKO mice (440 mmole/day Vs. 270 or less in other genotypes). Kidney H&E staining revealed no histological abnormalities, but BUN increased by ~300% in double KOs (p<0.05 Vs. other genotypes), consistent with dehydration. NKCC2 abundance in the thick limb increased by 50% whereas AQP2 abundance in medullary collecting duct decreased by ~70% in double KO mice. Contrary to the normal concordant regulation of AQP2 and NKCC2 in pathophysiologic states, this condition presents with discordant regulation of NKCC2 and AQP2.

Conclusions: In conclusion, combined deletion of pendrin and NCC causes polyuria, salt wasting, dehydration, and nephrogenic diabetes insipidus. These results indicate a major role for both NCC and pendrin in salt and water reabsorption and show that each transporter can provide compensation for loss of the other under basal conditions. We suggest that the role of pendrin and NCC in salt and water reabsorption at basal state in the distal nephron needs complete reevaluation.

Funding: NIDDK Support, Veterans Administration Support

SA-OR336

The A-kinase Anchoring Protein AKAP-KL (AKAP2) Is Localized in the Subapical Domain of All Collecting Duct Intercalated Cells and Is Regulated by Disturbance of Acid/Base Balance Annika Andersen,[1] Enno Klussmann,[2] Sebastian Frische.[1] *[1]Biomedical Institute, Aarhus University, Aarhus, Denmark; [2]Max-Delbrück-Centrum für Molekulare Medizin Berlin-Buch (MDC), Berlin, Germany.*

Background: Compartmentalized cAMP/protein kinase A (PKA) signalling mediated by A-kinase anchoring proteins (AKAPs) is an emerging paradigm. AKAP-KL (AKAP2) tethers PKA in lung and renal epithelial cells. However, the detailed localization and function of this AKAP in the kidney is unknown. This study:

a) describes the cellular and subcellular localization of AKAP-KL in the kidney

b) tests if the amount of AKAP-KL in collecting duct (CD) intercalated cells (ICs) correlates with IC subtype activity during acid/base disturbance.

Methods: Paraformaldehyde-fixed parraffin-embedded kidney tissue from untreated, NH_4Cl loaded and $NaHCO_3$ loaded rats were analyzed by immunohistochemistry using a mouse monoclonal antibody against AKAP-KL, fluorescence-coupled secondary antibodies and laser confocal microscopy. Co-labelling for the anion-exchangers pendrin and AE1 allowed identification of IC sub-types. For quantification of fluorescence, ICs were encircled manually and the fluorescences pertaining to AKAP-KL, pendrin and the nuclei were quantified using ImageJ. In total 2445 ICs were analyzed and >60 ICs were analyzed per animal.

Results: AKAP-KL was found in the brushborder of proximal tubule cells and in the subapical domain (predominantly) and cytoplasm (low amounts) of both type A and B CD ICs. In untreated rats (n=5), the fluorescence representing AKAP-KL was not significantly different in the two main classes of ICs (mean signal/cell ± SE: A: 52483±8353, B: 52155±5324 p=0.908). In NH_4Cl loaded rats (n=6), type A cells showed higher abundance of AKAP-KL than type B cells (A: 65003±4653, B: 45995±2674 p=0.002). In $NaHCO_3$ loaded rats (n=3) type B cells showed higher abundance of AKAP-KL than type A cells (A: 46352±3511, B: 58024±5314 p=0.036).

Conclusions: Type A and B-type ICs show inverse transport functions and acid/base transporter expression patterns. However, our studies indicate that in both cell-types, activation induced by disturbed acid/base balance involves compartmentalized signalling involving AKAP-KL.

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SA-OR337

Acidosis Induces Secretion of SDF-1 by Principal Cells Which Acts on β-Intercalated Cells (β-ICs) To Decrease HCO_3 and Increase H Secretion George J. Schwartz,[1] Jeffrey M. Purkerson,[1] Shuichi Tsuruoka,[2] Qais Al-Awqati.[3] *[1]Pediatrics, University of Rochester Medical Center, NY; [2]Nephrology, University of Tsukuba, Ibaraki, Japan; [3]Medicine, Columbia University College of Physicians & Surgeons, New York, NY.*

Background: Detection of elevated SDF-1 mRNA levels in kidney samples from acidotic mice by microarray and confirmed by RT-qPCR prompted us to study the role of the SDF-1/CXCR4 pathway in adaptation of the rabbit CCD to acidosis.

Methods: We performed immunofluorescence of sections and microdissected CCDs and in vitro microperfusion and incubation of CCDs at pH 7.4, pH 6.8, and ± 12.5 nM SDF-1α, or the CXCR4 receptor antagonist AMD3100 (1 mcM). Net HCO_3 flux was measured and expressed as the sum of H flux & HCO_3 flux, using Cl-free perfusate. 3D reconstructions of confocal images of incubated CCDs were stained for pendrin.

Results: Rabbit kidney sections confirmed expression of SDF-1 in CCD and double labeling with B1-V-ATPase indicated that SDF-1 is expressed primarily in principal cells. In microperfused CCDs the CXCR4 antagonist AMD3100 reduced the adaptive decrease in $JHCO_3$ and increase in JH induced by low pH (pH 6.8) by 34% and 36%, respectively, resulting in a 79% reduction in the adaptive change in Jnet. To determine whether SDF-1α is sufficient to induce changes in H/HCO_3 transport, microperfused CCDs were incubated with SDF-1α at pH 7.4 for 3 h. SDF-1 treatment resulted in an 81% reduction in net HCO_3 secretory flux that was comprised of a 23% reduction in $JHCO_3$ and a 32% increase in JH. Microdissected CCDs were incubated in *in vitro* culture medium aerated with 95%O_2/5%CO_2±SDF-1 @ 37 C for 3 h. Morphometric analysis of pendrin staining in images of 3D tubule reconstructions revealed that SDF-1 treatment promoted redistribution of pendrin from an apical cap to a more cytoplasmic distribution, similar to what was observed during incubation at pH 6.8.

Conclusions: Thus SDF-1/CXCR4 signaling is not cell autonomous; rather principal cells receive the acid signal releasing SDF1 which then diffuses to the ICs to change H/HCO_3 transport and regulate pendrin endocytosis from the apical membrane.

Funding: NIDDK Support

SA-OR338

Luminal Flow Modulates H^+-ATPase Activity in the Cortical Collecting Duct (CCD) Wen Liu,[1] Nuria M. Pastor-Soler,[2] Carlos Schreck,[1] Thomas R. Kleyman,[2] Lisa M. Satlin.[1] *[1]Mount Sinai School of Medicine, NY, NY; [2]University of Pittsburgh, PA.*

Background: ENaC-mediated Na^+ absorption and BK channel-mediated K^+ secretion in the cortical collecting duct (CCD) are modulated by flow, the latter requiring an increase in $[Ca^{2+}]_i$, microtubule integrity, and exocytic insertion of preformed channels into the apical membrane. As axial flow modulates HCO_3^- reabsorption in the proximal tubule due to changes in both luminal NHE3 and H^+-ATPase activity (Du Z. et al, Am J Physiol Renal Physiol 290:F289, 2006), we sought to test the hypothesis that flow also regulates H^+-ATPase activity in the CCD.

Methods: H^+-ATPase activity was assayed in individually identified cells in microperfused CCDs isolated from NZW rabbits, loaded with the pH-sensitive dye BCECF, and then subjected to an acute intracellular acid load (NH_4Cl prepulse technique). H^+-ATPase activity was defined as the initial rate of bafilomycin-inhibitable cell pH (pH_i) recovery in the absence of luminal K^+, bilateral Na^+ and CO_2/HCO_3^-, from a nadir pH of ~6.2.

Results: We found that (i) an increase in luminal flow rate from ~1 to 5 nl/min. mm stimulated H^+-ATPase activity (pH U/min) in both intercalated (IC; 0.059±0.009 to 0.146±0.023; p<0.03) and principal (PC; 0.050±0.009 to 0.126±0.004; p<0.03) cells, the latter identified by their selective labeling with the apical PC marker Dolichos Biflorus agglutinin, and (ii) flow-stimulated H^+ pumping was Ca^{2+} dependent and required microtubule integrity, based on its inhibition by pretreatment with BAPTA-AM and colchicine, respectively.

Conclusions: We conclude that luminal flow modulates H^+-ATPase activity in the rabbit CCD and that H^+-ATPases therein are present in both PC and IC. We speculate that flow stimulation of H^+ secretion in the distal nephron may contribute to diuretic-induced metabolic alkalosis.

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SA-OR339

Movement of NH_3 through the Human Urea Transporter B (UT-B) — A New Gas Channel R. Ryan Geyer,[1] Raif Musa-Aziz,[1,2] Walter F. Boron.[1] *[1]Dept of Physiol & Biophys, Case Western Reserve Univ School of Medicine, Cleveland, OH; [2]Dept of Physiol & Biophys, Univ of Sao Paulo, Sao Paulo, Brazil.*

Background: The urea transporters (UTs) mediate the facilitated diffusion of urea across the plasma membrane. UT-B (SLC14A1) is an integral membrane protein highly expressed in the kidney and red blood cell. A bacterial homolog has been crystallized as a homotrimer. Each monomer forms an independent urea channel. A fourth pore is formed at the three-fold axis of symmetry. Previously, the Boron laboratory showed that multimeric proteins (i.e. Aquaporins and Rh proteins) can function as gas (CO_2 and NH_3) channels. This study explores the CO_2 and NH_3 permeability of human UT-B expressed in *Xenopus* oocytes (*vs* H_2O-injected control oocytes).

Methods: We used microelectrodes to record the maximum transient change in surface pH (ΔpH_S) caused by exposing the oocyte to 5% CO_2/33 mM HCO_3^- (an increase) or 0.5 mM NH_3/NH_4^+ (a decrease). We computed osmotic water permeability (P_f) using video microscopy. Subtracting the respective values for day-matched, H_2O-injected control oocytes yielded channel-specific values (*). The magnitude of ΔpH_S* is a semiquantitative index of maximal CO_2 or NH_3 influx.

Results: $(\Delta pH_S^*)_{CO2}$ was not significantly different in UT-B vs H_2O oocytes (0.082 ± 0.007, n=18 vs 0.068 ± 0.003, n=17). However, with NH_3 addition, $(-\Delta pH_S^*)_{NH3}$ was twice as high in UT-B vs H_2O oocytes (0.058 ± 0.006, n=20 vs 0.030 ± 0.002, n=19). P_f was 8-fold greater in UT-B vs H_2O oocytes. $(-\Delta pH_S^*)_{NH3}$ and P_f* were each significantly greater than 0 for UT-B oocytes, indicating that UT-B can carry NH_3 and H_2O. To explore the pathway of NH_3 and H_2O movement through UT -B, we examined the effect of phloretin (UT inhibitor) and pCMBS (H_2O channel inhibitor). Phloretin (500 μM, 20-min pretreatment) reduced $(-\Delta pH_S^*)_{NH3}$ by 70% and P_f* by 50%. pCMBS (1 mM, 30-min pretreatment) reduced $(-\Delta pH_S^*)_{NH3}$ by 100% and P_f* by 30%.

Conclusions: Thus, our P_f* data confirm that UT-B has significant H_2O permeability and, using the pH_S approach, we show for the first time that UT-B has significant NH_3 but not CO_2 permeability. We propose that all NH_3 passes through the three monomers in UT-B—blocked by phloretin and pCMBS.

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SA-OR340

Acute Kidney Injury Following CABG Is Related to Long-Term Incidence of Myocardial Infarction Martin Holzmann, Linda Ryden Lujan. *Department of Emergency Medicine, Karolinska University Hospital, Internal Medicine Unit, Karolinska Institutet, Sweden.*

Background: Acute Kidney Injury (AKI) is strongly related to early mortality and postoperative complications following coronary artery bypass surgery (CABG). Recent studies indicate that AKI following CABG is related to long-term mortality. However, there is little information on the long-term risk of myocardial infarction (MI) associated with AKI.

Methods: From the Swedish Heart Surgery Register (Swedeheart), all patients undergoing a first, isolated CABG electively, during 1995-2008 in Sweden, with information on pre- and postoperative serum creatinine, and alive 60 days postoperatively, were included. Information about confounders was found in the Swedish Coronary Angiography and Angioplasty Register and from the Swedish Inpatient Register and Cause of Death Register,

information on incidence of MI and all-cause mortality, was assembled. Acute kidney injury was defined both as an absolute increase in postoperative serum creatinine of 0,3-0,5 mg/dL, 0,5-1,0 mg/dL or > 1,0 mg/dL, and according to AKIN criteria. Hazard ratios (HR) for different levels of AKI were calculated using Cox proportional hazards regression.

Results: The study population consisted of 45,002 patients, with a mean age of 67 years. During a mean follow-up of nine years there were 3,945 (8.8%) MIs and 11,157 (25%) deaths. After adjustment for confounders, the HRs for MI (95 percent confidence intervals) associated with a postoperative increase in serum-creatinine of 0,3-0,5 mg/dL, 0,5-1,0 mg/dL or > 1,0 mg/dL, were 1,52 (1,33-1,61), 1,85 (1,74-2,11) and 2,09 (1,78-2,31), respectively. The corresponding figures for death were 1,48 (1,39-1,53), 2,04 (1,83-2,21) and 3,84 (3,50-4,01), respectively. Similar associations were found when AKI was defined using AKIN criteria.

Conclusions: Acute kidney injury defined according to an absolute increase in postoperative values of serum creatinine or AKIN criteria is strongly associated with both long-term incidence of MI and death. Cardiologists or family doctors following patients with a history of CABG should take into account the long-term prognostic implications of AKI post-CABG.

SA-OR341

Temporal Relationship and Predictive Value of Urinary AKI Biomarkers after Cardiac Surgery Prasad Devarajan, Catherine D. Krawczeski, Stuart Goldstein, Yu Wang, Nuntawan Piyaphanee, Qing Ma, Michael R. Bennett. *Cincinnati Children's Hospital, Cincinnati, OH.*

Background: Acute kidney injury (AKI) occurs commonly after cardiopulmonary bypass (CPB). Serum creatinine (SCr) is a delayed marker for AKI. We evaluated the temporal pattern and predictive value of 4 most promising urinary AKI biomarkers: neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), liver fatty-acid binding protein (L-FABP), and kidney injury molecule-1 (KIM-1), for CPB-associated AKI.

Methods: Urine samples were obtained prospectively before and at several time points after CPB in 220 patients. AKI was defined as a ≥50% increase in SCr from baseline. Biomarker values were correlated with AKI severity and clinical outcomes. Logistic regression was used to assess AKI predictors. Biomarker predictive abilities were evaluated by area under the curve (AUC), net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

Results: AKI occurred in 27% of patients. NGAL first increased in AKI patients at 2h after CPB. IL-18 and L-FABP first increase at 6h and KIM-1 first increased at 12h. Biomarker elevations correlated with disease severity (RIFLE) and clinical outcomes (length of hospital stay, days on ventilator), and improved prediction of AKI above the clinical model. At 2h post-CPB, NGAL alone increased the AUC from 0.74 for the clinical model to 0.85 ($p<0.001$). At 6h post-CPB, NGAL, IL-18 and L-FABP each improved the AUC from 0,72 to 0.91, 0.84, and 0.77 respectively (all $p<0.05$). At the 6h time point, the combination of NGAL + IL-18 provided the best results in terms of AUC improvement, NRI, and IDI. At 12h post-CPB, the best predictive ability was obtained when all four biomarkers were combined.

Conclusions: This is the first large prospective study to (1) establish a temporal pattern of multiple sequential AKI biomarkers after CPB, which correlate with AKI severity and clinical outcomes, and (2) demonstrate the utility of biomarker combinations for the improved prediction of AKI beyond clinical models. Biomarker combinations hold tremendous promise for guiding the application of appropriately timed AKI therapies, based on the underlying pathophysiology.

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-OR342

Identification of Candidate Serum Biomarkers for Severe Septic Shock-Associated Kidney Injury Via Microarray Rajit K. Basu, Derek Wheeler, Prasad Devarajan, Stuart Goldstein, Hector R. Wong. *Center for Acute Care Nephrology, Cincinnati Children's Research Foundation, Cincinnati, OH.*

Background: Effective therapy for septic shock associated acute kidney injury (SSAKI), a disease process carrying significant morbidity and mortality, is lacking. A limitation for such therapy is that detection strategies for SSAKI, traditionally dependent on serum creatinine, are considerably varied and heterogeneous. While existing AKI biomarkers demonstrate relatively improved sensitivity for identification of SSAKI over creatinine, they may still be confounded by the unique pathophysiology of SSAKI.

Methods: We leveraged a previously generated genome-wide expression database of children with septic shock to address the need of identifying novel candidate biomarkers of SSAKI. Thirty-one patients with SSAKI (defined as a doubling of baseline serum creatinine) and 148 patients without SSAKI were identified. SSAKI persisted, to seven days after hospital admission.

Results: SSAKI patients were clinically similar to those without SSAKI but had higher mortality (45% versus 10%). 21 unique gene probes were up-regulated in SSAKI patients compared to patients without SSAKI. Using leave-one-out cross validation and class prediction modeling, the expression patterns of the 21 gene probes predicted SSAKI with a sensitivity of 98% (95% confidence interval (CI) 81-100) and a specificity of 80% (95% CI 72-86). Individual performance calculations were performed using two specific gene products (i.e. serum protein measurements) showing high sensitivity for predicting SSAKI: matrix metalloproteinase-8 (MMP-8) (89%, 95% confidence interval (CI) 64-98) and elastase-2 (83%, 95% CI 58-96). Both candidate biomarkers carried a negative predictive value of 95%. When applied to a separate validation cohort, both candidate biomarkers carried high sensitivity: MMP-8 (100%, 95% CI 68-100) and elastase-2 (100%, 95% CI 68-100).

Conclusions: We conclude that gene probes up-regulated in critically ill pediatric patients with septic shock may allow for the identification of novel candidate serum biomarkers for SSAKI prediction.

Funding: Other NIH Support - RC1HL100474

SA-OR343

Serum Angiopoetin-2 Correlates with Acute Kidney Injury and Outcome in Patients with Acute Liver Failure Alexander Lukasz,[1] Johannes Hadem,[3] Jan T. Kielstein,[1] Philipp Kümpers.[2] *[1]Department of Nephrology, Medical School of Hannover, Hannover, Germany; [2]Department of Nephrology, University Hospital Münster, Münster, Germany; [3]Department of Gastroenterology, Medical School of Hannover, Hannover, Germany.*

Background: Angiopoietin-2 (Ang-2), a circulating antagonistic ligand of the endothelial-specific Tie2 receptor, has been identified as a non-redundant facilitator of endothelial activation. We have recently shown that plasma Ang-2 levels correlate with the severity of Acute Kidney Injury (AKI) in critically ill patients. Moreover, administration of its endogenous competitor, the Tie agonist ligand Ang-1, protects against septic AKI in mice. In this study we ask if Ang-2 levels correlate with AKI, organ dysfunction and outcome in patients with primary acute liver failure (ALF).

Methods: We retrospectively identified 143 patients treated at the ICU of our institution between 1999 and 2009 with primary ALF and documented encephalopathy (secondary ALF due to sepsis etc were excluded). Serum samples obtained at ICU admission were available from 37 patients (Median age 34 years). Ang-2 was measured by in-house immuno-luminometric assay. 20 blood donors served as healthy controls.

Results: Patients who displayed any of AKI stages 1-3 showed significantly higher median [IQR] Ang-2 levels than those without AKI [34.2 (18.7-82.3) vs. 12.9 (6.1-17.2)ng/ml; $p<0,003$]. Ang-2 serum concentrations were increasingly high across healthy controls (1.4 [0.9-1.7] ng/mL), patients with transplant-free recovery (10.0 [4.7-12.1]ng/mL), and patients that died or received transplantation (16.8 [11.3-39.5]ng/mL). Ang-2 release correlated strongly with markers of organ dysfunction and disease severity scores, e.g. Sequential Organ Failure Assessment score [$r=0.492$, $p=0.002$]. Ang-2 was independently associated with the composite endpoint of death and/or Emergency Liver Transplantation on multivariate Cox regression analysis.

Conclusions: In addition to established marker or disease severity scores, circulating Ang-2 appears to be a relevant determinant of organ dysfunction, especially Acute Kidney Failure and outcome in patients with ALF and deserves more attention in patients with acute liver and kidney failure.

SA-OR344

A Prospective Trial Assessing the Effects of Clamp Ischemia during Partial Nephrectomy on Renal Function, Structure, and Biomarkers Dipen J. Parekh,[1] Barbara Ercole,[1] Kathleen Torkko,[2] William M. Hilton,[1] Prasad Devarajan,[3] Manjeri A. Venkatachalam,[1] Joel M. Weinberg.[4] *[1]UTHSC San Antonio; [2]Univ. of Colorado; [3]Univ. of Cincinnati; [4]Univ. of Michigan.*

Background: Tolerance of the human kidney to clamp ischemia (CI) during partial nephrectomy has been considered to be limited to 20-30 min. We have reassessed this question using intraoperative biopsies and have determined the utility of new biomarkers for following injury in this setting.

Methods: 40 patients undergoing open partial nephrectomy either without cooling (N=27, avg clamp time 32.3 min, range 15-53 min., 74% ≥ 30 min.) or with ice-slush cooling (N=13 avg clamp 48 min. range 30-61 min.) had biopsies of uninvolved areas of the kidney preclamp, during clamping. and then 5 min. after clamp release, along with serial measurements of standard renal functional parameters plus measurement of serum cystatin C and NGAL, and of urine NGAL, cystatin C, NAG, LFABP, NGAL, KIM-1 and IL-18.

Results: Serum creatinine transiently increased at 24 hours by 21.9±6.4% after warm CI and 27.2±7.9% after cold CI (Ps < .001), then recovered, but serum cystatin C did not change and serum NGAL was minimally affected. Urine biomarkers increased irrespective of whether they were factored for creatinine, with particularly large changes in KIM-1 and LFABP, but did not correlate with duration of ischemia, the change in creatinine or serum cystatin C, or the use of cold or warm ischemia. Ultrastructure and staining for actin, phosphotyrosine, β1 integrin, and ICAM-1 showed changes consistent with ischemia, but much milder than predicted from animal models and not strictly related to the duration or type of CI. Creatinine has remained stable in the patient cohort at up to 18 months of followup.

Conclusions: The data indicate that the insult to the clamped kidney from 30-60 minutes of clamp under conditions of open partial nephrectomy is well tolerated despite increases of urinary biomarkers, which may in part reflect local effects of the surgery itself. The work expands indications for partial nephrectomy in the management of renal cancers, and supports the use of CI as opposed to more complex procedures for partial nephrectomy.

Funding: NIDDK Support, Private Foundation Support

SA-OR345

Early Dynamic Changes in Serum Creatinine Predict Renal and Patient Outcome in Septic ICU Patients Claudio Rigatto, Manish M. Sood, Paul Komenda, Joe A. Bueti, Anand Kumar. *Internal Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: AKI predicts poor prognosis in patients with septic shock. It is not known whether early changes in serum creatinine (SCr) measured after ICU admission help predict renal outcome and survival in sepsis. An early drop in SCr may identify a good response to resuscitation and predict better outcomes.

Methods: Data was obtained from the Cooperative Antimicrobial Therapy of Septic Shock database comprising patients from 28 centres worldwide. 6504 had, serum creatinine measured at admission and 6 hours post . Patients requiring immediate initiation of RRT or with SCr > 400 mcmol/L on admission were excluded. The main exposure variable was % change in creatinine over the first 6 hours (PCr6); main outcome variables were need for dialysis and survival at 2 weeks. Multivariate Logistic Regression was used to compare predictive models with and without PCr6

Results: 4826 patients were analyzed. 343(7.1%) required dialysis and 1768 (36.6%) died within 2 weeks. Age, APACHE II, RIFLE stage on admission, centre, and fluid volume in the first 6 hours were the strongest predictors of need for dialysis (Base Dialysis Model). PCr6 was strongly associated with dialysis (OR 1.01 [1.006, 1.02]), was highly significant when added to the Base Dialysis Model (OR 1.02 [1.02,1.03]), and increased model discrimination (c-statistic 0.84 [0.82, 0.86] vs. 0.82 [0.80, 0.85]; p=0.001). Age, sex, Apache II, centre, and Gram negative sepsis were the strongest predictors of survival at 2 weeks post admission (Base Survival Model). Higher PCr6 was significantly associated with poor survival (OR 0.97 [0.97, 0.98]), was highly significant when added to the Base Survival Model, and improved discrimination (c-statistic 0.78 [0.77, 0.79] vs. 0.76 [0.74, 0.77]; p<0.0001). Patients who experienced a drop in creatinine > 10% at 6 hours had a much lower odds of needing dialysis (OR 0.44 [0.33,0.60] or dying (OR 0.41[0.35, 0.48].

Conclusions: In septic patients admitted to ICU, early dynamic changes in creatinine (in the first 6 hours) provide important prognostic information.

SA-OR346

Proximal and Distal Tubule Epithelial Cells Express CD133 during Repair after Acute Kidney Injury in Humans Ping L. Zhang,[1] Joseph V. Bonventre.[2] *[1]Dept of Pathology, William Beaumont Hospital, Royal Oak, MI; [2]Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Several recent studies have used CD133 (prominin-1) to identify and isolate renal progenitor cells in parietal epithelial cells (PEC) of glomeruli and proximal tubules in human kidney tissue. However, the "progenitor" status of the cells that express this protein has not been well established. In this study, we evaluated CD133 staining in normal fetal and adult human kidneys, and in various human renal diseases characterized by acute tubular injury (ATI).

Methods: Fetal kidneys (12-32 wks, n=64), normal adult kidneys (n=11) and renal biopsies from patients with ATI (primary or secondary, n=33) were evaluated for expression of CD133 using immunohistochemical techniques with two antibodies (ab)(monoclonal AC133 clone, 1:50, Miltenyi and polyclonal ab, 1:500, Biocare). CD133 expression was compared with expression of kidney injury molecule-1 (KIM-1, using AKG7 ab at 1:10), an injury marker of proximal tubules and cytokeratin-7, a distal-nephron tubular marker.

Results: Both types of CD133 antibodies showed a similar pattern of apical membrane staining. In fetal kidneys, CD133 stained primordial glomerular structures and medullary tubules. In normal kidney sections with negative KIM-1 staining, membrane CD133 staining was seen in PEC of the glomeruli and in less than 5% of proximal and distal tubular epithelial cells. In injured dedifferentiated proximal tubules, confirmed by positive KIM-1 staining, CD133 membrane expression along the apical surface was present in the majority of injured epithelial cells. With ATI, many distal tubule cells expressed both apical CD133 and cytokeratin-7. Tubular expression of CD133 in either proximal or distal tubules was significantly correlated with serum creatinine levels of adult patients.

Conclusions: Under normal conditions, CD133 is expressed in human fetal kidneys. In ATI, diffuse CD133 expression is seen in dedifferentiated injured tubular epithelial cell apical membranes implying that, rather than identify a specific "progenitor" population, CD133 is upregulated in dedifferentiated epithelial cells that are involved in repair of the epithelium after injury.

Funding: NIDDK Support, Clinical Revenue Support

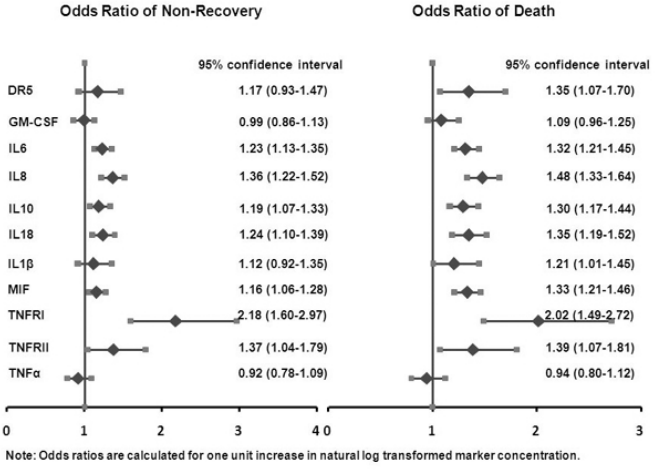
SA-OR347

Higher Concentrations of Inflammatory and Apoptotic Biomarkers Are Associated with Mortality and Nonrecovery of Kidney Function Xiao-Yan Wen,[1] Raghavan Murugan,[1] Lan Kong,[1,3] Nilesh Shah,[1,3] MinJae Lee,[1,3] Melinda J. Carter,[1] Michele M. Elder,[1] Paul M. Palevsky,[2,4] Mark L. Unruh,[2] John A. Kellum.[1,2] *[1]Department of Critical Care Medicine, Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), University of Pittsburgh, Pittsburgh, PA; [2]Department of Medicine, University of Pittsburgh, PA; [3]Department of Biostatistics, University of Pittsburgh Graduate School of Public Health, PA; [4]VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: Acute Kidney Injury (AKI) is a common complication of critical illness with high mortality rate despite renal replacement therapy (RRT). Although, higher circulating concentrations of inflammatory and apoptotic biomarkers are associated with AKI, whether these biomarkers predict outcomes in patients with severe AKI who are receiving RRT is unknown.

Methods: In a subset of 819 subjects from the Acute Renal failure Trial Network study, we measured 11 plasma inflammatory and apoptotic biomarkers on day-1 of RRT. Outcomes were 60-day mortality and renal recovery defined as alive and independent from RRT at day-60. We hypothesized that higher inflammatory and apoptotic biomarkers concentrations on day 1 would predict poor outcomes.

Results: 297 subjects (36.3%) died and 107 (13.1%) remained on RRT by day 60. 7 of the 11 plasma markers concentration were significantly lower in survivors and in subjects who recovered. Higher plasma concentrations of inflammatory and apoptotic markers were associated with a higher risk of renal non-recovery and death.



Conclusions: High circulating levels of plasma inflammatory and apoptotic biomarkers are strongly associated with non-recovery and mortality in critically ill patients receiving RRT.

Funding: NIDDK SupportBiological Markers of Recovery for the Kidney (BioMaRK) R01DK070910

SA-OR348

Endogenous Ouabain Is Implicated in Acute Kidney Injury in Cardiac Surgery and Critically Ill Patients Marco Simonini,[1] Nunzia Casamassima,[1] Chiara Lanzani,[1] Giorgio Slaviero,[1] Stephen S. Gottlieb,[2] Keyur B. Shah,[2] John Hamlyn,[2] Paolo Manunta.[1] *[1]Chair of Nephrology, San Raffaele Scientific Institute, Milan, Italy; [2]University of Maryland, Baltimore.*

Background: Endogenous Ouabain (EO) is an adrenal stress hormone with hemodynamic and renal effects. Acute Kidney Injury (AKI) is frequent post operative complication of major surgery. Preliminary studies have shown a rapid and early increase of EO in association with acute decrease in glomerular filtration rate. Our aim was to identify a new pre-operative marker for development of AKI in major surgery patients (pts) and to address the role in the development of kidney damage in Ouabain Hypertensive Rats (OHR).

Methods: We enrolled 425 consecutive adult pts admitted to elective cardiac surgery and 54 critical ill pts. The primary outcome was AKI according to RIFLE criteria. We also studied the prolonged ouabain effects on renal parameters in OHR.

Results: In cardiac surgery patients, AKI (4%, 7.3%, 12.9%, p=8.92E-08) and in hospital mortality (0.2%, 0.5%, 1.6%, p=0.018) increased with each incremental pre-operative EO tertile. In a linear regression analysis, circulating EO value before surgery resulted the strongest predictor of AKI (b=0.32, p=1.63E-06). Patients in the highest EO tertile had a relative risk of AKI that was 5.1 (p=1.16E-6) fold higher than in those in the lowest tertile. In 54 critically ill patients APACHE II score (11.8±7.4, 14.9±6.2, 19.9±10.2, p=0.02), AKI (11%, 39%, 61%, p=0.008), increased with each EO tertile. All the associations remained independently significant after adjustments for covariates. In the experimental OHR model, chronic infusion of low doses of ouabain lead to a reduction of creatinine clearance (-18%, p<0.05), an increase urinary protein excretion (+54%, p<0.05), associated with a reduction of expression of podocyte protein nephrin (-29%, p<0.01).

Conclusions: Pre-operative plasma EO levels are powerful biomarkers of AKI and post-operative complications within 24-48 hour. The elevated circulating levels of EO are strongly associated with the severity of illness, renal dysfunction, kidney damage and in-hospital mortality.

Funding: Government Support - Non-U.S.

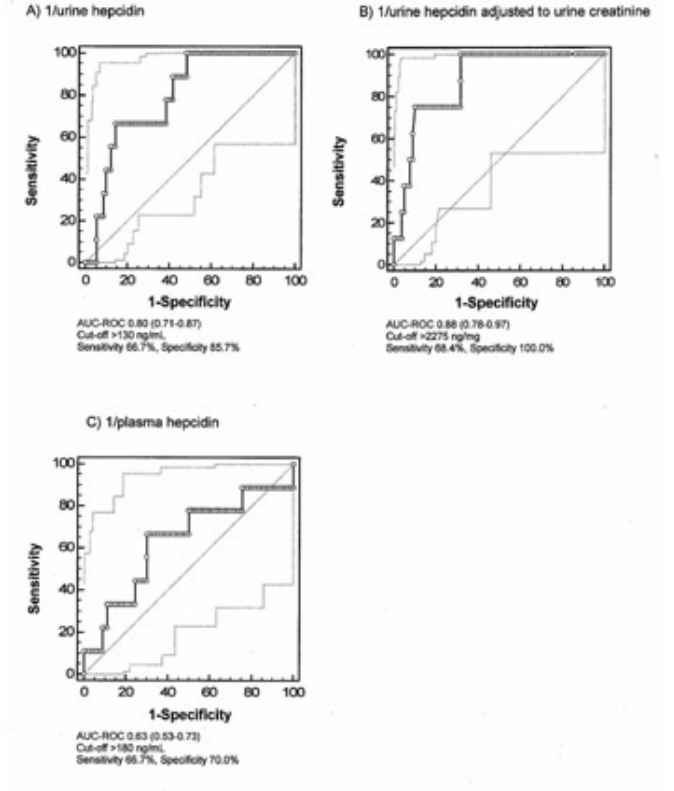
SA-OR349

Urine Hepcidin Has Additive Value in Ruling out Cardiopulmonary Bypass-Associated Acute Kidney Injury – An Observational Cohort Study Mark E. Westerman,[3] Michael Haase,[1] Anja Haase-Fielitz,[1] Peter R. Mertens,[1] John R. Prowle,[4] Rinaldo Bellomo.[2] *[1]Nephrology, Otto-von-Guericke University, Magdeburg, Germany; [2]Austin Hospital, Intensive Care, Melbourne, Australia; [3]Intrinsic Life Science, La Jolla; [4]Barts and NHS London Trust, London.*

Background: Conventional markers of acute kidney injury (AKI) lack diagnostic accuracy and are expressed only late after cardiac surgery with cardiopulmonary bypass (CPB). Recently, interest has focused on hepcidin, a regulator of iron homeostasis, as a unique renal biomarker.

Methods: We measured hepcidin (by ELISA) in 100 adult patients in the control arm of a randomized controlled trial (NCT00672334) that were identified to be at increased risk of AKI after cardiac surgery with CPB. AKI was defined according to RIFLE classification. Samples of plasma and urine were obtained simultaneously I) before CPB II) 6 hours and III) at 24 hours after start of CPB.

Results: At 6 and 24 hours after CPB, in AKI-free patients (N=91) urine hepcidin concentrations had largely increased and were 3 to 7 times higher compared to patients with subsequent AKI (N=9) in whom postoperative urine hepcidin remained at preoperative levels ($P = 0.004$, $P = 0.002$). Furthermore, elevated urine hepcidin and, even more so, urine hepcidin adjusted to urine creatinine at 6 hours after CPB discriminated patients who *did not* develop AKI (AUC-ROC 0.80 [95% CI 0.71-0.87]; 0.88 [95% CI 0.78-0.97]) (see Figure) or *did not* need renal replacement therapy (AUC 0.81 [95% CI 0.72-0.88]; 0.88 [95% CI 0.70-0.99]) from those who did. At 6 hours, urine hepcidin adjusted to urine creatinine was an independent predictor of ruling out AKI ($P = 0.011$). Plasma hepcidin did not predict the lack of AKI development.



Conclusions: Our findings suggest that urine hepcidin is an early predictive biomarker of ruling out AKI after CPB thereby contributing to early patients risk stratification.

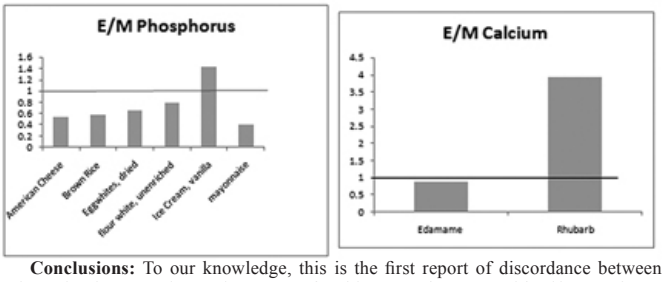
SA-OR350

Discordance between Measured Phosphorus (P) and Calcium (Ca) Content in Foods and Estimates from Nutrient Databases Anuja P. Shah,[1] Rachelle Bross,[2] Joel D. Kopple,[1] Rajnish Mehrotra.[1] *[1]Nephrology and Hypertension, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA; [2]General Clinical Research Center, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA.*

Background: Current clinical practice uses nutrient databases to counsel patients about dietary P and Ca content. Previous studies revealed that nutrient databases underestimate P content of prepared foods. There are limited data on accuracy of Ca content for any foods and P content of unprocessed foods.

Methods: P and/or Ca content of 34 food items was measured (M) using ICP-MS (inductively coupled plasma-mass spectroscopy) analysis at Michelson Laboratories Inc. (Commerce, CA), and data were compared to P and Ca content estimated (E) from nutrient databases (Nutrition Data System for Research, Minneapolis, MN). Confirmatory analysis of selected food items was performed at Medallion Laboratories, Minneapolis, MN.

Results: The E/M ratio for P content of foods varied widely from 0.55 to 1.40 (Figures). Wide variability was observed for both unprocessed and processed food items. The (E) P content in brown rice of 77 mg/100 g was significantly lower than the (M) 133 mg/100 g. Furthermore, the P content of coffee creamer, negligible by (E), was 107 mg/100 g by (M). The E/M Ca content in the three food items varied from 0.88 to 3.36, and the (M) content was discordant from both the database and the nutrition label. For example, the (E) Ca of 194 mg/100g in rhubarb was notably higher than the (M) 49 mg/100g.



Conclusions: To our knowledge, this is the first report of discordance between estimated and measured Ca and P content of a wide range of unprepared food items. Dietary recommendations for populations in which dietary P and Ca may need to be controlled should consider these discrepancies. Also, labeling of *measured* P and Ca content in foods may be useful.

Funding: Other NIH Support - Creff Grant and GCRC support, Pharmaceutical Company Support, Private Foundation Support

SA-OR351

Dietary Phosphorus Is Associated with Left Ventricular Mass: The Multi-Ethnic Study of Atherosclerosis Kalani T. Yamamoto,[1] Bryan R. Kestenbaum,[1] Cassianne Robinson-Cohen,[1] Marcia C. De Oliveira,[2] Alina Kostina,[1] Jennifer A. Nettleton,[2] Joachim H. Ix,[3] Ha T. Nguyen,[4] John Eng,[5] Joao A.C. Lima,[5] David Siscovick,[1] Noel Weiss.[1] *[1]University of Washington, Seattle, WA; [2]University of Texas Health Sciences Center, Houston, TX; [3]University of California, San Diego, CA; [4]Wake Forest University, Winston-Salem, NC; [5]Johns Hopkins University, Baltimore, MD.*

Background: Dietary phosphorus consumption has steadily increased in the US. Phosphorus alters key regulatory hormones and impairs vascular endothelial function, which may increase left ventricular mass (LVM). We investigated the association of dietary phosphorus intake with LVM in 4,494 individuals.

Methods: We studied participants from the Multi-Ethnic Study of Atherosclerosis, a community-based study of individuals free of cardiovascular disease. We quantified phosphorus intake using a 120-item food frequency questionnaire and measured LVM using cardiac MRI. We used linear and logistic regression models to estimate associations of dietary phosphorus intake with LVM and left ventricular hypertrophy (LVH), respectively.

Results: Mean dietary phosphorus intake was 1,167 mg/day in men and 1,017 mg/day in women. After adjustment for demographics, eGFR, total caloric and sodium intake, lifestyle factors, blood pressure, and other risk factors for LVH, each 20% greater dietary phosphorus intake was associated with an 1.1 gram greater LVM (95% CI 0.54, 1.63). The highest sex-specific quintile of dietary phosphorus intake was associated with an 6.2 gram greater LVM (95% CI 2.5, 9.8) and 2.1-fold greater odds of LVH (95% CI 1.1, 4.1) compared to the lowest sex-specific quintile.

Conclusions: Greater dietary phosphorus consumption is associated with greater LVM and a greater prevalence of LVH in a community-based cohort. If confirmed in future studies, dietary phosphorus may be a novel cardiovascular risk factor.

Funding: Other NIH Support - NIH NIDDK T32 “Research Training in Renal Disease”

SA-OR352

Effect of Bone Morphogenetic Protein-7 on Vascular Smooth Muscle Cells Calcification Induced by High Phosphorus Yi Yu. *Dept of Nephrology and Hemodialysis, Dongfang Hospital of Fujian Province, Fuzhou, Fujian, China.*

Background: This study is aimed to investigate the effect of Bone Morphogenetic Protein-7 (BMP-7) on vascular smooth muscle cells (VSMCs) calcification induced by high phosphorus in vitro by determining the expression of Pit-1, Cbfα-1 and calcium deposition.

Methods: The explants derived from thoracic aorta were used for primary culture of VSMCs and identification of cells were carried on by morphological identification and direct immunohistochemical staining of α-SMA. Passage 3 to 8 of VSMCs were used for experiments. Briefly, VSMCs were placed in various culture media, including normal phosphorus medium (Pi 1.3mmol/L), high phosphorus medium (Pi 2.6mmol/L), high phosphorus plus rhBMP-7 medium(Pi 2.6mmol/L+ rhBMP-7 100ng/ml), normal phosphorus plus rhBMP-7 medium (Pi 1.3mmol/L+ rhBMP-7 100ng/ml), calcium deposition was quantified and visualized by Alizarin stain method at day 6. Pit-1 mRNA levels was determined by RT-PCR, and Cbfα-1 expression was semi-quantified by RT-PCR and western blot at day 3. All of the experiments were repeated for 3 times at least.

Results: 1. VSMCs were characterized by the ribbon spindle shape. Immunohistochemical staining of α-SMA showed more than 95% positive staining in the cultured VSMCs. 2. Compared with normal phosphorus group, the calcium deposition was significantly increased in high phosphorus group (P<0.01), and BMP-7 reduced the increasing calcium deposition induced by high phosphorus (P<0.01), as were also shown in Alizarin stain. 3. Compared with normal phosphorus group, Pit-1 mRNA expression was significantly increased in high phosphorus group(P<0.01), and BMP-7 reduced the increasing Pit-1 mRNA expression(P<0.01). 4. As were shown in RT-PCR and western blot, Compared with normal phosphorus group, Cbfα-1 expression was significantly increased in high phosphorus group (P<0.01), and BMP-7 reduced the increasing Cbfα-1 mRNA(P<0.01) and protein (P=0.02) expression.

Conclusions: 1.Phosphorus induces VSMCs calcification in vitro. 2.BMP-7 inhibits the transformation of VSMCs into osteoblast-like cells induced by high phosphorus in vitro. 3.BMP-7 inhibits the calcium deposition of VSMCs induced by high phosphorus in vitro.

SA-OR353

MicroRNAs Induced by High Concentration of Phosphate and Calcium Are Involved in Vascular Smooth Muscle Cell Calcification Ting Gui, Yujing Sun, Aiko Shimokado, Shunji Itoh, Kosuke Oikawa, Yasuteru Muragaki. *First Department of Pathology, Wakayama Medical University, Wakayama, Japan.*

Background: MicroRNAs (miRNAs) are a recently discovered class of endogenous, small, non-coding RNAs that regulate the expression of protein-coding genes. The role of miRNAs in vascular calcification is currently unclear.

Methods: We examined the alteration of miRNAs in vascular smooth muscle cell (VSMC) calcification in vitro and in vivo to explore how miRNAs are involved in VSMC calcification. Using miRNA array analysis, we demonstrated that miRNAs are aberrantly expressed in the aortic media of 3 week-old klotho knock-out (KO) mice.

Results: The expression levels of miR-135a*, miR-712*, miR-714 and miR-762 in the aortic media of 2 week, 3 week and 4 week-old klotho KO mice were significantly higher than those in wild-type mice according to quantitative real-time polymerase-chain reaction (qRT-PCR). It was further confirmed using by qRT-PCR that these miRNAs were increased in cultured VSMCs treated with phosphate and calcium. We found in the miRNA database that the Ca2+ efflux proteins, PMCA1, NCX1 and NCKX4, frequently appeared as the potential targets of these miRNAs. Transfection of miRNA mimics to cultured VSMCs reduced the protein levels of each potential target as determined by western blot analysis. Conversely, miRNA inhibitors reduced the phosphate and calcium-induced VSMC calcification.

Conclusions: These results suggest that PMCA1, NCX1 and NCKX4 could be the potential targets for these miRNAs and that the reduction of these calcium transporters can cause the VSMC calcification.

SA-OR354

Correction of Hyperphosphatemia in Uremic Npt2b Knockout Mice Treated with Sevelamer Yves Sabbagh, Wen Tang, Christina M. Bracken, Stephen O'Brien, Susan Schiavi. *Endocrine and Renal Sciences, Genzyme Corporation, Framingham, MA.*

Background: Elevation in serum phosphorus even within the normal range is emerging as an important health risk in both normal and chronic kidney (CKD) populations as it is associated with increased incidence of vascular calcification and mortality. Although current CKD therapies such as phosphate binders effectively reduce serum phosphorus, management of individual biochemistry levels to that recommended by the current clinical guidelines is difficult. Limited understanding of the complexity associated with phosphate regulation has further prevented identification of optimal therapeutic strategies.

Methods: We have previously described generation of a conditional Npt2b knockout mouse ($Npt2b^{-/-}$) and demonstrated that ubiquitous deletion of Npt2b in adult mice leads to compensatory changes in critical hormones associated with phosphate regulation and increased expression of the renal transporter, Npt2a. We also demonstrated that Npt2b contributes to >90% of total active phosphate transport using the everted sac method to measure transport in ileum segments isolated from wild-type or $Npt2b^{-/-}$ mice. We therefore, investigated the role of Npt2b in mice with compromised renal function induced by adenine feeding.

Results: Uremic mice with the *Npt2b* deletion had significantly attenuated CKD associated hyperphosphatemia when compared to uremic wild-type mice (WT: 10.04±0.51 vs $Npt2b^{-/-}$: 8.21±0.56mg/dL, p<0.03). FGF23, an important regulator of phosphate was also decreased in uremic $Npt2b^{-/-}$ mice when compared to wild-type mice (WT: 5498±721.02 vs $Npt2b^{-/-}$: 3665.1±455.06pg/ml). We next evaluated whether treatment with the phosphate binder, sevelamer carbonate could further reduce hyperphosphatemia beyond deletion of Npt2b. Serum phosphate levels in the $Npt2b^{-/-}$ mice were normalized by sevelamer treatment (6.5±0.31mg/dl). In addition, serum FGF23 levels were further lowered in the sevelamer treated group compared to the untreated mice (1881.78±359.62pg/ml, p<0.05).

Conclusions: Taken together, these data provide further evidence that Npt2b is a critical component of phosphate homeostasis.

Funding: Pharmaceutical Company Support

SA-OR355

Successful Treatment with Ketoconazole of a Syndrome with Hypercalcemia, Hypercalciuria, Nephrolithiasis, Nephrocalcinosis, Elevated Serum Calcitriol and Mutations in the 24-Hydroxylase (*CYP24A1)* Gene Rajiv Kumar,[1] Peter Tebben,[2] Dawn S. Milliner,[1] Peter C. Harris,[1] Ron Horst,[3] John W. Foreman,[4] Paul Chelminski.[5] *[1]Mayo Clinic, Rochester, MN; [2]Mercy Healthcare, Edmond, OK; [3]Heartland Assays, Ames, IA; [4]Duke University, Durham, NC; [5]University of North Carolina, Chapel Hill, NC.*

Background: We describe the treatment of a novel syndrome in a Caucasian male characterized by hypercalcemia, hypercalciuria, elevated serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), undetectable serum 24, 25-dihydroxyvitamin D, nephrolithiasis, and reduced bone mineral density. Bilateral renal cysts and a 6 mm stone were present. The sequence of the 1-hydroxylase *(Cyp27b1)* gene was normal; the 24-hydroxylase (*Cyp24A1)* gene showed 2 intron-exon splice junction abnormalities that result in altered $1,25(OH)_2D$ 24-hydroxylase expression. Family history revealed an unaffected mother and three children among whom a daughter exhibited similar phenotypic features.

Methods: Because of persistent hypercalcemia and hypercalciuria, the patient was treated with ketoconazole, 200 mg orally every eight hours for one month. Relevant analytes were assayed in serum and urine.

Results: Serum $1,25(OH)_2D$ and calcium (Ca) decreased into the normal range ($1,25(OH)_2D$, pg/mL: *before*, 123, *after*, 40; serum Ca, mg/dL: *before*, 10.4, *after*, 9.5). Serum PTH concentration increased into the normal range, (PTH, pg/mL, *before*, 8.1, *after*, 27). Urinary Ca and P diminished (Ca, mg/24h: *before*, 316, *after*, 139; urinary P: *before*, 1525, *after*, 962). Serum inorganic P increased (P, mg/dL: *before*, 3.6, after, 4.4). 25(OH) D decreased (*before*, 50, *after*, 40 ng/mL).

Conclusions: In a syndrome characterized by intermittent hypercalcemia, hypercalciuria, elevated $1,25(OH)_2D$ and undetectable 24, 25 $(OH)_2$ D concentrations, nephrolithiasis, reduced BMD, and splice junction mutations of the *Cyp24A1* gene, treatment with ketoconazole reduces $1,25(OH)_2D$ serum concentrations with attendant decreases in serum Ca concentration and urinary Ca and P excretion. Ketoconazole is an effective treatment for hypercalciuria and hypercalcemia seen in patients with mutations of the *Cyp24A1* gene.

Funding: NIDDK Support, Other NIH Support - AR

SA-OR356

Vitamin D Repletion Does Not Increase Calcium Excretion among Patients with Kidney Stones David E. Leaf,[1] Mantu Gupta,[1] Ruslan Korets,[1] Eric N. Taylor,[2] John R. Asplin,[3] David S. Goldfarb,[4] Gary C. Curhan.[5] *[1]Columbia University Medical Center, New York, NY; [2]Nephrology and Transplantation, Maine Medical Center, Portland, ME; [3]Litholink Corporation, Chicago, IL; [4]Nephrology Section, New York Harbor VA Healthcare System, New York, NY; [5]Nephrology Division, Brigham and Women's Hospital, Boston, MA.*

Background: Despite the important role of vitamin D in maintaining bone health, as well as a variety of other physiological functions, many clinicians are reluctant to treat vitamin D deficiency in kidney stone formers because of the theoretical risk of increasing urinary calcium (UCa) excretion and the risk of calcium stone recurrence. We report on the effect of vitamin D repletion on UCa among stone-formers.

Methods: Patients were recruited from three metabolic stone clinics affiliated with Columbia University Medical Center. Enrollment criteria included: 1) history of nephrolithiasis; 2) UCa between 150 and 400 mg/day; 3) serum 25-hydroxyvitamin D (25(OH)D) level less than 30 ng/ml. Patients were given oral ergocalciferol 50,000 IU weekly for 8 weeks. Serum and 24-hour urine tests were repeated after 8 weeks.

Results: 30 patients were enrolled and 29 completed the study. Vitamin D repletion resulted in no significant change in UCa excretion. Other urinary parameters were similarly unchanged. Subgroup analysis on the basis of initial 25OHD and parathyroid hormone levels revealed no change in UCa excretion in any subgroup. No patient experienced any side effects, including kidney stone recurrence, while taking vitamin D.

Serum and 24hr urine parameters before and after vitamin D repletion (mean±SD)

	Baseline	Follow-up	p-value
U Calcium (mg)	257±54	255±88	0.91
U Oxalate (mg)	42±18	41±15	0.84
U Sodium (mmol)	228±94	202±65	0.23
U Creatinine (mg)	1896±479	1880±468	0.74
25(OH)D (ng/ml)	17±6	35±10	<0.0001
Serum Calcium (mg/dl)	9.3±0.4	9.4±0.4	0.69
PTH (pg/ml)	46±25	48±29	0.61

Conclusions: Among stone-formers with vitamin D deficiency and moderate levels of hypercalciuria, vitamin D repletion does not appear to increase UCa excretion. Vitamin D therapy, if indicated, should not be withheld on the basis of prior stone disease.

SA-OR357

Dietary Calcium Regulates SLC26A6-Mediated Oxalate Secretion Felix Knauf, Robert Brent Thomson, Peter S. Aronson. *Section of Nephrology, Yale University School of Medicine, New Haven, CT.*

Background: Dietary calcium intake is an important determinant of urinary oxalate excretion and risk for calcium-oxalate nephrolithiasis. Net intestinal absorption of dietary oxalate results from passive paracellular oxalate absorption as modified by SLC26A6-mediated back-secretion of oxalate. It has been postulated that dietary calcium restriction leads to increased oxalate absorption by reducing precipitation of oxalate with calcium in the lumen of the intestine, thereby increasing soluble oxalate available for absorption. We tested the hypothesis that dietary calcium also modulates net oxalate absorption by regulating SLC26A6-mediated oxalate secretion.

Methods: Wild-type mice were fed either a regular or low calcium diet. After 2 weeks the duodenum was harvested and probed for SLC26A6 protein expression normalized to actin by Western blot analysis. Active secretion of [14C]-oxalate was measured in vitro across isolated intestinal tissue mounted in Ussing chambers and bathed in standard calcium-containing Ringers solutions. To determine if the effect of lowering dietary calcium is specific for active oxalate secretion, active [14C]-glucose absorption was also assayed. Mannitol permeability and total tissue conductance were measured as markers of paracellular permeability.

Results: Mice fed a low calcium diet showed a 70% reduction of intestinal SLC26A6 protein abundance as compared with animals fed a regular calcium diet. Active oxalate secretion was reduced by 50% in intestinal tissue isolated from mice fed a low calcium diet compared to intestine from mice on a regular diet. Glucose absorption was not changed by modifying dietary calcium, indicating that the effect of a low calcium diet to reduce oxalate secretion is not the result of a general downregulation of intestinal transport processes. Paracellular permeability as measured by mannitol permeability and tissue conductivity was unaffected by dietary calcium.

Conclusions: We demonstrate that dietary calcium regulates SLC26A6-mediated oxalate secretion in the intestine. Downregulation of SLC26A6 may contribute to the increased net absorption of dietary oxalate that results from ingestion of a low calcium diet.

Funding: NIDDK Support, Private Foundation Support

SA-OR358

Increased Sensitivity to 1,25$(OH)_2D_3$ in Genetic Hypercalciuric Stone-Forming Rats Kevin K. Frick,[1] John R. Asplin,[2] Christopher D. Culbertson,[1] Daniel M. Asplin,[2] Nancy Krieger,[1] David A. Bushinsky.[1] *[1]Medicine, University of Rochester, NY; [2]Litholink Corporation, Chicago, IL.*

Background: Genetic hypercalciuric stone-forming (GHS) rats, bred to maximize urine (U) calcium (Ca) excretion, exhibit increased intestinal Ca absorption, increased bone resorption and reduced renal tubular Ca reabsorption, all leading to increased UCa compared to controls and all form kidney stones. GHS rats express an increased number of vitamin D receptors (VDR) at these sites of disordered Ca transport.

Methods: To determine if the excess VDR is biologically active, we fed GHS rats a Ca replete diet (NCD, 1.2% Ca) and injected 1,25$(OH)_2D_3$ (1,25D, 25 ng/d) or vehicle (veh) for 9 d. To determine if GHS rats would also show increased UCa in the absence of 1,25D-induced intestinal Ca absorption, GHS and SD rats were fed a low Ca diet (LCD, 0.02%) and again injected with 1,25D or veh for 9 d. To help understand the greater increase in UCa in response to 1,25D in GHS than SD, we measured kidney RNA expression of components of renal tubular Ca transport in the low Ca diet study.

Results: When fed NCD, with 1,25D, UCa in SD increased from 1.7±0.3 mg/d to 24.4±1.2 and in GHS from 10.5±0.7 to 41.9±0.7 and PTH was suppressed to undetectable levels in both groups (all groups n=8, mean ± SEM, all comparisons $p<0.01$). When fed LCD, with 1,25D, UCa in SD increased from 1.3±0.1 mg/d to 9.4±0.9 and in GHS from 4.7±0.3 to 21.5±0.9 and PTH was again suppressed in both groups (all n=8, all comparisons $p<0.02$). When fed LCD, expression of the distal luminal renal Ca transporter TRPV5 and the transcellular Ca transport protein calbindin D_{28k} were both elevated by 1,25D in SD but not GHS rats, consistent with less renal tubular Ca reabsorption in GHS than SD, resulting in greater hypercalciuria.

Conclusions: In both studies, the marked increase in UCa with 1,25D in GHS, which exceeded the increase in SD, indicates that the increased VDR in GHS results in greater biological response to administered 1,25D. In the absence of appreciable dietary Ca, the greater UCa in the GHS rats compared to SD may be secondary to a decrease in distal renal tubular Ca reabsorption mediated by TRPV5.

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SA-OR359

Randall's Plaques Are Aggregates of Spherical and Elongated Striated Units of Poorly Crystalline Biological Apatite Saeed R. Khan, Manoj Monga. *Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL.*

Background: Idiopathic calcium oxalate (CaOx) kidney stones develop by deposition of CaOx crystals on Randall's plaques (RP). Mechanisms involved in RP formation are still unclear. It is our hypotheses that RP formation is similar to vascular calcification involving components of extracellular matrix including membrane bound vesicles and collagen fibers. In order to verify our hypothesis we critically examined renal papillary tissue from stone patients obtained at time of stone removal.

Methods: Cold-cup biopsy of renal papilla was performed on 10 idiopathic stone patients undergoing PCNL. Kidney tissue was immediately fixed and processed for light and electron microscopy. All samples were examined by light and transmission electron microscopy (TEM). Some were also examined by scanning electron microscopy (SEM). Energy dispersive x-ray microanalyses and electron diffraction were utilized to identify the crystals.

Results: Multi-laminated spherulitic CaP deposits, the hallmark of RP's, were seen by TEM in interstitium as well as laminated basement membrane of tubular epithelia. In addition CaP crystals were also organized as bundles of elongated striated fibers. Both crystal types were associated with collagen fibers and membrane bound vesicles. Many such vesicles either contained or associated with needle shaped crystals. Some of the striated interstitial crystals were closely aligned with collagen fibers.

Conclusions: Randall's plaques comprise of two types of crystal deposits, the well known spherical units as well as bundles of striated fibers. Both types of deposits are associated with collagen fibers as well as membrane bound vesicles. The vesicles contained needle shaped crystals. We conclude that crystal deposition in renal papillae may start with membrane vesicle induced nucleation and grows by mineralization of interstitial collagen fibers. Similar mechanism has been proposed for calcium phosphate crystal deposition elsewhere in the body.

Funding: NIDDK Support

SA-OR360

Six1 Is a Key Mediator of TGF-beta Induced Renal Fibrosis Arthur Chi-Kong Chung,[1] Xiang Zhong,[1,2] Hui Y. Lan.[1,3] *[1]Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Shatin, NT, Hong Kong; [2]Department of Chemical Pathology, Chinese University of Hong Kong, Shatin, NT, Hong Kong; [3]Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, NT, Hong Kong.*

Background: TGF-β/Smad3 signaling is a key pathway that mediates renal fibrosis. However, mechanisms that control the fibrotic gene expression during fibrosis remained unclear. The present study tested the hypothesis that Six1 mediated Smad3-dependent renal fibrosis.

Methods: Six1 expression in tubular epithelial cells (TECs) after TGF-β treatment and diseased kidney was examined by Realtime PCR. The functional role of Six1 in renal fibrosis was determined in vitro by using overexpression and knockdown cell lines of Six1, and in vivo by delivering Six1 overexpression and knockdown plasmid in the obstructive kidneys by ultrasound-microbubble gene transfer system. Expression levels of the fibrotic markers were examined by Realtime PCR and Western blot analyses.

Results: Activation of TGF-β/Smad signaling was necessary for upregulation of Six1 in the fibrotic kidney and TECs in response to TGF-β. Enhanced TGF-β/Smad signaling in Smad7 knockout mice promoted Six1 expression and fibrosis in obstructive nephropathy. In addition, TGF-β induced Six1 expression in time- and dosage-dependent manner. This induction is mediated by Smad3, but not Smad2, because cells lacking Smad3, not Smad2, were protected from upregulation of Six1 and fibrosis in obstructive nephropathy, as well as in TGF-β1-stimulated renal TECs and mouse embryonic fibroblasts. Furthermore, overexpression of Six1 promoted but knockdown of Six1 expression suppressed TGF-β1-induced renal fibrosis in TECs. More importantly, delivery of Six1 knockdown plasmid ameliorated renal fibrosis in mouse model of obstructive kidney diseases, as evidence with reduction of expression of collagen I, fibronectin and α-smooth muscle actin.

Conclusions: Six1, a critical downstream mediator of TGF-β/Smad3 signaling, plays an essential role for renal fibrosis in response to TGF-β1. Targeting Six1 may be an effective therapeutic approach to combat renal fibrosis.

Funding: Government Support - Non-U.S.

SA-OR361

miR-302 Regulation of TGFβ/CTGF Signaling by TGFβ Type II Receptor Targeting: Prospective Data from the UUO Mouse Model Noel Faherty,[1] Simon Curran,[1] Helen O'Donovan,[1] Noelynn Oliver,[4] Catherine Godson,[1] Derek Brazil,[3] John Crean.[1] *[1]UCD Diabetes Research Centre, University College Dublin, Dublin, Ireland; [2]Centre for Vision and Vascular Science, Queen's University Belfast, United Kingdom; [3]FibroGen Inc, San Francisco, CA.*

Background: Signaling interplay between Connective Tissue Growth Factor (CTGF) and Transforming Growth Factor-β1 (TGFβ) is believed to play a role in the progression of glomerulosclerosis in diabetic nephropathy. We demonstrate a mechanism of regulation of TGFβ signalling via CTGF induced microRNA expression and present in vivo data on microRNA expression and signaling activity.

Methods: Expression of microRNAs in human mesangial cells (HMC) in response to treatment with CTGF was determined, target analysis identified putative gene targets. Expression of microRNA and signaling mediators in the 10 day unilateral ureteral obstruction (UUO) mouse model was investigated.

Results: TGFβ receptor II (TβRII) was determined to be a putative target of the miR-302 family, three members of which were increased in expression by CTGF. CTGF decreased expression of TβRII in HMCs concomitantly with increasing levels of miR-302d, as a proxy readout for miR-302 activity. We validated TβRII as a miR-302d target and inhibited miR-302d to attenuate CTGF dependent changes in TβRII levels. Further, we found a relevant signaling context for miR-302 family members, with attenuation of canonical Smad signaling and induction of non-canonical signaling associated with differential expression of miR-302d. In the UUO mouse model, miR-302d expression was increased with a coincident decrease in TβRII, suggesting pathophysiological significance. Decreased expression of the TGFβ type I receptor (TβRI) and an apparent dichotomy between decreased Smad2 and increased Smad3 phosphorylation was also observed.

Conclusions: Opposing levels of activated Smad2 and Smad3 lends support to the hypothesis that differential activity of both Smads is tacit in the progression of fibrosis. Further, the apparent lower levels of TβRI raise the intriguing possibility of chronic Smad3 phosphorylation and fibrotic damage in the UUO mouse being, at least in part, TGFβ receptor independent.

Funding: Government Support - Non-U.S.

SA-OR362

Direct Interaction of the Adapter Protein Dok-4 with Elk-4: A Novel Mechanism for Inhibition of Tyrosine Kinase-Induced Transcription Erika Hooker,[1] Cindy Baldwin,[1] Serge Lemay.[1,2] *[1]Experimental Medicine, McGill University, Montreal, QC, Canada; [2]Medicine (Nephrology), McGill University Health Centre, Montreal, QC, Canada.*

Background: Dok-4 is an adapter protein expressed in epithelial cells and one of 7 members of the Dok family. Whereas Dok-1, 2, and 3 inhibit Src family kinase (SFK) signaling in immune cells by recruiting inhibitory molecules (Csk, p120 RasGAP, and Ship1), the role and mechanism of action of Dok-4 remain obscure because the identity of its partner molecules is unknown. We previously showed that, in epithelial cells, Dok-4 can inhibit the transcription factor (TF) Elk-1, presumably through inhibition of the upstream Erk pathway.

Methods: To better define the mechanism of action of Dok-4 in renal epithelial cells, we sought to identify Dok-4-associated proteins and to define the impact of any such interactions on SFK signaling. We performed a yeast two-hybrid (Y2H) screen with Dok-4 as bait in a mouse kidney library. Subsequent validation was performed in transiently transfected 293 and MDCK cells using co-IP, immunofluorescence localization and a luciferase-based transactivation assay.

Results: Y2H screening identified 6 independent preys representing the C-terminal region of the Elk-1-related TF, Elk-4. Dok-4 and Elk-4 also co-immunoprecipitated in 293 cells. A Gal4-Elk-4 chimera was constructed to assess the impact of SFK and Dok-4 on Elk-4-mediated transactivation of a gal4-luciferase reporter. Elk-4 was exquisitely sensitive to activation by overexpressed SFKs and this was potently and dose-dependently inhibited by co-expression of Dok-4. Inhibition of Elk-4 by Dok-4 correlated with exclusion of Gal4-Elk-4 from the nucleus and its co-localization with Dok-4. Finally, we identified a canonical nuclear export signal in Dok-4, suggesting nucleo-cytoplasmic shuttling of Dok-4.

Conclusions: Elk-4 is a prominent target of activation by SFK and is potently inhibited by Dok-4, in part through a direct interaction with Dok-4. Direct interaction of Dok-4 with a TF represents a novel mechanism of adapter protein action in SFK signaling. Dok-4/Elk-4 interaction may serve to directly and specifically modulate tyrosine kinase-induced transcriptional responses in epithelial cells.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR363

Tyrosine Phosphorylation Sites Y4/8/10 of CD2AP Determine Binding to Nephrin Irini Schaefer, Beina Teng, Kirstin Worthmann, Hermann G. Haller, Mario Schiffer. *Nephrology, Medical School Hannover, Hannover, Germany.*

Background: CD2AP is an adaptor protein that can transmit intracellular signals involved in survival and cytoskeletal regulation of the cell. Until now it is unknown if the activation of CD2AP and its potential to interact with multiple downstream effectors is regulated by phophorylation. The aim of these studies was to identify tyrosine-phosphorylation sites of CD2AP and to analyze in detail if the phosphorylation of these residues are of fundamental importance.

Methods: First we analyzed a potential phosphorylation of CD2AP by 2D-gel-electrophoresis and immunoprecipitation. By alignment of CD2AP of different species we analyze potential tyrosine residues that are evolutionary conserved. To explore if these sites are important for nephrin binding we created CD2AP Tyrosine-mutants and performed immunoprecipitations. Furthermore we generated phospho-specific polyclonal antibodies for these sites.

Results: We can demonstrate a significant shift of the isoeletrical point of CD2AP after VEGF-stimulation. Endogenous immunoprecipitation of CD2AP showed specifically that CD2AP is tyrosine-phosphorylated. Highly conserved tyrosine residues are on position Y4/8/10, Y119 and Y273/280. Cotransfection of the generated CD2AP mutants with nephrin showed a significantly enhanced binding of nephrin to the triple mutant Y4/8/10 compared to WT-CD2AP and to the Y119 and Y273/280 mutants. Stimulation of podocytes with several cytokines showed a typical phosphorylation profile of phosphorylated CD2AP with the generated antibodies. Immunofluorescence of cryosections of mouse and human reveal that the phospho-specific antibodies against Y119 and Y273/280 showed a specific slit diaphragm staining whereas the phospho-specific antibody against Y4/8/10 showed a more cytosol-specific staining. Confocal microscopy with the phospho-specific antibody against Y4/8/10 on murine and human podocytes showed after stimulation with VEGF a colocalization of phospho-CD2AP spots with actin-endings especially at the leading edge.

Conclusions: These results indicate that CD2AP is a tyrosine phosphorylated protein and that phosphorylation on Y4/8/10 determine localization of CD2AP and thereby interaction with nephrin.

Funding: Government Support - Non-U.S.

SA-OR364

p38-alpha Phosphorylates Nephrin and Induces Its Endocytosis Via Association with beta-arrestin2 Magdalena Woznowski, Sebastian Alexander Potthoff, Eva Koenigshausen, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin, Ivo Quack. *Nephrology, Heinrich-Heine University, Duesseldorf, Germany.*

Background: Podocyte damage leads to foot process retraction, disruption of the slit diaphragm and proteinuria. Human acquired glomerulopathies and diabetic nephropathy are associated with TNF-alpha elevation. Especially the TNF-alpha activated MAPK p38 is known to play a critical role in mediating podocyte injury. Recent advances have also revealed crucial roles of slit diaphragm-associated proteins, including nephrin, podocin and beta-arrestin2. In this regard, beta-arrestin2 was shown to associate with nephrin, leading to nephrin endocytosis and thereby causing proteinuria.

Methods: GST-nephrin fusion proteins containing different fragments of the nephrin cytoplasmatic domain were used in a radioactive kinase assay with recombinant p38. In addition, nephrin mutants containing point mutations of putative p38 phosphorylation sites were generated and tested in these kinase assays. Expression plasmids for nephrin and its mutants as well as for beta-arrestin2 were transfected into HEK293T cells, which were subsequently treated with TNF-alpha. After cell lysis, coimmunoprecipitation with subsequent western blot analysis was performed.

Results: TNF-alpha treatment of podocytes or HEK293T cells leads to a rapid activation of the MAPK p38. Interestingly, nephrin is phosphorylated by p38alpha at Ser 1146. The nephrin mutant S1146A, which is not phosphorylated by p38 anymore, is impaired in its ability to associate with beta-arrestin2 and demonstrate less TNF-alpha-mediated endocytosis. On the other hand, a phospho-mimicry mutant S1146D shows a stronger interaction with beta-arrestin2 and increased endocytosis.

Conclusions: TNF-alpha induces nephrin/ beta-arrestin2 association and nephrin endocytosis. The MAPK p38 acts as a crucial mediator in this regard via phosphorylation of nephrin, thereby regulating its presence in the slit diaphragm. This could represent an important molecular mechanism responsible for TNF-alpha-mediated podocyte damage. In addition, this suggests that inhibition of p38 via pharmacological intervention could protect against inflammation-induced proteinuria.

SA-OR365

Functional Excitatory and Inhibitory Aminoacid Receptors in Primary Cultured Podocytes Maria Pia Rastaldi,[1] Anna Mondini,[1,2] Min Li,[1] Silvia Armelloni,[1] William J. Wilkinson,[2] Piergiorgio Messa,[1] Paul J. Kemp.[2] *[1]Renal Research Laboratory, Fondazione IRCCS Policlinico & Fondazione D'Amico, Milan, Italy; [2]School of Biosciences, Cardiff University, Cardiff, United Kingdom.*

Background: We showed that podocytes express several types of receptors, second messenger systems and proteins usually associated with synaptic communication between neurons. Such information suggests that podocytes have the potential to respond to external stimuli and transduce these signals into rapid, coordinated responses. Although there is molecular evidence for expression of these channels and receptors, almost no data are available which have functionally characterised them in primary podocytes.

Methods: By means of intracellular calcium imaging using the Ca2+-sensitive fluorescent dye Fura-2, we investigated the functional expression in primary cultured podocytes of a wide array of proteins typically involved in neuronal synaptic functions.

Results: Using this approach, we defined the functional expression of the three main subtypes of ionotropic glutamate receptors, by recording increases in [Ca2+]i in response to brief (10s) exposures of primary podocytes to: glutamate (100μM); kainate (100μM); N-methyl-D-aspartic acid (NMDA - 100μM); α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA - 50 μM). Furthermore, both GABAA and GABAB receptors were functionally present, since exposure of cells to GABA (γ-amino butyric acid - 300 μM) evoked an increase in [Ca2+]i which was only partially attenuated upon removal of extracellular Ca2+. Interesting, the rise in [Ca2+]i upon GABAA receptor activation shows that the result of opening Cl- channels in these cells is cell depolarisation and consequent Ca2+ entry through voltage-gated Ca2+ channels, a notion fully supported by the ability of 50 mM extracellular K+ to evoke a large increase in [Ca2+]i.

Conclusions: Our new data provide functional evidence for the existence of kainate, AMPA, NMDA, GABAA and GABAB receptors in primary podocytes and further support the idea that these cells have the potential to respond to external stimuli in a manner which appears similar to that normally associated with neuronal signalling.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR366

Signaling through the NF-kB Essential Modulator NEMO Triggers Podocyte Migration and Prolongs Proteinuria in a Nephrotoxic Nephrits Model Sebastian Braehler,[1] Henning Hagmann,[1] Christina Ising,[1] Friedrich Thaiss,[2] Stuart J. Shankland,[3] Bernhard Schermer,[1] Thomas Benzing,[1] Paul T. Brinkkoetter.[1] *[1]Department of Nephrology, University of Cologne, Germany; [2]Department of Nephrology, University Hospital Hamburg, Hamburg, Germany; [3]Department of Nephrology, University of Washington, Seattle, WA.*

Background: Inflammatory diseases lead to glomerular injury and are a major cause of proteinuria. The Nuclear Factor kappaB is one of the most important regulators of pro-inflammatory signaling. Its role in podocytes, however, is only poorly understood. In this study we inhibited NF-kB signaling in podocytes in vivo by specific ablation of the essential modulator Nemo.

Methods: Nemo flox/flox;Podocin cre/wt mice were viable and showed no proteinuria or changes in kidney morphology under non-stressed conditions. In a nephrotoxic nephritis model both KO and wild-type mice developed severe proteinuria within 3 days. At day 7 KO mice recovered faster and showed decreased levels of proteinuria compared to wild-type littermates. There were no differences in infiltrating inflammatory cells. However, immunohistochemistry and electronmicroscopy revealed restoration of the slit diaphragm morphology in KO mice while wild-type showed significant foot-process effacement.

Results: To address the mechanisms that underlie this beneficial phenotype we established a Nemo-knockdown podocyte culture system. Knockdown of Nemo resulted in reduced production of pro-inflammatory chemokines. In addition, reduced NF-kB activity led to decreased phosphorylation of ERK1/2-MAP-kinases in vitro and reduced cellular migration. Inhibitor studies were carried out to link inactivation of ERK1/2 to the stationary phenotype of Nemo-knockdown podocytes. Our results showed that inhibiting ERK1/2 activation blocked the migratory phenotype in wild-type cells comparable to Nemo-knockdown podocytes.

Conclusions: In conclusion, signaling through Nemo might not only be involved in the production of proinflammatory molecules in inflammatory conditions but also regulates podocyte mobility and structure through activation of MAPK. This pathway might represent a key regulator of podocyte migration in states of disease.

SA-OR367

Dietary Acid Reduction with Added Fruits and Vegetables or Oral Bicarbonate Preserves GFR in Subjects with CKD Stage 4 GFR Nimrit Goraya,[1,2] Chanhee Jo,[3] Donald E. Wesson.[1,2] *[1]Medicine, Texas A & M College of Medicine, Temple, TX; [2]Medicine, Scott & White Healthcare, Temple, TX; [3]Biostatistics, Scott & White Healthcare, Temple, TX.*

Background: Subjects with hypertensive nephropathy (HN) have progressive GFR decline despite blood pressure reduction with ACE inhibition but dietary acid reduction with oral alkali helps preserve GFR in HN patients with moderately reduced GFR. We explored if dietary acid reduction also preserves GFR in HN with severely reduced eGFR (CKD 4 = 15-29 ml/min) and if doing so with base-inducing fruits and vegetables (F+V) was as effective as $NaHCO_3$.

Methods: Subjects with CKD 4 on ACE inhibition were given F+V in an amount designed to reduce potential renal load by 50% (N=36) or oral $NaHCO_3$ at 1.0 meq/ kg lean body weight/day(N=35) and compared to time control subjects without dietary intervention (N= 35). Entry and follow up cystatin C-calculated eGFR (cysGFR), systolic blood pressure (SBP), and plasma potassium (P_K) were measured after one year.

Results: At entry, mean cysGFR was not different among groups (F+V=21.6 ± 4.6 ml/min, $NaHCO_3$ = 21.7 ± 3.4 ml/min, and time control = 21.5 ± 3.0 ml/min) but after 1 year, cysGFR was higher in both intervention groups (F+V =20.7 ± 4.7 ml/min, $NaHCO_3$ = 20.3 ± 3.2 ml/min) than time control (17.6 ± 2.5 ml/min, p<0.002 vs. F+V and $NaHCO_3$). SBP at study entry was not different among groups (F+V =136.3 ± 4.8 mmHg, $NaHCO_3$ = 136.1 ± 4.7 mm Hg and time control =137.0 ± 4.3 mmHg) but follow up SBP was lower than at entry in F+V (-4.6 ± 3.8 mmHg, p <0.001 vs. entry) and was not statistically different from the entry value at follow up for the remaining groups. Despite an increase in urinary potassium excretion with F+V, P_K was not statistically different at follow up compared to the entry value (net change = 0.01 ±0.12 meq/ml, p=0.593).

Conclusions: Dietary acid reduction is an effective adjunct to conventional therapy in preserving GFR in CKD stage 4 HN and doing so with F+V compared to $NaHCO_3$ does not increase P_K and appears to have the added cardiovascular benefit of SBP reduction.

Funding: Private Foundation Support, Clinical Revenue Support

SA-OR368

Estimated Net Endogenous Acid Production and Chronic Kidney Disease Progression in African Americans: The African American Study of Kidney Disease and Hypertension (AASK) Julia J. Scialla,[1] Lawrence J. Appel,[1] Brad C. Astor,[1] Edgar R. Miller,[1] Srinivasan Beddhu,[2] Mark Woodward,[3] Rulan S. Parekh,[4] Cheryl A. Anderson.[1] *[1]Johns Hopkins University; [2]University of Utah; [3]University of Sydney; [4]University of Toronto.*

Background: In CKD, increases in per nephron acid excretion needed to counteract the daily dietary acid load may promote renal injury and contribute to disease progression.

Methods: We evaluated the association between the net endogenous acid production (NEAP) determined by the diet, and rates of GFR decline in 632 African Americans with hypertensive CKD from the AASK Trial. Estimated NEAP was calculated from complete 24 hour urine specimens, collected between 12 and 36 months post-randomization, in participants not taking potassium or alkali supplements. Protein and potassium intake were estimated from 24 hour urea nitrogen and potassium excretion, respectively. Estimated NEAP was calculated as previously described: NEAP (mEq/d) = -10.2 + 54.5 [protein intake (g/d) / potassium intake (mEq/d)]. NEAP estimates were averaged to approximate baseline habitual dietary intake (49% of participants had 3 or more measures). I^{125}iothalamate glomerular filtration rate (iGFR) was measured every 6 months over follow up (average 6.9 iGFR measures per participant). The association between estimated NEAP and slope of iGFR from 12 months post-randomization was determined using linear mixed models with adjustment for randomized drug and blood pressure groups, and categories of age, body mass index, baseline GFR and proteinuria on iGFR slope.

Results: Median baseline iGFR was 48.6 (IQR 36.6 - 58.5) mL/min/1.73 m^2. In unadjusted and adjusted models there was a faster rate of decline in iGFR among those in higher quartiles of estimated NEAP (p-trend=0.05 and 0.01, respectively).

iGFR slopes (mL/min/1.73m2/year) by quartiles of estimated NEAP

Quartile (mEq/d)	Unadjusted absolute slope	Adjusted difference from Q1	p-value
1 (18.2 – 57.1)	-1.45	Ref	--
2 (57.2 – 72.8)	-2.11	-0.69	0.08
3 (72.9 – 89.5)	-2.25	-0.85	0.03
4 (89.6 – 232.5)	-2.34	-1.01	0.01
p-trend	0.05	0.01	--

Conclusions: In conclusion, a dietary pattern resulting in higher NEAP may contribute to faster rates of CKD progression.

Funding: NIDDK Support, Other NIH Support - National Center on Minority Health and Health Disparities, NCMHD, Pharmaceutical Company Support, Private Foundation Support

SA-OR369

Moderate Sodium Diet Potentiates the Renal and Cardiovascular Protective Effects of Angiotensin Receptor Blockers: A Post-Hoc Analysis of the RENAAL and IDNT Trials Hiddo Jan Lambers Heerspink,[1] Frank Holtkamp,[1] Eberhard Ritz,[2] Hans-Henrik Parving,[3] Gerjan Navis,[1] Dick de Zeeuw.[1] *[1]Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands; [2]Medical Endocrinology, University Hospital Copenhagen, Copenhagen, Denmark; [3]Internal Medicine, University of Heidelberg, Germany.*

Background: Dietary sodium restriction has been shown to enhance the short-term response of blood pressure and albuminuria to Angiotensin Receptor Blockers (ARBs). Whether this also enhances the long-term renal and cardiovascular protective effects of ARBs is unknown. We conducted a post-hoc analysis of the RENAAL and IDNT trials to test this hypothesis.

Methods: Patients with type 2 diabetes and nephropathy were randomized to ARB or non-Renin-Angiotensin-Aldosterone-System based antihypertensive therapy (control therapy). Treatment effects on renal and cardiovascular outcomes were compared in tertiles of dietary sodium intake, measured as on-treatment 24-hour urinary sodium:creatinine ratio (Na/Cr ratio).

Results: The analysis included 1177 subjects. Urinary sodium excretion was 152 (76), 179 (82) and 209 (90) mmol/24h in the tertiles of increasing Na/Cr ratio. ARB compared with control therapy had the greatest short-term effects in albuminuria and blood pressure in the lowest tertile of Na/Cr ratio. ARB also had the largest long-term effects on renal and cardiovascular events in the lowest Na/Cr tertile. The risk for renal events was reduced by 43% (95%CI 16 to 61), 0% (-42 to 30) and increased by 37% (-4 to 96) in subsequent Na/Cr tertiles respectively (p for trend <0.001). Cardiovascular events were reduced by 37% (8 to 57), and increased by 2% (-27 to 43) and 25% (-11 to 75), respectively (p for trend 0.021).

Conclusions: Reduced dietary sodium intake was associated with enhanced renal and cardiovascular protective treatment effects of ARB therapy as compared with control therapy in type 2 diabetic patients with nephropathy. Remarkably, the large difference in outcome occurred despite a liberal sodium intake even in the lowest tertile. This underscores the call for action to avoid high dietary sodium intake, particularly in type 2 diabetic patients who are receiving ARB therapy.

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SA-OR370

High Dietary Fiber (DF) Intake Is Associated with ↓Inflammation and Mortality in CKD: National Health and Nutrition Examination Survey (NHANES) III Vidya M. Raj Krishnamurthy,[1,2] Guo Wei,[2] Michel B. Chonchol,[3] Kalani L. Raphael,[1,2] Tom H. Greene,[1,2] Srinivasan Beddhu.[1,2] *[1]VA; [2]Univ Utah, SLC, UT; [3]Univ CO, CO.*

Background: Chronic kidney disease (CKD) is considered an inflammatory state and it is unknown whether high DF intake is associated with a ↓risk of inflammation and mortality in the CKD population.

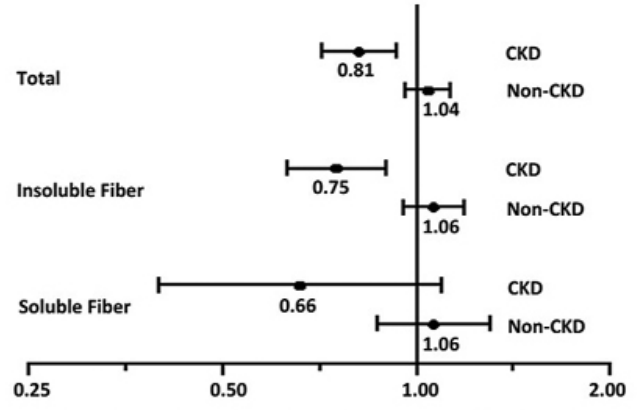
Methods: 14,543 NHANES III participants were studied. DF intake was assessed by standardized 24 h diet recall conducted by trained personnel. Mortality data were obtained by probabilistic matching with vital records.

Results: The mean age was 45.0 ± 15.8 yrs. 48% were men and 10% were black. 5.8% had CKD (eGFR < 60 ml/min/1.73 m2) and 25.7% had inflammation (serum CRP >3mg/L). The associations of DF in CKD and non-CKD groups with inflammation are summarized in the table and mortality in the figure.

Relationship of elevated serum CRP (> 3 mg/L) with DF in non-CKD and CKD

	Non-CKD	CKD
For each 10 g/d ↑ in total fiber	0.89 (0.83, 0.97)	0.62 (0.50, 0.77)
For each 10 g/d ↑ in insoluble fiber	0.87 (0.78, 0.96)	0.55 (0.42, 0.74)
For each 10 g/d ↑ in soluble fiber	0.72 (0.58, 0.90)	0.26 (0.13, 0.53)

Each cell represents a logistic regression model adjusted for demographics, MI, CHF, stroke, cancer, smoking, alcohol use, physical inactivity, SBP, DBP, calorie intake, protein intake, serum triglycerides, HDL and LDL cholesterol



Hazard Ratio for each 10 g/day increase in each type of fiber intake in CKD and non-CKD subpopulations

Models adjusted for age, gender, race, smoking, alcohol, leisure time physical inactivity, calorie intake and protein intake

The relationships of total fiber with elevated CRP and with mortality differed significantly between the non-CKD and CKD groups (p=0.002 for CRP and p=0.006 for mortality).

Conclusions: High DF intake is associated with lower risk of inflammation and mortality in CKD and these associations are stronger in magnitude in the CKD than in the non-CKD population. Increasing DF might decrease inflammation and mortality in the CKD population. Interventional studies are warranted to test this hypothesis.

SA-OR371

Lifestyle Factors and the Development of Stage 3 Chronic Kidney Disease in the Framingham Heart Study

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Background: Modifiable lifestyle factors may be targeted as part of stage 3 chronic kidney disease (CKD) prevention. We sought to investigate the association of these factors with the development of stage 3 CKD in the Framingham Offspring cohort.

Methods: Participants (n=1802, mean age 59 years, 54% women) attended Offspring Exams 7 (1998-2001) and 8 (2005-08) and were free of baseline stage 3 CKD (baseline estimated glomerular filtration rate [eGFR] ≥60mL/min/1.73m²). Lifestyle characteristics were measured at baseline and included diet quality assessed using the 2005 Dietary Guidelines for Americans adherence index (DGAI), physical activity index (PAI), alcohol intake (none, low-to-moderate, high), and current smoking status. The MDRD Study equation was used to determine eGFR; incident stage 3 CKD was defined as eGFR<60mL/min/1.73m² at Exam 8 and was modeled using logistic regression.

Results: Over 6.6 years of follow-up, 7.7% (n=139) of participants developed stage 3 CKD. Higher levels of dietary quality were associated with a decreased odds ratio [OR] of stage 3 CKD (Table; DGAI Quartile 4 vs. Quartile 1: OR 0.43, p=0.004; DGAI Quartile 3 vs. Quartile 1: OR 0.57, p=0.04). We did not observe associations with physical activity, smoking status, or alcohol intake with stage 3 CKD (Table, all p>0.25).

	OR (95% CI)	p
Dietary quality (DGAI)		
Quartile(Q) 1	1.0 (referent)	
Q2	0.71 (0.43-1.19)	0.20
Q3	0.57 (0.33-0.97)	0.04
Q4	0.43 (0.25-0.76)	0.004
Physical activity index (PAI)		
Q1	1.0 (referent)	
Q2	1.09 (0.64-1.86)	0.76
Q3	0.83 (0.48-1.45)	0.51
Q4	1.10 (0.65-1.88)	0.72
Current Smoker		
No	1.0 (referent)	
Yes	1.04 (0.53-2.04)	0.92
Alcohol Consumption		
None	1.0 (referent)	
Low-to-moderate	0.92 (0.61-1.39)	0.70
High	0.71 (0.39-1.27)	0.25

*Model covariates included all lifestyle factors simultaneously, age, sex, baseline eGFR, body mass index, hypertension, diabetes, and dipstick proteinuria.

Conclusions: Higher dietary quality is associated with a decreased risk of incident stage 3 CKD. Whether dietary modifications can prevent stage 3 CKD warrants further study.

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SA-OR372

Vitamin D Levels and Chronic Kidney Disease Progression: A CRIC Study

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Background: Studies of vitamin D receptor agonist (VDRA) therapy in animal models of kidney disease demonstrated reductions in albuminuria and amelioration of progression of podocyte injury. A recent randomized controlled trial demonstrated that VDRA therapy was associated with reductions in proteinuria in patients with diabetic nephropathy. The objective of this study was to examine the associations between vitamin D levels and chronic kidney disease (CKD) progression in CRIC participants.

Methods: Vitamin D levels were available in 1560 participants (44% diabetic) at Year 1 with a median (interquartile range [IQR]) age of 62 (55-68) years and eGFR of 45 (34-54) ml/min/1.73m^2. Cox proportional hazards models were censored at death.

Results: Median (IQR) serum 25(OH)D levels were 27 (16-37) ng/mL and 1,25$(OH)_2$D levels were 27 (18-37) pg/mL. During a median follow-up of 4.7 (IQR 3.8-5.5) years, 167 participants reached ESRD (24.1/1000 person-years), and 207 reached the composite of end-stage renal disease (ESRD) or halving of eGFR (29.8/1000 person years). Higher serum 1,25$(OH)_2$D levels were associated with lower risk of ESRD [hazard ratio (HR) per 10 pg/mL greater 1,25$(OH)_2$D level: 0.78 (95%CI 0.66-0.91), p<0.01) and the composite outcome [HR 0.79 (0.69-0.90), p<0.01], adjusted for age, race, diabetes, season, systolic blood pressure and eGFR (Model 1). After further adjustment for proteinuria, PTH and FGF-23 levels (Model 2), the associations remained significant for ESRD [HR 0.84 (0.71-0.99), p=0.04] and the composite outcome [HR 0.86 (0.75-0.99), p=0.03). Higher serum 25(OH)D levels were also associated with lower risk of ESRD [HR per 10 ng/mL greater 25(OH)D 0.83 (0.73-0.96), p=0.01] and the composite outcome [HR 0.84 (0.74-0.94), p< 0.01) in Model 1; however, the results for ESRD [HR 0.95 (0.80-1.11)] and the composite outcome [HR 0.96 (0.84-1.11)] were no longer significant after adjustment in Model 2.

Conclusions: Higher vitamin D levels are associated with less CKD progression. The persistent 1,25$(OH)_2$D association after adjustment for multiple measures of CKD severity (proteinuria, PTH and FGF-23 levels) suggested minimal confounding by CKD severity.

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SA-OR373

Chronic Kidney Disease Awareness and Healthy Behaviors in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Cohort

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Background: The association between chronic kidney disease (CKD) awareness and healthy behaviors is unknown. We examined whether CKD self-recognition is associated with behaviors that may slow CKD progression.

Methods: We conducted a cross-sectional analysis using baseline data from 3670 adults with CKD (eGFR <60 ml/min/1.73 m2) participating in REGARDS, a prospective cohort study of risk factors for stroke. CKD awareness was a "yes" answer to "Have you ever been told you have kidney disease?" Self-reported outcomes included current tobacco use, use of non-steroidal anti-inflammatory medications (NSAIDs), and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEI/ARB) use, based on pill review. Measured outcomes included blood pressure control (<140/<90 mmHg) and among self-reported diabetics, glycemic control (fasting glucose <126 mg/dl). Multivariable logistic regression adjusted for sociodemographics, co-morbidities, eGFR and albumin-creatinine ratio was used to estimate the odds of each behavior in individuals aware vs. unaware of their CKD.

Results: Only 10% of individuals were aware of their CKD. Those who were aware had nearly 60% lower odds of current tobacco use compared to those unaware [adjusted odds ratio 0.43, 95%CI (0.22-0.87)]. However, CKD awareness was not associated with reduced NSAID use [0.77 (0.38-1.55)], ACEI/ARB use [1.24 (0.87-1.76)], or blood pressure control [1.06 (0.76-1.48)]. Among diabetics, CKD awareness was not associated with glycemic control [0.81 (0.60-1.43)] or ACEI/ARB use [0.92 (0.60-1.43)].

Conclusions: Awareness of CKD was associated with decreased current tobacco use but was not associated with greater odds of other healthy behaviors. While the cross-sectional design and potential misclassification of awareness by survey question may limit interpretation, results suggest that a more nuanced understanding of the association among patient knowledge, awareness, and behaviors is needed.

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SA-OR374

Association of Obesity and Kidney Function Decline in Persons without Chronic Kidney Disease: Multi-Ethnic Study of Atherosclerosis (MESA)
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Background: Obesity has been associated with increased risk for chronic kidney disease (CKD) in some studies. Whether anthropometric measures of obesity are associated with renal function decline prior to onset of CKD (defined as estimated glomerular filtration rate (eGFR)<60ml/min/1.73m2) is not well known.

Methods: We studied the association of body mass index (BMI), waist circumference (WC), waist/hip ratio (WHR), and percent adulthood weight change with kidney function decline among 5160 White, Black, Hispanic and Chinese adults without CKD. GFR was estimated by creatinine (eGFRCr) and cystatin C (eGFRCys) with repeated measures over five years. We adjusted for age, sex, race, hypertension, and diabetes.

Results: Mean age was 60±10 years, 48% men, baseline mean eGFRCr was 82±13 and eGFRCys 95±17 ml/min/1.73m2. Mean eGFRCr decline was 1.55 (SD 2.85) and eGFRCys 1.12 (4.37) ml/min/1.73m2 per year. Persons with high WHR(≥0.9 men; ≥0.7 women) had 20-30% faster decline by eGFRCr (β -0.22, 95%CI -0.38 to -0.06) and eGFRCys (β -0.34, -0.57 to -0.12) after adjustment, including BMI, compared to persons with normal WHR. Persons with larger waist circumference (≥ 102cm for men; ≥ 90cm for women) had moderately faster rates of decline: (β -0.10, (-0.20 to -0.01) by eGFRCr, and β -0.07, (-0.21 to 0.07) by eGFRcys). In contrast, compared to persons with BMI 19 to 25 kg/m2, only persons with BMI ≥ 35 had faster decline by eGFRCr (β -0.19 ml/min/1.73m2 per year, 95%CI -0.37 to -0.004), but not by eGFRCys (β 0.11, -0.15 to 0.37). Weight gain or loss was not associated with eGFR decline (all p>0.05).

Conclusions: Central obesity, rather than weight or BMI, is more consistently associated with kidney function decline among persons without CKD. Further studies with imaging measures of fat distribution are needed to further evaluate these associations as potential modifiable risk factors for CKD.

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SA-OR375

Physical Activity, Death and Kidney Function Decline in Chronic Kidney Disease
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Background: Physical activity promotes diverse metabolic benefits that may counteract the toxic biochemical environment of chronic kidney disease (CKD). We tested the hypotheses that greater physical activity levels are associated with lower risks of death and kidney disease progression in a prospective cohort study of stage III-IV CKD patients.

Methods: We studied 294 participants from the Seattle Kidney Study, a Nephrology clinic-based study of CKD, who had an estimated glomerular filtration rate (eGFR) of 15-59 mL/min/1.73 m2. Participants self-reported type, frequency, and intensity of physical activity (PA), and we converted these responses to metabolic equivalent task (MET) min/week. PA levels of 100 MET-min/week are equivalent to leisurely walking for 30 minutes per week. We used proportional hazards models to quantify associations of PA with time to death and Poisson regression models to estimate associations of PA with a 25% decline in kidney function, defined using longitudinal serum cystatin C.

Results: Mean eGFR at baseline was 42 mL/min/1.73 m2) and mean age was 62 years. During a median 2.6 years of follow-up, 42 participants died ((14.3%) 5.7 deaths/100 person-years). After adjustment for demographics, eGFR, prevalent coronary artery disease and functional status, PA levels of 100-500 MET-min/week and >500 MET-min/week were associated with 35% (95%CI -69%,+37%) and 43% (95%CI -79%,+53%) lower risks of death, respectively, compared to PA levels <100 MET-min/week. Among 206 participants who completed at least one follow-up kidney function measurement, 53 (26%) lost ≥25% of GFR (11.7 events/100 person-visits). After adjustment, the estimated risk of GFR decline was 15%(95%CI -50%,+46%) lower in the highest PA group, relative to the lowest group.

Conclusions: Greater physical activity levels trended toward being associated with lower risks of death and kidney function decline in a longitudinal study of CKD. Physical activity, at even moderate levels, may be sufficient to confer health benefits among CKD patients and is emerging as one of few modifiable risk factors for major adverse health outcomes in this high risk population.

Funding: Private Foundation Support

SA-OR376

A One-Year Lifestyle Intervention Improves Myocardial Function and Exercise Capacity in Patients with Chronic Kidney Disease
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Background: Myocardial dysfunction (MD) is common in pts with chronic kidney disease. We sought the effect of a lifestyle intervention (LI) that included exercise training on MD and exercise capacity in CKD.

Methods: 83 pts with stage 3-4 CKD were randomized to standard care (control) or LI. LI included access to multidisciplinary care through a nurse practitioner led CKD clinic, which included a nephrologist, social worker, diabetes educator and exercise physiologist. LI involved 8 weeks supervised, gym-based individualized exercise training followed by home based training with ongoing telephone support and gym refresher sessions for 12m. Pts attended 4 week behavior-lifestyle change program led by a psychologist and dietician. MD was assessed at baseline and 12m using standard echo and tissue Doppler. Anthropometric, biochemical and fitness data were collected at baseline and follow-up. The effect of intervention was determined using analysis of covariance (ANCOVA). Change scores were compared between groups.

Results: 72 pts completed follow-up (LI=36, control =36). There were no baseline differences (Table 1). LI resulted in a significant (33%) increase in exercise capacity, with decreases in BMI. This group also showed improved diastolic function (increased e'). There were no changes in systolic function, blood pressure or biochemical parameters between groups.

Conclusions: The LI significantly improved exercise capacity and diastolic function in CKD. Lifestyle and exercise interventions may assist in managing the deleterious effects of reduced kidney function on the myocardium.

Table 1. Effect of one-year lifestyle intervention

	Control group		LI group		
Variable	Baseline	Change	Baseline	Change	P value
METs	7.3±3.0	-0.7±0.4	7.3±3.5	2.4±0.3*	<0.0001
BMI (kg/m2)	33.0±8.0	0.4	32.5±6.8	-0.6*	0.01
E/A	1.05±0.36	-0.17±0.04	1.01±0.3	0.11±0.07*	0.002
Deceleration time (ms)	240.0±39.7	16.2±7.5	240.6±52.2	-14.5±7.8*	0.002
S' (cm/s)	6.28±1.17	0.0±0.0	6.13±1.2	0.001±0.01	NS
E' (cm/s)	5.88±1.4	-0.47±1.0	5.59±1.5	0.75±1.16*	0.001
Myocardial Strain (%)	-19.5±2.8	1.3±0.5	-18.4±3.9	-0.4±0.7	NS

*p<0.05

SA-OR377

Reprogramming of Adult Proximal Tubular Epithelium to an Embryonic Nephron Progenitor State
Melissa H. Little, Caroline E. Hopkins, Norseha Suhaimi, Fiona Rae, Joan Li. *Institute for Molecular Bioscience, University of Queensland, St. Lucia, Brisbane, Queensland, Australia.*

Background: The mammalian kidney arises from the ureteric bud and the metanephric mesenchyme, the latter giving rise to a cap mesenchyme nephron progenitor population. This Six2 positive cap mesenchyme surrounds the ureteric tips of the developing kidney and contains self renewing nephron stem/progenitors. Lineage tracing has shown that Six2+ cells give rise to all segments of the uriniferous tubule except the collecting ducts(1). Nephron formation ends around birth via the terminal commitment of this progenitor field to nephron formation, hence this stem cell population does not persist postnatally. As a result, no new nephrons arise after birth and total nephron number is determined by the balance between cap mesenchyme self-renewal, differentiation and death.

Methods: In this study, we investigated whether the adult proximal tubular population could be reprogrammed to this earlier nephron progenitor state via the enforced reexpression of a cap mesenchymal transcriptional state. 15 key genes were cloned into lentiviral vectors and a combinatorial screen was performed via infection of the human proximal tubular cell line, HK2.

Results: Four stages of validation were performed, including i) epithelial to mesenchymal morphological change, ii) production of the cap mesenchyme-specific Cited1 protein, iii) re-expression of key cap mesenchyme genes and iv) successful integration into the cap mesenchyme of an ex vivo embryonic kidney reaggreagtion (2). In this way, we identified a combination of genes (Osr1, Six1, Six2, Hoxa11, Eya1, Snai2) capable of inducing a nephron progenitor state according to all four criteria. Approximately 0.875% of infected cells incorporated into cap mesenchyme in comparison to 0.05% for control HK2 cells cultured under identical conditions .

Conclusions: This is the first demonstration of reprogramming to the progenitor state of a solid organ and is the first evidence that it may be feasible to regenerate a cap mesenchyme population. These results open up the possibility of reinitiation of nephron formation but also to manipulation of the nephrogenic process to prolong nephron endownment.

Funding: Government Support - Non-U.S.

SA-OR378

Induced Pluripotent Stem Cells from Patients with Genetic Kidney Disease: Applications for Disease Modeling and Therapeutic Screening
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Background: The reprogramming of somatic cells to induced pluripotent stem (iPS) cells has attracted considerable attention for disease modeling, drug screening and regenerative medicine. We have recently shown that iPS cells can be derived from human kidney mesangial cells (JASN; May 2011).

Methods: In the present study, fibroblasts grown from skin biopsies of patients with Alport Syndrome and polycystic kidney disease (PKD) were reprogrammed by retroviral transduction using OCT4, SOX2, KLF4, and c-Myc. The DNA methylation profiles of skin-derived iPS cells were analysed by bisulfite sequencing using the OCT4 promoter, compared to kidney cell-derived iPS cells. The iPS cells were analysed for stem cell marker

expression using immunofluorescence microscopy and qPCR. The pluripotent capacity of the undifferentiated iPS cells was tested by the formation of teratomas following injection into immunodeficient mice and the directed differentiation into embryoid bodies.

Results: The disease-specific iPS cells resembled human embryonic stem cells (hES) in morphology and gene expression localising for OCT3/4, SSEA-4, TRA-1-60 and TRA-1-81 proteins. Using qPCR, the iPS cells expressed stem cell marker genes and exhibited silencing of the retroviral transgenes by passage four. DNA methylation profiles showed that fibroblast and mesangial-derived iPS cells had OCT4 methylation patterns similar to hES cells, but different to the primary target cells. Furthermore, iPS cells formed embryoid bodies and expressed markers of all three germ layers by immunostaining and RT-PCR. The injection of undifferentiated iPS colonies into immunodeficient mice formed teratomas, thereby demonstrating pluripotency.

Conclusions: The derivation of iPS cells from Alport Syndrome and PKD patients advances the potential of human pluripotent cells for modeling the genetic disorders, the directed differentiation of kidney cells and screening of new drug compounds.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR379

Identification of Early Stage Renal Progenitors in E9.5 Embryos by Using Osr1-GFP Knock-In Mice Atsuhiro Taguchi, Ryuichi Nishinakamura. *Kidney Development, Institute of Molecular Embryology and Genetics, Kumamoto, Kumamoto, Japan.*

Background: Though many groups are trying to induce renal progenitor cells from ES or iPS cells, it remains unclear to which in vivo stage those cells correspond. In this study, we propose the stepwise induction model by analyzing the in vivo developmental process of renal progenitor cells.

Methods: Osr1 is one of the earliest markers of the IM and most of the cell types within the kidney arise from the Osr1+ population. Thus we have generated Osr1-GFP knock-in mice and murine ES cells. By sorting Osr1-GFP+ cells from embryos and applying them to the colony- forming assay that promotes renal progenitor differentiation, we have confirmed the existence of renal progenitors in embryos at the early developmental stages.

Results: Consecutive application of Osr1-GFP+ kidney precursors to colony forming assay on Wnt4-expressing feeders, from E8.5 intermediate mesoderm to the E11.5 metanephric mesenchyme, revealed that the Osr1+ cells after E9.5 do contain colony-forming cells. Osr1-GFP+ cells at E8.5, however, formed no colonies. Further analyses of expressed genes of colonies derived from E9.5 implied that these colonies are progenitors of mesonephroi and distinct from metanephric progenitors at E11.5.

Conclusions: We have identified the functional difference of renal progenitor cells in the early stage embryo between E8.5 to E11.5 by colony-forming assay. Our data suggests a transition from the primitive to the definitive state between E8.5 and 9.5. These data imply the existence of cues which promote maturation of primitive progenitors to the functional progenitor cells between these developmental stages.

Funding: Government Support - Non-U.S.

SA-OR380

Developmental Regulators in Cap Mesenchyme Are Epigenetically Poised Nathaniel J.D. McLaughlin, Xiao Yao, Zubaida R. Saifudeen, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Background: In embryonic stem cells, the promoters of developmental regulators are held in a "poised" state by carrying activating (H3K4me3) and repressive (H3K27me3) chromatin domains. Upon receiving a differentiation signal, these bivalent domains resolve either into actively transcribed regions or silenced by packing into inaccessible heterochromatin. We hypothesized that progressive restriction of cell fate which occurs during differentiation of renal progenitors to epithelia involves conversion of bivalent chromatin into chromatin regions accessible or inaccessible to the transcription machinery.

Results: We show here that self-renewing Six2+ cap mesenchyme cells are enriched with polycomb protein Ezh2 (H3K27 methyltransferase) as compared to nascent nephrons, and exhibit a bivalent chromatin signature (H3K4me3/K27me3). This bivalency resolves during nephron differentiation via downregulation of repressive H3-K27me3 and acquisition of additional activating H3-R8me2/R17me2 marks. Cre-mediated deletion of *Ezh2* from the Six2 cell population in vivo eliminates H3K27me3 and compromises the size of this progenitor pool. ChIP-Seq of chromatin landscapes in progenitor and differentiating metanephric mesenchyme-derived cell lines revealed that bivalent chromatin domains in developmental regulators resolve during differentiation. Induction of *Pax8*, a known target of Wnt-β-catenin signaling, by exogenous Wnt3a is associated with recruitment of the H3K27 demethylase, KDM6b, and displacement of Ezh2, thus loss of repressive H3K27me3.

Conclusions: We conclude that "epigenetic poising" is not unique to pluripotent cells and represents a key mechanism for a cell fate decisive switch during nephron differentiation. Polycomb(Ezh2)-mediated silencing may represent an epigenetic mechanism to maintain the pool of renal progenitors.

Funding: NIDDK Support

SA-OR381

KIF3A Controls Nephrogenesis by Regulating FGF8 Lijun Chi, Norman D. Rosenblum. *Paediatrics, Prog Dev & Stem Cell Biology, Hosp Sick Child, U Toronto, Canada.*

Background: The primary cilium is critical for Hedgehog (HH)-dependent signaling in nonrenal tissues. The functional contribution of the primary cilium to early renal embryogenesis is undefined. We hypothesized that the primary cilium controls embryonic kidney morphogenesis by modulating HH signaling.

Methods: Mice with primary cilium deficiency were generated by inactivating *Kif3a*, which encodes a primary cilium motor protein, in the metanephric mesenchyme (*Kif3a-MM* null mice) using *Rarb2-Cre* and *Kif3a*^{loxP} mice. Metanephric and primary metanephric mesenchyme primary cultures were generated from WT and mutant mice.

Results: Confocal immunofluoresence imaging of WT and *Kif3a-MM* null kidney tissue demonstrated that KIF3A expression is restricted to the primary cilium. Nephrogenesis was reduced by 35% at P0 and 22% at E12.5 ($P<0.05$) in *Kif3a-MM* null mice. Surprisingly, HH signaling, assayed by expression of *Ptc1* mRNA and a *Ptc1-lacZ* reporter in E11.5-E17.5 kidney tissue, was similar to that in controls. In contrast, expression of *Fgf8* was almost undetectable by E15.5, at which stage the number of primary cilia was reduced by 70%. The significance of decreased *Fgf8* expression was determined in organ culture. Addition of FGF8 protein to *Kif3a-MM* null metanephroi, harvested at E11.5 and separated from the ureteric bud and then cultured on spinal cord, increased the number of PAX2-positive tubules 1.7-fold ($P<0.001$) compared to untreated *Kif3a-MM* controls. The functional contribution of KIF3A was investigated in cultured WT and *Kif3a-MM* null primary MM cells isolated from E11.5 WT and mutant kidneys. Cilia length was decreased 5-fold in *Kif3a-MM* null MM cells versus WT cells ($P=0.003$). Transfected *Kif3A-GFP* localized to the primary cilium and increased primary cilium length in null MM cells 1.7-fold compared to transfection with GFP alone ($P=0.04$). While transfected *Kif3A-GFP* exerted no significant effect on the expression of the HH response genes, *Ptc1* or *Gli1*, in *Kif3a-MM* null MM cells ($P<0.002$), expression of *Fgf8* mRNA increased 2.2-fold compared to controls ($P<0.002$).

Conclusions: We conclude that KIF3A acts in the primary cilium of metanephric mesenchyme cells to control nephrogenesis by regulating *Fgf8* expression.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR382

Structural Changes in the Cytoplasmic Domain of Integrin β1 Due to the Mutations in NPxY Motifs Induce Kidney Developmental Phenotypes Sijo Mathew,[1] Riya Jose Palamuttam,[1] Glenda Mernaugh,[1] Zhenwei Lu,[2] Leslie S. Gewin,[1] Nada Bulus,[1] Ambra Pozzi,[1] Charles R. Sanders,[2] Roy Zent.[1] *[1]Division of Nephrology and Hypertension, Vanderbilt University Medical Center; [2]Biochemistry and Center for Structural Biology, Vanderbilt University Medical Center, Nashville, TN.*

Background: Integrins are the principal receptors that regulate cell extracellular matrix interactions. β1 integrins are the major integrins found in the kidney and are required for its development and function. The two NPxY motifs in the cytoplasmic tail of the integrin β1 subunit are proposed to be critical for β1 integrin function. In this study, we defined the importance of the NPxY motifs in ureteric bud (UB)development.

Results: We generated mice that selectively express β1 integrin in the developing UB where the tyrosines (Y) in both NPxY motifs were mutated to alanines (A) or phenylalanines (F). The Y/A mutations caused severe developmental abnormalities; however, mice with the Y/F mutations developed normally, but were more susceptible to injury in comparison to wild type mice. Consistent with the *in vivo* data, collecting duct cells expressing the mutant β1 integrins had abnormalities in cell adhesion, migration, proliferation and tubule formation. Interestingly, only cells expressing the Y/A mutations failed to activate intracellular signaling mediated by GDNF or FGF, two growth factors critical for UB development. We further defined the molecular mechanism for the functional alterations induced by these mutations by solving the structure of the β1 integrin tail and transmembrane domain by NMR spectroscopy in DMPC/DHPC bicelles. We demonstrated there were significantly more structural disruptions in the Y/A compared to the Y/F mutants.

Conclusions: This study shows that the NPxY motifs in the integrin β1 tail regulate UB development by altering integrin dependent cell functions and growth factor signaling. Furthermore, it shows the structural alterations of the β1 integrin tail carrying mutations in these critical tyrosine residues. This observation has major implications in our understanding how the cytoplasmic tail of β1 integrins regulate cell and tissue function.

Funding: NIDDK SupportAmerican Heart Association

SA-OR383

Podocyte-Selective Loss of the Basic Helix-Loop-Helix Transcription Factor, Pod1/Tcf21 Results in Podocyte Defects and Massive Proteinuria Yoshiro Maezawa,[1] Tuncer Onay,[1] Chengjin Li,[1] Susan E. Quaggin.[1,2] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Department of Medicine and Division of Nephrology, St Michael's Hospital and University Health Network, Toronto, ON, Canada.*

Background: Podocytes are highly differentiated cells that are essential for function of the glomerular barrier. Although a number of transcription factors are known to be expressed in podocytes, their function(s) in orchestrating development of specialized features such as slit diaphragms and foot processes, is largely unknown.

Pod1/Tcf21 belongs to the bHLH family of transcription factors and is expressed in condensing metanephric mesenchyme, and immature and mature podocytes. We generated

a standard KO mouse that dies in the perinatal period with striking renal defects including severely disrupted and delayed glomerulogenesis, arrested differentiation of tubular epithelium, and abnormal branching of the ureteric bud. However, given the complexity of the Pod1 KO phenotype and early lethality, the precise role of pod1 in podocytes remains unclear.

Methods: To understand the role of Pod1 in podocyte development and function, we further analysed the standard KO mice, and developed podocyte-selective KO mice as well. A conditional floxed Pod1 allele was created using Bac cloning strategy and embryonic stem cell targeting, placing loxP sites around the first exon of Pod1. The floxed mice were bred with Podocin-Cre mice to delete the Pod1 gene specifically in podocytes.

Results: Analysis of conventional KO mice reveals defects in podocyte foot process formation. Podocyte-specific KO mice develop massive proteinuria at 4 weeks of age. Histological analysis reveals striking glomerulosclerosis and loss of podocytes at this stage. In contrast to conventional KO mice, glomerular tuft development and mesangial cell migration is normal, suggesting that these functions depend on Pod1 expression in the mesenchyme and NOT the podocyte lineage.

Conclusions: Together, these data demonstrate crucial but distinct role for Pod1 in renal mesenchymal and podocyte cell lineages. Furthermore, these mice represent a valuable tool to identify the transcriptional targets regulated by Pod1 in podocytes.

Funding: Government Support - Non-U.S.

SA-OR384

Deletion of Fgfr2 from the Peri-Wolffian Duct Stroma Leads to Induction Abnormalities, and Congenital Vesicoureteral Reflux Kenneth A. Walker,[1] Sunder Sims-Lucas,[1] Whitney Sunseri,[1] Caitlin M. Schaefer,[1] Feng Chen,[2] Carlton M. Bates.[1] *[1]Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]Medicine, Washington University School of Medicine, St Louis, MO.*

Background: Kidney development begins when the ureteric bud (UB) invades the metanephric mesenchyme (MM). The site of UB induction is strongly influenced by the peri-Wolffian duct stroma (ST). Previously, conditional deletion of fibroblast growth factor receptor 2 (Fgfr2) in both MM and ST was shown to cause ureteric induction defects, CAKUTs, and high rates of vesicoureteral reflux (VUR).

Methods: The purpose of this study was to elucidate the role of Fgfr2 signaling in the ST during kidney development through conditionally deleting *Fgfr2* in ST using Tbx18cre (*Fgfr2*$^{St-/-}$) mice; that drive cre expression in the ST, as well as in bladder mesenchyme but not in MM.

Results: Analysis at E11.5 showed *Fgfr2*$^{St-/-}$ mutants have a highly variable UB induction site compared to controls. At E10.5, during early UB induction, *Fgfr2*$^{St-/-}$ embryos exhibit reduced *Bmp4* and *Sprouty1* mRNA expression (67% and 59% respectively, of control expression levels). At birth, *Fgfr2*$^{St-/-}$ mice present with CAKUT phenotypes, and have higher rates of VUR (79%) than control mice (3%). Ureters from both genotypes show similar morphology however analysis of cultured E12.5 explants indicated that *Fgfr2*$^{St-/-}$ ureters display abnormal peristaltic contractions. Lastly, adult *Fgfr2*$^{St-/-}$ mice continue to have high rates of VUR (via live and euthanized cystogram methods) and often exhibit abnormal voiding behavior, megabladder and hydronephrosis compared to age-matched controls. In conclusion, Fgfr2 signaling in ST is required for normal induction of the UB (regulated by *Bmp4* and *Sprouty1*) and for normal ureteral peristalsis.

Conclusions: These combined ureteric induction and peristaltic defects have novel implications for understanding the developmental mechanisms of VUR. Furthermore, the bladder abnormalities (voiding dysfunction, megabladder and hydronephrosis) seen in mutants suggests critical roles for Fgfr2 in bladder development and could represent a novel biological/genetic link between VUR and voiding dysfunction; as is often associated in humans.

Funding: NIDDK Support

SA-OR385

Congenital Hydronephrosis and Delayed Distal Ureter Maturation in Discs-Large 1 (*Dlg1*) Null Mice Is Associated with Reduced Retinoic Acid Signaling and Impaired Apoptosis Via a Non-Epithelial Cell Autonomous Mechanism Sung Tae Kim,[1] Wojciech Swat,[2] Jeffrey H. Miner.[1] *[1]Renal Division, Washington University, St. Louis, MO; [2]Dept. of Pathology & Immunology, Washington University, St. Louis, MO.*

Background: The absence of Discs-large 1 (*Dlg1*), the mouse ortholog of the *Drosophila* discs-large tumor suppressor, results in congenital hydronephrosis characterized by urinary tract abnormalities, reduced ureteric bud branching, and delayed disconnection of the ureter from the common nephric duct.

Methods: To define the cellular requirements for *Dlg1* during urogenital development, we used a floxed *Dlg1* allele and Pax2Cre, Pax3Cre, and HB7Cre transgenes to generate tissue-restricted *Dlg1* mutants. In addition, we mated *Dlg1* heterozygote mice with Ret-GFP knockin, retinoic acid response element-LacZ transgenic mice, or HB7-GFP knockin to see specific compartments of urogenital system.

Results: Mutation in urothelium and collecting ducts (via by HoxB7Cre or Pax2Cre) and in nephrons (via Pax2Cre) resulted in no apparent abnormalities in ureteric bud branching or in distal ureter maturation and no hydronephrosis. Mutation in nephrons and ureteric smooth muscle (via Pax3Cre transgene) resulted in congenital hydronephrosis accompanied by reduced branching, a delay in distal ureter maturation, and smooth muscle orientation defects, phenotypes very similar to *Dlg1* null mice. *Dlg1* null mice showed reduced apoptosis and evidence of reduced retinoic acid signaling in the kidney, shown both by real-time PCR and immunofluorescence. These results were confirmed using Ret-GFP knockin and retinoic acid response element-LacZ transgenic mice.

Conclusions: Taken together, these results suggest that *Dlg1* expression in ureteric smooth muscle cells is essential for ensuring distal ureter maturation by facilitating retinoic acid signaling and apoptosis.

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SA-OR386

Obstructive Nephropathy in Megabladder Mice Is Due to a Novel Hypomorphic Myocardin Allele Brian Becknell,[1] Ashley R. Carpenter,[2] Michael L. Robinson,[3] Kirk M. McHugh.[2] *[1]Department of Pediatrics, Nationwide Children's Hospital, Columbus, OH; [2]Center for Molecular and Human Genetics, Nationwide Children's Hospital Research Institute, Columbus, OH; [3]Department of Zoology, Miami University, Oxford, OH.*

Background: Megabladder (mgb-/-) mice exhibit impaired bladder smooth muscle development, resulting in megacystis and hydronephrosis in utero and renal failure in adults. We previously identified the insertion of a portion of chromosome (chr) 16 into chr 11 of mgb-/- DNA, but its significance was unclear.

Methods: To further refine the genetic alteration in mgb-/- mice, DNA was subject to fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). RNA was evaluated by microarray, in situ hybridization, and QRT-PCR. Mice with a fully disrupted myocardin (myocd) allele (myocd+/-) were crossed with mgb-/- mice to generate myocd-mgb- animals.

Results: FISH demonstrated a megabase insertion of chr 16 into chr 11, inclusive of 4 genes on chr 16. CGH confirmed this and identified a 26 kilobase (kb) deletion of chr 11. This insertion/deletion occurred in a gene sparse region of chr 11, 300 kb upstream of myocd, a transcription factor that cooperates with the serum response factor (SRF) to initiate myogenesis. Transcriptome profiling of mgb-/- E15 bladders demonstrated intact expression of early markers of muscle patterning, but impaired expression of 27 downstream targets of the myocd/SRF transcriptional complex. Myocd expression was diminished in E15 mgb-/- bladders by in situ hybridization and QRT-PCR versus controls. Compound heterozygotes (myocd-/mgb-) phenocopied mgb-/- bladders. Myocd-/mgb- embryos showed a further reduction in myocd expression versus mgb-/- mice resulting in the development of patent ductus arteriosus (PDA) postnatally.

Conclusions: The mgb-/- phenotype occurs as a result of a long-range, position effect mutation on chr 11 that results in a hypomorphic myocd allele. Further reduction of myocd gene expression in myocd-/mgb- mice results in the emergence of PDA, providing the first evidence that decreased myocd gene dosage results in congenital anomalies involving these two disparate organ systems.

Funding: NIDDK Support

SA-OR387

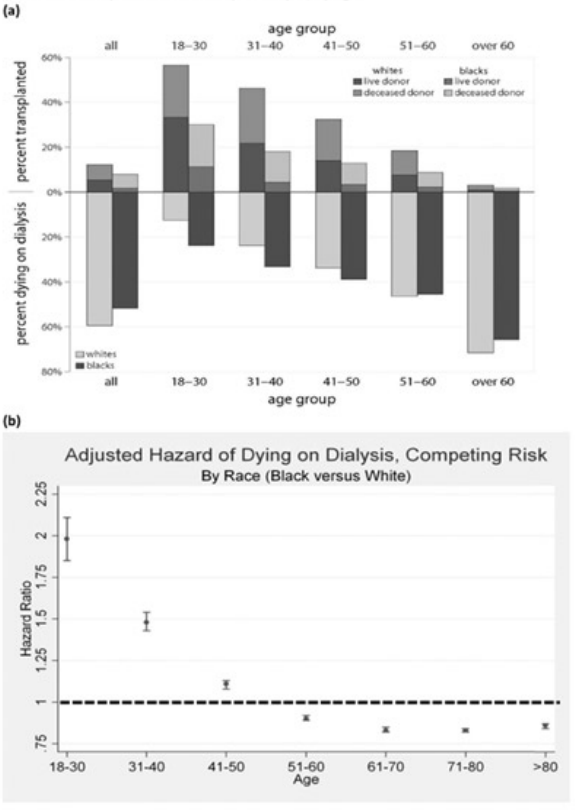
Age and Racial Disparities in Dialysis Survival Lauren M. Kucirka,[1] Morgan E. Grams,[2] Dorry L. Segev.[1] *[1]Surgery, JHU; [2]Medicine, JHU; [3]Epidemiology, JHU.*

Background: Many studies have reported that blacks survive longer on dialysis than whites. This observation is paradoxical given racial disparities in access to and quality of care, and is inconsistent with observed lower survival among blacks with chronic kidney disease. The perception of improved dialysis survival has affected clinical decision-making and engendered complacency about the low rates of kidney transplantation among blacks. We hypothesized that age and the competing risk of transplantation modify survival differences by race. The goal of our study was to estimate death on dialysis by race, accounting for age as an effect modifier and kidney transplantation as a competing risk.

Methods: We studied 909,841 incident end-stage renal disease patients captured in USRDS between 1995 and 2005. Multivariate age-stratified Cox proportional hazards and competing risk models were constructed to examine death on dialysis comparing black and white patients.

Results: Similar to previous studies, blacks had an overall 17% lower death rate on dialysis compared with whites (adjusted hazard ratio (aHR) 0.83, $p<0.001$). However, stratifying by age blacks under 50 had significantly higher mortality than their white counterparts (Figure 1a). This finding persisted in adjusted analyses treating kidney transplant as a competing risk (aHR 1.98 for 18-30; aHR 1.51 for 31-40; aHR 1.10 for 41-50; $p<0.001$, Figure1b); only those over 50 had lower death rates (aHR 0.87 for 51-60; 0.85 for 61-70; 0.83 for 71-80; 0.86 for >80; $p<0.001$).

Figure 1: (a) Percent of Incident ESRD Patients Who Received a Transplant (Upper Panel) or Died on Dialysis (Lower Panel) During the Study Period, by Race and Age Category, and (b) Relative Adjusted Hazard of Death (Black versus White) on Dialysis, by Age



Conclusions: The commonly cited dialysis survival advantage among blacks applies only to older adults. Blacks under the age of 50 die on dialysis at as much as twice the rate of their white counterparts.

Funding: NIDDK Support

SA-OR388

End-of-Life Care Intensity in Older Adults on Chronic Dialysis Susan P.Y. Wong, William Kreuter, Ann M. O'Hare. *University of Washington.*

Background: Survival after initiation of chronic dialysis is often limited in older adults, fostering a growing interest in advance care planning in this population. Little is known about their treatment choices at the end-of-life (EOL) and the major determinants of their choices.

Methods: Using data from USRDS, we performed a retrospective mortality study on 43,768 Medicare beneficiaries age ≥65 years who initiated chronic dialysis from 6/1/2005-12/31/2007 and died within 1-year of initiation. Using linked Medicare inpatient claims, we examined the following EOL care measures during the last 6-months of life: hospitalization, intensive care unit (ICU) admission, and receipt of an intensive procedure (mechanical ventilation, feeding tube placement, and cardiopulmonary resuscitation [CPR]). We examined the adjusted association of these measures with patient characteristics and regional patterns of healthcare spending (end-of-life expenditure index or EOL-EI).

Results: Most (79.5%) patients were hospitalized at least once in the last 6-months of life. Of these, 31.5% received at least one intensive procedure. Receipt of an intensive procedure was associated with Black race (24.8% v. 14.7%; OR 1.56, 95%CI 1.44-1.68) and age ≤75 years (48.1% v. 38.5%, 1.37, 1.29-1.46). Sex, cause of ESRD, and co-morbidities did not show strong and consistent associations with receipt of an intensive procedure. After adjustment for differences in patient characteristics, we found a stepwise increase in the likelihood of ICU admission and receipt of an intensive procedure during the last 6-months of life from regions with the lowest to highest healthcare spending (Figure 1).

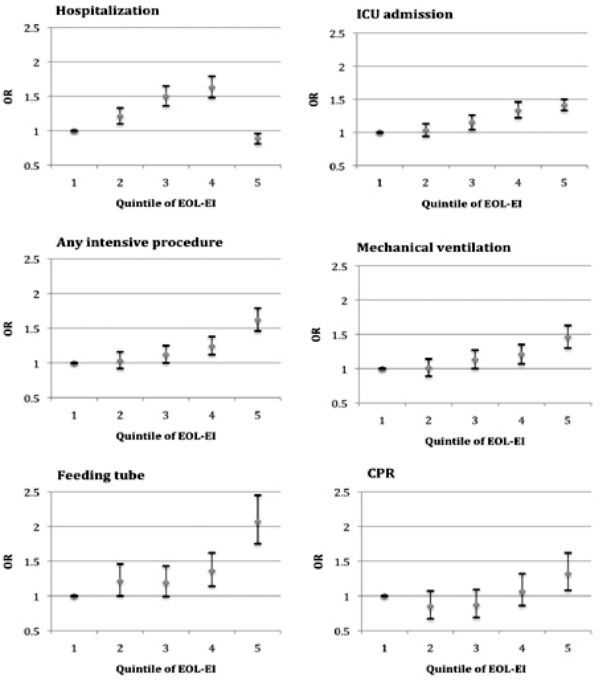


Figure 1. Adjusted OR for hospitalization, ICU admission and receipt of an intensive procedure, including mechanical ventilation, feeding tube, and CPR in the last 6-months of life according to quintile of EOL-EI.

Conclusions: Hospitalization, ICU admission and receipt of intensive procedures are common at the EOL among older dialysis patients. In general, utilization of intensive procedures in this population seems to be shaped more by regional treatment patterns than by patient characteristics.

SA-OR389

Lower Dialysate Na+ Impacts Weight Gain & Fluid Overload Hospitalizations Eduardo K. Lacson,[1] John Rogus,[1,2] Peter Kotanko,[2] Nathan W. Levin,[2] Raymond M. Hakim.[1] *[1]Fresenius medical Care, North America, Waltham, MA; [2]Renal Research Institute, New York, NY.*

Background: Interdialytic weight gain (IDWG) and fluid overload hospitalizations (FOH) increased with greater dialysate to serum sodium (sNa+) gradient. However, many physicians keep the base dialysate sodium (dNa+) formulation constant in a facility. We hypothesized that lowering dNa+ will decrease IDWG and FOH in prevalent hemodialysis (HD) patients.

Methods: A phased roll-out (Jan-Jun 2009) of dialysate formulation for Fresenius Medical Care, North America facilities decreased base dNa+ from 140 to 137 mEq/L. We identified 592 facilities with median dNa+ drop of 3.0 meq/L and 188 facilities where physicians kept the base sodium unchanged, when comparing 6-months before (Jul-Dec 2008) to 6-months after (Jul-Dec 2009) the conversion period. We identified all adult, in-center HD patients that survived for the entire 18-month study period divided into 25,653 patients with lower facility dNa+ and 9,613 controls that maintained dNa+. We estimated the change in key variables for each patient between the baseline and post-conversion periods to determine if they differed between cases and controls.

Results: We verified that post-conversion mean dNa+ was lower in study patients vs. controls (mean: 137 vs. 139 mEq/L, $p<0.0001$). Mean IDWG decreased in facilities with lowered dNa+ by 0.14 kg vs. controls' 0.03 kg ($p<0.0001$), accompanied by a larger decline in pre-HD weight (0.74 vs.0.57 kg, $p=0.01$) respectively. At baseline, FOH was higher in study patients (5% vs. 4%, RR=1.18, $p=0.002$). Post-conversion, overall FOH rate increased in both groups but the difference between groups lost significance (13.3% vs. 12.5%, RR=1.06, $p=0.055$). No significant differences in period changes between groups were noted in pre-HD sNa+, albumin and hemoglobin, pre- and post-HD systolic blood pressure (SBP) or post-HD weight.

Conclusions: Lowered dNa+ was accompanied by a greater decline in IDWG and pre-HD weight along with attenuation of the propensity for greater FOH over time. No accompanying differential changes in sNa+, SBP, post-HD weight, albumin or hemoglobin were observed.

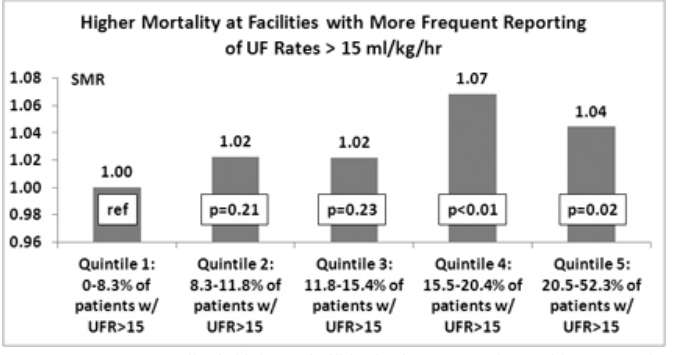
SA-OR390

Dialysis Facility Use of High Ultrafiltration Rates Is Associated with Higher Standardized Mortality Joseph M. Messana,[1] Dori Bilik,[2] Brett Lantz,[2] Robert A. Wolfe,[2] Jeffrey Pearson,[2] Rajiv Saran.[1] *[1]University of Michigan Kidney Epidemiology and Cost Center, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Background: Observational patient-level studies have associated hemodialysis (HD) ultrafiltration rates (UF) above 10-13 ml/kg/hr with more intradialytic hypotension, unstable sessions and higher mortality. The current study validates this using the % of pts at a dialysis facility with UF>15 ml/kg/hr data from the July-December 2009 CROWNWeb collection period.

Methods: Facility-level UF practice pattern was calculated from monthly patient treatment fluid removal and treatment time data. Facility % of patient-months with UF>15 were related to the 2009 standardized mortality ratio (SMR) using Poisson regression. To avoid patient-level treatment-by-indication bias, we analyzed death rates among all patients at a facility, categorizing facilities by their UF practice pattern. The SMR used CMS data sources adjusted for case-mix including patient age, sex, race, ethnicity, cause of ESRD, time since start of ESRD, comorbidities at ESRD incidence, nursing home status, and state general population death rate.

Results: 3,153 dialysis facilities were grouped into quintiles according to the % UF>15. Mortality was higher for facilities in the two highest quintiles relative to the lowest quintile. Findings were similar after adjustment for other facility practice patterns (achieved hemoglobin, URR, vascular access type).



Conclusions: Mortality is higher at facilities having more patients with UF>15. The reasons for prescribing high UF and approaches to reducing/optimizing UF requirements in dialysis patients deserve urgent attention by the dialysis community. Death rates could plausibly be lowered by 4-7% at the 40% of facilities with the highest % of pts UF>15 if those facilities adopt the practice facilities in the lowest qunitile.

Funding: Other U.S. Government Support

SA-OR391

Effects on Ventricular Volumes by Frequent Hemodialysis: Results from the Frequent Hemodialysis Network (FHN) Trials Christopher T. Chan, Gerald J. Beck, Glenn M. Chertow, John T. Daugirdas, Tom H. Greene, Peter Kotanko, Brett Larive, Nathan W. Levin, Ravindra L. Mehta, Michael V. Rocco, Javier Sanz, Brigitte Schiller, John B. Stokes, Alan S. Kliger. *FHN Trials Group, NIDDK.*

Background: Increased cardiac ventricular volume is an independent risk factor for death in patients with end-stage renal disease (ESRD). As part of the FHN trials, the impact of daily in-center hemodialysis (DHD) and nocturnal home hemodialysis (NHD) on left and right ventricular (LV, RV) volumes and function were investigated. We hypothesized that frequent hemodialysis would lower LV and RV volumes.

Methods: The FHN trials randomized 245 patients to 12 months of DHD or conventional hemodialysis (CHD), and 87 patients to 12 months of NHD or CHD. LV and RV volumes and function were ascertained by cardiac MRI at baseline and at the end of the study.

Results: The absolute (mean± SD) intradialytic weight loss per session achieved during the Daily and Nocturnal Trials were: DHD (2.11 ± 0.86kg) and conventional HD (3.10 ± 1.04kg), p<0.001 and NHD (2.04 ± 0.87kg) and conventional HD (2.55 ± 1.02kg), p <0.01 respectively. Table 1 shows changes from baseline to 12 months. Reductions in LV and RV volumes were higher with DHD than with CHD. Changes in cardiac volumes were not significantly different between NHD and CHD.

Mean Change From Baseline to 12 Months in Cardiac Indices

Variables	CHD (Daily Trial)	DHD (Daily Trial)	CHD (Nocturnal)	NHD (Nocturnal)
Intradialytic weight loss (kg)	-0.1 ±0.1	-1.0±0.1*	0.2±0.2	-0.3±0.1*
LVEDV (ml)	-1.9±4.0	-21.9±3.7*	6.4±7.8	0.0±8.0
LVESV (ml)	-2.2±2.9	-10.8±2.7	5.0±5.8	-1.4±6.0
Left cardiac output (L/min)	0.075±0.22	-0.55±0.20*	0.21±0.36	0.39±0.37
LV ejection fraction (%)	0.2±1.0	1.5±1.0	-1.3±2.1	1.0±2.2
RVEDV (ml)	-15.0±5.1	-33.4±4.8*	-13.5±7.4	-9.0±7.6
RVESV (ml)	-9.1±3.4	-16.5±2.7*	-6.2±4.5	-6.1±4.6
Right cardiac output (L/min)	-0.38±0.24	-1.04±0.22*	-0.56±0.37	0.054±0.38
RV ejection fraction (%)	1.1±1.2	1.1±1.1	-0.8±2.2	2.0±2.3

*p<0.05 between CHD and DHD or CHD and NHD, ESV = end-systolic volume, EDV = end-diastolic volume

Conclusions: DHD lowered left and right ventricular volumes. The pathophysiological importance of these reductions in intracardiac volumes requires further investigations.

Funding: NIDDK Support

SA-OR392

Increasing Diffusive Sodium Removal Improves Blood Pressure Control through Volume-Independent Effect in Hemodialysis Patients Yi-Lun Zhou, Jing Liu, Li-Jie Ma, Fang Sun, Yang Shen, T.G. Cui. *Department of Nephrology, Chao-yang Hospital, Capital Medical University, Beijing, China.*

Background: Sodium overload is a predominant factor in the pathophysiology of hypertension in end stage renal disease. Lowering dialysate sodium concentration has been demonstrated to improve blood pressure (BP) control, and this phenomenon is considered to be a result of an improvement in volume status via increasing sodium removal. However, sodium, apart from volume, may have an independent effect on BP regulation.

Methods: 16 non-diabetic stable hemodialysis patients were recruited, who are hypertensive, have achieved their dry weight assessed by both clinical and bioimpedance methods, without intradialytic symptoms, with their pre-dialysis plasma sodium levels slightly higher than the facility dialysate sodium concentration 138mmol/L. After 1 month period with standard dialysate sodium concentration of 138mmol/L, the patients were followed up for a 4 months period with dialysate sodium set at 136mmol/L, without changes in instructions to patients about dietary sodium control. During the period of study, the dry weight was adjusted monthly under the guidance of bioimpedance spectroscopy (BCM, Fresenius Medical Care), to maintain post-dialysis volume status within normal range.

Results: Along with lowering dialysate sodium, systolic and diastolic home-monitored BPs progressively decreased and reached statistical significance by the end of month 2. There was a significant decrease (-10 mmHg and -6mmHg) in 44-hour ambulatory systolic and diastolic BP by the end of the trial as well. Interdialytic weight gain adjusted to the estimated dry weight mildly but significantly decreased (4.81±1.51% vs 4.36±1.37%, p=0.047). No changes in monthly incidence of dialysis-related symptoms and pre-dialysis plasma sodium concentration were observed. The post-dialysis volume parameters were maintained in a steady state throughout the study period.

Conclusions: In selected hypertensive hemodialysis patients without volume overload, increasing diffusive sodium removal by lowering dialysate sodium concentration resulted in significant BP decrease. It is most likely due to the volume-independent effect.

SA-OR393

Aldosterone Deficiency as the Cause of Intradialytic Hypotension and Its Successful Management with Fludricortisone Daniel L. Landry,[1] Seyedeh S. Hosseini,[2] Osazee J. Osagie,[2] Arley F. Diaz,[1] Benjamin J. Freda,[1] Gregory Lee Braden.[1] *[1]Renal Division, Baystate Medical Center, Springfield, MA; [2]Internal Medicine, Kindred Parkview Hospital, Springfield, MA.*

Background: Intradialytic hypotension (IDH) occurs in many patients on maintenance hemodialysis. While there is evidence to suggest that aldosterone (Aldo) has non-genomic effects on vascular tone independent of sodium retention in oligo-anuric dialysis patients, the presence of Aldo deficiency as a cause of IDH has not been defined.

Methods: Twelve chronic hemodialysis patients with refractory IDH despite aggressive medical management had plasma cortisol, renin and Aldo levels measured randomly before dialysis. Those patients that failed to have an Aldo level above 5 ng/dL were then given fludrocortisone (FC) 0.1 mg bid. Pre- and post-dialysis blood pressures (BP), average ultrafiltration volumes and frequency of IDH (SBP < 100 mmHg) were measured on 12 separate occasions prior to and after FC initiation.

Results: Of 12 patients with severe IDH, five had low random plasma Aldo levels in the setting of symptomatic IDH. None of the patients had cortisol deficiency on cosyntropin stimulation testing. All 5 patients with Aldo deficiency had improvement in pre-dialysis (110.1±2.2 v. 133.0±2.0, p<.0001) & post-dialysis systolic BP (103.8±1.4 v. 123.2±2.4, p<.0001) & pre-dialysis diastolic BP (61.0±1.4 v. 73.0±2.0, p<.0001) & post dialysis diastolic BP (56.1±1.2 v. 66.6±2.0 mmHg, p<.0001) after the initiation of FC. Ultrafiltration volumes (2.8 ± 0.26 v. 3.6 ± 0.40 liters, P=.0012) and the frequency of IDH events (2.9 ± 0.27 v. 0.7 ± 0.13 events per treatment, p<0.0001) improved as well. Three of the 7 patients with normal adrenal hormones were also empirically tried on FC with no improvement in any of the above-mentioned parameters.

Conclusions: Aldo deficiency is a clinical entity present in some chronic hemodialysis patients suffering from IDH that is readily treatable. Furthermore, this study supports the premise that Aldo – in the absence of changes in sodium balance in oligo-anuric dialysis patients - has direct effects on vascular resistance and deserves further investigation in the chronic dialysis population.

SA-OR394

Acetate-Free Biofiltration Reduces Intradialytic Hypotension in the Long-Term: A Randomized Controlled European Multicenter Trial Nicola Tessitore,[1] Giovanni O. Panzetta,[2] Antonio Santoro,[3] Rafael Perez-Garcia,[4] Albino Poli.[5] *[1]Nephrology, AOUI, Verona, Italy; [2]Nephrology, Trieste, Italy; [3]Nephrology, Policlinico S.Orsola Malpighi, Bologna, Italy; [4]Hospital Infanta Leonor, Madrid, Spain; [5]Public Health Dpt, AOUI, Verona, Italy.*

Background: Intradialytic hypotension (IH) is a common complication of conventional bicarbonate hemodialysis (BD) and it may contribute to the high cardiovascular morbidity and mortality among dialysis patients, a risk that can be contained by the use of convective therapies.

Methods: We have analysed data from a controlled randomised trial to evaluate whether acetate-free biofiltration (AFB), a hemodiafiltration technique with buffer-free dialysate, a high-flux membrane and sterile bicarbonate infusion in post-dilution mode which improved dialytic cardiovascular stability in short-term nonrandomized studies, may influence long-term IH rate, predialysis Sistolic Blood Pressure(SBP), Left Ventricular Mass Index(LVMI), cardiovascular morbidity and mortality by comparison with BD.

Results: The study enrolled 371 patients, 194 on BD and 177 on AFB. During a 3-year follow-up, the IH rate did not change for BD (3.2 to 3.1 session/pt-month,p=ns) and dropped significantly for AFB (4.1 to 2.5 session/pt-month,p<0.0001), equal to a significant IH risk reduction for AFB (incidence rate ratio 0.81[95% CI 0.76-0.86],p=0.0001). Median predialysis SBP did not change for BD, and dropped by 7 mmHg for AFB(p=0.01). Median LVMI increased for BD (4.2 g/m^2), and decreased for AFB (2.2 g/m^2), with no significant differences within or between each group. There was no difference in annual cardiovascular morbidity and mortality between BD and AFB (8.8% and 6.9% vs 9.0% and 5.9%,p=ns). AFB, however, reduced long-term case fatality (cardiovascular death among patients who had a nonfatal cardiovascular event) by comparison with BD (27% vs 59%,p=0.019).

Conclusions: In conclusion, compared to BD, AFB reduced both the IH rate and predialysis SBP in the long-term. It does not affect, however, LVMI, cardiovascular morbility and mortality, but it contains the risk of cardiovascular death after a nonfatal cardiovascular event, possibly thanks to the better intradialytic cardiovascular stability.

SA-OR395

Short-Term Versus Long-Term Effects of Depressive Symptoms on Cardiovascular and Non-Cardiovascular Mortality in Incident End-Stage Renal Disease Patients Tessa O. Van den Beukel,[2,3] Marion Verduijn,[2] Friedo W. Dekker,[2] Adriaan Honig,[4] Carl E.H. Siegert,[3] Elisabeth W. Boeschoten,[5] Raymond T. Krediet,[6] Sandra Van Dijk.[1] *[1]Dept of Medical Psychology, Leiden University Medical Center; [2]Dept of Clinical Epidemiology, Leiden University Medical Center; [3]Dept of Nephrology, Sint Lucas Andreas Hospital; [4]Dept of Psychiatry, Sint Lucas Andreas Hospital; [5]Hans Mak Institute; [6]Dept of Nephrology, Academic Medical Center, Netherlands.*

Background: Dialysis patients with depressive symptoms (DS) have a higher mortality rate compared to patients without DS, even after adjustment for a wide array of covariates. It is unknown whether short-term mortality differs from long-term mortality and whether this depends on the cause of mortality. The objective of this study is to assess whether DS in incident hemodialysis and peritoneal dialysis patients are related to short-term (0-6 months), medium-term (6-24 months), and long-term (24-52 months) cardiovascular and non-cardiovascular mortality.

Methods: 1528 patients participating in the NECOSAD study, a prospective multicenter cohort study of dialysis patients in the Netherlands, completed the Mental Health Inventory, which was used as a measure of DS (cut-off point of ≤ 52), at the starting period of dialysis therapy. Cox regression analyses were used to examine survival.

Results: The adjusted hazard ratio for mortality (HR) for patients with DS compared to patients without DS was 1.43 (95% CI, 1.08-1.88) for cardiovascular mortality and 2.07 (95% CI, 1.62-2.64) for non-cardiovascular mortality. DS posed a strong risk factor for non-cardiovascular mortality at the short-term (HR=2.82, 95% CI, 1.58-5.05), medium-term (HR=2.02, 95% CI, 1.36-3.01) and long-term (HR=1.74, 95% CI, 1.18-2.58), whereas the association between DS and cardiovascular mortality was not observed during the first 6 months of follow-up (HR=1.03, 95% CI, 0.49-2.15).

Conclusions: DS at the start of dialysis therapy pose a risk factor for cardiovascular and / or non-cardiovascular mortality, depending on the length of follow-up. The cause-specific mortality rates over time may help clinicians to understand the association between DS and survival in dialysis patients.

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SA-OR396

An Important Novel Association between Circulating Endothelial Progenitor Cells and Residual Kidney Function in Peritoneal Dialysis Patients Angela Yee Moon Wang,[1] Sharon Shee Yin Wong,[1] Ruijie Li,[2] Qing Shang,[2] Wai Kei Lo,[3] Cheuk-Man Yu,[2] Kar Neng Lai.[1] *[1]Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong; [2]Medicine & Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong, Hong Kong; [3]Medicine, Tung Wah Hospital, Hong Kong, Hong Kong.*

Background: Bone marrow-derived circulating endothelial progenitor cells (EPCs) plays an important role in vascular repair & maintaining vascular homeostasis. Given the increasing recognition of the significance of residual kidney function (RKF) in end-stage renal disease (ESRD) patients receiving long-term peritoneal dialysis (PD) , this study aimed to determine the association, if any, between circulating EPCs cells (defined as cells that are both CD34+ and kinase insert domain receptor [KDR]+) and RKF in PD patients.

Methods: Forty-one ESRD patients (mean age: 58 ± 8 years) receiving long-term PD treatment (mean ± SD duration on PD: 22 ± 27 mos) were recruited with flow cytometric analysis of circulating CD34+KDR+CD45$^-$ cells & assessment of RKF, urea (Kt/V) and creatinine clearance (CrCl), clinical and biochemical data.

Results: The median (interquartile range) residual glomerular filtration rate (GFR) was 2.5 (0.7, 4.9)ml/min per 1.73m^2. The total weekly Kt/V and CrCl was 2.17 ± 0.51 and 87 ± 41 L/wk per 1.73m^2, respectively. On univariate analysis, circulating CD34+KDR+CD45$^-$ cells showed significant association with total CrCl (β=0.40; P=0.009) and was contributed by its association with RKF (β=0.41; P=0.008) but not with PD CrCl (β = -0.19; P=0.23). Using multiple linear regression analysis and adjusting for confounding covariates, a significant and independent association remained between circulating CD34+KDR+CD45$^-$ cells and RKF (β=0.40; P=0.005).

Conclusions: RKF but not PD clearance showed an independent, positive association with circulating CD34+KDR+CD45$^-$ cells in PD patients. These data provide important novel evidence that reduced level of circulating EPCs may be the missing link between loss of RKF and increased cardiovascular events and mortality in PD patients. The exact cause and effect relationship between circulating EPCs and RKF in PD patients warrant further investigation.

Funding: Government Support - Non-U.S.

SA-OR397

Low Vitamin E Levels, Cerebrovascular Events and Mortality in Hemodialysis Patients Christiane Drechsler,[1] Katharina Espe,[2] Christoph Wanner.[1] *[1]Dept of Medicine 1, Div of Nephrology, University of Wuerzburg, Germany; [2]Institute of Nutritional Science, Potsdam, Germany; [3]Center for Cardiovascular Research, Charité, Berlin, Germany.*

Background: Hemodialysis patients suffer from increased oxidative stress and experience an exceedingly high mortality as compared to the general population. Trials with the antioxidant vitamin E have shown controversial results. Considering the different causes of death in the dialysis population, this study investigated the effect of vitamin E on specific clinical outcomes in hemodialysis patients.

Methods: In 1046 diabetic hemodialysis patients (participants of the 4D study) alpha-tocopherol was measured by RP-HPLC. By Cox regression analyses, hazard ratios (HR) were determined for pre-specified endpoints according to baseline alpha-tocopherol levels: sudden death (n=134), myocardial infarction (n=172), stroke (n=89), combined cardiovascular events (n=398), fatal infection (n=107), and all-cause mortality (n=508).

Results: Patients had a mean age of 66 (30-83) years and mean alpha-tocopherol level was 22.8 (3.35-87.1)µmol/L. Patients with lower alpha-tocopherol levels in the first quartile (<18.3µmol/L) had an adjusted 2fold higher risk of stroke than those in the fourth quartile (>29.1µmol/L) (HR 2.00, 95% confidence interval 1.11-3.61). Especially patients without a history of cerebrovascular events in the past were affected [HR 3.01 (1.43-6.33)]. Low alpha-tocopherol levels particularly increased the risk of ischemic stroke by 3fold [HR 2.95 (1.43-6.08)], while they did not associate with hemorrhagic stroke [HR 1.10 (0.17-7.32)]. Furthermore, all-cause mortality was significantly increased [HR 1.31 (1.01-1.69)].

Conclusions: Low alpha-tocopherol was strongly associated with stroke, particularly ischemic stroke, in diabetic hemodialysis patients, and contributed to an increased mortality. Whether vitamin E supplementation decreases the occurrence of ischemic stroke, requires further evaluation.

SA-OR398

Oxidized High-Density Lipoprotein as a Risk Factor for Cardiovascular Events in Prevalent Hemodialysis Patients Hirokazu Honda,[1] Masashi Ueda,[2] Shiho Kojima,[2] Shinichi Mashiba,[2] Tetsuo Michihata,[3] Keiko Takahashi,[4] Kanji Shishido,[4] Tadao Akizawa.[1] *[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan; [2]Ikagaku Co. Ltd, Kyoto, Japan; [3]Ebara Clinic, Tokyo, Japan; [4]Kawasaki Clinic, Kawasaki, Japan.*

Background: The present study assessed the impact of oxidized high-density lipoprotein (oxHDL), dysfunctional HDL, on mortality and cardiovascular disease (CVD) events in prevalent HD patients and compared oxHDL to interleukin-6 (IL-6), a strong predictor for CVD events in HD patients.

Methods: This prospective study examined a cohort of prevalent HD patients (n=412). Blood samples were obtained at baseline to measure lipids, high sensitive-C reactive protein (hsCRP), IL-6, oxidized low-density lipoprotein, N-terminal pro B-type natriuretic peptide, inter-cellular adhesion molecule 1 (ICAM-1), myeloperoxidase, adiponectin, and oxHDL.

Carotid intima-media thickness (CIMT) was assessed at baseline and the 3-year follow-up. Nutritional status was assessed by subjective global assessment (SGA), body mass index, and geriatric nutritional risk index (GNRI). After the baseline assessment, study patients were prospectively followed-up (mean observational period, 40 months).

Results: At baseline, patients with high oxHDL had a worse nutritional state and higher HDL-cholesterol (chol), ICAM-1, and adiponectin levels and a higher oxHDL/HDL-chol ratio than low oxHDL patients. A combination of high oxHDL and high IL-6 was significantly associated with increased CIMT at baseline and a larger increase in CIMT at the 3-year follow-up. High oxHDL did not predict all-cause mortality; however, it was significantly associated with CVD-related mortality and composite CVD events, particularly with concomitant high IL-6. Those associations were confirmed in multivariate Cox hazard models adjusted with confounding variables.

Conclusions: A high oxHDL level may be associated with CVD events and CVD-related mortality, particularly with concomitant high IL-6 in prevalent HD patients.

SA-OR399

The Activation of mTOR Pathway Induced by Inflammation Accelerates the Progression of Atherosclerosis in Hemodialysis Patients Kun Ling Ma, Jing Liu, Min Gao, Xiaoliang Zhang, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: Chronic inflammation is an independent risk factor in the progression of atherosclerosis (AS) in hemodialysis patients. Our previous studies *in vivo* and *in vitro* demonstrated that inflammation accelerated the progression of AS via the dysregulation of low lipoprotein receptor (LDLr) pathway, which was inhibited by Rapamycin, an inhibitor of mammalian target of Rapamycin (mTOR). This study was to investigate whether inflammation exacerbates lipid accumulation in the tissues of radial arteries in hemodialysis patients and its underlying mechanisms.

Methods: Thirty-one hemodialysis patients receiving arteriovenostomy were divided into two groups by the plasma level of C-reactive protein: Control (n=16), inflamed group (n=15). Hematoxylin-eosin staining and Oil Red O staining were used to check foam cell formation and lipid droplets accumulation using the tissues surgically removed from radial artery. Immunohistochemistry and immunofluorescent staining were used to check protein expressions related with intracellular cholesterol trafficking.

Results: There was a significant increasing of lipid accumulation in the radial artery in inflamed group compared to control, which was correlated with the increased protein expressions of LDLr, sterol regulatory element binding protein-2 (SREBP-2), and SREBP cleavage-activating protein (SCAP). Confocal microscopy observation showed that inflammation enhanced the translocation of SCAP escorting SREBP-2 from the endoplasmic reticulum to the Golgi, thereby activating LDLr gene transcription. Further analysis showed that dysregulation of LDLr pathway induced by inflammation was associated with the increased protein expression of mTOR, especially with the enhanced co-expression of mTOR and SREBP-2.

Conclusions: This study suggested that inflammation accelerated the progression of atherosclerosis in hemodialysis patients through the disruption of LDLr pathway, which could be partly through the activation of mTOR pathway.

Funding: Government Support - Non-U.S.

SA-OR400

Pharmacologically-Relevant Concentrations of Intravenous Iron Preparations Promote Endothelial Injury and Monocyte Adhesion/Infiltration Vaijinath (Vijay) S. Kamanna,[1,2] Shobha H. Ganji,[1,2] Nosratola D. Vaziri.[1] *[1]Department of Medicine, University of California, Irvine, CA; [2]Medical Research Service, VA Health Care System, Long Beach, CA.*

Background: Intravenous iron (IV Fe) preparations are widely used in the management of anemia in ESRD populations. With recent implementation of bundling reimbursement policy, the use of these agents in ESRD patients has dramatically increased to lower the cost of anemia treatment by limiting the use of expensive ESA products. In many instances iron preparations are administered on a routine basis with insufficient attention to the status of total body iron stores or the underlying inflammation which can be aggravated by iron. Endothelial injury and dysfunction are critical steps in the pathogenesis of atherosclerosis, thrombosis and cardiovascular disease. Non-transferrin-bound iron avidly promotes oxidative stress which is a major mediator of endothelial damage and dysfunction. In fact, administration of these agents has been shown to raise markers of oxidative stress in ESRD patients. This study was undertaken to assess the effect of pharmacologically-relevant concentrations of IV iron preparations on endothelial cells and monocytes and interaction thereof in vitro.

Methods: Morphological changes in human aortic endothelial cells (HAEC) were examined by phase contrast microscopy. Cell viability was tested by a cell growth kit. Monocyte adhesion to HAEC was done using fluorescently labeled monocytes.

Results: In contrast to the control HAEC which showed normal morphology, cells treated with iron sucrose (50 and 100 µg/ml) for 4 h showed loss of normal morphological characteristics with cellular fragmentation, shrinkage, detachment, and features of apoptosis including nuclear condensation/fragmentation. Iron sucrose (10-100 mg/ml) robustly increased monocyte adhesion to HAEC by 5-25 folds.

Conclusions: The study documents the potential adverse effects of IV iron preparations on endothelial cells and their interactions with monocytes. These events can contribute to endothelial dysfunction, atherosclerosis, thrombosis and cardiovascular disease which are the major cause of premature death in ESRD population.

Funding: Private Foundation Support

SA-OR401

Intradialytic Protein Supplementation Attenuates Dialysis-Associated Inflammation and Reduces Co-Morbid Disease Risk Emily Tomayko,[1] Pei-Tzu Wu,[1] Hae Ryong Chung,[1] Peter Fitschen,[1] Brandon Kistler,[1] Barbara Yudell,[1] Elizabeth Jeanes,[2] Shane Phillips,[2] Ken Wilund.[1] *[1]Kinesiology and Community Health, University of Illinois, Urbana, IL; [2]Physical Therapy, University of Illinois, Chicago, IL.*

Background: Inflammation may be both cause and consequence of protein malnutrition, cardiovascular disease, and bone disease in dialysis patients; improving nutritional status may reduce inflammation and the risk for these conditions. This study examines the effects of intradialytic protein intake on circulating inflammatory markers and disease risk factors during a single dialysis session and also over a 6-month period.

Methods: Hemodialysis patients were randomly assigned to the following groups: whey protein (n=20), soy protein (n=15) or placebo (n=10). Blood was drawn immediately after start of dialysis treatment and three hours later on two days one week apart and analyzed for plasma interleukin-6 (IL-6) levels. On day 1, patients did not receive a study beverage but on day 2 consumed their assigned beverage before treatment. A subset of 19 patients continued with the beverage at every dialysis session for 6 months; blood was collected monthly and analyzed for standard clinical chemistries.

Results: All groups had a day 1 increase in IL-6 levels during dialysis indicating an acute inflammatory effect of a single dialysis session; this increase was attenuated in the groups consuming protein on day 2 compared to the placebo group ($p<0.05$). For the 6 month intervention, regression modeling showed a significant increase in albumin levels over time in the whey protein group ($p<0.05$). Alkaline phosphate levels declined in the protein groups compared to placebo ($p<0.05$) suggesting improvement in bone and cardiovascular disease risk. Furthermore, there were no significant group differences for plasma phosphorus, calcium, or potassium, suggesting neither soy nor whey protein intake during dialysis treatment adversely affects these clinically-relevant minerals.

Conclusions: These data support the safety and efficacy of intradialytic protein supplementation for attenuating increases in dialysis-associated inflammation and reducing co-morbid disease risk over a six-month period.

Funding: NIDDK Support

SA-OR402

Effect of Brazilian Nut Supplementation on Antioxidant and Inflammatory Status in Hemodialysis Patients Denise Mafra,[1] Milena Barcza Stockler-Pinto,[2] Julie Lobo,[2] Cristiane Moraes,[1] Najla Elias Farage,[3] Gilson Teles Boaventura,[1] Denis Fouque,[4] Maria Thereza Batista Wady,[1] Wellington Seguins da Silva,[5] Olaf Malm.[2] *[1]Clinical Nutrition, Federal University Fluminense, Niteroi, Rio de Janeiro, Brazil; [2]Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]Nutrition, RenalCor Clinic, Rio de Janeiro, Brazil; [4]Nephrology, Hôpital Edouard Herriot- Université Claude Bernard, Lyon, Rhone, France; [5]Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil.*

Background: Cumulative evidence indicates that oxidative stress and inflammation frequently occurs in hemodialysis (HD) as a result of a decrease of antioxidant defenses and an overproduction of reactive oxygen species (ROS). Dietary intake of selenium (Se) has been associated with increased activity of glutathione peroxidase (GSH-Px) and, Se also plays an important role as an anti-inflammatory agent. The richest known food source of Se is the brazilian nut (Bertholletia excelsa, family Lecythidaceae), found in the Amazon region. The aim of this study was to evaluate the effect of Brazil nut supplementation on GSH-Px, TNF-α and IL-6 levels in HD patients.

Methods: Forty HD patients (57.5% men, 53.3 ± 16.1 yr) from RenalCor Clinic at Rio de Janeiro, Brazil were studied. All patients received 1 nut (around 5g) a day for three months. The GSH-Px, TNF-α and IL-6 levels were determined by ELISA.

Results: The cytokines levels were above the normal values and, the activity of GSH-Px was below the normal values before nut supplementation. After 3 months supplementation, cytokines levels decreased and the activity of GSH-Px increased significantly ($p< 0.001$).

Parameters before and after nut supplementation

Parameters	Before supplementation	After supplementation
GSH-Px (nmol/ml/min)	34.06 ± 5.2*	40.3 ± 8.4
IL-6 (pg/mL)	67.1 (21.1-80.2)*	13.5 (12.8-22.2)
TNF-α (pg/mL)	21.0 ± 0.3*	13.9 ± 8.7

*p<0.001

Conclusions: Our results indicate that brazilian nut increases the antioxidant status and plays an important role as an anti-inflammatory agent and in HD patients.

Funding: Government Support - Non-U.S.

SA-OR403

Effect of Phosphate Binders on Serum Inflammatory Profile, Soluble CD14 and Endotoxin Levels in Hemodialysis Patients: A Prospective, Randomized, Controlled Trial

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Background: Hyperphosphatemia and subclinical endotoxemia are important sources of inflammation in hemodialysis (HD). Pro-inflammatory cytokines are strong correlates of soluble CD14 (sCD14) concentrations, an independent predictor of mortality in this population. We evaluated the effects of calcium acetate and Sevelamer hydrochloride on serum inflammatory profile, endotoxin concentrations and sCD14 levels in HD patients.

Methods: This prospective, randomized, open-label, parallel design trial included 59 stable HD patients, 30 receiving Sevelamer and 29 calcium acetate. Serum levels of inflammatory parameters (high-sensitive C-reactive protein (hs-CRP), tumor necrosis factor-α, interleukin (IL)-1, 6, 10, and 18), as well as serum endotoxin and sCD14 concentrations were measured at baseline and after 3 months of therapy.

Results: Serum IL-6 increased in patients receiving calcium acetate, whereas hs-CRP and IL-6 significantly decreased in subjects treated with Sevelamer, with IL-10 experienced a trend to increase (p=0.052). Serum endotoxin and sCD14 levels did not change after treatment with calcium acetate. However, these parameters decreased by 22.6% and 15.2%, respectively (p<0.01), in patients receiving Sevelamer. Multiple regression analysis showed that variation in serum endotoxin concentrations was the strongest factor associated with IL-6 change, whereas the only variables independently associated with changes in sCD14 levels were the variations in serum IL-6 and endotoxin concentrations.

Conclusions: Administration of the non-calcium phosphate binder Sevelarmer to maintenance HD patients is associated with a significant decrease in hs-CRP and IL-6. In addition, Sevelamer therapy was also associated with reductions of serum endotoxin levels and sCD14 concentrations.

SA-OR404

Mechanism of Increased Renal Angiotensin II in Glomerular Disease

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Background: Earlier studies by others demonstrated that intrarenal angiotensin II (AII) is increased in various kidney diseases independently of plasma AII.

Methods: We explored the mechanism of this using 2 types of tissue-specific angiotensinogen (Agt) knockout (KO) mice.

Results: Kidney Agt KO showed 85.6% decrease in renal Agt mRNA; however, renal Agt protein was unaffected. Nevertheless, urinary Agt/creatinine was significantly decreased to 47%, indicating that Agt protein synthesized in the kidney is immediately secreted into the urine.

Liver Agt KO mice were near-completely deficient in Agt mRNA and protein in the liver, and in plasma Agt, as well. Remarkably, in the kidney of liver Agt KO, Agt protein was undetectable. Immunostaining of Agt in mosaic proximal tubule-specific megalin KO mice revealed that Agt uptake is completely dependent on megalin. Prior to assessing AII content next, we verified the RIA method by observing undetectable AII in whole body Agt KO. With this method, we found that renal AII content is similar in kidney KO (n=9, 286±38 fmol/g) and control mice (n=8, 315±15), whereas both liver KO (n=8) and kidney/liver dual KO (n=10) have lower AII (110±16, 74±4, both p<0.05).

We, then, assessed the impact of glomerular sieving dysfunction on renal Agt and AII using the immunotoxin-inducible podocyte injury model. After selective podocyte injury, renal Agt protein was dramatically increased. Renal AII content was also increased upon podocyte injury (364±33 fmol/g, n=10), compared with mice without injury (155±27, n=10, p<0.05).

Conclusions: These results indicate that 1) liver Agt is the major source of renal AII, 2) a fraction of circulating Agt, which is originated in the liver, is filtered through the glomerulus, delivered into the urinary space and reabsorbed by PTCs via megalin, and 3) glomerular damage increases the leakage of Agt into the urinary space, which leads to increase in both renal Agt and AII. The data also offer one mechanistic explanation for the notion that proteinuria is a significant risk factor for the progression of glomerular diseases.

Funding: Government Support - Non-U.S.

SA-OR405

Mesenchymal Stem Cells Infusion after Revascularization of Atherosclerotic Renal Artery Stenosis Improves Renal Tubular Transport Function

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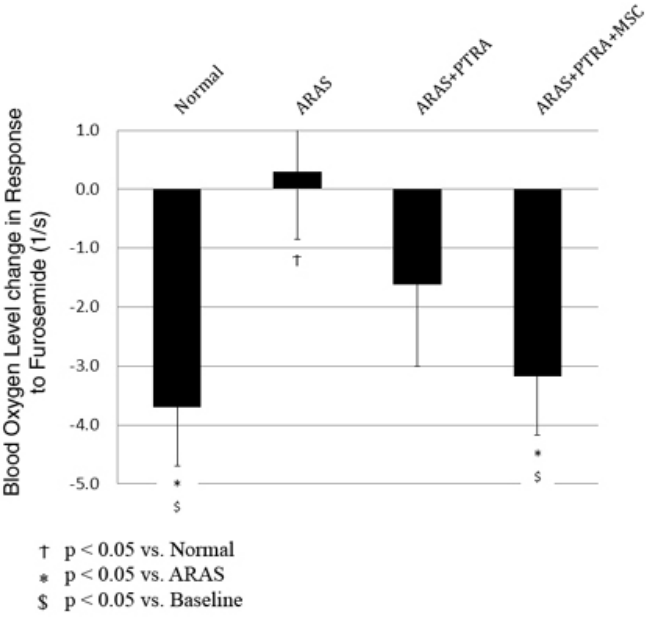
Background: Atherosclerotic renal artery stenosis (ARAS) leads to reduced blood flow, reduced GFR, and fibrosis, which often fail to recover after percutaneous transluminal renal angioplasty (PTRA). We have shown ARAS is characterized by impaired tubular function and mesenchymal stem cells (MSC) decrease renal injury in untreated ARAS.

Methods: Pigs studied after 10 weeks of untreated unilateral ARAS, treated 4 weeks earlier with PTRA and stenting, w/wo adjunct intra-renal MSC infusion (10^7); normal pigs served as controls (n=4 each group). Medullary oxygenation in stenotic kidneys (R2*) and changes in transport-related oxygen consumption were assessed using blood oxygen-level-dependent (BOLD) magnetic resonance imaging (MRI) before and 15 min after administering furosemide (0.05 ml/kg IV).

Results: Blood pressure was increased in ARAS but decreased in ARAS+PTRA and ARAS+PTRA+MSC (-14.5 and -12 mmHg). In normal kidneys medullary R2* declined after furosemide (Figure, -12.5±7%). This response was blunted in stenotic ARAS medulla (1.0±2.6%), and remained limited in pigs with PTRA (-4.0±7.5%). In contrast, ARAS+PTRA+MSC restored the ΔR2* (-8.5±4.8%) to normal levels.

Conclusions: These data demonstrate MSC infusion immediately after technically successful PTRA allows recovery of measured furosemide-inhibited solute transport beyond that achieved with revascularization alone. Our results support MSC administration as an adjunct intervention for improved viability of the stenotic kidney.



Volume, flow and GFR, 10 weeks after ARAS or sham.

	Volume (cc)	Renal Blood Flow (ml/min)	GFR
Normal	130.2±11.8	534.7±74.7	92.1±7.3
ARAS	100.3±8.8*	385.3±19.2*	57.7±7.3*
ARAS+PTRA	110.4±5.7	438.0±28.1*	63.4±3.1
ARAS+PTRA+MSC	130.8±6.8	508.6±23.8	85.3±8.6

* P<0.05 vs Normal

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SA-OR406

Silencing of Endothelial Cell Enriched MicroRNA-126 Is Associated with Elevated SDF-1 Levels and the Increase of Circulating Sca-1$^+$ Progenitor Cells after Ischemia Reperfusion Injury

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Background: Stromal cell-derived factor-1 (SDF-1) expression has been shown to be upregulated in the kidney following ischemia reperfusion injury and may serve a reparative response by recruiting CXCR4-positive cells to the kidney. As SDF-1 mRNA contains a potential seed sequence for microRNA-126 (miR-126), we investigated the regulatory role of this endothelial cell (EC) enriched miR in SDF-1 expression and peripheral progenitor mobilization in mouse models of ischemia-reperfusion.

Methods: Using miR-reporter constructs and Western blots, we demonstrated that SDF-1 mRNA is a direct target of miR-126 in HUVEC. Using a transwell system and conditioned medium harvested from HUVEC incubated with antagomir-126 or scramblemir we assessed the effect of miR-126 on chemotaxis of CD34$^+$ cells. Finally, we injected mice with antagomir-126 and performed either hind limb ischemia (HLI) or ischemia-reperfusion injury of both kidneys (IRI) to assess the effect of miR-126 silencing on progenitor cell mobilization.

Results: *In vitro* experiments showed that silencing of miR-126 in HUVEC enhanced SDF-1 expression and stimulated migration of CD34$^+$ cell. After IRI or HLI, qRT-PCR on whole kidney lysates and immunohistochemistry in the ischemic hind limb displayed elevated levels of SDF-1 in the animals injected with antagomir-126. In both models of ischemic injury, FACS-analysis revealed a marked upregulation of the number of circulating Sca-1$^+$/Lin$^-$ progenitor cells in the antagomir-126 treated mice. In contrast, in the absence of ischemia silencing of miR-126 had no effects on progenitor cell mobilization.

Conclusions: We demonstrate that silencing of endothelial miR-126 can enhance the expression of SDF-1 in an ischemia-dependent way, leading to the mobilization of Sca-1$^+$/Lin$^-$ progenitor cells into the circulation.

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SA-OR407

Chronic Perturbation of the Endothelial Surface Glycocalyx Results in Proteinuria without Morphological Changes of the Glomerulus Martijn Dane,[1,2] Daniel R. Potter,[2] Ton J. Rabelink,[1] Hans Vink,[2] Bernard Van Den Berg.[1] *[1]Nephrology, LUMC, Leiden, Zuid-Holland, Netherlands; [2]Fysiology, MUMC, Maastricht, Limburg, Netherlands.*

Background: The glomerular filtration barrier consists of podocytes, glomerular basement membrane and the endothelial layer with its surface bound glycocalyx. Although the sequence of events that can result in proteinuria is not known for cardiovascular conditions such as T2D, the endothelial glycocalyx has been shown to be damaged in proteinuric T2D. We hypothesize that damage of the glycocalyx can initiate proteinuria.

Methods: In C57BL/6J mice, vascular surface hyaluronan is enzymatically degraded through chronic infusion of hyaluronidase (hyal). After two weeks, systemic vascular glycocalyx volume, urine prot/creat- and dex500/dex40 ratios, as well as dry/wet kidney ratio and blood pressure were determined. EM analysis was performed for ultrastructural changes in the glomerular barrier. After four weeks, localization of the lectins LEA, WGA and BSI (binding N-acetyl-β-D-glucosamine, N-acetyl-β-D-glucosaminyl and N-acetyl-α-D-galactosaminyl, resp.) within the glomerulus were analyzed according to CD31 position using confocal microscopy.

Results: After two weeks hyal glycocalyx volume was decreased ($P<0.05$) to(mean 0.27 ml ± 0.04 SD vs. hyal-inact 0.51 ml ± 0.11). Pooled urine samples after hyaluronidase showed a 5-fold increase in prot/creat ratio and accompanied by an increased Dex500/Dex40 leakage (0.22 ± 0.06 vs. 0.62 + 0.26, $P<0.05$). After 4 weeks hyal, luminal endothelial LEA and BSI staining was decreased compared to hyal-inact (LEA: 4%vs. 75%; BSI: 11%vs 65%, of positive staining). WGA staining was only present outside of the CD31 perimeter (GBM, podocytes) and remained unchanged. No change in blood pressure and heart rate was observed during two weeks of infusion.

Conclusions: Chronic hyaluronidase treatment damaged the glomerular endothelial glycocalyx and was associated with development of proteinuria. This insult was without changes in extravascular carbohydrate content, or with overt histopathological changes, such as microaneurisms, GBM thickening or podocyte flattening. Loss of endothelial glycocalyx function can cause the onset of proteinuria.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR408

In End-Stage Renal Disease Serum Amyloid A Accumulates in Plasma and HDL of Patients Leading to Diminished Anti-Inflammatory Capacity of HDL Tao Huang, Markus Tolle, Mirjam Schuchardt, Markus van der Giet. *Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany.*

Background: The reduction in renal function correlates with an increase in cardiovascular mortality. Epidemiological studies demonstrate that high density lipoprotein (HDL) loses its anti-inflammatory properties. The chemokine monocyte chemoattractant protein-1 (MCP-1) production is crucial for inflammatory response in the vascular wall by recruitment of monocytes. The aim of this study was to investigate structural modifications in HDL of ESRD patients leading to reduced protective function.

Methods: HDL was isolated from serum of donors via ultracentrifugation Separation of HDL in lipid/protein fraction was done by isopropanol/hexan method. Rat vascular smooth muscle cells (VSMCs) were used. MCP-1 expression was detected by real-time PCR and secretion by Luminex™ technology. Serum amyloid A (SAA) level was quantified by ELISA.

Results: HDL isolated from healthy donors as well as from ESRD patients dose-dependently reduced the thrombin-induced MCP-1 expression and secretion in VSMCs, but with a reduced capacity of HDL from patients. HDL from ESRD patients significantly induced MCP-1 secretion, whereas HDL from healthy controls had no effect. Therefore, HDL from ESRD patients was separated in protein/lipid fraction and the stimulatory potential of each fraction was analyzed. The protein fraction strongly induced MCP-1, whereas the lipid fraction showed basically no activity. Mass-spectrometry analysis of the fraction leading to a strong MCP-1 induction identified SAA. Enrichment of SAA in HDL from healthy donors showed comparable reduced anti-inflammatory potential of HDL. SAA levels were markedly increased in both serum and HDL of ESRD patients compared to healthy controls. There is a correlation of SAA content in HDL and the reduced anti-inflammatory capacity.

Conclusions: Here we present evidence that HDL from ESRD patients rendered pro-inflammatory by SAA accumulation. SAA enrichment leads to dysfunctionality of the particle HDL. The decreased anti-inflammatory properties of HDL may substantially contribute to the excessive cardiovascular microinflammation in ESRD patients.

SA-OR409

Knockout of the Vascular Endothelial Glucocorticoid Receptor Accelerates Atherosclerosis in a Mouse Model Julie Goodwin,[1] Xinbo Zhang,[2] Jun Yu.[2] *[1]Pediatrics, Yale University School of Medicine, New Haven, CT; [2]Cardiology, Yale University School of Medicine, New Haven, CT.*

Background: Cardiovascular complications, and in particular atherosclerosis, are a major source of morbidity and mortality in patients with chronic kidney disease. The therapeutic potential of alterations in glucocorticoid metabolism in cardiovascular disease has recently become a focus of investigation. We hypothesize that the endothelial glucocorticoid receptor is a critical mediator of atherosclerosis through changes in eNOS metabolism that promote a pro-inflammatory state.

Methods: Mice with knockout of the endothelial glucocorticoid receptor were bred onto an ApoE knockout background. Control and knockout animals were fed an atherogenic diet for 14 weeks. Then mice were sacrificed and atherosclerotic lesion development was assessed in the aorta and brachiocephalic artery by Oil Red O and H & E staining. Total cholesterol and triglycerides were also measured at baseline and after feeding periods in each group.

Results: Knockout animals had higher mortality with only 35% surviving until the end of the feeding period compared to 85% of the wild-type animals ($p=0.039$). Knockouts also demonstrated more severe atherosclerotic lesions in both the aorta (69.8±8.2% vs. 44.8±2.3%, $p=0.019$) and brachiocephalic artery (70±10.3% vs. 33±10.8%, $p=0.031$) compared to controls. There was no difference in cholesterol or triglyceride levels between the groups either at baseline or after 14 weeks of atherogenic diet. Lesion analysis in knockout animals fed for only 5 weeks appears similar to that of wild-type animals fed for 14 weeks.

Conclusions: These studies suggest that:

(1) knockout of the endothelial glucocorticoid receptor results in a more severe atherosclerotic phenotype

(2) differences in lipid levels cannot account for the observed phenotype

(3) the endothelial glucocorticoid receptor may be a critical mediator of local inflammation in this model.

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SA-OR410

Type-I Interferon Enhances Arterial Intimal Hyperplasia Mark S. Segal, Pui Lee, Laura Sautina, Yanpeng Diao. *Medicine, University of Florida, Gainesville, FL.*

Background: System Lupus Erythematosis (SLE) patients have a significantly increased risk of coronary heart disease and premature atherosclerosis. Interestingly, approximately half of SLE patients have an elevated serum level of Type-I Interferon (INF-I) and IFN-I has been shown to induce the production of pro-atherosclerotic chemokines. We hypothesized that IFN-I would accelerate arterial intimal hyperplasia (IH) by altering the ratio of endothelial progenitor cells (EPC) to smooth progenitor muscle cells (SMPC).

Methods: In-vitro, SMPC and EPC were cultured with or without INF-I. In-vivo, femoral artery injury was performed in a mice expressing INF-I via electroporation of an INF-I expression plasmid into the gastrocnemius and analyzed 4 weeks after injury.

Results: SMPC number was significantly higher after the treatment of INF-1. However, no difference was observed in EPC clone number. In mice with elevated systemic levels of INF-I, there was a greater degree of lumen narrowing than in controls. Meanwhile, while there was a significant increase in proliferating SMC within the neointima in INF-I treated animals, but there was no difference in media SMC proliferation and endothelial cell proliferation between INF-I treated and control animals.

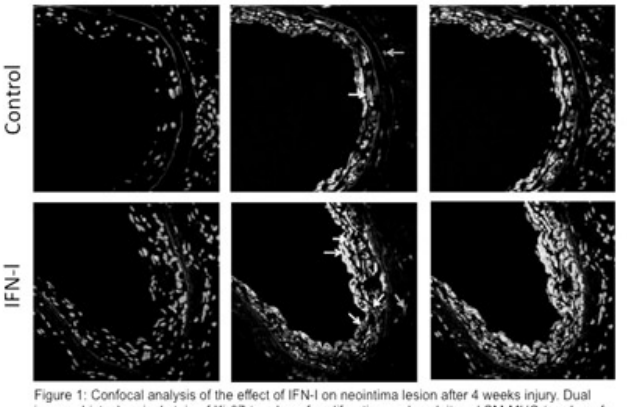

Figure 1: Confocal analysis of the effect of IFN-I on neointima lesion after 4 weeks injury. Dual immunohistochemical stain of Ki-67 (marker of proliferation; red nuclei) and SM-MHC (marker of SMC; green cytosol) indicates that an increased number of proliferating (yellow arrows) SMC within neointima in INF-I treated animals (lower panels) than control (upper panels). Note: Only the cells with red nuclei and green cytosol were identified as proliferating SMC, most of the red nuclei without green cytosol identified as leukocytes (CD45 staining cells; orange arrows), data not shown. All sections were counterstained with DAPI (blue). Object: 40X

Conclusions: Our finding that IFN-I accelerates IH in an arterial injury model has profound implications for patients with SLE. The mechanisms appear to involve altering the balance of EPC and SMPC, but may also affect progenitor SMC within the arteries itself. These studies shed light on the increased cardiovascular risk in SLE patients.

Funding: Private Foundation Support

SA-OR411

MicroRNA-21 and TGF-beta in Diabetic Glomerulopathy Jennifer Yi-Chun Lai,[1] Viji Nair,[1] Wenjun Ju,[1] Jinghui Luo,[1] Stuart Orkin,[2] Robert G. Nelson,[3] Matthias Kretzler,[1] Markus Bitzer.[1] *[1]Medicine, University of Michigan, Ann Arbor, MI; [2]Dana Farber Cancer Institute, Boston, MA; [3]NIDDK, National Institutes of Health, Phoenix, AZ.*

Background: Glomerulopathy is an early finding in Diabetic Nephropathy (DN) associated with activation of TGFβ signaling and podocyte apoptosis. Pima Indians exhibit high rates of type 2 diabetes mellitus and DN. MicroRNAs (miRs) are implicated in mediating development of DN. We previously showed that miR-21 inhibits renal cell apoptosis in cultured renal cells and in vivo. We examined regulation of miR-21 expression in human and murine models of DN and further explored miR-21 functions in miR-21 null mice.

Methods: Total RNA was extracted from micro-dissected glomeruli of kidney biopsy tissues from 26 diabetic Pima Indians. Small RNA fraction was obtained from the flow-through of silica-membrane RNA columns. MiR-21 expression was quantified using Taqman qrt-PCR (Applied Biosystems) and correlated with urinary albumin-creatinine ratio (ACR) using Pearson correlation. Mice ubiquitously deficient for miR-21 generated by cre-mediated deletion of the floxed pre-miR-21 coding sequence were crossed with Albumin-TGFβ1 transgenic mice. PAS-staining was used for histologic analysis. Albuminuria in mice was determined semi-quantitatively.

Results: MiR-21 exhibited high relative expression in glomeruli and excellent correlation with ACR (Correlation coefficient = 0.8, FDR = 0, $p < 0.001$). TGFβ1/miR-21 null mice exhibited strongly increased deposition of PAS-positive material, and loss of glomerular cells and open capillary loops. In TGFβ1/miR-21 null mice albuminuria was already increased at 2 weeks of age and exceeded 5g albumin/1g creatinine at 4 weeks of age in over 50% of mice but was less than 0.5g/g in all wildtype littermates.

Conclusions: MiR-21 expression is abundant in glomeruli and associated with ACR in human diabetic nephropathy. Loss of miR-21 results in progressive glomerular injury induced by TGFβ1. We propose that miR-21 expression increases with injury as an attempt by glomerular cells to limit damage. Studies exploring the underlying mechanisms are underway.

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SA-OR412

DNA Aptamers Raised Against AGEs Improve Diabetic Nephropathy in KK/Ay-Ta Mice Yusuke Kaida,[#1] Kei Fukami,[#1] Seiji Ueda,[#1] Sho-Ichi Yamagishi,[#2] Seiya Okuda.[#1] *[1]Division of Nephrology, Department of Medicine, Kurume University, Kurume, Fukuoka, Japan; [2]Department of Pathophysiology and Therapeutics of Diabetic Complications, Kurume University, Kurume, Fukuoka, Japan.*

Background: Diabetic nephropathy (DN) is one of the most common causes of end-stage renal disease and accounts for the morbidity and mortality in these patients. There is a growing body of evidence that advanced glycation end products (AGEs) and their receptor (RAGE) system plays a role in DN. In this study, we selected AGEs-DNA aptamer directed against AGEs *in vitro*, and examined their protective effect on DN in KKAy/Ta mice, an animal model of type 2 diabetes.

Methods: We identified high-affinity DNA aptamers directed against AGE-human serum albumin (AGEs-aptamers) using combinatorial chemistry *in vitro*. AGEs-aptamers (CCGAAACCAGACCACCCCACCAAGGCCACTCG GTCGAACCGCCAACACTCACCCCA) and control-aptamers were administrated to KKAy/Ta mice and non-diabetic control C57BL/6J mice (Ctrl mice) for 8 weeks using an osmotic mini pump. Urinary albumin excretion (UAE) was measured by enzyme-linked immunosorbent assay (ELISA). RAGE expression was evaluated by real-time PCR. Glomerular injury and fibrosis were evaluated by periodic acid-Schiff staining (PAS) and Masson-trichrome staining, respectively.

Results: Plasma levels of glucose and HbA1c were increased by about 1.5-2-folds in 16-week diabetic mice compared with Ctrl mice (plasma glucose; 298+/-59.6 mg/dl, HbA1c; 5.8+/-0.52 % in 16-week diabetic mice). Serum levels of blood urea nitrogen (BUN), creatinine (Cr) and UAE were significantly increased in diabetic mice, all of which were inhibited by the administration of AGEs-aptamers (BUN; 33.1+/-12.6 vs 27.5+/-1.7 mg/dl, $p<0.05$, Cr; 0.15+/-0.061 vs 0.09+/-0.003 mg/dl, $p<0.01$, UAE; 709+/-43 vs 234+/-21 mg/mgCr, $p<0.01$). Further, AGEs-aptamers suppressed RAGE expression and improved glomerular injury and sclerosis index in DM mice.

Conclusions: We demonstrated for the first time that administration of AGEs-aptamers significantly prevented the progression of DN in KKAy/Ta mice. The present study suggests that AGEs-aptamers may be a novel therapeutic tool for the treatment of DN.

SA-OR413

Epigenetic CpG Methylation of Tight Junction Protein, Claudin-1 through Sirt1 and Dnmt1 Controls Albuminuria in Diabetic Nephropathy Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi. *Keio University.*

Background: We previously reported that our **Tg mice with kidney-specific overexpression of Sirt1** protected against **AKI** (JBC 2010). However, a correlation between renal Sirt1 and **CKD** is unclear. Here, we explore the role of renal Sirt1 in **diabetic nephropathy (DN)** and **the regulatory mechanism** of the expression of claudin-1 by Sirt1.

Methods: WT or Tg mice were rendered DN by the treatment with streptozotocin (STZ). We also utilized cultured **podocytes** and human renal epithelial cell, **HRE cells** to explore **epigenetic** regulatory mechanism by Sirt1 by performing bisulfite sequences, methylation-specific PCR (MSP), real-time MSP and ChIP assay.

Results: WT+STZ mice were presented with prominent albuminuria with podocytes foot process effacement. Sirt1 expression was decreased in proximal tubules as well as podocotyes. Among various molecules, tight junction protein claudin-1, a parietal epithelial cell marker, was ectopically upregulated in podocytes. Conversely, these findings were attenuated in **Tg+STZ**. In podocytes with the direct **transfection of claudin-1**, the expressions of slit diaphragm proteins, podocin and synaptopodin were downregulated and albumin permeability was significantly increased. Notably, we identified a claudin-1 CpG island. In HREs, **high glucose (HG)** decreased the expression of Sirt1 and reduced the methylation of claudin-1 CpGs, concomitant with the increased claudin-1 expression. Contrarily, **Sirt1 cDNA transfection** induced hypermethylation in claudin-1 CpGs and this increase was blocked by DNA methyl transferase (Dnmt)-1 silencing. Additionally, Sirt1 transfection protected against both HG-induced hypomethylation of claudin-1 CpGs and upregulation of claudin-1 expression. Finally, ChIP revealed that Sirt1 deacetylated histone H3 H4 and methylated H3K9 within claudin-1 CpGs, which activated Dnmt1.

Conclusions: In **non-DN** state, Sirt1 downregulated claudin-1 expression through Dnmt1-dependent epigenetic regulation. Oppositely, in **DN**, HG-induced Sirt1 downregulation increased the claudin-1 expression leading to the downregulation of slit diaphragm proteins and to the increase in podocytes' albumin permeability. This novel epigenetic mechanism contributes to albuminuria in DN.

SA-OR414

Proximal Tubules-Specific Sirt1-Deficient Mice Aggravates Albuminuria in Diabetic Nephropathy by Accelerating De-Diffrentiation of Podocytes Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi. *Keio University.*

Background: NAD-dependent deacetylase, Sirt1 confers protective effects in various tissues. We previously reported that proximal tubules (PT)-specific Sirt1 transgenic mice protected against acute kidney injury (JBC 2010) and diabetic nephropathy (DN) (oral presentation at ASN 2010). In this study, we newly developed **PT-specific conditional Sirt1-deficient mice** to investigate if its role in the initiation and progression of DN.

Methods: We generated PT-specific Sirt1 deficient mice (conditional knockout mice, CKO) by crossing $Sirt1^{flox/flox}$ mice with KAP-Cre mice. Wild-type (WT) or CKO mice were injected with saline (control) or streptozotocin to be rendered DN. The phenotypes of four groups of mice, **WT+Sal, CKO+Sal, WT+STZ, CKO+STZ** were analyzed 24 weeks after the treatment.

Results: Sirt1 expression was specifically reduced in PTs in CKO. Immnostaining showed that Sirt1 expression in PTs and podocytes was reduced in WT+STZ compared with that in WT+Sal. This reduction was further prominent in CKO+STZ. Urinary analysis demonstrated that the increase in **urinary albumin excretion** in WT+STZ was further augmented in CKO+STZ. PAS staining and electron microscopy showed the increase in mesangial matrix accumulations or GBM thickness in WT+STZ mice were not altered in CKO+STZ. However, the increase in **podocyte foot process effacement** in WT+STZ as compared in WT+Sal was further augmented in CKO+STZ. Real time PCR assay after laser microdissection of glomerus demonstrated that **a tight junction protein, claudin-1** was elevated in WT+STZ as compared with WT+Sal and that this increase was further enhanced in CKO+STZ. Conversely, **slit-diaphragm proteins**, nephrin, podocin, synaptopodin were further decreased in CKO+STZ as compared with reduction in WT+STZ.

Conclusions: The present study revealed that deficiency of Sirt1 in PT had unfavorable effects on the phenotype of podocytes including the foot process effacement, the downregulation of slit diaphragram proteins and the upregulation of tight junction protein, claudin-1 in DN. Sirt1 in PT is the safeguard against the initiation and progression of DN-induced **podocytes' de-diffrentiation** though the novel mechanism of **PT-podocyte communication**.

SA-OR415

Human Adipose-Derived Adult Stem Cells Attenuates Podocyte Injury of Rats with Diabetic Nephropathy Li Zhang, Xiangfei Liu, Bo Fu, Dian Geng Li, Qinggang Li, Xiang-Mei Chen. *Chinese PLA Institute of Nephrology, Chinese PLA General Hospital, Beijing, China.*

Background: Diabetic nephropathy (DN) is a common complication of diabetes mellitus. Once diabetic nephropathy becomes overt, there is no curative therapy, and most patients will eventually progress towards end-stage renal disease. Adipose-derived adult stem cells (ADSCs) have been shown to possess self-renew and pluripotent capabilities, and to protect against acute renal injury. This study was conducted to investigate the effects of ADSCs on DN in rats.

Methods: ADSCs were isolated from adipose tissue of patients undergoing tumescent liposuction. Adult male Sprague-Dawley rats underwent right nephrectomy followed by streptozotocin (STZ) injection intraperitoneally (65mg/kg) two weeks later. Diabetic rats received daily subcutaneous injections of protamine zinc insulin to maintain blood glucose level (BG) between 16.7-33.3mmol/L. 12 weeks after STZ injection, the rats were divided into two groups, control group (NX+STZ) and hADSCs group (NX+STZ+ hADSCs). 5×10^6 hADSCs were intravenously injected into tail vein of the rats in hADSCs group once every four weeks for 20 weeks, while PBS was injected as control. 32 weeks after STZ injection, the rats were sacrificed and samples of 24-h urine, blood, kidney and pancreas were collected.

Results: Treatment with hADSCs had no effect on blood glucose levels and pancreatic histological damage in the DN rats, but did lower 24-hour urine protein excretion, lighten kidney weight, reduce glomerular hypertrophy (mean cross-sectional area of glomerular

tuft) and podocyte loss, ameliorate inflammatory cell infiltration and renal interstitial fibrosis. Both in vivo and in vitro assay demonstrated that hADSCs could decrease podocyte apoptosis and upregulate podocyte VEGF protein expression. Real-time PCR indicated that the expression of IL-1 and IL-4 was downregulated, while IL-6 and IL-10 was upregulated.

Conclusions: These results suggested that hADSCs therapy can protect against diabetic renal injury through attenuating podocyte injury and modulating inflammatory response.

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SA-OR416

BMP4 Regulates Podocyte Injury in the Diabetic Nephropathy Tatsuya Tominaga,[1] Hideharu Abe,[1] Seiji Kishi,[1] Kojiro Nagai,[1] Otoya Ueda,[2] Kou-Ichi Jishage,[2] Naoshi Fukushima,[2] Toshio Doi.[1] *[1]University of Tokushima; [2]Chugai Pharmaceutical Co. Ltd,.*

Background: We have shown that bone morphogenetic protein 4 (BMP4) signaling leads mesangial matrix hyperplasia in diabetic nephropathy (DN). Degeneration and loss of podocyte provide the important pathological mechanism for DN.

Methods: In order to analyze the molecular mechanism of podocyte injury, the effect of Bmp4 in vitro was investigated in the cultured podocyte. We studied the role of Bmp4 in streptozotocin (STZ)-induced diabetic mice. The induced-Bmp4 transgenic mice (Bmp4 tgm) were generated by the tamoxifen-regulated Cre-loxP system in order to examine the role of Bmp4 in vivo. Finally diabetic Bmp4 heterozygous knockout mice (Bmp4+/-) were analyzed to investigate suppressant effect of Bmp4 in vivo.

Results: Cultured podocyte showed the activation of p38 by Bmp4 treatment. The expression of nephrin was decreased by Bmp4 treatment in a dose dependent manner in vitro. Diabetic mice represented the positive expression of Bmp4, which degrees were associated with the levels of glomerular matrix hyperplasia in STZ mice ($r = 0.927$, $p < 0.01$). Bmp4 tgm were indicated significant mesangial matrix expansion and thickening of glomerular basement membrane, which showed similar glomerular alteration to diabetic glomerulosclerosis. Albuminuria was dramatically increased in Bmp4 tgm compared with non-inducible mice. Moreover, we confirmed podocyte injury in Bmp4 tgm. The Bmp4 tgm decreased both the expression of nephrin and the count of WT-1 positive cells. The glomerular matrix hyperplasia progressed in accordance with the decreased number of WT1 positive cells. The number of podocyte also decreased inversely with an increase of the albuminuria. To confirm the direct evidence of Bmp4 signaling in vivo, we investigated whether the reduction of Bmp4 expression improved the diabetic glomerular changes by using Bmp4+/- mice. The diabetic Bmp4+/- mice showed to improve the nephrin expression compared with diabetic wild mice. Proteinuria also decreased in diabetic Bmp4+/- mice compared with diabetic wild mice.

Conclusions: These data suggest that BMP4 plays an essential role for development of podocyte injury in DN.

Funding: Government Support - Non-U.S.

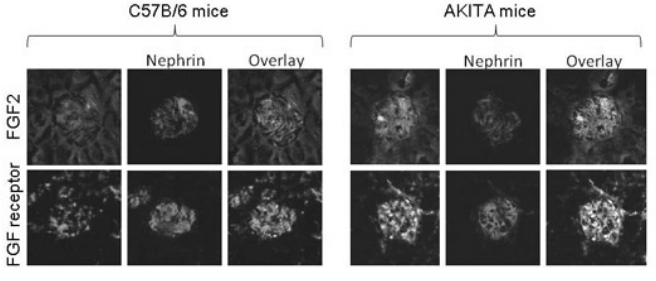
SA-OR417

FGF Signaling Predisposes Podocyte Injury in Diabetic Nephropathy (DMN) Koji Okamoto, Tomoko Ishizu, Kenjiro Honda, Kent Doi, Takehiko Wada, Toshiro Fujita, Eisei Noiri. *Departments of Nephrology & Endocrinology, Hemodialysis & Apheresis, University Hospital, The University of Tokyo, Japan.*

Background: Genome-wide association study for DMN revealed FGF2 pathway related genes as DMN susceptibility genes (*Nature Genetics 2011*). However, the relationships between FGF2 signaling and DMN was not fully elucidated.

Methods: FGF2 ligands and FGFRs expression were studied in mice model (AKITA mice vs. sib control mice) and mice podocyte cell line. To analyze the resistance to FGF2 in mice model, exogenous FGF2 (5 μg/animal/day i.v., day0-3) was administered to AKITA mice or sib control mice. To analyzed the efficacy of FGF antagonist to mice model, puromycin aminonucleoside and/or FGF antagonist (50 μg/animal/day i.p. day 0-4) was injected in AKITA mice.

Results: FGF2 level of renal cortex was increased in AKITA compared with control mice, (AKITA 0.86 ± 0.08 vs. sib control 0.46 ± 0.02 pg/μg protein). In addition, mRNA expression of FGFRs in total renal cortex (187%), and intraglomerular protein expression of FGFRs were increased in AKITA.



In vitro assay, high glucose medium significantly induced mRNA expression of FGFRs in mouse podocyte cell line (138%). Microalbuminuria in AKITA+FGF2 was significantly increased on day 10-15 (231.7 ± 64.8 / 88.6 ± 11.7 mg/gCr). In contrast, microalbuminuria was not induced in control mice. Next, the FGF antagonist was evaluated as a therapeutic agent to the accelerated diabetic nephropathy using puromycin aminonucleoside and AKITA. When PAN (10 mg/animal/day i.v., day0) was administered to AKITA, microalbuminuria in AKITA+PAN was significantly increased on day 5-10 (AKITA+FGF2 527 ± 64.8 mg/gCr). In contrast, microalbuminuria was not increased in those treated by FGF antagonist.

Conclusions: Both FGF2 and FGF receptors were induced under hyperglycemia and play a crucial role of podocyte injury in DMN.

Funding: Government Support - Non-U.S.

SA-OR418

Translationally Controlled Tumor Protein Is Associated with Podocyte Hypertrophy in a Mouse Model of Type 1 Diabetes Dong Ki Kim,[1] Yon Su Kim,[1] Shin-Wook Kang.[2] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.*

Background: Translationally controlled tumor protein (TCTP) is thought to be involved in various intracellular processes by regulating mammalian target of rapamycin complex 1 (mTORC1) signaling. Because diabetes characteristically induces hypertrophy in podocytes in the early stage of the disease and mTORC1 signaling has been implicated in this process, TCTP may have a role in the pathogenesis of podocyte hypertrophy in diabetic nephropathy.

Methods: We examined the changes in TCTP expression in experimental diabetic glomeruli. To characterize the role of TCTP in podocyte hypertrophy, we conducted in vivo gene silencing of TCTP through the hydrodynamic injection of TCTP shRNA expressing lentivirus (LV-shTCTP).

Results: Glomerular expression of TCTP were higher in DM mice compared with C mice. Double immunostaining for TCTP and synaptopodin revealed that podocytes were the main cells responsible for this increase. TCTP knockdown by RNA interference ameliorated the activation of mTORC1 downstream effectors and the overexpression of cyclin dependent kinase inhibitors (CKIs) in diabetic glomeruli. Light microscopic examination revealed a marked glomerular hypertrophy in DM mice compared with C mice. In contrast, transduction of LV-shTCTP in DM mice attenuated the glomerular hypertrophy. The mean volume of podocyte measured by an unbiased stereological technique using optical disector were significantly higher in DM mice compared with control, whereas numerical density of podocytes were lower in diabetic mice. These morphologic changes were significantly ameliorated by the LV-shTCTP treatment. Electron microscopy of glomeruli revealed that podocytes of DM mice were enlarged with mild thickening of GBM, whereas the foot process was mostly intact. However, the diabetes-induced hypertrophy of podocytes, but not the GBM thickening, was substantially reduced in DM+LV-shTCTP1 mice.

Conclusions: These findings suggest that TCTP might play an important role in the process of podocyte hypertrophy under diabetic conditions via the regulation of mRNA translation and the induction of cell cycle arrest.

Funding: Private Foundation Support

SA-OR419

Transcriptome Analysis of Human Diabetic Kidney Disease Ae Seo Deok Park,[1] Karolina Woroniecka,[1] Davoud Mohtat,[1] David B. Thomas,[2] Katalin Susztak.[1] *[1]Albert Einstein College of Medicine, Bronx, NY; [2]Nephrocor, Uniondale, NY.*

Background: Diabetic kidney disease (DKD) is the single leading cause of kidney failure in the United States. The aim of this study was to provide an unbiased catalogue of gene expression changes in human diabetic kidney samples.

Methods: Affymetrix expression array was used to identify differentially regulated transcripts in 22 microdissected human renal glomerular and 22 tubular samples. Stringent statistical analysis was used to identify differentially expressed transcripts in control and diseased glomeruli and tubuli. Two different web-based algorithms were used to define differentially regulated pathways.

Results: The DKD samples were significant for racial diversity, decreased eGFR (DKD glomeruli 31.08±13.36 ml/min vs. Control 80.91±23.42; DKD tubuli 21.85±11.54 ml/min vs. Control 73.77±21.08), increased proteinuria, serum creatinine, and BUN, and presence of diabetes and hypertension. Histological changes showed consistent with DKD, increased glomerulosclerosis, tubular atrophy, interstitial fibrosis, vascular sclerosis, and mesangial matrix expansion. The phenotype was significant for advanced CKD (Stage IV).

Statistically significant, 1,700 differentially expressed probesets in DKD glomeruli were identified, and 1,831 in DKD tubuli. And 330 probesets were commonly differentially expressed in both compartments. Almost all podocyte specific transcripts showed decreased expression and there was excellent correlation between the protein and mRNA level data.

Pathway analysis highlighted the regulation of the complement system, actin cytoskeleton pathways like RhoA and Cdc42, extracellular matrix (ECM)-receptor interaction like integrin and ILK, and VEGF signaling in DKD glomeruli among other 22 canonical pathways. The DKD tubulointerstitial compartment showed strong enrichment for inflammation related pathways. The canonical complement signaling pathway was determined to be statistically differentially regulated in both DKD glomeruli and tubuli, and was associated with increased glomerulosclerosis.

Conclusions: Our studies identified multiple novel genes and pathways that may play a role in the pathogenesis of DKD or could serve as biomarkers.

Funding: Other NIH Support - NIH (US. Government), Private Foundation Support

SA-OR420

MiR-21 Is a Key Mediator in the Development of Diabetic Kidney Injury in db/db Mice and In Vitro under Diabetic Conditions Xiang Zhong,[1,3] Arthur Chi-Kong Chung,[3] Haiyong Chen,[2,3] Xiaoming Meng,[2,3] Yuan Dong,[3] Rong Li,[1,3] Xiao Ru Huang,[3] Hui Y. Lan.[2,3] *[1]Chemical Pathology, Chinese University of Hong Kong, Hong Kong, China; [2]Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong, China; [3]Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China.*

Background: Our recent studies found that miR-21 plays a pathological role in TGF-β/Smad3-driven renal fibrosis. In the present study, we hypothesized that miR-21 may also act as a key mediator and therapeutic target for diabetic nephropathy.

Methods: miR-21 expression was examined in diabetic kidney of db/db mice. The functional role and therapeutic potential of miR-21 in diabetic kidney injury was examined in db/db mice by an ultrasound-microbubble-mediated anti-miR-21 gene transfer. In addition, role and mechanisms of miR-21 in diabetic renal injury was examined in vitro under diabetic conditions in a rat mesangial cell (MC) line / NRK52E tubular epithelial cell (TEC) line by overexpressing or down-regulating of miR-21.

Results: Renal miR-21 was markedly upregulated (two folds increased) in db/db mice, which was associated with the development of renal fibrosis including collagen I and IV (a 1.5-fold increased), and the severity of microalbuminuria ($p<0.01$). Similar results were also evidenced in high glucose-stimulated MC and TEC. The functional role of miR-21 was further examined in vitro that knockdown of miR-21 suppressed, but overexpression of miR-21 enhanced, high glucose-induced Col I, Col IV, and fibronectin expression ($p<0.05$). More importantly, ultrasound-microbubble-mediated gene transfer of miR-21 knockdown plasmid into the diabetic kidney of db/db mice at week 10 resulted in a significant improvement of microalbuminuria ($p<0.01$) and renal fibrosis ($p<0.01$) on weeks 20, revealing a therapeutic potential for diabetic nephropathy by targeting miR-21.

Conclusions: In conclusion, miR-21 is a key mediator for the development of diabetic kidney disease. Targeting miR-21 may represent a novel and effective therapy to combat diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-OR421

Identification of the Site of Endothelin A Receptor Antagonist-Induced Fluid Retention Donald E. Kohan, Deborah Stuart, Kevin A. Strait, Peter K. Stricklett, Mark Chapman. *University of Utah Health Sciences Center, Salt Lake City, UT.*

Background: Endothelin (ET) receptor antagonist-induced fluid retention has been responsible for discontinuation and/or failure of several large clinical trials. ETB receptor inhibition causes fluid retention though reduction of the known natriuretic effects of renal ET-1, however how ETA receptor (ETAR) antagonism elicits fluid retention is uncertain.

Methods: The relatively ETAR-selective antagonist, ambrisentan (ETA:ETB selectivity 29-4000:1 depending on the assay), was administered to mice in which ETAR were selectively deleted in cardiomyocytes (CM ETA KO), vascular smooth muscle (VSM ETA KO), or the renal collecting duct (CD ETA KO) as well as mice with intact ETAR.

Results: All gene targeting utilized mice with loxP-flanked ETAR and Cre recombinase expressed in a tissue-specific manner. Mice were given a 4% NaCl diet to exacerbate Na retention. Animals were treated with vehicle alone for 1 week, followed by 2 weeks of 100 mg/kg/day ambrisentan or vehicle by oral gavage (N=5-10 per group). Baseline radiotelemetric blood pressure and pulse were not different between groups, nor did ambrisentan treatment alter these parameters. Two weeks of ambrisentan treatment increased body weight in control and CM ETA KO, but not in VSM ETA KO or CD ETA KO. Ambrisentan decreased hematocrit in control, CM ETA KO and VSM ETA KO, but not in CD ETA KO animals. Body fluid volume compartments were determined by impedence plethysmography. Ambrisentan tended to increase extracellular fluid volume in all mouse groups except in CD ETA KO mice. At the conclusion of the experimental study, plasma volume was determined by Evan's blue dye dilution; no alterations in plasma volume were detected, although the technique was unlikely to detect the magnitude of predicted changes.

Conclusions: In summary, ambrisentan causes fluid retention in controls and animals deficient in ETAR in the heart or vasculature, however absence of CD ETAR prevented the ambrisentan response. These data suggest that the CD is the site responsible for ETAR antagonist induced fluid retention; concurrent treatment with diuretics targeting the CD may mitigate ETAR-induced fluid retention.

Funding: NIDDK Support, Other NIH Support - NHLBI, Pharmaceutical Company Support

SA-OR422

The Role of mTOR and SGK1 in Mediating Aldosterone Regulation of ENaC In Vivo Atif A. Kidwai,[1] Chih-Jen Cheng,[2] Jian Wang,[1] Michel G. Baum,[2] David Pearce.[2] *[1]Division of Nephrology, UCSF, San Francisco, CA; [2]University of Texas Southwestern Medical Center, Dallas, TX.*

Background: SGK1 is a key mediator of aldosterone activation of ENaC in the kidney tubules. The protein kinase mammalian target of rapamycin, mTOR, has been shown in cultured cells and *in vitro* to phosphorylate SGK1, and stimulate ENaC.

Methods: To determine the importance of this effect *in-vivo*, we monitored Na+ balance in mice following intraperitoneal injection of the mTOR inhibitor PP242 (30mg/kg; n = 10). To better understand the effect of mTOR on current in the native collecting duct, we examined the effect of PP242 using *in-vitro* microperfusion of collecting ducts. Cortical collecting ducts were harvested and subjected to microperfusion according to standard techniques. Potential difference (PD) measurements were made following treatment with PP242 (1 μM, N = 7) or rapamycin (0.1 μM, N = 5).

Results: We found that urine volume (ul) was increased by 56% (609 +/- 55 vs 953 +/- 152 ul $p = 0.048$), sodium (mmol/L) was increased by 79% (19.1 +/- 3.51 vs 53.4 +/- $p = 0.034$), total sodium excreted (mmol) was increased by 167% (11.7 +/- 2.46 vs 42.9 +/- 11.1 $p = 0.014$). The Urine sodium to potassium ratio increased by 62% (0.20 +/- 0.03 vs 0.53 +/- 0.13 $p = 0.026$), suggesting ENaC inhibition, possibly due to decreased SGK1 phosphorylation. In the microperfusion data, PD (mV) decreased from 9.1 +/- 2.76 to 4.92 +/- 1.29 with PP242 treatment ($p= 0.03$ paired t-test vs control), and recovered to 6.98 +/- 1.71 ($p = 0.11$). Effects were amiloride sensitive, indicating ENaC dependence. Rapamycin had no effect on PD.

Conclusions: Together with cell culture and in vitro data, these data strongly suggest that mTOR —in particular mTOR complex 2—regulation of SGK1 plays a physiologically important role in ENaC regulation in the kidney tubules. (NIH DK085101 NIH DK056695)

Funding: NIDDK Support

SA-OR423

Impaired Phosphorylation of Na-K-2Cl Cotransporter by OSR1 Deficiency Manifests Hypotension and Bartter-Like Syndrome Sung-Sen Yang,[1] Pauling Chu,[1] Shinichi Uchida,[2] Sei Sasaki,[2] Shih-Hua P. Lin.[1] *[1]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, and National Defense Medical Center, Taipei, Taiwan; [2]Department of Nephrology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Na^+-(K^+)-(2)Cl^- cotransporters [N(K)CCs], including NKCC1 and renal-specific NKCC2 and NCC, play pivotal roles in the regulation of blood pressure and renal NaCl reabsorption. Oxidative stress-responsive kinase-1 (OSR1) is a known upstream regulator of N(K)CCs.

Methods: We generated and analyzed global and kidney tubule-specific (KSP-) OSR1 knockout mice to elucidate the physiologic role of OSR1 *in vivo*, particularly on blood pressure and kidney function.

Results: Although global $OSR1^{-/-}$ mice were embryonically lethal, $OSR1^{+/-}$ mice had lower blood pressure associated with reduced phosphorylated (p-)NKCC1 abundance in aortic tissue but intact renal Na^+ reabsorption. KSP-$OSR1^{-/-}$ mice were normotensive but primarily exhibited impaired Na^+ reabsorption in the thick ascending loop (TAL) on a low Na^+ diet accompanied by remarkably decreased expression of p-NKCC2 and a blunted response to furosemide, a NKCC2 inhibitor. The expression of total SPAK and p-SPAK was significantly increased in parallel to that of total NCC and p-NCC despite unchanged total NKCC2 expression. KSP-$OSR1^{-/-}$ mice also manifested hypokalemia with renal K^+ wasting and hypercalciuria, associated with reduced ROMK1, enhanced ENaC (β), and epithelial Ca^{2+} channels (TRPV5 and 6) expression.

Conclusions: These results suggest that globally, OSR1 is involved in the regulation of blood pressure and renal tubular Na^+ reabsorption mainly via the activation of NKCC1 and NKCC2. In the kidneys, NKCC2 but not NCC is the main target of OSR1 and the reduced p-NKCC2 in KSP-$OSR1^{-/-}$ mice may lead to a Bartter-like syndrome.

Funding: Government Support - Non-U.S.

SA-OR424

A SPAK/OSR1 Isoform Network Regulates Renal Sodium Transport James A. McCormick,[1] Kerim Mutig,[2] Joshua N. Curry,[1] Eric J. Delpire,[3] Sebastian C. Bachmann,[2] David H. Ellison.[1] *[1]Department of Nephrology, Oregon Health & Science University, Portland, OR; [2]Department of Anatomy, Charité Universitätsmedizin, Berlin, Germany; [3]Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, TN.*

Background: SPAK (Ste20-related Proline Alanine rich Kinase) and the related OSR1 (Oxidative Stress Responsive 1) kinase phosphorylate several renal ion cotransporters, including NCC, NKCC2 and NKCC1, increasing cotransporter activity. Knock-in of a dominant-negative SPAK mutant (Thr243Ala) in mice suppresses activity of both NCC and NKCC2. However, targeted disruption of SPAK *reduces* NCC activity, but *increases* NKCC2 activity. We recently cloned a novel SPAK isoform that lacks the majority of the kinase domain including the T-loop activation residue (Thr243); we hypothesized that it acts as a dominant-negative inhibitor of full-length SPAK and OSR1.

Results: First, we confirmed that this isoform is expressed almost exclusively in kidney by performing RT-PCR on a broad panel of tissues, and named it KS-SPAK. Next, we performed immunofluorescence on kidney sections using an antibody against the C-terminus of SPAK, which recognizes both full-length SPAK and KS-SPAK, and an antibody against the N-terminus of SPAK, which recognizes only full-length SPAK. We found that the majority of SPAK expressed in the thick ascending limb is truncated at the N-terminus, while in the distal convoluted tubule, most SPAK is full-length; OSR1 is expressed in both segments. *In vitro* kinase assays showed that KS-SPAK inhibits phosphorylation of the N-terminus of NKCC2 by full-length SPAK or OSR1. Western blotting of kidney lysates from mice showed that changing extracellular fluid volume (by sodium restriction or NCC knockout), but not potassium loading or aldosterone infusion, leads to a striking shift in the ratio of KS-SPAK to full-length SPAK.

Conclusions: Taken together, these data suggest KS-SPAK acts as an inhibitor of NKCC2 activity *in vivo*, providing an explanation for the opposing effects of SPAK manipulation on NKCC2 activity in mice, and identify a novel kinase network that modulates responses to changes in extracellular fluid volume.

Funding: NIDDK Support

SA-OR425

T60M Mutation in Thiazide-Sensitive Na^+-Cl^- Cotransporter (NCC) Causes Defective NCC Expression and Reverses Gordon Syndrome Sung-Sen Yang,[1] Sei Sasaki,[2] Shinichi Uchida,[2] Shih-Hua P. Lin,[1] Pauling Chu.[1] *[1]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [2]Department of Nephrology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Defective phosphorylation of the thiazide-sensitive sodium-chloride cotransporter (NCC) on T60 residue, the most important phosphoacceptor site of SPAK/OSR1 kinases, abolished its functional activity without affecting surface expression in Oocytes. T60M-NCC mutation is common in Asian patients with Gitelman's syndrome (GS).

Methods: To explore the pathophysiological importance T60 residue of NCC *in vivo*, we created T58M-Ncc knock-in mice to model human GS with T60M -NCC.

Results: $Ncc^{T58M/T58M}$ mice exhibited typical features of GS with a blunted response to thiazide, confirming that Ncc function was markedly diminished. In the kidneys, total and p-Ncc (T53, T58, and S71) of $Ncc^{T58M/T58M}$ mice were remarkably reduced despite normal abundance of Ncc mRNAs, and immuno-gold staining also showed that T58M Ncc almost located in the sub-apical region of the DCT cells, suggseting that phosphorylation of T60 may play a pivotal role in the NCC trafficking/sorting. In MDCK cells, NCC interacts with adaptor protein (AP)-3, a lysosomal sorting-related protein. An increased T60M-NCC-AP3 interaction with an attenuated T60M-NCC expression which could be reversed by AP3 knockdown was observed. Furthermore, phenotype of $Wnk4^{D561A/+}$ knock-in mice, a Gordon syndrome animal model with an activated Spak/Osr1-Ncc phosphorylation signaling and increased p-Spak/Osr1/Ncc expression featuring the mirror image of GS, was reversed by Ncc T58M mutation accompany with a reduced total and p-Ncc expression.

Conclusions: Our results indicated that phosphorylation of NCC T60 is involved in its trafficking/sorting and thus activity *in vivo*. Defective NCC phosphorylation by T60M could reduced its expression by an enhanced AP3-associated lysosomal degradation, and reverses the phenotype of Gordon syndrome.

Funding: Government Support - Non-U.S.

SA-OR426

The Calcineurin Inhibitor Tacrolimus Activates the Renal Sodium Chloride Cotransporter To Cause Hypertension Ewout J. Hoorn,[1] Stephen B. Walsh,[2] James A. McCormick,[3] Antje Furstenberg,[2] Chao-Ling Yang,[3] Tom Roeschel,[3] Alexander Paliege,[4] James P. Conley,[3] Sebastian C. Bachmann,[4] Robert J. Unwin,[2] David H. Ellison.[3] *[1]Erasmus Medical Center, Rotterdam, Netherlands; [2]UCL, London, United Kingdom; [3]OHSU, Portland; [4]Charite University, Berlin, Germany.*

Background: Calcineurin inhibitors (CNIs) are immunosuppressive drugs, which are used widely to prevent rejection of transplanted organs and treat autoimmune disease. Hypertension, hyperkalemia and acidosis often complicate their use. These side effects resemble familial hyperkalemic hypertension, a genetic disease characterized by over-activity of the renal sodium chloride co-transporter (NCC) and caused by mutations in WNK kinases. We hypothesized that CNIs induce hypertension by stimulating NCC.

Methods: The effects of the CNI tacrolimus on NCC were studied in mice and kidney transplant patients by characterizing renal tubular function, and by using immunoblotting and immunofluorescence.

Results: In wild-type mice, tacrolimus caused salt-sensitive hypertension, hyperkalemia, and acidosis. In kidney homogenates, tacrolimus increased phosphorylated NCC and the NCC regulatory kinases WNK3, WNK4, and SPAK. Immunofluorescence confirmed that NCC co-localized with calcineurin. The functional importance of NCC was demonstrated by showing that tacrolimus had no effect on blood pressure in NCC knockout mice. Moreover, hydrochlorothiazide reversed the hypertension induced in wild-type mice. In contrast, the hypertensive response to tacrolimus was exaggerated in mice over-expressing NCC. Kidney transplant recipients who were treated with tacrolimus and who had hypertension, hyperkalemia and/or acidosis demonstrated a higher fractional chloride excretion in response to a thiazide and had increased NCC abundance and phosphorylation in their biopsies compared to CNI-free patients or healthy controls.

Conclusions: These findings indicate that tacrolimus-induced hypertension is largely mediated by NCC activation. WNK and SPAK kinases may link NCC with calcineurin, which is a phosphatase. Finally, our data suggest that thiazide diuretics may be especially effective in preventing the complications of CNI treatment.

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SA-OR427

***In Vivo* Activation of NCC by Angiotensin II Requires Integrity of the WNK4-SPAK Pathway** Maria Castañeda-Bueno,[1] Luz Graciela Cervantes-Perez,[1] Norma Hilda Vázquez,[1] Norma O. Uribe-Uribe,[1] Norma Bobadilla,[1] Dario Alessi,[2] Gerardo Gamba.[1] *[1]Nephrology, Instituto Nacional de Nutrición SZ and UNAM, Mexico City, Mexico; [2]Dundee University, Scotland, UK.*

Background: We have previously reported, using *Xenopus laevis* oocytes and mpkDCT cells, that NCC activity is modulated by angiotensin II (AngII) through the WNK4-SPAK pathway, suggesting that PHAII-type mutations in WNK4 imitate the AngII effect on NCC leading to arterial hypertension (PNAS, 2009). Additionally it has been shown in vivo that AngII promotes NCC phosphorylation independently of aldosterone.

Methods: To test this hypothesis *in vivo*, a complete WNK4-knockout mouse strain was generated by targeted disruption by Pfizer Inc and donated to our Unit. Control littermates and $WNK4^{-/-}$ mice of 14 weeks were characterized and exposed to regular vs. low salt diet. In addition, under normal diet, animals were also infused for 4 days with AngII at non-pressor dose. WNK4 expression was assessed by real time PCR. NCC and SPAK expression and phosphorylation (pNCC-T58; pSPAK-S373) were assessed using specific antibodies.

Results: $WNK4^{-/+}$ and $^{-/-}$ mice born at expected Mendelian rates and grew normally. No transcripts for WNK4 were detected from $WNK4^{-/-}$ renal mRNA. $WNK4^{-/-}$ mice exhibited a mild hypokalemia and metabolic alkalosis, with normal glomerular filtration rate, blood pressure and calciuria, together with increased plasma renin activity and decreased aldosterone. Control littermates exhibited increased expression and phosphorylation of NCC and increased phosphorylation of SPAK after nine days of low salt diet or four days of AngII infusion. In contrast, WNK4-/- mice exhibited lower level of NCC expression, while SPAK expression was similar to control. Immunohistochemistry revealed normal morphology of distal convoluted tubule, with lower NCC expression. Under low salt diet or AngII infusion, no increased expression or phosphorylation of pNCC-T58 or pSPAK-S373 was observed.

Conclusions: Our observations reveal that absence of WNK4 *in vivo* precludes the NCC and SPAK phosphorylation promoted by low salt diet or AngII infusion, suggesting that this peptide hormone action on NCC occurs by signaling through a WNK4 dependent mechanism.

Funding: Private Foundation Support

SA-OR428

Role and Mechanism of WNK1-OSR1/SPAK Cascades in the Regulation of Sodium Homeostasis in Mice Jian Xie, Joonho Yoon, Zhen Liu, Thao Truong, Chou-Long Huang. *Department of Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: WNK1 is a ubiquitous protein kinase of which increased expression causes hypertension at least partly by increasing renal Na reabsorption. *In vitro*, WNK1 phosphorylates and activates Ste20-related protein kinases OSR1 and SPAK, which in turn activate NCC and NKCC2. Alternatively, others reported that WNK1 activates these transporters independently of OSR1/SPAK. We examine the *in vivo* role and mechanism of WNK1 regulation of Na transport.

Methods: Mice with global inactivation of Wnk1 are embryonic lethal from cardiovascular (CV) developmental defects. We generated mice with conditional knockout of WNK1 in the kidney by crossing homozygous exon2-floxed mice with transgenic mice carrying a kidney-specific Cre. Mouse lines that allow conditional tissue-specific expression of constitutive-active (CA) OSR1 or SPAK were generated by targeting respective cDNA preceded by floxed transcriptional terminator to the ROSA26 locus.

Results: Mice with conditional KO of WNK1 in the kidney exhibited increased 24-h urinary Na excretion (168 ± 26 vs 131 ± 12 $_{\mu}$mol, $p < 0.01$) and lower blood pressure (SBP 114 ± 1 vs 123 ± 3 mmHg, $p < 0.05$) versus wild type when fed a normal Na diet *ad lib*. Conditional KO mice had defect in conserving Na during transition from pair-fed normal Na diet (0.49%) to Na-deficient diet (0.01%) (24-h Na excretion 1 and 2 days after Na-deficient diet: 20 ± 4 and 3 ± 0.1 in KO vs 13 ± 0.8 and 1.3 ± 0.3 $_{\mu}$mol in WT, $p < 0.01$). Mice with global deletion of Osr1 exhibited CV developmental defects similar to mice with global Wnk1 deletion, but with ~0.5 day delay in onset (begins at embryonic day E10.5 vs E11). Endothelial-specific expression of CA-OSR1 rescued lethality from global Osr1 deletion.

Conclusions: These results indicate that WNK1 plays an important role in regulation of renal Na reabsorption. Similar timing and developmental defects between Wnk1 and Osr1 deletion indicate potential interactions of WNK1 and OSR1 signaling cascades. Further studies will investigate which ion transporters are regulated by WNK1 and OSR1, and whether CA-OSR1/SPAK can complement developmental and renal defects of WNK1 deficiency.

Funding: NIDDK Support

SA-OR429

Pathophysiology of Familial Hyperkalemic Hypertension Related to Deletions of the First Intron of WNK1 Juliette Hadchouel,[1] Emmanuelle Vidal-Petiot,[1] Christelle Soukaseum,[1] Veronique Baudrie,[1] Lydie Cheval,[2] Xavier Jeunemaitre.[1,3] *[1]Paris Centre for Cardiovascular Research (INSERM U970), Paris, France; [2]Cordeliers Research Centre (INSERM U872), Paris, France; [3]Hôpital Européen Georges Pompidou, Paris, France.*

Background: Mutations in the serine-threonine kinase WNK1 are responsible for Familial Hyperkalemic Hypertension (FHHt), an autosomal dominant form of hypertension associated with hyperkaliemia and hyperchloremic metabolic acidosis. WNK1 gives rise to a long ubiquitous isoform, L-WNK1, and a shorter isoform lacking a functional kinase domain and expressed exclusively in the distal nephron, KS-WNK1. FHHt mutations are large deletions of the first intron of the gene, but the consequences of these deletions on the expression of L-and KS-WNK1 and on renal ion transport are unclear.

Methods: In order to elucidate the mechanisms underlying the deregulation of renal ion handling in WNK1-related FHHt patients, and thereby the role of WNK1 on blood pressure and ion homeostasis, we generated WNK1+/Δi1 knock-in mice harboring a deletion of the first intron of WNK1.

Results: WNK1+/Δi1 mice display high blood pressure, hyperkalemia and hyperchloremic metabolic acidosis. We show that L-WNK1 expression is increased in the Distal Convoluted Tubule (DCT) and, to a lesser extent, in the connecting tubule, whereas KS-WNK1 expression is not modified upon deletion of WNK1 first intron. As

expected from in vitro studies, the modification of L-WNK1 expression leads to increased expression and activity of the sodium-chloride cotransporter NCC. In addition, the blood pressure and metabolic disorders are reversed by administration of hydrochlorothiazide. Finally, the increased sodium transport in the DCT is associated with a decreased activity of the Epithelial sodium Channel (ENaC), as illustrated by a blunted diuretic response to amiloride, which could explain the hyperkalemia and the acidosis.

Conclusions: Our study demonstrates that, by increasing L-WNK1 expression in the DCT, the deletion of WNK1 first intron leads to the stimulation of NCC activity and the development of FHHt. The contribution of L-WNK1 activation in the CNT remains to be defined.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR430

RDX5791, a Non-Systemic NHE3 Inhibitor, Normalizes Blood Pressure and Reduces Salt-Induced Organ Damage in Uremic Rats Andrew G. Spencer,[1] Marc Navre,[1] Craig F. Plato,[2] Kristin M. Joly,[2] Eric Daniel Labonte,[1] Jeffrey Jacobs,[1] Dominique Charmot.[1] *[1]Ardelyx, Inc., Fremont, CA; [2]Plato BioPharma, Inc., Westminster, CO.*

Background: Excess dietary Na+ exacerbates hypertension and accelerates cardiac and renal dysfunction in CKD patients. RDX5791 is a potent, non- systemic inhibitor of the intestinal Na+ antiporter NHE3 and shifts Na+ excretion from urine to feces. The current study evaluated the effects of RDX5791 on systemic hemodynamics and organ damage in uremic rats fed a 4% NaCl diet for four weeks.

Methods: At study inception, 5/6th nephrectomized rats were functionally matched to a vehicle group (n=24) or one of three groups of prophylactically dosed RDX5791 (0.3, 1, or 3 mg/kg/d; n=12/group). At mid-study, half the vehicle group was functionally matched to continue vehicle treatment or begin 3 mg/kg/d RDX5791 treatment (therapeutic arm). Diastolic and systolic blood pressure (DBP, SBP) and serum and urine chemistry were measured weekly or biweekly. Extracellular fluid volume (ECFV) and total body water (TBW) were monitored via bioimpedance spectroscopy. At endpoint (day 28), rats were sacrificed and morphological analyses of heart and kidney were performed.

Results: Compared to vehicle rats, prophylactic RDX5791 (1 mg/kg/d) reduced DBP by 26% (144 to 107 mmHg), SBP by 21% (204 to 161 mmHg), albuminuria by 78% (97.1 to 21.4 mg/d), left ventricle:tibia length ratio by 20% (27.7 to 22.1 mg/mm), ECFV/TBW (43.5% to 41.5%), and weight of remnant kidneys, (1.88 to 1.43 g) which were analyzed for the extent of fibrotic injury. Importantly, the cardiorenal effects of RDX5791 were dose-dependent. In the therapeutic arm, RDX5791 reduced established albuminuria, hypertension and the expanded ECFV to values similar to those in prophylactically-treated animals.

Conclusions: RDX5791 provides robust antihypertensive, cardiac and renal protective effects in uremic rats fed a high sodium diet. Those effects are likely mediated by the shift in sodium clearance from the kidneys to the GI tract. RDX5791 replicates many of the clinical benefits of low sodium intake in hypertension and CKD; it may act synergistically with RAAS antagonists to improve patient outcomes.

SA-OR431

Renal Allograft Rejection and Delayed Graft Function Regulated by miRNAs Julia Wilflingseder,[1,2] Alexander Kainz,[1,2] Rainer Oberbauer.[1,2] *[1]Nephrology, KH der Elisabethinen, Linz, Austria; [2]Nephrology, Medical University of Vienna, Vienna, Austria.*

Background: Aim of this study was the identification of deregulated miRNAs in renal biopsies from patients who developed acute vascular rejection, acute interstitial rejection, antibody mediated rejection (ABMR) and delayed graft function (DGF).

Methods: Sixty-five selected post-transplant FFPE biopsy samples (30 acute cellular rejection (15 Banff-1, 15 Banff-2), 11 ABMR, 14 DGF, 10 protocol biopsies) were analyzed. Affymetrix GeneChip® miRNA Arrays holding 904 unique small RNA sequences based on the Sanger miRBASE version 11 were used for miRNA profiling. Differentially regulated miRNAs were identified by Student's t-test and Bonferroni correction. First experimentally validated targets according to mirTarBase were used as basis for functional interpretation. Second predicted target genes were identified using in-silico approaches (miRror – combination of databases like TargetScan and mirRanda, miTALOS) and also interpreted on the functional level.

Results: Patients with acute rejection (AREJ), ABMR and DGF discriminate from the control group (protocol biopsies) in the unsupervised cluster analysis and also in the principal component analysis suggesting miRNAs play an important role in rejection and DGF. Seven miRNAs are up-regulated in DGF. Four up-regulated and 17 down-regulated miRNAs are identified in acute rejection episodes compared to control group and six miRNAs are up-regulated in ABMR. Mir-21 and mir-182 are up-regulated in DGF and ABMR and mir-663 is up-regulated in both rejection groups.

Conclusions: These preliminary data suggest that a distinct miRNA signature is associated with rejection and delayed graft function. A detailed analysis of the miRNAs and target genes will be presented at the meeting. The identified miRNAs and target genes may provide novel insights in the molecular regulation of acute rejection and delayed graft function and the identified molecules may serve as novel diagnostic markers and therapeutic targets.

Funding: Government Support - Non-U.S.

SA-OR432

Urinary miR-210 as a Mediator of Acute T-cell Mediated Rejection in Renal Allograft Recipients Johan M. Lorenzen, Hermann G. Haller, Wilfried Gwinner, Thomas Thum. *Nephrology, Hanover Medical School, Hannover, Germany.*

Background: microRNAs (miRNAs) are small ribonucleotides regulating gene expression. microRNAs are present in the blood in a remarkably stable form. We tested whether miRNAs are also detectable in urine and may serve as new predictors of outcome in renal transplant patients with acute rejection.

Methods: We profiled urinary miRNAs of stable transplant patients and transplant patients with acute rejection. The results were validated in a validation cohort of 62 patients with acute rejection, 19 control transplant patients without rejection and 13 stable transplant patients with urinary tract infection by quantitative RT-PCR.

Results: miR-10a, miR-10b and miR-210 were strongly deregulated in urine of patients with acute rejection. We confirmed these data in urine of a validation cohort of 62 patients with acute rejection, 19 control transplant patients without rejection and 13 stable transplant patients with urinary tract infection by quantitative RT-PCR. miR-10b and miR-210 were down-regulated and miR-10a up-regulated in patients with acute rejection compared to controls. Only miR-210 differed between patients with acute rejection when compared to stable transplant patients with urinary tract infection or transplant patients before/after rejection. Low miR-210 levels were associated with higher decline in GFR one year after transplantation.

Conclusions: Selected miRNAs are strongly altered in urine of patients with acute renal allograft rejection. miR-210 levels identify patients with acute rejection and predict long-term kidney function. Urinary miR-210 may thus serve as a novel biomarker of acute kidney rejection.

Funding: Government Support - Non-U.S.

SA-OR433

Impact of Inflammation in Protocol Biopsies Taken at Week 6 after Transplantation on Fibrosis in Biopsies Taken at One Year Willy Aasebo,[1] Hallvard Holdaas.[2] *[1]Akershus University Hospital; [2]Oslo Universtity Hopsital - Rikshospitalet.*

Background: To assess if inflammation in protocol biopsies taken 6 weeks after kidney transplantation predict inflammation and fibrosis one year after transplantation.

Methods: All single kidney transplant recipients that had a protocol biopsy taken at week 6 and week 52 (n = 157) were included. Recipients with donor specific antibodies at transplantation, AB0 incompatibility transplantations and recipients with indication biopsies taken between week 4 and 8 were excluded. The immunosuppressive protocol consisted of induction therapy with two doses of basiliximab, thereafter calcineurine inhibitors + mycophenolate + steroids.

All biopsies were classified according to Banff criteria. Recipients with rejection in protocol biopsies at week 6 received treatment with Solu-medrol and temporarily increased oral steroids dosages.

Results: Of the 100 biopsies that were negative at week 6, 69 were also negative at one year (69%), 26 had borderline and 5 had rejection. In the Borderline-group at week 6 (n = 43), 20 were negative at one year (47%), 19 had borderline and 4 had rejection. Of the 14 recipients with subclinical rejection at week 6 (9%), 10 had negative biopsies at one year (71%), 3 had borderline and 1 had rejection.

Fibrosis/atrophy in biopsies at one year according to inflammation in protocol biopsies taken at week 6 after transplantation. N = 157.

	Classification of biopsies at week 6.					
	Negative	Borderline	Rejection	P	P	P
At one year	N = 100	N = 43	N = 14	Neg vs. bord	Neg vs. rej	Bord vs. rej
Interst Inflam.	0.80 (0.71)	0.91 (0.43)	0.71 (0.47)	0.36	0.66	0.17
Tub. atrophy	1.03 (0.63)	0.98 (0.34)	0.71 (0.46)	0.60	0.073	0.028

Mean (SD)

Conclusions: Inflammation in protocol biopsies at week 6 does not predict interstitial fibrosis or tubular atrophy at one year in low-risk single kidney transplant recipients.

Recipients with subclinical rejections at week 6 had less tubular atrophy than the other groups and low degree of inflammation at one year. This finding indicates that identifying and treating these 9% of the recipient may be important.

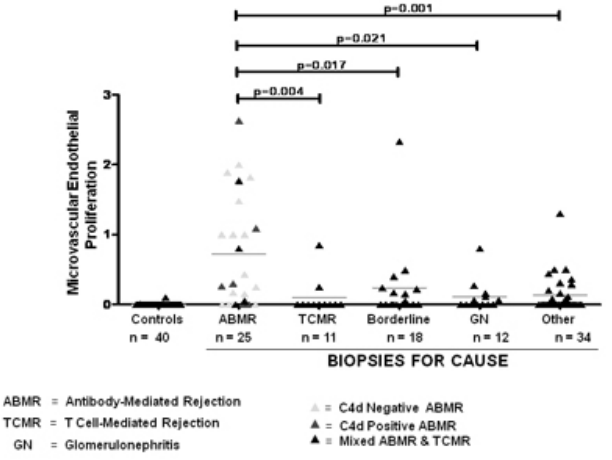
SA-OR434

Microcirculation Endothelial Cell Cycling Is Selectively Increased in Antibody-Mediated Rejection of Kidney Transplants, but Not in Other Diseases Stephen Adebayo Osasan, Declan G. de Freitas, Jessica Chang, Michael Mengel, Philip F. Halloran, Banu Sis. *ATAGC, University of Alberta, Edmonton, AB, Canada.*

Background: Antibody-mediated rejection (ABMR) is dominated by microcirculation inflammation and endothelial injury, caused by donor specific antibodies. We hypothesized that ABMR is associated with a greater endothelial repair response than other diseases in kidney transplants.

Methods: We related microcirculation endothelial cell cycling(MECC) to histopathology lesions, diagnoses, and whole-genome microarrays in 100 kidney transplant and 40 normal implantation biopsies. We performed double immunostaining for Ki-67 and CD31 to identify capillaries with cycling endothelial cells. We quantified MECC by counting number of Ki-67+CD31+ glomerular and peritubular capillaries in the entire cortical area of the biopsies.

Results: Transplant biopsies showed higher numbers of capillaries with cycling endothelial cells than controls (p=0.003). MECC was higher in ABMR than other diseases.



Increased MECC correlated with donor specific HLA antibody (p=0.003), microcirculation lesions (glomerulitis, capillaritis, transplant glomerulopathy), and C4d staining (p<0.05). Furthermore, transcript sets representing the molecular burden of active ABMR (endothelial cell, NK-cell, and macrophage-associated transcripts as well as interferon gamma regulated transcripts) correlated with increased MECC (p<0.05). Within the ABMR biopsies, increased MECC correlated with higher grades of glomerulitis (p=0.01) and peritubular capillaritis (p=0.08). Interestingly, endothelial cycling was not increased in TCMR, suggesting that T cells cross the microcirculation in TCMR without damaging the capillaries.

Conclusions: The endothelial repair response is selectively increased in kidneys with ABMR, reflecting the burden of active microcirculation injury.

SA-OR435

Ischaemic Glomeruli Are Increased in Protocol Biopsies after Transplantation, in Association with Peritubular Capillary (PTC) Loss, Inflammation and IF/TA Floortje Steegh,[1] Marielle Gelens,[2] Mat Daemen,[3] Ernest Van Heurn,[4] Maarten H.L. Christiaans,[2] Carine Peutz-Kootstra.[1] *[1]Pathology, Academic Hospital Maastricht, Maastricht, Netherlands; [2]Nephrology, Academic Hospital Maastricht, Maastricht, Netherlands; [3]Pathology, Academic Hospital Amsterdam, Amsterdam, Netherlands; [4]Surgery, Academic Hospital Maastricht, Maastricht, Netherlands.*

Background: Chronic transplant dysfunction is a major cause of renal allograft loss, preceded by inflammation and IF/TA in the renal biopsy. Recently we showed that PTC loss occurs within the three months after transplantation, and predicts lower renal function at 1 year. We hypothesized that capillary loss also occurs in the glomerular capillary bed, resulting in more ischemic glomeruli.

Methods: Protocol biopsies of 89 patients taken 0, 3 and 12 months (M0, M3, M12) after renal transplantation were studied. Recipients received kidneys from living (n=23), deceased after brain death (n=26) or deceased after cardiac death (n=40) donors. Biopsies were scored according to Banff09 with a total inflammation (ti) score. Ischemic glomeruli were scored as percentage of the total glomerular number. In a subgroup of 48 patients PTC number was determined as described (Steegh et al. JASN 2011). Statistical analysis was performed by T-test, Pearson correlations and multivariate analysis.

Results: Compared to M0, the mean number of ischemic glomeruli at M3 and M12 increased significantly from 7.86% to 20.64% and 23.79% (0-3 months p<0.001, 0-12 months p<0.001). The progression of ischemic glomeruli correlates with PTC loss at M3 (r=0.397, p=0.030). Increase in ischemic glomeruli correlates with ti and IF/TA scores at M3 and M12. Significant predictors for increased ischemic glomeruli at M12 are donortype and donor age (r2=0.165,p=0.005).

Conclusions: This study shows for the first time that the loss of PTCs is associated with an increase of ischemic glomeruli at M3 after transplantation. We hypothesize that interstitial and glomerular microvasculature loss is interrelated and may be initiated by similar mechanisms, leading to decreased renal function and worse prognosis for allograft survival.

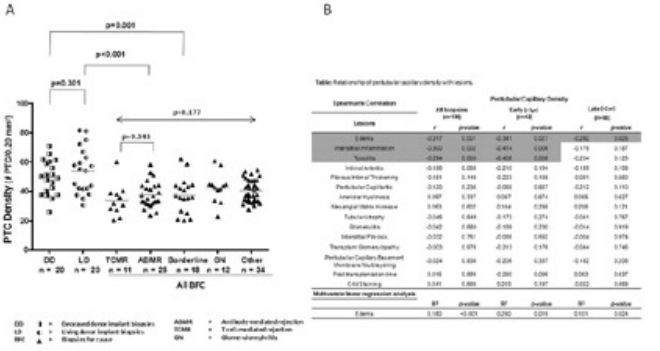
SA-OR436

Kidney Transplant Fibrosis Is Not Dependent on Peritubular Capillary Loss Stephen Adebayo Osasan, Jessica Chang, Declan G. de Freitas, Michael Mengel, Philip F. Halloran, Banu Sis. *ATAGC, University of Alberta, Edmonton, AB, Canada.*

Background: It has been suggested that progressive loss of peritubular capillaries (PTC) contributes to kidney fibrosis. We hypothesized that kidney transplant fibrosis is associated with and dependent on microcirculation loss.

Methods: We related PTC density to histopathology lesions, diagnoses, and post-transplant time in 100 kidney transplant biopsies for cause with 40 normal implantation biopsies as control. We performed CD31 immunostaining to identify the PTCs and counted total number of CD31+ PTCs in the entire cortical area of the biopsies. The PTC density was calculated by dividing the total number of PTCs by the number of ocular grid areas (0.2mm2).

Results: The PTC density was lower in transplant biopsies compared to controls, but did not differ among diagnoses in the transplant cases.



In early (<1 year) transplant biopsies, PTC density was lower in those with increased edema and tubulointerstitial inflammation. In late (>1 year) cases, only edema correlated with decreased PTC density. However, increased interstitial fibrosis, tubular atrophy, arterial fibrous intimal thickening, transplant glomerulopathy, peritubular capillary basement membrane multilayering, and time post-transplant did not relate to reduced PTC density. In multivariate regression analysis, edema was the only determinant of reduced PTC density in both early and late transplant biopsies.[Figure1B]

Conclusions: Contrary to our predictions, our results indicate that atrophy and fibrosis were not accompanied by reduction in capillary density. The histologic feature that correlated with reduced capillary density was edema, not fibrosis. Edema expands the peritubular interstitium, giving a false impression of reduced PTC density in biopsies. Thus kidney transplant fibrosis and nephron loss are not due to microcirculation loss.

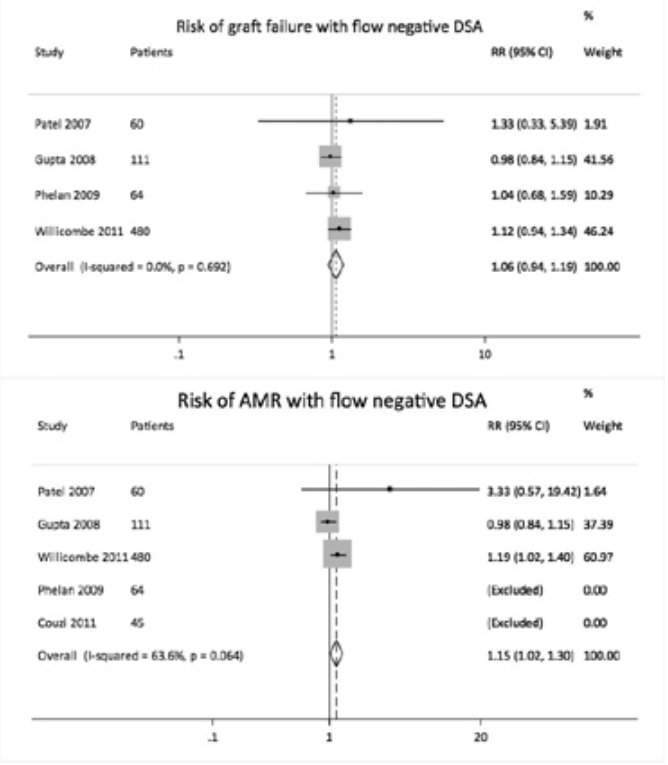
SA-OR437

Systematic Review & Meta-Analysis of Graft Outcomes Associated with Donor Specific Antibodies (DSA) & Negative Crossmatch Amudha Palanisamy, Sumit Mohan, Russell J. Crew, Bekir Tanriover, Geoffrey K. Dube, David J. Cohen, Jai Radhakrishnan. *Columbia University.*

Background: The introduction of solid phase methods provides increased sensitivity and specificity for the detection of DSA. However, the clinical relevance of DSA in the presence of negative CDC and flow crossmatches is unclear.

Methods: We identified 415 studies for review using the search term "(antibodies OR solid-phase assay OR luminex OR antibody mediated rejection) AND kidney AND crossmatch" which investigated allograft outcomes in patients with DSA detected by Luminex but not by cytotoxic or flow crossmatches. In addition, abstracts presented at the ASN & NKF between 2005 – 2010 were hand-searched for inclusion. 54 studies were identified for further review and 17 studies were extracted for review of full text. Ultimately, 5 retrospective studies with data comparing the incidence of AMR and graft survival in DSA positive and DSA negative groups detected by Luminex alone were pooled for analyses.

Results: All 5 studies reported the incidence of AMR, but only 4 studies reported the occurrence of graft failure. The time to follow-up was variable (range 9 months-4 years). Our pooled analysis included 104 DSA positive patients and 676 patients without DSA. There was an overall increased risk of AMR associated with the DSA positive group compared to patients without DSA (RR=1.149, 95% CI 1.017–1.299, p=0.026). The heterogeneity between the studies was not significant(I^2= 63.6%,p= 0.06). However, no increased risk of graft failure was detectable (RR=1.06, 95% CI 0.944–1.91, p=0.325) and there was no heterogeneity between studies(I^2=0%,p=0.69).



Conclusions: Our systematic review and pooled analysis underlines the paucity of outcomes data associated with preformed DSA detected by luminex but suggests an increased risk of AMR and no significant impact on overall graft survival in the short term.

SA-OR438

The Serum *p*-Cresol and Indoxyl Sulfate Correlated with Cardiovascular Disease in Mild-to-Moderate Chronic Kidney Disease of Renal Transplant Recipients Cheng-Hsu Chen,[1,2,3] Chi-Hung Cheng,[1,4] Ming-Ju Wu,[1,4] Tung-Min Yu,[1,2] Ya-Wen Chuang,[1] Kuo-Hsiung Shu.[1,4] *[1]Division of Nephrology, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung City, Taiwan; [2]School of Medicine, China Medical University, Taichung City, Taiwan; [3]Department of Life Science, Tunghai University, Taichung City, Taiwan; [4]Department of Medicine, Chung Shan Medical University, Taichung, Taiwan.*

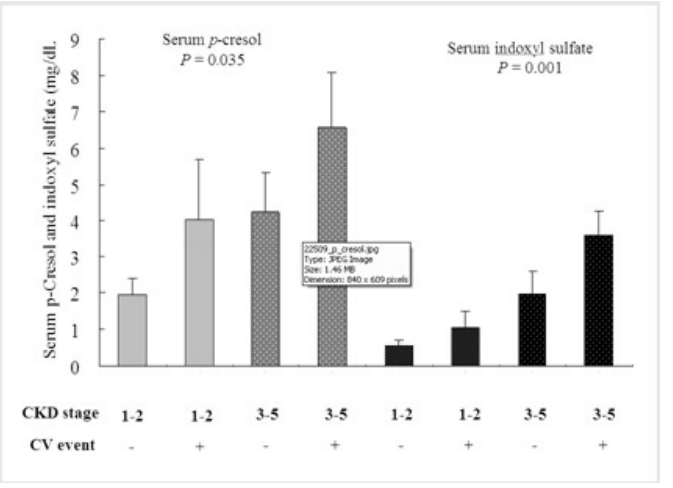
Background: Cardiovascular disease (CVD) is the major cause of death after renal transplantation (RTx). The *p*-cresol (pCS) and indoxyl sulfate (IS), anionic uremic toxins, markedly accumulated in serum and deteriorated of chronic kidney disease (CKD), are associated with CVD.

Methods: We recruited 95 RTx recipients with mean follow-up duration 6.3 ± 4.9 years. Their mean age was 48.7 ±14.1 years; and 56.8% (54/95) male patients. According the eGFR staging, we divided RTx recipients into stage 1-2 group and stage 3-5 group, then, examined pCS and IS and CVD events in these two group RTx recipients.

Results: The CKD staging is significantly different in the follow-up duration (5.0 ± 3.2 vs. 7.1 ± 5.6; P = 0.037). The serum pCS (2.4 ± 3.1 vs. 5.2 ± 6.8 mg/dL, P = 0.020) and IS (0.7 ± 1.0 vs. 2.7 ± 3.4 mg/dL, P = 0.001) was also significantly different between groups.

	Stage 1-2 (n = 37)	Stage 3-5 (n = 58)	P value
Age of RTx (yrs)	43.3 ± 15.2	41.9 ± 13.8	0.644
Follow-up duration (yrs)	5.0 ± 3.2	7.1 ± 5.6	0.037
Hb (mg/dL)	13.4 ± 1.6	11.8 ± 2.2	0.000
Albumin (mg/dL)	4.4 ± 0.3	4.1 ± 0.4	0.000
Serum p-cresol	2.4 ± 3.1	5.2 ± 6.8	0.020
Serum indoxyl sulfate	0.7 ± 1.0	2.7 ± 3.4	0.001

Interestingly, the CV event was correlated with the serum levels of pCS and IS between the CKD groups.



Conclusions: These findings suggest that serum pCS and IS may help to predict CVD risk in different CKD stage recipients. Whether pCS and IS is a modifiable cardiovascular risk factor in RTx recipients remains to be proven.

SA-OR439

Longer CMV Prophylaxis May Prevent Low Level CMV Reactivation but May Not Prevent Primary CMV Infection Christine M. Ribic,[1] Sundus A. Lodhi,[3] Jon A. Gregg,[3] Kenneth E. Lamb,[3] Herwig-Ulf Meier-Kriesche.[3] *[1]Medicine, McMaster University, Hamilton, ON, Canada; [2]Medicine, University of Florida, Gainesville, FL.*

Background: Whether longer valganciclovir (VG) prophylaxis prevents primary CMV disease after kidney transplantation(tx) is still debatable even after the published IMPACT trial and especially considering the subsequent editorials. For this reason, we analyzed data from our own center where we have been using extended VG prophylaxis since 2005.

Methods: This single centre retrospective study evaluated 690 tx recipients who received VG prophylaxis with a mean follow-up of 2.6 (SD+/-2.35) years. CMV event-free survival was calculated from the time of completion of prophylaxis and the primary endpoint was defined as positive CMV viremia identified by quantitative PCR (≥200 copies/ml) or positive pp65 antigenemia.

Results: There was no significant difference in CMV event-free survival (p=0.68) in high-risk (D+/R-) tx recipients who received prophylaxis for a median duration of 227 (n=55) versus 125 days (n=63) (Fig 1A). Likewise, when changing the endpoint to different CMV PCR thresholds (100, 200, 250, 500, 1000, 5000 or 10,000 copies/ml of plasma), there was also no significant difference between long and shorter prophylaxis.

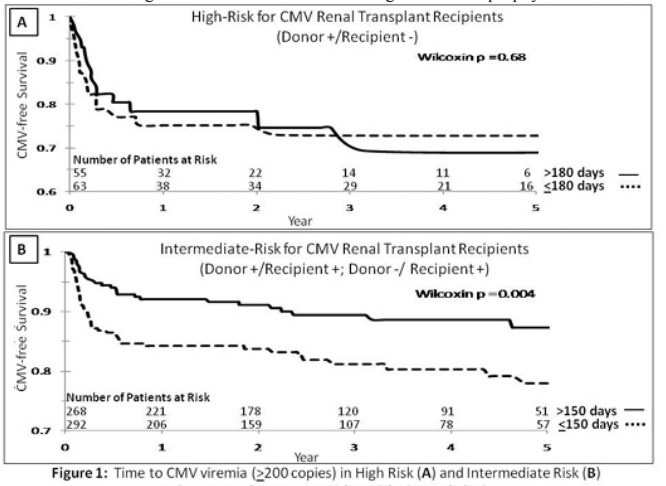


Figure 1: Time to CMV viremia (≥200 copies) in High Risk (**A**) and Intermediate Risk (**B**) patients up to 5 years post Valganciclovir prophylaxis.

Conversely in intermediate-risk patients, there were significantly (p= 0.004) higher rates of CMV viremia in those patients who received shorter prophylaxis for a median duration of 186 (n=263) versus 116 (n=292) days (Fig 1B). However this effect was driven by low level viremia results as the reduction in events in the longer prophylaxis group did not persist for the endpoint of CMV PCR ≥500 copies/ml.

Conclusions: In summary, our data suggests that longer prophylaxis may not impact the cumulative incidence of primary CMV infection but may prevent low level CMV reactivation.

SA-OR440

Significance of Persistent Asymptomatic EBV Viral Load in Pediatric Renal Transplant (TX) Recipients Asha Moudgil,[1] Karen Martz,[2] Therese Moore,[3] William E. Harmon,[4] Vikas R. Dharnidharka.[5] *[1]Nephrology, Children National Medical Center, Washington, DC; [2]NAPRTCS DCC, EMMES Corporation, Rockville, MD; [3]Nephrology, Mayo Clinic, Rochester, MN; [4]Pediatric Nephrology, University of Florida, Gainesville, FL; [5]Nephrology, Children Hospital, Boston, MA.*

Background: Children, in particular EBV naïve at TX, are at risk of developing post-transplant lymphoproliferative disorder (PTLD). Currently, many centers are prospectively monitoring EBV viral load (VL) by real time quantitative PCR (qPCR) and intervening (reducing immunosuppression {IS}, therapy with antiviral drugs in response to + VL to prevent PTLD. However, acute rejection (AR) may occur due to reduction in IS causing graft failure (GF). Outcome of these strategies is not clear.

Methods: EBV asymptomatic VL subregistry was created within NAPRTCS to study prevalence of PTLD on AR and GF in pts with persistent + VL for 6 months and in those with high VL ($>10^2$ times over the number detected by qPCR) in comparison to EBV -VL pts from the same centers.

Results: 14 centers enrolled 425 children (63 {14.8%} with +VL and 362 (85.2%} with -VL) from 2005 onwards. Of 60 with +VL and complete data, 40% were <5yrs age at TX, 31 M: 29F, 68.3% Caucasians and 8.3% Blacks, 79.3% and 75% were EBV and CMV naïve at TX. Of donors, 3.3% and 45% were EBV and CMV naïve. Thymoglobulin induction was used in 43.3% and 35% in EBV +VL and -VL pts (p=ns). Asymptomatic +VL developed 6.4± 6.5 months after TX, 50% with +VL had reduction in IS and 10% treated with antivirals. PTLD developed in 3/60 pts with +VL and 4/362 pts with -VL, 6.7-8.1 and 5.9-8.8 months after TX (5% vs 1.1% P=0.06). Of pts with high VL 3/39 (7.7%) and 0/21 with low VL developed PTLD (high VL vs low VL, p=ns and high VL versus -VL, p 0.02). The hazard ratio for time to AR and GF using Cox regression model with EBV +VL as a time-varying co-variate were 1.5(95% CI 0.8 to 2.9, p=ns) and 0.61(95% CI 0.2 to 2.01, p=ns).

Conclusions: Children with persistent high EBV viral load may be at higher risk for development of PTLD, but not at higher risk for AR or GF.

Funding: Pharmaceutical Company Support

SA-OR441

Aberrant mTOR Signaling Contributes to Cardiac Remodeling and Renal Fibrosis in Fibroblast Growth Factor Receptor 2 Conditional Knockout Mice Christina R. Nguyen, Sunder Sims-Lucas, Carlton M. Bates. *Nephrology, Children's Hospital of Pittsburgh, Pittsburgh, PA.*

Background: Cardiovascular disease is the leading cause of early death in pediatric chronic kidney disease (CKD), but a clear mechanism connecting the heart and kidney pathology is not understood. Mice with conditional knockout of fibroblast growth factor receptor 2 in the ureteric bud ($Fgfr2^{UB-/-}$) result in a 75% reduction in nephron number and progressive cardiac remodeling, mimicking pediatric structural CKD.

Methods: Serial serum studies, echocardiograms, and renal ultrasounds were obtained at 2, 6, and 9 months in $Fgfr2^{UB-/-}$ mice and littermate controls. Telemetric blood pressure and inulin clearance were assessed in males and tissue was examined via histology and Western blot at 9 months in both genders.

Results: At 2 months, $Fgfr2^{UB-/-}$ mice appear similar to controls. By 5 months, mutants have loss of corticomedullary differentiation, reduced kidney size (p=0.004), and higher blood urea nitrogen (BUN) (p=0.0045). At 5 and 9 months, the left ventricular mass (LVM)/body weight of mutant males is greater than controls (p=0.008, 0.03). Inulin GFR is lower in mutant males. Histological assessments reveal progressive fibrosis in mutant hearts and kidneys. Western blots suggest increased phospho-Akt (pAkt), phospho-p70S6 kinase, and phospho-tuberin (components of the mTOR pathway) in mutant hearts and kidneys.

Conclusions: $Fgfr2^{UB-/-}$ mice are a model of progressive structural chronic kidney disease leading to cardiac remodeling with normal blood pressure. Preliminary results suggest aberrant signaling through the mTOR pathway in both the kidney and heart may contribute to progressive fibrotic changes. This model will be useful in understanding the pathophysiology of normotensive chronic kidney disease and in providing therapeutic targets to prevent cardiac and renal fibrosis.

SA-OR442

The Hedgehog Pathway Is Strongly Activated in Stromal Cells during Renal Fibrosis Steven L. Fabian,[1] Radostin Penchev,[1] Petra Sipilä,[2] Anjali N. Rao,[1] Andrew P. McMahon,[3] Benjamin D. Humphreys.[1] *[1]Renal, Brigham and Women's Hospital, Boston, MA; [2]Mouse Biology and Genetics, University of Turku, Finland; [3]Molecular and Cellular Biology, Harvard University, Cambridge, MA.*

Background: The Hedgehog (Hh) pathway is best known for its role in regulating tissue patterning during development but can be reactivated in cancer and tissue injury. Although Hh plays a role in renal development, nothing is known about its role in adult kidney. Here we report strong activation of epithelial to stromal Hh signaling in renal fibrosis.

Methods: Hh pathway member expression was defined using reporter mice, in situ hybridiztaion, qPCR and immunofluorescence in two renal fibrosis models: unilateral ureteral obstruction (UUO) and unilateral ischemic reperfusion injury (UIRI).

Results: Indian Hedgehog (Ihh) reporter mice demonstrated Ihh expression in outer medullary proximal tubules while lineage analysis of Sonic Hedgehog (Shh) cells disclosed papillary collecting duct expression of Shh. These results were confirmed by in situ hybridization and qPCR. Gli1 reporter mice showed Gli1 expression in the papilla and in PDGFRβ+, CD31-, F4/80- interstitial pericytes in the outer medulla with enrichment around vasculature. A similar pattern using reporter mice was found for the Hh effector Gli2. Gli1 is a readout of Hh pathway activity, and in UUO and UIRI was increased in pericytes 14 and 25-fold respectively, with increased Gli2, Gli3 and Ptch1 expression. Ihh expression also increased 4-fold in UUO. Over the course of fibrosis, Gli1+ cells increased 11-fold and acquired the myofibroblast marker αSMA. In the pericyte-like cell line 10T1/2, Hh agonists triggered cell proliferation.

Conclusions: These results are the first to document strong activation of Hh signaling during renal fibrosis, and they suggest paracrine signaling of epithelial Hh ligand to stromal pericytes and myofibroblasts. The ability of Hh to trigger pericyte proliferation in culture suggests a possible role for Hh signaling in pericyte and myofibroblast proliferation in the early phases of renal fibrosis. These studies introduce the Hh signaling pathway as a novel therapeutic target in renal fibrosis.

Funding: NIDDK Support

SA-OR443

Endothelial HIF Signaling Modulates Renal Fibrosis Pinelopi P. Kapitsinou,[1] Mark Michael,[1] Timothy A. Sutton,[2] Volker H. Haase.[1] *[1]Nephrology, Vanderbilt University, Nashville, TN; [2]Nephrology, Indiana University, Indianapolis, IN.*

Background: Hypoxia and increased activity of hypoxia-inducible factor (HIF) is commonly found in chronic kidney disease. We previously identified tubular epithelial HIF-1 as a promoter of renal fibrosis, however the role of HIFs in other renal cell types remains unclear. Here we used a genetic approach to investigate the role of endothelial HIF-1 and HIF-2 in health and in response to chronic renal injury.

Methods: To delete HIF-1 and -2 in endothelial cells Vecadherin (Cdh5)-Cre transgenic mice were crossed to mice carrying conditional HIF-1A and/or HIF-2A alleles (Cdh5Cre;Hif1f/f,Hif2f/f: double mutants, Cdh5Cre;Hif1f/f, Cdh5Cre;Hif2f/f). To assess the HIF deficient vasculature, mutant transgenics were crossed to a reporter line. Unilateral ureteral obstruction (UUO) was used as a renal fibrosis model.

Results: Endothelial HIF-1/HIF-2 double mutants developed and aged normally, had no defects in renal morphology and function and possessed intact renal vasculature. When we exposed double mutants to 12 days of UUO, we found a 32% increase in collagen deposition compared to controls based on Sirius Red staining (n=10-11, P=0.03). Capillary density assessed by CD31 immunohistochemistry was reduced by 25% in double mutants compared to controls (n=4-6, P=0.03). This was associated with a 2.4-fold reduction in CD31 mRNA levels (n=4-6, P=0.009). In addition, mutant UUO kidneys showed a 25% increase in F4/80+ve cell infiltration. No difference in collagen accumulation was found when UUO experiments were performed with either endothelial HIF-1 or HIF-2 single mutants. However, capillary density by CD31 and cablin staining was decreased by 32% in endothelial HIF-2 single mutants compared to controls (n=5-6, P=0.03).

Conclusions: Our data show that endothelial HIF-1 and HIF-2 are dispensable under physiologic conditions, whereas both endothelial HIF-1 and HIF-2 in concert suppress the fibrotic response to chronic kidney injury. Furthermore, we identified HIF-2 as a critical regulator of the capillary maintenance during fibrosis. Our data provide new insights into the cell type-specific role of individual HIF transcription factors in renal disease.

Funding: NIDDK Support

SA-OR444

Macrophage HIF Suppresses Renal Inflammation in Chronic Kidney Disease Hanako Kobayashi,[1] Detlef O. Schlondorff,[2] Volker H. Haase.[1] *[1]Medicine, Vanderbilt University, Nashville, TN; [2]Medicine, Mount Sinai Medical Center, New York, NY; [3]Medicine, Vanderbilt University, Nashville, TN.*

Background: Hypoxia has been implicated as an important microenvironmental factor involved in the development of fibrosis in chronic kidney disease (CKD), and hypoxia inducible factor (HIF) has been shown to be a key regulator in this process. Our previous studies showed that deletion of HIF-1a in proximal tubule epithelial cells decreases renal fibrosis, indicating that HIF-1 activation in epithelial cells is fibrogenic. However systemic pharmacologic HIF activation in certain animal models can slow CKD progression indicating cell-type dependent functions of HIF with regard to renal fibrogenesis.

Methods: We used a genetic approach and studied the effect of HIF activation (HIF-1 and HIF-2) in the unilateral ureteral obstruction (UUO) mouse model. HIF was activated by global deletion of von Hippel-Lindau tumor suppressor gene (*Vhl*) using inducible Cre-loxP-mediated gene targeting system (*Ubc-Cre*).

Results: Unexpectedly, global activation of HIF ameliorates renal fibrosis following UUO and is associated with a marked decrease in macrophage (MØ) infiltration. To test the hypothesis that HIF activation of MØ changes their responses and thereby fibrosis, we performed UUO in mice in which *Vhl* is deleted in MØ ($LysM\text{-}Vhl^{-/-}$). We found that MØ infiltration is decreased by 40% in the $LysM\text{-}Vhl^{-/-}$ mice compared to cre-negative littermate controls following UUO. Moreover, we found that *Ccr2*, the major receptor for Monocyte/MØ (M/M) chemotaxin, is downregulated in cultured M/M by *Vhl* deletion. However, despite the marked decrease in the MØ infiltration, there is no reduction in collagen accumulation in $LysM\text{-}Vhl^{-/-}$ mice.

Conclusions: The decrease in inflammation that is associated with reduced MØ infiltration in $Ubc\text{-}Vhl^{-/-}$ UUO kidneys is partly due to the effect of MØ HIF; however, it is not sufficient to reduce renal fibrosis, and suggests that HIF activation in other cell types modulates collagen production in this model. Detailed analysis of MØ regulation by HIF will be further discussed.

Funding: NIDDK Support

SA-OR445

Iron Facilitates Kidney Inflammation in a Model of Chronic Renal Failure Induced by Persistent Expression of Kidney Injury Molecule-1 Dhruti D. Patel,[1,2] Liangying Gan,[1] Ningning Wang,[1] Takaharu Ichimura,[1] Benjamin D. Humphreys,[1] Joseph V. Bonventre.[1] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]School of Medicine, University of North Carolina, Chapel Hill, NC.*

Background: Chronic renal failure is characterized by tubular degeneration, fibrosis and often tissue iron deposition. In humans and rodent models of progressive kidney disease, Kidney Injury Molecule-1 (Kim-1) is persistently expressed in the proximal tubule (PT) and released into the urine.

Methods: A transgenic mouse was created, using Cre-Lox technology, which overexpresses Kim-1 primarily in the PT. Tissues were stained for iron using Prussian blue. Tubular Kim-1 expression and surrounding inflammatory cells were characterized by immunofluorescence. Primary PT cells were treated with media containing bovine Holo-Transferrin and 100 μM ferric ammonium citrate to model tissue iron overload. MHC-II expression was evaluated by flow cytometry. RAW macrophage activation was evaluated by TNFα production after exposure to conditioned media from Kim-1 expressing cells.

Results: The Kim-1 transgenic mouse develops progressive kidney disease with fibrosis, anemia, cardiac hypertrophy and suffers a uremic death. Urinary transferrin levels, tubule-interstitial inflammation and heme oxygenase-1 are increased. There are large amounts of iron deposition in PT cells and surrounding macrophages. We studied the role of this iron maldistribution in the modulation of inflammatory changes leading to renal failure. In primary cultures of mouse PT cells, addition of transferrin upregulated MHC-II levels. Iron loading of cells also eventually resulted in cell death of PT cells. Conditioned media from transferrin-treated tubular cells produced a marked induction of TNFα production by macrophages.

Conclusions: Increased PT cell iron is associated with upregulation of MHC-II in vivo. In vitro, increased cellular iron uptake results in increased cellular MHC-II expression, and eventually leads to cell death. Iron-enriched PT cells release pro-inflammatory agents which increase activation of macrophages and may contribute in an important way to progression of chronic kidney disease.

Funding: NIDDK Support, Private Foundation Support

SA-OR446

Platelet-Derived Growth Factor Receptor Signaling Activates Pericyte-Myofibroblast Transition in Progressive Kidney Fibrosis Yi-Ting Chen,[1,2] Fan-Chi Chang,[1] Ching-Fang Wu,[1] Yu-Hsiang Chou,[1] Wen-Chih Chiang,[1] Yung-Ming Chen,[1] Jeremy S. Duffield,[3] Shuei-Liong Lin.[1] *[1]Department of Medicine, National Taiwan University Hospital, Taipei, Taiwan; [2]Department of Medicine, E-DA Hospital, Kaohsiung, Taiwan; [3]Institute of Stem Cell & Regenerative Medicine, University of Washington, Seattle.*

Background: Pericytes are the major source of scar producing myofibroblasts following kidney injury; however, the mechanisms of this transition are unclear.

Methods: We examined Collagen 1 (α1)-GFP reporter mice (pericytes and myofibroblasts express GFP) following ureteral obstruction or ischemia-reperfusion injury and focused on the role of platelet-derived growth factor (PDGF)-receptor (PDGFR) signaling in these two different injury models.

Results: Pericyte proliferation was noted after injury with reactivation of α-smooth muscle actin expression, a marker of the myofibroblast phenotype. PDGF expression increased in injured tubules, endothelium, and macrophages after injury, whereas PDGFR subunits α and β were expressed exclusively in interstitial GFP-labeled pericytes and myofibroblasts. When PDGFR α or β activation was inhibited by receptor-specific antibody following injury, proliferation and differentiation of pericytes decreased. The antibodies also blunted the injury-induced transcription of PDGF, transforming growth factor β1, and chemokine CCL2. They also reduced macrophage infiltration and fibrosis. Imatinib, a PDGFR tyrosine kinase inhibitor, attenuated pericyte proliferation and kidney fibrosis in both fibrogenic models.

Conclusions: PDGFR signaling is involved in pericyte activation, proliferation and differentiation into myofibroblasts during progressive kidney injury. Hence, pericytes may be a novel target to prevent kidney fibrosis by means of PDGFR signaling blockade.

Funding: Government Support - Non-U.S.

SA-OR447

Angiotensin II Induces Progressive Renal Injury by Activating the TGF-beta/Smad Pathway through Activation of Sustained EGFR-ERK Signaling Jianchun Chen, Jian-Kang Chen, Raymond C. Harris. *Medicine, Vanderbilt University, Nashville, TN.*

Background: Although it is well recognized that angiotensin II can mediate progressive renal fibrotic injury, the underlying mechanisms are not completely understood.

Methods: We generated renal proximal tubule cell-specific EGF receptor knockout mice (*EGFR*^{ptKO}) by crossing EGFR$^{flox/flox}$ mice with gGT-Cre mice and chronically infused angiotensin II (Ang II) into these mice and their wild type littermates (*WT*). Blood pressures were similar in the two groups.

Results: In *WT*, Ang II infusion increased TGF-beta expression and $Smad_{2/3}$ phosphorylation in isolated renal proximal tubule epithelial cells, decreased E-cadherin expression, up-regulated vimentin, N-cadherin and snail expression and increased tubulointerstitial fibrosis. Ang II also markedly induced EGFR phosphorylation at Tyr845, a Src-dependent phosphorylation site. In Ang II-infused *WT*, Tyr845-phosphorylated EGFR associated with the adaptor proteins, Shc and Grb2 and increased ERK phosphorylation. In LLCPKcl4 cells stably transfected with AT1R (*AT1/Cl4*), Ang II stimulated ROS production, TGF-beta *de novo* synthesis and $Smad_{2/3}$ phosphorylation. Antioxidants or siRNA knockdown of Src kinase or EGFR blocked Ang II-induced ERK activation, inhibited TGF-beta/Smad2/3 signaling. *In vivo*, *EGFR*^{ptKO} mice had decreased ERK signaling, TGF-beta expression and $Smad_{2/3}$ phosphorylation, and significantly less tubulointerstitial fibrosis in response to Ang II perfusion. Administration of a specific EGFR tyrosine kinase inhibitor, erlotinib, to the wild type FVB/NJ mice, had a similar inhibitory effect on Ang II infusion-induced renal cortical tubules injury.

Conclusions: This study demonstrates that ROS-dependent Src activation-mediated prolonged-EGFR-ERK signaling, inducing TGF-beta de novo synthesis and Smad2/3 signaling activation, is a novel molecular mechanism underlying Ang II-induced progressive renal injury.

Funding: NIDDK Support, Veterans Administration Support

SA-OR448

NADPH-Oxidase 4 Knock-Out Mice Display Increased Tubular Apoptosis and Interstitial Fibrosis in the Unilateral Obstruction Model Stellor Nlandu Khodo,[1] Eva Bernabeu Dizin,[1] Pierre-Yves F. Martin,[1] Eric Feraille,[1] Karl-Heinz Krause,[2] Sophie M. De Seigneux.[1] *[1]Nephrology, University of Geneva, Switzerland; [2]Pathology, University of Geneva, Switzerland.*

Background: Kidney interstitial fibrosis is correlated with chronic kidney disease (CKD) progression. NOX4 is the major kidney NADPH-oxidase expressed mostly in the tubular compartment. In the kidney NOX2 is expressed at lower levels. NOX isoforms are involved in apoptosis and prosurvival pathways as well as in hypoxia signaling and may therefore play a role in fibrosis progression.

Methods: We studied unilateral urinary obstruction (UUO) in wild type and NOX4 knock-out (KO) mice as well as in NOX2/4 double-KO mice to decipher the role of these enzymes in kidney fibrosis progression. mCCDcl1 cells were specifically used to examine their role in apoptosis.

Results: NOX4 was expressed in the proximal tubule and collecting duct whereas NOX2 was expressed at low levels along the nephron. Kidney development was not altered by the absence of NOX2 and NOX4. Interstitial fibrosis assessed by Sirius red staining and collagen-I Western blot after 7 and 14 days UUO was two times higher in NOX4 KO and NOX2/NOX4 mice than in wild type mice. Tubular apoptosis was significantly enhanced in NOX4 and NOX2/NOX4 KO mice compared to wild type. Peritubular capillary density and VEGF expression assessed by Western blot were significantly lower in UUO kidneys of NOX4 and NOX2/NOX4 KO mice compared to wild type. Oxidative stress was increased in the interstitium of obstructed kidneys of NOX4 KO mice whereas it was attenuated in NOX2/NOX4 KO animals. In mCCDcl1 cells NOX4 si-RNA silencing led to apoptosis in the presence of TGF-b1.

Conclusions: We demonstrate that NOX4 deletion is deleterious in the UUO model and increases tubular cell apoptosis under conditions of tubular cell stress. NOX4 deletion decreases kidney peritubular vascularisation via decreased tubular VEGF production in UUO. NOX2 has a different role from NOX4 and does not compensate for the absence of NOX4.The role of NOX4 in tubular cell might be different from other cell types. These effects of NOX4 may explain enhanced kidney fibrosis in UUO NOX4 KO mice.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR449

Inhibition of microRNA-21 as a Therapeutic Strategy for Kidney Fibrosis Jeremy S. Duffield,[1] Deidre Mackenna,[2] Phong T. Tran,[2] Kara Kersjes,[2] Aaron Chang,[2] B. Nelson Chau.[2] *[1]Medicine, University of Washington, Seattle, WA; [2]Regulus Therapeutics, Lo Jolla, CA; [3]Medicine, Harvard Medical School, Boston, MA.*

Background: Fibrosis is an underlying pathology involved in disease progression and is associated with poor prognosis in a variety of chronic kidney diseases (CKD). microRNAs are ~22 bp evolutionarily conserved non-coding RNAs that regulate multiple mRNA targets and their dysregulation has been implicated in multiple disease processes including fibrosis. In particular, miR-21 is up-regulated in cardiac, lung & liver fibrosis & oligonucleotide-mediated inhibition of miR-21 is effective in mouse models of heart & lung fibrosis. We investigated if antagonizing miR-21 could represent a novel therapeutic approach for CKD.

Methods: We used mouse models of kidney injury with fibrosis, genetic and oligonucleotide silencing of miR21 in vivo, and bioinformatics to study miR21 in kidney disease.

Results: miR-21 was upregulated in two different models of kidney fibrosis: unilateral ureteral obstruction (UUO) and unilateral ischemia-reperfusion injury (IRI). Genetic deletion of miR-21 prevented kidney fibrosis in both models as measured by fibrotic gene expression, quantitative histology and biochemical analyses. To assess the therapeutic potential of inhibiting miR-21, high affinity oligonucleotide-based anti-miRs were administered to mice prophylactically or therapeutically in UUO or IRI models. These compounds delivered effectively to renal tubule epithelium and were detected in various interstitial cell types, including pericytes and interstitial myofibroblasts. Anti-miR-21 improved fibrosis in both models that largely phenocopied miR-21-/- mice. To investigate the role of miR-21 in fibrosis, we evaluated mRNA expression profiles of miR-21 deficient kidneys. Surprisingly, the expected global de-repression of potential miR-21 target mRNAs

was only observed when miR-21 deficient mice were subjected to stress (UUO or IRI). Such global analysis has revealed key miR-21 targets and corresponding downstream pathways that may mediate the anti-fibrotic effect miR-21 inhibition.

Conclusions: miR21 is an important new therapeutic target in kidney disease

Funding: NIDDK Support, Pharmaceutical Company Support

SA-OR450

T Lymphocytes Lacking Sphingosine Kinase 2 Attenuate Folic Acid Induced Kidney Fibrosis in Mice

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Background: Epidemiological studies indicate some survivors of AKI who regain renal function have a progressive decline in kidney function leading to end stage renal disease-ESRD. Sphingosine-1-phosphate (S1P) is generated by phosphorylation of sphingosine by sphingosine kinases (SphK1 and SphK2).

Methods: WT, SphK1$^{-/-}$ and SphK2$^{-/-}$ mice were administered Folic Acid-FA (250 mg/kg, ip) and followed for 14 days. mRNA changes were measured with RT-PCR and protein changes with IHC and western blots.

Results: SphK2$^{-/-}$ mice exhibited lower fibrosis (trichrome staining) compared to WT or SphK1$^{-/-}$ mice at day 14 post FA. Compared to kidneys of FA treated SphK1$^{-/-}$ mice, SphK2$^{-/-}$ mice expressed lower levels of mRNA encoding TGF-β (15.79%, $p<0.001$), α-SMA (30.8%, $p<0.01$) and fibronectin (36.9%, $p<0.01$). Likewise, kidney sections of FA-treated SphK2$^{-/-}$ mice displayed lower levels of collagen and fibronectin immunoreactivity compared to SphK1$^{-/-}$ mice. FA-treated SphK2$^{-/-}$ mouse kidneys had reduced infiltration of macrophages (45.1%, $p<0.05$)) and neutrophils (27.9%, $p<0.01$) compared to SphK1$^{-/-}$ mice. SphK2$^{-/-}$ CD4^{+} T cells have a hyper-proliferative response with significantly higher amounts of IFN-γ compared to WT or SphK1$^{-/-}$ cells. In fibrosis, IFN-γ is anti-fibrotic; therefore, we tested the role of CD4^{+} T cells from SphK2$^{-/-}$ mice transfered into WT and SphK1$^{-/-}$ mice. Transfer of SphK2$^{-/-}$ CD4^{+} T cells to either WT or SphK1$^{-/-}$ mice attenuated the progression of fibrosis at day 14 in the recipients.

Conclusions: We conclude that: 1) FA induces fibrosis to a similar degree in WT and SphK1$^{-/-}$ mice, 2) the lack of SphK2 attenuates FA-induced fibrosis possibly due to hyper-proliferative T cells. Understanding the function of SphK2 may contribute further to our understanding of the pathogenesis of fibrosis and development of a SphK2 selective inhibitor may lead to a new therapeutic agent that impede the progression of kidney disease to ESRD.

Funding: NIDDK Support

SA-OR451

Risk of Vascular Access Events in the FHN Daily Trial

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Background: Daily hemodialysis (HD) improves several patient outcomes, but the potential risks are unclear. We evaluated the risk of vascular access complications in the Frequent Hemodialysis Network (FHN) Daily Trial.

Methods: We randomized 245 patients with end-stage renal disease to receive daily HD (6 days/wk, 1.5-2.75 hrs/session) or conventional HD (3 days/wk, 3-4.5 hrs/session) for 12 months. All patients were dialyzed in-center. Using proportional hazards regression, we compared the time from randomization to first access event (repair or loss) between groups. Fistula and graft repairs included angioplasty, stenting, thrombolysis, thrombectomy, and surgical revision. Catheter repairs included fibrin sheath stripping. Accesses that were removed or could no longer be used were defined as losses. Catheter removals performed electively once a fistula or graft was in use were not counted as events. We adjusted for age and diabetes. In a secondary analysis, we evaluated fistulae and graft events separately from catheters. For this, we considered the first catheter and fistula/graft that was used for each patient at or after randomization.

Results: After mean followup of 8.8 months, there were 75 events in 245 patients (daily group: 33 repairs, 14 losses; conventional group: 17 repairs, 11 losses). Compared to conventional HD, the hazard ratio of first access event with daily HD was 1.77 (95% CI 1.11-2.84 p=0.017). In the 220 patients with arteriovenous fistulae/grafts, the HR of first access event with daily HD was 1.69 (95% CI 1.002-2.78, p=0.049). A similar trend was observed in the 52 patients who had catheters (HR with daily HD=1.40, 95% CI 0.47-4.24, p=0.54).

Conclusions: In the FHN trial, patients receiving daily HD had an increased risk of vascular access events, and this did not differ by access type. Whether this risk is due to increased HD frequency or heightened surveillance is the subject of further study.

Funding: NIDDK Support

SA-OR452

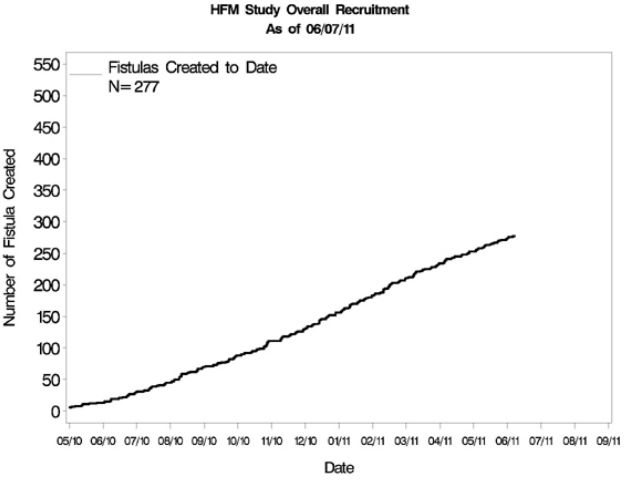
Progress of the Hemodialysis Fistula Maturation (HFM) Study

Gerald J. Beck,[1] Alfred K. Cheung,[2] Laura M. Dember,[3] Harold I. Feldman,[4] Jonathan Himmelfarb,[5] Thomas S. Huber,[6] John W. Kusek,[7] Prabir Roy-Chaudhury,[8] Miguel A. Vazquez,[9] Charles E. Alpers,[2] Michelle L. Robbin,[10] Joseph Vita,[5] The HFM Study Group.[7] *[1]Cleveland Clinic; [2]U Utah; [3]Boston U; [4]U Penn; [5]U Washington; [6]U Florida; [7]NIDDK; [8]U Cincinnati; [9]U Texas Southwestern; [10]U Alabama Birmingham.*

Background: The NIDDK-sponsored HFM Study is designed to identify predictors and causes of AVF maturation failure. HFM will enroll 600 patients undergoing AVF creation. The study group includes 6 clinical centers (Boston U, U Cincinnati, U Florida, U Texas Southwestern, U Utah, U Washington), a Data Coordinating Center (Cleveland Clinic), and 3 Cores: Ultrasound (U Alabama Birmingham), Vascular Function (Boston U) and Histology (U Washington).

Methods: The protocol includes 1) pre-operative assessment of vascular anatomy and function with measurement of flow-mediated dilation, pulse wave velocity, and venous capacitance; 2) detailed intra-operative documentation of surgical procedures; 3) serial post-operative ultrasounds, and 4) uniform characterization of AVF function. Maturation is declared after 4 weeks of successful use under routine clinical care. Follow-up continues until AVF abandonment. Serum, plasma, DNA and vein tissue are collected for biomarker and mechanistic studies.

Results: Enrollment began in 5/2010 and study completion is expected in 2013.



As of 6/7/2010, 277 participants have had AVF creation: 180 were receiving dialysis before surgery; 97 had not yet initiated dialysis. 26 others have not yet had AVF surgery. Baseline characteristics are: 70% male, median age 56 yr, 48% white, 43% black, 57% have DM.

Conclusions: The HFM Study is enrolling participants at a steady rate and successfully collecting multifaceted data that should identify early predictors of AVF maturation and elucidate underlying mechanisms.

Funding: NIDDK Support, Other U.S. Government Support

SA-OR453

Modern Era Access Outcomes: Comparison of Fistulas (AVF) vs. Grafts (AVG) after Year 2000

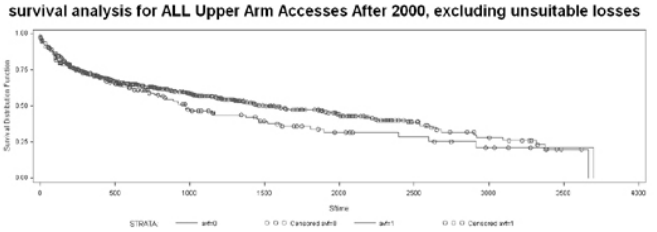
Charmaine E. Lok,[1] Cynthia B. Bhola,[1] Louise M. Moist,[2] Dheeraj Rajan.[1] *[1]Toronto General Hospital, Toronto, ON, Canada; [2]London Health Sciences Centre, London, ON, Canada.*

Background: Fistulas have been promoted to have longer patency and lower complication rates compared to AVGs based on data prior to year 2000 which largely excluded AVFs that were not suitable for dialysis.

We sought to compare the cumulative patency of AVFs and AVGs created after 2000 from the time of their creation to their final abandonment.

Methods: Cumulative patency was compared with Kaplan Meier curves (log rank test). Further analysis of vascular accesses (VA) were based on whether they 1) were first or subsequent VA and 2) failed to mature (FTM) or not. Intervention rates were compared using the exact binomial test (SAS, v9.2).

Results: 1740 VA created (1375 AVF; 365 AVG) between Jan 2000-June 2010 were followed. The mean age was 59 yrs, 63% male, >50% Caucasian, 42% had DM and 68% had HTN. There was no difference in cumulative patency between AVFs (median 7.1months) and AVGs (11.3<INS cite=mailto:t67medc dateTime=2011-06-08T12:23> </INS>months) p= 0.12 when FTM was included. AVFs had superior patency (53.8 months) compared with AVGs (27months) when FTM was *excluded*. This finding of AVF superiority was due to forearm VA only. No differences were found in comparisons of upper arm VA (n=850), regardless of whether AVF that FTM were included or not, or if they were first or subsequent VA.



Grafts had a higher rate of angioplasties (3.18/1000 days) compared to AVF (1.36/1000 days) to maintain patency after cannulation (p<0.001). Grafts also required more thrombolysis vs. AVF (0.98/1000 days vs. 0.06/1000 days; p<0.0001). The intervention rates to attain AVF maturation is not included in this analysis.

Conclusions: AVFs and AVGs created after year 2000 have similar cumulative patency rates. Factors important in VA choice, such as patient preference, complications, interventions to attain function should be considered.

SA-OR454

Pre-Existing Venous Calcification Prior to Dialysis Vascular Access Surgery

Timmy C. Lee, Nida Nida Safdar, Meenakshi J. Mistry, Yang Wang, Begoña Campos, Prabir Roy-Chaudhury. *Internal Medicine, University of Cincinnati, OH.*

Background: Vascular calcification is present in arterial vessels used for dialysis vascular access prior to surgical creation of the access. Calcification in the veins used to create a new vascular access has not previously been documented. The objective of this study was to describe the prevalence of calcification in vein samples collected at the time of new vascular access creation.

Methods: 67 vein samples, collected at the time of new access creation, were studied. Routine immunohistochemistry, using a von Kossa stain, was performed to quantify calcification. A semiquantitative scoring system from 0-4+ was used to quantify the percentage positive area (brown or black) as a fraction of total area (0 = 0-10% positive; 1+ =11-25%; 2+ = 26-50%; 3+=51-75%; and 4+=76-100% positive) for each vascular layer (endothelium, intima, media, and adventitia).

Results: 22/67 (33%) samples showed evidence of calcification.

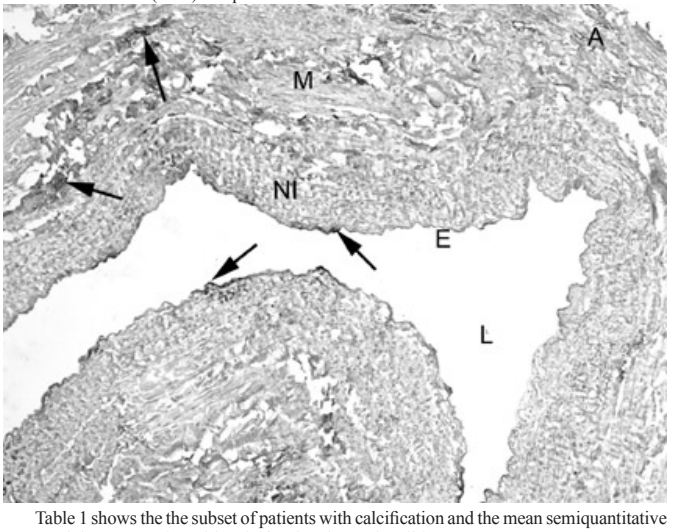


Table 1 shows the the subset of patients with calcification and the mean semiquantitative scores of the 22 samples with calcification for each cell layer.

Table 1:Proportion of Vascular Calcification and Semiquantitative Score by Cell Layer

	Endothelial	Intima	Media	Adventitia
Proportion of Vascular Calcification	4/22 (18%)	19/22 (86%)	22/22 (100%)	7/22 (32%)
Semiquantitative Score	00.18±0.39	1.2±0.66	1.6±0.59	0.36±0.58

Conclusions: Our results are the first to demonstrate that vascular calcification is present within veins used to create new dialysis vascular access. In samples with vascular calcification, the calcification was present predominately within the intimal and medial layers. Important questions that remain to be answered include the pathogenetic role of uremia in the development of venous calcification and clinical impact of vascular calcification on vascular access stenosis and outcomes.

Funding: NIDDK Support

SA-OR455

Primary Arteriovenous Fistula Patency Is Dependent on Venous Distensibilty, Not on Proinflammatory, Procoagulant Markers or Vascular Functional Parameters

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Background: Role of vascular functional parameters, procoagulant and proinflammatory factors that influenced success of primary arteriovenous fistula for hemodialysis (AVF) patency were not elucidated completely. The aim of the study was to explore their correlation with outcome of AVF 4 weeks after creation.

Methods: Prospective, observational, cross sectional study was performed on 114 patients (59 male, age 55.8±15.5) who underwent primary non-dominant forearm AVF creation for hemodialysis. Cephalic vein (CVr) and radial artery radius (RAr), venous distensibility (VD), resistance index (RI) and endothelial function by flow mediated dilatation (FMD) were determined by Doppler sono examination. Serum levels of tissue plasminogen activator (tPA), vascular endothelial growth factor (VEGF), vascular cell adhesion molecule (VCAM) and interleukin 6 (IL-6) were determined by ELISA. Their concentrations and blood vessels examinations were compared between groups based on outcome after 4 weeks of maturation: 1–primary AVF success (n=76), group 2–secondary success (n=30), group 3–AVF creation failure (n=8).

Results: Primary AVF success was reached in 66.7% of patients, secondary in 26.3%. Only 7% patients failed to mature AVF. Comparison between groups showed significant difference in CVr (p=0.039), RAr diameter (p=0.006) and venous distensibility (p=0.044), unlike RI, FMD, serum tPA, VEGF, VCAM and IL-6 levels (p>0.05). Significant correlation was found between outcome and CVr (p=0.028), VD (p=0.017) and RAr (p=0.002). RI correlated with age (p=0.018), and inversely with CVr (p>0.001) and RAr (p=0.013). Significant correlation was found between IL-6 and tPA, VEGF and VCAM (all p<0.05), VEGF and VCAM (p=0.002). Linear regression was only significant for VD (B=0.517, 95% CI 0.0.054-0.981, p=0.029).

Conclusions: Venous distensibility showed to be the most important factor for primary AVF patency after 4 weeks of maturation. Significant correlation was found among procoagulant and proinflammatory biomarkers, but their impact was not significant for an early AVF outcome.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

SA-OR456

Clinical vs. Surgical Fistula Failure Risk Assessment

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Background: Fistulas (AVF) are preferred for vascular access (VA), but ≈ 50% fail. We derived and validated a prediction equation (Risk Score) for AVF failure risk based on age, ethnicity, presence of coronary artery disease (CAD) and peripheral vascular disease (PVD) (Lok, 2006). It is unknown how the Risk Score compares with the surgeon's preopertive evaluation (SPE).

This study compared their predictive ability.

Risk of FTM (%)	FTM Risk Score	Surgeon Assess
Low (25%)	<2.0	Excellent
Moderate (35%)	2.0-3.0	Good
High (50%)	3.1-7.9	Marginal
Very high (70%)	>=8.0	Poor

Methods: AVFs created (2006-2011) were followed for 6 mos to ascertain maturity and need for intervention (n=467). The Risk Score and the SPE were determined by the clinician and surgeon, respectively. The Risk Score was independently validated using strict definitions of CAD and PVD via medical records (V_Score). Kappa statistics compared the Score vs. V_Score and the agreement between the Risk Score and the SPE.

Results: The failure outcomes of 322 AVF by the Risk Score were: low risk-33%; moderate risk-43%; high risk-55%; and very high risk-73% (p=0.001). The Risk Score had a Kappa agreement of 0.85 with the V_Score (15% difference). The AVF failure outcomes by the SPE were: low risk-14%; moderate risk-27%; high risk-69%; and very high risk-70% (p<0.0001). There was fair correlation between the Risk Score and SPE for AVF outcomes, yet both predicted failure well. Despite the ability of both to accurately predict AVF failure, 71% of patients with a >50% risk of AVF failure still underwent AVF creation and 70% of them failed. Of all AVFs created, 36% had interventions; of those AVFs that failed, 42% had interventions.

Conclusions: The FTM Risk Score is strengthened when definitions of CAD and PVD are strictly followed. The surgeon's evaluation correlates poorly with the FTM Risk Score, suggesting that they use parameters not accounted for by the Risk Score and may be complimentary. High failure risks indicated by these two evaluations should prompt serious consideration for an alternate VA. There is need to determine patient eligibility for AVF creation.

SA-OR457

Effect of Recombinant Human Type 1 Pancreatic Elastase (PRT-201) Treatment on Fistula Patency Bradley S. Dixon,[1] Eric K. Peden,[2] David B. Leeser,[3] Mahmoud T. El-Khatib,[4] Prabir Roy-Chaudhury,[4] Jeffrey Lawson,[5] Matthew Menard,[6] Marc H. Glickman,[7] Laura M. Dember,[8] Steven K. Burke.[9] *[1]U Iowa, IA; [2]Weill Cornell MC, NY; [3]Methodist Hospital, TX; [4]U Cincinnati, OH; [5]Duke U, NC; [6]Brigham & Womens' Hospital, MA; [7]Sentara Heart Hosp, VA; [8]Boston U, MA; [9]Proteon Therapeutics, MA.*

Background: Stenosis is a common cause of fistula (AVF) failure. We conducted a phase 1/2 randomized, double-blind, dose-escalation trial to determine if PRT-201 treatment was safe, would promote dilation & prevent failure of newly created AVFs.

Methods: A single dose of PRT-201 (N=45) or placebo (N=21) in 2.5 mL of saline was dripped onto the outside of the AVF over 10 min immediately after creation. Doses were aggregated into low (LD, 0.003, 0.010 & 0.033 mg), medium (MD, 0.1, 0.33 & 1 mg), & high (HD, 3, 6, 9 mg) groups (N=16, 17, 12). Patients were followed for up to 12 mon. Blinded duplex Doppler ultrasound evaluation was done at 6 weeks, & 3 & 6 months. Primary outcomes were safety & immediate change in outflow vein diameter (VD) & blood flow (BF). Secondary efficacy endpoints were AVF maturation (lumen VD ≥4 mm & BF ≥500 ml/min at 6 weeks), & time to primary patency loss (AVF occlusion or procedures required to maintain or restore patency).

Results: No safety concerns occurred. There was a modest but statistically significant immediate change in PRT-201 treated VD (5±6%, p<0.01). Lumen VD and BF increased in all groups with no difference in maturation at 6 weeks. Primary patency at 6 months was not statistically different among groups (Placebo 60%, LD 81%, MD 76%, HD 73%, p=NS). If immediate post-surgical complications that caused patency loss were excluded from the analysis (N=3 patients in LD group) patency loss was reduced in LD versus placebo (HR 0.28, 95% CI=0.08-0.93, p=0.04) and versus HD (HR 0.07, 95% CI=0.01-0.52, p=0.01). Angioplasty was the most common reason for patency loss (placebo 43%, LD 12%, MD 35%, HD 33%), with a trend toward less hemodynamically significant lumen stenosis in LD (placebo 47%, LD 31%, MD 62%, HD 50%).

Conclusions: PRT-201 was safe. At LD, PRT-201 might decrease lumen stenosis & prolong fistula patency but an adequately powered trial is needed.

Funding: Pharmaceutical Company Support

SA-OR458

Far Infrared Therapy Improves Endothelial Function and Access Flow of Newly-Created Arteriovenous Fistula in Patients with Stage 5 Chronic Kidney Disease Chih-Ching Lin.[1,2] *[1]School of Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Divsion of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.*

Background: Endothelial dysfunction plays a significant role in the pathogenesis of malfunction of vascular access. The aim of this study is to evaluate the effect of far infrared (FIR) therapy on endothelial function and access flow (Qa) of newly created AV fistula.

Methods: We enrolled 75 patients (in stage 5 CKD) who were randomly allocated to treatment group (receiving 40 minutes of FIR therapy three times weekly for 3 months postoperatively, N=37) and control group (without FIR therapy, N=38). Access flow of AV fistula was measured by Doppler ultrasonography at 4 timings, including 2 days, 1 month, 2 and 3 months after vascular surgery. Markers of Endothelial function, including asymmetric dimethyl arginine (ADMA) and L-arginine, were measured both immediately before and 3 months after the creation of AV fistula.

Results: Finally, 67 patients completed the study, including 33 in FIR group and 34 controls. In comparison with controls, patients in FIR group had lower values of incremental change of ADMA as well as higher values of Qa at all of the 4 timings and incremental change of L-arginine, thus leading to a higher incremental change in the ratio of L-arginine to ADMA 3 months later (as shown in table 1).

Comparison of the endothelial function and access flow of AVF between HD patients with and without FIR therapy for 3 months

	Control group	FIR group	P value
Case number completing study	34	33	
Qa0 (ml/min)	259.1±85.5	337.9±111.3	0.002
Qa1 (ml/min)	597.9±230.1	805.2±315.7	0.003
Qa2 (ml/min)	681.8±275.2	925.8±354.6	0.002
Qa3(ml/min)	770.6±344.0	987.3±375.1	0.016
Δ L-arginine 3-0 (μM)	-0.7± 2.9	2.2± 5.2	0.007
ΔADMA 3-0 (μM)	0.01±0.05	-0.05±0.07	<0.001
(L-arginine3/ADMA3)/ (L-arginine0/ ADMA0)	0.98± 0.08	1.09±0.12	<0.001

Qa0 /Qa1/ Qa2/ Qa3: Qa measured at 2 days, 1, 2, or 3 months after AVF creation; ADMA0/3 or L-arginine0/3 indicates the concentration measured 1 day before/3 months after AVF creation

Conclusions: In conclusion, 3 months of FIR therapy improves endothelial function and access flow of newly created AVF in patients with stage 5 CKD.

Funding: Government Support - Non-U.S.

SA-OR459

Improved Arteriovenous Fistula Maturation with Intra-Operative Implant of a Perianastomotic Sirolimus Eluting Collagen Membrane (Coll-R) Maria V. DeVita,[1] Eric S. Chemla,[4] Kipshidze Nickolas,[2] Surendra Shenoy,[3] Sriram Iyer.[1] *[1]Lenox Hill Hospital, New York, NY; [2]Center of Angiology and Vascular Surgery, Tbilisi, Georgia; [3]Washington U School of Medicine, St Louis, MO; [4]St. George's Healthcare NHS Trust, London.*

Background: Nonmaturation of arteriovenous fistulae (AVF) remains a major limiting factor for their use in hemodialysis patients(pts). Etiologies include perianastomotic stenosis and neointimal hyperplasia of the draining vein. Since there is no proven solution, novel approaches are needed. The Coll-R, an investigational product (Vascular Therapies, NJ, USA) is indicated and designed for perivascular implantation. It consists of collagen, a topical hemostat and sirolimus, an anti-proliferative agent with proven efficacy for suppressing neointimal tissue growth when delivered locally to the vascular wall. The goal of this study was to evaluate the performance and safety of the Coll-R when applied around the anastomotic site and outflow vein during creation of AVF. In this first in human study, endpoints were freedom from Coll-R related adverse effects and time to maturation.

Methods: Pts. from 2 hospitals inTbilisi, Georgia, scheduled for AVF creation were invited to participate. Venous mapping was performed to assure suitable vascular anatomy. The Coll-R was implanted intra-operatively during AVFcreation. Data collected included technical success of the implantation, wound healing, time to unassisted maturation and whole blood sirolimus levels.

Results: Thirty pts, 17 male, mean age 51 years (range 25-77) underwent radiocephalic (n=22) or brachiocephalic(n =8) AVF creation with implantation of the Coll-R (675μg of sirolimus). All completed a minimum of 11 weeks of follow up. There were no Coll-R related technical failures or adverse events. AVF matured in 26 pts. (87%); mean maturation time was 27±18 days; 4 pts thrombosed in <4 weeks; mean peak sirolimus level was 4.13 ng/ml seen 6 hr post-op. Two late AVF failures occurred at 119 and 170 days respectively.

Conclusions: Coll-R implantation during AVF creation does not cause systemic immunosuppression and improves fistula maturation. Use of this novel therapy may provide an unmet clinical need.

Funding: Pharmaceutical Company Support

SA-OR460

Frequent Hemodialysis Fistula Infectious Complications Charmaine E. Lok,[1] Sarah Daisy Kosa,[1] Christopher T. Chan,[1] Deborah Lynn Zimmerman.[2] *[1]Toronto General Hospital, Toronto, ON, Canada; [2]Ottawa Hospital, Ottawa, ON, Canada.*

Background: Frequent hemodialysis (FHD) is associated with many beneficial clinical outcomes. Fistulas (AVF) are the preferred access for FHD; however there is a paucity of data on the impact of frequent cannulation and infectious complications. We compared the rate of infections in patients on FHD who cannulated their AVF using buttonhole (BH) vs. stepladder (SL) techniques. A second comparison was made with conventional intermittent HD (CIHD) patients using SL cannulation.

Methods: Patients who received short daily hemodialysis (SDH)(≥5x/wk) and nocturnal hemodialysis (NHD) (≥3 x/week, ≥5 hrs/session) who were dialyzed with an AVF were prospectively followed for infectious complications between Jan 2001-Dec 2010. Rates of bacteremia and BH site infections were compared using the exact binomial test.

Results: Forty-six patients had SDH and 128 had NHD. For self cannualtion, 50% of SDH and 72% of NHD used BH technique. In total, the BH technique was used on 198,910 fistula days while SL cannulation was performed on 99,681 fistula days. There were 39 BH-related bacteremias and at least 2 local BH-site infections (BHI). The BH bacteremia rate was 0.196/1000 fistula days. Staphylococcus aureus accounted for 85% of bacteremias. There were 5 related hospitalizations, and 3 metastatic infections (endocarditis, septic arthrisit, mycotic aneurysim and loss of fistula). In comparison, there was 1 possible fistula related infection in CIHD between Jan 1 2000-dec 31 2010 (rate of 0.002/1000 fistula days).

Conclusions: The rate of BH related infection is high in patients who self cannulate on FHD. The rate of bacteremia is >50 times that on CIHD. The majority of bacteremias are due to s. aureus and the consequences are serious with 13% requiring hospitalization and 10% with metastic spread or loss of access. The risks and benefits of BH cannulation require individual consideration with careful monitoring, prophylaxis and management.

Funding: Clinical Revenue Support

TH-PO001

Response of the Human Kidney to Clamp Ischemia Dipen J. Parekh,[1] Barbara Ercole,[1] Claudia Schrimpf,[2] Manjeri A. Venkatachalam,[1] Joel M. Weinberg.[3] *[1]UTHSC San Antonio; [2]Harvard Institutes of Medicine; [3]University of Michigan.*

Background: Structural changes in tubule cells during clamp ischemia are well characterized for animal models, but their timing and extent in the human kidney has not been established and may differ significantly, depending on the clinical setting.

Methods: We biopsied uninvolved areas of kidney in patients undergoing open partial nephrectomy for renal masses before renal artery clamping, then during periods ranging from 15 to 60 min. of warm and cold ischemia (80% > 30 min.), and then after 5 minutes of reflow for ultrastructure (N=39) and for immunofluorescence and rhodamine phalloidin staining (N=22).

Results: During the clamp period, apical membrane structure was remarkably well preserved with only patchy brush border clubbing, fragmentation, desquamation and blebbing and not in all patients. Mitochondria developed progressive swelling, which paradoxically was more prominent in distal than proximal tubule cells. This resolved during the 5 minutes of reflow in most cells in most patients, but persistence of swelling and development of matrix condensation occurred occasionally. Using a composite 0-5 scale covering the full spectrum of ultrastructural changes, average scores were: Preclamp 1.02±0.07, End clamp 2.18±0.07, Post clamp 1.86±0.09. Consistent with the ultrastructure, staining for F-actin with rhodamine phalloidin was well preserved. Immunostaining for phosphotyrosine, which reflects cellular ATP content, was decreased in 68.4% of the clamp biopsises and 52.6% of the postclamp biopsies with larger changes in proximal tubules, however β1 integrin was decreased in only one post clamp biopsy. ICAM-1 expression in peritubular capillaries was increased in 46.7% of the clamp biopsies and 66.7% of post clamp biopsies. None of the patients developed acute kidney injury.

Conclusions: These data provide the first detailed analysis of the structural response of the human kidney to clamp ischemia and document many of the expected structural alterations based on prior animal work, but indicate a greater than expected resistance to injury in this commonly used clinical application of clamp ischemia.

Funding: NIDDK Support, Private Foundation Support

TH-PO002

MicroRNA-687 Targets PTEN To Regulate Hypoxic/Ischemic Kidney Injury Kirti Bhatt, Qingqing Wei, Zheng Dong. *Department of Cellular Biology and Anatomy, Georgia Health Science University and Charlie Norwood VA Medical Center, Augusta, GA.*

Background: MicroRNAs are small, endogenous, non-coding RNAs that are critical regulators of gene expression in various pathophysiological conditions. Recent studies have suggested an emerging role of microRNAs in both renal development and pathophysiology. Targeted deletion of Dicer (a key enzyme for microRNA biogenesis) from proximal tubules protects against renal ischemia-reperfusion injury (IRI), demonstrating a pathogenic role of microRNAs in renal IRI. Despite these findings, the specific microRNA species that contribute to renal IRI are not known.

Methods: To identify the pathogenic microRNAs, we analyzed microRNA expression by microRNA microarray.

Results: Out of 220 detectable microRNAs, 138 were decreased to varying degrees while 70 microRNAs were induced during renal IRI in C57BL/6 mice. Among these microRNAs, microRNA-687 (miR-687) was most dramatically up-regulated. miR-687 was also induced by hypoxia in cultured renal proximal tubular cells. Bioinformatic analysis suggested that the tumor suppressor PTEN is a top candidate target of miR-687. The 3'UTR of PTEN gene contains a miR-687 binding sequence, which was shown to be a target sequence of miR-687 in a luciferase reporter assay. Consistently, miR-687 induction during hypoxia in cultured cells and renal ischemia-reperfusion in mice was followed by a concomitant decrease in PTEN expression. Notably, blockade of miR-687 with anti-miR-687-LNA could prevent PTEN decrease under these conditions. Functionally, anti-miR-687-LNA reduced apoptosis and induced cell cycle arrest in hypoxic cells.

Conclusions: Together, this study has identified miR-687 as a novel regulator of PTEN during renal hypoxia and ischemia-reperfusion injury. Targeting specific microRNAs may offer a new strategy for renoprotection.

Funding: NIDDK Support, Veterans Administration Support

TH-PO003

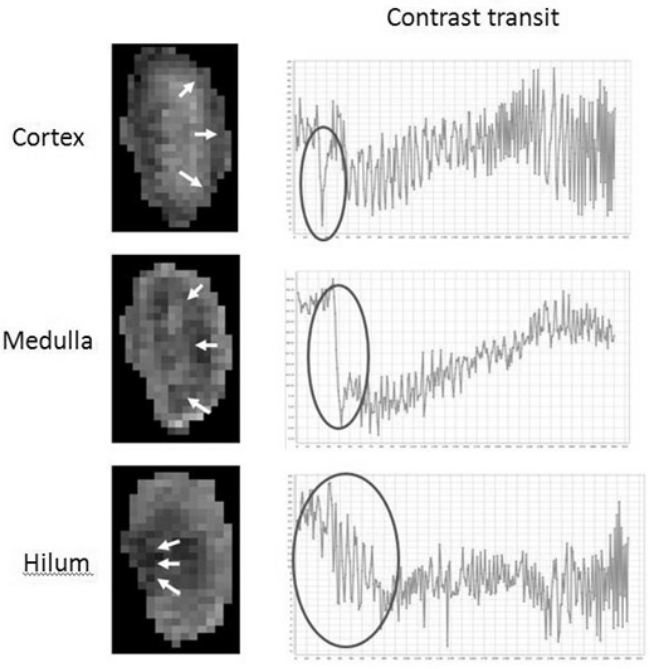
Utility of Local Arterial Input Function in Dynamic Susceptibility Magnetic Resonance Imaging To Identify Regional Renal Perfusion Defect Andrew M. Siedlecki. *Internal Medicine, Washington University in St. Louis, St. Louis, MO.*

Background: Rapid acquisition of magnetic resonance signal in tissue may offer a highly sensitive platform for the study of renal blood flow. We hypothesize that dynamic susceptibility contrast-magnetic resonance imaging (DSC-MR) can accurately measure regional renal blood flow in the kidney when the standard global arterial input function is replaced by per voxel local arterial input function (LAIF).

Methods: We performed mild ischemic injury in mice and measured renal perfusion after injury by means of magnetic resonance imaging and Doppler flowmetry. For MR studies, a multi-dimensional integral was constructed to account for the properties of gadolinium contrast flow, within single voxels of space. Posterior probabilities were derived from conditional probabilities by applying Bayesian evidential theory. Invasive measurement of cortical and medullary blood flow was performed by Doppler flowmetry.

Results: Invasive measurement of surface cortical blood flow by Doppler flowmetry returned to baseline within 35 minutes following mild (15 minute) ischemic injury (IRI, n=5; sham, n=5). In a separate cohort (IRI, n=5; sham, n=5), MR imaging showed a delayed transit in contrast through the kidney vasculature at a more distant time point, 45 minutes following ischemic injury. Perturbed blood flow in procedurized animals compared to shams was manifest in a significatnly prolonged contrast outflow, β, and mean transit time (MTT)(seconds) ($p<0.01$).

Conclusions: We apply for the first time the concept of convolution-free local arterial input function in the kidney using nuclear magnetic resonance technology. This study is an advancment in the analysis of renal blood flow as it obviates computational deconvolution and offers a non-invasive assessment of regional blood flow.



TH-PO004

Over-Expression of cGMP-Dependent Protein Kinase I (PKG-I) Attenuates Ischemia Reperfusion Induced Kidney Injury Shuxia Wang. *Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY.*

Background: cGMP-dependent protein kinase (PKG) is a multifunctional protein. Whether PKG plays a role in ischemia/reperfusion induced kidney injury (IRI) is unknown.

Methods: In this study, using an in vivo mouse model of renal IRI, we determined the effect of renal IRI on kidney PKG-I levels and also evaluated whether overexpression of PKG-I attenuates renal IRI.

Results: Our studies demonstrated that PKG-I levels (mRNA and protein) were significantly decreased in the kidney from mice undergoing renal IRI. Moreover, PKG-I transgenic mice had less renal IRI, showing improved renal function and less tubular damage as compared to their wild type littermates. Transgenic mice in renal IRI group had decreased tubular cell apoptosis accompanied with decreased caspase 3 activity and increased expression of Bcl-2, Bag-1 and p-AKT. In addition, transgenic mice undergoing renal IRI demonstrated reduced macrophage infiltration into the kidney and reduced production of inflammatory cytokines. In vitro studied showed that peritoneal macrophages isolated from transgenic mice had decreased migration as compared to control macrophages.

Conclusions: Taken together, these results suggest that PKG-I protects against renal IRI, at least in part through inhibiting inflammatory cell infiltration into the kidney, reducing kidney inflammation, and inhibiting tubular cell apoptosis.

Funding: NIDDK Support, Other NIH Support - NCRR P20

TH-PO005

Thrombin and PAR-1 Regulate Sphingosine Kinases 1 and 2 and Sphingosine-1-Phosphate Receptors Following Renal Ischaemia and Reperfusion Injury Jonathan H. Erlich,[1,2] Anthony Chuang,[1] Sean E. Kennedy,[3] Dian Purnamasari.[3] *[1]Prince of Wales Clinical School, University of New South Wales, Sydney, NSW, Australia; [2]Nephrology, Prince of Wales Hospital, Randwick, NSW, Australia; [3]School of Women's & Children's Health, University of New South Wales, Sydney, NSW, Australia.*

Background: Recent studies have suggested sphingosine-1-phosphate signaling (S1P) via the S1P receptor-1 (S1Pr1) contributes to protection following renal ischaemia reperfusion injury (IRI). S1P generation via expression and activation of sphingosine kinase (SPHK) has been suggested to be a potentially important downstream secondary signaling system for protease activated receptor-1 (PAR-1).

Methods: In an established model of renal IRI using 25 min bilateral renal ischaemia and varying times of reperfusion (2, 5 and 24h) we examined the effects of PAR-1 deficiency, PAR-1 inhibition using hirudin and reduced thrombin generation using low-TF mice. As previously shown, all strategies that reduced PAR-1 signaling resulted in reduced renal injury.

Results: In wild type (WT) mice SPHK-1 mRNA was slightly induced at 2 and 5 h with just over a doubling by 24h (2.31 ± 0.35 units). In contrast SPHK-2 showed a similar level of induction at 2h but decreased at 5 and 24 (0.76 ± 0.35 units) compared to sham control. PAR-1 deficient mice had mild induction of both SPHK-1 and -2 mRNA at 2h with 5 to 10-fold induction at 5h and in excess of 100-fold by 24h. Of note PAR-1 deficient mice had higher basal SPHK-2 mRNA expression. Hirudin treated and low-TF mice had elevated basal SPHK-1 and -2 mRNA that initially decreased but subsequently increased by 24h reperfusion. At all times those mice had higher levels of SPHKs than WT mice. WT and PAR-1 deficient mice showed different patterns of S1P receptor mRNA induction with WT showing early induction of S1Pr1 and latter reductions compared to PAR-1 deficient mice that showed no induction of S1P r1 and r3 at 2h but dramatic up regulation at 5 and 24h.

Conclusions: Taken together the data suggests that thrombin negatively regulates SPHK and S1P receptor expression via PAR-1 signaling. This suggests at least some of the protective effect of PAR-1 deficiency on renal IRI may be via increased S1P signaling.

Funding: Clinical Revenue Support

TH-PO006

The Role of Fibrinogen in Ischemic Acute Kidney Injury Inga Soerensen, Nathan D. Susnik, Hermann G. Haller, Roland Schmitt. *Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: Fibrinogen (Fib) has heterogeneous roles in tissue injury, inflammation and repair. We studied the role of Fib in ischemic acute kidney injury (AKI).

Methods: Intrarenal Fib was analyzed in post-ischemic mouse kidneys using immunohistochemistry, immunoblot, PCR and In situ hybridization. Fib -/- and +/- mice that underwent unilateral or bilateral renal clamping were followed for survival and renal function. Post-ischemic kidneys were analyzed by quantification of damage markers, inflammatory infiltrates, apoptosis and cell proliferation. In transplant experiments kidneys from Fib +/- and -/- mice were transplanted into -/- recipients. In vitro, primary renal tubular epithelial cells and proximal tubular epithelial cells lines were stimulated with different conditions to test for Fib expression by qPCR.

Results: .27 min of transient renal ischemia was associated with more than a 10-fold increase in intrarenal Fib levels. While Fib signal was confined to the intravascular space in control kidneys, it was found throughout the tubulointerstitium in postischemic kidneys. In agreement with recent microarray data, Fib de novo expression was also found in a subset of epithelial cells of postischemic kidneys by immunofluorescence and in situ hybridization. A significant renal mRNA up-regulation of Fib alpha, beta and gamma subunits was confirmed by qPCR. In vitro, Fib was induced in renal tubular epithelial cells by exposure to homogenate from ischemic kidneys, Oncostatin-M and hyper-IL6. In vivo, Fib -/- mice subjected to renal ischemia showed reduced survival and worse renal function but levels of tubular injury markers Kim-1 and NGAL were significantly lower than in Fib +/- kidneys. To address discrepancies between local and systemic effects of Fib deficiency, kidneys were transplanted from Fib -/- and +/- mice into Fib -/- recipients.

Conclusions: Our data indicate that Fib is expressed in small but significant amounts by tubular epithelial cells in response to inflammatory signals in AKI. Although genetic deletion of systemic Fib is associated with higher mortality, our results suggest that the deletion of intrarenal Fib expression may be protective against post-ischemic damage.

Funding: Government Support - Non-U.S.

TH-PO007

Tumor Necrosis Factor alpha Downregulates Tamm-Horsfall Protein (Uromodulin) Expression in Acute Kidney Injury Tarek M. El-Achkar,[1] Ruth A. McCracken,[1] Soline Bourgeois,[3] Ziyad Al-Aly.[2] *[1]Saint Louis University and Saint Louis VA Medical Center; [2]Saint Louis VA Medical Center; [3]University of Zurich.*

Background: Acute kidney injury (AKI) is a common disease with serious health implications. We recently described a protective role for Tamm-Horsfall protein (THP) against AKI. THP is expressed exclusively in the thick ascending limbs (TAL) of the kidney, but the factors that regulate the expression of THP in AKI are still unknown. In this study, we investigate whether tumor necrosis factor alpha (TNFα), a cytokine expressed by TAL and up-regulated during AKI, is a major regulator of THP in AKI.

Methods: We used a mouse model of ischemia-reperfusion injury (IRI) achieved by bilateral pedicle clamping. We also performed in vitro IRI on a pure thick limb cell line (MKTAL) using an established model of mineral oil layering.

Results: Using real-time PCR, we show that THP mRNA is transiently down-regulated in the kidney after IRI. Concomitantly, we observed an increase in TNFα mRNA levels after injury. Using immunofluorescence microscopy, we localized this TNFα up-regulation to TAL and S3 segments of the outer medulla. Similarly, in MKTAL cell culture, IRI increased TNFα expression while suppressing THP expression. Incubating MKTAL cells with TNFα suppresses THP mRNA in a dose dependent manner, and reproduces the findings seen in ischemic injury in vivo. Blocking TNFα after IRI significantly abrogates the suppression of THP expression.

Conclusions: In conclusion, our data suggest that the surge of TNFα at the onset of AKI down-regulates THP expression. Because of the protective function of THP, modulation of THP expression by TNFα blockade could present a therapeutic opportunity to prevent and attenuate AKI.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO008

Nitric Oxide Induced Regulation of Renal Organic Cation Transport after Renal Ischemia/Reperfusion Injury Reinhard Schneider,[1] Boris Betz,[1] Kerstin Moeller-Ehrlich,[2] Christoph Wanner,[1] Hermann Koepsell,[3] Christoph Sauvant.[4] *[1]Dept. Medicine I, Div. Nephrology, University Hospital, Wuerzburg; [2]Center of Experimental Molecular Medicine, Wuerzburg; [3]Instiute of Anatomy I, Bay. Julius-Maximilians University, Wuerzburg, Germany; [4]Clinic for Anaesthesiology, University Hospital, Halle (Saale), Germany.*

Background: Renal organic cation transporters (Oct) are downregulated by inflammatory nitric oxide (NO) production in rat endotoxemia. NO generated by inducible NO synthase (iNOS) is substantially increased in renal cortex after renal ischemia/reperfusion (I/R) injury. Therefore, we investigated effects of iNOS-specific NO inhibition on the expression of the organic cation transporters rOct1 and rOct2 (Slc22a1, Slc22a2) after I/R injury; both *in vivo* and *in vitro*.

Methods: *In vivo*, I/R injury was induced by bilateral clamping of the renal arteries for 45 min in SD-rats, while I/R injury was simulated *in vitro* in renal proximal tubular cells (NRK-52E) by a condition of oxygen depletion, aglycemia and acidosis for 120 min. N^6-(1-iminoethyl)-L-lysine (L-NIL) was applied as specific iNOS inhibitor.

Results: *In vivo*, L-NIL inhibited NO generation after I/R injury. Moreover, L-NIL abolished I/R-induced downregulation of rOct1 and rOct2 as determined by qPCR and western blotting. Functional evidence was obtained by measuring fractional excretion (FE) of the endogenous organic cation serotonin. Concordant with the expression of the rate-limiting Oct, the FE of serotonin decreased after I/R injury and was abolished by L-NIL. *In vitro*, I/R downregulated both rOct1 and rOct2, which were also abolished by L-NIL; the same was true for the uptake of the organic cation MPP.

Conclusions: We showed that renal I/R injury downregulates rOct1 and rOct2 expression as well as concomitant transport function; both most probably mediated via NO. In principle, this may be an autocrine effect of proximal tubular epithelial cells. Moreover, we conclude that rOct1, or rOct1 and rOct2 limit (also NO-dependent) the excretion rate of endogenous serotonin; this may have additional clinical impact due to its potential of inducing vascular damage.

Funding: Government Support - Non-U.S.

TH-PO009

Effect of Gender Differences on Renal Inflammatory Response after Renal Ischemia-Reperfusion Injury in Mice Kyung Pyo Kang, Jung Eun Lee, Aesin Lee, Yujin Jung, Ki Dong Lee, Sik Lee, Sung Kwang Park, Won Kim. *Department of Internal Medicine, Research Institute of Clinical Medicine and Diabetes Research Center, Chonbuk National University Medical School, Jeonju, Republic of Korea.*

Background: Male gender is associated with more rapid progression in renal disease, while female gender is slowly. This gender disparity may be influenced by sex hormonal milieus. The purpose of this study is to investigate the effect of gender differences on the renal inflammatory responses after acute ischemia-reperfusion injury in mice.

Methods: Experiments were performed in male and female C57BL/6 by bilateral renal ischemia-reperfusion injury. In other study, orchiectomy or ovariectomy was carried out 14 day before I/R injury. Different groups of animals were treated by testosterone and 17β-estradiol. Histologic examination and qRT-PCR for TNF-α, IFN-γ, and MCP-1 were performed.

Results: In acute ischemia-reperfusion kidney injury model, female gender was more resistance to the kidney injury compared to the male gender. The tubular injury score and the number of F4/80(+) macrophages were increased in male mice compared to the female mice. The expressions of TNF-α, IFN-γ, and MCP-1 were increased at 2 d of AKI in male mice compared to female. Orchiectomised male mice were more resistance to renal injury compared to the normal male mice. But, treatment of testosterone to the orchiectomised mice increased renal injury and infiltration of F4/80(+) macrophages. Ovariectomized female mice were prone to renal injury and associated with increased infiltration of F4/80(+) macrophages. The expressions of TNF-α, IFN-γ, and MCP-1 were decreased in the orchiectomised male mice, while increased in the ovariectomized female mice compared to the normal male and female mice. Hormone replacement such as testosterone and estrogen reverse these cytokine expressions.

Conclusions: These results suggested that male gender is more susceptible to the ischemia-reperfusion renal injury. The testosterone may be one of causes of increased susceptibility, while estrogen may be protective in renal inflammatory response after ischemia-reperfusion injury.

Funding: Government Support - Non-U.S.

TH-PO010

ADAM-10 Is the Major Sheddase of Membrane-Associated Meprin A Release during Acute Kidney Injury Christian Herzog, Sudhir V. Shah, Gur P. Kaushal. *Medicine, UAMS, Little Rock, AR.*

Background: Meprin A, comprised of α and β subunits is a potent membrane-bound metalloproteinase highly expressed in proximal tubules of the kidney cortex where it is bound by its β subunit to the brush-border membrane. In acute kidney injury (AKI) meprin A is released and redistributed to the cytosol and may become deleterious to the kidney. The present study has identified the protease involved in the release of meprin A during AKI.

Methods: Meprin A localization was determined by double-immunofluorescence staining and Western blot of mouse kidneys exposed to cisplatin (CP) or ischemia-reperfusion (IR) injury. Stable transfectants of meprin β and cotransfectants of α+β subunits in HEK cells served an in vitro model to investigate the protease involved in meprin A shedding. Broad-spectrum inhibitors to serine-, metallo-, acidic-proteases and the ADAM family of proteases followed by highly specific inhibitors were examined for their ability to prevent shedding of meprin A in the presence / absence of the inflammatory agent phorbol myristate acetate (PMA). Knockdown experiments with siRNAs to ADAM-9, -10 and -17 and overexpression of ADAM-10 with a plasmid construct confirmed the putative sheddase.

Results: Meprin A localization was altered from the brush-border membrane towards the cytosol and the basolateral membrane in mice subjected to CP and IR injury. Broad-spectrum inhibitors of various classes of proteases indicated involvement of proteases of the ADAM family. Since ADAM-9, -10 and -17 are known for ectodomain shedding we determined wether one or all of them are involved in the shedding. GI-254023X, a highly potent inhibitor of ADAM-10 and to a lesser degree of ADAM-17, reduced meprin shedding significantly in HEK cells stably transfected with meprin β and αβ. SiRNA to ADAM-10 reduced shedding to less than 50% whereas siRNAs to ADAM-9 and -17 were much less effective (-30% or -10%). Overexpression of ADAM-10 by transient transfection ± PMA led to a 2-fold increase in shedding in both the meprin β and αβ transfectant.

Conclusions: Our results demonstrate that ADAM-10 is the major sheddase for meprin A release and both meprin A and ADAM-10 can therefore be valuable therapeutic targets in the prevention of AKI.

Funding: NIDDK Support, Veterans Administration Support

TH-PO011

SFKs Regulate Both Pore and Leak Pathways of Paracellular Permeability in Renal Epithelial Cells Devin S. Caswell, Shuchie Jaggi, Danielle Janosevic, Victoria V. Rohring, Josephine Axis, Kurt Amsler. *Biomedical Sciences, New York College of Osteopathic Medicine, Old Westbury, NY.*

Background: Regulation of renal epithelial cell paracellular permeability is critical for normal renal tubular function. Dysregulation is associated with many renal pathogenic states, including renal ischemia/reperfusion injury, radiocontrast-induced nephropathy, and exposure to calcium oxalate crystals. We have begun investigating the role of src Family Kinases (SFKs) in regulating steady state renal epithelial cell paracellular permeability.

Methods: Renal cells were grown on semipermeable membranes. Paracellular permeability was quantitated by measuring calcein flux and TER. Protein content was quantitated by Western blot.

Results: As reported previously, under steady state conditions renal epithelial cells (LLC-PK1, mIMCD3, and MDCK) maintain a barrier to the paracellular movement of both small ions (pore pathway; measured as TransEpithelial Resistance (TER)) and large solutes (leak pathway; measured as flux of calcein and fluorescein-dextran4000). PP1 and PP2 (well-characterized, broad spectrum SFK inhibitors) but not PP3 (an inactive analog) produced a concentration-dependent increase in both pore and leak pathway permeabilities without producing significant cell death (Trypan Blue-staining nuclei). This effect was observed within 1-2 hours. Neither basal nor the PP1/2-mediated increase in paracellular permeability was markedly affected by c-src knockdown or expression of either dominant-negative or wild type c-src. PP1/2 treatment did not produce changes in the cellular contents of occludin, ZO-1, ZO-2, or claudin-1. PP1/2 treatment also did not affect claudin-1 partitioning between Triton X-100 soluble and insoluble fractions.

Conclusions: These results demonstrate that SFK activity regulates renal epithelial cell paracellular permeability through both the pore and leak pathways under steady state conditions. This regulation is not dependent on c-src activity, implicating another SFK (c-yes or fyn).

TH-PO012

miR-127 Regulates Trafficking in Proximal Tubule Cells Trough Its Target KIF3B in Response to Ischemia/Reperfusion Elia Aguado Fraile, Edurne Ramos, Nuria Villegas, Elisa Conde, Ignacio Blanco Sanchez, Laura Garcia-Bermejo. *Pathology, Hospital Universitario Ramón y Cajal.*

Background: Acute renal failure and renal trasplant are clinical situations associated to ischemic insult. In kidney, ischemia/reperfusion (I/R) provokes proximal tubule cell adhesion alterations and dysfunction, including cell trafficking. Cellular transport is mainly regulated by tubuline and adaptors proteins such as kinesin and kinesin-associated proteins, including KIF3B. miRNAs are key regulators of the cell response to stress and we have previously reported that miR-127 is a mediator of proximal tubule cells response to ischemia.

The aim of this work is to identify miRNAs and their targets mediating the proximal tubule cell response to ischemia

Methods: Rat proximal epithelial cells (NRK-52E) were submitted to hypoxia and nutrient deprivation (H/R), mimicking I/R. Monolayer impedance was estimated by RTCA device. Pinocytosis was determined by cell incubation with DEXTRAN-FITC. KIF3B expression was measured by western blot and qRT-PCR. Cytoskeleton and tight junctions (TJ) organization was studied by immunofluorescence.

Results: In this work, we demonstrated using pre/anti-miR-127 transfection that this miRNA is promoting cell adhesion after H/R. miR-127 overexpression increases monolayer impedance and maintains ZO-1 localization in cell membrane assuring TJ integrity. Moreover, tubulin cytoskeleton alterations during H/R are reduced by miR-127 overexpression. On the other hand, NRK-52E cells pinocytosis capacity is increased when miR-127 is inhibited, indicating that cell trafficking is controlled by this miRNA. Therefore, we have identified KIF3B as a real target of miR-127. Indeed, overexpression and inhibition of this miRNA leads to KI3FB mRNA and protein modulation.

Conclusions: These results demonstrate that KIF3B is a real target of miR-127 in NRK-52E cells, modulating proximal tubule pinocytosis function. These miRNA is involved also in cell-cell adhesion maintenance, playing a critical role in preservation of epithelial barrier function. Thus, miR-127 appears as a key regulator of tubular response to ischemia and could be considered as a potential therapeutic target in renal ischemic injury.

Funding: Government Support - Non-U.S.

TH-PO013

Acute Kidney Injury but Not Bilateral Nephrectomy Increases Metabolism, Reactive Oxygen Species, and Mitochondrial Mass in Tubular and Mesenchymal Stem Cells Jon D. Ahlstrom,[1] Daniel Oldroyd,[1] Florian Togel,[2] Zhuma Hu,[1] Ping Zhang,[1] Christof Westenfelder.[1,3] *[1]Medicine, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Medicine, Cornell College of Medicine, New York, NY; [3]Physiology, University of Utah, Salt Lake City, UT.*

Background: Acute kidney injury (AKI) is a treatment-resistant syndrome in which complex mechanisms mediate injury and repair. We demonstrated that mesenchymal stem cells (MSC) are highly renoprotective in rats with AKI, and promising in a Phase I Trial (*Nature Rev Nephrol* 2010). "Uremic" changes in the internal milieu caused by I/R-induced AKI result in pathological manifestations in virtually all major organs.

Methods: To further investigate in rats the systemic effects of comparable levels of acute azotemia, induced either by bilateral IR-AKI or bilateral nephrectomy (NPHX), we exposed cultured normal rat kidney cells (NRK, proximal) and rat MSC to 10% serum obtained from rats 24 hrs post either 50 min IR-AKI, NPHX or from shams. Both AKI and NPHX resulted in similar increases in serum creatinine (AKI: 4.9; NPHX: 4.8 mg/dL).

Results: Exposure of cells to 10% AKI sera x 48 hrs caused, compared to incubation with NPHX or sham sera, increased metabolic activity (CellTiter-Blue/Alamarblue cell viability assay), greater reactive oxygen species activity (CM-H2DCFDA, flow cytometry), and increased mitochondrial mass (Mitotracker Green, flow cytometry).

Conclusions: Summary: At comparable levels of azotemia (AKI vs. NPHX), AKI induced distinct changes in the internal milieu that adversely and similarly affected NRK and MSC. This indicates that the injured kidney per se generates harmful signals that target NRK and MSC, and, by inference, renal and extra-renal cells in vivo. Conclusion: These data identify yet inadequately understood pathogenic signals that are generated by the injured kidney, and whose nature, once defined, should advance our understanding of the acute uremic state and how it affects renal and other organ functions. And, these initial observations provide the basis for the development of interventions that are both renoprotective and that optimize MSC-based therapies of AKI.

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TH-PO014

Collecting Duct Specific Mitochondrial Injury Aggravates Unilateral Ureteral Obstruction Renal Injury in Mice Young Tai Shin,[1] Jin Young Jeong,[1] Bo Ra Lee,[1] Won Ik Jang,[1] Hyunjun Ju,[1] Yoon-Kyung Chang,[2] Seong Suk Kim,[3] Dae Eun Choi,[1] Ki Ryang Na,[1] Kang Wook Lee.[1] *[1]Internal Medicine, Chungnam National University, Daejeon, Korea; [2]Internal Medicine, Daejeon Saint Mary Hospital, Daejeon, Korea; [3]Internal Medicine, Sun Hospital, Daejeon, Korea.*

Background: In various renal injuries including toxins, drugs, and ischemia, mitochondrial injury plays important role in renal injury. There are few model of mitochondrial injury in kidney. Also, there is few study for roles of mitochondria on collecting ducts. We generated collecting duct specific mitochondrial injury model. And, we evaluated the effects of mitochondrial dysfunction on collecting ducts in unilateral ureteral obstructed mice kidney.

Methods: For generation of collecting duct specific mitochondrial injury mice, CRIF flox/flox mice were bred with Hoxb7-Cre mice. For evaluation of influence of CRIF1 deletion on mitochondrial function, we measured O2 consumption and membrane potential in control and silencing RNA treated mIMCD cells. For evaluation of effect on UUO induced renal injury, We divided mice into the following 4 groups: CRIF1flox/flox(WT) group; CRIF1 flox/flox-Hob7 Cre (CRIF1-KO) group; WT UUO group; and CRIF1-KO UUO group. We evaluated MCP-1, osteopontin, F4/80, TGF-b, a-SMA. We measured 8-OHDG for oxidative stress.

Results: Inhibition of Crif1 mRNA in mIMCD cell reduced O2 consumption and membrane potential. There are no significant differences in blood and urine chemistry including Na, K, Cl, urea nitrogen, and creatinine between WT and CRIF1-KO mice. Renal expression of MCP-1, OPN, Numbers of F4/80 positive cells, TGF-b, a-SMA, and Masson Trichrome stained area were significantly increased in CRIF1-KO-UUO kidneys compared

with WT UUO kidneys., Urinary 8-OHDG was increased in CRIF1-KO-mice compared with WT mice. Also, Crif1-KO mice showed significantly increase of 8-OHDG-positive cell recruitment compared to WT mice. CRIF1-KO-UUO-kidneys were shown more increase recruitment of 8-OHDG-positive cells compared to WT-UUO-kidneys.

Conclusions: Collecting duct specific mitochondrial injury increases oxidative stress. Oxidative stress induced by mitochondrial injury aggravates UUO induced renal injury.

Funding: Government Support - Non-U.S.

TH-PO015

Chronic Nicotine Exposure Induces p66shc Expression in Renal Proximal Tubule Cells: Impact on Oxidative Stress and Injury

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Background: We reported that chronic NIC exposure exacerbates oxidative stress and consequent injury in a mice model of AKI. Since the adaptor protein p66shc mediates ROS release via cytochrome c binding we tested the hypothesis that chronic NIC exposure aggravates oxidative stress-linked ROS production and injury through increase in cytochrome c-bound p66shc in proximal tubule cells.

Methods: Wild type (w.t.), p66shc knockdown (k.d.) or cytochrome c binding deficient (W134F-p66shc) mouse renal proximal tubule cells were treated with 200 μM NIC for 24 hours prior to treatment with 200 μM H_2O_2 and ROS production as well as LDH release was determined. Immunoprecipitation studies were performed to determine mitochondrial as well as cytochrome c binding of p66shc. The fluorescent dye JC-1 was used to determine mitochondrial depolarization.

Results: Chronic NIC treatment increased H_2O_2-induced mitochondrial and cytochrome c-binding of p66shc and also exacerbated H_2O_2-induced ROS production, depolarization of the mitochondria and LDH release. Conversely, NIC+H_2O_2-induced ROS production and LDH release was attenuated in p66shc k.d or W134F cells compared to w.t. cells. Ser36 phosphorylation of p66shc –which is necessary for its mitochondrial localization- was increased by NIC+H_2O_2 but not by NIC treatment. Surprisingly, chronic NIC treatment increased expression of p66shc through dose-dependent induction of its promoter. Importantly, increased expression of p66shc was detected in the kidneys of mice that were chronically exposed to NIC.

Conclusions: Chronic NIC treatment increases expression of p66shc, which in turn, Ser36 phosphorylated via oxidative stress. The result is augmented mitochondrial/cytochrome c binding of p66shc and consequent ROS production/injury through opening the mitochondrial permeability transition pore. This mechanism may be responsible for the augmented oxidative stress in the ischemic kidney of mice with chronic nicotine exposure.

Funding: NIDDK Support

TH-PO016

Role of Nonesterified Fatty Acids in Respiratory Impairment and Mitochondrial Deenergization of Proximal Tubules Secondary to Hypoxia/Reoxygenation

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Background: Hypoxia/reoxygenation (H/R) of proximal tubules leads to persistent ATP depletion due to decreased mitochondrial membrane potential (MMP) resulting from nonesterified fatty acid (NEFA)-mediated uncoupling that is paradoxically accompanied by respiratory inhibition rather than the stimulation expected for uncoupled states.

Methods: Since NEFA have been reported to directly inhibit electron transport in some settings we assessed respiratory function in tubules after H/R as a function of NEFA availability.

Results: Compared to respiration supported by the complex II-dependent substrate, succinate, which was highly uncoupled after H/R but relatively well preserved (ADP-stimulated respiration (S3) of permeabilized tubules 71.0±8.5% of normoxic control (NC)), respiration supported by complex I-dependent substrates that normally predominate in cells was also uncoupled, but S3 was reduced to 26.9±3.3% of NC, $P < 0.001$ vs. succinate, N=5. With complex I substrates, acutely lowering NEFA after permeabilization improved coupling but only minimally increased S3. In contrast, lowering NEFA during 60 min. of reoxygenation prior to permeabilization increased S3 supported by complex I substrates, but it remained lower (55.7±7.5% of NC) than with succinate after the same treatment, 80.0±4.8%, $p < 0.02$. MMP at the end of H/R was much lower with complex I substrates (30.7±9.2% NC) than with succinate (67.4±4.5%), $P < 0.004$. Lowering NEFA during 60 min. of reoxygenation strongly improved recovery and decreased the MMP difference between complex I substrates (73.3±5.1% of NC) and succinate (83.4±6.6%).

Conclusions: The studies indicate that selectively impaired utilization of complex I substrates to support respiration after H/R promotes NEFA-induced deenergization and is only minimally improved by acutely removing NEFA. In the presence of NEFA, the higher efficiency of complex I substrates to support electron transport does not mitigate the impact of the impaired respiration on MMP. However, lowering NEFA within cells for 60 min. allows strong recovery of MMP despite persistence of some respiratory impairment.

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TH-PO017

Transcription Factor Nrf2 Regulates CYP2E1 Mediated Rhabdomyolysis Induced Acute Kidney Injury

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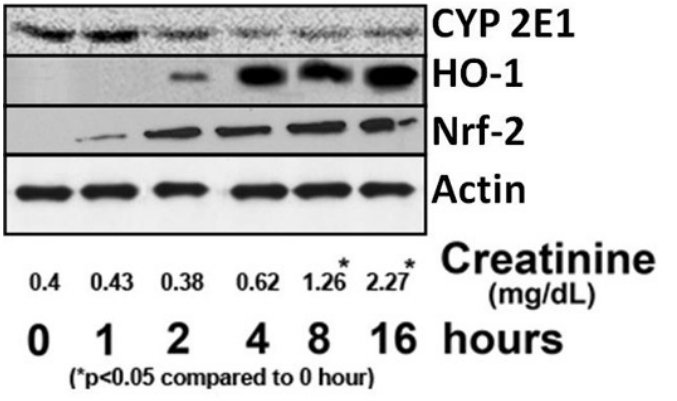
Background: CYP2E1 is a member of the Cytochrome P450 family that plays an important role in the rhabdomyolysis induced AKI (JASN 21: 289A, 2010). Nfr2 regulates the expression of anti-oxidative and other cytoprotective genes against variety of insults. The purpose of the current study was to 1. Evaluate the role of Nrf2 in the up regulation of anti-oxidative protective factors generated in response to CYP2E1 induced oxidative stress and 2. Examine the ability of Nrf2 to protect against the cytotoxic actions of CYP2E1.

Methods: Male S-D rats were injected with 50% glycerol following an overnite deprivation of water. Specific CYP2E1 inhibitor Chlormethiazole (CMZ) was administered prior to glycerol injection. Both mRNA and protein expression of Nrf2, heme-oxygenase-1 (HO-1) including other Nfr2 regulated enzymes were studied invivo and in LLC-PK1 cells exposed to myoglobin. Cytotoxicity was measured by the LDH release.

Results: Increases in the Nrf2 mRNA and nuclear protein including upregulation of the HO-1 and other Nrf2 regulated genes were observed in the kidney in the glycerol treated rats with the breakdown of the CYP2E1 protein and prior to AKI as measured by SCr.



Administration of CMZ significantly decreased oxidative stress and blocked the increases in the Nrf2 mRNA and nuclear protein including HO-1. Pre-treatment of the LLC-PK1 cells with an activator/inhibitor of Nrf2 prior to myoglobin exposure significantly decreased/increased ROS generation and cytotoxicity.

Conclusions: Nrf2 plays a key role in the adaptive response against increased oxidative stress caused by CYP 2E1. Inhibition of CYP2E1 coupled with prior activation of Nrf2 may be a valuable approach to reduce CYP2E1 mediated rhabdomyolysis induced AKI.

TH-PO018

Activation of Protein Kinase Cα Promotes Mitochondrial Function and Cell Survival in Renal Proximal Tubular Cells Injured by Oxidant

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Background: Previously, we have shown that protein kinase C (PKC) activation promotes recovery of mitochondrial function and active Na^+ transport following oxidant injury in renal proximal tubular cells (RPTC). However, the specific PKC isozyme involved in the recovery of mitochondrial function is unknown. This study investigated the role of PKCα in decreases and recovery of mitochondrial functions and cell survival in RPTC injured by an oxidant.

Methods: Wild-type PKCα and inactive PKCα mutants were overexpressed in primary cultures of RPTC prior to oxidant exposure (0.35 mM *tert*-butyl hydroperoxide, TBHP; 45 min). PKCα activation, mitochondrial functions associated with oxidative phosphorylation, production of reactive oxygen species (ROS), and cell viability were determined after TBHP treatment.

Results: TBHP exposure in RPTC was followed by PKCα activation and translocation to mitochondria. State 3 respiration in TBHP-injured RPTC decreased to 48% of controls. Mitochondrial membrane potential increased 50% at 4h and decreased to 48% of controls at 24h following TBHP injury. F_0F_1-ATPase activity and ATP content decreased to 59% and 60% of controls, respectively, in TBHP injured RPTC. Oxidant exposure increased ROS production by 200% and induced 47% RPTC lysis. Blocking PKCα activation by overexpressing inactive PKCα exacerbated oxidant-induced decreases in state 3 respiration, blocked the increases in ROS production, but had no effect on mitochondrial hyperpolarization, decreases in F_0F_1-ATPase activity and ATP content, and increases in RPTC lysis. In contrast, increasing activation of PKCα by overexpressing wild-type PKCα improved state 3 respiration and mitochondrial coupling, prevented mitochondrial hyperpolarization, and restored F_0F_1-ATPase activity and ATP content in TBHP-injured RPTC. PKCα activation did not block TBHP-induced increases in ROS production but reduced RPTC lysis to 17%.

Conclusions: We conclude that: 1) activation of PKCα during oxidant injury in RPTC promotes mitochondrial functions and decreases cell death and 2) blocking ROS production does not prevent oxidant-induced RPTC death.

Funding: NIDDK Support

TH-PO019

Carbonyl Stress Induced Protein Modification in Snake-Bite Mediated Acute Renal Failure – A Pathogenesis Link Pinaki Mukhopadhyay,[1] Debarati Mukherjee,[2] Raghwendra Mishra,[2] Monoj Kar.[2] *[1]Nephrology, NRS Medical College, Kolkata, West Bengal, India; [2]Biochemistry, NRS Medical College, Kolkata, West Bengal, India.*

Background: To identify the role of damaged proteins in association with oxidative stress (OS) and carbonyl stress (CS) in the pathogenesis of snakebite mediated acute renal failure (SARF).

Methods: Oxidative stress index, MG (methylglyoxal), thiobarbituric acid reactive substances (TBARS), AOPP, AGE, pentosidine and dityrosine were measured in 41 CRF and 58 SARF patients and compared with 36 normal control subjects. One way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis was performed to check any statistical difference, significance level was considered at $p< 0.05$. Pearson correlation coefficient (r) was evaluated between the studied parameters from the aggregate of patients and control. Receiver-operating characteristic (ROC) curve analyses were performed regarding all parameters of CRF and SARF groups.

Results: SARF had significantly elevated level of oxidative stress index (OSI) compare to normal control ($p=0.020$) and CRF ($p=0.019$).This may be attributed to increased total oxidative stress (TOS) level in SARF compare to control ($p=0.007$) and CRF ($p=0.009$). Both MG and TBARS level were found to be significantly raised ($p<0.001$) in SARF and CRF group compare to normal controls. AOPP level of SARF was higher than that of CRF ($p=0.002$) and control ($p<0.001$).SARF group has significantly higher level of AGE ($p=0.002$), dityrosine ($p<0.001$) and pentosidine ($p=0.001$) than control. CRF shows significantly higher level of all protein damage markers than control ($p<0.001$). The studied protein damage markers were found to be positively correlated with the surrogate renal failure marker, i.e. serum creatinine, oxidative cellular damage marker TBARS and the carbonyl stress marker MG.

Conclusions: The toxins of snake venom induce protein damage associated with carbonyl and oxidative stress. Although it is still uncertain that whether these damages are cause or effect of SARF, this study indicates that they might play a pivotal role in pathogenesis of SARF in a similar way which occurs in CRF with only difference in the time course of the disease.

TH-PO020

Adenosine A1 Receptor Agonism as an Activator of Mitochondrial Biogenesis in the Kidney Sean Robert Jesinkey, Jason A. Funk, Lauren P. Wills, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: Adenosine receptor agonism is a beneficial physiological stress response observed in multiple organs. Recently, we have shown that mitochondrial biogenesis is important in the recovery of renal cells from injury. The goal of this study was to determine if adenosine receptors play a role in mitochondrial biogenesis.

Methods: Using a mitochondrial biogenesis assay that incorporates the Seahorse Biosciences analyzer and FCCP-uncoupled oxygen consumption with primary cultures of renal proximal tubular cells (RPTC), we determined the efficacy of pharmacological agonists and antagonists for both adenosine Gi/o receptor subtypes (A1 and A3) to induce mitobiogenic signaling. We further examined changes in mRNA levels of mitochondrial genes in renal cortices of C57BL/6 mice exposed to CCPA (0.1mg/kg) for 24 h via real-time PCR. Immunoblot analysis was performed to determine alterations of electron transport chain proteins.

Results: We discovered that the specific adenosine A1 receptor agonist 2-chloro-N6-cyclopentyladenosine (CCPA) (20 nM) increased FCCP-uncoupled respiration by 21% after 24 h. However, treatment with the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) resulted in no change of FCCP-uncoupled respiration. In addition, exposure to various A3 receptor agonists and antagonists, such as Hemado and MRS1334, respectively, had no effect on basal and uncoupled oxygen consumption. The master regulator of mitochondrial biogenesis PGC-1α, the complex 1 proteins NDUFB8 (nuclear-encoded), COX1 (mitochondrial-encoded) and ND6 (mitochondrial-encoded), and ATP synthase β (nuclear-encoded), all displayed increased gene expression following CCPA exposure. Furthermore, immunoblot analysis revealed CCPA also increased COX1 and NDUFB8 protein levels in mouse renal cortical tissue.

Conclusions: These novel results provide evidence that the adenosine A1 receptor is linked to mitochondrial biogenesis and that activation of this receptor results in renal mitochondrial biogenesis.

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TH-PO021

Preconditioning with Meclizine Protects the Kidney Against Ischemia Reperfusion Injury Liangying Gan,[1] Gabriela Campanholle,[1] Vishal M. Gohil,[2] Venkata Sabbisetti,[1] Vamsi K. Mootha,[2] Takaharu Ichimura,[1] Joseph V. Bonventre.[1] *[1]Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

Background: Renal ischemia-reperfusion (I/R) injury is a major cause of acute kidney injury (AKI). Preconditioning is a powerful way to protect the kidney against subsequent ischemic injury. It would be of great importance to be able to mimic ischemic preconditioning by a pharmacologic intervention. Meclizine has anti-histaminergic and anti-muscarinic activities and has been reported to reduce mitochondrial oxygen consumption.

Methods: C57bl/6 mice were pretreated with two i.p. injections of 100 mg/kg of meclizine, or vehicle, at 17 and 3 hrs before renal I/R injury. Serum, urine and kidney tissue samples were collected at 24 hrs after surgery for further evaluation. Cytoprotective effects of meclizine on renal tubular cells subjected to hydrogen peroxide injury was also evaluated in vitro.

Results: Meclizine pretreatment protected mice from I/R injury in a dose dependent fashion, as reflected by a significantly lower serum creatinine, BUN and Kim-1 levels. Meclizine pretreatment also reduced tubular necrosis, neutrophil and macrophage infiltration and decreased expression of inflammatory cytokines IL-1β, TNF-α, MCP-1 and IL-6. These protected kidneys expressed lower levels of injury associated iNOS and heme oxygenase-1. Meclizine did not protect kidneys from I/R when administrated after injury and did not protect from aristolochic acid or cisplatin nephrotoxic kidney injury. Meclizine reduced oxygen consumption of isolated kidney mitochondria. In vitro, Meclizine-preconditioning of renal tubular cells against oxidative injury was confirmed.

Conclusions: Meclizine preconditioning protects the kidney against I/R injury and proximal tubular cells in vitro. There is no protection against nephrotoxic injury. The protection is both functional as well as morphological with suppression of the inflammatory response and injury-associated gene expression. Meclizine may be an important candidate for a pharmacological renal preconditioning agent.

Funding: NIDDK Support

TH-PO022

A Novel Class of Small Molecule Mitochondria-Targeted ROS Scavenger Attenuates Cisplatin Induced Nephrotoxicity Dae Eun Choi,[1] Jin Young Jeong,[1] Bo Ra Lee,[1] Bong-Hyun Ahn,[2] Soon Ha Kim,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National University, Daejeon, Korea; [2]LG Life Sciences, Daejeon, Korea.*

Background: Although cisplatin is highly effective anti-cancer drug, nephrotoxicity is a major clinical problem. Mitochondrial injury and reactive oxygen species (ROS) generation play a role in cisplatin induced nephrotoxicity. LG life sciences developed a novel class of small molecule mitochondrial ROS scavenger NecroX series that can reduce mitochondrial reactive oxygen species and improve cell survival. We therefore investigated the effect of NecroX-7 on nephrotoxicity in mice for development of a therapeutic candidate of renal injury.

Methods: We used male C57BL/6 mice. Mice were divided into 4 groups; normal control group (n=5), NecroX-7 treated control group (n=5), vehicle with cisplatin (15mg/kg, intraperitoneal injection) treated group (n=9), and NecroX-7 (2mg/kg, intraperitoneal injection) with cisplatin treated group (n=9). In mice, we measured BUN and serum creatinine. We examined 8-OH deoxyguanosine in kidney tissue. We examined TUNEL and light microscopic findings (H&E and PAS stain).

Results: The levels of BUN and serum creatinine in NecroX-7 with cisplatin treated mice were significantly lower than that of vehicle with cisplatin treated mice (BUN: 143.7±9.3 vs. 102.1±14.9 mg/dL, serum creatinine: 0.38± 0.03 vs. 0.29±0.03 mg/dL, $p<0.05$ respectively). In microscopy, NecroX-7 significantly reduced renal tubular epithelial cell necrosis and detachment. NecroX-7 significantly reduced 8-OH deoxyguanosine positive cells in cisplatin treated mice.

Conclusions: In conclusion, NecroX-7 attenuates cisplatin induced nephrotoxicity

TH-PO023

Pharmacological and Genetic Blockade of Autophagy Sensitizes Kidneys to Acute Injury Man Jiang, Zheng Dong. *Georgia Health Sciences University and Charlie Norwood VA Medical Center.*

Background: Autophagy is induced during cell stress and may either contribute to cell death or serve as a survival mechanism. Recent studies have demonstrated autophagy in renal tubular cells following acute kidney injury (AKI). However, how autophagy is induced and what role it plays in renal pathophysiology is not well understood.

Methods: Here we have examined the role of autophagy in AKI by using pharmacological inhibitors and a conditional Atg-7-knockout model.

Results: Chloroquine, a pharmacological inhibitor of autphagy, blocked autophagic flux and notably, enhanced cisplatin and ischemia-reperfusion -induced kidney injury in C57Bl/6 mice. To gain definitive evidence, we established a proximal tubule specific Atg7-knockout (PT-Atg7-KO) mouse model. Knockout of Atg7 led to the impairment of autophagy-conjugation systems, resulting in the loss of LC3-II and accumulation of p62 (an autophagy-selective substrate) during cisplatin treatment. Importantly, compared with their wild-type littermates, PT-Atg7-KO mice showed significantly more severe kidney injury and rapid progression of renal failure. Five days after cisplatin treatment, wild-type mice had 1.15mg/dl serum creatinine and 244 mg/dl BUN, which were increased respectively to 3.24mg/dl and 351mg/dl in PT-Atg7-KO mice. Consistently, kidney tissue damage and tubular apoptosis were aggravated in PT-Atg7 KO mice. Nonetheless, cisplatin-induced p53 activation was not affected by Atg7 deficiency, suggesting that autophagy protects kidneys without blocking upstream signaling. Primary proximal tubular cells isolated from PT-Atg7 KO mice were more sensitive to cisplatin-induced caspase activation and apoptosis than the cells from wild-type mice. Finally, PT-Atg7 KO mice were also more sensitive to renal ischemia-reperfusion injury than their wild-type littermates.

Conclusions: Together, these results demonstrate compelling evidence for a renoprotective role of autophagy during acute kidney injury.

Funding: NIDDK Support, Veterans Administration Support

TH-PO024

Selective Stabilization of HIF-1α in Renal Tubular Cells by 2-Oxoglutarate Analogues Gunnar Schley,[1] Bernd Klanke,[1] Yohannes Hagos,[2] Kerstin U. Amann,[3] Birgitta C. Burckhardt,[2] Kai-Uwe Eckardt,[1] Carsten Willam.[1] *[1]Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Center of Physiology and Pathophysiology, University Göttingen, Göttingen, Germany; [3]Institute of Pathology, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: 2-oxoglutarate dependent enzymes, which comprise e.g. the hypoxia inducible factor (HIF), represent promising therapeutic targets to prevent progressive renal fibrosis or to preserve renal function in acute kidney injury. Lipophilic 2-oxoglutarate analogues (2OGAs), which are widely taken up in many cells of most organs, have been explored as inhibitors of these enzymes. Given the selective expression of organic anion transporters (OAT) in renal tubular cells we hypothesized that hydrophilic 2OGAs could be used to specifically target these cells.

Methods: HIF stabilization and HIF target gene induction by hydrophilic and lipophilic 2OGAs were analyzed in different cell lines and in C57BL/6N mice. Mice were subjected to bilateral renal ischemia-reperfusion 6h after ip injection of 2OGAs and kidney function and morphology were evaluated 3 days later.

Results: In vitro analyses showed that in contrast to the lipophilic 2OGA, HIF stabilization and target gene induction by the hydrophilic 2OGA was dependent on the expression of OAT1. In vivo the functional effects of hydrophilic 2OGAs are largely limited to the kidney, particularly in renal proximal tubular cells expressing OAT1. Lipophilic 2OGAs resulted in HIF accumulation in tubular and interstitial renal cells and extra-renal tissues. Both lipophilic and hydrophilic 2OGAs induced HIF-dependent genes expressed in tubular cells, but only lipophilic 2OGAs induced erythropoietin synthesis. Preconditional application of the lipophilic 2OGA protected the kidney against ischemia-reperfusion injury, surprisingly the hydrophilic 2OGA did not, suggesting that HIF stabilization in proximal tubular cells is insufficient to achieve organ protection.

Conclusions: This study provides proof of concept for selective drug targeting of proximal tubular cells. This may expand the options for therapeutic use of 2OGAs by limiting undesired side effects.

Funding: Government Support - Non-U.S.

TH-PO025

Pharmacological Modulation of Soluble Epoxide Hydrolase Activity Attenuates Ischemia Reperfusion Injury in Kidney Yun Jung Oh,[1] Jung Pyo Lee,[1,2,3] Seung Hee Yang,[1,3] Dong Ki Kim,[1,3] Chun Soo Lim,[1,2,3] Yon Su Kim.[1,3] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea; [3]Seoul National University Kidney Research Institute, Seoul, Korea.*

Background: Soluble epoxide hydrolase (sEH) in endothelial cells determines the plasma level of epoxyeicosatrienoic acids (EETs), which may control the vascular tone as one of the vasoactive materials. We hypothesized that the regulation of sEH activity may have a therapeutic value in the prevention of acute kidney injury by the control of EETs level.

Methods: Ischemia-reperfusion injury (IRI) was induced in C57BL/6 mice, and sEH activity was controlled by the intraperitoneal administration of 12-(3-adamantan-1-ylureido)-dodecanoic acid (AUDA).

Results: The deterioration of kidney function induced by IRI was partially moderated and prevented by AUDA treatment. In addition, AUDA treatment significantly attenuated tubular necrosis induced by IRI, especially inner medulla area. sEH was expressed prominently in the intraglomerular capillary loops and periglomerular arterioles under normal condition. Ischemic injury induced the down-regulation of sEH, while AUDA administration did not generate an impact on the expression pattern of sEH induced by IRI. in-vivo activity of sEH was assessed by the measurement of substrate (EpOME) and its metabolite (DHOME) using LC/MS/MS. Ischemic injury did not change the plasma levels of EpOME and DHOME, but sEH inhibition by AUDA significantly elevated plasma EpOME and EpOME/DHOME ratio. The protective effect of sEH inhibitor was derived from the suppression of proinflammatory cytokines and the up-regulation of regulatory cytokines. AUDA treatment prevented the intrarenal infiltration of inflammatory cells, but promoted the endothelial cell migration and neovascularization.

Conclusions: Taken together, these data provide evidence that the control of sEH activity might be a feasible therapeutic strategy to prevent acute kidney injury.

TH-PO026

Preservation with Cardiotrophin-1 Prevents Cold Ischemia Injury and Inflammatory Response and Improves Survival and Graft Function in a Syngeneic Rat Kidney Transplant Model Jose M. Lopez-Novoa,[1,3] María Begoña García-Cenador,[2] Maria Pilar Perez de Obanos,[4] Juan Ruiz,[4] Francisco Javier García-Criado.[2] *[1]Department of Physiology and Pharmacology, University of Salamanca, Spain; [2]Department of Surgery, University of Salamanca, Spain; [3]Bio-inRen S.L., Salamanca, Spain; [4]Digna Biotech S.L., Madrid, Spain.*

Background: Reperfusion injury (RI) in grafted kidneys due to warm and cold ischemia (CI) is clinically manifested as a high incidence of delayed graft function. Cardiotrophin-1 (CT-1), a member of the interleukin-6 (IL-6) family, has been shown to have a protective effect on myocardial and liver damage induced by RI. The purpose of the present study has been to assess the effect of CT-1 added to perfusion and preservation fluid on renal inflammation induced by CI and in renal function in the Fisher-Fisher rat model of syngenic kidney transplantation (KTx).

Methods: Once extracted, kidneys were perfused with University of Wisconsin (UW) fluid at 4ºC containing CT-1 (0.2 mg/L) and immersed in 15 ml of cold UW with or without CT-1(0.2 mg/L). Orthotopic transplantation of the left donor kidney was performed after 24 h of cold storage as above reported. The receptor's right kidney was removed at the time of KTx. At different time points after CI or KTx, kidneys were obtained, frozen and several markers of inflammation (superoxide anion (SOA) production, TNF-α release, iNOS expression, serum levels of soluble ICAM-1 and VCAM-1 and NFκB activation) were evaluated.

Results: 24 or 48 h after CI without CT-1, there was a marked increase in SOA and TNF-α production, iNOS, and VCAM-1 expression and NFκB activation. The presence of CT-1 in the UW fluid completely prevented these increases. When kidneys were grafted after 24 h preservation, the addition of CT-1 to the UW fluid reduced reperfusion injury (lower serum creatinine and higher creatinine clearance), improved early) and long-term survival, and reduced renal SOA production, iNOS expression and NFκB activation, and serum levels of TNF-α, soluble ICAM-1 and VCAM-1.

Conclusions: Preservation with CT-1 of kidneys to be grafted improves short- and long-term outcomes and inflammatory response after CI and syngeneic KTx in rats.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

TH-PO027

Klotho Ameliorates Cisplatin Nephrotoxicity Monica Chang Panesso,[1] Mingjun Shi,[1] Han Ju Cho,[1] Dihua Zhang,[1] Makoto Kuroo,[2] Orson W. Moe,[1] Ming Chang Hu.[1] *[1]Charles and Jane Pak Center for Mineral Metabolism and Clinical Research, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX; [2]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.*

Background: Cisplatin is a powerful anti-cancer agent but nephrotoxicity is one serious side effect which limits its use. Klotho was shown to protect the kidney from ischemia-reperfusion injury, but there is no information about the Klotho effect on nephrotoxic agents. We tested whether Klotho protects the kidney from cisplatin.

Methods: 1) Cisplatin (10 mg/Kg) was intraperitoneally injected into *WT*, Klotho deficient (*Kl*$^{-/+}$) or transgenic overexpressing (*Tg-Kl*) mice. 2) Normal rat kidney (NRK) epithelial cells, were treated with cisplatin and cytotoxicity was examined. 3) The effect of Klotho was compared to blocker of cisplatin uptake (cimetidine: inhibitor of organic cation transporter, OCT) on cisplatin cytotoxicity.

Results: Cisplatin injection increased plasma creatinine and induced histological renal damage. These changes were exaggerated in *Kl*$^{-/+}$ mice; and ameliorated in *Tg-Kl* mice. NGAL expression (a marker of renal damage) in the kidney was significantly higher in *Kl*$^{-/+}$ and lower in *Tg-Kl* compared to *WT* mice. Likewise, the addition of Klotho to NRK cells decreased cisplatin-induced LDH release and apoptosis. Next, we examined cisplatin uptake by NRK cells to examine if Klotho protection is mediated by suppression of cisplatin uptake. Klotho inhibited cisplatin uptake from basolateral compartment and suppressed cisplatin cytotoxicity, similar to cimetidine. However, unlike cimetidine which only suppressed cisplatin uptake, Klotho had an additional protective effect on NRK cells even when Klotho was added to NRK cells 20 minutes after cisplatin addition when uptake is already completed.

Conclusions: Our results indicate that Klotho has dual beneficial effects on cisplatin nephrotoxicity: (1) Suppression of basolateral uptake of cisplatin; (2) Direct cytoprotective effect. Klotho may present a useful agent to prevent cisplatin-induced nephrotoxicity and can potentially remove this dreaded complication from patients who benefit from cisplatin.

Funding: NIDDK Support

TH-PO028

Renoprotective Effect of Sulfotransferase (SULT) Inhibitors on Ischemia/Reperfusion (IR)-Induced Acute Kidney Injury (AKI) Rats Megumi Komori,[1] Misato Yoshimura,[1] Masataka Sagata,[1] Kazuhiko Nishi,[2] Akinobu Hamada,[1,3] Hideyuki Saito.[1,3] *[1]Clinical Pharmaceutical Sciences, Kumamoto Univ Grad Sch of Pharm Sci, Kumamoto, Japan; [2]Hemodialysis and Apheresis, Kumamoto Univ Hosp, Kumamoto, Japan; [3]Pharmacy, Kumamoto Univ Hosp, Kumamoto, Japan.*

Background: Since AKI appears to be the high risk disease state developing into chronic kidney disease, early detection of AKI and prompt treatments are required. However, adequate therapeutic strategies and/or medications have not been established due to the lack of sufficient information for effective treatments of AKI. IR-induced AKI is evoked by diverse pathophysiological or surgical situations. In this study, we explored the biotoxicological features of these uremic toxins and evaluated renal preventive effects of AST-120 and SULT inhibitors, which obstruct hepatometabolic generation of indoxyl sulfate (IS) and p-cresyl sulfate (PCS), in IR-AKI rats.

Methods: AKI was produced by clamping isolated arteries of right and left kidneys for 30 min, followed by reperfusion of the kidneys. IS and PCS in serum and urine were assayed by HPLC technique. Kidney injury molecule-1 (Kim-1) was measured by ELISA kit.

Results: IR evoked severe renal injury with the marked increases in Scr (12.6-fold vs sham-operated rats) and BUN (13.6-fold), in association with a significant accumulation of both IS (23.2-fold) and PCS (>105.6-fold) in the serum, and the urinary excretion of Kim-1. Oral administration of AST-120 to IR rats resulted in the remarkable restoration of Scr and BUN levels with the marked decline of serum levels of IS, PCS and urine levels of Kim-1. Administration of quercetin, a typical SULT inhibitor, appeared to suppress serum

levels of both uremic toxins, i.e., 0.39- and 0.23-fold decline vs those levels in untreated rats for IS and PCS, respectively, accomapnied with a significant alleviation of renal injury (Scr, 0.45-fold and BUN, 0.52-fold).

Conclusions: In conclusion, the sulfate-conjugated uremic toxins could be key metabolic factors influencing the development or progression of IR-AKI, therefore proposing suppression of their serum accumulations might be a possible renopreventing treatment against derangements in AKI.

Funding: Government Support - Non-U.S.

TH-PO029

Therapeutic Potential of Anti-Transforming Growth Factor-β Antibody on Acute Tubulo-Interstitial Injury in Aristolochic Acid Nephropathy Laetitia Giordano,[1] Nathalie Caron,[1] Thomas Baudoux,[2] Eric De Prez,[2] Marie-Hélène Antoine,[2] Steven R. Ledbetter,[3] Joelle L. Nortier,[2] Agnieszka Anna Pozdzik.[2] *[1]Physiology-NARILIS, University of Namur, Belgium; [2]Experimental Nephrology Unit, ULB Erasme, Brussels, Belgium; [3]Cardio-Metabolic and Renal Research, Genzyme Corporation, New-York.*

Background: Aristolochic acid nephropathy (AAN) characterized by rapidly progressing renal fibrosis of toxic origin is primed by acute injury of proximal tubular epithelial cells (PTEC). Anti-transforming growth factor-β (TGF-β) antibody has been shown to improve renal fibrosis in various models of glomerular diseases, but its roles in primary tubulo-interstitial nephropathies are not yet well known.

Methods: We studied the efficacy of a murine pan-specific anti-TGF-β monoclonal antibody (1D11) in an acute phase of AAN. Weight matched rats were daily sc. injected with AA (15 mg/kg/day) or vehicle (polyethylene glycol-PEG) from day 0 to day 5. Four groups (n=6/group) were randomly assessed: PEG+1D11; AA alone; AA+1D11 and AA+control isotype (13C4). The anti-TGF-β and control isotype antibodies (5 mg/kg) were administered ip. at days -1, 0, 2 and 4.

Results: After 5 days of treatment, renal function and morphology remained normal in the control group PEG+1D11. Anti-TGF-β antibody statistically attenuated AA induced acute kidney injury, as attested by less increased creatininemia and urinary excretion of N-acetyl-β-glucosaminidase, less severe necrosis of PTEC from S3 segment and reduced macrophages infiltration (ED-1 staining) in outer medulla. Intrarenal regulatory T cells (CD4+CD25+ highFoxp3+) infiltration and neo-angiogenesis (FVIII staining) around the injured areas were also reduced by 1D11 as compared with 13C4. The proliferation of PTEC from S1-3 segments (neutral endopeptidase/proliferating nuclear cell antigen double staining) did not seem to be affected by 1D11.

Conclusions: Our results demonstrate that anti-TGF-β antibody significantly attenuates acute PTEC injury, reduces macrophages infiltration and modulates the regulatory T cells immune response. Therefore, 1D11 could be a potential, new renoprotective therapy interfering with early fibrogenesis events after a toxic insult.

TH-PO030

Apolipoprotein A-I Mimetic Peptide 4F Attenuates Kidney/Heart Injury and Endothelial Dysfunction in Sepsis Roberto De Souza Moreira,[1,2] Maria Irigoyen,[2] Maria Heloisa M. Shimizu,[1] Niels O.S. Camara,[3] Rildo A. Volpini,[1] Antonio C. Seguro,[1] Lucia Andrade.[1] *[1]Nephrology, University of São Paulo, Brazil; [2]Heart Institute, USP, Brazil; [3]Immunology, USP, Brazil.*

Background: Kidney injury, heart injury and cytokine-induced vascular hyperpermeability are associated with high morbidity and mortality in sepsis. Although the mechanism is unknown, 4F reduces inflammation and protects HDL, the levels of which are reduced in sepsis. We hypothesized that 4F also protects kidneys and hearts in a rat model of cecal ligation and puncture (CLP).

Methods: We divided Wistar rats into control (sham-operated), CLP and CLP+4F (10mg/kgBW, i.p., 6 h after CLP) groups. At 24 h post-CLP, we evaluated cardiac function, mean arterial pressure (MAP), heart rate (HR), baroreflex sensitivity, total cholesterol (TC), LDL, HDL and inulin clearance (ml/min/100gBW), as well as performing immunoblotting for protein regulators of vascular permeability (Slit2 and Robo4) and eNOS in kidney tissue. We also measured serum IL-6, IL-10, IL-18, and TNF-α. Data are mean±SEM.

Results:

Group[a]	CO (ml/min)	MPI	EF (%)	LVEDP (mmHg)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	73±2.3	0.45±0.04	75 ±2.7	7.5±0.5	65±3.0	42±2.3	11±1.4
CLP	50±6.2[b]	0.64±0.02[c]	67.6±2.8[b]	4.6±0.4[c]	38±4.1[c]	19±1.7[c]	18±2.7[b]
CLP+4F	77±8.1[d]	0.37±0.03[c]	79±2.0[d]	7.9±0.6[c]	51±3.8[b,d]	34±2.9[b,e]	8±2[d]

CO: cardiac output; MPI: myocardial performance index; EF: ejection fraction; LVEDP: left ventricular end-diastolic pressure. [a]n=10/group; [b]p<0.05 vs. control; [c]p<0.001 vs. control; [d]p<0.05 vs. CLP; [e]p<0.001 vs. CLP.

Although there were no intergroup differences in MAP, HR was significantly higher in CLP rats than in control and CLP+4F rats. Baroreflex sensitivity was worsened by CLP and completely restored by 4F. GFR was lower in CLP rats than in control rats (0.5±0.06 vs. 0.8±0.03, p<0.01) and was completely restored in CLP+4F rats (0.8±0.06, p<0.01). All cytokines were lower in CLP+4F rats than in CLP rats. CLP reduced Slit2, Robo4 and eNOS expression by 30%, 40% and 60%, respectively, and 4F completely restored such expression.

Conclusions: 4F inhibits inflammatory responses and strengthens the vascular barrier, protecting kidneys and hearts in an HDL-dependent manner.

Funding: Government Support - Non-U.S.

TH-PO031

Cardiotrophin-1 Protects the Kidney from Contrast Agent-Induced Nephropathy Jose M. Lopez-Novoa,[1,2] Yaremi Quiros-Luis,[2] Penelope D. Sanchez-Gonzalez,[2] Maria Pilar Perez de Obanos,[4] Juan Ruiz,[4] Francisco J. Lopez-Hernandez,[1,2,3] Ana Isabel Morales Matin.[4] *[1]Department of Physiology and Pharmacology, University of Salamanca, Spain; [2]Bioinren S.L., Salamanca, Spain; [3]IESCYL, Hospital Universitario de Salamanca, Salamanca, Spain; [4]Digna Biotech S.L., Madrid, Spain.*

Background: Cardiotrophin-1(CT-1) is a cytokine of the IL-6 family that protects liver and heart from chemical damage. Contrast nephropathy (CN), although generally reversible, is often associated to longer hospitalization time, dialysis and higher incidence of cardiovascular events. The purpose of this study was to assess if CT-1 administration could protect the kidney function in an experimental model of CN in rats receiving previously a low doses of gentamicin as a predisposing agent.

Methods: The study has been performed in 6 groups of Wistar rats: Control group (C): rats receiving saline solution; Gentamicin group (G): rats receiving G (50mg/kg/day, ip) for 6 days; Contrast agent gastrographin (Gg) group: rats receiving a single dose of Gg (3.7mg/Kg i.v.); Cardiotrophin group (CT-1): rats receiving CT-1 (100 µg/Kg/day i.v.) for 6 days; G + Gg group: rats receiving G for 6 days and then Gg as above described. G +CT-1 + Gg group: rats receiving G and CT-1 for 6 days and then Gg (as described). CN severity was assessed by measuring plasma urea (pU) and creatinine (pCr), creatinine clearance (CrCl), glomerular filtration rate (GFR), renal blood flow (RBF), urinary excretion of tubular damage markers (NAG, KIM-1 and PAI-1), renal cell apoptosis and lipid peroxidation (TBARS) and by histological studies.

Results: G, CT-1 or Gg alone did not produce any change in the parameters studied. In the group G +Gg there was an increase in pCr and pU, a decrease in CrCl, a lower GFR and RBF, a high urinary excretion of NAG, KIM-1 and PAI-1, and marked tubular necrosis. These increases were markedly lower in the group that also received CT-1 (G+ CT-1+Gg). The number of apoptotic cells and the amount of TBARS were markedly lower in the kidneys from the group G+CT-1 +Gg than in those from the G +Gg group

Conclusions: CT-1 administration prevents most of the alterations in kidney function and structure associated to this model of CN.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

TH-PO032

Cardiotrophin-1 Protects from Gentamicin- and Cisplatin-Induced Acute Kidney Injury Jose M. Lopez-Novoa,[1,2] Yaremi Quiros-Luis,[2] Penelope D. Sanchez-Gonzalez,[2] Rebeca Nunez-Lozano,[2] Maria Pilar Perez de Obanos,[3] Juan Ruiz,[3] Francisco J. Lopez-Hernandez,[1,2,4] Ana Isabel Morales Matin.[1] *[1]Department of Physiology and Pharmacology, University of Salamanca, Spain; [2]Bio-inRen S.L., Salamanca, Spain; [3]Digna Biotech S.L., Madrid, Spain; [4]Unidad de Investigacion, IESCYL-Hospital Universitario de Salamanca, Salamanca, Spain.*

Background: Gentamicin and cisplatin are widely used drugs whose major limitation is nephrotoxicity. Cardiotrophin-1 (CT-1) is a cytokine belonging to the IL-6 family that has been demonstrated to protect liver and heart from chemical and ischemic damage. The purpose of this study has been to assess if CT-1 administration could protect the kidney function in experimental models of gentamicin and cisplatin-induced acute kidney injury (AKI) in rats.

Methods: The study has been performed in 5 groups of Wistar rats: Control group (C): rats receiving saline solution i.p. for 6 days. Cardiotrophin group (CT-1): rats receiving CT-1 i.v. (100µg/Kg/day) for 6 days; Gentamicin group (G) rats receiving gentamicin (150mg/kg/day, i.p.) for 6 days; Cisplatin group (Csp), rats receiving a single dose of cisplatin (6 mg/kg i.p); CT-1 + G group: rats receiving CT-1 and G at the doses above described. CT-1 + Csp group: Rats receiving CT-1 and Csp at the doses above described. AKI severity was assessed by measuring plasma urea (pU) and creatinine (pCr), creatinine clearance (CrCl), urinary flow, urinary protein excretion, urinary excretion of total proteins and AKI markers (NAG, KIM-1 and N-GAL), and by histological studies.

Results: Gentamicin or Csp administration at these doses induced a marked AKI characterized by an increase in urinary flow, and in pCr and pU, a decrease in CrCl, and an increase in urinary excretion of proteins, NAG KIM-1 and N-GAL. Administration of CT-1 in G-treated (CT-1 + G)or Csp-treated (CT-1 + Csp) rats induced a significant reduction in pCr, pU, and urinary excretion of proteins, NAG, KIM-1 and N-GAL and an increase in CrCl, compared with the G group. CT-1 also prevented the structural kidney alterations induced by G or Csp.

Conclusions: Our results demonstrate that CT-1 protects rats from the AKI induced by either gentamicin or cisplatin administration.

Funding: Government Support - Non-U.S.

TH-PO033

Probenecid Prevents Acute Tubular Necrosis and Interstitial Fibrosis in a Mouse Model of Aristolochic Acid Nephropathy Thomas Baudoux,[1] Agnieszka Anna Pozdzik,[1] Volker Manfred Arlt,[2] Jean-Michel Hougardy,[1] Eric De Prez,[1] Nathalie Quellard,[3] Jean-Michel Goujon,[3] Joelle L. Nortier.[1] *[1]Experimental Nephrology Unit, Fac. Medicine, Univ. Libre de Bruxelles, Brussels, Belgium; [2]Section of Mol. Carcinogenesis, Inst. Cancer Research, Sutton, United Kingdom; [3]Dept of Pathol & Electron Microscopy, CHU La Miletrie, Poitiers, France.*

Background: Aristolochic acid nephropathy (AAN) is characterized by early tubulo-interstitial (TI) injury (i.e necrosis of proximal tubular epithelial cells [PTEC] and inflammatory infiltrate) and chronic lesions (i.e. tubular atrophy and interstitial fibrosis). In vitro, probenecid (PBN) inhibits AA entry through organic anion transporters (OATs), thereby reducing the formation of specific AA-DNA adducts and preserving cellular viability.

Methods: To confirm these results in vivo, 4 groups of C57BL/6 mice were injected ip with AA (dissolved in polyethylene glycol [PEG]); AA+PBN ; PEG+PBN or PEG. AA (5 mg/kg bw) or equivalent vehicle volume were given once a day and PBN (150 mg/kg bw) twice a day. Plasma creatinine level (Pcr), TI lesions, DNA repair processes (PCNA immunostaining) and AA-DNA adduct formation were studied in 6 mice/group after 2, 4, 5 and 8 days.

Results: No structural and functional abnormalities were found in mice receiving PEG or PEG+PBN. In contrast, AA induced severe necrosis of PTEC (days 4-5) leading to a transient acute kidney injury (day 5). Mononuclear cells infiltration, tubular atrophy and interstitial fibrosis were prominent on day 8. In the AA+PBN group, no Pcr elevation was observed. TI injury scores and PCNA positive cell were significantly reduced as soon as day 5. AA-DNA adduct levels were significantly lower at day 8. PBN reduced both the extent and the severity of ultrastructural lesions induced by AA (i.e. loss of brush border, mitochondrial edema and disappearance of mitochondrial crest).

Conclusions: Our data show that PBN, as an OATs inhibitor, reduces AA-DNA adduct formation and AA-induced tubulotoxicity, possibly by attenuation of mitochondrial injuries. This study demonstrates for the first time the nephroprotective effect of PBN towards acute PTEC toxicity and interstitial fibrosis in a mouse model of AAN.

Funding: Private Foundation Support

TH-PO034

Sildenafil and N-Acetylcysteine Role in Renal Ischemia Reperfusion Syndrome Is Time Dependent Maria De Fatima Vattimo,[1] Cassiane Dezoti Fonseca,[1] Mirian Watanabe,[1] Fernanda Teixeira Borges.[2] *[1]School of Nursing, University of Sao Paulo, SP, Brazil; [2]Nephrology Division, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Severity related to time of ischemia can determine the effect of the renoprotective agents and the prognosis of acute kidney injury (AKI). Sildenafil citrate (SIL) has shown a role in vasoconstriction damage while N-acetylcysteine (NAC) increases intracellular glutathione and scavenges reactive oxygen species (ROS). This study evaluated the effect of SIL and NAC in a time dependent ischemic AKI model.

Methods: Adult, male, Wistar rats (260–310 g) were divided into groups: ***SHAM***, ***Ischemia 30*** (Isch, renal pedicles clamping, RPC, for 30 min), ***Isch 30 + SIL*** (0.25 mg/kg 60 min before RPC), ***Isch 30 + NAC*** (150 mg/kg before and after RPC), ***Isch 45*** (45 min RPC), ***Isch 45 + SIL***, ***Isch 45 + NAC***. Renal function (RF, creatinine clearance, crCl); oxidative injury (urinary peroxides, UP; TBARS; nitric oxide, NO and thiols in renal tissue); expression of inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1) (western blotting) and tubulointerstitial (TI) injury were evaluated.

Results: table 1: Renal function,oxidative profile and TI.

Groups(n)	crCL/100g (ml/min)	UP (nmol/ gcr)	Thiol (nmol/ mg protein)	TBARS (nmol/g cr)	NO (µmol/g cr)	TI
SHAM (7)	0.97±0.02	1.7±0.1	170±16	57±7	30±2	0.2±0.1
Isch 30 (6)	0.12±0.01a	5.9±0.2a	117±10a	114±16a	77±7a	2.5±0.2a
Isch 30+Sil (6)	0.55±0.07abc	2.3±0.3bc	111±11a	48±2b	36±4b	1.8±0.3a
Isch 30+NAC (6)	0.57±0.08abc	3.2±0.5bc	89±4a	58±7b	31±2b.1	1.6±0.4ab
Isch 45 (5)	0.04±0.01a	9.7±1.8ab	129±17a	117±33a	76±15a	3.2±0.2a
Isch 45+Sil (5)	0.10±0.01a	4.0±0.6c	68±12ac	45±7bc	47±5c	2.2±0.4ac
Isch 45+ NAC (5)	0.04±0.01a	7.8±1.6a	104±9a	117±13a	83±5a	3.6±0.2a

a p<0.05 versus SHAM;b p<0.05 versus Isch 30; c p<0.05 versus Isch 45

Conclusions: SIL and NAC treatment increased RF, decreased oxidation, NO levels and induced iNOS absence and HO-1 expression in 30 min RPC. In the 45 groups, only SIL protected those paramethers. Histology results were in favor of SIL, once it was able to reduce TI at 30 and 45 min RI. It can be concluded that SIL renoprotecive action is more effect than NAC´s for severe ischemia reperfusion syndromes and that HO-1 may be considered a cellular protecting mediator.

Funding: Government Support - Non-U.S.

TH-PO035

Therapeutic Effects of GLP-1 Agonist in Gentamicin-Induced Kidney Injury in Rats Joon Seok Choi, Chang Seong Kim, Jeong-Woo Park, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Internal Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea.*

Background: Glucagon-like peptide-1 (GLP-1) has various extra-pancreatic actions. Although the kidney also has GLP-1 receptors, a role GLP-1 in aminoglycoside-induced nephropathy has not been examined. We investigated effect of exendin-4 in gentamicin (GM)-induced nephropathy in rats.

Methods: Two groups of rats were treated with GM (100 mg/kg/day), one of which was cotreated with exendin-4 (5 µg/kg/day) for 14 days and the other was not. The control group was treated with vehicle only. We evaluated renal function and the expression of inflammatory cytokines and adhesion molecules. In another series of experiment, effects of exendin-4 were determined in human proximal tubular cells (HK-2 cells) cultured in the presence of GM.

Results: Fasting blood glucose levels, blood pressure, creatinine clearance and albuminuria were not affected by exendin-4 in GM-treated rats. There was an upregulation of inflammatory cytokines such as IL-1β, IFN-r and TNF-α, which was prevented by exendin-4. Exendin-4 also effectively reversed the increases of ICAM-1, VCAM-1and MCP-1 mRNA expression in GM-treated rats. The expression of ED-1 and i-NOS was increased by GM, of which magnitude was attenuated by exendin-4. In HK-2 cells, GM increased connective tissue growth factor(CTGF) and iNOS expression compared to the control, and this effect was inhibited by exendin-4 treatment. Exendin-4 treatment attenuated the expression of phosphorylated ERK1/2.

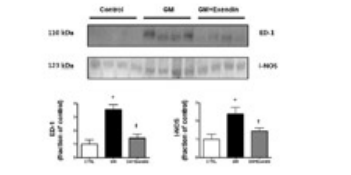

Figure 1. Effects of exendin-4 on inflammatory cell infiltration and inducible nitric oxide synthase (iNOS) expression. Semiquantitative immunostaining of ED-1 and i-NOS. Each column represents the mean±s.e.m. (n=4). * p<0.05 compared to the control † p<0.05 compared to GM treated rat

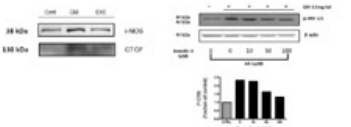

Figure 3. In vitro analysis of i-NOS, CTGF and p-ERK 1/2. Semiquantitative immunoblotting of iNOS, CTGF and p-ERK 1/2 expression in HK-2 cells incubated with GM (0.5 mg/ml) in the absence or presence of exendin-4.

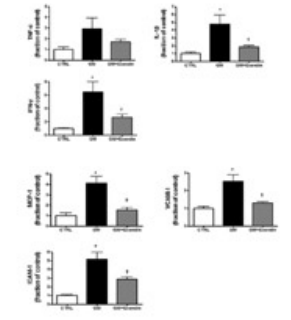

Figure 2. Effects of exendin-4 on inflammatory cytokines and adhesion molecules. Real-time polymerase chain reaction(PCR) was used to examine the expression of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β, monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1. Each column represents the mean±s.e.m. (n=5). * p<0.05 compared to the control † p<0.05 compared to GM treated rat

Conclusions: Exendin-4 has reno-protective effects through its anti-inflammatory actions. Its underline mechanism may include inhibition of MAPK signaling pathway.

TH-PO036

Acute Testosterone Supplementation Improves Renal Ischemia/Reperfusion-Induced Acute Kidney Injury (I/R-AKI) Andrea P. Soljancic,[1] Arnaldo F. Lopez-Ruiz,[1] Kiran B. Chandrashekar,[1] Rodrigo Maranon,[1] Ruisheng Liu,[2] Luis A. Juncos.[1] *[1]Medicine-Division of Nephrology, University of Mississippi Medical Center, Jackson, MS; [2]Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS.*

Background: Testosterone (TST) administration has beneficial effects during certain conditions (*e.g.* stroke, myocardial infarction) due to its antinflammatory property and perhaps by improving local blood flow. Because renal I/R-induced AKI is associated with renal hemodynamic impairment, inflammation and reduced vascular endothelial growth factor (VEGF) expression (which may have cytoprotective effects), we tested the hypothesis that TST improves AKI and preserves the abovementioned renal parameters in established AKI.

Methods: We randomized 3 groups of adult male SD rats (n=5-6): **1)** Sham (S), **2)** I/R-AKI Control (CT), **3)** I/R-AKI + testosterone propionate (TP). Renal I/R was induced by clamping both renal pedicles for 40min. TP (20 ng/kg/min x 10 min IV) was administered using a femoral catheter 3 hours after reperfusion. Animals were followed for 48 hours post ischemia, during which plasma creatinine, urine protein excretion and a urinary marker of AKI (KIM-1) were monitored at 24 hours. At the end of the study, outer medullary blood flow (OM-RBF) was measured by laser Doppler in anesthetized animals and then kidneys were harvested to evaluate renal inflammation (TNFα) and renal cytoprotective factor (VEGF).

Results:

Groups	PLCreat(mg/dl)	OM-BRF (TPU)	UrKIM-1(pg/ml)	UrProt(mg/24h)	TNF(pg/ml)	VEGF pg/ml)
S	0.54±0.04	19.3±1.4	712±20	30.95±3.4	16.4±0.3	376±5.7
CT	2.46±0.3*	11.4±0.3*	4576±75*	129.2±8.7*	61.8±1.1*	105±2.3*
TP	1.4±0.07*#	19.9±2.1#	1722±29*#	67.8±6.3*#	31.3±0.2*#	230±7.3*#

Data: Mean±SEM. *p<0.05 vs S, #p<0.05 vs CT

Conclusions: Our data suggest that TST improves AKI as demonstrated by: 1) reversing of OM-RBF impairment, 2) attenuation of renal injury and inflammation (Proteinuria, KIM-1, TNFα), 3) blunting of AKI-induced VEGF reduction. In conclusion, acute TST infusion improves renal hemodynamics, function, injury, and VEGF expression after I/R-induced AKI.

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TH-PO037

Preconditioning Effects of Rituximab on Subsequent Ischemia-Reperfusion Injury in Mouse Kidney Hyeonseok Hwang,[1,2] Sang Ju Lee,[1] Suk Young Kim,[1] Chul Woo Yang.[1] *[1]Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; [2]Clinical Research Institute, DaeJeon St. Mary's Hospital, Korea.*

Background: The adaptive immune system contributes to robust inflammatory response after Ischemia-reperfusion (I/R) renal injury. There is growing evidence of the roles of B-cell in postischemic kidney, and we investigated preconditioning effects with rituximab against subsequent I/R injury in mouse kidney.

Methods: Male C57BL/6 mice were used. Rituximab (5, 10 and 40 mg/kg) was administered 7 days before I/R injury by clamping both renal pedicles for 22 min. The effect of rituximab pretreatment was evaluated in terms of renal function, tubular necrosis score, serum immunogologulin (Ig) G and IgM levels.

Results: Pretreatment with 10 and 40 mg/kg rituximab decreased blood urea nitrogen and serum creatinine at 1, 3 and 10 days postischemia. Renal function was not improved in mice treated with 5 mg/kg rituximab. Tubular necrosis scores were lower in mice with 10 mg/kg rituximab treatment than in control mice. The serum IgG level was not different between control and rituximab-treated mice, but serum IgM level was tended to be reduced in rituximab-treated mice.

Conclusions: Our study demonstrates that preconditioning with more than 10 mg/kg rituximab exerts a protective effect against subsequent renal I/R injury.

Funding: Private Foundation Support

TH-PO038

Gamma-Tocotrienol Protects Against Oxidative Damage in Renal Proximal Tubule Cells Corey J. Hayes, Diana Bakajsova, Cesar M. Compadre, Grazyna Nowak. *Dept. of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: *Alpha, beta, gamma,* and *delta* tocopherols and tocotrienols are well known for their antioxidant properties. Oxidative stress is a major mechanism of acute kidney injury, diabetic nephropathy, and chronic kidney disease. This study tested if gamma-tocotrienol (GT3) protects against oxidative stress and mitochondrial dysfunction in renal cells.

Methods: Primary cultures of renal proximal tubular cells (RPTC) were treated with *tert*-butyl hydroperoxide (0.35 mM TBHP; 45 min), a model oxidant. Some RPTC were pre-treated with 5 μM GT3 prior to TBHP exposure. Production of reactive oxygen species (ROS), mitochondrial functions, and cell viability were measured after TBHP exposure.

Results: ROS production in injured RPTC increased 200% and was followed by decreases in state 3 respiration (50%) and respiratory control ratio (47%). Mitochondrial membrane potential decreased 39% and 82% at 4h and 24h, respectively, in TBHP-injured RPTC. Oligomycin-sensitive respiration declined 63%, F_0F_1-ATPase activity decreased 31%, and ATP content decreased 65% in TBHP-injured RPTC. Cell death increased to 22% and 52% at 4h and 24h, respectively, after TBHP exposure. Pretreatment of RPTC with GT3 blocked production of ROS, prevented the decreases in state 3 respiration, and maintained the respiratory control ratio in TBHP-injured RPTC. TBHP-induced decreases in oligomycin-sensitive respiration and F_0F_1-ATPase activity in mitochondria were prevented by GT3. Further, GT3 ameliorated decreases in ATP content after TBHP exposure. Finally, pretreatment with GT3 blocked cell lysis at 4h and reduced it from 52% to 13% at 24h after injury.

Conclusions: This is the first report demonstrating the protective properties of GT3 in renal cells. GT3 protects against RPTC injury by: 1) decreasing production of ROS, 2) improving mitochondrial respiration and coupling, 3) maintaining mitochondrial membrane potential, 4) blocking decreases in F_0F_1-ATPase function, 5) maintaining intracellular ATP levels, and 6) preventing RPTC lysis and death. This study suggests that GT3 could be used as a potential treatment to prevent renal injury associated with oxidative stress.

Funding: NIDDK Support

TH-PO039

Role of Cilastatin Against Cisplatin-Induced Nephrotoxicity and Inflammation in Rats Alberto Lázaro Fernández,[1] Blanca Humanes Sanchez,[1] Sonia Camaño Paez,[1] José Antonio Lázaro Manero,[1] Jose Manuel Lara Martinez,[2] Alberto Tejedor Jorge.[1] *[1]Laboratory of Renal Physiopathology, Hospital General Universitario Gregorio Marañón, Madrid, Spain; [2]Department of Pathology, Hospital General Universitario Gregorio Marañón, Madrid, Spain.*

Background: Cisplatin is a major antineoplastic drug for the treatment of solid tumors, but its nephrotoxicity is a major complication and a dose limiting factor for anticancer theraphy. Several evidences have shown that inflammation contribute to the pathogenesis of cisplatin-induced acute renal failure. In this study, we have investigated the potential use of cilastatin, a renal dehydrodipeptidase I inhibitor as nephroprotector on cisplatin-induced renal injury and inflammation in rats.

Methods: Male Wistar rats were divided into 4 groups: control rats, cilastatin-control rats, cisplatin-injected rats, cilastatin-treated cisplatin-injected rats. Nephrotoxicity was assessed 5 days after cisplatin treatment, by measuring serum creatinine, blood urea nitrogen (BUN), glomerular filtration rate, proteinuria and renal morphology. Inflammation was measured by electrophoretic mobility assay (EMSA) and immunohistochemical studies.

Results: Cisplatin-treated rats showed significant elevations in BUN, creatinine, and proteinuria and decreased the glomerular filtration rate when compared with control rats. Cisplatin rats also exhibited severe morphological changes such as necrosis and extensive vacuolization of the proximal tubule and inflammatory mediators were increased. Cilastatin significantly prevented partial or totally these changes in renal function and ameliorated histological damage in cisplatin-treated animals. Cilastatin also reduced serum tumor necrosis factor-alpha (TNF-a) levels, nuclear factor κB activation and ED1 (monocytes/macrophages) positive cells.

Conclusions: This study provides evidence that cilastatin reduces in vivo cisplatin nephrotoxicity by preventing inflammation and might represent a novel strategy in the prevention of cisplatin-induced acute renal injury.

Funding: Government Support - Non-U.S.

TH-PO040

CD73-Deficiency Protects in Kidney Ischemia Reperfusion Injury: The Role of Adenosine A1, A2A, A2B and A3 Receptors Siddharth V. Rajakumar,[1] Bo Lu,[1] Simon C. Robson,[2] Anthony J. d'Apice,[1] Peter J. Cowan,[1] Karen M. Dwyer.[1] *[1]Immunology Research Centre, St Vincent's Hospital, Melbourne, Victoria, Australia; [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

Background: Adenosine, acting at A1, A2A, A2B and A3 receptors, is protective in numerous models of ischemia reperfusion injury (IRI). Extra-cellular adenosine is generated by the successive hydrolysis of nucleotides by the ecto-enzymes CD39 and CD73. CD39 over-expression protects in kidney IRI while CD73 deficiency and inhibition also confer protection. Given CD73-deficiency results in reduced adenosine generation, the mechanism by which this protection is achieved remains unclear. We aim to determine the role of A1, A2A, A2B and A3 receptors in the protective effect of CD73-deficiency in kidney IRI.

Methods: Wild-type (WT) and CD73-deficient (CD73KO) mice underwent right nephrectomy and 18 minutes left renal ischemia. After 24 hour reperfusion, renal function (serum creatinine, umol/l) and histological renal injury (scored S:1-9) were assessed. Mice were pre-treated with specific A1, A2A, A2B or A3 receptor antagonists.

Results: Compared to WT mice [Cr=96,S:3.6,n=5], CD73KO mice are protected [Cr=45,S:0.8,n=5,p<.05]. A1-antagonist treatment has no significant effect on WT mice (trend toward protection) [Cr=64,S:2.7,n=4] and protection is maintained in CD73KO mice [Cr=59,S:1.0,n=4,p<.05]. A2A-antagonist treatment worsens renal function and histological damage in both WT [Cr=145,S:4.8,n=4, trend:p=.08] and CD73KO mice [Cr=82,S:2.8,n=6,p<.05], but its detrimental effect is still attenuated in CD73KO mice [p<.05]. A2B-antagonist treatment has minimal effect on WT mice [Cr=85,S:4.0,n=3] but significantly worsens renal dysfunction in CD73KO mice [Cr=146,S:5.3,n=5,p<.01]. A3-antagonist treatment abrogates injury in WT mice [Cr=49,S:1.5,n=4,p=.05] and protection in CD73KO mice is maintained [Cr=43,S:0.5,n=4,p<.05].

Conclusions: CD73-deficiency and A3 receptor inhibition protect in kidney IRI. Protection mediated by CD73-deficiency is not dependent on A1, A2A or A3 receptor signaling and is dependent on A2B receptor signaling. The A2B receptor plays a protective role in states of lower adenosine generation.

Funding: Government Support - Non-U.S.

TH-PO041

Increased Angiopoietin-Like Protein 4 (Angptl4) Expression and Hyperlipidemia in Acute Kidney Injury Syed M. Ali,[1] Shenyang Li,[1] Neriman Gokden,[2] Didier Portilla.[1] *[1]Nephrology, Internal Medicine, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, Little Rock, AR; [2]Pathology, .*

Background: In the present study we examined the mechanisms by which cisplatin causes hyperlipidemia and the potential role of hyperlipidemia in nephrotoxicity. We studied whether changes on Lipoprotein Lipase(LPL) activity, the enzyme responsible for the hydrolysis of triglycerides(TG), and changes in the expression of Angptl4, a potent inhibitor of LPL account for CP-mediated hyperlipidemia.

Methods: Wild type mice fed a normal diet or a diet containing peroxisome proliferator activated receptor-alpha ligand WY received a single intraperitoneal injection of saline or CP(25 mg/kg BW). Serum, white epididymal adipose tissue, liver and kidney tissue were collected after CP exposure.

Results: CP caused a time-dependent reduction in white epididymal adipose tissue mass by 40% at day 3, that was accompanied by focal necrosis and increased lymphocytic infiltration. LPL activities measured in serum and adipose tissue were inhibited by 70% when compared to saline treated mice. CP also inhibited kidney tissue LPL activity by 40%. Angptl4 mRNA levels were increased by 9-fold in liver and kidney tissue and by 2-fold in adipose tissue of CP-treated mice. Treatment of adipose tissue cells in culture with 25 micromolar CP for 24 hrs led to 70% reduction in LPL activity and a two-fold increased expression of Angptl4 mRNA levels. CP also increased the accumulation of TG in serum and kidney tissue. Renal function, CP-mediated inhibition of LPL activity, increased expression of Angptl4, and accumulation of TG in kidney tissue were ameliorated by the use of a PPARalpha ligand.

Conclusions: 1) Cisplatin mediated hyperlipidemia is caused by direct effects of CP on adipose tissue LPL activity, 2) Inhibition of LPL activity and increased Angptl4 gene expression in adipose, liver, and kidney tissue represent a common biochemical mechanism

involved in CP-mediated hypertriglyceridemia, 3) Fibrates ameliorate renal function and prevent the accumulation of TG in kidney tissue by preventing CP-mediated inhibition of LPL activity via reduced expression of Angptl4 in the proximal tubule.

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TH-PO042

Regulation of Nrf2 Mediated Signaling during Acute and Chronic Hypoxic Stimulation of Human Proximal Tubular Epithelial Cells

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Background: Acute kidney injury (AKI) caused by ischemia reperfusion (IR) is a major clinical problem in both native and transplanted kidney, but mechanisms by which IR contributes to AKI are largely undefined. We have previously shown that genetic deletion of Nrf2 enhances susceptibility to ischemic AKI in mice. Because Nrf2-dependent transcriptional response is essential for mitigating cellular stress, we investigated whether hypoxic reoxygenation impairs activation of Nrf2 signaling in kidney epithelial cells, thereby worsening tubular injury in AKI.

Methods: Human renal proximal tubular epithelial cells, HK2, were exposed to acute (2 h) and chronic (12 h) hypoxic conditions with and without re-oxygenation for up to 6 h. The expression levels of *Nrf2*, *Keap1* (an endogenous inhibitor of Nrf2), and several antioxidant genes, as well as Nrf2 recruitment to the endogenous antioxidative genes were evaluated.

Results: Gene expression analysis revealed a distinct and dynamic regulation of expression levels of *Nrf2* and *Keap1* in response to acute and chronic hypoxic-reoxygenation conditions. Hypoxia alone stimulated significantly *Nrf2* mRNA expression, whereas re-oxygenation had a modest effect. However, immunoblot analysis revealed enhanced levels of Nrf2 after 6 h post-reoxygenenation. Hypoxia and reoxygenation also altered the expression level of Keap1. There was persistently elevated level of *Hmox1* expression during reoxygenation, while *Nqo1* expression was transiently induced. The analysis of Nrf2 recruitment to the antioxidative gene promoters using chromatin immunoprecipitation (ChIP) assays revealed an increased Nrf2 binding to the antioxidant response elements (AREs) of *Hmox1* and *Nqo1* promoters during acute hypoxic-reoxygenation condition, but not in the chronic exposure.

Conclusions: Our data demonstrate a dynamic and distinct regulation of Nrf2 activation by hypoxia in kidney epithelial cells. Modulation of Keap1 expression may play a role in regulating ARE-mediated gene expression during hypoxic-reoxygenation in AKI.

Funding: NIDDK Support

TH-PO043

Renal Dysfunction Associated with Increased Intra-Abdominal Pressure in Experimental Heart Failure: Nephroprotective Effects of Phosphodiesterase-5 Inhibition

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Background: The deleterious effects of elevated intra-abdominal pressure (IAP) on the kidneys are widely recognized in abdominal compartment syndrome, visceral edema and laparoscopic surgery. Previously, we demonstrated that rats with congestive heart failure (CHF) exhibited exaggerated sensitivity to the adverse renal effects of elevated IAP compared with sham controls. In the present study we tested whether IAP induces acute kidney injury (AKI), and whether phosphodiesterase-5 (PDE5) inhibition ameliorates the adverse renal effects of elevated IAP in rats with CHF.

Methods: Following a baseline period, rats with high- and low-output CHF induced by the placement of aorto-caval fistula or LAD ligation, respectively, and sham-controls were subjected to consecutive IAPs of 7, 10, or 14 mmHg for 45 min each by CO_2 insufflation. Urine flow (V), Na+ excretion (UNaV), glomerular filtration rate (GFR), renal plasma flow (RPF) and NGAL excretion were determined. The effects of pretreatment with Tadalafil (10 mg/day, PO) on the adverse renal effects of elevated IAP were examined in these rats.

Results: While IAP of 7 mmHg in sham-controls did not affect V, UNaV, GFR and RPF, IAPs of 10 and 14 mmHg produced dose-dependent reductions in these parameters. Basal kidney function and renal hemodynamics were lower in both low- and high-output CHF rats. When subjected to 10 and 14 mmHg, CHF rats exhibited exaggerated declines in V, UNaV, GFR, RPF and increased NGAL excretion compared to sham controls. Pretreatment with Tadalafil ameliorated the deleterious renal effects of high IAP in both CHF models.

Conclusions: Rats with CHF are vulnerable to the adverse renal effects of pneumoperitoneum. Tadalafil abolishes renal dysfunction and AKI induced by high IAP, supporting a therapeutic role for PDE5 inhibition in laparoscopic surgery in CHF states.

Funding: Government Support - Non-U.S.

TH-PO044

Role of Autophagy for Tubular Maintenance, Aging and Stress Adaptation

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Background: Autophagy is a major pathway that delivers damaged proteins and organelles to lysosomes to maintain cellular homeostasis. Recent studies indicate an upregulation of autophagy in tubular cells under stress conditions. However, the precise function of tubular autophagy in vivo remained unclear. To determine the role of autophagy in tubular cells, we generated Atg5 flox/flox: Pax8 rtTA: Teto-Cre mice and Atg5 flox/flox: Ksp-Cre mice where Atg5 is deleted in all tubular segments or only the distal tubular compartment, respectively. Atg5 deletion in the complete tubular system resulted in accumulation of p62-positive protein aggregates and increased serum creatinine 4 months after doxycycline administration. Under pathophysiological condition such as ischemia/reperfusion injury, proximal tubules displayed upregulated autophagic activity and autophagy-deficient mice exhibited significantly increased signs of tubular injury. Otherwise, Atg5 deletion restricted only to the distal tubular system did not result in histological abnormalities or increased serum creatinine levels for up to 1 year of age. These data suggest that autophagy is a critical regulator of proximal tubular homeostasis and protects tubular cells from acute kidney injury.

Funding: Government Support - Non-U.S.

TH-PO045

The Impact of Protein Kinase C λ/ι Deficiency in Proximal Tubule Epithelial Cells

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Background: Podocyte specific deletion of atypical protein kinase C λ/ι (PKCλ/ι), in mice, leads to a severe glomerular phenotype with mislocated slit diaphragms, atypical cell-cell junctions and proteinuria. Because of the great effect of PKCλ/ι on podocyte polarity we wanted to investigate the role of PKCλ/ι in the highly polarized kidney tubules using a transgenic mouse model.

Methods: We specifically deleted PKCλ/ι in proximal tubule epithelial cells by breeding transgenic Sglt2-Cre mice with PKCλ/ι floxed mice. Offspring of these mice were tested for renal function before and after treatment with aristolochic acid to induce the murine model of acute kidney injury aristolochic acid nephropathy (AAN). Sections of kidney were examined for proliferation by Ki67 staining. Furthermore, cDNA of WT and KO kidneys was tested via realtime PCR for the gene expression of acute kidney injury markers, NGAL and Kim-1. To investigate the PKCλ/ι deficiency in vitro we isolated proximal tubule epithelial cells from WT and KO kidneys and analyzed them in a Ca^{2+} switch assay.

Results: The histology of the PKCλ/ι Sglt2-Cre mice revealed no obvious renal phenotype up to 6 month after birth. After injection of aristolochic acid, both WT and KO mice developed acute renal injury marked by an increase of serum creatinine and serum urea at day 3-6. On day 14 we sacrificed the animals, and compared the histology of WT and KO by HE stainings. We detected a different amount of tubular damage by a semiquantitative damage scoring. Realtime PCR analysis confirmed the damage score with the expression measurements of NGAL and Kim-1. In stainings we found an increase of Ki67 positive cells in the KO mice.

In isolated PKCλ/ι deficient proximal tubule epithelial cells we could demonstrate a disturbed expression pattern of the junction protein E-cadherin, especially in the Ca^{2+} switch assay.

Conclusions: In summary, we found that atypical PKCλ/ι is an important factor for the reorganization of proximal tubular epithelial cells after acute kidney injury.

Funding: Government Support - Non-U.S.

TH-PO046

TWEAK Modulates Proliferation, Matrix Remodeling and Migration in Renal and Embryonic Fibroblasts through Ras Signaling

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Background: TWEAK is a TNF superfamily member involved in different processes. In the kidney, TWEAK induces proliferation and an inflammatory phenotype in renal tubular cells. TWEAK blockade ameliorates renal damage in mice AKI models, but there is scarce information about the role of TWEAK in tissue repair after injury. Renal fibroblasts and their matrix-related properties are key players in tissue remodeling after injury and in renal fibrosis.

Methods: TWEAK induced proliferation in cultured renal fibroblasts (TFB cells) through ERK pathway. Looking for the link between TWEAK signaling and ERK, we tested Ras/ERK pathway. TWEAK treatment of renal fibroblasts resulted in an early activation of Ras and ERK. Ras inhibition blocked TWEAK-induced activation of ERK and proliferation. These results represent the first evidence of Ras involvement in TWEAK signaling. Murine embryonic fibroblasts (MEF) also increased proliferation in response to TWEAK, whereas MEF cells lacking N- and H-Ras isoforms did not, supporting the Ras involvement.

Results: Renal fibroblasts are characterized by an active role in extracellular matrix remodeling. TFB cells stimulated with TWEAK drastically reduced collagen type I and fibronectin synthesis and secretion. By contrast, TWEAK increased the activity of MMP-9 and PAI-1 production. Similar effects over collagen type I and fibronectin were observed in MEFs and seemed to be Ras-dependent. These results suggested that TWEAK modulates tissue remodeling through the Ras pathway, so we tested the potential functionality of this pathway in a wound healing assay. Wound-closure time was smaller in MEF with active Ras in the presence of TWEAK. This effect disappeared in Ras-deficient MEFs.

Conclusions: TWEAK regulates fibroblast proliferation, matrix proteins production and wound closure capacities depending on Ras. These results suggest that TWEAK/Ras pathway may contribute to tissue remodeling during tissue repair.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO047

Signalling Pathways Responsible for Proximal Tubule Cell Adhesion Promotion during I/R by Darbepoetin α: New Therapeutic Targets for a New Use of Darbepoetin α

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Background: Several therapeutic approaches to AKI assayed during last ten years did not showed a great impact in clinical practise. It has been described that EPO exerts cytoprotection against ischemic injury in several tissues. We previously reported that Darbepoetin α (DPO) treatment in mice protected animals from renal ischemic injury (Blanco-Sanchez et al., ASN 2009). Renal structure and function, altered during reperfusion, are restored after DPO treatment since DPO protects proximal tubule cell adhesion. We studied several signalling pathways underlying these effects, in particular cytoskeleton organization regulators.

Methods: We used a mice model of I/R and an *in vitro* model of hypoxia/reoxygenation (H/R) in mouse proximal tubule cells (MCT) where DPO is administered after ischemic insult. LIMK phosphorylation was studied by WB, Rac1, RhoA and PKCa traslocation were observed by immunofluorescence and cell adhesion was estimated by cell impedance studies.

Results: DPO promotes LIMK phosphorylation *in vitro,* in a dose dependent manner. LIMK can be regulated by Rho-GTPAses, thus we determined localization of RhoA and Rac1 in cells submitted to H/R and treated with DPO. Our results indicated that Rac-1 was traslocated to the cell membrane during reoxygenation, indicating Rac1 activation, and this traslocation is promoted by DPO treatment. In contrast, RhoA traslocation is not increased by DPO. Finally, we checked PKCa activity *in vitro* and *in vivo* finding that DPO does not exert any effect on its traslocation. Moreover, *in vitro* use of GO9760, a specific PKCa inhibitor, did not affect cell adhesion.

Conclusions: DPO exhibited a beneficial effect in experimental acute kidney injury activating Rac-1-LIMK pathways, avoiding cytoskeleton reorganizations without PKCa contribution. New targets for DPO in cytoprotection have been identified and they may be relevant for a new therapeutic use of EPO.

Funding: Government Support - Non-U.S.

TH-PO048

Renal Ischemia Reperfusion Injury Promotes Mid-Term Tubular Senescence

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Background: Renal ischemia reperfusion injury (IRI) leads to acute kidney injury, but the changes have not been studied so much after mid-to-long term of this injury. Levels of serum creatinine and urea nitrogen in this model increased within a day, but recovered some days after injury. And morphologically, most tubular damages repaired after a month or later, but fibrosis was observed depending on the magnitude of ischemia. It is unknown that tubulointerstitial areas other than the fibrotic areas remain normal or not. Here we revealed the enhanced senescence status in renal tubules in outer medulla 15-30days after IRI.

Methods: We evaluated histological changes in rat kidney 1, 5, 15, 30 days after IRI (n=6). Senescence status was also evaluated with senescence-associated β galactosidase (SABG) staining, immunohistochemistry of p21, quantitative PCR of p21, and immunoblot of p53 and p16. Mitochondrial DNA copy number was also calculated by quantitative PCR of mitochondrial DNA of the kidney.

Results: After IRI, repairing tubules were observed within day 5, and the repair was completed and patchy fibroses were observed 15 and 30 days after IRI. Fibrotic areas significantly increased in a time-dependent manner after IRI (0% in control, 12% in IRI-day 30). SABG positive areas in outer medulla also significantly increased with the days after IRI (2% in control, 10% in IRI-day 30). The SABG positive areas were limited mainly in tubules. Tubules were positively stained for p21 from 5 days after IRI until 30 days. Transcript levels of p21 after 30 days of IRI increased by 8 fold to control. Protein levels of p53 and p16 increased from day 5 to day 30 after IRI. Mitochondrial DNA copy number, which is parallel with mitochondrial number, decreased in a time-dependent manner after IRI (100% in control, 70% in IRI-day 30).

Conclusions: Renal IRI promotes mid-term tubular senescence. This alteration may be causative of long-term deterioration of renal function after AKI.

TH-PO049

Hypothermia Attenuates Ischemia-Reperfusion Renal Injury Via Preservation of ERK Phosphorylation

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Background: It is known that hypothermia during ischemic period is protective against ischemia reperfusion renal injury. Although it is thought that decrease of metabolic demand and free radical generation may involve in hypothermia, the exact explanation is not known. We evaluate whether molecular pathway might be involved in renal protection against IR renal injury at hypothermia (32°C).

Methods: C57Bl/6 mice were divided into six groups; sham operatative mice, 32°C temperature (cold) IR mice, 37°C temperature (warm) IR mice (reperfusion 27 minutes after clamping of both renal artery and vein) and PD98059 with 32°C temperature IR mice, L-NAME with 32°C temperature IR mice. Kidneys were harvested at 10min and 27min after both renal artery ischemia and 24hr after IR injury. Renal ERK, iNOS, eNOS, caspase-3 activation was evaluated by western blot. We examined BUN, serum creatinine (s-Cr), TUNEL and light microscopic findings.

Results: Serum level of BUN and s-Cr in cold IR mice was significantly lower than that of warm IR mice ($p<0.01$). Hypothermia decreased tissue injury score and TUNEL positive cells in IR kidney. pERK and eNOS expression were increased in cold IR mice at ischemic kidney(10min and 27min after both renal artery clamping). Inhibition of p-ERK using PD98059 elevated the levels of BUN and s-Cr in cold IR mice than control cold IR mice. Also it increased tissue injury score and TUNEL positive cells that were decreased in cold IR kidney. However, there were no differences of BUN, s-Cr and tissue injury score between cold IR mice and L-NAME with cold IR mice.

Conclusions: In conclusion, the results of the present study suggest that hypothermia has reno-protective effect on IR injured mice. Renoprotective effect of hypothermia may be involved in ERK phosphorylation

TH-PO050

Hypoxia-Inducible Factor Mediates Hypoxia-Induced Up-Regulation of microRNA miR-21 in Human Renal Epithelial Cells

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Background: Hypoxia-inducible factor (HIF) is an important transcriptional regulator in cellular response to hypoxia. We have found that miR-21 contributes to the protective effect of delayed ischemic preconditioning on renal ischemia-reperfusion injury in mice. In the present study, we examined the role of HIF in the regulation of miR-21 expression in primary cultures of human renal epithelial cells (HRE).

Methods: HRE cells were treated with cobalt chloride (300uM for 4h) or hypoxia (2% O_2 for 24h). HIF decoy oligodeoxynucleotides (40nM) were used to block the activity of HIF. miR-21 and PDCD4 levels were analyzed by real-time PCR.

Results: Treatment of HRE cells with cobalt chloride or hypoxia, which are classical inducers of HIF activation, caused significant up-regulation of miR-21 by 183% ± 9% (vs. control, n=6, $P<0.05$) and 168% ± 22% (vs. normoxia, n=6, $P<0.05$), respectively. Blockade of HIF activity with a decoy significantly reduced miR-21 levels in HRE cells treated with cobalt chloride by 42% ± 13%, compared to scrambled oligonucleotides (n=6, $P<0.05$). The up-regulation of miR-21 expression by cobalt chloride was correlated with down-regulation (by 57% ± 8%, $P<0.05$) of mRNA levels of programmed cell death protein 4 (PDCD4), a pro-apoptotic target gene of miR-21. The HIF decoy significantly blunted the down-regulation of PDCD4 ($P<0.05$).

Conclusions: These findings suggest a new mechanism in which HIF activation mediates up-regulation of miR-21. The mechanism may be relevant to renal protection against ischemic injury.

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TH-PO051

Predictive Performance of Biomarkers in Acute Kidney Injury (AKI) Using AKI Network (AKIN) Serum Creatinine or Urine Output Criteria

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Background: There is limited information on the value of biomarkers to diagnose acute kidney injury (AKI) according to AKIN serum creatinine compared to urine output criteria.

Methods: We conducted a prospective, multicenter observational study to compare the usefulness of urine neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C, osteopontin, interleukin-18 (IL-18) and N-acetyl-beta-D-glucosaminidase (NAG) levels to diagnose AKI in 51 critically ill patients using the AKIN urine output and serum creatinine criteria. Biomarkers samples were collected every 12 hours after intensive care unit (ICU) admission for a ≥48 hours and up to 10 days.

Results: The Area Under the Curve (AUC) of the different biomarkers to predict AKI are included in the table:

	AKIN serum creatinine (n=17)*	AKIN urine output alone (n=23)	AKIN urine output and/or serum creatinine (n=40)
urine NGAL	0.598 (0.489-0.707)	0.302 (0.180-0.425)	0.342 (0.230-0.455)
urine KIM-1	0.783 (0.680-0.886)	0.523 (0.388-0.658)	0.601 (0.481-0.720)
urine cystatin C	0.710 (0.619-0.801)	0.432 (0.303-0.561)	0.497 (0.376-0.617)
urine osteopontin	0.630 (0.510-0.749)	0.540 (0.410-0.670)	0.543 (0.422-0.664)
urine IL-18	0.545 (0.423-0.668)	0.684 (0.558-0.810)	0.655 (0.541-0.769)
urine NAG	0.582 (0.431-0.734)	0.708 (0.568-0.847)	0.694 (0.562-0.826)

*11 patients also met urine output criterion

Conclusions: In our cohort, urine NGAL, KIM-1, cystatin C and osteopontin offered their best performance when used to predict AKI according to AKIN serum creatinine criteria while urine IL-18 and NAG were best using AKIN urine output criteria. The different performances of these biomarkers may represent their propensity for distinguishing structural injury vs functional change. These results can be helpful to plan further studies using biomarkers in AKI.

Funding: Pharmaceutical Company Support, Private Foundation Support, Clinical Revenue Support

TH-PO052

Long-Term Outcome of the Hemolytic Uremic Syndrome: Observations from a Prospective Multicenter Study Alejandra Rosales,[1] Johannes Hofer,[1] Magdalena Riedl,[1] Therese C. Jungraithmayr,[1] Dorothea Orth-Höller,[3] Burkhard Toenshoff,[2] Reinhard Würzner,[3] Helge Karch.[4] *[1]Department of Pediatrics I, Innsbruck Medical University; [2]University Children's Hospital Heidelberg; [3]Division of Hygiene and Medical Microbiology, Innsbruck Medical University; [4]University of Münster.*

Background: Hemolytic uremic syndrome (HUS) is one of the most common causes of acute renal failure in childhood. The long-term prognosis of patients with HUS is not well delineated to date.

Methods: Over a six-year period, 619 patients under 21 years of age with the clinical diagnosis of HUS were registered in Austria and Germany in the largest prospective multicenter study so far.

Results: An infection with enterohemorrhagic E. coli (EHEC) was confirmed in 79% of cases. 70% of EHEC+ patients totally recovered after 5 years. Long-term sequelae such as proteinuria (18.3%), hypertension (8.6%) and/or decreased GFR (6.8%) were present in 30% of EHEC+ HUS patients and were also observed secondarily after apparent complete recovery. Neurological symptoms dropped from 25% during acute phase to 4% after 1 year, not increasing afterwards. Certain risk factors for presenting HUS after an infection with EHEC such as more virulent serotypes, Shiga toxin 2 production or *eae* positivity were not associated with long-term sequelae. The use of antibiotic therapy in the diarrheal phase was not associated to poor outcome (p=0.95). Plasma treatment was more frequently used in severe cases (presenting hypertension, neurological symptoms and needing dialysis; all p<0.005). In these high-risk EHEC+ HUS patients, the use of plasma therapy was associated with poor long-term outcome at all follow up points (all p< 0.05). Patients presenting long-term problems required dialysis for longer periods than patients who did not present sequelae (median dialysis duration 15 day vs. 9 days; p=0.001).

Conclusions: Because of frequent long-term sequelae (30%), which partly develop after apparent full recovery, follow-up investigations for at least 5 years are recommended. Early identification of the cause of disease is mandatory and has great influence on the selection of the best treatment option.

Funding: Government Support - Non-U.S.

TH-PO053

Biomarkers of Kidney Injury and Risk of AKI Progression Following Cardiac Surgery Jay L. Koyner, Amit X. Garg, Steven G. Coca, Kyaw Sint, Heather Thiessen Philbrook, Uptal D. Patel, Michael Shlipak, Chirag R. Parikh. *TRIBE AKI Consortium.*

Background: Acute kidney injury (AKI) following cardiac surgery is associated with poor patient outcomes.

Methods: Using samples from the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE AKI) study, we evaluated whether biomarkers of kidney function and injury could forecast the severity of AKI at the time of a clinical diagnosis of AKI. Among 426 individuals who developed at least Acute Kidney Injury Network (AKIN) Stage 1 AKI, serum creatinine (Scr), urinary IL-18, urinary albumin to creatinine ratio (ACR) and urinary and plasma NGAL were measured on the day of AKI diagnosis. The primary end point was progression of AKI, defined by worsening of AKIN Stage (e.g. from Stage 1 to Stage 2) prior to hospital discharge. We categorized each biomarker into quintiles (Q) and grouped them [Low (Q1 & Q2), Intermediate (Q3 & Q 4) and High Q5]. Using multivariable logistic regression, we determined the adjusted odds of AKI progression.

Results: The 50 subjects (11.7%) who progressed beyond their original AKIN Stage needed more dialysis, had longer ICU and hospital stays and higher inpatient mortality (p<0.0001 for all). After adjustment for clinical predictors, when compared to Q1 and Q2, the highest quintile of each biomarker remain associated with AKI progression; [Odds ratio (95% CI)] percent change SCr 3.6 (1.6-8.2), IL-18 3.5(1.5-8.0), ACR 4.6 (1.8-11.7), urine NGAL 2.2 (0.99- 4.7), and plasma NGAL 11.64 (4.2-32.4). After including post operative changes in SCr in the clinical model, IL-18 3.2 (1.4-7.6), ACR 4.0 (1.5-10.4) and plasma NGAL 9.7 (3.4-27.5) were all independently associated with AKI progression. Each biomarker provided improvement in risk classification over the clinical model alone with plasma NGAL performing the best: Net Reclassification Index 0.33 (p<0.0001) and Integrated Discrimination Improvement 0.11 (p<0.0001).

Conclusions: Biomarkers of AKI at the time clinical creatinine rise improve risk stratification and identify patients with newly diagnosed AKI who are at the highest risk for the most severe forms of AKI and the worst patient outcomes.

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TH-PO054

Multiple Nephrotoxic Events and Associations with Acute Kidney Injury and Mortality Katherine Rausa, Ladan Golestaneh, Michal L. Melamed. *Nephrology, Albert Einstein College of Medicine - Montefiore Medical Center, Bronx, NY.*

Background: Acute kidney injury (AKI) occurs in 15% of hospitalized patients and is associated with significant morbidity and mortality. We hypothesized that patients would encounter multiple potentially nephrotoxic events, and that those experiencing more events would be at higher risk of AKI and mortality.

Methods: We investigated all patients greater than 18 years old admitted between 2008 and 2010 at Montefiore Medical Center, a tertiary medical center in the Bronx, NY. We examined 16 potentially nephrotoxic exposures including systolic blood pressure < 90 mm Hg, ICD-9 diagnoses of sepsis, and exposure to different medications (e.g. NSAIDs, iv contrast). AKI was defined as a 50% increase in serum creatinine over a pre-admission creatinine. Mortality data was collected via linkage to the Social Security Death Index.

Results: There were 46,754 admissions meeting our eligibility criteria. 2,111 patients developed AKI (5%). Those developing AKI were more likely to have higher Charlson scores (2.3 versus 2.0, p=0.002) ,diabetic complications (5.8% versus 4.4%, p<0.001) or an eGFR that was < 60 ml/min/1.73 m(2) (37% versus 22% p<0.001).

In a multivariable model, NSAID use was associated with a higher risk of AKI (OR 1.58, 95% CI: 1.40, 1.78), and statin use was associated with a lower risk (OR 0.86, 95% CI: 0.77, 0.96).

The median number of potential nephrotoxic events was 2 (IQR 1 to 3). Each additional renal insult increased the multivariable-adjusted odds risk of AKI by 4% (95% CI 1%, 7%, p=0.006).

A total of 4788 patients died. Of those that died, 22% had a prior episode of AKI compared to 11% mortality without previous AKI (p<0.001). AKI was associated with a multi-variable adjusted higher risk of mortality (OR 1.5, 95% CI 1.3, 1.8). Each additional potential nephrotoxic event was associated with a 21% higher odds of mortality (95% CI 19%, 24%) even after adjusting for AKI.

Conclusions: In hospital acquired AKI, a greater number of potential nephrotoxic events is associated with a higher risk of AKI and mortality. Future research should focus on trying to minimize the number of nephrotoxic events in hospitalized patients.

TH-PO055

Acute Kidney Injury Time as a Predictor of Adverse Clinical Outcomes in Critically Ill Hospitalized Patients Guillermo G. Garcia, Jonathan Chavez, Jaime Briseño. *Nephrology, Hospital Civil de Guadalajara, Guadalajara, Mexico.*

Background: Acute kidney injury (AKI) is a complex and frequent complication of many diseases with a high mortality rate. Recent evidence shows that in addition to AKIN staging of severity, the duration of kidney failure is associated to poor outcomes. Prognostic classification systems such as RIFLE and AKIN do not take into account the etiology, duration or recovery time of AKI. Studies in diabetic patients have suggested that the time resolution of AKI is an independent prognostic factor for mortality. We report a similar finding in a population of critically ill hospitalized patients.

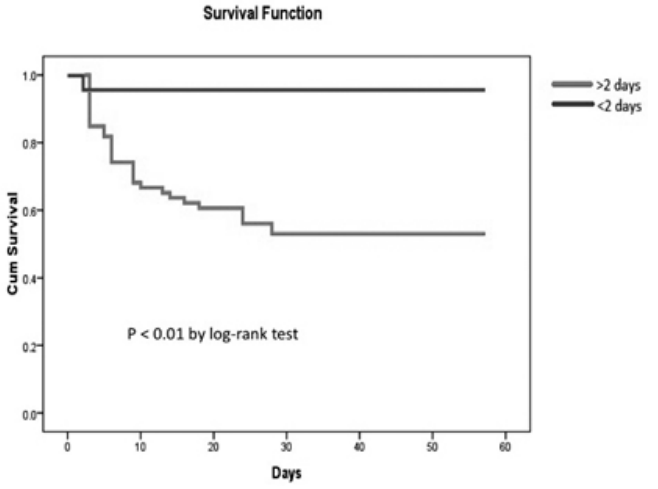
Methods: To determine the association between length of AKI and adverse clinical prognosis, we performed a cohort study in a hospitalized patients at the Hospital Civil de Guadalajara, from January 2009 to January 2010 that met the criteria for AKI by AKIN. Association was determined by Pearson's X2 or t-test depending on the nature of the variable. Correlation of data was analyzed using Pearson's correlation coefficient. Survival was determined by Kaplan-Meier method. A p <0.05 value was considered statistically significant.

Resutls:

Length of AKI and outcomes

	≤ 2 days n=47	> 2 days n=66	RR (IC 95%)	p
Age (y)	47.16±12.32	66.24±17.6		0.01†
Mechanical Ventilation, %	4	27	4.82 (1.27-18.3)	0.02
Vasopressors, %	11	41	3.31 (1.44-7.62)	<0.01
RRT, %	0	12		
Death, %	4	47	9.28 (2.38-36.05)	<0.01
Final S creatinine >1.5 mg/dl	2	52	9.48 (2.26-39.47)	<0.01

†t-test



Conclusions: AKI duration regardless of stratification by AKIN, is independently associated with poor outcomes. It could provide additional prognostic information than the rise of serum creatinine alone. These data needs to be validated by prospective studies of AKI. Finally, the longer duration of renal failure is associated with a risk of a high final serum creatinine. The latter could be a risk factor for CKD in the long term.

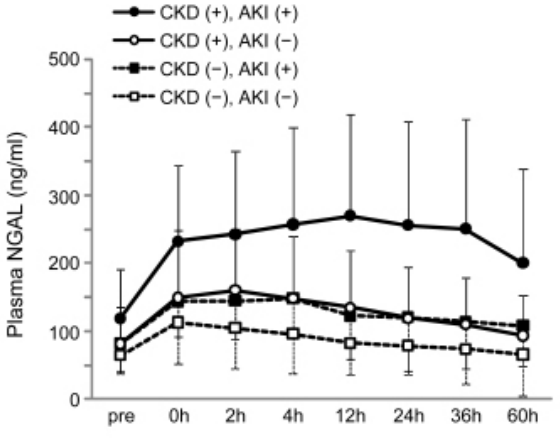
TH-PO056

Plasma Neutrophil Gelatinase-Associated Lipocalin Predicts Acute-on-Chronic Kidney Injury after Adult Cardiac Surgery: A Multicenter Prospective Study Kent Doi,[1] Masahiro Urata,[2] Daisuke Katagiri,[1] Kousuke Negishi,[1] Toshiro Fujita,[1] Seiichiro Murata,[2] Motoyuki Hisagi,[3] Minoru Ono,[3] Eisei Noiri.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Japan; [2]Cardiovascular Surgery, Itabashi Central General Hospital, Japan; [3]Cardiothoracic Surgery, University of Tokyo, Japan.*

Background: Plasma NGAL is a recently developed new AKI biomarker and previous clinical evaluations for plasma NGAL focused on only AKI occurred in patients without CKD. It is unclear whether plasma NGAL can predict acute-on-chronic kidney injury after cardiac surgery, because CKD significantly increases plasma NGAL levels in stable condition and may hamper the prediction of AKI by plasma NGAL.

Methods: The present study prospectively evaluated 143 adult patients who had cardiac surgery at two general hospitals. Plasma NGAL was measured before surgery, at ICU arrival (0 hr), and 2, 4, 12, 24, 36, 60 hr after. AKI was diagnosed by the AKIN criteria. 67 patients (46.9%) were complicated with CKD (eGFR<60) before surgery.

Results: Of 143 patients, 54 patients (37.8%) developed AKI after surgery and a multiple logistic regression analysis revealed that complication of CKD was significantly associated with AKI occurrence. Plasma NGAL before surgery, at 0, 2, 4, 12, 24, and 36 hr after ICU arrival in AKI were significantly higher than non-AKI both in CKD and non-CKD populations (Figure). ROC analysis showed the highest area under the ROC curve with plasma NGAL at 4 hr in CKD and non-CKD populations (CKD 0.738, non-CKD 0.809). The cutoff values of AKI prediction were 158 ng/ml in CKD and 97 ng/ml in non-CKD.



Conclusions: Plasma NGAL measured at pre-surgery and early time points in the post-surgery (4 hr) will predict AKI not only in non-CKD patients but CKD patients when the cutoff values properly determined.

Funding: Government Support - Non-U.S.

TH-PO057

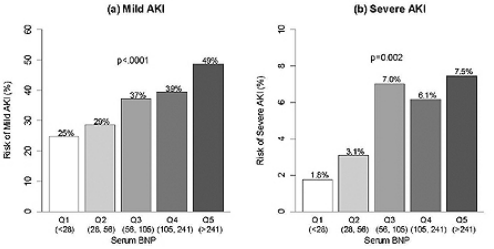
Pre-Operative Serum Brain Natriuretic Peptide and Risk of Acute Kidney Injury after Cardiac Surgery Uptal D. Patel, Amit X. Garg, Harlan M. Krumholz, Michael Shlipak, Steven G. Coca, Kyaw Sint, Heather Thiessen Philbrook, Jay L. Koyner, Madhav Swaminathan, Cary Steven Passik, Chirag R. Parikh. *TRIBE-AKI Consortium.*

Background: Acute kidney injury (AKI) following cardiac surgery is associated with poor outcomes and is difficult to predict. We conducted a prospective study to evaluate whether pre-operative brain natriuretic peptide (BNP) levels predict postoperative AKI among patients undergoing cardiac surgery.

Methods: The TRIBE-AKI Consortium enrolled 1,139 adults undergoing cardiac surgery at six hospitals from 2007-2009, who were selected for high AKI risk. Pre-operative BNP was categorized into quintiles (Fig).

Results: AKI was common using AKI Network definitions; at least mild AKI was a ≥0.3mg/dL or 50% rise in creatinine, n=407 (36%), and severe AKI was either a doubling of creatinine or the requirement of acute renal replacement therapy, n=58 (5.1%). In analyses adjusted for pre-operative characteristics, pre-operative BNP was a strong and independent predictor of mild and severe AKI. Compared with the lowest BNP quintile the highest quintile had significantly higher risk of mild AKI (risk ratio [RR] 1.87; 1.40-2.49) and severe AKI (RR 3.17; 1.06-9.48). After adjustment for clinical predictors, addition of BNP improved the area under the curve to predict mild (0.67 to 0.69, p=0.02) and severe AKI (0.73 to 0.75, p=0.11). Compared with clinical parameters alone, BNP also improved risk prediction of AKI cases into lower and higher risk [net reclassification index was 22.8% (p 0.0003) for mild AKI and 38.0% (p 0.0049) for severe AKI].

Figure. Incidence of Mild AKI (a) and Severe AKI (b) by Quintiles of Pre-operative BNP



AKI defined as during entire hospitalization, at least mild AKI defined as ≥50%, ≥0.3 mg/dL or dialysis; severe AKI defined as ≥100% or dialysis. BNP measured in pg/mL. *Abbreviations:* Acute kidney injury (AKI), brain natriuretic peptide (BNP)

Conclusions: Pre-operative BNP level is a strong independent predictor of post-operative AKI in high-risk patients undergoing cardiac surgery. If confirmed in other types of patients and surgeries, pre-operative BNP levels may improve risk stratification and discrimination among surgical candidates.

Funding: Other NIH Support - The research reported in this article was supported by the American Heart Association Clinical Development award, the grant R01HL-085757 from the National Heart, Lung, and Blood Institute. The study was also supported by CTSA Grant Number UL1 RR024139 from the National Center for Research Resources (NCRR). The plasma BNP assay was donated by Biosite. The granting agencies and Biosite, Inc. did not participate in the protocol development, analysis and interpretation of the results.

TH-PO058

PCreatinine Production Is Reduced in Septic Patients on Continuous Renal Replacement Therapy Pei-Chen Wu,[1] Ravindra L. Mehta.[2] *[1]Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [2]Medicine, University of California San Diego, San Diego, CA.*

Background: Doi *et al*. reported that sepsis resulted in reduced creatinine production (Pc) in a cecal ligation and puncture of nephrectomized mouse model (*J Am Soc Nephrol*. 2009; 20(6): 1217-21). Reduced Pc may hinder the elevation of serum creatinine (SCr) and thus delay the diagnosis of acute kidney injury (AKI). This finding has not yet been validated in humans.

Methods: This is a retrospective study of patients treated with CVVHDF from June 2008 to August 2010 at an academic medical center. Sepsis was defined as ≥ 2 SIRS signs suspected or known to be caused by a bacterial or fungal infection. All patients had serum and effluent samples analyzed for urea nitrogen and creatinine every 12 hours during therapy. Once patients were in steady state, usually at least 48 hours after CVVHDF initiation, we computed daily Pc using an equation published previously (*Am J Kidney Dis.* 1998; 32**:** 444-453). Pc of the first 4 days in steady state was recorded and analyzed. Every SCr was adjusted with the cumulative daily fluid balance.

Results: There were total 221 treatment-days in steady-state in 65 patients (mean age ± SD was 55.7 ± 14.9 years, 81.5% was male). Pc was lower in septic patients.

Pc (mg/day)	Septic (N = 143)	Non-Septic (N = 31)	p-Value
Mean ± SD	536.0 ± 361.8	710.5 ± 230.4	0.011

During the first 4 days of steady state, Pc remained lower in septic group in spite of antibiotic treatment. Higher Pc was significantly associated with male gender (R = 0.23, p = 0.003), African Americans, non-ESRD CKD, HIV infection (R = 0.27, $p < 0.001$), and higher BW (R = 0.16, p = 0.041), but not with inotropic use (p = 0.268), intubated status (p = 0.069), or age (p = 0.460).

Conclusions: Our results show that creatinine production is altered in septic patients requiring CVVHDF. Additional studies are required to ascertain the mechanisms for reduced creatinine production. Serum creatinine levels may underestimate the severity of renal dysfunction in septic patients requiring dialysis and should be used with caution as a marker of AKI in sepsis.

Funding: Government Support - Non-U.S.

TH-PO059

The Utility of Fractional Excretion of Sodium and Urea in Advanced Chronic Kidney Disease Tarik Noureldeen, Joel Topf. *Nephrology, St John Hospital and Medical Center, Detroit, MI.*

Background: The most common etiologies of acute renal failure are prerenal azotemia and acute tubular necrosis (ATN). The fractional excretion of sodium (Fe Na) and urea (Fe urea) are often used to differentiate between these two entities. Little is known regarding the effect of advanced chronic kidney disease on the validity of these measurements.

Methods: We retrospectively analyzed 33 patients with acute renal failure. Data elements included Fe Na, Fe urea, baseline creatinine, estimated glomerular filtration rate, age, diuretic use and time from diagnosis to measurement of acute renal failure indices. Patients were classified to be ATN or pre-renal azotemia based on response to therapy, judgment of the treating nephrologist and objective data including ultrasound, microscopic urine analysis and biochemical findings. Data were analyzed using χ^2 and ROC analysis.

Results: Fe urea did not assist in differentiating pre-renal azotemia from ATN. The data shows, however, that as kidney function deteriorates the fractional excretion of sodium increases independent of diuretic use. Using ROC analysis we found that a cut point of 2.4% rather than 1% increased the accuracy of Fe Na in discriminating pre-renal azotemia vs. ATN in our population of patients with advanced chronic kidney with a mean glomerular filtration rate of 39 ml/min.

Conclusions: Fe urea was not a reliable indicator of pre-renal azotemia vs. ATN. However, Fe Na was a useful test using a cut point of 2.4% in our population with advanced kidney disease presenting with acute renal failure.

TH-PO060

Plasma and Urine Neutrophil Gelatinase-Associated Lipocalin (pNGAL and uNGAL) as a Novel Indicator for Early Identifying Critically Ill Acute Kidney Injury (AKI) Patients Who Subsequently Require Replacement Therapy Khajohn Tiranathanagul, Sukgasem Amornsuntorn, Paweena Susantitaphong, Somchai Eiam-Ong. *Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*

Background: The high mortality in critically ill AKI patients still persisted despite the advancement in renal replacement therapy (RRT). This might be explained by a delay in initiating RRT caused by using current traditional indications. The levels of pNGAL and uNGAL which were correlated with the degree of tubular cell injury might be utilized as the novel indicator for early initiation of RRT. This prospective cohort study was conducted to determine the accuracy of using pNGAL as a predictor in early identifying the AKI patients who subsequently needed RRT.

Methods: Forty seven critically ill AKI patients with RIFLE stage II-III who did not reach the traditional indications for RRT were enrolled in this study. The pNGAL and other parameters were determined in each patient daily for two consecutive days during the seven day follow-up. The primary endpoint was the RRT initiation according to the traditional indications within the further seven days.

Results: Of forty-seven critically ill AKI patients (31 males) with mean age 63.0±18.1 years had APACHE II score 18.8±7.7. The serum creatinine level at enrollment was 2.35±0.93 mg/dL. pNGAL could predict further RRT requirement with area under ROC 0.813 ($p<0.001$, 95%CI 0.66-0.90). At cut-point of 960 ng/mL provided sensitivity and specificity of 72.2 and 89.6% as well as positive and negative predictive of 81.25 and 83.8%. The uNGAL which could be obtained from thirty-three non-anuric patients were provided slightly lower area under ROC 0.806 ($p=0.005$, 95%CI 0.63-0.98). At cut-point of 2600 ng/mL provided sensitivity and specificity of 54.5 and 90.9% as well as positive and negative predictive of 75.0 and 80.0%.

Conclusions: The pNGAL is an excellent early biomarker for RRT-initiation in critically ill AKI patients. Moreover, the cut-point 960 ng/mL might be used as the early new indicator for early initiation of RRT that might improve patient survival.

TH-PO061

Evaluation of the Effect of Renin Angiotensin Aldosterone System (RAAS) Blockade on Glomerular Hemodynamic and Renal Function in Patients Undergoing Intravenous Contrast Studies Shitij Arora, Prachi Aggarwal, Anubhav Kaul, Ammar Husan, Shivtej Kaushal, Manu Bansal, Ajay Gupta, Ajith K. Chickaballapur Narayanaswamy, Frantz M. Duffoo. *Internal Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.*

Background: Contrast induced nephropathy (CIN) is the third leading cause of acute renal failure. Along with other preventive measures, there are studies suggestive of improvement in renal hemodynamics and function with the use of RAAS blockade. Current recommendations are to withhold ACEI and ARB 48 hours prior to administration of contrast. Our study aims to evaluate the effect of RAAS blockade on renal function in patients with intravenous (IV) contrast exposure.

Methods: In a retrospective study, 239 patients who had received IV contrast and hydration as per protocol were selected. Group I (study group) included patients on RAAS blockade and group II (control group) patients were without RAAS blockade. Creatinine clearance was calculated using the Cockcroft-Gault (C-G) formula before and 48-72 hours after contrast exposure. CIN was defined as 25% or 0.5mg/dl increase in creatinine from baseline.

Results: The prevalence of CIN was 14.6% in patients within the control group versus 17% in the study group, demonstrating no significant difference. Patients in the study group had a mean change in GFR of 2.77 ± 2.11 ml/min compared to 8.24 ± 2.70 ml/min in the control group, demonstrating neither significant difference between the two groups ($p=0.112$) nor any decline in renal function post IV contrast administration. Linear regression analysis identified no major independent variables predicting change in GFR; however, the presence of hypertension had a partial correlation of -0.227 ($p=0.35$) with change in GFR in the study group; whereas age had a partial correlation of -0.180 ($p=0.036$) with change in GFR in the control group.

Conclusions: Our study demonstrates that there was no significant difference in the serum creatinine and GFR post contrast administration with or without RAAS blockade. Therefore, we theorize the fact there is no beneficial effect in withholding RAAS therapy prior to contrast exposure.

TH-PO062

Evaluation of 4 Urinary Biomarkers in Assessing Renal Failure in Cirrhosis. Role for Urinary Neutrophil Gelatinase-Associated Lipocalin Cláudia Fagundes, Marie-Noelle Pepin, Monica Guevara, Elisabet Garcia Lopez, Gregori Casals, Manuel Morales, Gustavo Henrique Santos Pereira, Ezequiel Rodriguez, Elsa Sola, Rogelio Barreto, Wladimiro Jimenez, Vicente Arroyo, Pere Gines. *Hospital Clinic, Barcelona, Spain.*

Background: Urinary biomarkers are useful in assessing acute renal failure and outcome prediction in various clinical settings. There is little information on their relevance in cirrhosis

Methods: Aim: evaluate the usefulness of 4 urinary biomarkers in the differential diagnosis of renal failure and evaluate prognosis in cirrhotic patients. Urinary neutrophil gelatinase-associated lipocalin(uNgal), Kidney injury molecule (KIM1), ß2-microglobulin, and N-Acetyl glucosaminidase(NAG) were measured in 241 patients.

Results: 71 without ascites, 88 with ascites without renal failure, and 82 with ascites and renal failure (defined by serum creatinine > 1.5mg/dL) due to different causes. All biomarker levels, except ß2-microglobulin, were higher in patients with renal failure compared to those without renal failure. Of all biomarkers, uNGAL was the best to discriminate among the different causes of renal failure. Patients with acute tubular necrosis(ATN, n=11) and infection-associated renal failure(n=12) had higher values (324(195-1018) and 284(73-486) ng/mg creatinine, respectively) median (IQR), while patients with hypovolemia-related renal failure(n=16) and hepatorenal syndrome(n=22) had lower values (30(20-59) and 74(43-147) ng/mg creatinine, respectively). Neither KIM-1 nor NAG were able to discriminate between causes of renal failure.ß2-microglobulin was similar in all causes except for higher levels in patients with ATN. Patients with high uNgal (≥44 ng/mg creatinine) had lower 3mth survival compared to patients with low uNgal (67% vs 85%, respectively; $p=0.0038$). A logistic model performed to estimate the effect of biomarkers on 3mth survival showed that only uNgal and the presence of renal failure had independent prognostic value

Conclusions: In conclusion, only uNgal was capable of distinguishing between causes of renal failure and to predict mortality. Measurement of uNgal could be useful in assessing renal failure in cirrhosis.

Funding: Government Support - Non-U.S.

TH-PO063

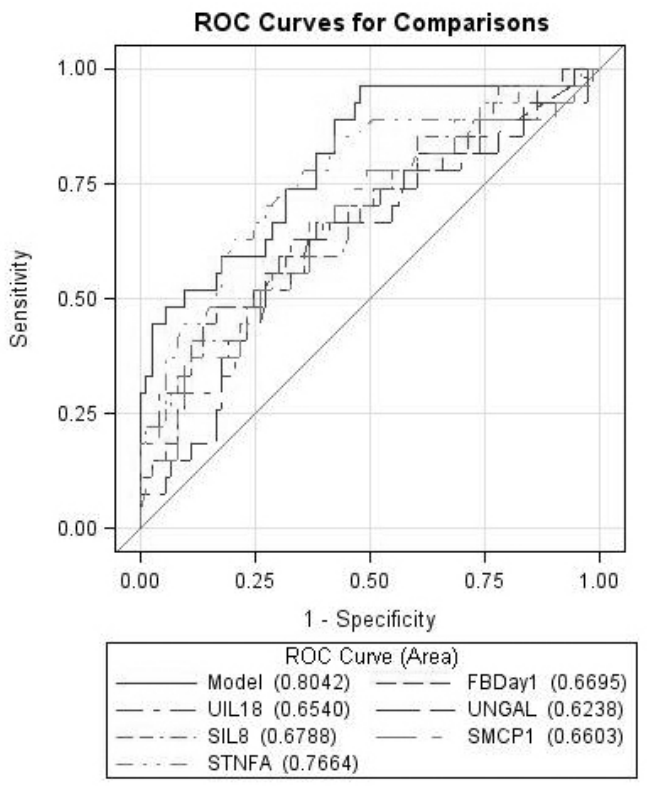
Comparison of Clinical, Biochemical and Biomarker Predictors of Acute Kidney Injury in Cardiac Surgery Ganesh Kambhampati, Mourad Alsabbagh, Gurjit Dhatt, Abdo Asmar, Uma Krishna Pakkivenkata, A. Ahsan Ejaz. *Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, FL.*

Background: We investigated the utility of clinical, biochemical and novel biomarkers to predict acute kidney injury in cardiac surgery.

Methods: Data from a prospective observational study were analyzed to compare the utility of positive fluid balance, serum MCP-1, TNF-alpha, IL-8, urine NGAL and urine IL-18 to predict acute kidney injury as measured by serum creatinine.

Resutls: 100 patients were analyzed. Receiver operating characteristic (ROC) curve analyses were used to determine each test's overall accuracy, as measured by the area under the curve

The diagnostic performance of the various tests as measured from the AUC (95%CI) is as follows- AUC for 24 hr FB was 0.6695 (0.5434-0.7955, $p= 0.004$), uIL-18 was 0.6540 (0.5247 0.7833, $p= 0.0100$), uNGAL was 0.6238 (0.4952-0.7523, $p=0.2114$), sIL-8was 0.6788(0.5554 0.8023, $p= 0.0042$), sMCP1 was 0.6603 (0.5339-0.7867, $p=0.0195$) and sTNF-alpha was 0.7664 (0.6556-0.8771, $p= 0.0004$). At 24hours, positive FB provided similar information as urine NGAL and urine IL18 to predict acute kidney injury. The predictive power of sIL-8, sMCP-1 and sTNF-alpha was better compared to 24hr fluid balance.



Conclusions: Positive FB at 24-hours following cardiac surgery may be an early and simple diagnostic tool to predict AKI. It is as good as the novel biomarkers such as urine NGAL and urine IL18. sIL-8, sMCP-1 and sTNF-alpha were better predictors of AKI compared to positive FB.

TH-PO064

Iodine/eGFR Is a Simple, Useful Indicator for Determining the Safe Contrast Media Dosage To Avoid Contrast-Induced Nephropathy during Coronary Angiography Moon-Jae Kim.[1] *[1]Division of Nephrology & Hypertension, Inha University Hospital, Incheon City, Korea; [2]Division of Nephrology, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon-Si, Korea.*

Background: Contrast-induced nephropathy (CIN) has been commonly defined as a sudden, rapid deterioration in renal status after study of iodinated contrast medium (CM) in the absence of any other causes. To avoid the risk of developing contrast-induced nephropathy (CIN), it has been suggested that patients be subjected to a minimal necessary dose of contrast medium (CM-dose).

Methods: The ratio of CM-dose to eGFR in predicting the risks of CIN was assessed and sought to determine the safe level of CM-dose/eGFR in patients undergoing coronary angiography (CA). We enrolled a total of 226 patients and calculated the ratio of CM-dose using grams of iodine (g-I) to eGFR, thus expressing it as g-I/eGFR. Among the CIN patients, those requiring dialysis were also evaluated.

Results: Overall, there were 16 cases (7.1%) of CIN. On univariate and multivariate regression analysis, g-I/eGFR alone was found to be an independent predictor for CIN (hazard ratio=10.73, p<0.001). In an receiver operating characteristic analysis, fair discrimination for CIN was found at a g-I/eGFR level of 1.42 (C statics=0.867), and at this value, the sensitivity and specificity were 81.3% and 80%, respectively. Of patients (n=51) with g-I/eGFR ≥1.42, 23.6% (13/51) and 7.8% (4/51) developed, while those with g-I/eGFR <1.42 (n=171) had a lower incidences of CIN (1.8%, 2/171, p<0.001).

Conclusions: G-I/eGFR is a simple, useful indicator for determining the safe CM-dose based on the pre-CA eGFR values. Furthermore, g-I/eGFR might have a close relationship with the development of renal failure requiring dialysis as well as CIN not-requiring dialysis.

TH-PO065

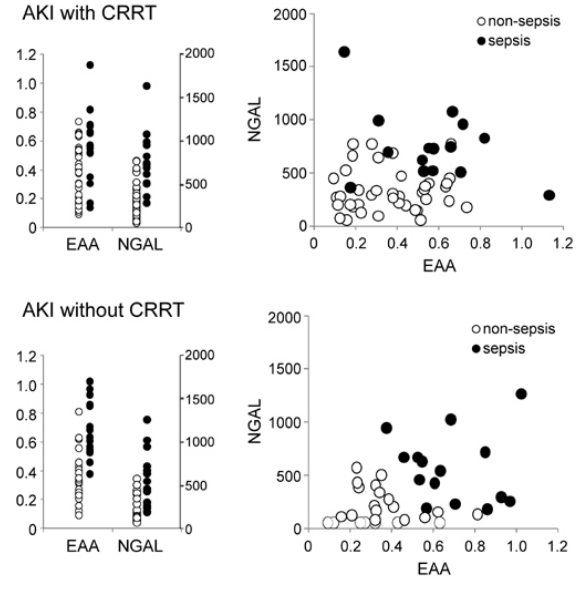
A Biomarker Panel of Plasma Neutrophil Gelatinase-Associated Lipocalin and Endotoxin Activity Assay in Septic Acute Kidney Injury Daisuke Katagiri,[1] Kent Doi,[1] Kousuke Negishi,[1] Takehiro Matsubara,[2] Naoki Yahagi,[2] Toshiro Fujita,[1] Eisei Noiri.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Japan; [2]Critical Care Medicine, University of Tokyo, Japan.*

Background: Plasma NGAL and endotoxin activity assay (EAA), which is a rapid ex vivo diagnostic test using the biological response of patient neutrophils to an immunological complex of endotoxin, are predictive for sepsis. This study evaluated a biomarker panel consisting of these two markers in AKI.

Methods: We evaluated two cohorts of 55 AKI patients who needed CRRT and 40 AKI patients who did not receive CRRT in ICU. Plasma NGAL and endotoxin activity (EA) in whole blood were measured using the Triage NGAL Device (Alere, USA) and the EAA system (Spectral Diagnostics, Canada).

Results: Plasma NGAL and EA values in septic AKI patients were significantly higher than in the other AKI patients (Table). A scatter diagram of plasma NGAL and EAA demonstrated the combination of these biomarkers enhanced the accuracy over each biomarker (Figure). The combination can detect sepsis in AKI with high AUC-ROC values both in the CRRT and the non-CRRT group (AUC-ROC 0.92 and 0.98, respectively).

		AKI with RRT		AKI without RRT	
		Sepsis (n=15)	Non-sepsis (n=40)	Sepsis (n=15)	Non-sepsis (n=25)
NGAL (ng/ml)	0 hr	748±333*	334±199	568±324*	194±158
	24 hr	705±230*	333±186		
	48 hr	652±255*	313±156		
EAA	0 hr	0.56±0.25*	0.37±0.19	0.69±0.20*	0.34±0.17
	24 hr	0.48±0.20*	0.30±0.16		
	48 hr	0.48±0.31*	0.26±0.13		



Conclusions: Sepsis and septic AKI are extremely complex and have multiple pathophysiological mechanisms. Therefore, combinations of biomarkers reflecting different pathways might be necessary for these complicated conditions. The present report describes a new biomarker panel of septic AKI with plasma NGAL (AKI and neutrophil activation) and EAA (endotoxemia), which is a useful diagnostic tool for septic AKI.

Funding: Government Support - Non-U.S.

TH-PO066

Plasmapheresis Rescue Therapy in Progressive ANCA-Associated Systemic Vasculitis Anoek A.E. Joode,de, Johannes S. Sanders, Coen A. Stegeman. *Internal Medicine/Nephrology, University Medical Center Groningen, Groningen, Netherlands.*

Background: We evaluated outcome of 25 patients with ANCA-associated vasculitis (AAV) with progressive disease despite treatment with cyclophosphamide and high dose steroids treated with additional plasmapheresis (PPh). Outcome was compared with 47 matched-controls (C).

Methods: Patients newly diagnosed with AAV from January 1990 until December 2009 were evaluated (n= 272). Patients were included when PPh was not started at diagnosis but was added for progressive disease during the course of initial standard therapy (n=25). We selected controls matched for BVAS and creatinine at diagnosis. Primary endpoint was eGFR or death.

Results: Plasmapheresis was added in 25 patients at 22 (range 2 to 51) days after start of therapy. In 20 of 25 patients a rise in serum creatinine > 30% despite induction therapy led to PPh. In 14, renal biopsy showed ongoing disease-activity, in 6 the decision was

made on clinical ground. Progressive pulmonary disease and progressive necrotic lesions were other indications.

Renal involvement was present in all patients; in the PPh- group, 5 patients temporarily needed dialysis,1 patient needed dialysis in the C-group. At 6 months and 5 years, 2 and 5 patients had died in both groups (RR 1.29; 95% CI 0.37-4.75).

At baseline mean eGFR was 46 ml/min/1.73m2 in PPh-group versus 43 in C-group, while at start of PPh eGFR was 25 ml/min/1.73 m2. At 6 months mean eGFR in the PPh-group had significantly improved (p<0.0001) and was 49 and 50 respectively in both groups. During long-term follow-up there was no difference in renal function between the groups at 12 months and 5 years.

Relapses occurred in 13 patients in PPh-group and in 20 in C-group (p=0.44). However, time to first relapse differed significantly between the PPh- and C- group (p=0.0084).

Conclusions: Patients with progressive disease despite induction therapy with cyclophosphamide and steroids in whom plasmapheresis was added, had significant improvement in renal function and similar long-term outcome as matched controls. Research is needed to determine factors that identify patients early in the course of therapy in whom plasmapheresis should be instituted.

TH-PO067

Proteinuria Independently Associate with the Development of Acute Kidney Injury in Intensive Care Unit Fen Jiang, Xinling Liang, Wei Shi, Yuan Han Chen, Wenjian Wang, Penghua Hu. *Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, GuangDong Province, China.*

Background: Proteinuria as a very important factor for the increasing mortality of chronic kidney injury(CKD),but the role of proteinuria for acute kidney injury(AKI) is still unclear. The purpose for the study is to analyze the relationship between proteinuria and AKI in adute Intensive care unit(ICU).

Methods: From October 2009 to July 2010 patients(≥18 years) who in ICU were enrolled and retrospectivelyevaluated .All patients were free of chronic dialysis for three months before admission and both of the levels of serum creatinine and urinary protein in the first 48 hours after admitted in ICU were recorded. Both RIFLE and AKIN criteria were employed for the diagnosis and staging of AKI in ICU.Dip-stick was used to detect proteinuria,andcoverthreecategory:no-proteinuria,mild-proteiuriaand heavy-proteinuria by the level of urine protein .

Results: Five hundred and twenty-four patients were included in this study. Ninety-nine patients were diagnosied as AKI by RIFLE criteria,while the number increased to one hundred and five according to AKIN criteria One hunderd and fifty-eight patients had proteinuria. Fifty-one patients with proteinuria developed to AKI by RIFLE criteria, while more seventeen patients with proteinuria had AKI according to AKIN criteria. The AKI patients had a different level of urinary protein compared with the no-AKI patients according to the AKIN criteria (P<0.05). Multiple logistic regression analysis suggested that heavy proteinuria was a risk factor for AKI in ICU regardless by RIFLE (odds ratio [OR] 3.478; 95%CI 1.544-7.839; p=0.003) or AKIN criteria(OR 3.589; 95%CI 1.679-7.668; p=0.001).Whlie proteinuria showed no direct effect on hospital mortality for AKI patients (OR1.451; 95%CI 0.906-2.322; p=0.121).

Conclusions: Proteinuria appears to be a key factor for the development of AKI in ICU, and the dip sticks maybe an effective method for early diagnosis of AKI.

Funding: Government Support - Non-U.S.

TH-PO068

Utility of Urinary HGF for Differential Diagnosis of Acute Kidney Injury in Cirrhotic Patients Won K. Han, John R. Fontanilla. *Medicine/Nephrology, Thomas Jefferson University Hospital, Philadelphia, PA.*

Background: To test the utility of a single measurement of urinary biomarkers in distinguishing acute tubular injury from prerenal azotemia (PRA), a prospective study was conducted in 90 cirrhotic patients with or without acute kidney injury (AKI) at a single institution.

Methods: Urine samples were collected within 48 hours from initial admission and/or at the time of renal consultation during hospitalization (63 AKI and 27 non-AKI). Urine concentrations of hepatocyte growth factor (HGF), kidney injury molecule-1 (KIM-1), and *N*-acetyl-b-D-glucosaminidase (NAG) were measured. AKI was defined as a greater than 50% increase in serum creatinine from baseline. Patients were followed during their hospital course through clinical and laboratory data to determine etiology of AKI.

Results: Patients with acute tubular injury (n=21) had a significantly elevated median urinary HGF level compared with PRA and non-AKI groups (n=48) (1.81 ng/mg urine creatinine, P<0.0001). At a cutoff value of 0.88 ng/mg urine creatinine, sensitivity and specificity of HGF for detecting acute tubular injury were 0.86 (95% CI, 0.64 to 0.97) and 0.83 (95% CI, 0.70 to 0.93), respectively. These values were superior to those for KIM-1 and NAG. Urinary KIM-1 was not a sensitive biomarker for distinguishing AKI in this study.

Conclusions: In conclusion, our results demonstrate that a single measurement of urinary HGF helps to distinguish acute tubular injury from normal function and PRA in patients with cirrhosis.

TH-PO069

Urinary Cystatin C as a Marker for Tubular Dysfunction Associated with Sepsis in Patients with Severe Sepsis and Septic Shock Francisco Ortuño Anderiz,[1] Alberto Barrientos,[2] Antonio Cruceyra,[3] Gonzalo Navarro,[1] Marta Cubells,[1] Carmen Gijon,[1] Enrique Morales,[1] Antonio Blesa,[1] Miguel Angel Gonzalez,[1] Fernando Martinez,[1] Sonia Vazquez,[1] Miguel Sanchez Garcia.[1] *[1]Critical Care, Hospital Clinico San Carlos, Madrid, Spain; [2]Nephrology, Hospital Clinico San Carlos, Madrid, Spain; [3]Biochemistry, Hospital Clinico San Carlos, Madrid, St. Helena.*

Background: Preliminary data in heterogeneous patient groups suggest that urinary cystatin C (UCysC) increases as a function of renal tubular injury, independent of glomerular filtration rate. We prospectively studied the correlation of UCysC levels with renal function and the need for renal replacement therapy (RRT) in patients presenting to our ICU with severe sepsis or septic shock (SS/SS).

Methods: Consecutive patients with SS/SS were included if plasma creatinine (Pcr) < 2 mg/dl. We also studied a control group of simultaneous patients admitted without SS/SS. Cases were followed for 5 days infno acute kidney injury (AKI) ensued and until recovery of renal function or for a maximum of 30 days. We used univariate analysis with Student's t-test or non-parametric tests as appropriate.

Results: We enrolled 53 cases, 50 (84.7%) in septic shock, and 44 controls, 9 (20.5%) in non-septic shock, over 20 months. Age, sex and admission group were similar between groups, 80% required mechanical ventilation. Mean APACHE II (16.5±6.1 *vs* 12.8±7.7, p=0.01) and SOFA scores (7.8±3.1 *vs* 3.5±2.7, p=0.001) and plasma creatinine (Pcr) (1.3±0.4 *vs* 1±0.4, p=0.001) were significantly higher in cases compared to controls, although ICU mortality was similar, 19 (35.8%) *vs* 10 (22.7%) (NS), respectively. UCysC was significantly increased in all septic patients on ICU admission compared to the non-septic control group (6.17±9.92 vs. 0.76±2.82 mg/creatinine in gram, p=0.001), irrespective of renal function, however (Pcr 1.3±0.3 mg/dl, urea 68±39.6 mg/dl, urea fractional excretion 32±15%). This difference was maintained in patients of both groups with normal renal function (5.89 ± 10.4 vs 0.82 ± 2.93 mg/g, p = 0.003). There was a poor association between plasma CysC and UCysC (r = 0.164, p = 0.022).

Conclusions: Our findings confirm that a high urinary excretion of CysC may occur, independent of renal function or subsequent development of AKI, and suggest a role for UCysC as an early marker of sepsis. In the subset of patients with SS/SS, in particular, elevated UCysC concentrations may be an early and sensitive sign of "septic tubular dysfunction", undetectable by routine laboratory tests.

Funding: Government Support - Non-U.S.

TH-PO070

Early Detection of Acute Kidney Injury (AKI) in Severe Sepsis and Septic Shock. The Role of Plasma Cystatin C Francisco Ortuño Anderiz,[1] Alberto Barrientos,[2] Antonio Cruceyra,[3] Gonzalo Navarro,[1] Marta Cubells,[1] Carmen Gijon,[1] Enrique Morales,[1] Antonio Blesa,[1] Miguel Angel Gonzalez,[1] Fernando Martinez,[1] Sonia Vazquez,[1] Miguel Sanchez Garcia.[1] *[1]Critical Care, Hospital Clinico San Carlos, Madrid, Spain; [2]Nephrology, Hospital Clinico San Carlos, Madrid, Spain; [3]Biochemistry, Hospital Clinico San Carlos, Madrid, Spain.*

Background: Cystatin C (CysC) has been studied as a biomarker for early detection of acute kidney injury (AKI) in heterogeneous clinical settings. In the critically ill patient AKI is most frequently associated with sepsis. Given the prognostic implication of septic AKI, we studied the potential of plasma CysC (PCysC) for early prediction in patients with severe sepsis or septic shock (SS/SS).

Methods: We included patients with SS/SS and ICU-admission plasma creatinine (Pcr) < 2 mg/dl. RIFLE criteria, blood chemistry and PCysC measurements were obtained daily. Cases were followed for 5 days if no AKI ensued or until recovery of renal function (follow-up censored at 30 days). We used Student's t-test or non-parametric tests as appropriate, and logistic regression model to assess the association between CysC and mortality, including variables with p <0.05 on bivariate analysis.

Results: 50 cases were enrolled over 20 months. PCysC concentrations were determined in 232 plasma samples. 29 were male, 21 female, mean age was 61.3±16.7, 56% were medical, 40% surgical and 4% trauma patients, 44 (88%) were in septic shock and 40 (80%) required mechanical ventilation. Mean APACHE II score was 16.3±6 and SOFA score 7.5±3. ICU mortality was 36%. AKI occurred in 20 (40%) patients (RIFLE criteria: R 8, I 5, F 7), with 7 (14%) requiring renal replacement therapy (RRT). PCysC on day 1 was significantly higher in patients later developing RIFLE-F (2.11 ± 1.27 mg/L) than in those who did not (1,15 ± 0,57) (p=0,041), while Pcr was not (1.48 ± 0.44 mg/dl *vs* 1.28 ± 0.32; p=0,2). PCysC had an area-under-ROC curve of 0.76 for AKI and of 0.82 for RIFLE-F. The PCysC-Pcr correlation was r = 0.246 (p=0.003). PCysC level on day 1 was associated with 30-day survival (1.1 ± 0.07 mg/L *vs* 1.5 ± 0.6 in non-survivors, p = 0.009), OR 0.167, 95% CI 0.033-0.851 (p=0.031,). Values > 1 mg/L on day 1 were associated with decreased ICU and hospital-survival (p=0.01 and 0.001, respectively).

Conclusions: In patients with SS/SS PCysC rises earlier than Pcr and predicts severe renal dysfunction, need for RRT and death in patients admitted to ICU for SS/SS.

Funding: Government Support - Non-U.S.

TH-PO071

The Value of Urine LFABP Level as a New Biomarker after Cardiac Surgery in Adults Liu Shang, Miaolin Che, Renhua Lu, Zhaohui Ni, Jia Qi Qian, Yucheng Yan. *Renal Division, Renji Hospital,Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: Acute kidney injury (AKI) is one of the most common and serious complications of cardiac surgery associated with substantial morbidity and mortality. A good biomarker can help early detection of AKI and perhaps, interventions. This study is to evaluate the value of uL-FABP of predicting the development and the prognosis of AKI following cardiac surgery.

Methods: By examining serum creatinine and uL-FABP corrected by urine creatinine at preoperative, 0h and 2h postoperative time points, we compared the differences between AKI and non-AKI groups, complete and uncomplete renal recovery groups, patient survival and death groups. ROC curves were used to evaluate their predictive accuracy.

Results: As a Result, We found the levels of urine L-FABP were both significantly higher in AKI patients compared to non-AKI at 0h and 2h after cardiac surgery (7247.68 vs 174.43 ng/mg and 3478.99 vs 118.71 ng/mg,both P<0.001),and the AUCs at 0h and 2h postoperative to predict AKI and AKI stage II-III were 0.844,0.818 and 0.832,0.805 respectively. Patients with uncomplete renal recovery at discharge had higher levels of urine L-FABP at 2h postoperative(12051.90 vs 2870.11ng/mg,P<0.05),and it showed good predictive values for detecting renal prognosis with the AUCs of 0.775. Similar results were found the level of uL-FABP at the two time points postoperation might be useful in predicting hospital mortality (14093.98 vs 292.11ng/mg and 7528.33 vs 200.68ng/mg,both P<0.05),with the AUCs of 0.893 and 0.873.

Conclusions: In conclusion, urine L-FABP which increased at the early stage after cardiac surgery might be an alternative biomarker for the early detection of AKI and composite end points.

Funding: Government Support - Non-U.S.

TH-PO072

A Comparison of Clinical Outcomes between the Selective Cytopheretic Device and Case-Matched Historical Controls from the PICARD Database Ravindra L. Mehta,[1] Alexander S. Yevzlin,[2] James A. Tumlin,[3] Lakhmir S. Chawla,[4] Ashita J. Tolwani,[5] John J. Dillon,[6] Kevin W. Finkel,[7] J. Ricardo Da Silva,[8] David Humes.[9] *[1]UCSD; [2]University of Wisconsin; [3]SERRI; [4]George Washington University; [5]University of Alabama; [6]University of Texas; [7]Cytopherx, Inc.; [8]University of Michigan.*

Background: Dialysis-dependent acute kidney injury (AKI) is a significant complication in ICU patients and is associated with hospital mortality rates between 50-60%. A recent pilot study of the Selective Cytopheretic Device (SCD), a novel cartridge that is able to selectively bind and deactivate neutrophils as an adjunct to CRRT therapy, showed improvement in kidney function and mortality rate.

Methods: We conducted a retrospective analysis of the PICARD database and the SCD pilot study, comparing mortality and dialysis dependence in both groups. 35 subjects from PICARD were matched to 35 subjects from the SCD pilot study using the diagnosis of AKI, CRRT therapy, and SOFA score. Logistic regression was used to compare mortality as the dependent variable and age, SOFA score, and presence of SCD therapy as independent variables.

Results: Mean age did not differ significantly between the two groups: 60.2 vs. 56.9 in PICARD vs. SCD, respectively (t-test, p=0.54). Mean SOFA score did not differ significantly between the two groups: 12.38 vs. 11.3 in PICARD vs. SCD, respectively (t-test, p=0.80). The in-hospital mortality rate was 62.9% in PICARD vs. 31.4% 60 day mortality in SCD (chi square, p=0.008). Logistic regression revealed odds ratio of death to be 1.02 (CI 0.9804 to 1.0535, p=0.38) for age, 1.22 (1.0205 to 1.4635, p=0.03) for SOFA score, and .28 (0.0974 to 0.7971, p=0.02) for SCD therapy. 7.7% (1/13) of survivors from the PICARD group were dialysis dependent at day 60 vs. 0% (0/24) from the SCD group (t-test, p=0.12).

Conclusions: The management of critically ill patients with AKI may have changed since the PICARD study. Nevertheless, this study suggests that SCD therapy is associated with significant improvement in mortality in critically ill patients with AKI receiving CRRT therapy.

TH-PO073

Renal Function Follow-Up Evaluation Using Cystatin C in Neonates Prenatally Diagnosed for Congenital Anomalies of the Kidney and Urinary Tract Paloma Parvex,[1] Christophe Combescure,[2] Jacques Birraux,[3] Alexandra Wilhelm-Bals,[1] Eric Girardin.[1] *[1]Pediatric Nephrology, Universitiy Hospital, Geneva, Switzerland; [2]Clinical Epidemiology, University Hospital, Geneva, Switzerland; [3]Pediatric Surgery, Universitiy Hospital, Geneva, Switzerland.*

Background: Congenital abnormalities of the kidney and urinary tract (CAKUT) account for 20% of all significant anomalies detected on prenatal ultrasound. Despite this frequent occurrence, no reliable method to measure renal function (RF) is validated in neonates. Cystatin C (CysC) has been proposed to be an accurate renal marker for the neonatal period. The *aims* of this study were to assess long term RF prospectively from birth in neonates prenatally diagnosed with CAKUT.

Methods: Among 47 neonates with CAKUT diagnosed prenatally, 21 pts with severe kidney malformation (KM) had renal function follow-up with the measure of CysC and creatinine on the same day. Median follow-up was of 235 (137-739) days. KM were repartee as follow: 12 pelvic dilatations >10mm; 5 hypo-dysplastic or ectopic kidney (2 withTCF2 mutation); 3 posterior urethral valves; 1 uretherocele; 1 megabladder. 6 pts underwent interventions. One of pts was start on dialyses and exclude from analyses. Factors influencing CysC were analyzed performing a linear mixed model to take account of the repeated measures.

Results: In our 20 pts, CysC decreases rapidly in the first month (M) (16.2%) p<0.001), slower between 1 M and 1 year (y) (3.9% per month, p<0.001) and stabilizes after 1 y (0.2% per month, p=0.83). CysC was significantly increased in pts with bilateral KM compared to pts with unilateral KM (p=0.02) and in TCF2 pts (p=0.002). The decrease of the CysC over time was less pronounced in pts with bilateral KM (p=0.04) and in TCF2 pts (p<0.001), these pts therefore presenting a worse prognosis in RF. Creatinine decrease with age, rapidly the first M (p=0.0001) and then stabilized.

Conclusions: Renal function follow-up in pts diagnosed with CAKUT, using CysC showed a worse prognosis over time in pts with bilateral kidney malformation or TCF2 mutation.

TH-PO074

Early Urinary Levels of Cell Death Markers Predict AKI after Cardiopulmonary Bypass Michael R. Bennett, Nuntawan Piyaphanee, Catherine D. Krawczeski, Prasad Devarajan. *Cincinnati Children's Hospital Medical Center.*

Background: Acute kidney injury (AKI) occurs commonly after cardiopulmonary bypass (CPB), often as a result of ischemia reperfusion injury (IRI). Serum creatinine (SCr) is an inadequate marker for AKI. Studies of animal models of IRI have demonstrated that the apoptotic pathway plays a significant role in the development of AKI. We set out to determine if apoptotic markers could be detected in the urine of patients undergoing CPB to determine if they could predict AKI, and to help pinpoint the timing of kidney cell death in these patients.

Methods: Urine samples were obtained prospectively before and at intervals after CPB in 391 patients. AKI was defined as a ≥50% SCr rise within 48h of CPB. For this pilot - we selected 35 patients, 18 of which developed AKI, and measured caspace-cleaved cytokeratin 18 (M-30, a marker of apoptosis), as well as total cytokeratin (M-65, total cell death) using commercially available ELISAs. Measurements were made at 0, 4 and 12 hours post CPB. These markers have recently been found to be elevated in urine of patients with chronic kidney disease.

Results: Defined by pRIFLE criteria, the AKI group consisted of R (n=8), I (n=8) and F (n=2). AKI and non-AKI groups did not differ significantly by race, sex or length of CPB. The median age of the AKI group (0.5 yrs IQR=0.4-1.8) was significantly lower than the non-AKI group (p=0.001; 7 yrs IQR=4.7-13.6). M30 levels were not significantly different between AKI and non-AKI groups at any time point. Markers of total cell death, however, were markedly increased at 4 hours post CPB in the AKI group (median 1467 U/L, IQR=429-3060) vs the non-AKI group (p=0.003; 158 U/L, IQR=62-507). Total cell death levels did not differ at 0 or 12 hours post CPB.

Conclusions: While urinary levels of the apoptotic marker M30 were not different between AKI and non-AKI groups, total cell death was greatly increased at as little as 4 hours post CPB. This indicates that significant cellular damage occurs very early after CPB and makes early diagnosis and intervention imperative. This data also indicates that urinary markers of cell death may be valuable early biomarkers of AKI.

Funding: NIDDK Support

TH-PO075

Efficacy of Urine Neutrophil Gelatinase-Associated Lipocalin as a Predictive Marker for Acute Kidney Injury in Different Patient Populations Kianoush Banaei-Kashani,[1] Laura N. Hanson,[2] Otto Stephan Schwarz Vignolo,[3] John C. Lieske.[1] *[1]Nephrology and Hypertesnion, Mayo Clinic, Rochester, MN; [2]Mayo Validation Support Services, Mayo Clinic, Rochester, MN; [3]Pulmonary Critical Care, Atlanta Pulmonary Group/Critical Care of Atlanta, Atlanta, GA.*

Background: Increased urine NGAL (uNGAL) excretion is a promising biomarker to predict subsequent Acute Kidney Injury (AKI). In this study we evaluated its utility among specific patient populations within a single center prospective cohort at a tertiary medical center.

Methods: All ICU admissions were screened and high risk patients with at least 30 minutes of hypotension were evaluated for possible inclusion. All patients who met RIFLE criteria at enrollment were excluded. After consent, 204 patients were followed for seven days or until hospital discharge for AKI as defined using RIFLE criteria (I or greater). ROC curves were prepared to assess uNGAL at ICU admission as a predictor of subsequent AKI, stratified by patient characteristics.

Results: Baseline serum creatinine was an overall poor predictor of AKI. uNGAL performed reasonably well in medical but not surgical ICU patients (AUC 0.71 vs 0.53, Table 1). When stratified by diagnosis (Table 2), in a univariate analysis uNGAL performed better in patients with congestive heart failure and sepsis (AUC 0.70 and 0.66) as compared to those with diabetes (AUC 0.53).

Conclusions: uNGAL performance as a biomarker of AKI varies between patient groups. When assessing biomarkers for detection of AKI, it is important to take into account the diagnoses, type of patients and comorbidities of the patients being evaluate.

		AUROC (p-Value)	
	Number of patients	Serum Creatinine	uNGAL
All ICU patients	204	0.55 (0.4)	0.62 (0.027)
Medical ICU	73	0.60 (0.2)	0.71 (0.008)
Surgical ICU	80	0.59 (0.2)	0.53 (0.7)
Congestive heart failure	34	0.46 (0.4)	0.70 (0.053)
Diabetes	41	0.68 (0.1)	0.53 (0.7)
Sepsis	43	0.61 (0.3)	0.66 (0.1)

Funding: Private Foundation Support

TH-PO076

Comparison of the Recognition and Management of Early Acute Kidney Injury Compared to Delayed AKI in Hospitalised Patients Hannah R. Wilson, Jennifer R. Joslin, Amy Irvine, Scott R. Henderson, Hannah E. Wilkinson, Bernard Freudenthal, Deepasree Bangaru-Raju, Thomas Sanctuary, Mark T. Kinirons, Maria Ostermann. *Guy's and St Thomas' NHS Foundation Trust.*

Background: Acute kidney injury (AKI) is associated with significant morbidity, mortality and healthcare costs. In 2009, a National Confidential Enquiry into Patient Outcomes and Death (NCEPOD) report identified significant failings in the recognition and management of hospitalised patients with AKI and issued guidance on early management. We aimed to explore whether patients with AKI on admission to hospital were managed better than patients who developed AKI whilst in hospital.

Methods: During a 7 day period in May 2011, electronic patient records of all level 1 medical and surgical patients in a London teaching hospital were screened. Medical notes of patients with AKI as defined by the KDIGO criteria were reviewed by doctors independent from the treating team to assess compliance with NCEPOD recommendations.

Results: 50 patients had AKI on admission (52% male; mean age 73 years; 66% medical, 34% surgical). 94% had risk factors for AKI (median 2 [range 0-5]). 24 patients developed AKI in hospital (50% male; mean age 72 years; 58% medical, 42% surgical). 92% had risk factors for AKI (median 2 [0-4]).

AKI was recognised on the first day of onset in 50% of both cohorts and more often in medical specialties. When recognised, early management strategies were initiated in <50% of patients (Table 1).

Management of Early Versus Late AKI

	AKI on admission to hospital (1st day AKI)	AKI developed as inpatient (1st day AKI)
Recognised by team	50%	50%
Documented plan if recognised	72%	100%
Senior review if recognised	84%	92%
Fluid balance chart complete	20%	33%
Clinical fluid status assessed	42%	42%
Fluids given if hypovolaemic	75%	67%
Nephrotoxic drugs stopped	36%	10%
Urine dipstick if recognised	52%	50%

Conclusions: Despite increasing publicity, AKI is poorly recognised both when present on admission and when developed as an inpatient. Even when recognised, adherence with NCEPOD recommendations is poor. More awareness and better tools are necessary to recognise AKI early and guide management.

TH-PO077

In Acute Kidney Injury; NGAL in Contrast to Procalcitonin Is a Biomarker of Injury Irrespective of Sepsis; Whereas the Latter of Sepsis Regardless of Injury Ahmed G. Adam,[1] Amr Soliman,[1] Muhamad Alsawy,[3] Ahmed Aglaan.[2] *[1]Internal Medicine - Nephrology, Dialysis, Transplantation Unit, Faculty of Medicine; [2]ER, Faculty of Medicine, University of Alexandria; [3]Clinical Pathology, Faculty of Medicine.*

Background: Acute Kidney Injury is associated with a mortality rate which varies according to definition that of severe injury (RIFLE category F) approximates 50%. Although, it has been argued that patients die with AKI rather than of AKI and that AKI is simply an expression of illness severity, consistent and strong evidence supports the notion that AKI has an independent impact on outcome.

Methods: This study was done to illustrate the incidence of septic and non septic acute renal failure in patients admitted to Alexandria main university hospitals. 170 patients were included over 6 months. NGAL and Procalcitonin beside serum creatinine was measured.

Results: 3 main categories of diagnosis: AKI group 57.7% , Acute on top of CKD group 25.4%, GN group 16.9%. According to prognosis 35.2% Died, 25.4% CKD, 16.9% ESRD, 16.9%Recovered, 5.6%Unknown. As regard sepsis, 42.25% of the patients were septic while 57.75% of the patients were non septic. Urinary NGAL was significantly higher in the patient group. There was no statistically significant difference between the level of uNGAL in the different groups of diagnosis. There was no statistically significant difference between the level of uNGAL in the different groups of prognosis. Serum Procalcitonin was significantly higher in the septic patients than that in the non septic patients. The serum Procalcitonin level was the highest in the group of septic patients who died..

Conclusions: The correlation between serum creatinine and serum procalcitonin (r = 0.108, p = 0.649) is weak and insignificant preserving the diagnostic capability of procalcitonin as a marker of sepsis even in the setting of AKI , also the correlation between uNGAL and serum procalcitonin (r = 0.094, p = 0.655) is weak and of no statistical significance. From the fact that procalcitonin is a well established marker for sepsis and the weak correlation between urinary NGAL and serum Procalcitonin we can conclude that uNGAL is indeed unique for AKI regardless of sepsis.

TH-PO078

Novel Biomarkers of AKI: Multicenter Prospective Cohort Study Kianoush Banaei-Kashani,[3] Daniel Laskowitz,[1] Ludwig Wagner,[2] Aysegul Ilhan,[2] John C. Lieske.[3] *[1]Department of Medicine, Duke University School of Medicine, Durham, NC; [2]Department of Medicine, University of Vienna, Austria; [3]Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Novel biomarkers are needed for early diagnosis of AKI to allow early initiation of new and established therapeutic options.

Methods: All ICU admissions in three tertiary medical centers were screened, and high risk patients with at least 30 minutes of hypotension, or sepsis were evaluated for possible inclusion. All patients who met RIFLE criteria at enrollment were excluded. After consent, 342 patients were followed for seven days or until hospital discharge for AKI as defined using RIFLE criteria (I or F). Blood and urine samples were collected at enrollment, 12 and 24 hour and then daily up to 7 days to measure serum creatinine, other known AKI biomarkers, and more than 140 novel biomarker candidates.

Results: Urinary NGAL predicted AKI 24 hours before RIFLE criteria with a concordance statistic (C-stat) of 0.72, which was the best among known AKI biomarkers. However, by multivariate logistic regression analysis identified a panel of 2 urine biomarkers that performed even better (C-stat of 0.8). The addition of uNGAL to the 2 biomarker panel did not improve the model.

Conclusions: We have identified two novel biomarkers that together predict AKI stages I and F 24 hours before RIFLE criteria. These biomarkers need to be validated in a second prospective cohort, and in other patient populations.Multivariate logistic regression analysis

	p-Value	C Stat
Model 1 (n=342)		
Log(10) Urine ngal/1000	< 0.0001	0.72
Model 2 (n=342)		
Marker 1	<0.0001	0.8
Marker 2	0.0005	
Model 3 (n=342)		
Marker 1	0.0001	0.79
Marker 2	0.0152	
Log(10) Urine ngal/1000	0.0751	

Funding: Private Foundation Support

TH-PO079

Epidemiology of Acute Kidney Injury in Newborns Born with Perinatal Depression David J. Askenazi, Hayden Hundley, Angela Montesanti, Pushkar Pawar, Namasivayam Ambalavanan. *Pediatrics, University of Alabama at Birmingham, AL.*

Background: Infants born with perinatal depression are at risk for AKI. Based on studies conducted in the 1990's, the incidence of AKI in infants with 5 minute Apgar scores ≤ 6 is reported between 40-60%. Fluid overload has been shown to be independently associated with poor outcomes in pediatric and adult populations. This association has not been described in neonates.

Methods: Between February 2010 and May 2011, we screened 146 infants of which 58 met inclusion criteria (birth weight (wt) > 2000 grams, gestational age > 34 weeks and 5 minute Apgar ≤ 7) consented to the study. Serum creatinine (SCr) was measured daily for the first 4 days of life. AKI was defined as a rise in SCr of > 0.3 mg/dl or persistent SCr above 1.5 mg/dl.

Results: AKI occurred in 9 / 58 (15.6%) subjects. AKI was associated with higher birth wt, male infants, lower Apgar scores at 5 minutes, lower cord pH, and intubation in the delivery room. Infants of mothers with pre-eclampsia were protected against AKI. Fluid status, [(birth wt / wt at day of life 3) – 1]*100, during the first 3 days of life differed between those with AKI (net gain of 12.0 ± 14.4%) an those without AKI (net loss of 2.8 ± 7.0) (p<0.001). Survival was worse in those with AKI [7/9 (77.8%)] as all infants without AKI survived (p<0.02).

Conclusions: The incidence of AKI in this tertiary hospital was lower than those reported previously, possibly due to improved care of infants shortly after birth. Risk factors for AKI in this population are described. Infants with AKI are more likely to die and have a net positive fluid balance over the first few days of life which could be a potentially modifiable variable in the course of AKI.

Funding: Other NIH Support - Pilot and Feasiblity Grant from the UAB/ UCSD O'Brien Center for Acute Kidney Injury Research

TH-PO080

Impact of Radiocontrast-Induced Nephropathy Following Coronary Angiography in Hospitalized Patients Javier Neyra,[1] Sunay Shah,[1] James E. Novak.[2] *[1]Department of Internal Medicine, Henry Ford Hospital, Detroit, MI; [2]Division of Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Background: Radiocontrast-induced nephropathy (RCIN) is the third leading cause of hospital-acquired acute kidney injury in the United States and is associated with unfavorable outcomes.

Methods: We retrospectively utilized a population-based linked administrative database of hospitalized patients who underwent coronary angiography from January 2008 through December 2009 to analyze in-hospital and long-term outcomes associated with RCIN. RCIN was defined as an increase in serum creatinine > 25% from baseline, absolute increase in serum creatinine ≥ 0.5 mg/dL, or decrease in estimated glomerular filtration rate (eGFR) ≥ 25% within 72 hours after contrast exposure. Patients with end-stage kidney disease and those exposed to other contrast medium or acute dialysis before the procedure were excluded.

Results: A total of 1,168 patients were included in the study. RCIN occurred in 82 of 382 patients (17.7%) with eGFR < 60 mL/min/1.73m² and in 108 of 596 patients (15.3%) with eGFR ≥ 60 mL/min/1.73m². In the group with lower eGFR, the in-hospital mortality for those who developed RCIN was 12.2% (10/82) vs. 2.9% (11/382) for those without RCIN (P = 0.0002) but the 1-year mortality was not significantly different (6.9% [5/72] vs. 6.2% [23/371], P = 0.812). Additionally, patients with lower eGFR had more frequent irreversible increase in serum creatinine (> 0.5 mg/dL) and outpatient dialysis initiation when they developed RCIN (23.6% [17/72] vs. 10.8% [40/371], P = 0.0029 and 5.6% [4/72] vs. 1.1% [4/371], P = 0.009, mean follow up of 16 months, respectively). In the group with higher eGFR, both in-hospital and 1-year mortality were significantly higher in those who developed RCIN (5.6% [6/108] vs. 0.5% [3/596], P < 0.0001 and 8.8% [9/102] vs. 2.5% [15/593], P = 0.0013, respectively).

Conclusions: The occurrence of RCIN following coronary angiography in hospitalized patients is associated with higher in-hospital mortality independently of baseline eGFR. Patients with impaired baseline eGFR who develop RCIN tend to have more frequent long-term kidney function deterioration and subsequent dialysis initiation.

TH-PO081

Quality of Care and Outcomes in Hospitalized Patients with Acute Kidney Injury (AKI) Emmett D. Ratigan,[1] Ravindra L. Mehta.[1] *[1]Medicine, University of California San Diego, San Diego, CA; [2]Medicine, University of California San Diego, San Diego, CA.*

Background: AKI is common in hospitalized patients and is associated with adverse outcomes including in-hospital mortality, development of chronic kidney disease (CKD), progression to end-stage renal disease (ESRD), and long-term mortality. A recent cohort from the UK (NCEPOD; 2009) demonstrated significant lapses in quality of care (QC) for hospitalized patients with AKI. We assessed the QC in a consecutive cohort of 100 hospitalized patients discharged with an ICD-9 coded diagnosis of AKI. We hypothesized that the recognition and management of AKI would be variable and influenced by time of development of AKI.

Methods: We reviewed charts and extracted data on timing of AKI, rapidity of diagnosis, nephrotoxic administration, management of AKI complications, and in-hospital outcome. All survivors were reviewed to one year post-discharge for incidence of nephrotoxic exposures, re-hospitalizations, repeat AKI, development of ESRD, and all-cause mortality. Patients were characterized having AKI at admission (AKIADMIT, n= 75) and those developing it during the hospitalization (AKIPOST, n=25).

Results: All patients were verified to have AKI by the acute AKIN and RIFLE serum creatinine criteria. Amongst the cohort, the mean age was 59, 65% were male, 47% had hypertension, 43% CKD, and 35% diabetes. QC parameters were different for AKIADMIT vs AKIPOST.

	Delayed Diagnosis	Nephrotoxin Administration	Prompt Treatment of Hyperkalemia	Length of Stay (Days)	In-hospital Mortality
AKIADMIT	9%	49%	82%	10.2	12%
AKIPOST	40%	76%	50%	22.6	36%
P value	<0.01	0.04	0.04	<0.01	<0.01

Of the 82 patients surviving the index admission, variable follow-up occurred in 66, and at one year post-discharge, repeat AKI was 33%, readmission 53%, ESRD 3%, and all-cause mortality 15%. At six years post-discharge, 63% of the initial 100 patient cohort had died

Conclusions: Despite advances in the understanding of AKI, in-hospital management for AKI has room for improvement. The timing and quality of follow-up care of AKI survivors is highly variable and may contribute to the lack of recognition and development of CKD post AKI.

TH-PO082

The Risk Factors and Impact of AKI Following Stroke Rupesh Raina, Charbel A. Salem, Martin J. Schreiber, Sevag Demirjian. *Nephrology, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Acute Kidney Injury (AKI) has been shown to be an independent predictor of long-term mortality and cardiovascular outcomes following acute stroke. However, these studies were not based on strict definitions of AKI and the risk factors of AKI in this population were not assessed before.

Methods: We ran a retrospective analysis on 628 patients who were admitted with their first ever acute stroke. AKI was defined as a rise in Cr of ≥0.3 mg/dl or a percent increase of ≥50% from baseline or a reduction in Urine output as defined by AKIN criteria. Hospital discharge disposition was dichotomized to poor (death, nursing facility, ventilator weaning facility) or good (acute rehabilitation or home). Multivariable models were used to assess the correlation between outcomes (AKI, persistence of AKI) and the risk factors (age, gender, race, HTN, CKD, DM, CAD, HPL). Using chi-square test, outcomes were compared for patients with AKI and those with no AKI.

Results: The mean age was 63, and 50.6 % were females. 90 patients developed AKI and seventy-one patients had persistent AKI. The results of the regression analysis for AKI on admission are shown in the table.

Factor	OR (p-value)	95% Confidence Limits
Male	4.01 (<0.001)	2.2 to 7.5
Hypertension	3.09 (0.005)	1.4 to 6.8
CKD	42.98 (<0.001)	20.2 to 89.9

The risk factors for AKI persistence were very similar with CKD remaining the leading risk factor. 32 patients with AKI (36%) were discharged to acute rehab, while 32 (36%) were discharged to home, and 9 (10%) died. Patients with persistent AKI on discharge were 2.4 times more likely to have a poor discharge disposition than those without AKI (p<0.01)

Conclusions: Using a strict definition of AKI following stroke, we identified the risk factors behind the development and persistence of AKI. We also showed a significant impact of AKI on short-term outcomes. CKD was the most significant risk factor for both AKI on admission and its persistence on discharge, followed by HTN and age. Patients with these risk factors and acute stroke should be monitored closely and renal protective measures should be applied.

TH-PO083

Acute Kidney Injury Is Associated with Increased Odds of CKD and Death among Patients Who Undergo Revascularization for Peripheral Vascular Disease Pradeep Arora,[1,2] Pooja Mahajan,[1] Nauman Tahir,[1] James W. Lohr,[1,2] Nader Nader,[3] Hassan H. Dosluoglu.[4] *[1]Department of Medicine, SUNY at Buffalo, Buffalo, NY; [2]Divison of Nephrology, VAMC, Buffalo, NY; [3]Department of Anesthesiology, VAMC, Buffalo, NY; [4]Department of Surgery, VAMC, Buffalo, NY.*

Background: Several databases, sub-analyses of existing studies and single center studies have shown that acute kidney injury (AKI) is associated with development of chronic kidney disease (CKD) in long term follow up. However, the impact of AKI on long term survival has not been studied. We determined the impact of AKI on survival in peripheral vascular disease patients undergoing vascular procedures/surgery.

Methods: Study population included 740 peripheral vascular disease patients undergoing lower extremity revascularization as the primary procedure at VA Western New York Healthcare System between January 1, 2001 and December 31, 2009. All data were collected prospectively. Kaplan Meier and Cox proportional analyses were performed to study the impact of AKI on survival. Logistic regression was performed to evaluate the impact of AKI on development of CKD.

Results: 70 patients developed AKI as defined by AKIN criteria. 39 of these patients had died by the last follow up date. 188 of 670 patients died among those who did not have AKI. Results of Cox regression are shown in the table. 149 patients developed CKD by the last follow up date(analysis was done after excluding pre procedure CKD patients). 50% of those patients who had AKI subsequently developed CKD, compared to 22% who did not have AKI. Logistic regression results are shown in the table.

Cox Proportional Hazards Method and Logistic Regression Analyses

Parameter	Hazard ratio	Odds ratio
Age	1.05 (1.04-1.07)	3.01 (2.51-3.63)
AKI	2.24 (1.58-3.20)	3.20 (1.75-5.84)
CAD	1.61 (1.21-2.15)	1.51 (1.03-2.22)
DM	1.34 (1.02-1.76)	1.88 (1.29-2.74)
HTN	0.94 (0.69-1.26)	0.84 (1.29-2.74)
Pre Creatinine	1.49 (1.08-2.07)	
Type of surgery	1.00 (0.75-1.32)	

Hazard ratio is for survival model and OR is for development of CKD

Conclusions: AKI following vascular interventions in peripheral vascular disease patients are associated with a significant increase in CKD and mortality.

TH-PO084

Long-Term Risk of Mortality for Acute Kidney Injury in HIV-Infected Patients Mário Raimundo, Maria Joao Melo, Antonio Gomes da Costa, Jose António Lopes. *Nephrology and Renal Transplantation, Hospital de Santa Maria- CHLN, Lisbon, Portugal.*

Background: Acute kidney injury (AKI) is a common complication in human immunodeficiency virus (HIV)-infected patients and it is associated with increased in-hospital mortality. The impact of AKI on long-term mortality of hospitalized HIV-infected patients has however not been extensively addressed. The aim of the present study was therefore to evaluate the influence of AKI on long-term mortality of HIV-infected patients who are hospitalized and survive to acute episode.

Methods: Three hundred ninety nine HIV-infected patients (mean age: 41 years; 265 male; 288 caucasian) who were hospitalized in the Department of Infectious Diseases of our Hospital (Hospital de Santa Maria, Lisboa, Portugal) between January 2005 and December 2007 and were discharged alive were studied retrospectively. Acute kidney injury was defined according to Risk Injury Failure Loss End-stage kidney disease (RIFLE) classification based on serum creatinine. The outcome measure was mortality at 2 years of follow-up. Kaplan-Meier method was used to determine survival curves and log-rank test was employed to evaluate statistical differences between the survival curves. Cox regression method was used to determine independent predictors of 2-year mortality. Risk factors were assessed with univariate analysis, and variables that were statistically significant (P<0.05) in the univariate analysis were included in the multivariate analysis.

Results: Patients who had AKI within the hospitalization (N=59) had higher 2-year mortality than those patients who did not develop AKI (N=340) (22% versus 10.9%; log-rank P=0.008; unadjusted hazard ratio 2.3, 95% confidence interval 1.2 to 4.3, P=0.01). After

adjusting for other covariates with prognostic importance, AKI still remained associated with increased 2-year mortality (adjusted hazard ratio 2.1, 95% confidence interval 1.1 to 3.9, *P*=0.024).

Conclusions: Acute kidney injury was independently associated with increased 2-year mortality of hospitalized HIV-infected patients who were discharged alive.

TH-PO085

Validation of the Acute Kidney Injury Risk Index for Patients Undergoing General Surgery at the Philippine General Hospital Rommel P. Bataclan. *Section of Nephrology, Dept. of Medicine, Philippine General Hospital, Manila, Philippines.*

Background: Post-operative Acute Kidney Injury (AKI) is one of the most common cause of hospital morbidities, and an independent risk factor for in-hospital mortality. A General Surgery AKI Risk Index can be used to stratify pre-operatively, patients at high risk to develop AKI post-operatively.

Methods: This is a validation study of an AKI Risk Index Score. All adult patients who underwent general surgery at the Philippine General Hospital from June 1, 2010 to May 31, 2011 will be included. They will grouped into a particular Risk Group depending on the presence of certain risk factors. It will then be determined if AKI happened in the patient using the RIFLE Criteria. Based on the incidence of AKI in the Risk Groups, Sensitivity, Specificity, Positive & Negative Predictive Values will be computed. A Receiver Operating Curve (ROC) will also be constructed.

Results: 824 general surgeries were included. Frequency of AKI among patients who underwent general surgery was 3.4% (n=28) and the mortality rate of patients with AKI was 17%(n=5), with overall mortality of 0.6%. Based on the scoring system, patients under Class I (presence of 0-2 risk factors), Class II (3 risk factors), Class III (4 risk factors), Class IV (5 risk factors) and Class V (6-8 risk factors) and were associated with risk of developing AKI of 0.05%, 0.10%, 2.91%, 3.88% and 6.15% respectively. Overall, sensitivity and specificity were 82.2% and 51.1% respectively. Discrimination test by receiving operating characteristics (ROC) curve showed an area under the curve (AUC) of 0.731.

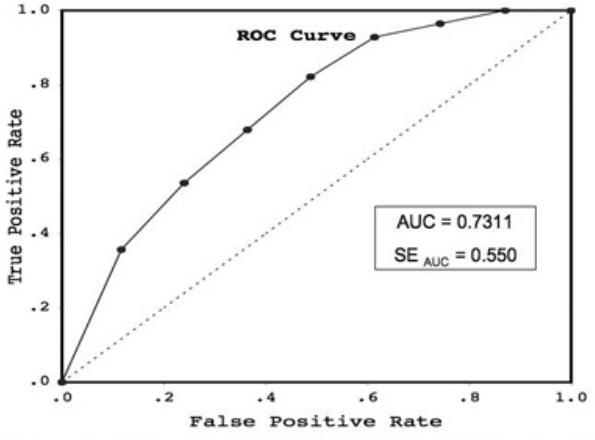


Figure 1. ROC curve for the prediction of AKI after General Surgery using the Scoring System by Kheterpal et. al

Conclusions: The General Surgery Acute Kidney Injury Risk Index can be used in our local setting to predict AKI among stable chronic kidney disease patients after general surgery.

TH-PO086

Risk Factors and Outcomes of Acute Kidney Injury (AKI) Associated with Outpatient Parenteral Anti-Infective Therapy (OPAT) Charuhas V. Thakar,[1, 2] Sashi Ameneni,[1] Loretta Simbartl,[2] Stephen Kralovic,[1, 2] George Smulian.[1, 2] *[1]Internal Medicine, University of Cincinnati, OH; [2]Medical Service, Cincinnati VA, Cincinnati, OH.*

Background: Treatment of infections in hospitalized patients frequently requires OPAT. Incidence, risk factors and outcomes of AKI associated with OPAT are not well studied.

Methods: We studied 244 acute care discharges between 2006 and 09 requiring OPAT in a VA healthcare system. We examined the association of patient characteristics and drug exposure with AKI (defined by AKIN criteria). In patients recieving vancomycin (Vanc), average trough levels before meeting AKI criteria were used for analysis. Chi-square, t-tests and logistic regression was used for analysis.

Results: Sample was 99% male, with mean age of 61 [standard deviation (SD) 10.9], and mean baseline creatinine of 1.1 mg/dl (SD 0.5). 71% of cases received Vanc based regimen. 111/244 (45%) patients developed AKI (Stages I – 63%, II – 12%, III – 25%); of whom 10 required dialysis. Of the AKI cases 63% met AKI criteria after discharge. Older age (p = 0.014), high Vanc trough (p = 0.0002), high baseline creatinine (p = 0.005), diabetes (p = 0.001), ICU admission (p < 0.0001), proteinuria (p = 0.004) diuretic use (p = 0.02), and aminoglycoside use (0.004) were associated with AKI by univariate analysis. In a multivariate model, ICU stay [odds ratio (OR): 4.1, 95% confidence interval (CI), 1.1 – 14.1], proteinuria (OR: 3.3, 95% CI, 1.1 – 10.2), and Vanc trough levels (OR per unit increase: 1.08, 95% CI, 1.01 – 1.15) were associated with AKI. In a separate model that included patients recieving Vanc based regimens, an average trough of ≥ 20 vs < 20 was associated with AKI (OR 3.8, 95% CI, 1.6 - 8.9)

Re-admission at 90-days was 57% in AKI vs 38% in no AKI group (p = 0.003). Creatinine at 2-years was higher in AKI versus no AKI group [1.54 (SD, 0.92) versus 1.09 (SD, 0.72) respectively]. In 7/10 patients dialysis was deemed permanent.

Conclusions: AKI is a significant and under-recognized complication associated with OPAT, frequently occurs after discharge, and may result in irreversible damage. Risk stratification and monitoring of these patients is needed to reduce kidney toxicity.

Funding: Veterans Administration Support

TH-PO087

Determination of Renal Function Improves Significantly the Prediction of Survival in Palliative Care Patients Scott O. Grebe,[1] Oliver Schmalz,[1] Ruediger Trebst,[1] Thomas F. Mueller,[2] Manfred F. Weber.[3] *[1]Medizinische Klinik 1, HELIOS-Klinikum Wuppertal, Univ. of Witten/Herdecke, Wuppertal, Germany; [2]Div. of Nephrology and Transpl. Immunology, Univ. of Alberta, Edmonton, AB, Canada; [3]Medizinische Klinik 1, Klinikum Köln-Merheim, Univ. of Witten/Herdecke, Cologne, Germany.*

Background: One of the most important challenges in palliative care medicine is an exact estimation of patient survival. For this purpose various clinical scoring systems such as the palliative prognostic score (PPS) are used. Despite impaired renal function is a known risk factor for patient outcome in general, its influence on the survival of palliative care patients has not yet been studied.

Methods: All patients admitted to our palliative care unit in 2008 were included in the study. The relation between renal function and survival was prospectively analyzed and compared to currently used scores. Besides clinical routine measurements serum creatinine (S-Crea), the eGFR using the simplified MDRD-formula, dipstick proteinuria (U-Prot), the Karnofsky-Index (KI) and the PPS were determined at admission. In addition to descriptive statistics the influence of these parameters on survival was tested using Kaplan-Meier estimates and Wilcoxon scores. In order to identify the most important prognostic factors a multiple regression analysis based on the Cox proportional hazards model was performed.

Results: Overall 308 patients (mean age=70.2±12.9 years, 58.4% male, mean survival 45±96 days) were included. The mean values of S-Crea and eGFR were x=1.5±1.6 mg/dl and x=86.2±65.8 ml/min, resp. The percentage of patients with significant U-Prot was 35.2%. The median KI and PPS were 40% (Range:10-90%) and 30-50% (Range:<30%->70%), resp. In the univariate survival analysis only age (p=0.0016), KI (p<0.0001), PPS (p<0.0001), S-Crea (p=0.0074) and eGFR (p=0.0213) showed a significant association with patient outcome. The strongest predictors in the age-adjusted stepwise multiple regression model were PPS (p<0.0001), KI (p=0.0002) and eGFR (p=0.0053). None of the other parameters met the 0.05 significance level.

Conclusions: The determination of the eGFR adds significantly to a reliable prediction of survival in palliative care patients.

TH-PO088

Identification of Potentialy Controllable Riskfactors for AKI after Cardiac Surgery Sanne Vellinga, Gert A. Verpooten, Karin Janssen van Doorn. *Nephrology-Hypertenson, University Hospital Antwerp, Edegem, Antwerp, Belgium.*

Background: Acute kidney injury (AKI) in the immediate postoperative period after successful cardiac surgery was proven to be of major influence on morbidity and mortality. Our study investigates the potential prognostic value of perioperative packed-cell transfusion on the development of AKI.

Methods: Retroscpectively, we reviewed 565 patients who underwent coronary artery bypass grafting (CABG). In the perioperative period, following parameters were examined: demographic characteristics, diabetes, history of congestive heartfailure, urgency of surgery, lowest perioperative hematocrit, intra- and postoperative packed cell transfusion, serumcreatinine (at admission, every 24h minimum 48h during 7 days and after 8 weeks), eGFR (MDRD-formula), postoperative administration of furosemide, duration of cardiopulmonary bypass and crossclamping and need for renal replacement therapy. AKI was defined by the AKIN-criteria.

Results: A total of 83 patients (14,7%) evolved to AKI in the post-operative period. Two patients needed renal replacement therapy in the first seven days and both died. AKI was associated with age (mean 66,5y ± 9,4 No-AKI vs 69,6y ± 10,2 AKI p<0,05), pre-existing renal function (mean eGFR 86,7 ± 51,8 mL/min/1,73m² vs 70,6 ± 24,0 mL/min/1,73m², p<0,01), urgency of surgery (5,3% vs 13,4%, p=0,03), both intra- and postoperative packed cell transfusion (9,9% intra-operative transfusion vs 25,3%, p<0,01 and 16,3% postoperative transfusion vs 42,2%, p<0,01) and postoperative administration of furosemide (60,0% vs 84,3%, p<0,01). In multivariate analysis pre-operative creatinine (p<0,01), postoperative packed cell transfusion (p<0,01) and postoperative administration of furosemide (p<0,01) remained significant.

Conclusions: Our study clearly shows that pre-existing renal disease, postoperative packed cell transfusion and postoperative administration of furosemide are important risk factors for the development of AKI after CABG. As two of these factors are easily controllable our results advocate further exploration of these factors and their potential to reduce AKI related morbidity.

TH-PO089

Improved Survival in Patients Requiring Dialysis after Pediatric Hematopoietic Cell Transplantation (HCT) Jurat S. Rajpal, Rachel I. Vogel, Clifford E. Kashtan, Angela R. Smith. *University of Minnesota, Minneapolis, MN.*

Background: HCT, a critical therapy for many children with life threatening illnesses, is associated with a substantial risk of acute kidney injury (AKI) requiring dialysis. The purpose of this study is to compare survival in these patients in two decades, 1990-1999 and 2000-2009.

Methods: 1427 patients <21 years old who had their first HCT at the University of Minnesota between January 1990 and December 2009 were reviewed using the center database. Kaplan-Meier estimates and 95% confidence intervals for 100 days and 1 year post-HCT overall survival (OS) are reported by dialysis group and treatment decade (1990-1999 vs. 2000-2009) and compared using the log-rank test.

Results: The incidence of AKI requiring dialysis was not significantly different between the two cohorts (1990-99: n=65/778, 8.35% vs. 2000-09: n=62/649, 9.55%, p = 0.42).

Proportion of patients surviving by decade based on need for dialysis

	100 days post transplant			1 year post transplant		
	Dialysis	No dialysis	p value	Dialysis	No dialysis	p value
1990-1999	0.18 (0.10, 0.29)	0.85 (0.82, 0.87)	<0.0001	0.11 (0.05, 0.20)	0.64 (0.61, 0.68)	<0.0001
2000-2009	0.42 (0.30, 0.54)	0.92 (0.89, 0.94)	<0.0001	0.23 (0.13, 0.34)	0.79 (0.76, 0.82)	<0.0001

Patients requiring dialysis in the 2000-2009 cohort had significantly higher survival than those in the 1990-1999 cohort at both +100 days and at 1 year (+100 days: 42% vs. 18%, p = 0.0090; 1 year: 23% vs. 11%, p = 0.0010).

Conclusions: AKI requiring dialysis is an important complication of pediatric HCT that has shown no change in incidence over the last two decades. Survival has significantly improved at both 100 days and 1 year post-transplant, regardless of dialysis status. Despite a recent reduction in mortality for those who require dialysis, mortality remains significantly higher than for patients who do not need dialysis.

Uni- and multi-variate analyses of risk factors for AKI requiring dialysis in pediatric HCT recipients are in progress. Development of effective preventative strategies will likely lead to reduced mortality in this patient population.

TH-PO090

The SAFE-T Consortium: A Collaborative Approach for the Qualification of Novel Kidney Biomarkers with the Regulatory Authorities Joe Keenan,[1] Ralf Schindler,[3] Patrick T. Murray.[4] *[1]SAFE-T Consortium, Argutus Medical Ltd, Dublin, Ireland; [2]SAFE-T Consortium, AstraZeneca, Wilmington, DW; [3]SAFE-T Consortium, Charite, Berlin, Germany; [4]SAFE-T Consortium, UCD Mater, Dublin, Ireland; [5]SAFE-T Consortium, Hoffman La Roche, Basel, Switzerland.*

Background: Drug-induced kidney injury is not an uncommon adverse event in drug development. The greatest issue is the late identification of Acute Kidney Injury due to the current standards (i.e. serum creatinine (sCr) and blood urea nitrogen (BUN)) which are delayed indicators of injury and may not be significantly changed until 2/3 of the kidneys function has already been lost. Over the last three years there has been progress with preclinical qualification processes for kidney biomarkers (PSTC and HESI qualification with EMA and FDA). These landmark qualifications mean that drug companies may now use certain novel preclinical markers for real decision making within their qualification context.

Methods: The principal objective of this new project is to collect and generate sufficient clinical data from a number or candidate kidney biomarkers, that will provide convincing evidence for the health authorities to endorse these biomarkers for the detection and monitoring of drug induced kidney injuries in specific clinical situations. 22 kidney biomarker have been selected and are being analytically validated by a number of technologies (bead-based, electrochemiluminescence, LC/MS, and standard microtitre ELISA) by the participants of the consortium. A number of patient clinical studies have been started in key areas (Cisplatin toxicity, Contrast induced nephropathy and acute GN) and these samples will provide the basis for the exploratory phase of the project.

Results: SAFE-T have gained regulatory feedback on this project and are actively recruiting patients and collecting samples. More than half of the 22 kidney markers have been analytically validated through a series of internal bar meetings and this list will be displayed.

Conclusions: A SAFE-T status update including regulatory strategy, assay validation and study designs will be provided.

TH-PO091

Safety and Tolerability of a Novel siRNA To Prevent Acute Kidney Injury in Cardiac Surgery: Initial Results from a Double-Blind, First-in-Man Study Sevag Demirjian,[1] Gorav Gorav Ailawadi,[2] Michel Burnier,[3] Dani Bitran,[4] Shuli Silberman,[4] Stanton Shernan,[5] Victor Baum,[2] Lakhmir S. Chawla.[6] *[1]Nephrology, Cleveland Clinic, Cleveland, OH; [2]Surgery and Anesthesiology, University of Virginia, Charlottesville, VA; [3]Nephrology, Hôpitaux Univérsitaire de Lausanne, Lausanne, Switzerland; [4]Shaare Zedek Medical Center, Jerusalem, Israel; [5]Anesthesiology, Brigham & Womens' Hospital, Boston, MA; [6]Anesthesiology, George Washington University, Washington, DC.*

Background: Acute kidney injury (AKI) results in appreciable mortality after cardiac surgery and is most commonly due to ischemia-reperfusion injury (IRI). IRI is thought to lead to renal dysfunction via p53-mediated apoptosis. An analogue of QPI-1002, a synthetic small interfering RNA (siRNA), has been shown in preclinical models to temporarily silence p53 gene expression and attenuate renal dysfunction. We report safety results from a first-in-man (FIM) study of QPI-1002 in pts undergoing cardiac surgery under cardiopulmonary bypass (CPB).

Methods: A total of 16 pts (4/cohort) at variable risk for AKI were enrolled at 6 centers globally. Pts were randomized 3:1 to receive an IV bolus injection of either placebo or QPI-1002, 0.5, 1.5, 5.0, or 10.0 mg/kg in dose escalation fashion 4 hrs post-CPB. The primary outcomes measured were safety and tolerability [dose-limiting toxicities (DLTs)] through 30 days post-dose.

Results: No DLTs were observed. Initial safety data included 107 adverse events (AEs); 90(84.1%) were severity grades 1-2 [scale: 1 (mild)-5]. Those most frequently reported were hypotension, anemia, pleural effusion, atrial fibrillation and leukocytosis (in 43.8%,37.5%,37.5%,31.3%,and 31.3%, resp.). No relationship between dose and any AE was seen.

Conclusions: In this FIM Phase I dose escalation study, there were no reported DLTs or evidence of a dose-dependent change in the number or severity of AEs. The observed AE profile appeared consistent with that expected for cardiac surgical patients post-operatively. The safety data support continued evaluation of QPI-1002 for prophylaxis of AKI in pts undergoing cardiac surgery.

(Unblinded data will be available at presentation.)

Funding: Pharmaceutical Company Support

TH-PO092

Age-and-Gender-Adjusted eGFR To Estimate Baseline Creatinine for RIFLE Classification Simone Spoorenberg,[1] Sabine Meijvis,[1] Gerjan Navis,[2] Douwe Hedde Biesma,[1] Jan Grutters,[1] Willem Jan W. Bos.[1] *[1]St Antonius Hospital; [2]University Medical Centre Groningen.*

Background: Risk, Injury, Failure, Loss and End-Stage kidney failure (RIFLE) criteria, based on rise in serum creatinine compared to baseline value, are used to classify acute kidney injury. If baseline creatinine is not available, ADQI recommends to estimate it, using a fixed eGFR of 75 mL/min/1.73m2. As GFR declines over age, we investigated RIFLE classification using age-and-gender-adjusted eGFR.

Methods: In 271 patients with community-acquired pneumonia, we compared RIFLE classification using either fixed eGFR of 75 mL/min (creatinine-75) or age-and-gender-adjusted eGFR (creatinine-age), with RIFLE classification based on actual baseline creatinine value(creatinine-actual).

Results: Use of creatinine-75 resulted in RIFLE over-classification of 46/271 patients and under-classification of 19/271 patients (p<0.001). Use of creatinine-age resulted in over-classification of 29/271 patients and under-classification of 20/271(p=0.04).

RIFLE classification for all patients

RIFLE Class (creatinine-75)

RIFLE Class (creatinine-actual)	0	1	2	3	Total n (%)
0	**199 (73)**	24 (8.9)	10 (3.7)	4 (1.5)	237 (88)
1	16 (5.9)	**5 (1.8)**	5 (1.8)	3 (1.1)	29 (11)
2	1 (0.4)	2 (0.7)	**2 (0.7)**	0 (0.0)	5 (1.8)
3	0 (0.0)	0 (0.0)	0 (0.0)	**0 (0.0)**	271
Total n (%)	216 (80)	31 (11)	17 (6.3)	7 (2.6)	271 (100.0)

Over-classification: 46 pt
Correct classification: 206 pt
Under-classification: 19 pt

RIFLE Class (creatinine-age)

RIFLE Class (creatinine-actual)	0	1	2	3	Total n (%)
0	**215 (79)**	16 (5.9)	3 (1.1)	3 (1.1)	237 (88)
1	18 (6.6)	**4 (1.5)**	4 (1.5)	3 (1.1)	29 (11)
2	1 (0.4)	1 (0.4)	**3 (1.1)**	0 (0.0)	5 (1.8)
3	0 (0.0)	0 (0.0)	0 (0.0)	**0 (0.0)**	0 (0.0)
Total n (%)	234 (86)	21 (7.7)	10 (3.7)	6 (2.2)	271 (100.0)

Over-classification: 29 pt
Correct classification: 222 pt
Under-classification: 20 pt

RIFLE classification calculated with actual creatinine ("gold standard") compared to RIFLE classification based on use of a fixed eGFR of 75 mL/min/1.73m^2 (creatinine-75) (p<0.001), and compared to RIFLE classification based on use of an age-and-gender-adjusted eGFR (creatinine-age) (p=0.04).

Next, we compared presence (RIFLE 1-3) and absence (RIFLE 0) of acute kidney injury. Using creatinine-75, 216/271 patients were classified correct, 38/271 were over-classified,

and 17/271 were under-classified (p=0.007). The use of creatinine-age classified 230/271 correct, over-classified 22/271 and under-classified 19/271 (p=0.76).

For patients >76 years use of creatinine-75 resulted in over-classification of 27/96 patients, and under-classification of 4/96 (p<0.001). Using creatinine-age resulted in over-classification of 12/96 patients, and under-classification of 6/96(p=0.10).

Conclusions: Use of an age-and-gender-adjusted eGFR improves RIFLE classification compared with use of a fixed eGFR, especially in elderly. If actual baseline creatinine is not available, we propose to use an age-and-gender-adjusted baseline creatinine for RIFLE classification.

TH-PO093

Proteinuria in Community-Acquired Pneumonia as a Prognostic Marker for Outcome Simone Spoorenberg,[1] Sabine Meijvis,[1] Gerjan Navis,[2] Jan Grutters,[1] Douwe Hedde Biesma,[1] Henk J.T. Ruven,[1] Johannes C. Kelder,[1] Willem Jan W. Bos.[1] *[1]St Antonius Hospital; [2]University Medical Centre Groningen.*

Background: Acute Kidney Injury (AKI), classified according to the Risk, Injury, Failure, Loss and End-Stage kidney disease (RIFLE) criteria, is occasionally seen in patients with Community Acquired Pneumonia (CAP). It is known that RIFLE criteria may underestimate the occurrence of AKI. We investigated the incidence and predictive value of proteinuria in patients with CAP, and compared the results with the RIFLE criteria.

Methods: We retrospectively investigated RIFLE criteria and proteinuria in patients hospitalized with CAP. Proteinuria, defined as total-protein/creatinine ratio>23mg/mmol, was measured in a urine sample from the day of admission. Primary outcome was length of hospital stay (LOS). Secondary outcomes were in-hospital mortality and one-year mortality.

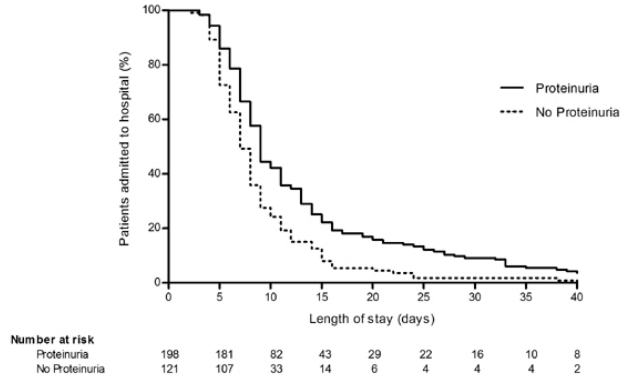
Results: In 319/496 patients (64%) a urine sample was available. 198 patients (62%) had proteinuria. Patients with proteinuria had a significant longer LOS and an increased in-hospital mortality. RIFLE class was a predictor for LOS, in-hospital mortality and one-year mortality. In multivariate analysis, proteinuria only was an independent predictor for LOS.

	Proteinuria		RIFLE 1-3	
	OR or HR	95% CI	OR or HR	95% CI
Length of stay				
Unadjusted[2]	1.63	1.28-2.06	1.55	1.12-2.15
Adjusted[2]	1.38	1.08-1.75	1.21	0.86-1.71
In-hospital mortality				
Unadjusted[1]	5.59	1.27-24.63	3.81	1.40-10.35
Adjusted[1]	3.27	0.69-15.45	1.81	0.62-5.27
One-year mortality				
Unadjusted[2]	1.74	0.94-3.22	3.06	1.69-5.55
Adjusted[2]	1.09	0.56-2.10	1.81	0.97-3.37

OR=[1]: Odds ratio and HR=[2]: Hazard ratio

Conclusions: The incidence of proteinuria during CAP is high. Proteinuria and RIFLE criteria are both associated with adverse outcome in CAP. Only proteinuria is an independent predictor for LOS. Proteinuria can be a marker for outcome and might be used to assess the severity of CAP.

Figure 1: Length of stay for patients with proteinuria (total protein >23 mg/mmol creatinine) and patients without proteinuria (Log Rank p<0.001).



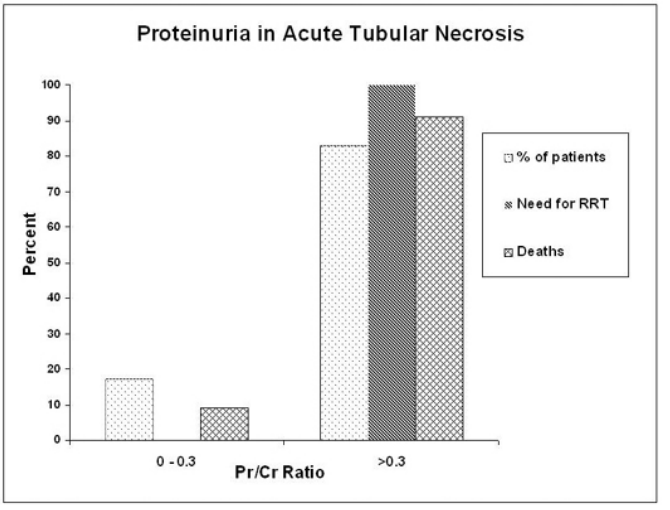
TH-PO094

Proteinuria in Acute Tubular Necrosis Kevin K. Pandya, Kavitha Potluri, David J. Leehey, Alexander R. Chang, Holly J. Kramer. *Divison of Nephrology, Loyola University Medical Center, Maywood, IL.*

Background: Acute kidney injury (AKI) as defined by well-established criteria is observed in about 5-7 percent of hospitalized patients and 66% of intensive care patients. ATN accounts for nearly 50% of AKI cases in hospitalized patients. Because of tubular damage in ATN, the normal mechanism of protein reabsorption is likely to be altered, with receptor- mediated endocytosis of albumin expected to decrease, leading to overt proteinuria. However, data on the incidence of proteinuria in the setting of ATN are sparse. We examined the changes in urine protein excretion after an episode of ATN in adults without protienuria.

Methods: This is an observational study focused on patients age > 18 years who experienced an episode of AKI as defined by AKIN/RIFLE criteria. Criteria to select patients with ATN included FENa > 2%, serum urea nitrogen-to-creatinine ratio < 20, urine-to-plasma creatinine ratio < 20, as well as the nephrologist's clinical determination based on available data. Patients with known proteinuria (UA showing >1+ proteinuria) were excluded from the study. Proteinuria (based on Pr/Cr ratio) was measured at time of diagnosis of ATN. All study patients were followed during the hospitalization for secondary endpoints: mortality and need for dialysis.

Results: A total of 32 patients with absence of proteinuria prior to ATN episode were identified over an approximately 7 months time period. 83% of the patients (24/32) had urine Pr/Cr > 0.3 (g/g). Of the patients who required dialysis, 100% (13/13) were noted to have Pr/Cr > 0.3. Lastly, of the patients who died within the selected cohort, 91% (10/11) had Pr/Cr ratio > 0.3.



Conclusions: The majority of patients with ATN in this study had a Pr/Cr > 0.3 g/g and these patients had higher mortality and need for RRT. Urine Pr/Cr may be a simple and readily available biomarker to predict outcome in ATN.

TH-PO095

The Capacity of Nephrology and Critical Care Services in England and Wales To Manage Acute Kidney Injury Ben Bray,[1] Beverley Matthews,[2] Donal O'Donoghue.[3] *[1]NHS Greater Glasgow and Clyde, Glasgow, United Kingdom; [2]NHS Kidney Care, London, United Kingdom; [3]Salford Royal Foundation NHS Trust, Salford, United Kingdom.*

Background: Acute Kidney Injury is increasingly recognised as an important quality and safety challenge in all health economies. In England, coded admissions for AKI are rising by over 10% per annum (Department of Health) and a national audit of AKI deaths (NCEPOD 2009, *Adding Insult to Injury*) highlighted access to specialist nephrology and critical care specialist services as a factor in many deaths from AKI. Here we report the result of a survey of the capacity of nephrology and critical care units in England and Wales to meet the demand for managing AKI.

Methods: A web based questionnaire was sent to clinicians representing all hospitals in England and Wales offering inpatient nephrology and critical care services. The questionnaire included items relating to staffing, facilities, use of guidelines and AKI related bed occupancy on World Kidney Day 2011. The AKIN classification of AKI was used.

Results: Complete results were obtained from 41/59 (69%) nephrology centres and 45/175 (26%) critical care centres in England and Wales. There was variation in the model of AKI management between centres – only 29% of nephrology centres had dedicated high dependency facilities. Bed occupancy in nephrology centres was 97%. 23% of nephrology beds were occupied by patients with AKI (Stage I-III), 57% of whom had RRT dependent AKI. The prevalence of RRT dependent AKI amongst critical care patients was 9%. Although the majority of nephrology centres have agreed guidelines for the clinical management of AKI (63%) and contrast nephropathy (89%), only 37% have protocols concerning the transfer and referral of patients from hospitals without nephrology services.

Conclusions: Delays in access to specialist nephrology and critical care services may lead to worse outcomes for patients with AKI. High bed occupancy in nephrology services in England and Wales suggests that there is little scope for increasing access to these services without increasing efficiency or increasing capacity. One suggested solution would be better use of protocols to define planned pathways of care for patients with AKI.

TH-PO096

Stress-Induced Hyperglycemia and Acute Kidney Injury in Critically Ill Children Roberto Gordillo,[1] Robert Woroniecki.[2] *[1]Pediatrics, University of Illinois College of Medicine at Peoria, IL; [2]Pediatrics, Albert Einstein College of Medicine, Bronx, NY.*

Background: Stress-induced hyperglycemia (SIH) is common in critically ill patients and has been associated with increased mortality and morbidity. However, it is not clear if SIH is associated with acute kidney injury (AKI) in children. We hypothesized that SIH is associated with intensity of AKI.

Methods: We analyzed the records of children with SIH admitted between 01/2005-01/2010 to the pediatric intensive care unit (PICU). Subjects with prior kidney disease and diabetes were excluded. Intensity of AKI was defined by pediatric RIFLE criteria. Serum glucose (SG) and estimated glomerular filtration rate (eGFR) on admission, 48 hours, and at 7 days after admission were obtained. Delta glycemia (DG) was defined as the difference between the peak and the lowest SG during PICU stay.

Results: Out of 37 subjects, 13 (35%) had AKI. Results are shown in Table. We found no correlation between SG and eGFR at 0hr, 48hr and 7days. Subjects with SIH and AKI stayed longer in PICU and hospital, and we found a positive correlation between DG and length of stay in PICU (p=0.0003, r2=0.3) and length of hospital stay (p=0.0005, r2=0.34).

	AKI (n=13)	No-AKI (n=24)	p
Age (years)	4.1 ± 1.1	6 ± 0.9	0.88
Caucasian (%)	31	45.8	0.49
Female (%)	46	41	0.99
Serum glucose on admission (mg/dl)	148 ± 22.49	135.8 ± 13.2	0.6
Peak Glucose (mg/dl)	235 ± 19.9	212 ± 66.31	0.2
Days of hospital stay	31.23 ± 3.7	24.1 ± 3.7	0.05
Days of PICU stay	20 ± 2.6	16.25 ± 2.99	0.06
Death	1	0	

Conclusions: In children with SIH, SG and DG is not associated with intensity of AKI, but it does correlate with length of hospital and PICU stay.

TH-PO097

Natriuretic Peptide and Loop Diuretic Agents in Cardiac Surgery Associated Acute Kidney Injury Prevention Sagar U. Nigwekar, Khuloud Shukha, Sushrut S. Waikar. *Brigham and Women's Hospital.*

Background: In animal studies, natriuretic peptide (NP) and loop diuretics (LD) are shown to prevent cardiac surgery associated acute kidney injury (CSAKI). However, randomized controlled trials (RCTs) in humans have shown inconsistent results and are underpowered for evaluation of hard outcomes such as renal replacement therapy requirement (CSAKI-R) and mortality. There also remains a question regarding their safety. Our study aimed to systematically review these RCTs to ascertain efficacy and safety of these agents in CSAKI prevention.

Methods: We searched MEDLINE, EMBASE, and Google Scholar for RCTs evaluating NP or LD for CSAKI prevention. Outcomes analyzed were CSAKI (defined per the individual RCT), CSAKI-R, mortality and durations of intensive care unit (ICU) and hospital stay. Statistical analyses were performed using the random effects model and results expressed as relative risks (RR) for dichotomous outcomes and weighted mean difference (WMD) for continuous outcomes, with 95% confidence intervals (CI). Methodological quality assessment was performed by Jadad score.

Results: Fourteen RCTs (12 NP, 2 LD) involving 1,599 patients met the inclusion criteria. NP dose administered in these RCTs was noted to be lower than that administered in Auriculin Anaratide Acute Renal Failure Study. Pooled analysis of RCTs evaluating NP showed reduction in CSAKI (RR 0.48, [0.23 to 0.96]), CSAKI-D (RR 0.23, [0.08 to 0.70]) , mortality (RR 0.50, [0.27 to 0.93]), ICU length of stay (WMD -0.44 days, [-0.82 to -0.06 days]) and hospital length of stay (WMD -4.08 days, [-6.32 to -1.83 days]) in the NP group. There was no statistical heterogeneity in these analyses. Statistical analysis could not be performed due to limited number of RCTs evaluating LD. Both NP and LD agents were well tolerated. Methodological quality of the RCTs was variable; however, sensitivity analysis of high quality studies did not change the findings.

Conclusions: NP agents, in low doses, are well tolerated and may prevent CSAKI and its associated complications. Appropriately powered RCT is urgently needed to confirm these results. Role of LD agents is not adequately studied in this population.

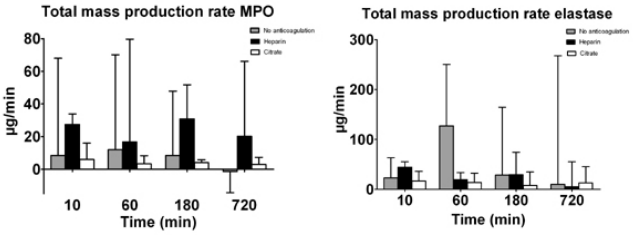
TH-PO098

Regional Citrate Anticoagulation Decreases the Degranulation of Neutrophils in Continuous Venovenous Hemofiltration in Critically Ill Patients Louise Schilder,[1] Shaikh Azam Nurmohamed,[1] Albertus Beishuizen,[2] Pieter M. Ter Wee,[1] Johan Groeneveld.[2] *[1]Nephrology, VU University Medical Center, Amsterdam, Netherlands; [2]Intensive Care, VU University Medical Center, Amsterdam, Netherlands.*

Background: During continuous venovenous hemofiltration in critically ill patients, anticoagulation is administered to prevent clotting of the hemofilter. In case of a high bleeding tendency, regional anticoagulation using citrate is an option. Citrate creates an almost calcium-free environment in the extracorporeal circuit, not only preventing coagulation, but potentially also downregulating the degranulation of neutrophils, as indicated by decreased plasma levels of myeloperoxidase (MPO) and elastase levels.

Methods: Three anticoagulation regimes were compared: no anticoagulation (n=13), unfractionated heparin (n=7) and trisodium citrate (n=17). The data were obtained during prospective observational and randomised studies. At initiation of CVVH (cellulose triacetate membrane, t=0) prefilter blood was collected. Thereafter, blood samples were collected from pre- and postfilter at 10, 60, 180 and 720 min. MPO and elastase were determined by ELISA.

Results: Plasma levels of MPO and elastase increased over time in the patients during heparin-CVVH relatively to citrate-CVVH (P<0.001 resp P=0.015). The production rates of MPO and elastase in the extracorporeal circuit were lowest when citrate was administered. For MPO, citrate was superior to heparin in this respect (P=0.002) and for elastase, citrate was superior to no anticoagulation (P=0.04) and heparin (trend, P=0.07). High plasma levels of MPO and elastase were associated with higher mortality rates (P=0.006 resp P<0.001).



Conclusions: Regional citrate anticoagulation is superior to heparin and to no anticoagulation in CVVH with respect to biocompatibility in terms of neutrophil degranulation. This effect of citrate could favour patient survival.

TH-PO099

Selective A2B Adenosine Receptor Activation Protects from Kidney Injury in a Model of Auto-Immune Disease Gabriela E. Garcia,[1] Luan D. Truong,[2] Almut Grenz,[1] Holger Eltzschig,[1] Richard J. Johnson,[1] Lili Feng.[3] *[1]Medicine, University of Colorado Denver, Aurora, CO; [2]Pathology, The Methodist Hospital, Houston, TX; [3]Medicine, Baylor College of Medicine, Houston, TX.*

Background: A_{2B} adenosine receptor ($A_{2B}R$) activation has been previously implicated in attenuating tissue inflammation under conditions of limited oxygen availability. In addition, this adenosine receptor can modulate tissue protection by stimulating angiogenesis. We investigated the role of $A_{2B}R$ in a model of severe, macrophage-mediated and cytokine-dependent anti-glomerular basement membrane antibody-associated glomerulonephritis (GN).

Methods: We activated $A_{2B}R$ with the selective $A_{2B}R$-agonist BAY 60-6583 during GN to determine its effects on kidney injury, expression of cytokines/chemokines, and recruitment of inflammatory infiltrates.

Results: Normal kidneys had little $A_{2B}R$ mRNA expression; however, its expression was increased in nephritic kidneys. Activation of $A_{2B}R$ in the acute inflammatory phase of GN did not attenuated $CD8^+$ infiltrates but increased $ED1^+$ accumulation compared with the control group. However, necrotizing lesions and glomerular hypercellularity were markedly attenuated in the $A_{2B}R$-agonist treated group. As a result of the attenuation of kidney damage, reduced proteinuria and serum creatinine were observed in the $A_{2B}R$ agonist-treated group. The expression of CX_3CL1, CCL2, CCL4, CCL5, CCL19, and CCL22 chemokines, was not different between both groups. In contrast, the expression of the anti-inflammatory cytokine, TGF-β1 was significantly induced in the $A_{2B}R$ agonist-treated group. In addition, activation of $A_{2B}R$ reduced the expression of osteopontin-1 a pro-inflammatory and anti-angiogenic mediator. Notably, in both groups macrophages were predominantly MI phenotype, however, in the control group 71% of macrophages were M1 phenotype compared with 51% in the $A_{2B}R$ agonist-treated group.

Conclusions: These findings suggest that $A_{2B}R$ could protect from kidney injury by modulating pro-and-anti-inflammatory mediators. Similarly, markedly reduction in necrotizing lesion by $A_{2B}R$ activation suggests a potential angiogenic capillary repair for this adenosine receptor.

Funding: NIDDK Support, Private Foundation Support

TH-PO100

EP2-Receptor Deficiency Ameliorates the Course of Crescentic Glomerulonephritis in Mice Gunther Zahner, Malte A. Kluger, Ulf Panzer, Rolf A. Stahl, Andre Schneider. *III. Medizinische Klinik, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany.*

Background: Prostaglandin E2 (PGE2) has important immune modulatory roles in various diseases in different organs. PGE2 mediates its effects through four different EP-receptors, named EP1 to EP4. Different intracellular signals of the receptor-subtypes and different combinations of subtypes on the cell surface may partly be responsible for the diverse effects of PGE2 in inflammation.

Methods: Clinical parameters: measurement of BUN and albuminuria.

Histological assessment of renal damage on PAS-stained tissue sections and immunohistochemistry against CD3 and F4/80.

Quantitative PCR of Cox-2, CCL2, IL-6 and TNFalpha.

FACS-based intracellular staining of IL-17 and IFN gamma.

Results: In order to elucidate the potential role of the EP2 receptor in glomerulonephritis, the nephrotoxic-serum-nephritis (NTN) was induced in EP2 deficient (EP2-/-) and in wildtype (WT) mice (C57bl6 background). Succesful onset of the disease was evaluated functionally at day 4 after NTN-injection. No differences between EP2-/- and WT mice was detected in this early phase of the disease.

10 days after induction of the nephritis (during the T-cell dependent autologous phase), EP2-/- mice developed less severe nephritis in terms of significantly better preserved kidney function, less glomerular damage, reduced renal infiltration of CD3+ T-cells and F4/80 positive macrophages/dendritic cells and less RNA-expression of the pro-inflammatory mediators Cox-2, CCL-2, IL-6 and TNFalpha when compared with nephritic WT-mice. Interestingly, renal Th-17 immune response seemed to be impaired in nephritic EP2 -/- mice.

Conclusions: Thus, the EP2 receptor seems to play an important role in the development of NTN.

Funding: Government Support - Non-U.S.

TH-PO101

Suppression of Hyaluronan Synthesis with 4-Methylumbelliferone in NZB/W F1 Mice Is Associated with Reduced Renal Inflammation and Renal Function Improvement Daniel Tak Mao Chan, Wan Wai Tse, Mel Chau, Susan Yung. *Department of Medicine, University of Hong Kong, Hong Kong.*

Background: We previously demonstrated that glomerular hyaluronan (HA) expression is increased in lupus nephritis, but its role in pathogenesis remains to be determined. This study investigated the effect of 4-methylumbelliferone, a specific inhibitor of HA synthesis, on disease manifestations in a lupus mouse model.

Methods: Female NZB/W F1 mice with established nephritis and proteinuria greater than 3g/l were randomized to receive treatment with sterile PBS, vehicle alone (1% Arabic Gum in PBS) or 4-methylumbelliferone in 1% Arabic Gum (MU, 3g/kg/day) for 2, 4, 8 and 12 weeks (n=6 for all time points for each group), after which the mice were sacrificed, blood collected and kidneys harvested for assessment of histology and expression of inflammatory mediators. Spot urine was obtained for the measurement of albumin-to-creatinine ratio.

Results: Serum HA levels increased in a time-dependent manner as disease progressed in PBS and vehicle treated mice. This was associated with increased periglomerular and tubulo-interstitial HA expression. MU treatment for 12 weeks reduced serum HA level by 30% and decreased renal HA expression to near normal levels. Urine albumin-to-creatinine ratio and serum levels of urea and creatinine increased in both PBS and vehicle treated mice with progressive disease, but were significantly decreased in mice after 12 weeks of MU treatment ($P<0.05$ for all, compared to both control groups). MU-treated mice also showed reduced glomerular deposition of IgG and C3, IL-6 and TNF-α expression, and glomerular infiltration by $CD4^+$ T cells, $CD19^+$ B cells and macrophages compared to controls.

Conclusions: These results suggest that HA plays an important role in the pathogenesis of lupus nephritis and suppression of HA synthesis can ameliorate disease manifestations.

Funding: Government Support - Non-U.S.

TH-PO102

Connective Tissue Growth Factor (CTGF) Induces a Chronic Th17 Inflammatory Response in the Kidney Raquel Rodrigues-Diez,[1] Raúl R. Rodrigues Díez,[1] Sandra Rayego-Mateos,[1] Carolina Lavoz,[1] Matilde Alique Aguilar,[1] Alberto Ortiz,[2] Jesus Egido,[2] Marta Ruiz-Ortega.[1] *[1]Universidad Autonoma; [2]ISCFJD, Madrid, Spain.*

Background: Several studies have suggested that plasma or urine CTGF levels is a useful risk marker for renal damage in chronic kidney diseases (CKD). However, the renal effects of CTGF has not been fully investigated. Until now, CTGF has been considered as a profibrotic mediator, although growing evidences suggest that could also participate in inflammation. In vivo CTGF induces an acute renal inflammatory response in mice, observed at 24 hours and characterized by upregulation of Th1/Th2 cytokines and chemokines. However, there is no studies at longer times. IL-17-producing CD4(+) T helper (Th17) cells have been identified as novel effector immune cells. IL-17, besides participating in immune diseases, is involved in chronic inflammatory diseases, including CKD.

Methods: Systemic administration of CTGF (2.5 ng/g BW) was done in mice. Blockade of Th17 response was done by a neutralizing antibody against IL-17.

Results: CTGF caused a sustained Th17 inflammatory response in the kidney, observed at 5 days and remained elevated until 15 days. At 10 days, CTGF-treated mice presented focal interstitial infiltration of monocytes/macrophages and T lymphocytes, including CD4+/IL-17 expressing cells, and upregulation of chemokines. In contrast, there was no fibrosis or proteinuria. Elevated renal gene and protein levels of IL-17 were found in CTGF-treated mice, compared to controls, while tissue levels of INF-γ and IL-4 (Th1 and Th2 hallmark cytokines, respectively) were not changed between groups. The main factors involved in the regulation of Th17 cell differentiation in mice (IL-6 and RORγt) were elevated in the kidney of CTGF-treated mice. The blockade of Th17 response, by a neutralizing antibody against IL-17, diminished CTGF-induced inflammatory response at 10 days (infiltrating cells and upregulation of proinflammatory factors), clearly demonstrating the involvement of Th17 response in CTGF-mediated renal damage.

Conclusions: Our findings indicate that CTGF is a potential therapeutic target for CKD, as it promotes chronic kidney inflammation by activating Th17 response.

Funding: Government Support - Non-U.S.

TH-PO103

Fully Human Anti-Macrophage Migration Inhibitory Factor (MIF) Antibody Is Effective in Treatment of Established Experimental Glomerulonephritis Frederick W.K. Tam,[1] Gurjeet Bhangal,[1] Jennifer Smith,[1] John P. McDaid,[1] Dirk Voelkel,[2] Manfred Rieger,[2] H. Terence Cook,[1] Charles D. Pusey,[1] Scheiflinger Friedrich,[2] Randolf Kerschbaumer.[2] *[1]Imperial College Kidney and Transplant Institute, Imperial College London, London, United Kingdom; [2]Baxter Innovations GmbH, Vienna, Austria.*

Background: The macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine placed high upstream in the cytokine cascade. Early studies have shown that MIF is upregulated in glomerulonephritis in humans and administration of murine anti-MIF antibodies reduced the severity of experimental glomerulonephritis. A clinical therapy targeting MIF using fully human anti-MIF antibodies is of major interest.

Methods: A highly diverse panel of fully human MIF-specific monoclonal antibodies (mAbs) was selected from a phage display library and antibodies were produced as human IgGs. The effect of mAbs with pronounced in vitro neutralizing properties was assessed in a series of pilot studies using the model of nephrotoxic nephritis in Wistar Kyoto rats. The mAb BaxB01 was chosen for further studies. Treatment was started after onset of proteinuria (day 4) to assess the antibody in a clinically relevant design. Rats were randomised into three treatment groups (isotype control, 20 mg/kg and 40 mg/kg anti-MIF mAb) and treated on day 4 and day 6.

Results: The severity of glomerular injury was assessed on day 8. Proteinuria, glomerular macrophage infiltration and glomerular crescents were reduced dose dependently following treatment with anti-MIF mAb [Table 1]. In addition, urinary levels of proinflammatory cytokines like TNFα and IL-1β were reduced.

mAb	isotype control	BaxB01 (20 mg/kg)	BaxB01 (40 mg/kg)
Proteinuria (mg/day)	129.7±6.5	105.6±8.7	85.4±4.9 **
Macrophage (glomerular cross section)	30.5±1.5	22.0±1.4 *	19.3±1.5 ***
Crescent (%)	93.9±0.7	86.3±1.4*	78.3±3***

*p<0.05, ** p<0.005, ***p<0.001

Conclusions: A fully human anti-MIF mAb was able to neutralize the proinflammatory effects of MIF in vitro and was effective for treating antibody-mediated experimental glomerulonephritis even after onset of proteinuria. This antibody has important potential for clinical use in nephritis and other MIF-related diseases.

Funding: Pharmaceutical Company Support

TH-PO104

The Cross-Talk between NKT Cell and Th17 Response in the Experimental Autoimmune Glomerulonephritis Ji In Park,[1] Seung Hee Yang,[1] Ran-Hui Cha,[2] Jung Pyo Lee,[1] Dong Ki Kim,[1] Yon Su Kim.[1] *[1]Department of Internal Medicine, Seoul National University College of Medicine; [2]Department of Internal Medicine, National Medical Center.*

Background: Th17 cells are emerging as a major player in several autoimmune diseases such as multiple sclerosis and rheumatoid arthritis which were known as Th1 mediated diseases. NKT cells, which have a role of linkage between the innate and adaptive immune response, are also associated with autoimmune diseases. But the cross-talk between NKT cell and Th17 has not been evaluated in the context of autoimmune disease. In present study, we examined the role of NKT cell/Th17 response in autoimmune glomerulonephritis (AGN) utilizing a murine model of chronic graft-versus-host disease.

Methods: AGN was induced by the adoptive transfer of lymphocytes from (C57BL/6 x DBA/2J) F1 hybrids into wild C57BL/6, type I/II NKT cell deficient, and type I NKT cell deficient mice. The transferred lymphocytes infiltrated into the glomeruli inducing cellular proliferation.

Results: The severity of AGN, confirmed by deterioration of kidney function, proteinuria, and renal pathology, was attenuated with the absence of NKT cells compared to wild type mice. Complement 3 deposition and T cell infiltration were consistent with the severity of AGN. NKT cells were recruited into glomerulus with the induction of AGN. The systemic immune responses as measured by splenic T cell activation, intracellular IL-17, and inflammatory cytokines, are enhanced with the induction of AGN. But the absence of NKT cells, especially both of type I and II NKT cells, reduced the systemic immune responses. The intrarenal immune response induced by AGN was paralleled with systemic responses. Moreover, intrarenal STAT3 phosphorylation, which is the major transcription factor for Th17 response, was significantly attenuated in NKT cell deficient hosts. NKT cells secreted IL-17 as well as inflammatory cytokines (TNF-α, IL-6, IL-12, IFN-g) when activated. Blocking of IL-17 signaling reduced the mesangial cell responses against the stimuli induced by the nephritogenic lymphocytes.

Conclusions: Taken together, the cross-talk between NKT cell and Th17 response might be a pivotal linkage for the development of AGN.

TH-PO105

CXCL5/CXCL1-CXCR2 Axis Drives Neutrophil Infiltration in Murine Experimental Glomerulonephritis in a Time and Compartment Specific Manner Erik M. Disteldorf,[1] Markus Koch,[4] Mischo Mischo Kursar,[4] Jan-Eric Turner,[1] Hans-Joachim Paust,[1] Joachim Velden,[3] Hans-Willi Mittrücker,[2] Rolf A. Stahl,[1] Stefan H.E. Kaufmann,[4] Ulf Panzer.[1] *[1]III. med. Klinik, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany; [2]Immunology, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany; [3]Pathology, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany; [4]Institut für Infektionsbiologie, Max Planck Institut, Berlin, Germany.*

Background: Crescentic glomerulonephritis is characterized by the recruitment of leukocyte subsets including neutrophils that cause renal tissue damage. The mechanisms of neutrophil migration in glomerulonephritis are not well characterized. In this study we investigate the role of the chemokine receptor CXCR2 and its ligands CXCL1, CXCL2 and CXCL5 in renal neutrophil trafficking and organ injury.

Methods: Experimental glomerulonephritis was induced in mice.

Results: Induction of nephritis resulted in early (12 hours) up-regulation of glomerular CXCL1 and CXCL2 mRNA expression that preceded glomerular neutrophil accumulation. CXCR2-deficiency resulted in reduced neutrophil infiltration and less damage at day 3, demonstrating the importance of the CXCL1/2 - CXCR2 axis during the early course of nephritis. In contrast, CXCL5 expression peaked at day 14 and was localized to the tubulointerstitial area. At this time neutrophils migrate into the same compartment. To investigate the functional role of CXCL5, nephritis was induced in CXCL5-/- mice. CXCL5-/- mice developed an ameliorated course of disease as a consequence of reduced tubulointerstitial neutrophil infiltration at day 14, whereas at day 3 neutrophil accumulation and disease severity was unaltered. In vitro experiments identified the cytokine IL-17A

as a strong inductor of CXCL5. IL-17A expression in NTN peaked at day 7-10 preceding renal CXCL5 expression. In line, CXCL5 expression was downregulated in nephritic IL-17A-/- mice.

Conclusions: In conclusion, our study shows that the CXCL5/CXCL1-CXCR2 axis orchestrates renal neutrophil recruitment in a time and compartment specific manner. CXCL5 mediated neutrophil recruitment is driven by IL-17 and contributes to renal tissue injury in crescentic glomerulonephritis.

TH-PO106

IFN-αand IFN-β Specifically Affect Renal Progenitors and Podocytes In-Vitro and In-Vivo A. Migliorini,[1] C. Sagrinati,[2] M.L. Angelotti,[2] E. Parente,[2] L. Ballerini,[2] E. Ronconi,[2] A. Peired,[2] L. Lasagni,[2] E. Lazzeri,[2] Paola Romagnani,[2] Hans J. Anders.[1] *[1]Medizinische Poliklinik, LMU, Munich, Germany; [2]Excellence Centre for Research, DENOthe, University of Florence, Florence, Italy.*

Background: Viral infections can be associated with glomerulonephritis in various ways which is likely to involve antiviral immunity. IFNα and IFNβ orchestrate antiviral defence and potentially contribute to GN. For example, lack of the IFNα-R ameliorates murine immune complex disease complicated by glomerulonephritis but local effects of IFNα/β on glomerular pathology remain speculative.

Methods: C133+CD24+ human renal progenitors and podocytes cultured +/- hIFNα and hIFNβ. SCID mice injected with adriamycin +/- recombinant mIFNα and mIFNβ.

Results: IFNα and IFNβ both activated renal progenitors to express several interferon-related (antiviral) genes such as MX1, IFIT1 and CXCL10. IFN-α significantly decreased the proliferation of human renal progenitors, by contrast IFNβ inhibited differentiation of renal progenitors towards podocytes as determined by induced nephrin expression. Next we compared the impact of recombinant IFNα and IFNβ injections on adriamycin-induced nephropathy in SCID mice which allowed us to exclude IFN-dependent effects on adaptive immunity. Both IFNs increased proteinuria as compared to control mice injected with adriamycin only. Quantitative morphometry by confocal microscopy revealed that IFNβ injections had specifically reduced the number of WT1+/nephrin+ podocytes while IFNα injections specifically reduced the numbers of proliferating parietal epithelial cells, respectively. In summary, both type I IFNs can aggravate glomerular pathology, albeit in different ways. IFNα impairs the proliferation of potential podocyte progenitors, while IFNβ mostly impairs progenitor differentiation in vitro.

Conclusions: These data propose that IFNα and IFNβ contribute to glomerulosclerosis and proteinuria by specifically affecting homeostasis of podocytes and their potential repair by local progenitors, a mechanism that may contribute to in viral infection-associated glomerular pathology.

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TH-PO107

Human Bone Marrow Derived Mesenchymal Stromal/Stem Cells Attenuate Tubular Inflammation upon Albumin Challenge Hao-Jia Wu, Wai Han Yiu, Joseph C.K. Leung, Loretta Y.Y. Chan, Lian Qizhou, Miao Lin, Hung-Fat Tse, Kar Neng Lai, Sydney C.W. Tang. *Department of Medicine, University of Hong Kong, Hong Kong, Hong Kong.*

Background: Emerging evidence indicates that bone marrow derived mesenchymal stem cells (BM-MSC) protect against many forms of chronic renal diseases (CKD). The mechanism underlying this effect is not completely understood. We have previously shown the tubular expression of proinflammatory mediators induced by proteinuria play a vital role in the pathogenesis of CKD. This study aims to explore whether BM-MSC exerted anti-inflammatory effects in renal proximal tubular cells (PTEC) under milieu mimicking proteinuric nephropathy.

Methods: PTEC were treated with human albumin serum (HSA) and co-cultured with BM-MSC for 6 hours and 24 hours. Transcription and secretion of proinflammatory mediators were measured by real-time qPCR and ELISA, respectively. NF-κB signalling was assessed by western blot.

Results: Real-time qPCR revealed that co-culture with BM-MSC significantly reduced the up-regulated mRNA transcripts including IL6, CCL2, CCL5, IL8, TNF-alpha, IL1-beta, and ICAM-1 in PTEC exposed to HSA. The suppression of proinflammtory genes translated into reduced secretion of IL-6, CCL2, CCL5, IL8 and TNF-alpha proteins as detected by ELISA. In addition, the reduction of these proinflammtory cytokines and chemokines by BM-MSC was associated with attenuation of HSA induced I-κB phosphorylation in PTEC. To dissect the potential mechanism, we detected that the anti-inflammatory genes, HGF and IL1RN (IL1 receptor antagonist), were significantly induced in BM-MSC during co-culture with PTEC under protein overload condition.

Conclusions: Our *in vitro* data suggest an anti-inflammatory role of BM-MSC in HSA-elicited PTEC inflammation, probably through paracrine effects of HGF and IL1RN via NF-κB signaling.

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TH-PO108

Macrophage-Specific Thymosin β4 Knockout Protects Against LPS-Induced Inflammation Li-Jun Ma,[1] Bridgette Corsa,[1] Yiqin Zuo,[1] Paul Riley,[2] Agnes B. Fogo.[1] *[1]Pathology, Vanderbilt University Medical Center, Nashville, TN; [2]Molecular Medicine Unit, UCL Institute of Child Health, London, United Kingdom.*

Background: Macrophages are a rich source of thymosin β4 (Tβ4), but little is known about the role of Tβ4 in macrophage response. We investigated whether Tβ4 modulates macrophage function.

Methods: Floxed Tβ4 short hairpin RNA (T4shRNAflox) mice were crossed with lysMcre mice to generate macrophage-specific Tβ4 KO (Mac-Tβ4 KO) mice. Peritoneal thioglycolate-elicited macrophages from wild type (WT, n=3) and Mac-Tβ4 KO (n=3) mice were isolated and exposed to LPS (50 ng/ml) or buffer for 48 hrs to induce inflammation. Expressions of Tβ4 protein, phospho-c-Jun and total cJun in macrophages were examined by Western Blot. Unilateral ureteral obstruction (UUO) was performed in adult male WT (n=5) and Mac-Tβ4 KO (n=4) mice. Macrophage infiltration was assessed at day 7 after UUO. Data are expressed as mean±SE.

Results: Macrophage Tβ4 protein level was markedly diminished in Mac-Tβ4 KO vs WT by 87%. P-c-Jun expression was robustly induced by LPS in WT macrophages, but was significantly reduced by 52±8 % in macrophages from Mac-Tβ4 KO mice. UUO-induced kidney macrophage infiltration was significantly inhibited in Mac-Tβ4 KO vs WT by 28.5%.

Conclusions: Macrophage Tβ4 deletion protects against LPS-induced inflammation, and decreases macrophage infiltration in obstructed kidneys. Our data suggests that targeting macrophage Tβ4 may offer novel treatment of inflammation.

Funding: NIDDK Support

TH-PO109

Mice Fed Adenine-Containing Diet Is Optimal Model of Tubulointerstitial Damage with Renal Dysfunction Izumi Yamamoto,[1] Keitaro Yokoyama,[1] Ichiro Ohkido,[1] Taketo Uchiyama,[1] Ichiaki Ito,[2] Akiko Murayama,[2] Jun Yanagisawa,[2] Tatsuo Hosoya.[1] *[1]Department of Internal Medicine, Division of Kidney and Hypertension, Jikei University School of Medicine, Tokyo, Japan; [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaragi, Japan.*

Background: Adenine is produced endogenously as a by-product of the polyamine pathway and is salvaged by adenine phosphoribosyltransferase (APRT:EC 2.4.2.7). APRT deficiency leads to the accumulation of 2,8-dihydroxyadenin(DHA)crystals in human(OMIM:102600). Mice fed the overload of the adenine-containing diet are mimic to APRT deficiency mice and both were used as human DHA disease model that showed DHA crystals in the tubular lumens with fibrosis and renal dysfunction.

Methods: The aim of this study is to evaluate the protocol for the optimal mice model of tubulointerstitial damage (TID) with renal dysfunction induced by adenine feeding. Inbred male C57BL/6 mice (9 weeks old) were fed a standard laboratory powder diet or a powder diet containing 0.25% adenine (0.25%Ad). Mice fed 0.25%Ad were euthanized on days 3, 7, and 14 (Group 1,3,5), and mice fed the control diet were euthanized on days 3, 7, 14 (Group 2,4,6). Additionally, we analysed mice fed 0.25%Ad for 7 days with subsequent control diet euthanized on days 14 (Group7).

Results: Mice fed 0.25%Ad showed nephrolithiasis, extensive tubular dilation, inflammation, necrosis and fibrosis (Masson's Trichrome stain) with the elevation of serum creatinine, urea and TID area (%) in a time-dependent manner. The discrepancy of serum creatinine and urea possibly due to dehydration observed in Group 3 were ameliorated in Group 7. The observation of Group 3 and 7 showed that once 0.25%Ad stopped, renal dysfunction recovered and TID did not advance.

Conclusions: These datum showed that mice fed 0.25%Ad with short term exposure is one of the candidate of optimal mice models of tubulointerstitial damage and repair with renal dysfunction.

	Group 1	Group 3	Group 5	Group 7	P value
Creatinine(mg/dl)	0.21±0.03	0.3±0.03	0.47±0.05	0.24±0.02	<0.05
Urea(mg/dl)	40.7±3.1	71.0±6.3	100.2±9.9	34.3±2.3	<0.05
TID (%)	13.3±4.9	19.7±5.7	54.3±9.9	19.8±11.0	<0.05

Funding: Government Support - Non-U.S.

TH-PO110

Reduction of Renal Fibrosis and TGF-β Production by Late Treatment with a Spleen Tyrosine Kinase Inhibitor in Experimental Glomerulonephritis John P. McDaid,[1] Jennifer Smith,[1] Gurjeet Bhangal,[1] Mei-Ching Yu,[1] Stephen Paul McAdoo,[1] H. Terence Cook,[1] Charles D. Pusey,[1] Esteban S. Masuda,[2] Frederick W.K. Tam.[1] *[1]Kidney and Transplant Institute, Imperial College, London, United Kingdom; [2]Rigel Pharmaceuticals, Inc, San Francisco, CA.*

Background: Spleen tyrosine kinase (Syk) is important in antibody mediated inflammation. Treatment with fostamatinib, a selective Syk inhibitor, is effective in reducing inflammation in experimental glomerulonephritis (GN). However, the effect on fibrosis has not been investigated. The aim of this study is to investigate whether inhibition of Syk at the fibrotic stage will reduce renal fibrosis and glomerular synthesis of profibrotic factors.

Methods: Fostamatinib (40 mg/kg) or vehicle was given daily to Wistar Kyoto rats from day 14 to 28 after nephrotoxic serum. Renal injury was assessed by creatinine clearance

(CrCl), proteinuria and histology. Effect of Syk inhibition on TGF-β production was investigated by incubation of glomeruli *ex vivo* in the presence of R406 (active metabolite of fostamatinib) or control medium.

Results: On day 14, all 20 rats had GN with proteinuria. By day 28, rats treated with fostamatinib had better renal function (CrCl) and less glomerular α-smooth muscle actin (SMA), interstitial fibrosis, infiltrating macrophages and crescents (table) than those treated with vehicle only. One rat in the vehicle group died. Proteinuria was unaffected in treated animals. Incubation of nephritic glomeruli *ex vivo* with R406 reduced the glomerular production of TGF-β by 68 % ($p<0.01$).

	CrCl (ml/day)	Proteinuria (mg/day)	Interstitial Collagen (IU)	Glomerular αSMA (IU)	Glomerular Crescent (%)	Interstitial Mø (IU)	Glomerular Mø (GCS)
Vehicle (n=9)	0.33±0.07	165±18	1.72±0.2	0.26±0.02	99.6±3.5	1.05±0.12	3.4±0.45
Fostamatinib (n=10)	0.92±0.04***	175±7.6 ns	0.9±0.9 **	0.08±0.01***	84.4±3.1***	0.59±0.4*	0.7±0.25***

* p<0.05, **p<0.01, *** p<0.001, ns not significant; IU imaging analysis unit; Mø macrophage; GCS glomerular cross section

Conclusions: To our knowledge, this is the first report showing that Syk inhibition reduces glomerular TGF-β synthesis and renal fibrosis. Syk is a potential therapeutic target in prevention of antibody mediated renal fibrosis.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

TH-PO111

The Blockade of IL-17 Ameliorates the Hypertension and the Upregulation of Profibrotic and Proinflammatory Mediators in Kidney of DOCA-Salt Rats

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Background: Inadequate levels of aldosterone cause hypertension, inflammation and fibrosis in the kidney. Recent findings have implicated the Interleukin-17 (IL-17) pathway in hypertension, and we showed that aldosterone promotes autoimmune damage and Th17 inflammatory response in experimental Autoimmune Encephalomyelitis. We hypothesized that hypertension and the upregulation of profibrotic/proinflammatory mediators induced by DOCA-salt in the kidney are dependent on the increase of IL-17.

Results: First, we evaluated if in the DOCA-salt model the Th17 response is caused by high-blood pressure per-se. Uninephrectomized DOCA-salt rats (0.5mg/0.1kg + 0.9% NaCl/0.3% KCl in drinking water; n=4, 16 days) presented high systolic blood pressure (140 mmHg vs. vehicle 96 ± 1mmHg, $p<0.05$), increased renal abundance of IL-17 and of IL-23, a cytokine that maintains the IL-17 phenotype (IL-17; 11 fold vs. control, IL-23; 2 fold vs. control, $p<0.05$ mRNA/protein). Changes in blood pressure were prevented by spironolactone (SPIRO; 50mg/kg/d) and anti-hypertensive Triple Therapy (TT; 0.32mg/kg/d reserpine, 6.5mg/kg/d hydralazine, 4mg/kg/d hydrochlorothiazide). However, the increase of IL-17 and IL-23 caused by DOCA-salt was prevented only by SPIRO but not by TT (n=4, N.S.). In a second set of experiments, we studied if fibrotic/inflammatory damage in kidney of DOCA-salt rats is dependent on IL-17. We treated rats with DOCA-salt for 16 days, and then with Anti-IL-17antibody (100μg/day), control IgG (100μg/day) or only DOCA-salt for the last 12 days of treatment. Anti-IL-17 ameliorated hypertension (112 ± 3mmHg vs. DOCA-salt 138 ± 6mmHg or IgG groups $p<0.05$). Moreover, Anti-IL-17 reverted the upregulation of Collagen I, Monocyte chemoattractant protein-1, Osteopontin and NADPH oxidase.

Conclusions: Our results suggest that the mineralocorticoid receptor activation but not hypertension induces the Th17 response, and that IL-17 is implicated in hypertension and renal damage due to high-salt+mineralocorticoids in rats.

Funding: Government Support - Non-U.S.

TH-PO112

Loss of Extracellular Adenosine Induced by Knock Out of the CD73/Ecto-5'-nucleotidase in Mice Leads to Age Dependent Proteinuria

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Background: Adenosine is relevant for the normal physiology of the kidney and protective within ischemia and inflammation. We addressed the role of extracellular adenosine formed by the ectoenzyme CD73 on proteinuria and renal inflammation using CD73-deficient ($CD73^{-/-}$) mice and wild-type (WT) controls. The $CD73^{-/-}$mutant exhibits a lack of adenosine in renal tissue to appr. 70 % and a vascular proinflammatory phenotype.

Methods: Renal function and proteinuria were screened 4 weekly in young CD73-/-mice. At the onset of significant proteinuria as compared to WT mice, kidneys were harvested and analysed by electrone microscopy and immune fluorescence for inflammatory cells, IgG, C3, podocytes. Serum chemokines and cytokines were screened using bioplex protein assays.

Results: Already 13 weeks old $CD73^{-/-}$mice showed increased proteinuria (0.1 ± 0.13 vs. 0.04 ± 0.004 g/g creatinine in WT), increasing to 3-fold at 6 months (0.29 ± 0.03 vs. 0.05 ± 0.02 g g/g creatinine). Glomerulitis and peritubular capillaritis observed in $CD73^{-/-}$mice were associated to glomerular deposition of IgG, C3 and to the presence of CD11b, CD8, CD25 and GR-1-positive cells in the renal cortex. Serum total IgG and the cytokine IL-18 were up-regulated as compared to WT-mice. In $CD73^{-/-}$ mice kidneys, a reduced number of podocytes per glomerulus was associated to decreased expression of synaptopodin and nephrin. Ultrastructural analysis showed endotheliosis and reduced fenestration of the glomerular endothelium.

Conclusions: Our findings indicate that lack of extracellular adenosine formed by the 5´ectonucleotide nucleotidase CD73 age-dependently causes kidney injury with proteinuria ongoing with a loss of podocytes and a proinflammatory renal phenotype. Importantly, increased serum IL-18 votes for an autoimmune phenotype known to promote glomerulonephritis.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO113

Characterization of Pathogenic Macrophages and CCL2 in Diabetic Nephropathy

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Background: Recent evidence suggests macrophage-mediated inflammation and expression of the macrophage chemoattractant protein CCL2 in the glomeruli may be critical in the development of diabetic nephropathy. However, the phenotype of the macrophages infiltrating diabetic kidneys, the critical effector molecules they produce, and whether interaction of CCL2 with its major receptor, CCR2, affects this phenotype has not been determined. Therefore, a thorough understanding of the phenotype of kidney-infiltrating macrophages in the presence and absence of intact CCR2 signaling is a necessary first step to developing more targeted and safer therapies.

Methods: Using multi-color flow cytometry, we have characterized the surface phenotype of the macrophage populations present in whole kidneys, purified glomeruli, and peripheral blood in non-diabetic, wild-type (C57BL/6) mice. We are now comparing these populations to those present in the kidneys and peripheral blood of mice with diabetic nephropathy, with or without intact CCR2 signaling, using two models of the disease, namely streptozotocin-injected eNOS-/- or db/db mice.

Results: Compared with non-diabetic mice, albuminuric mice had increased macrophage numbers in both the kidneys and blood, with the major population expressing the surface markers CD11b, F4/80 and CD11c. This increase was inhibited by the administration of a CCR2 antagonist prior to albuminuria development, and this correlated with a decrease in disease severity in the db/db model.

Conclusions: These results suggest this macrophage population may be responsive to CCR2 signaling and involved in the pathogenesis of diabetic nephropathy. Studies are now underway to determine whether these macrophages are concentrated in the glomeruli of diabetic kidneys, the cytokines these cells produce that may have nephrotic potential, and the effect of CCR2 inhibition on cytokine production.

Funding: Pharmaceutical Company Support

TH-PO114

Adrenocortocortropin (ACTH) Gel Suppresses Renal Tubulointerstitial Inflammation and Injury by Direct Stimulation of the Melanocortin 1 Receptor (MC1R)

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Background: ACTH is a pituitary neuro-immuno-endocrine hormone and a prototype agonist of the melanocortin system. Clinical and experimental evidence demonstrates that ACTH has beneficial actions in chronic kidney disease; however, the mechanism(s) remain uncertain. This study examined the effect of ACTH gel (Acthar Gel, Questcor Pharmaceuticals, Inc.) on progressive renal tubulointerstitial injury.

Methods: Following unilateral ureteral obstruction (UUO) surgery with or without simultaneous adrenalectomy, rats were administrated ACTH gel (10 IU/kg) or saline (CON) every other day for 2 weeks. Renal histology was assessed. Expression of cognate receptors of ACTH was evaluated *in vivo* and in cultured murine renal tubular epithelial cells (TEC).

Results: Compared to CON, ACTH gel prevented enlargement of the obstructed kidney as assessed by the increase in kidney to body weight ratio (1.13±0.14 *vs* 0.67±0.09 g/100g *wt*, $P=0.02$). Morphologically, renal interstitial fibrosis and tubular atrophy were significantly ameliorated by ACTH gel. In addition, renal inflammation, marked by ED-1 positive macrophage infiltration, and UUO induced expression of chemokines MCP-1 and RANTES in tubules, were also attenuated by ACTH gel treatment. Although somewhat diminished, the beneficial effects of ACTH gel were still evident in UUO rats that underwent adrenalectomy, suggesting a steroid independent mechanism. Consistently, abundant expression of MC1R was observed in renal tubules of rat kidneys and in cultured TEC. *In vitro*, ACTH gel markedly suppressed TNF-α induced proinflammatory events in cultured TEC, including NFκB activation and downstream target gene expression. This effect was significantly diminished in TEC in which MC1R expression was silenced by RNAi, demonstrating that ACTH gel induced anti-inflammatory signaling in TEC requires MC1R.

Conclusions: ACTH gel markedly suppresses tubulointerstitial inflammation, tubular atrophy and fibrosis in progressive chronic kidney disease due, at least in part, to direct, anti-inflammatory effects on TEC. By silencing MC1R expression for the first time in kidney cells, we have also shown that these effects are mediated *via* the MC1R.

Funding: Pharmaceutical Company Support

TH-PO115

Effects of Rho Kinase Inhibitor, Fasudil on Spontaneously Hypercholesterolemic Rats Taketoshi Kushiyama,[1] Takashi Oda,[1] Takahiro Uchida,[1] Atsushi Watanabe,[1] Kojiro Yamamoto,[1] Keishi Higashi,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Soichiro Miura,[1] Hiroo Kumagai.[1] *[1]Nephrology, National Defense Medical Collage, Tokorozawa, Saitama, Japan; [2]Preventive Medicine and Public Health, National Defense Medical Collage, Tokorozawa, Saitama, Japan.*

Background: Recent reports indicate the renoprotective effects of rho kinase inhibitor, fasudil by the inhibition of macrophage infiltration. Therefore we examine the long term therapeutic effects of fasudil on the spontaneously hypercholesterolemic (SHC) rat, paying special attention to the phenotypic changes in macrophages.

Methods: Eight-week-old male SHC rats were randomly assigned to 6 groups (n=7 each): 1) the vehcle-treated group (Ve), 2) the low dose fasudil-treated group (f30, 30mg/kg/day), 3) the high dose fasudil-treated group (f100, 100mg/kg/day), 4) the OL-treated group (OL, 5mg/kg/day), 5) the combination of low dose of fasudil and OL treated group (c30), 6) the combination of high dose of fasudil and OL treated group (c100). As a healthy control group, SD rats were treated with vehicle alone (n=7). Rats were killed after treating for 24 weeks.

Results: Urinary protein excretion in f100, OL and c30 groups was significantly less than that in Ve group. Surprisingly urinary protein excretion level in c100 decreased to the same level as control group. The total collagen content was significantly decreased in the c100 group compared with Ve group. Prominent interstitial ED-1 positive cells observed in the Ve group were significantly attenuated in the all treatment groups. The CD80 and CCL3 (M1 macrophage marker) mRNA level relative to the GAPDH mRNA level was significantly decreased in c100 group. The relative expressions of CD206 (M2 macrophages marker), as assessed by the ratio of the CD206 mRNA to CD68 mRNA levels, was significantly greater in c100 than other treatment groups.

Conclusions: Fasudil showed significant beneficial effects on the interstitial fibrosis of SHC rats, the mechanism of which may be associated with antiproteinuric effects and increment in M2 macrophages.

TH-PO116

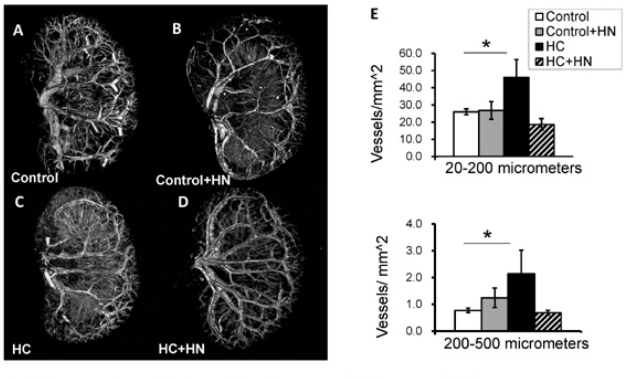
Humanin Prevents Intra-Renal Microvascular Remodeling, Inflammation, and Fibrosis in ApoE Knockout Mice Xin Zhang,[1] Victor Urbieta Caceres,[1] Caitlin C. Bell,[1] John A. Crane,[1] Hui Tang,[1] Kyra L. Jordan,[1] Yun Kyu Oh,[2] Pinchas Cohen,[3] Amir Lerman,[2] Lilach O. Lerman.[1] *[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Division of Cardiovascular Disease, Mayo Clinic, Rochester, MN; [3]Department of Pediatrics, UCLA, Los Angeles, CA.*

Background: Atherosclerosis promotes chronic kidney disease. Humanin (HN) is an endogenous mitochondria-derived cytoprotective peptide, but its role in atherosclerosis-induced kidney damage is unknown.

Methods: Ten C57BL/6 and 12 ApoE KO mice were fed 16 wks of normal or high cholesterol (HC) diet, respectively, supplemented daily with Intraperitoneal Injection of saline (Control, n=4, HC, n=6) or +HN (4 mg/kg/d, Control+HN, n=6, HC+HN, n=6). Microvascular remodeling was assessed ex-vivo with micro-CT and alpha-SMA staining. Angiogenesis, inflammation and apoptosis were evaluated in kidney tissue by Western-blots, and fibrosis by trichrome staining.

Results: ApoE KO mice had elevated serum cholesterol but normal creatinine levels. Cortical microvascular spatial density and media/lumen ratio were significantly increased in HC and restored in HC+HN.

Figure1



A-D Representative 3-dimensional tomographic images of kidney microvasculature; E HN decreased the spatial density of both small (20-200μm) and large (200-500μm) cortical microvessels that proliferated in ApoE KO HC kidneys. * p<0.05 vs. normal.

VEGF (P<0.05) and angiopoietin-1 (P=0.07) expression increased in HC+HN, but HN also significantly upregulated anti-angiogenic angiostatin and thrombospondin-1. HN downregulated the expression of inflammatory cytokines MCP-1 and TNF-alpha in HC+HN. Furthermore, pSTAT3 significantly declined in HC, and HN restored pSTAT3 and attenuated Bax in HC+HN, suggesting blunted apoptosis, and HN blunted a trend for renal fibrosis observed in HC.

Conclusions: HN attenuates murine HC-induced renal microvascular proliferation and remodeling by upregulating anti-angiogenic factors, and mitigates inflammation, apoptosis, and fibrosis. These findings suggest HN as a novel therapeutic target in atherosclerosis.

Funding: Private Foundation Support

TH-PO117

Role of mTOR Signaling in Interstitial Inflammation and Kidney Fibrosis Guochun Chen,[1] Lin Sun,[1,2] You-Ming Peng,[1] Hong Liu,[1] Guanghui Ling,[1] Li Xiao,[1] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]Departments of Pathology and Medicine, Northwestern University, Chicago, IL.*

Background: Progressive inflammation and fibrosis are responsible for chronic kidney failure, however, the molecular mechanism remains obscure. Recent studies have highlighted the mammalian target of rapamycin (mTOR) as a regulator of inflammatory reaction and collagen expression. In this study, we revealed a role of mTOR signaling in the progression of interstitial infiltration and fibrosis after kidney injuries.

Methods: Ischemic-reperfusion injury (IRI) or unilateral ureteral obstruction (UUO) mouse models were used in the experiment. Daily administration of Rapamycin was performed to inhibit mTOR signaling in designated groups. Immunohistochemical staining and western blot were performed to evaluate the activation of mTOR signaling. Expression of inflammatory chemokines was analyzed by realtime PCR.

Results: mTOR signaling was significantly up-regulated 24-48 hours post-injuries in rodent models of either IRI or UUO. Confocal imaging confirmed that pS6K, a downstream target of mTOR signaling, was expressed in de-differentiated epithelial cells, myofibroblasts and macrophages in mouse models of severe IRI and UUO. Up-regulation of SMA, vimentin and collagen-I correlated well with the expression of pS6K in fibrotic kidneys. Interestingly, although high expression of pS6K was observed in macrophages, little could be detected in migrated T cells or neutrophils, which suggested macrophages may be primary effectors of mTOR signaling that triggered the inflammatory cascade at the onset of kidney fibrosis. Administration of rapamycin, a specific mTOR complex inhibitor, significantly reduced renal fibrotic production (SMA and collagen I) and interstitial inflammation in a mouse UUO model, including chemokines production (MCP-1,TNF-a,IL-1beta and CXCL-1) and cells infiltration.

Conclusions: Taken together, our data suggest that activation of mTOR signaling mediates persistent tubulointerstitial inflammation and fibrosis after kidney injuries. Inhibition of mTOR signaling may be potential of anti-fibrosis in chronic kidney diseases.

Funding: Government Support - Non-U.S.

TH-PO118

Ischemic Preconditioning Lowers Renal Reperfusion Injury and Inflammatory Stress Leonella Luzardo,[2] Elia Ripoll,[1] Marcel.la Franquesa,[1] Liliana Gadola,[2] Nuria Lloberas,[1] Inmaculada Herrero,[1] Ana Panuncio,[2] Josep M.B. Grinyo,[1] Joan Torras.[1] *[1]Laboratory of Experimental Nephrology, IDIBELL. Hospital Universitari de Bellvitge, Spain; [2]Departamento de Fisiopatología, Hospital de Clínicas, Hospital de Clínicas Facultad de Medicina, Montevideo, Uruguay.*

Background: Acute kidney injury is associated with major in-hospital morbidity and mortality. In addition to ischemia, inflammation linked to reperfusion injury amplifies tissue damage. The aim of the study was to evaluate the effect of ischaemic preconditioning (IP) on a kidney ischemic-reperfusion injury model.

Methods: Male Wistar rats were randomly distributed in four groups (n=8 each). 1) SHAM; 2)ISCHEMIA, both renal pedicles occluded for 40 minutes; 3)IP-10 ten minutes of ischaemic preconditioning, 10 minutes of reperfusion and 40 minutes of ischemia; 4)IP-15 fifteen minutes of ischaemic preconditioning, 10 minutes of reperfusion and 40 minutes of ischemia. Under tiopental i/p, laparotomy was performed and renal pedicles were occluded using Aescula Braun clamps. Ischemia and reperfusion were confirmed visually. A blood sample was obtained (tail vein) prior to surgery and 24 hs later. 48 hs after procedure, animals urine and blood samples were taken. Left kidney tissue sections were obtained for immune-histochemistry using anti-nitrotyrosine antibody. On right kidney tissue we measured CD40, IL6, IL10, TNF-α, TGF-β, TLR 3 and TLR 4 by RT-PCR. Values are reported as mean ± SD and statistical analysis was performed by ANOVA test.

Results: IP vs ISCHEMIA groups had significantly lower plasma urea levels at 48 hs (37.6 vs 112.5; P<0,0002) and creatinine levels at 24 hs (0.76 vs 2.06; P=0.004) and at 48 hs (0.57 vs 1.17; P=0.002). PI groups showed lower expression of pro-inflammatory molecules CD40, TNF-α and TLR3 (P<0.05) but not TLR4 (P=0.06). IL6, IL10 and TGF-β expression was no significantly different. IP groups showed less anti-nitrotyrosine staining than ISCHEMIA group (IP-10: 0.29±0.19; IP-15: 0.52±0.14; ISCHEMIA: 1±0.13; P=<.0001)

Conclusions: Ischaemic preconditioning, either 10 or 15 minutes, showed a protective effect from a further prolonged ischemic injury. This could be explained, at least in part, due to a lower inflammatory stress.

TH-PO119

Activation of Type II NKT Cells Mediates Kidney Ischemia/Reperfusion Injury Li Li,[1] Liping Huang,[1] Steven P. Song,[1] Hong Ye,[1] Diane L. Rosin,[2] Mark D. Okusa.[1] *[1]Division of Nephrology, Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia, Charlottesville, VA; [2]Department of Pharmacology, University of Virginia, Charlottesville, VA.*

Background: CD1d-restricted type I and type II natural killer T (NKT) cells bridge innate and adaptive immune responses in kidney ischemia-reperfusion injury (IRI). Although type II NKT cells have not been as extensively studied as type I NKT cells, these cells have been shown to play a critical role in cancer and autoimmune diseases. We hypothesized that type II NKT cell activation is also involved in the immune response of kidney IRI.

Methods: We subjected mice to bilateral pedical clamp for 24 min followed by 24 hrs reperfusion, and plasma were collected for creatinine measurement.

Results: Compared to WT mice, CD1dKO mice (both type I and type II NKT cell deficient) were markedly protected, while Ja18KO mice (type I NKT cell deficient) showed an intermediate degree of protection ($P<0.05$). Plasma creatinine was (mg/dl): 1.38±0.19, 0.73±0.17 and 0.29±0.02, respectively. An optimal dose of sulfatide (1 μg/g) significantly and specifically activates type II NKT cells and promoted mild kidney IRI; plasma creatinine was 1.11± 0.10 vs. 0.47±0.04, respectively ($P<0.01$). IL-12(p35/p40) and IL-23 pathways are required for sulfatide-induced inflammation in kidney IRI as a protective effect was shown in sulfatide treated IL-12p35KO, IL-12p40KO and IL-23p19KO mice ($P<0.01$). IL-17AKO and IL-17RKO mice protected kidneys from sulfatide-mediated kidney injury in IRI ($P<0.01$), while IFN-γKO mice did not ($P>0.05$). Taken together with our prior studies, this indicates that IL-17A/R and IFN-γ contribute differentially to type I and type II NKT cell activation in kidney IRI. Co-stimulatory molecules CD40 and OX40L are required to mediate type II NKT cell activation, as CD40KO mice or mice injected with anti-OX40L mAb were resistant to sulfatide-induced kidney inflammation compared with WT mice (P all < 0.01).

Conclusions: Understanding the pathogenic role of NKT cells in the initiation of the immune response will provide new information for designing novel therapeutic strategies for AKI.

Funding: NIDDK Support, Other NIH Support - Genzyme (Genzyme Renal Innovations Program), American Heart Associate National Scientist Development Grant 0835258N

TH-PO120

Mannose-Binding Lectin Mediates Renal Ischemia Reperfusion Injury Independent of Complement Activation Cees van Kooten,[1] Pieter van der Pol,[1] Nicole Schlagwein,[1] Danielle Van Gijlswijk,[1] Stefan P. Berger,[1] Ingeborg M. Bajema,[2] Gregory L. Stahl,[3] Johan W. De Fijter,[1] Mohamed R. Daha.[1] *[1]Nephrology, LUMC, Leiden, Netherlands; [2]Pathology, LUMC, Leiden, Netherlands; [3]Center for Experimental Therapeutics, Harvard Instirute of Medicine, Boston.*

Background: Ischemia/reperfusion injury (IRI) is an inevitable event in kidney transplantation and has a major impact on short- and long-term graft survival. Complement activation, a central component of innate immunity, is one of the hallmarks of renal IRI. Genetically-determined low serum levels of Mannose Binding Lectin (MBL) have been associated with improved renal allograft survival.

Methods: We used an experimental rat model of IRI by 45 minute ischemia of uni-nephrectomized Lewis rats and investigated the role of MBL-mediated complement activation using blocking antibodies against MBL or C5, followed by functional and histological analysis. In vitro cultured human proximal tubular epithelial cells (PTEC) were used to investigate direct effects of MBL on tubular integrity

Results: Transient inhibition of MBL completely preserves renal function and tubular integrity and prevents influx of neutrophils and macrophages following experimental renal IRI in rats. Although complement deposition (C3 and C5b-9) is observed from 24 hours onwards in non-treated rats, inhibition of the down stream complement mediators C3 and C5 was not protective. Histological signs of tubular injury were already observed within hours after reperfusion, accompanied by vascular leakage of MBL into the interstitium, reaching tubular epithelial cells in ischemic but not control kidneys. Exposure of in vitro cultured human PTEC to purified MBL resulted in specific binding and internalization of MBL, followed by a dose- and time-dependent induction of cell death.

Conclusions: In conclusion, we demonstrate a novel role for MBL in the pathophysiology of IRI, independent of its capacity to activate the lectin pathway of complement. As a consequence of vascular leakage, exposure of renal tubular cells to MBL results in a rapid induction of tubular cell death, one of the earliest events in IRI, making MBL an interesting target for therapy.

Funding: Government Support - Non-U.S.

TH-PO121

The Role of Toll-Like Receptors Adaptor Protein MyD88 and Activation of Nrf2 in Ischemic/Hypoxic Injury of the Kidney Min Li, Altaf-M Khan, Eric E. Simon, Vecihi Batuman. *Department of Medicine/Nephrology, Tulane University School of Medicine, New Orleans, LA.*

Background: Inflammation is a critical component of innate immunity that is triggered by Toll-like receptors through activation of its adaptor protein MyD88 in acute kidney injury (AKI). We previously demonstrated the pivotal role of MyD88-dependent mechanisms in the coordination of innate immune responses to ischemic/hypoxic acute renal tubular injury.

Methods: In the present study, we found that the exposure of human proximal tubule epithelial cells (HK-2) to hypoxia and wild-type mice kidneys to ischemia activated NF-E2-related factor 2 (Nrf2), a basic leucine zipper transcription factor that regulates inflammation, leading to expression of Nrf2-regulated genes including heme oxygenase-1 (HO-1).

Results: Suppression of MyD88 by specific shRNA transfection in HK-2 cells and MyD88-deficient murine kidneys elevated the base level of Nrf2 expression. The activation of Nrf2 and tissue production of HO-1 was significantly increased in MyD88 knockdown cells subjected to hypoxia in vitro and in MyD88-/- mice after ischemia/reperfusion in vivo. However, compared with the normal HK-2 cells and sham operated mice, the production of IL-6 and MCP-1 was still increased but was markedly lowered in MyD88 knockdown cells and in MyD88-/- mice subjected to ischemic/hypoxic injury. By using pharmacological and genetic analyses, we found that activation of Nrf2 in response to hypoxia/ischemia is dependent on MyD88 for the production of pro-inflammatory cytokines.

Conclusions: Our results show that MyD88 dependent signaling tempers activation of Nrf2 in response to ischemic/hypoxic injury resulting in exaggerated injury. In contrast, blocking MyD88 is associated with more brisk Nrf2 response leading to higher levels of HO-1, reduced levels of IL-6 and MCP, and better preservation of kidney after ischemia/reperfusion injury.

Funding: Other U.S. Government Support

TH-PO122

Acute Kidney Injury in Cisplatin Nephrotoxicity Is Dependant on Mast Cell Degranulation and TNF Release Shaun A. Summers,[1] Poh-Yi Gan,[2] David J. Nikolic-Paterson,[1] Oliver M. Steinmetz,[2] A. Richard Kitching,[1] Stephen R. Holdsworth.[1] *[1]Department of Medicine and Nephrology, MMC and Monash University; [2]Department of Medicine, Monash University.*

Background: Mast Cells (MCs) are pluripotent innate immune cells, which contain a large number of vasoactive and inflammatory molecules, which are released after degranulation. Previously we demonstrated that compared to C57BL/6 wild type (WT) mice, mast cell deficient, KitWsh/Wsh mice, are protected from cisplatin nephrotoxicity (CN) . In these studies we sought to define the pathophysiological role of MCs in CN.

Methods: Initially we assessed the role of MC degranulation after cisplatin treatment. To determine the role of MCs in AKI we administered cisplatin to WT and KitWsh/Wsh mice. We assessed kidney TNF mRNA expression and serum TNF production as well as pro-inflammatory chemokines. Subsequently we reconstituted KitWsh/Wsh mice with MCs from WT and TNF-/- mice, and then administered cisplatin. Renal injury and leukocyte recruitment was assessed 4 days later.

Results: Administration of cisplatin resulted in an increase in serum (control 33.4±2.7 vs. cisplatin treated 53.2±7.1 ng/ml tryptase, $P<0.05$) and kidney (control 1.7±0.7 vs. cisplatin treated 7.6±1.5 ng/ml tryptase , $P<0.01$) MC degranulation. Serum TNF levels (84.4±7.4 vs. 60.5±4.9pg/ml) were lower in KitWsh/Wsh mice. Intrarenal TNF mRNA expression, as well as mRNA for key T cell (RANTES/CCL5, IP-10/CXCL10), and neutrophil chemokines (KC/CXCL1 and MIP2/CXCL2) were also decreased in KitWsh/Wsh mice. To define the mechanism of MC-mediated AKI, KitWsh/Wsh mice, reconstituted with MCs derived from WT or TNF-/- mice, were given cisplatin. Compared to KitWsh/Wsh mice reconstituted with WT MCs, renal injury was attenuated in KitWsh/Wsh mice reconstituted with TNF-/- MCs (BUN 100.3±16.2 vs. 41.6±15.9mmol/l, $P<0.01$; histological injury assessed semiquantatively 3.3±01 vs. 2.2±0.2, $P<0.001$). Compared to mice reconstituted with WT MCs there was a decrease in serum TNF production (WT MCs 104.8±5.7 vs. TNF-/- MCs 50.9±8.9 pg/ml, $P<0.001$) and kidney neutrophil recruitment.

Conclusions: Acute kidney injury induced by cisplatin is dependent on MC degranulation and TNF release.

Funding: Government Support - Non-U.S.

TH-PO123

Autoantibodies to LAMP-2 in ANCA Negative Pauci-Immune Focal Necrotizing Glomerulonephritis (piFNGN) Andrew J. Rees, Renate Kain. *Clinical Department of Pathology, Medical University of Vienna, Vienna, Austria.*

Background: piFNGN occurs characteristically in people with ANCA-associated vasculitis. However, ANCA assays are negative in up to 10% of patients; antibodies to MPO and PR3 cannot be detected thus leaving the cause of injury obscure. We reported a high prevalence of antibodies to LAMP-2 in patients with active piFNGN (including one ANCA negative individual) and showed they could induce piFNGN in rats. The purpose of this study was to ascertain the frequency of antibodies to LAMP-2 in ANCA negative piFNGN more generally and to determine why the antibodies concerned fail to bind to LAMP-2 in neutrophils by indirect immunofluorescence.

Methods: Eleven ANCA negative patients were studied (10 with isolated piFNGN and 1 with renal, lung and skin involvement). We confirmed standard fluorescent ANCA assays and ELISA for MPO and PR3 were negative in all 11. All 8 patients' autoantibodies also bound glycosylated hLAMP targeted to the cell surface and expressed in CHO cells. Neutrophil LAMP-2 has a unique glycosylation pattern with complex poly-lactosamine chains and we confirmed that this carbohydrate moiety resulted in the contrasting binding to human LAMP-2 in neutrophils and CHO cells.

Results: We showed that: (i) removal of poly-lactosamines with endo-β-galactosidase from neutrophil LAMP-2 decreased its molecular mass to that of LAMP-2 purified from glomeruli or expressed in CHO cells; and (ii) ANCA assays became positive for all 8 patients when the ANCA slides were pre-treated with this enzyme. This treatment had no effect in control slides. Lastly, we confirmed immunoblot that antibodies to LAMP-2 in ANCA negative patients bind native LAMP-2 purified from glomeruli.

Conclusions: In summary, we demonstrated that some ANCA negative patients with piFNGN have antibodies to LAMP-2 that bind glomerular LAMP-2 but not that of neutrophils. We suggest that these autoantibodies contribute to pathogenesis in the absence of canonical ANCA.

By contrast, 8 of the 11 (73%) had circulating autoantibodies that bound to unglycosylated human recombinant LAMP-2 extracellular domain in our standard ELISA and by immunoblot.

Funding: Government Support - Non-U.S.

TH-PO124

Expression of the PTPN22 Gain-of-Function Variant Results in ERK Inhibition Leading to Down-Regulation of IL-10 Expression Yali Cao,[1,2] Jia Jin Yang,[1] Susan L. Hogan,[1] Yichun Hu,[1] Caroline E. Jennette,[1] Elisabeth Berg,[1] J. Charles Jennette,[1] Ronald J. Falk,[1] Gloria A. Preston.[1] *[1]UNC Kidney Center, UNC-CH, Chapel Hill, NC; [2]Nephrology, China-Japan Friendship Hospital, Beijing, China.*

Background: We predicted that cellular signaling dynamics would be disrupted by expression of the disease-associated allele of the protein tyrosine phosphatase PTPN22 (R620W), conferring a gain-of-function phenotype. This change negatively affects SRC activation due to loss of CSK sequestration. Signaling through RAS is also affected through GRB2-PTPN22 (R620W) interactions.

Methods: Phosphatase activity was measured in leukocytes from patients with anti-neutrophil cytoplasmic autoantibody (ANCA) disease, comparing patients with the gain-of function allele to patients with the normal allele. PTPN22 protein was captured using an anti-PTPN22 antibody on two separate microtiter plates. One was analyzed for total protein captured and the other for activity status of the captured protein, reported as an activity/protein ratio. Activation of downstream signaling pathways was determined by western blot analysis. IL-10 transcription was quantitated by TaqMan PCR.

Results: Basal activity of PTPN22(R620W) variant was persistently elevated in leukocytes (p<0.0001), neutrophils (p=0.0004) and lymphocytes (p=0.0003), while undetectable in leukocytes expressing normal PTPN22. Inappropriate activity of the gain-of-function phosphatase resulted in downregulation of ERK, opposite to controls. Instead, p38 MAPK was up-regulated. *IL-10* transcription, which is reliant on the ERK pathway, was negatively affected by expression of the variant with reduced levels compared to controls (n=26, p<0.0001). Over the course of disease, patients expressing variant PTPN22(R620W) did not show a spike in IL-10 transcription as they entered remission in contrast to controls, implying that environmentally triggered signals were blunted (p<0.0001).

Conclusions: Sustained activity of PTPN22, due to the gain-of-function mutation, acts as a dominant negative regulator of ERK activity leading to blunted cellular responsiveness to environmental stimuli and expression of protective cytokines.

Funding: NIDDK Support

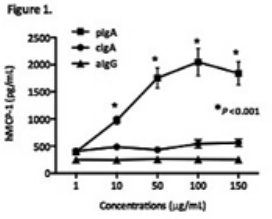
TH-PO125

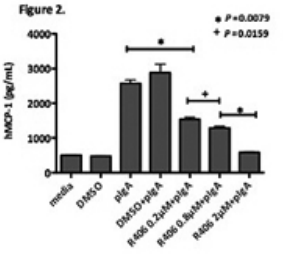
Spleen Tyrosine Kinase Is Involved in the Production of Pro-Inflammatory Cytokines by Human Mesangial Cells Following Stimulation with Polymeric IgA1 Isolated from IgA Nephropathy Patients Min Jeong Kim,[1] Jonathan Barratt,[2] Karen Molyneux,[2] Esteban S. Masuda,[3] Charles D. Pusey,[1] Frederick W.K. Tam.[1] *[1]Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Dept. of Infection, Immunity & Inflammation, Univ. of Leicester, United Kingdom; [3]Rigel Pharmaceuticals, South San Francisco.*

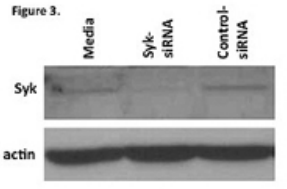
Background: Polymeric IgA1 (pIgA) isolated from the serum of IgAN patients stimulates human mesangial cells (HMC) to produce pro-inflammatory cytokines. The aim of our study was to determine if spleen tyrosine kinase (Syk) is involved in HMC in the downstream signaling pathway of IgA receptors, leading to the production of pro-inflammatory cytokines.

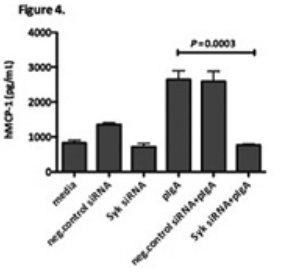
Methods: HMC were incubated with pIgA, control IgA from healthy human (cIgA), or heat aggregated IgG (aIgG). We measured MCP-1 by ELISA and additional 26 cytokines by multiplex cytokine assay from the culture supernatant after 24h. We then incubated HMC with a Syk inhibitor, R406, 1h before stimulation with pIgA (50μg/mL) and performed real-time RT-PCR and ELISA. HMC were then transfected with Syk siRNA or negative control siRNA, 72h before stimulation with pIgA. Transfection efficiency was proved by Western blot.

Results: MCP-1 was significantly higher upon stimulation with pIgA than cIgA or aIgG in a dose dependent manner, and this was inhibited significantly by R406 (Fig1,2). The same was true for other cytokines (IL-6, IL-8, IL-9, IP-10 and RANTES). The MCP-1 mRNA was also reduced significantly. The Syk expression was effectively suppressed and MCP-1 protein level after stimulation with pIgA was significantly reduced upon transfection with Syk siRNA (Fig3,4).









Conclusions: Our data strongly suggest the involvement of Syk in HMC in the production of pro-inflammatory cytokines upon stimulation with pIgA, and its role in the pathogenesis of IgAN. Syk may be considered as a potential target in the treatment of IgAN.

TH-PO126

HIV Induces Activation of Renin Angiotensin System in Lymphocytes through Downregulation of Vitamin D Receptor Nirupama Chandel, Hersh Goel, Shabina Rehman, Ashwani Malhotra, Mohammad Husain, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra medical School, Great Neck, NY.*

Background: Interstitial lymphocytes aggregation is frequently associated with renal fibrosis. Activation of the renin-angiotensin system (RAS) has been demonstrated to play a key role in the progression of HIV-associated nephropathy (HIVAN). Interstitial lymphocytes serve as a source for profibrotic cytokines and vasoactive agents such as Ang II. Vitamin D receptor (VDR) has been reported to be a negatively regulator of renin transcription. In the present study, we evaluated the effect of HIV infection on lymphocyte VDR expression and activation of the RAS.

Methods: Lymphocytes (LY) were isolated from human blood obtained from the New York Blood Bank (n=5). LYs were incubated with X4 virus or buffer for two hours and then washed and re-incubated in media for 24 hours. RNA and proteins were extracted from lymphocytes. Immunoblotting and real time PCR studies were performed for expression of VDR, angiotensinogen (Agt) and renin. Ang II ELISA was carried out on lymphocytes prepared under similar conditions. To establish a causal relationship between VDR and the RAS activation, lymphocytes with or without silenced VDR (siRNA-VDR/LY) were evaluated for the expression of Agt and renin and production of Ang II. To confirm the relationship between HIV and VDR, LYs and HIV/LY were treated with Vitamin D2 analogue for 24 hours; subsequently, VDR, renin, and Agt expression was determined by immunoelectrophoresis.

Results: HIV/LY showed attenuated (P<0.01) expression of VDR but displayed enhanced expression of Agt and renin (P<0.01) . Moreover, HIV/LY showed 2.5 fold increased Ang II production (P<0.01) when compared to control lymphocytes. On the the other hand, Vit D treated HIV/LY not only showed upregulation of VDR but also displayed attenuated expression of renin and diminished (P<0.05) production of Ang II.

Conclusions: These findings indicate that HIV enhances lymphocyte RAS activation through VDR downregulation. The present study provides mechanistic insight into the role of lymphocytes in the activation of the RAS in HIVAN.

Funding: NIDDK Support

TH-PO127

Angiotensin II Stimulates S100A12 Production in Macrophages Eiko Matsuoka,[1] Yasukiyo Mori,[1] Yayoi Shiotsu,[1] Atsushi Kosaki.[2] *[1]Department of Cardiology and Nephrology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Department of Medicine II, Kansai Medical University, Osaka, Japan.*

Background: S100A12, formerly called EN-RAGE, is an endogenous ligand for the receptor for advanced glycation end products (RAGE). Our working hypothesis is that S100A12 protein might contribute to the development of atherosclerosis. We recently found that plasma S100A12 level was an independent factor associated with the prevalence of cardiovascular disease in hemodialysis patient (Shiotsu Y and Mori Y et al. Clin J Am Soc Nephrol 2011; 6: 718-723). S100A12 is reported to be abundantly expressed and secreted from neutrophils and monocytes/macrophages in human. However, the detail of regulatory mechanism of S100A12, especially in chronic kidney disease, remains unknown. In this study, we evaluate the effect of Angiotensin II (Ang II) on S100A12 transgenic mice (S100A12tg).

Methods: We exploited the fact that S100A12 is not present in mice and generated the mice (C57BL6/J) expressing human S100A12 in myeloid cells under the control of CD11b promoter. RT-PCR using the specific primer for human S100A12 and ELISA for human S100A12 protein, which was developed by our laboratory, were used to evaluate the S100A12 transcripts and its protein. Macrophages were collected from peritoneal cavities of S100A12tg. Because we found the expression of S100A12 in HepG2, the cell line from human hepatic carcinoma, in the preliminary experiment, HepG2 was used as a reference.

Results: In HepG2, Ang II increased the level of S100A12 mRNA in a time-dependent(8h: 1.2-fold, 16h: 1.6-fold). The stimulating effects were abolished by pretreatment with Olmesartan, Ang II type 1 receptor blocker. The effect of AngII on S100A12 was greater in peritoneal macrophages from S100A12tg. Ang II increased the level of S100A12 transcripts by 6-fold. Although the peritoneal macrophages from wild type mice had no transcripts as well as protein of S100A12, S100A12 proteins in culture medium of peritoneal macrophages from S100A12tg was detectable (1.44ng/mL) after the treatment with Ang II.

Conclusions: These findings suggest that Ang II stimulates S100A12 production in macrophages.

TH-PO128

Effects of Uremic Toxins on the Cross-Talk between Leukocytes and the Vessel Wall in an In Vivo Rat Model Anneleen Pletinck, Griet L.R.L. Glorieux, Eva Schepers, Wim Van Biesen, Raymond C. Vanholder. *Internal Medicine, Nephrology, University Hospital Ghent, Ghent, Belgium.*

Background: The major cause of death in CKD-patients is cardiovascular disease. Accumulation of uremic toxins is partly involved in this condition. *In vitro* models showed that p-cresylsulfate (pCS) and indoxylsulfate (IS) have an effect on leukocyte activation and/or vascular integrity. These models however miss the complicated cross-talk between different cell systems as present *in vivo*. This study evaluated the effects induced by acute exposure to uremic toxins on the recruitment of circulating leukocytes in the rat peritoneal vascular bed, using an intravital microscopic model.

Methods: The peritoneum of rats was exposed to HBSS or HBSS containing an uremic toxin at a concentration relevant in uremia. Leukocyte-endothelial interactions, bloodflow rate and vessel diameter were analyzed in postcapillary venules during 120 min.

Results: In the control condition,no effect on the number of rolling and adhering leukocytes was observed. The number of extravasated leukocytes increased moderately, but significantly after 20 minutes. Exposure to pCS significantly increased the number of rolling leukocytes already after 2 min ($p<0.05$). Adhering was significantly increased at 120 min ($p=0.011$). The number of extravasated leukocytes was significantly increased from 20 min on ($p<0.05$),which became significantly higher compared to the control group after 30 and 60 min. Exposure to IS only caused a strong and fast extravasation of leukocytes after 10 min of exposure ($p<0.05$). This became significantly higher compared to the controls after 30 min ($p<0.05$). In 5/7 rats, an interruption of blood flow was observed after ± 30-60 min exposure to IS. Vasoconstrictive properties of IS were tested with a wire myograph, but results were negative.

Conclusions: These results provide clear evidence that pCS and IS exert a proinflammatory effect that could contribute to the propensity for vascular disease in uremic patients. Tissue factor will be investigated to reveal a possible role of the coagulation-cascade in the interrupted blood flow, observed after IS-exposure. Other toxins (SDMA,ADMA,p-cresylglucuronide) are currently being analyzed.

TH-PO129

Aberrantly Glycosylated IgA1 Diminishes Adiponectin Expression in Glomerular Mesangial Cells *In Vitro* and *In Vivo*: A Novel Anti-Inflammatory Mechanism in IgA Nephropathy Hitoshi Sugiyama, Tatsuyuki Inoue, Masashi Kitagawa, Keiichi Takiue, Hiroshi Morinaga, Yoko Kikumoto, Ayu Ogawa, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Okayama University Graduate School, Okayama, Japan.*

Background: The pathogenesis of IgA nephropathy (IgAN) may be associated with the mesangial deposition of aberrantly glycosylated IgA1. However, the effect of desialylated and degalactosylated (deSial/deGal) IgA1 on human mesangial cells (HMCs) *in vitro* and *in vivo* still remains unknown.

Methods: Enzymatically modified deSial/deGal IgA1was generated to identify mediators affected by aberrantly glycosylated IgA1 in cultured HMCs. The presence of deglycosylated IgA1 was confirmed by lectin binding to *Helix aspersa* (HAA) and *Sambucus nigra agglutinin* (SNA). The deposition of deglycosylated IgA1 was investigated with dual staining of HAA and IgA in human renal biopsy specimens from patients (n=53) including IgAN and lupus nephritis. The urinary HAA/SNA ratio was determined in spot urine samples from patients with IgAN (n=78) and other kidney diseases (n=142).

Results: A cytokine array analysis revealed that 52 proteins were upregulated and 34 were downregulated in cultured HMCs after stimulation with deSial/deGal IgA1. Among them, the secretion of adiponectin was suppressed in HMCs after stimulation with deSial/deGal IgA1. HMCs expressed mRNAs for adiponectin and its type 1 receptor, but not the type 2 receptor. There was a significant downregulation of adiponectin expression in the glomeruli of renal biopsy specimens from patients with IgAN in comparison to those with lupus nephritis. In addition, the deposition of aberrantly glycosylated IgA1 was observed in the mesangium of patients with IgAN by dual staining of HAA and IgA. Moreover, the urinary HAA/SNA ratio of lectin binding was significantly higher in IgAN in comparison to other kidney diseases.

Conclusions: In conclusion, these results indicate that aberrantly glycosylated IgA1 downregulated the adiponectin expression in HMC *in vitro* and in the glomeruli of human IgAN *in vivo*. Glomerular lectin binding assays using HAA demonstrated the presence of aberrantly glycosylated IgA1 in the glomeruli, predominantly in IgAN.

TH-PO130

Stimulation of Wnt/beta-Catenin Signaling by Paricalcitol Prevents oxLDL-Induced Tregs Apoptosis and Restores Their Function Pascal Meier. *Service of Nephrology, RSV - Hôpital du Valais, Sion, Switzerland.*

Background: OxLDL induce Tregs cell cycle arrest and apoptosis affecting their suppression capacity and finally promote a constant micro-inflammatory state in patients with ESKD. This is partly due to the down-regulation of the Wnt/beta-catenin signaling that promotes phosphorylation of beta-catenin leading this latter to its ubiquitinylation and degradation by the proteasome. Because vitamin D analogs can modulate beta-catenin in other tissues, we tested whether the vitamin D analog paricalcitol could ameliorate Tregs response by stimulating Wnt/beta-catenin signaling in presence of oxLDL.

Methods: Tregs from healthy blood donors were incubated for up to 72 h in RPMI alone with oxLDL (100 µg/mL) before washing and then treated with paricalcitol (1.5 ng/ml). To better analyze the effects of paricalcitol, Tregs were stimulated with Abs to CD3/CD28 (10 µg/mL). RT-PCR and Western blot analyses were performed. Cell viability was measured using a MTT assay and apoptosis indirectly assessed by Fas staining (flow cytometry) and confirmed by DNA fragmentation. To address their suppressive capacity, activated Tregs were analyzed in co-culture with $CD4^+/CD25^-$ T cells (T_{eff}) in presence of APC.

Results: Compared with vehicle-treated Tregs, paricalcitol significantly upregulated beta-catenin in activated Tregs after oxLDL treatment. Quantitative determination showed a more than 150-fold and 80-fold induction of beta-catenin protein over the controls and vehicle-treated Tregs, respectively. Immunohistochemical staining confirmed the beta-catenin upregulation in cytoplasm and nucleus. The expression of the anti-apoptotic protein, Bcl-x_L an upstream mediator of the Wnt/beta-catenin signaling in Tregs was examined. Bcl-x_L was markedly down regulated in oxLDL-treated Tregs; and administration of paricalcitol largely stimulated nuclear Bcl-x_L synthesis in parallel with the lower expression of cell-surface apoptotic marker Fas and less DNA fragmentation.

Conclusions: Thus paricalcitol improves the cytosolic and nuclear expression of beta-catenin that in its turn favors Bcl-x_L transcription protecting Tregs to enter into apoptosis; and finally, significantly ameliorates oxLDL-treated Tregs function.

Funding: Clinical Revenue Support

TH-PO131

Neutrophilic Granulocytes Modulate T Lymphocytic Response in Peritonitis in Mice and Humans Sibylle Von Vietinghoff,[1,2] Gerhard Wingender,[2] Marcus Hiss,[1] Klaus Ley,[2] Hermann G. Haller,[1] Mitchell Kronenberg.[2] *[1]Medicine, Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]La Jolla Institute for Allergy and Immunoloy, La Jolla, CA.*

Background: In patients undergoing peritoneal dialysis, peritonitis is a common complication and can limit this treatment. Under resting conditions most peritoneal leukocytes are T lymphocytes, but neutrophilic granulocytes invade the peritoneal cavity in large numbers during peritonitis. Here we investigated how neutrophilic granulocyte accumulation alters cellular antigen-specific host response by glycolipid specific invariant natural killer T (iNKT) lymphocytes.

Methods: Leukocytes were recovered from peritoneal fluid from stable patients on peritoneal dialysis and patients with acute peritonitis. Neutrophilic peritonitis was induced in mice by thioglycollate injection. T lymphocytes were co-incubated with granulocytes in vitro. iNKT cells were stimulated with the specific antigen alpha-Galactosyl-ceramide. Multi-color flow cytometry and cytokine ELISA were used for analysis.

Results: Neutrophilic inflammation in experimental peritonitis in mice decreased iNKT cell transcription factor expression and antigen-induced cytokine production in vivo. This was reverted by blockade of neutrophil mobilization. Transcription factor expression was decreased in glycolipid-reactive iNKT cells from the neutrophil rich bone marrow compared to spleen in mice. In vitro, the function of both mouse and human iNKT cells was inhibited by co-incubation with neutrophils. This required cell-cell contact with live neutrophils. During peritonitis, transcription factor T-bet expression was reduced in peritoneal compared to peripheral blood iNKT lymphocytes in patients on peritoneal dialysis.

Conclusions: Our data reveal a novel regulatory axis whereby neutrophils reduce iNKT lymphocyte responses. This may be important in shaping the extent of inflammation in both host defense and destructive inflammation.

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TH-PO132

Lipoxin A4 Reprogrammes CD16+ Mononuclear Cells Eileen Nolan,[1,2] Debra F. Higgins,[1,2] Paula Maderna,[1,2] Yvonne M. O'Meara,[2,3] Catherine Godson.[1,2] *[1]UCD Diabetes Research Centre, UCD Conway Institute of Biomolecular and Biomedical Research; [2]UCD School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4; [3]Mater Misericordiae University Hospital, Dublin 7, Ireland.*

Background: The diversity of macrophage influence on kidney disease is related to their phenotypic and functional heterogeneity. $CD16^+$ macrophages (M1) are pro-inflammatory, contributing to renal fibrosis and progression of CKD, whereas $CD16^-$ cells (M2) are anti-inflammatory and pro-resolving. Monocytes exhibit similar non-uniformity,

with upregulation of a $CD16^+$ subset occurring in haemodialysis, and contributing to increased cardiovascular risk. Lipoxins are endogenously-produced eicosanoids, with established anti-inflammatory and pro-resolving properties. The purpose of this study was to determine whether lipoxin A_4 (LXA_4) would prove effective in reducing pro-inflammatory mononuclear cell populations.

Methods: The human monocyte cell line THP-1 was differentiated into a macrophage-like phenotype, by treatment with PMA (10 nM) for 48 hours. Both THP-1 monocytes and THP-1-derived macrophages were treated with LXA_4 (10nM) or vehicle (0.1% ethanol) for 30 minutes, before exposure to pro-inflammatory stimuli (LPS 100ng/ml or IFN-γ 10ng/ml). Cells were then stained with CD16 antibody and analysed by flow cytometry. Supernatants were retained for cytokine analysis by ELISA.

Results: Treatment of both monocytes and macrophages with LXA_4 resulted in a significant ($p < 0.05$) decrease in CD16 expression, as compared with IFN-γ treated cells, suggesting that LXA_4 reduces the monocyte and macrophage inflammatory subsets, and promotes the macrophage M2 phenotype. Analysis by ELISA of supernatants revealed significantly reduced macrophage production of the pro-inflammatory cytokines IL-6 and TNF-α ($p < 0.05$), following treatment with LXA_4, again suggesting a role for this agent in directing macrophage phenotype.

Conclusions: Lipoxin A_4 may have therapeutic potential in reprogramming pro-inflammatory monocytes and macrophages, thereby inducing a diminution of their detrimental influence, but a beneficial promotion of the anti-inflammatory and pro-resolving activities of M2 macrophages.

Funding: Government Support - Non-U.S.

TH-PO133

Interactions between Complement and Kidney Cells: Implications in Renal Diseases Isabell Kopka,[1] Peter F. Zipfel,[1] Gunter B. Wolf.[2] *[1]Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany; [2]Internal Medicine III, University of Jena, Jena, Germany.*

Background: The complement system is an enzyme cascade of more than 60 proteins and activation products which are involved in innate immune activation and regulation that leads to effector functions including the deposition of the C3b, release of the anaphylatoxin C3a, and formation of the terminal complement complex (TCC). Recently, complement has been implicated in the pathogenesis of primary renal disease and renal transplant rejection. These are caused by complement gene mutations of components, changes in complement levels in the circulation, or presence of complement components in renal tissues. This study focuses on the expression and activity of complement components and regulators of renal cells and their modification during lysis.

Methods: Since kidney cells are damaged when exposed to normal human serum (NHS) in most kidney diseases, human renal proximal tubular cells (HK-2) were challenged with NHS and viability, complement activation, TCC deposition, and presence of complement membrane regulators were assayed using flow cytometry, real time RT/PCR and viability assays.

Results: Exposure to increasing amounts of NHS caused damage revealed by C3b deposition, C3a generation, surface deposition of TCC and decreased viability of the renal cells. However, complement regulators CD46, CD55 and CD59 were detected on the surface of the cells. The complement regulator CD59 was also upregulated in HK-2 cells upon NHS incubation as shown in real time PCR. Moreover, HK-2 cells produced C3 which could be cleaved by Kallikrein and Thrombin, two members of the coagulation system.

Conclusions: The generated C3a acts as an effector molecule and as a cytokine and initiates the induction of tissue factor expression primarily on macrophages which induces an anti-inflammatory response of lysed kidney cells. These results show that exposure to NHS affects viability of renal cells due to activation of the complement system. However, renal cells respond by expressing membrane complement regulators like CD59. Thus, the complement system plays an essential role in the pathogenesis of renal diseases.

Funding: Government Support - Non-U.S.

TH-PO134

Overexpression of Human Liver-Fatty Acid-Binding Protein (L-FABP) Endogenously Enhances Peroxisome Proliferator-Activated Receptor (PPAR)-α Activity Possibly Via Inducing Hepatocyte Nuclear Factor (HNF)-4α in Immortalized Mouse Proximal Renal Tubular Cells (mProx) Hideki Kimura,[1] Daisuke Mikami,[1] Kazuko Kamiyama,[1] Kenji Kasuno,[1] Naoki Takahashi,[1] Takeshi Sugaya,[2] Haruyoshi Yoshida.[3] *[1]Div of Nephrol, Dept of General Med, Sch of Med, Univ of Fukui, Fukui, Fukui, Japan; [2]CMIC Co, Ltd, Tokyo, Japan; [3]Dept of Med, Obama Municipal Hosp, Obama, Fukui, Japan.*

Background: PPAR reportedly exerts anti-inflammatory and anti-fibrotic actions in proximal renal tubular cells (PRTCs) under inflammation and hypoxia. L-FABP abundantly expressed in PRTCs of human kidney may modulate PPAR activity via their molecular interaction as well as serves as an anti-oxidant. However, no information has been known about the effects of L-FABP induction on PPAR activity in PRTCs.

Methods: Human L-FABP-overexpressed mProx (hL-FABP-mProx) was generated by transfection of the genomic DNA, and analyzed for specific factors involved in PPAR pathway and inflammatory effector molecules, because mouse L-FABP is scarcely expressed in mProx.

Results: In hL-FABP-mProx, hL-FABP expression was up-regulated by over 300-fold as compared with mProx. PPAR-α expression was increased by 10-fold, while PPAR-γ was down-regulated by 5-fold. Palmitate-induced PPRE-luciferase activity (PPAR-α activity) was increased by 2-fold, while PPAR-γ activity was unchanged. mRNA levels of mouse L-FABP, a target gene of PPAR-α, were 40-fold greater in hL-FABP-mProx than in mProx, which was not further augmented by an exogenous PPAR-α activator (fenofibrate), while those of heart-FABP, a target gene of PPAR-γ were unchanged. Concerning known stimulators for PPAR-α expression, hL-FABP overexpression enhanced HNF-4α expression by over 100-fold, but had no affect on Lipin 1 expression. Hypoxia decreased HNF-4α and PPAR-α expression by 40-50% in hL-FABP-mProx. Finally, hL-FABP mProx showed not only reduced basal expression of MCP-1, PAI-1 and CTGF, but also reduced TNF-α-stimulated expression of MCP-1 by 40-70% as compared with mProx.

Conclusions: hL-FABP overexpression in mProx endogenously enhances PPAR-α activation possibly via inducing HNF-4α expression. PPAR-α and HNF-4α induction may cause the reduced inflammatory response in hL-FABP mProx.

TH-PO135

Uremic Priming of a Macrophage Pro-Inflammatory Response Neal B. Blatt,[1] Patricia L. Christopherson,[1] Timothy Cornell,[2] Thomas P. Shanley.[2] *[1]Pediatrics - Nephrology, University of Michigan, Ann Arbor, MI; [2]Pediatrics - Critical Care, University of Michigan, Ann Arbor, MI.*

Background: Monocytes from ESRD patients demonstrate increased cellular activation including increased pro-inflammatory cytokine production. These findings implicate these cells as a key player in the development of ESRD-mediated chronic inflammatory state and excess cardiovascular disease. However, the majority of monocyte samples have been obtained from patients on dialysis (with bioincompatible cuprophane membranes), making it difficult, if not impossible to distinguish the impact of dialysis from the impact of ESRD itself on monocyte function. Our goal is to use a mouse model of chronic renal failure to determine the impact of uremia on macrophage function.

Methods: 129 SvJ mice underwent subtotal nephrectomy (SNx) or a sham procedure. Bone marrow-derived macrophages (BMDM) were isolated from sham and SNx mice and then stimulated with the Toll-like receptor (TLR) ligands lipoteichoic acid (LTA) or lipopolysaccharide (LPS).

Results: SNx mice developed an approximate doubling in BUN values within 4 weeks post-surgery that remained stable for at least 6 months (SNx: 47 ± 9 vs Sham: 24 ± 3 mg/dL, $P<0.001$) indicating the development of uremia. BMDM from SNx mice show increased TNF-α production in tissue culture supernatants following stimulation with both LPS (SNx: 661 ± 74 vs sham: 394 ± 42 pg/mL, $P<0.05$) and LTA (SNx: 2248 ± 100 vs sham: 1808 ± 62, $P<0.05$). At the mRNA level, SNx BMDM show 50% less TNF-α transcript in unstimulated conditions (SNx 0.5 ± 0.1 vs sham 1.0 ± 0.1 fold-induction, $P<0.05$), however upon LPS stimulation, the TNF-α transcript increases in parallel to what is seen at the protein level (SNx: 40.5 ± 8.7 vs sham: 5.9 ± 6.7 fold-induction, $P<0.05$).

Conclusions: We have utilized a well-established mouse model of chronic renal failure to demonstrate that macrophages from uremic mice are primed to respond to TLR ligands. These findings suggest that uremia by itself is able to reprogram the bone marrow to foster the development of a chronic inflammatory state. Additional experiments are in progress to look at epigenetic regulation of these pro-inflammatory macrophage cytokine responses.

Funding: Other NIH Support - NICHD Child Health Research Career Development Award

TH-PO136

Thrombin Stimulates Production of Colony Stimulating Factors in Proximal Tubular Epithelial Cells – Possible Role for Tubulointerstitial Injury Via Chemokine Regulations Michiko Shimada, Yuko Shimaya, Hideaki Yamabe, Yoshiko Shutto, Takeshi Fujita, Norio Nakamura, Ken Okumura. *Department of Nephrology, Hirosaki University, Hirosaki, Japan.*

Background: Colony-stimulating factors (CSFs) are well-known hematopoietic growth factors. However, recent studies revealed that CSFs are involved in many inflammatory conditions. In the experimental anti-glomerular membrane glomerulonephritis, both glomerular lesions and tublointerstitial lesions were attenuated in the granulocyte-macrophage CSF (GM-CSF) knockout mice. Whereas, it is suggested that Granurocyte-CSF (G-CSF) may attenuate cisplatin-induced acute kidney injury by accelerated re-generation and decreased apoptosis of tubular cells. However, the local productions of CSFs and its regulation in the kidney is not well elucidated.

Methods: We used primary human proximal tubular epithelial cells (PTEC) to test the effect of thrombin for CSFs production, since thrombin is suggested to induce tubulointerstitial injury. PTEC were incubated with thrombin (0.5-5U/ml) and the effect on the production of GM-CSF, G-CSF, macrophage-CSF (M-CSF), interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) were measured in the cell supernatant by enzyme-linked immunosorbent assay (ELISA) and mRNA expressions were analyzed by quantitative RT-PCR. Also, we used direct thrombin inhibitor argatroban to verify the specific effect of thrombin. Furthermore, we assessed the role of CSFs for the chemokine expressions, using transient blockade of CSFs by siRNA technique.

Results: Thrombin stimulated the production of CSFs (GM-CSF, G-CSF, M-CSF) and chemokines (MCP-1 and IL-8) demonstrated by ELISA for protein expression and by quantitative RT-PCR for mRNA expressions. These effects of thrombin were significantly reduced by argatroban demonstrating specific effects of thrombin. Besides, induction of MCP-1 by thrombin was attenuated by M-CSF knockdown, and IL-8 induction was attenuated by G-CSF knockdown, showing chemokine expressions in PTEC were at least in part regulated by CSFs produced locally by PTEC.

Conclusions: Thrombin stimulates the production of CSFs by PTEC. Locally produced CSFs at least in part regulates chemokine expressions in PTEC.

TH-PO137

PGA-Peptoid 1 Inhibits Apoptosis and Inflammation in Renal Cells Alvaro C. Ucero,[1] Sergio Berzal,[1] Monica Sancho,[3] Carlos Ocaña,[1] Marta Ruiz-Ortega,[1] María Jesús Vicent,[2] Alberto Ortiz,[1] Adrian Mario Ramos.[1] *[1]Nefrología Experimental, IIS-FJD/Univ. Autonoma de Madrid, Madrid, Spain; [2]Peptide and Protein Lab, Centro de Investigación Príncipe Felipe, Valencia, Spain; [3]Polymer Therapeutics Lab, Centro de Investigación Príncipe Felipe, Valencia, Spain.*

Background: Tissue injury is an unwanted adverse effect of inflammation. Inflammatory stimuli may induce cell injury and death and, in turn, injured cells may promote inflammation. Thus, a therapeutic agent that targets both cell death and inflammation may be of particular interest.

Results: A family of Apaf-1 inhibitory nanomedicines that modulate apoptosis includes PGA-peptoid 1 (QM56). We now report that QM56 inhibits apoptosis in renal tubular cells co-stimulated by the lethal cytokine cocktail TWEAK/TNFa/INFγ. In addition, QM56 inhibited MCP-1 and Rantes chemokine expression in response to this stimulus. The anti-inflammatory effect of QM56 was independent from protection of apoptosis, since it also inhibited the inflammatory response to TWEAK (a cytokine that alone promotes tubular cell proliferation but not apoptosis) in tubular cells as well as renal fibroblasts. In addition, the anti-inflammatory effect showed by QM56 was independent from Apaf-1, since it was observed in MEF-Apaf-1$^{-/-}$ cells. TWEAK-induced MCP-1 and Rantes synthesis is NF-kB-dependent and is prevented by the NF-kB inhibitor parthenolide. TWEAK also activated the JAK/STAT pathway. JAK2/STAT3 activation was required for the full expression of TWEAK-induced chemokine mRNA, but not for p65 nuclear translocation. In this regard, QM56 also failed to inhibit p65 nuclear translocation and DNA binding in response to TWEAK while it was inhibiting the JAK2/STAT3 phosphorylation/activation and hence chemokine expression elicited by the cytokine. Along with the inhibition of chemokine synthesis, blockade of the JAK2/STAT3 pathway by QM56 was also corroborated in MEF-Apaf-1$^{-/-}$ treated with TWEAK.

Conclusions: We conclude that the anti-inflammatory activity of QM56 was independent of its role as inhibitor of apoptosis and that it could have potential therapeutic relevance.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO138

Serum CRP and IgA1 O-Glycosylation as Predictors of Disease Progression in IgA Nephropathy Karen Molyneux,[1,2] Demetris Christou,[1] Javeria Peracha,[1] John Feehally,[1,2] Alice C. Smith,[1,2] Joanna Boyd,[1] Chee Kay Cheung,[1] Jonathan Barratt.[1] *[1]John Walls Renal Unit, Leicester General Hospital, Leicester, Leicestershire, United Kingdom; [2]Department of Infection, Immunity and Inflammation, University of Leicester., Leicestershire, United Kingdom.*

Background: IgA nephropathy (IgAN) is an inflammatory glomerulonephritis which is progressive in some patients. We have shown that abnormal O-glycosylation of serum IgA1 early in the disease is associated with risk of progression. C-reactive protein (CRP) may also be associated with progression but the relationship between CRP and IgA1 O-glycosylation has not been investigated.

Methods: We studied sera from 70 patients with biopsy-proven IgAN obtained early in the course of their disease while renal function was normal, and 70 healthy controls. The patients were classified as progressors (serum creatinine increased by >100% during follow up, n=32), or non-progressors (serum creatinine remained normal after a minimum ten years follow up, n=38). CRP was measured by ELISA. IgA1 O-glycosylation was previously measured.

Results: In our study, the mean serum CRP was higher in patients than controls (IgAN 2.86±0.49μg/ml, controls 1.78±0.27μg/ml, p=0.012). Furthermore, the progressors had higher CRP than non-progressors (progressors 3.18±0.52μg/ml, non-progressors 2.59±0.79μg/ml, p=0.023). However, there was no correlation between IgA1-HA and CRP in progressors, non-progressors or controls. We compared the number of progressors and non-progressors with high or low CRP and IgA1-HA (defined as above or below median respectively). Combining the two markers, 12 progressors and 6 non-progressors had both high IgA1-HA and high CRP, compared to 4 progressors and 15 non-progressors with both low IgA1-HA and low CRP (p=0.008).

Conclusions: Our results confirm that both high IgA1-HA and high CRP at an early stage of IgAN are associated with subsequent development of progressive renal disease. However, neither could individually account for progression. We found no correlation between IgA1-HA binding and CRP, indicating that they are independently associated with progression in IgAN. Combining them gave a better indication of a subsequent course of the disease.

TH-PO139

Enhanced VASP Expression during Human Glomerulonephritis and Diabetic Nephropathy Bernd Hohenstein, Christian Hugo. *Division of Nephrology, Department of Internal Medicine III, University Hopsital Carl Gustav Carus, Dresden, Germany.*

Background: Vasodilator stimulated Phosphoprotein (VASP) is a central cytoskeletal protein that has been involved in platelet adhesion, migration and endothelial barrier function and integrity in several experimental disease models including passive crescentic nephritis by our group (JASN 2005 Apr;16(4)) but not in human kidney disease such as glomerulonephritis (GN) or diabetic nephropathy (DN).

Methods: We performed immunohistochemical staining in 120 human renal biopsies with minimal-change GN, FSGS, MGN, IgAN, lupus nephritis, vasculitis, Schoenlein-Henoch, endocapillary GN, MPGN or crescentic nephritis and 50 biopsies with DN. We investigated VASP in glomerular and tubulointerstitial (TI) compartments regarding expression and distribution and compared data to biopsies without apparent renal disease (n=8).

Results: Compared to controls where VASP expression was low, biopsies from most GN demonstrated enhanced VASP expression in glomeruli ($p<0.05$ for IgAN, vasculitis, MPGN, lupus N, crescentic nephritis, endocapillary GN) and also in the TI ($p<0.05$ for crescentic N, vasculitis, MGN, MPGN). In most biopsies there was a prominent endothelial expression pattern of VASP in glomerular as well as peritubular capillaries. Immigrating inflammatory cells also expressed relevant amounts of VASP. Endothelial VASP was prominent in mild diabetic lesions but demonstrated no further increase with ongoing injury. In GN, TI VASP expression correlated with the decline in GFR as estimated by the MDRD equation ($P<0.0001$, $r=0.34$).

Conclusions: VASP as a known important regulator of EC barrier and function shows a prominent endothelial expression pattern in human GN and DN, where EC function is markedly disturbed. The correlation of TI VASP and falling GFR also indicates this functional link.

TH-PO140

Upregulation of Serum Amyloid A in ESRD Patients Is Associated with the Presence of a Unique Biomarker Identified by Protein Chip Array Analysis Vinod K. Bansal,[1] Debra Hoppensteadt,[2] Walter Jeske,[3] Jawed Fareed.[2] *[1]Nephrology, Loyola University Medical Center; [2]Pathology, Loyola University Medical Center; [3]Throacic and Cardiovascular Institute, Loyol University Medical Center.*

Background: Serum amyloid A (SAA) is an acute phase reactant which is regulated by IL1, IL-6 and TNFα. The circulating levels of serum amyloid A are markedly increased in inflammatory conditions. SAA is a 104 amino acid polypeptide with a molecular mass of 12-14 KDa. Protein Chip Array methods have been used to identify unique biomarkers in ESRD patients. The purpose of this study was to determine the relationship of a previously reported biomarker in ESRD with circulating levels of SAA.

Methods: Plasma samples from 117 ESRD patients, over the age of 18 on maintenance hemodialysis and 50 age matched controls (healthy volunteers) were included in this study. SAA levels were measured by using a sandwich ELISA method (Abazyme, Needham, MA). Proetin Chip Array profiling was carried out utilizing surface enhanced laser desorption (SELDI; BioRad Corp, Hercules, CA) utilizing gold chips.

Results: The plasma SAA levels were markedly elevated in ESRD (41.8+12.6; range 4.6-98.1 μg/ml) in contrast to the normals (4.5+1.2; range 1.1-9.8 μg/ml). SELDI analysis revealed a unique biomarker signal in 78 out of 117 (67 %) in the ESRD patients and 2 out of 50 (4%) in the normal individuals. The presence of the 11.6 KDa biomarker correlated with the high SAA levels.

Conclusions: These studies suggest that SAA level upregulation is associated with the presence of the 11.6 KDa biomarker. Protein Chip Array profiling may be useful in the risk stratification of ESRD patients.

Funding: Private Foundation Support

TH-PO141

In Situ Immune Complex (MPO-Anti MPO Antibody) & Complement 3 Cause Glomerular Capillary Injury in Human MPO-ANCA Associated Glomerulonephritis Soko Kawashima, Yoshihiro Arimura, Yoshinori Komagata, Shinya Kaname, Akira Yamada. *First Department, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.*

Background: Myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA) associated glomerulonephritis(GN) is characterized by pauci-immune necrotizing glomerulonephritis(NCGN). MPO-ANCA has been thought to be involved in the activation of neutrophils in the pathogenesis of NCGN. Recent studies suggest that immunoglobulins precipitated on the glomerular capillaries might play some role in the pathogenesis of MPO-ANCA associated GN. Here, we investigated a possible role of MPO, IgG, complements and MPO-positive cells in the pathogenesis of MPO-ANCA associated GN.

Methods: Renal specimen including 317 glomeruli obtained from 20 patients with MPO-ANCA associated GN were analyzed. Glomerular infiltration of MPO-positive cells, deposition of extracellular MPO and endothelial cell injury were analyzed and the number of infiltrating MPO-positive cells was scored in each glomerulus, especially in early stage of the disease. Colocalization of MPO, IgG, C3 and CD34 deposition was analyzed. Immunofluorescence staining for triple staining (MPO, IgG and CD34) were performed for samples of renal biopsies.

Results: All of 20 patients showed a weak but significant staining for IgG (pauci-immune GN), which was often accompanied by MPO deposition along the glomerular capillary walls. Triple positive (MPO, IgG & C3) deposition was detected in 15 glomeruli (5%) mainly with low activity and chronicity. CD34 staining was lost around the area where MPO and IgG were detected, suggesting the endothelial injury may be induced by MPO- and IgG-associated pathogenic mechanism. Deposition of MPO, IgG and C3 was observed along the glomerular capillary walls predominantly in the early stage of MPO-ANCA associated GN.

Conclusions: These results indicate that not only the activation of neutrophils, but also MPO and the immune complexes composed of MPO-anti MPO antibody may play some direct roles in the pathogenesis of glomerular capillary injury in the early phase of human MPO-ANCA associated GN.

TH-PO142

The Activation of Renin-Angiotensin System Is Involved in Hyperlipidemia Mediated Renal Injuries in Apolipoprotein E Knockout Mice Jie Ni, Kun Ling Ma, Jing Liu, Wenjin Huang, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: The activation of renin-angiotensin system (RAS) and hyperlipidemia play crucial roles in the progression of chronic kidney disease (CKD). Whether there is the interaction of hyperlipidemia with RAS activation in accelerating the progression of CKD remains unknown. This study was to investigate the role of RAS activation in hyperlipidemia mediated renal injuries using an atherosclerotic mouse model from apolipoprotein E knockout mice (apoE-/- mice).

Methods: Male apoE-/- mice were respectively fed with high fat diet (HF group, containing 40% fat and 0.15% cholesterol, n=8) and normal chow diet (control, n=8) for 8 weeks. Lipid profile in the plasma was analysed by clinical biochemistry assay. The plasma levels of prorenin, rennin, and angiotensin II were checked by radiommunoassay. The protein expressions of angiotensinogen, angiotensin II, renin, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT2) in kidneys of apoE-/- mice were checked by immunohistochemical staining and Western Blot. The extracellular matrix deposition in kidneys was evaluated by Masson-staining.

Results: It was fiund that mice fed with high fat diet developed hyperlipidemia with significant extracellular matrix deposition in renal tubular interstitium of kidneys compared to the controls . The plasma levels of renin and angiotensin II didn't present signifciant changes between groups except that plasma prorenin level was significantly reduced in HF group. The protein expressions of angiotensinogen, angiotensin II, rennin, ACE, AT1 and AT2 in the kidneys in HF group were significantly increased, which was significantly associated with the extracellular matrix deposition.

Conclusions: Our study demonstrated that the activation of intra-renal RAS might be involved in hyperlipidemia mediated renal injuries, suggesting a new potential mechanism for the synergistic effect between hyperlipidemia and RAS activation in accelerating the progression of CKD.

Funding: Government Support - Non-U.S.

TH-PO143

Role of Mineralocorticoid Receptor and Rac1 in Inflammatory Cytokine Production in Rat Peritoneal Macrophages Kenichi Ishizawa, Miki Nagase, Shigetaka Yoshida, Wakako Kawarazaki, Toshiro Fujita. *Department of Nephrology and Endocrinology, University of Tokyo Graduate School, Japan.*

Background: Mineralocorticoid receptor (MR) plays an important role in the progression of target organ damage, possibly via induction of inflammation, fibrosis, macrophage infiltration, and oxidative stress. Recent reports demonstrated that macrophage-specific MR knockout mice are protected from DOCA/salt-induced myocardial fibrosis, and from angiotensin II/L-NAME/salt, suggesting the role of MR signaling in macrophage function. We reported that small G protein Rac1 activates MR directly in a ligand-independent pathway. In the present study, we examined whether MR and Rac1 signalings affect pro-inflammatory cytokine production using rat peritoneal macrophages.

Methods: Rat resident peritoneal macrophages were collected by peritoneal lavage after injection of phosphate-buffered saline. The cells were resuspended in RPMI1640 medium with 10% FBS, and plated on culture dish. After vigorous washing, the attached macrophages were incubated in the presence or absence of lipopolysaccharide (LPS). Gene expression was analyzed using quantitative real-time PCR, and protein level was analyzed by immunoblotting and immunocytochemistry. Rac1 activation assay was performed using GST pull-down assay.

Results: Most of the attached cells were positive for a pan-macrophage marker ED-1. LPS increased TNFα, IL-6,and IL-1β generation in peritoneal macrophages, and Rac-specific inhibitor markedly inhibited the responses. Macrophage Rac1 was activated by LPS treatment. MR mRNA and protein were detected in peritoneal macrophages. LPS-induced TNFα, IL-6,and IL-1β production was dramatically suppressed by treatment with spironolactone.

Conclusions: Our findings suggest that MR participates in the inflammatory cytokine secretion in peritoneal macrophages, implicating the cross-talk between Rac1 and MR cascades.

TH-PO144

Perivascular Immune Cell Infiltration in Aging Kidneys and Identification of Associated Genes Yuan Huang,[1,2] Gerda A. Noordmans,[1] Jan-Luuk Hillebrands,[1] Peter Heeringa,[1] Ron Korstanje,[3] Harry Van Goor.[1] *[1]Department of Pathology and Medical Biology, University Medical Center Groningen, Netherlands; [2]Department of Pathology, Fudan University, China; [3]The Jackson Laboratory.*

Background: Dysregulated immune responses play a major role in age-related organ deterioration. The study of immune alterations in the aging kidney and the genes involved in this process may pave the way for the discovery of new strategies for healthy aging.

Methods: Kidneys from aged mice (www.jax.org/phenome) sacrificed at 2 years were collected. Females from 23 strains and males from 20 strains were evaluated. PAS staining and immunohistochemical staining of CD45 and CD3 were performed. The size of the perivascular immune cell clusters was measured in all mice on PAS staining. Haplotype Association Mapping (HAM) was used to identify genetic loci associated with perivascular infiltration.

Results: CD45 staining showed marked renal perivascular immune cell clusters. 90% of the clusters were positive for the T cell marker CD3 in all mice. Females from strains (C57BR/CDJ, C57L/J, NON/LtJ, P/J) had larger clusters ($P < 0.05$). Quantitative data were used for HAM analysis in females. Significant association with loci on Chr1, Chr5, Chr14, distal Chr17, and proximal Chr17 were detected. We identified candidate genes *Mapk14 (*associated with macrophage infiltration), and *Man2a1 (*associated with autoimmune disease mimicking human SLE). In male mice, strains (129S1/SvImJ, C57L/J, C57BL/6J, LP/J, NON/LtJ, P/J) had significantly larger clusters. Binary data were used for HAM analysis in males. Significant association with loci on Chr1, Chr2, Chr8, and Chr14 were detected. *Wisp2* showed the highest peak. *Wisp2* encodes the protein WISP2 (WNT1 inducible signaling pathway protein 2), which is associated with proliferation, extracellular matrix regulation, angiogenesis and fibrosis.

Conclusions: Our study identified strain differences with respect to the presence and number of perivascular immune cell clusters in aged kidneys. A genetic analysis identified novel candidate genes that might participate in renal aging and provide novel means to prevent aging-related diseases.

TH-PO145

Continuous Monitoring of Hemodialysis with Pulse-Wave Analysis Rita L. McGill,[1] Jan K. Berkow,[2] Richard J. Marcus.[1] *[1]Division of Nephrology, West Penn Allegheny Health System, Pittsburgh, PA; [2]iNTELOMED, Inc., Pittsburgh, PA.*

Background: Blood pressure (BP) and pulse (HR) monitoring can't always predict symptomatic circulatory stress during hemodialysis (HD), which doesn't always indicate simple hypovolemia. In pulse-wave analysis (PWA), pulse strength from a pulse-oximetry waveform analyzes the combined hemodynamic effects of cardiac ejection and arterial distensibility, expressed as effective circulatory flow (eCf). Comparing eCf to HR estimates hemodynamic stress and provides a real-time estimate of autoregulatory reserve capacity.

Methods: We studied 24 subjects with known heart disease or frequent symptoms with the CV Insight™, a novel PWA device, during 33 HD. PWA data was compared to continuous HR and BP monitoring, with clinical observation to detect dialysis symptoms such as cramps and nausea. We analyzed observations post-hoc to develop algorithms to predict events/symptoms.

Results: 16/33 HD had PWA patterns resembling volume depletion in lower body negative pressure experiments. eCf fell progressively with volume removal. HR typically increased when eCf had decreased to <60% of initial values; hypotension or dialytic symptoms often followed within 25-45 min. Such patients typically felt cold during stress. In 6 HD starting with marked volume expansion, eCf increased and HR decreased during the first 1-2 hours, before a typical pattern emerged. In 7 HD, eCf was consistently high, even with increased HR and symptoms; these patients typically felt uncomfortably hot under stress, suggesting inappropriate peripheral vasodilation. In 2 HD, arrhythmia interfered with interpretation of PWA.

Conclusions: PWA is a novel non-invasive technique yielding valuable information about circulatory stress and autoregulatory capacity during HD. This pilot study shows distinct patterns of compartment hydraulics which may explain patient variability in ultrafiltration tolerance. PWA profiling may assist clinicians in assessing optimal target weights & providing comfortable ultrafiltration. Further study is warranted to refine predictive algorithms that anticipate symptoms and hemodynamic insufficiency, which may improve evaluation of strategies to support circulation during HD.

Funding: Private Foundation Support

TH-PO146

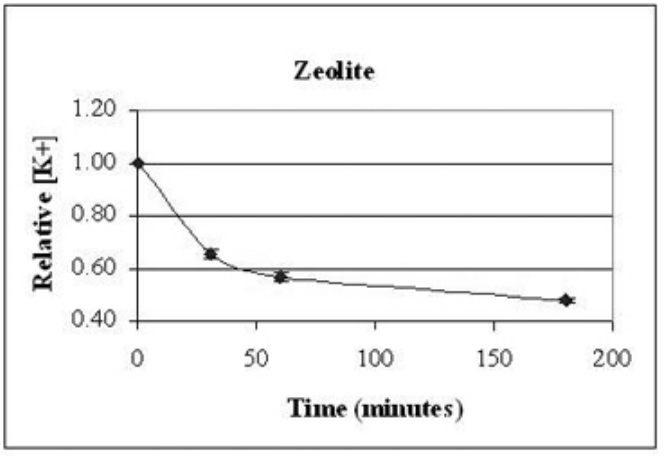
Tests with Novel Adsorbents In Vitro Maarten Wester,[1] Frank Simonis,[2] Jeroen Kooman,[3] Jaap A. Joles.[1] *[1]Nephrology & Hypertension, University Medical Center Utrecht, Netherlands; [2]Nanodialysis, Eindhoven, Netherlands; [3]Nephrology, Maastricht University Medical Center, Maastricht, Netherlands.*

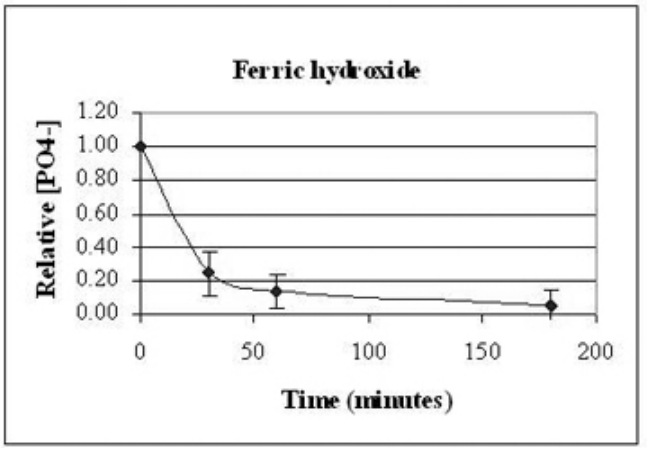
Background: As part of the Dutch/European consortia iNephron/Nephron+ we are involved in the development of an adsorbent-based wearable artificial kidney device (WAKD). With this WAKD we aim to replace renal function continuously 24h/d. Currently we tested adsorbance by a zeolite targeting K+, a ferric hydroxide targeting PO4-, and a modified carbon targeting protein-bound substances, such as PAH.

Methods: Plasmapheresis plasma of Goodpasture and anti-GBM nephritis patients (N=3) was exposed to 100mg of each matrix for 1 to 3 hours.

Results: After one hour K+ decreased from 4.11±0.30 to 2.51±0.30 mM ($P<0.001$). Relative PO4- concentration from 100% to 14±10% ($P<0.001$), and the relative PAH concentration from 100% to 50±31% ($P<0.001$).

The saturation of the zeolite and ferric hydroxide was tested by extending the exposure time up to 3 hours.





Most of the adsorbance occurred in the first hour of exposure, reaching saturation after 3 hours.

Conclusions: In conclusion, the daily production of toxins should be readily removed by these adsorbents. Preclinical in vivo experiments will be performed in goats.

Funding: Private Foundation Support

TH-PO147

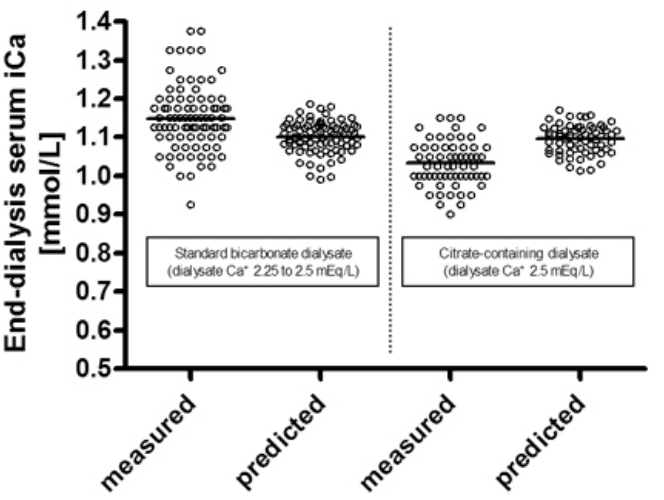
Independent Validation of a Versatile Citrate Dialysis Model Stephan Thijssen,[1] Len A. Usvyat,[1] Jeffrey J. Sands,[2] Nathan W. Levin,[1] Peter Kotanko,[1] Jose A. Diaz-Buxo.[2] *[1]Renal Research Institute, New York, NY; [2]Fresenius Medical Care, Waltham, MA.*

Background: Citrate dialysis (CD) offers many advantages over heparin dialysis, but calcium kinetics in CD are complex. We recently introduced a versatile mathematical model of CD (Thijssen S et al., Blood Purif 2010). The goal of this study was to validate a refinement of this model in a new setting using data from an independent clinical trial.

Methods: Data from a recently completed trial on the effects of citrate-containing dialysate on heparin requirements (Sands et al., ASN 2010) were used. Briefly, patients were studied prospectively on standard bicarb dialysate as well as on Citrasate® dialysate (citrate, 2.4 mEq/L; Ca, 2.5 mEq/L). Pre- and post-HD serum iCa was simulated with our model and compared to measured values. For post-HD simulations, starting iCa levels in the model were adapted to measured values to assess the intra-HD simulation quality.

Results: Pre-HD iCa was underestimated by about 0.12±0.06 mmol/L with both dialysates. This was fairly consistent across the spectrum of iCa levels. End-HD iCa was underestimated by 0.046±0.074 mmol/L on bicarb dialysate and overestimated by 0.077±0.046 mmol/L on Citrasate® (Fig. 1). The prediction errors with both dialysate types were less variable with this refined model than our previously published model.

Conclusions: Average prediction quality for serum iCa was good. Efforts have to be directed towards further explaining and reducing the spread of the prediction error, e.g. by taking into account individual pre-HD serum citrate levels, pH levels and shifts, and better estimates of bone Ca buffering. Although prediction errors were small on average, the model should be adjusted to avoid underestimation of end-HD serum iCa in regular bicarb dialysate treatments and overestimation with use of citrate-containing dialysate.



TH-PO148

***p*-Cresol Sulfate and Indoxyl Sulfate Induce Similar Cellular Inflammation and Immune Response in Cultured Proximal Renal Tubular Cells** Chiao-Yin Sun. *Nephrology, Chang Gung Memorial Hospital, Keelung, Taiwan.*

Background: Uremic toxins, *p*-cresol sulfate (PCS) and indoxyl sulfate (IS), have important roles in the progression of CKD. Previous study has shown that IS induces cellular oxidative stress.The aim of this study is to find the cellular inflammation responses to PCS and IS.

Methods: Cultured mouse proximal renal tubular cells (PKSV-PRs) treated with PCS or IS at concentrations of 0, 1, and 5 mg/L were analyzed by PCR array with inflammation and immune panel. The gene-annotation enrichment analysis and functional annotation clustering were analyzed with the DAVID v6.7. The functional networks of target genes were analyzed by the GeneMANIA.

Results: There were 12 and 30 genes up-regulated by PCS at concentrations of 1 and 5 mg/L greater than 1.5 × respectively. There were 13 and 32 genes up-regulated by IS at concentrations of 1 and 5 mg/L greater than 1.5 × *vs.* control respectively. Ccr2, Csf2 and Ptprc were down-regulated by PCS and IS. Sixteen up-regulated functional annotation clusters of cells treated with PCS or IS at a concentration of 5 mg/L were noted by functional annotation clustering analysis. Eleven common functional annotation clusters of up-regulated genes were noted in cells treated with PCS or IS. The calculated function networks were similar between the PCS and IS. Tgfb1, Fasl, Il6/15, Il15, Csf1/3 and Cxcl10 were the major cytokines in the functional networks of PCS and IS. Stats, Smads, Nfkb2, Ikbkb, Bcl2 and Bax were the major intracellular signals for PCS and IS. Real-time PCR results showed that PKSV cells treated with PCS or IS had significantly increased Tgfb1 expression. The molecular functional networks with the highlight of Tgfb1 for the cells treated with PCS and IS at a concentrations of 5 mg/L were analyzed. In both PCS and IS, Col4a5, Cxc10, Fasl, Stat1 and Ikbkb were the target genes in the predicted molecular functional networks with the Tgfb1.

Conclusions: PCS and IS stimulated significant cellular inflammation reactions. The cellular inflammation and immune response induced by PCS and IS were similar in cultured proximal renal tubular cells.

Funding: Government Support - Non-U.S.

TH-PO149

Distribution of Hydrogen Sulfide (H_2S)-Producing Enzymes and the Roles of H_2S in Diabetic Nephropathy Jun-Ichiro Yamamoto,[1,2] Waichi Sato,[1] Tomoki Kosugi,[1] Shoichi Maruyama,[1] Seiichi Matsuo,[1] Yukio Yuzawa.[1,3] *[1]Division of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi-Ken, Japan; [2]Division of Nephrology, Tsushima City Hospital, Tsushima, Aichi-Ken, Japan; [3]Division of Nephrology, Fujita Health University School of Medicine, Toyoake, Aichi-Ken, Japan.*

Background: Hydrogen sulfide (H_2S) has recently been found to play beneficial roles in ameliorating several diseases. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), the main enzymes in the transsulfuration pathway, catalyze H_2S production in mammalian tissues. However, the distributions and precise roles of these enzymes in the kidney have not yet been identified.In this study,we examined the localization of CSE and CBS in kidney and the effect of H_2S donor, NaHS in renal peritubular capillary (PTC) blood flow velocity, diameter and blood flow.

Methods: The normal mice (nTg) and pancreatic β-cell-specific calmodulin-overexpressing transgenic mice (CaMTg) as a model of diabetes at 3 month old were used. Immunohistochemical studies examined localize of CSE and CBS expression in nTg kidneys. We compared with the expression levels of CSE and CBS in CaMTg and nTg by Western blot analysis. And then we examined effect of NaHS on PTC blood flow velocity,diameter in nTg and CaMTg by obtained using an intravital video CCD.

Results: In the nTg kidney, we detected expression of both CBS and CSE in the brush border and cytoplasm of the proximal tubules, but not in the glomeruli or distal tubules.CSE

expression was markedly reduced under diabetic conditions, whereas CBS expression was unaffected. Administration of the NaHS increased PTC diameter and blood flow. Progressive diabetic nephropathy showed vasoconstriction and a loss of blood flow in PTCs that was ameliorated by NaHS treatment.

Conclusions: The biological profile of CSE resembles that of endothelial nitric oxide synthase (eNOS). These findings suggest that CSE expression in the proximal tubules may also regulate tubulointerstitial microcirculation via H_2S production. H_2S could represent a target of treatment to prevent progression of ischemic injury in diabetic nephropathy.

TH-PO150

***In Vitro* and *In-Vivo* Characterization of New Pumping System in a New Hemodialysis Device**

Matthew R. Muller,[1] Neil Tiwari,[1] Martin Desch,[3] Jeff McKee,[2] Benjamin Brooks,[2] Angelito A. Bernardo.[1] *[1]Baxter Healthcare Corporation, McGaw Park, IL; [2]Baxter Healthcare Corporation, Roundlake, IL; [3]DEKA Research and Development, Manchester, NH.*

Background: Hemodialysis devices typically use peristaltic pumping technology. A new hemodialysis device under development uses a pneumatically controlled diaphragm pump (PDCP). The pump contains a fluid and air compartments with flexible diaphragm separating the two sides. Positive or negative pressure is applied to the air compartments to move fluid into and out of the fluid compartment of the pump.

Methods: *In vitro* studies (n=12) were performed during simulated hemodialysis treatments (4 h, blood flow Qb 400 ml/min) with bovine blood and donated human blood in the new device and hemodialysis device with peristaltic roller pump (PRP). For bovine blood, PCDPs were heat aged for 3-year shelf life (70° C, 40 days, 65±5% humidity) per American Society for Testing and Materials standards (ASTM F1984). Hemolysis was evaluated by modified index of hemolysis (MIH) per ASTM F1841-97. *In vivo* studies in sheep (n=6) were done with the PCDP device during simulated 4 h dialysis at Qb 400 ml/min. Hemolysis was assessed by mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), plasma free hemoglobin, and lactate dehydrogenase (LDH) levels.

Results: The MIH in the heat aged PCDP was 0.68±1.32 and 1.86±0.53 for the PRP (p=0.01). When human blood was used, the MIH was 0.55±0.09 for PCDP in contrast to 1.16±0.36 for PRP (p=0.0007). Using the sheep model during simulated dialysis the PCDP device showed stable levels of MCV, MCH and MCHC during the 4 h treatment. The LDH levels and free hemoglobin levels were maintained at baseline levels throughout the 4 h session.

Conclusions: The new device using PCDP demonstrated significantly reduced hemolysis as compared to the device with PRP *in vitro* using bovine and human blood. Data from sheep dialysis showed stable markers of hemolysis. Therefore, the PCDP in the new hemodialysis device may provide an alternative option for hemodialysis. Additional testing will be performed in human clinical trials.

Funding: Pharmaceutical Company Support

TH-PO151

Autologus Adipose Tissue Depots To Inhibit Arteriovenous Graft (AVG) Stenosis

Christi M. Terry,[1] William G. Sanders,[2] Huan Li,[1] Yuxia He,[1] Alfred K. Cheung.[3,1] *[1]Medicine, Univ. of UT, SLC; [2]Pharmaceutics, Univ. of UT, SLC; [3]Medicine, VASLHCS, SLC, UT.*

Background: AVG often fail after clotting due to underlying neointimal hyperplasia (NH). Adipose tissues (AT) produce adiponectin (ADPN), that inhibits smooth muscle cell (SMC) proliferation, a contributor to NH. The anti-diabetic vasculoprotective glitazones induce ADPN from AT. The transplant of autologous AT mixed with glitazone to the AVG perivascular surface to serve i)as an *in situ* bioreactor of ADPN, and ii) as a sustained delivery depot of glitazones, was examined as a novel means to inhibit AVG stenosis.

Methods: Human AT was mixed with dry glitazone (rosiglitazone (ROS) or pioglitazone (PGZ)) and cultured. Culture media was analyzed by ELISA for ADPN and monocyte chemotactic protein-1 (MCP-1), and for drug by HPLC-MS/MS. The effect of conditioned media on platelet-derived growth factor (PDGF)-induced SMC proliferation (CyQuant), and LPS-induced release of TNFα from monocytes was assessed (ELISA). The feasibility and safety of a perivascular AT-PGZ depot was examined in a porcine model.

Results: ADPN release was similar from AT-ROS and untreated AT (ND-AT) at day 2 but increased 4-fold from AT-ROS over ND-AT by day 8. In contrast, ADPN release from AT-PGZ was 4-fold above ND-AT at day 2 but dropped to levels similar to ND-AT by day 8. MCP-1 release from ROS-AT or PGZ-AT was attenuated compared to ND-AT ($p<0.005$). LDH release, a marker of toxicity, was similar between treated and untreated AT. Forty percent of drug was released from ROS-AT by day 13. The conditioned media from day 6 ROS-AT or ND-AT inhibited SMC proliferation (~55% or 35%, respectively); however only media from ROS-AT attenuated TNF release from LPS-treated monocytes implicating glitazones in the monocyte inhibition. An autologous AT-PGZ(100μM) depot was applied perivascularly to the jugular vein in a pig. No overt inflammation or necrosis was noted when explanted at 6 days.

Conclusions: ADPN release was enhanced from glitazone-AT and prolonged release of drug from the depot was observed. In vivo transplant of the PGZ/AT was well-tolerated. These data support the testing of glitazone-AT perivascular transplants for the prevention of AVG stenosis in the porcine model.

Funding: Other NIH Support - NIH RO1 HL067646-04A1, Veterans Administration Support, Private Foundation Support

TH-PO152

Enhancement of Middle-Molecular-Weight Solute Clearance by Mechanical Vibration: *In Vitro* Study

Mauro Neri,[1,2] Jeong Chul Kim,[1,2] Francesco Garzotto,[1] Massimo de Cal,[1] Dinna N. Cruz,[1,2] Federico Nalesso,[1] Alessandra Brendolan,[1] Claudio Ronco.[1,2] *[1]Nephrology, San Bortolo Hospital, Vicenza, Italy; [2]International Renal Research Institute Vicenza, Italy.*

Background: Phenomena such as concentration polarization and protein gel layering on the dialyzer membrane surface can significantly reduce middle molecule clearance. Previous simulation studies showed that high wall shear stress and vortices induced by a shaking motion could reduce the development of resistance layers on membrane surfaces. We analyzed the effects of mechanical shaking of hemodialyzers on the clearance of β-2 microglobulin (β-2MG) in HD mode using 2 different membranes (PS vs PAN).

Methods: We collected spent dialysate from a patient with high initial concentration of β-2MG and concentrated this using HF with a low flux dialyzer. We added urea to check for a mass balance error. We applied HD treatment in a crossover study with a mechanical shaker (driven by a crank-slide mechanism) at 0,1,2,3,4 Hz (cycles/sec) of lateral shaking frequency and 20mm of amplitude. Samples were taken every 4 minutes at blood inlet, blood outlet and dialysate outlet for urea and β-2MG measurements.

Results: The blood side clearance (Kb) of β-2 MG increased in PS while in PAN the dialysate side clearance (Kd) increased. Vibration frequency was not linearly proportional to β-2 MG clearances in PS. It increased desorption of proteins into blood and dialysate compartments in PAN, so the concentrations in blood outlet and dialysate outlet increased. (Kb-Kd) indicates the removal by adsorption.

f	Kb in PS	Kd in PS	Kb-Kd in PS	Kb in PAN	Kd in PAN	Kb-Kd in PAN
0	87.9	84.9	3	130.2	2.5	127.7
1	94.8	93	1.8	110.8	53.4	57.2
2	104.5	83.7	20.8	105,7	11.2	94.5
3	95.7	90.8	4.9	90.1	18.5	71.6
4	95	94.2	0.8	88.6	21.4	67.2

Conclusions: Shaking motions of PS and PAN hemodialyzer could increase the clearance of β-2 MG. To apply this dynamic dialysis method to clinical practice, appropriate selection of membrane material and optimal shaking conditions should be investigated considering hemolysis and mechanical fracture of membrane.

TH-PO153

A Biodegradable Perivascular Wrap for Controlled and Directed Drug Delivery To Prevent Arteriovenous Graft (AVG) Stenosis

William G. Sanders,[1] Paul C. Hogrebe,[4] Alfred K. Cheung,[3,2] Christi M. Terry.[2] *[1]Pharmaceutics, Univ. of UT, SLC; [2]Medicine, Univ. of UT, SLC; [3]Medicine, VASLHCS, SLC, UT; [4]Bioengineering, Univ. of UT, SLC.*

Background: Stenosis of AVG due to neointimal hyperplasia (NH) at the anastomoses is common. Sunitinib decreases smooth muscle cell (SMC) proliferation and angiogenesis; both processes are integral for NH formation. We report the development of a biodegradable perivascular wrap that provides unidirectional diffusion of sunitinib towards the graft and vascular walls, and should minimize drug loss to the extravascular tissues while inhibiting NH.

Methods: Two formulations were created: In "A", sunitinib was dissolved in a non-porous poly(lactic-co-glycolic acid) (PLGA) wrap. In "B", dry sunitinib was mixed into a hyaluronic acid (HA) hydrogel which was then infused into a porous PLGA network. A pliable non-porous PLGA backing without drug was incorporated onto both formulations to promote unidirectional diffusion. The in vitro drug release from the formulations into the media was assayed by HPLC-tandem mass spectrometry. Unidirectional drug release was tested using a modified co-culture chamber with the non-porous backing as the separating insert. Drug traffic from "A" into tissue was tested with an explanted porcine artery in an ex vivo flow chamber.

Results: Formulation "A" yielded a slow release rate, with drug exhausted after 40 days. Formulation "B" produced a high initial burst with almost complete release by 14 days. The non-porous PLGA backing effectively prevented drug diffusion into the lower co-culture chamber. The sunitinib concentration within the artery wall tissue at 24 h after wrap placement was 17.8 nM, corresponding to three times the in vitro EC50 for SMC inhibition and 0.4% of the total drug load. Concentrations in the circulating media were 0.72 nM (0.014% of total load) after 24 h, suggesting some trafficking of the drug through the vessel wall into the lumen.

Conclusions: Our biodegradable formulation "A" polymer wrap provided a directed, sustained release of sunitinib that rapidly achieved high vascular wall tissue drug concentrations. This data supports the further characterization of the formulation in vivo in a porcine model of AVG stenosis.

Funding: Other NIH Support - 2RO1 HL067646, Veterans Administration Support, Private Foundation Support

TH-PO154

Prototyping a Peritoneal Dialysis (PD)-Based Automated Wearable Artificial Kidney (AWAK): A Progress Report Christian G. Bluchel,[1] Martin Roberts,[2-4] Jui Pin Er,[1] David B. Lee.[2-4] *[1]Temasek Engineering School, Temasek Polytechnic, Singapore; [2]VAGLA Healthcare System, Los Angeles, CA; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]AWAK Technologies, Singapore.*

Background: AWAK is designed to provide round-the-clock, dialysis-on-the-go for patients with end-stage renal disease (ESRD). Based on sorbent technology, and tidal peritoneal dialysis (TPD), AWAK continuously regenerates spent dialysate and recycles fresh dialysate at rates up to 96 L/day. This is an update on our prior reported prototype.

Methods: The first prototype consists of a disposable and a non-disposable assemblies. TPD is conducted by first draining the tidal volume (TV) out of the peritoneal cavity (PC) into the disposable assembly (outflow mode, OM). Following regeneration of the spent dialysate, the fresh dialysate is returned into the PC (inflow mode, IM). Cycles of OM-IM continued until the time for sorbent cartridge (SC) replacement. The PC is then completely drained into an UF receptacle. A volume equal to TV plus RV is returned to the PC and the remainder fluid is discarded as UF. Several improvements have been made on this prototype and using the new set up we have validated the regeneration and reconstitution of spent peritoneal dialysate.

Results: In the updated design all wetted components are completely housed in the disposable assembly, which is replaced with each cartridge exchange, i.e. every 7 or 12h, depending of the type of cartridge used. A common non-disposable assembly controls all fluid movements. It runs no contamination risk, given its never wetted by the dialysate. The configuration using the "regular", 7h SC weighs less than 1kg and is easily wearable, e.g., in a shoulder bag, without attracting undue attention. It regenerates and reconstitutes fresh dialysate (similar in composition to the "2 bag bicarbonate dialysate") from both a synthetic spent peritoneal dialysate and spent dialysate from PD patients. The intermittent UF phase with complete drainage of the PC makes the "overfill"syndrome unlikely

Conclusions: Further safety measures and miniaturization have been successfully engineered into the AWAK prototype for animal and clinical trials.

Funding: Private Foundation Support

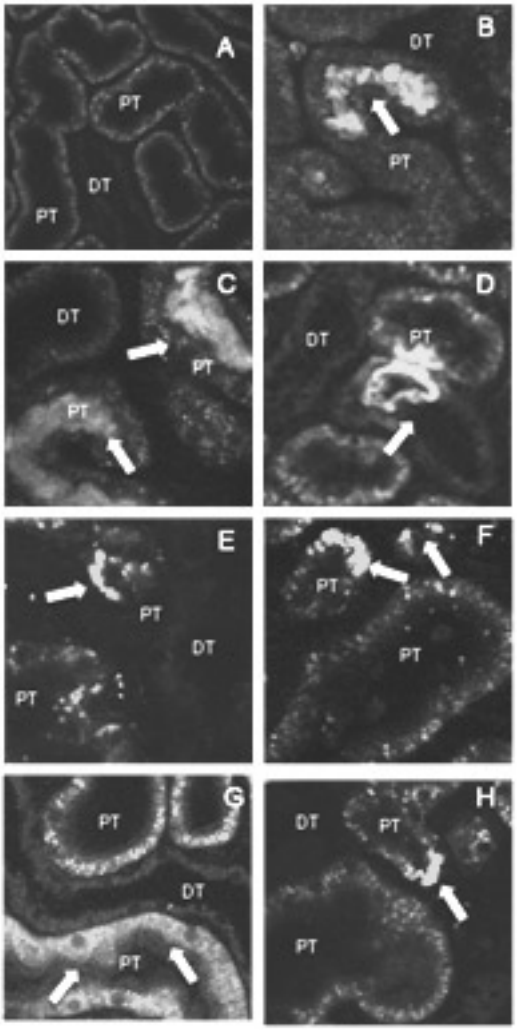
TH-PO155

Hydrodynamic-Mediated Transgene Expression of Baculovirus Vectors in Live Mammalian Kidneys Peter R. Corridon, George Rhodes, Simon J. Atkinson, Robert L. Bacallao. *Medicine, Indiana University, Indianapolis, US.*

Background: Viral vectors have been investigated for their use in mammalian gene transfer for decades. While gene transfer has been difficult to accomplish in the kidney, reports have indicated that hydrodynamic cavitation may be used to facilitate renal gene transfer.

Methods: We present a method that utilizes tissue cavitation to deliver bacuolvirus vectors for relatively rapid renal transfection in live animals. In devising this approach we hypothesized that the hydrodynamic forces generated from pressured injections were sufficient to facilitate both the passage of baculoviral particles across epithelial and endothelial layers, and ultimately their endocytic cellular uptake. We tested this hypothesis using Green fluorescent protein (GFP) and GFP-actin chimeric bacuolvirus vectors.

Results: GFP and GFP tagged actin expressing baculovirus vectors were introduced into 30 rodent kidneys using renal vein guided, retrograde pressurized injections. Transgene incorporation and expression was examined using intravital multiphoton fluorescence microscopy. This method produced detectable levels of protein expression in various tubular, glomerular and vascular segments, throughout a period of 3 weeks. Figure 1 shows intravital multiphoton microscopy images taken from kidney proximal tubules (PT) and distal tubules (DT) from Sprague-Dawley rats that received GFP-actin baculovirus vectors. The images were taken across a 3 week time frame, post viral delivery: (A) tissue autofluorescence, (B) Day 1, (C) Day 2, (D) Day 3 (E) Day 4, (F) Day 5, (G) Day 6 and (H) Day 21. Arrows are used to indicate regions with transgene expression. Moreover, we found that transgene expression was stable for 21 days.



Conclusions: These results outline a potential approach for renal gene transfer in live animals employing a simple, reproducible delivery system.

Funding: NIDDK Support

TH-PO156

Physiologic Assessment of Arteries for Arterio-Venous Fistula (AVF) Creation for Hemodialysis Access Rosa M. Marticorena,[1] Cesar Ginocchio,[1] Arash Jaberi,[2] Niki Dacouris,[1] Vern Malcolm Campbell,[1] Sandra A. Donnelly.[1] *[1]Nephrology, Keenan Research Centre Li Ka Shing Knowledge Institute, St. Michael' Hospital, University of Toronto, Toronto, ON, Canada; [2]Radiology, Ottawa Hospital, Ottawa, ON, Canada.*

Background: Achieving adequate flow for hemodialysis treatment is the primary goal of fistula creation. Arterial dilatation is essential to increase radial artery flows from 20-30 mL/min to 600-1200 mL/min. Arterial size is insufficient to determine if fistula maturation will occur as evident by a 40% rate of failure to mature in spite of having the requisite 2mm diameter and the successful creation of fistula in children. The increased blood flow provides the necessary hemodynamic change in venous wall shear stress that is a key determinant of outflow vein dilatation. The objective of this work was to determine if healthy arteries defined by normal physiologic parameters of pulse wave velocity (PWV), augmentation index (AIX) and elasticity was associated with adequate vascular remodeling to generate successful AVF maturation.

Methods: End stage renal disease patients of a tertiary care academic hemodialysis program were studied between June 2009 and Dec 2010. Patients who had functioning fistulas (Group I n=28) were compared to patients who had a failed fistula (Group II n=10). Blood pressures and radial artery waveform were recorded with HDI/Pulsewave CR-2000 Research CardioVascular Profiling Systems (Hypertension Diagnostics, Inc,Minnesota, USA) and large and small artery elasticity were estimated by a modified Windkessel model. Carotid, radial and femoral pulse wave velocity and waveform were evaluated with SphygmoCor® (AtCor Medical, Inc., IL, USA).

Results:

	Group I	Group II
Age (years)	52±14	59±11
AIX	26.0±10.2*	35.2±11.1
PWV (M/sec)	8.6±1.3*	7.2±1.3
Mean±SD *p<0.05 Group I vs Goup II		

In multivariate analysis, successful AVF maturation was negatively correlated with AIX and DM and was positively correlated with PWV and male sex. Measures of large artery stiffness and small artery elasticity were not associated with successful AVF maturation.

Conclusions: These preliminary observations suggest that physiologic assessment of arteries may enhance vessel selection for AVF creation.

TH-PO157

Generation of Infusible Dialysate through a New Hemodialysis System

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Background: A new hemodialysis system under development generates online infusible dialysate for priming, bolus and rinse back eliminating the need for bagged solutions. To meet the American Association for Medical Instrumentation (AAMI) standards for infusible dialysate, the system is required to achieve 9-log reduction in bacteria and at least 2-log reduction in endotoxin. The system uses sequential ultrafiltration via an endotoxin and bacterial retentive ultrafilter and a polyethersulfone (PES) dialyzer as the redundant filter. The system also employs high temperature non-chemical heat disinfection to maintain high level of microbial control over time.

Methods: The ultrafilters and PES dialyzers were exposed to 90 and 30 cycles respectively of 75 ± 5° C hot water for 45 min to simulate maximum heat stress. This system was challenged in triplicate with at least 40 liters of bacterial suspension containing 10^6 CFU/ml and endotoxin content of 10^2 IU/ml. American Type Culture Collection isolates of *B. cepacia, P. aeruginosa, B. diminuta* and *M. abscessus* were used for microbial challenge. During repeated simulated dialysis sessions, bacterial and endotoxin levels were measured pre- and post-ultrafilter and post-dialyzer (venous line sample).

Results: Dialysate fluid post ultrafilter, met AAMI ultrapure dialysate standards with bacteria levels of <0.1 CFU/ml, and endotoxin level of <0.03 IU/ml. The quality of the post-dialyzer venous blood line samples (for priming, bolus and rinse back) met the AAMI standards for infusible dialysate with no bacteria being cultured (0 CFU/L) from all samples representing 9 log reduction and endotoxin levels of <0.03 EU/ml, representing greater than 2 log reduction in endotoxin.

Conclusions: A new hemodialysis system using sequential ultrafiltration via endotoxin and bacterial retentive filter and PES dialyzer is capable of generating infusible dialysate when challenged with markedly high levels of bacteria and endotoxin. Therefore, generation of infusible quality dialysate has been shown using a robust *in vitro* testing procedure.

Funding: Pharmaceutical Company Support

TH-PO158

Effect of Serum Thyroid Stimulating Hormone Levels on Glomerular Hemodynamics Examined by Inulin and Para-Amino-Hippuric Acid Clearance

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Background: Glomerular hemodynamics can be examined by the method of Gomez's formula, in which both inulin and para-amino-hippuric acid (PAH) clearance are simultaneously measured. Although the effect of drugs is examined, few have examined the effect of intrinsic hormones on glomerular hemodynamics. Hypothyroidism is reported to reduce glomerular filtration rate (GFR), although its mechanism is unknown. In the present study, we examined the effect of serum levels of thyroid stimulating hormone (TSH), which has been demonstrated to affect peripheral arterial resistance (J Clin Endocrinol Metab 89:3455, 2004), on glomerular hemodynamics.

Methods: Twenty eight patients (60.6 12.8 years, 9 males and 19 females, serum creatinine < 1.0 mg/dl) with mild IgA glomerulonephritis (n = 5) or type 2 diabetes (n = 23) ,who agreed with the study, were examined. All patients were of euthyroidism. Inulin and PAH clearance were simultaneously measured according to the methods of Horio et al (Clin Exp Nephrol 13:50, 2009). Resistance of afferent arteriole (R-a) and efferent arteriole (R-e) was calculated with use of Gomez's formula.

Results: Both R-a and R-e correlated with age (r = 0.352, p = 0.0353; r = 0.311, p = 0.0647, respectively). There was a significant positive correlation between serum TSH and R-a (r = 0.531, p = 0.0063), but not between serum TSH and R-e. Renal plasma flow (RPF), renal blood flow (RBF), and GFR tended to negatively correlate with serum TSH levels (r = - 0.351, p = 0.0850; r = - 0.354, p = 0.0821; r = - 0.389, p = 0.0547, respectively). In a multiple regression analysis, serum TSH was significantly and positively associated with R-a (= 0.459, p = 0.0240), after adjustment of blood pressure and age (R^2 = 0.330, p = 0.0350), although it was not with R-e.

Conclusions: Using inulin and PAH clearance, present study indicates that TSH, which causes increased peripheral arterial resistance, increases R-a, possibly being a cause of decrease in RBF, RPF, and GFR. Subclicical hypothyroidism is suggested to affect renal hemodynamics.

TH-PO159

Usability Testing of an Educational Website for Patients with Chronic Kidney Disease: Results from Safe Kidney Care

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Background: Web-based technology is critical to the future of healthcare. As part of a larger study (Safe Kidney Care [SKC]) evaluating patient safety in chronic kidney disease (CKD), we sought to determine how well a representative sample of patients with CKD could interpret and use the SKC website (www.safekidneycare.org), an informational website aimed at providing disease-specific safety information to patients with CKD.

Methods: Participants enrolled from CKD clinics at the University of Maryland underwent formal usability testing administered to each participant independently by a single interviewer with a second recording observer. Each participant was provided a list of 21 tasks to complete as part of formal computer-based testing, including items such as "Go to the internet", "What kinds of information do you think you could get from this website?", "Point to 2 places where you can find a calculator to estimate your kidney function", and "Click on the link to a page that talks about safety concerns with fluids." Tasks were rated as either "easily completed or a non-critical error", or "critical error" (user cannot complete a task without significant interviewer intervention).

Results: 8 participants representative of the CKD population completed formal usability testing. Median completion time for all tasks was 17.5 minutes (range 10-39 minutes). In total there were 32 critical errors in 162 tasks (19%), with the highest proportion of critical errors occurring when participants were asked to find the website on the internet, increase font size, scroll to the bottom of the webpage, or find information on treatments that may damage kidneys. Participants were generally satisfied with the content and usability of the website.

Conclusions: Web-based educational materials for patients with CKD should target a wide-range of computer literacy levels and anticipate variability in competency in use of the computer and internet.

Funding: NIDDK Support

TH-PO160

Predicting the Risk of Type I Diabetic Nephropathy with Single Nucleotide Polymorphisms Using Support Vector Machines

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Background: In a prior genome wide association study (GWAS) of Type 1 diabetic nephropathy (GoKinD study), Pezzolesi et al identified 13 SNPs associated with disease utilizing logistic regression (LR) models. Support vector machine (SVM) is a novel machine learning classifier that has been shown to be superior for multidimensional data such as GWAS data.

Methods: We utilized the PLINK whole genome association analysis toolset, for performing quality control of the GoKinD dataset. We used WEKA, an open source data mining package that provides multiple classifiers, to run both logistic regression and SVM with the Radial Base Function (RBF) kernel model.

Results: We identified 11 markers at a threshold of p<10-5, two of which (rs1411766, rs10868025) overlapped with SNPs identified using LR (Pezzolesi et al), and a third, rs10868025 was in compete linkage disequilibrium with rs1411766; we also noted that p-values were roughly similar, using both approaches. Higher p-value thresholds using larger feature spaces, improved the sensitivity and specificity of SVM prediction over the LR approach.

Comparison of p-values generated between our results and Pezzolesi et al. (2009)

SNP	P value Pezzolesi et al. (2009)	Our P-value
rs39059	5.0 x 10-6	1.9 x 10-4
rs10868025	5.0 x 10-7	1.27 x 10-7
rs739041	6.4 x 10-6	6.3 x 10-5
rs451041	3.1 x 10-6	3.6 x 10-5
rs1041466	3.1 x 10-6	1.5 x 10-5
rs1411766	1.8 x 10-6	4.0 x 10-6
rs64922208	6.1 x 10-6	1.8 x 10-5
rs7989848	7.0 x 10-6	2.3 x 10-5
rs9521445	2.9 x 10-6	1.0 x 10-5

Conclusions: Using SVMs, we were able to identify several novel SNPs correlated to type 1 diabetic nephropathy. Taking a wide net approach and testing all highly correlated SNPs will generate more accurate models. We intend to validate the models on an external data set (DCCT-EDIC).

TH-PO161

In Vivo siRNA Targeting to the Podocyte John N. Vassiliadis,[1] Stephen O'Brien,[1] Pradeep K. Dhal,[2] Susan Schiavi,[1] Steven R. Ledbetter,[1] Cynthia M. Arbeeny,[1] Stefan Wawersik.[1] *[1]Renal and Endocrine Sciences, Genzyme Corporation, Framingham, MA; [2]Polymer Chemistry, Genzyme Corporation, Waltham, MA.*

Background: Development of methods for rapid in vivo knockdown of gene function by siRNA would greatly aid in the validation of therapeutic targets for drug discovery. Because of the central role of the podocyte in glomerular disease, siRNA targeting to these cells is of particular interest. Here, we test the ability of polymer-bound siRNA to reduce gene expression in podocytes, focusing on the slit diaphragm protein nephrin. In the kidney, nephrin is expressed solely in the podocyte, and loss of nephrin function leads to rapid increase in urinary protein excretion.

Methods: Nephrin-specific siRNA was complexed with a poly(ethylene glycol)-poly(L-lysine) block copolymer and the complex was administered intraperitonially.

Results: After 72 hours, quantitative real-time PCR showed a 70% decrease in nephrin mRNA and a 68% decrease in nephrin protein levels when compared to animals injected with a non-targeting siRNA. In addition, we observed a doubling of urinary albumin levels after nephrin siRNA administration.

Conclusions: We are currently testing whether multiple doses of nephrin siRNA can further knock down gene expression and induce more severe proteinuria, as well as evaluating the targeting of siRNA to other tissues through this method. Taken together, these data show a rapid and effective method to target gene expression in the podocyte which may drastically reduce the time and cost of validating new therapeutic targets for glomerular disease.

Funding: Pharmaceutical Company Support

TH-PO162

Quantification of Interstitial Fibrosis by Second Harmonic Imaging in Rat Kidney Tissue Sections Hu Sheng Qian,[1] Damian C. Matera,[1] Steven M. Weldon,[1] Chung-Wein Lee,[1] Agnes B. Fogo,[2] Glenn A. Reinhart.[1] *[1]CardioMetabolic Disease Research, Boehringer Ingelheim Pharmaceutics, Inc, Ridgefield, CT; [2]Department of Pathology, Vanderbilt University School of Medicine, Nashville, TN.*

Background: Interstitial fibrosis is a powerful indicator of nephropathy in chronic kidney diseases. However, accurate quantitative analysis of fibrosis by Sirus Red morphometry (SRM) remains a challenge. Our aim was to establish an imaging technique that would enable us to easily and accurately quantitate fibrillar collagen in rat kidney tissues.

Methods: We used a Multiphoton System to develop the novel methodology of Second Harmonic Generation (SHG) for imaging renal fibrosis. 3μm unstained sections of rat kidney at day 5 after unilateral ureteral obstruction (UUO) were assessed for cortical fibrosis. The Two Photon Excitation Fluorescence (TPEF) allowed for the visualization of kidney background and tubular organization, while SHG was used for the display of the architecture of fibrillar collagen.

Results: To validate the specificity of the fibrillar components detected by the SHG in renal fibrosis, the relationship between SHG signal and immunohistochemistry for collagens Type I, III, and IV was analyzed. Our results showed that the SHG signal strongly co-localized with fibrillar collagens I and III, but not with collagen IV. To examine relationship between SHG imaging and traditional SRM analysis of global fibrosis, kidneys from rats at day 5 after UUO treated with ACEI (n=6 /group) were also assessed by both methods with similar trends of quantitative fibrotic area. However, SRM showed 23.2% to 38.9% reduction of fibrotic area by ACEI, while SHG showed 36.1% to 50.2% reduction of fibrosis. Our results demonstrate that SHG imaging can be used for specific detection of the main types of fibrillar collagens involved in renal fibrosis, and can offer more sensitive and a greater dynamic range for evaluation of the therapeutic effects on interstitial fibrosis.

Conclusions: We conclude that combination of SHG and TPEF imaging of unstained kidney tissues provide a novel alternative to traditional SRM and is a powerful tool for the quick visualization and quantitative assessment of renal fibrosis

TH-PO163

Assessment of Renal TSPO Expression Using Micro PET Imaging in Mice Mohammed Noor Tantawy,[1] H. Charles Manning,[1] Keiko Takahashi,[2] Hiroki Fujita,[3] Christopher Chad Quarles,[1] Raymond C. Harris,[2] John C. Gore,[1] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN; [3]Akita University Graduate School of Medicine, Akita, Japan.*

Background: Translocator protein18-kDa (TSPO) is a crucial mitochondrial protein involved in various cellular functions, including cholesterol metabolism, steroidogenesis, and apoptosis. TSPO is abundantly expressed in kidney tissue; however, its involvement in renal pathology is poorly understood. To effectively characterize the TSPO expression in mouse models of kidney diseases, here we evaluated a TSPO PET imaging assay for mouse kidney using a novel radioligand, [^{18}F]PBR06.

Methods: Mice were injected with a radioligand, [^{18}F]PBR06. 37 MBq i.v. and imaged by a microPET system for 90 min in a dynamic sequence. The time-activity curves (TAC) of the kidney were recorded over the duration of the scans. Specificity of binding was evaluated by displacement of [^{18}F]PBR06 with excess PBR06. Adding to wild type mice, 20-wks old db/db or non-diabetic db/m mice were subjected to the imaging assay. TSPO protein levels in kidney tissues were assessed by Western blot.

Results: The TACs plateaued in kidney at 50-80 min, indicating a state of equilibrium, and the cold ligand displaced ~75% of the radiotracer. The probe was not present in bladder at this time, indicating that the kidney signals resulted from binding of [^{18}F]PBR06 to renal TSPO but not excretion. Interestingly, [^{18}F]PBR06 binding was remarkably reduced in db/db kidney compared to db/m kidney, while no difference was observed in liver: % injection dose per kidney; db/m 10.5 ± 0.2% vs. db/db 6.2 ± 0.5%. Similar reduction in renal TSPO protein was also observed in db/db mice by Western blot.

Conclusions: Renal TSPO expression can be assessed by *in vivo* PET imaging using [^{18}F] PBR06. Reduced expression in db/db mice suggests a significant role of TSPO in diabetic nephropathy.

Funding: NIDDK Support

TH-PO164

α3 Chain Type IV Collagen as an Non-Invasive Optical Biomarker for Chronic Glomerular Diseases Kapil Chaudhary, Nino Kvirkvelia, Maggie McMenamin, Michael P. Madaio. *Department of Medicine, Georgia Health Science University, Augusta, GA.*

Background: Type IV collagen, a major structural component of the GBM, with relatively limited expression including the kidney. Expression of α3 chain of type IV collagen, α3(IV) is altered during the course of chronic kidney disease. Hence, irrespective of the underlying etiology, quantification of α3(IV) expression has a potential as a biomarker of kidney disease progression. For this purpose, we hypothesized that ex vivo quantification of human anti- α3(IV)NC1 antibody (Ab) binding, in real time, could serve this role.

Methods: Human mAb F1.1 against α3(IV)NC1 was conjugated with amine reactive near infrared fluorophore (Dylight 750 or 800). Effective conjugation was determined by 20% SDS PAGE with antigenic specificity of the conjugate maintained. Following injection of conjugated mAb F1.1 into normal mice, live animal imaging was performed by XenogenTM camera; under anesthesia. Kidney specific fluorescence was quantified by software provided with the live imaging system. Pharmacokinetics of fluorophore-mAb F1.1 was studied by imaging the mice at 6,12,24,36 & 72 hrs after injection in the prescence of suitable positive and negative controls. Tissue specificity of fluorophore-mAb F1.1 was evaluated by analysis of formalin fixed kidney, liver, lung, spleen and heart under XenogenTM camera. Glomerular specificity of human mAb F.1.1- fluorophore conjugate was further evaluated by IF on 4 μm kidney tissue sections from same experiment.

Results: Human mAb F1.1 was effectively conjugated with fluorophore Dylight 750 and 800. After injection of conjugated F1.1, kidney specific fluorescence was highest at 24-36 hrs and was detectable after even 72hrs. mAb F1.1-fluorophore conjugate is able to provide non-invasive quantification of α3 chain, type IV collagen in kidney.

Conclusions: The results provide the potential to pursue human mAb F1.1 as an optical biomarker of progressive glomerular disease in experimental kidney disease models by providing non-invasive quantification of type IV collagen. If successful, this approach and reagents/antibody will have a potential application to patients with many forms of chronic kidney disease.

Funding: NIDDK Support, Private Foundation Support

TH-PO165

Optimization of High Field MRI Methods for the Mouse Kidney Imaging Feng Wang,[1] Rosie T. Jiang,[2] Mohammed Noor Tantawy,[1] Keiko Takahashi,[2] Raymond C. Harris,[2] Christopher Chad Quarles,[1] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.*

Background: MRI could complement the physiological information obtained from conventional assays of kidney function and facilitate better understanding of pathological process in kidney disease. However, the application of MRI techniques to mouse renal models is highly limited due to the small size and motion artifacts. In this study, high-field MRI methods were optimized for the imaging of mouse kidney, and applied to serial assessment of UUO injured kidney.

Methods: T1W, T2W and MTC imaging protocols were optimized on 7T MRI system to enhance the structural delineation, signal to noise, and image contrast. Several rapid acquisition MRI methods were tested to minimize motion artifacts, including coherent gradient echo and multi-slice inversion-recovery fast gradient echo. Respiratory gating and fat saturation were also considered.

Results: We demonstrate that a coherent GE sequence is superior to IR fast GE for T1W. T1 contrast was optimized with a ~35° flip angle at TR 45ms. A fast spin echo sequence yielded reliable T2W and optimal contrast was achieved with a TE of ~50 ms (TR 2000ms). The MT images were optimized using the GE sequence similar to that used for T1W and the greatest improvement was observed when fat saturation was employed. High-field T1W, T2W and MTC images enabled the qualitative and quantitative evaluation of structural (size, shape, contrast, thickness) variations in mouse UUO model. Structural abnormalities were observed in inner medulla and papilla of UUO kidney as early as 3 hrs after surgery. Deformation of medullary rays of UUO kidney became evident at day 3. In T2W and MTC, the ratio of intensity between cortex and medulla decreased as the disease progressed, while it was not altered in T1W.

Conclusions: We have developed the optimal MRI protocols to allow the visualization of fine renal structural details, achieving resolutions of 0.1 x 0.1 x 0.5 mm^3. These methods should be effectively used for evaluating renal structural integrity in mouse models of kidney diseases.

Funding: NIDDK Support

TH-PO166

MRI Assessment of Mouse Kidney Blood Volume at 7T Feng Wang,[1] Rosie T. Jiang,[2] Mohammed Noor Tantawy,[1] Keiko Takahashi,[2] Raymond C. Harris,[2] Christopher Chad Quarles,[1] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.*

Background: MRI mapping of relative blood volume (RBV) is widely used to assess pathological differences in tissue vascular density. However, this method has not been applied to renal disease. In this study, we optimized MRI acquisition methods for RBV mapping in mouse kidney and assessed the vascular property in UUO model.

Methods: Multi-slice T2-weighted fast spin echo images (TR = 2000ms, TE = 48ms, RARE-factor = 8, 256^2 matrix, 25.6 mm^2 FOV, 0.5 mm slice thickness) of the mouse kidneys were acquired before and after the injection of an iron oxide contrast agent (CA) at 7T MRI. Navigator and respiratory gating was employed to minimize motion artifacts. RBV was calculated pixel by pixel from the steady-state T2-weighted images using a standard formula. The dose of CA was optimized based on the CNS value. Reproducibility of the RBV data was assessed by measuring RBV (n=10) on consecutive days. Renal RBV maps was evaluated in UUO mice (n=6) at 3hrs, 1, 3, 6, and 10 days.

Results: The dose of CA was optimized at 6 mg/kg for RBV mapping in mouse kidney. The mean kidney RBV measured on consecutive days was 19.97 ± 1.50 and 19.86 ±1.62, yielding a concordance correlation coefficient of 0.94, indicating that this approach is highly reproducible. The RBV values of medulla were rapidly reduced in UUO kidney, corresponding to the destructive morphology. The RBV values of cortex was also progressively declined in UUO kidney (~10% at day3, ~20% at day 6, ~50% at day 10), while those of contra-lateral kidney was gradually increased (~10% at day 6).

Conclusions: MRI measurements of renal RBV provide a valuable assay to characterize vascular density in renal disease.

Funding: NIDDK Support

TH-PO167

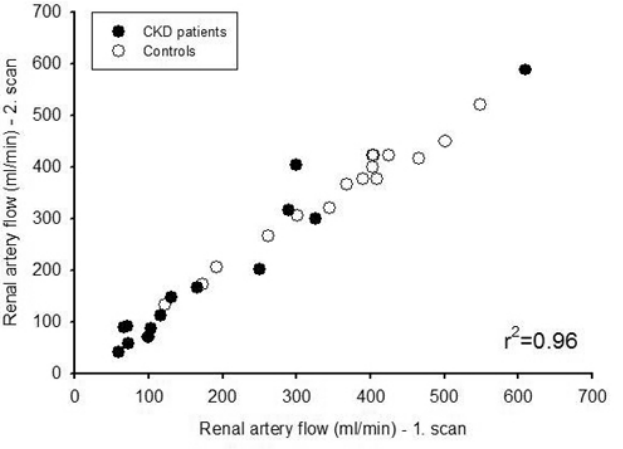
Reproducibility of Renal Artery Blood Flow and Intrarenal Oxygenation Measurements Using Magnetic Resonance Imaging in Patients with Chronic Kidney Disease and Healthy Controls Dinah Sherzad Khatir,[1] Michael Pedersen,[2] Steffen Ringgaard,[2] Bente Jespersen,[1] Niels Henrik Buus.[1] *[1]Department of Renal Medicine, Aarhus University Hospital, Skejby, Aarhus N, Denmark; [2]MR Research Center, Aarhus University Hospital, Skejby, Aarhus N, Denmark.*

Background: To evaluate the reproducibility of measurements of renal artery blood flow (RBF) and renal oxygenation by magnetic resonance imaging (MRI) in patients with chronic kidney disease (CKD) and healthy controls.

Methods: RBF and oxygenation in both kidneys were determined on two occasions with 1-2 weeks interval in 7 CKD patients (mean 65 years, eGFR = 30 ml/min) and 8 healthy volunteers (mean 43 years, eGFR >90 ml/min). All investigations were performed in a 1.5 Tesla Siemens Avanto MRI system. RBF was measured by phase-contrast sequence with velocity gradients applied orthogonally to the renal artery and data were acquired with ECG gating. RBF was calculated as the product of the cumulative vessel blood velocity and the diagonal vessel area. Regions of interest were drawn according to the vessel, and automatic segmentation was done with manual correction. Blood Oxygen Level Dependent (BOLD) MRI using R2*-sensitive echo planar imaging sequence was used as an estimate of renal oxygenation.

Results: Both scan procedures were successful in all subjects. RBF (ml/min/kidney) for patients were 189,5±153,2 and 190,4±158,1 and for controls 356±119,3 and 348,4±107,3 (P<0.05 vs. patients) for first and second scans(figure). In both patients and controls RBF measurements were highly reproducible with a linear correlation coefficient of r2=0.96. Renal medulla R2* (ms-1) for patients were 29,9±2,2 and 26,8±4,4 and for controls 25,9±2,5 and 26,1±2,2 (P>0.05 vs. patients) for first and second scans (r2=0.44).

Conclusions: MRI-based determinations of RBF and R2* are reproducible in CKD patients and healthy controls.



Funding: Government Support - Non-U.S.

TH-PO168

The Usefulness of Contrast-Enhanced Ultrasound (CEUS) for Diagnosis of Renal Cell Carcinoma by Employing a Time-Intensity Curve (TIC): The Characteristics of Microcirculation in Renal Cell Tumor Conformed by Renal Tumor Biopsy under CEUS Tokunori Yamamoto, Hideki Mizuno, Yasushi Yoshino, Momokazu Gotoh. *Urology, Graduate School of Medical Sciences, Nagoya University, Nagoya, Aichi, Japan.*

Background: To evaluate the usefulness of contrast-enhanced ultrasound (CEUS) for diagnosis of renal cell carcinoma by employing a time-intensity curve (TIC).

Methods: From May 2008 to October 2009, CEUS was performed prior to surgery in 30 patients with renal masses. 10 of the 30 patients had cystic renal masses. The final diagnoses of all patients were pathologically confirmed. Contrast enhancement as a function of time was measured in two (tumor or solid component of cystic lesions and normal parenchyma) regions of interest (ROI) and TICs were obtained. The time to the contrast enhancement peak (TTP), intensity change from the baseline to peak (ΔI), and ΔI / TTP of the tumor and the normal parenchyma were measured from the TIC. In adition, we performed renal tumor biopsy under CEUS to conform the characteristics of the TIC

Results: Pathological diagnoses were renal cell carcinoma in 30 patients. The TTP of the cancer was shorter than that of the normal parenchyma in all cases (6.0 ± 2.0 s vs. 10.4 ± 3.0 s; $p < 0.0001$). The ΔI did not differ between the cancer and normal parenchyma (21.3 ±5.9 db vs. 20.9 ± 7.0 db; $p = 0.68$); the ΔI / TTP of the cancer was significantly higher than that of the normal parenchyma (3.9 ± 1.4 db/s vs. 2.2 ± 0.94 db/s; $p < 0.0001$). TIC patterns of solid cancer and cystic cancer were very similar.TIC of the normal tissue and the cancer in pathology conformed by renal biopsy for the tumor under CEUS also demonstrated similar to the characteristics of the TIC, respectively.

Conclusions: An objective and quantitative diagnosis of renal cell carcinoma by CEUS using a second-generation ultrasound contrast agent can be made by employing a TIC. The TIC patterns of solid and cystic cancers were very similar, despite their morphological and vascular differences. CEUS using a TIC is a promising tool in the diagnosis of cystic renal cancer.

TH-PO169

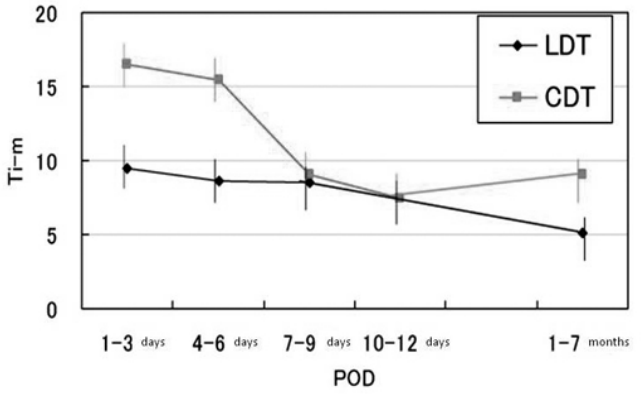
The Usefulness of Contrast-Enhanced Ultrasound (CEUS) for Evalutaion of Transient Time from Lobular Artery Via Glomerular Capillary to Outer Medulla by Employing a Time-Intensity Curve (TIC) Tokunori Yamamoto, Hideki Mizuno, Yasushi Yoshino, Momokazu Gotoh. *Urology, Graduate School of Medical Sciences, Nagoya University, Nagoya, Japan.*

Background: The usefulness of contrast-enhanced ultrasound (CEUS) for evalutaion of the graft function in transient time from lobular artery via glomerular capillary to outer medulla by employing a time-intensity curve (TIC)

Methods: From May 2008 to October 2009, CEUS was performed prior to operation in 12 patients with cadaveric(n=6) and living renal transplantations(n=6) (Figure1). Contrast enhancement as a function of time was measured in two (lobular artery lesions and outer medulla) regions of interest (ROI) and TICs were obtained, respectively. The rise time of defference between lobular artery lesions and outer medulla lesion were measured from the TIC.

Results: In living renal transplantations, initial urine appeared imidiately after the operation in all cases. The transient time are less than 10 sec.

In cadaveric renal transplantations with early ATN, initial urine appeared postoperative days (POD) from 7 to 10 days in all cases. The transient time are less than 10 sec since 10 POD.



Conclusions: An objective and quantitative diagnosis of renal cell carcinoma by CEUS using a second-generation ultrasound contrast agent can be made by employing a TIC. The transient time from lobular artery via glomerular capillary to outer medulla by employing a time-intensity curve (TIC) may be reflect the graft function including ATN.

TH-PO170

SPECT Imaging Method for the Assessment of Glomerular Number Mohammed Noor Tantawy,[1] Christopher Chad Quarles,[1] Donald D. Nolting,[1] Rosie T. Jiang,[2] Keiko Takahashi,[2] Mark P. De Caestecker,[2] Agnes B. Fogo,[2] John C. Gore,[1] Raymond C. Harris,[2] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center.*

Background: Total nephron endowment and thereby glomerular number varies widely between individuals in normal populations. This is of clinical importance as reduced glomerular number is strongly associated with hypertension and CKD. However, there is no non-invasive technique to estimate the total number of renal glomeruli *in vivo*. This study was aimed to develop a non-invasive imaging technique which enables glomerular counting in living subjects (mice).

Methods: 1) Dextran-500 was characterized for glomerular labeling by injecting (i.v.) FITC-Dextran-500 (10mg/kg) to mice. 2) The labeling method to conjugate ^{99m}Tc to Dextran-500 was developed and a novel radiotracer, [^{99m}Tc] Dextran-500, was generated for SPECT imaging. 3) [^{99m}Tc] Dextran-500 was retroorbitally injected to mice and its glomerular binding was assessed by NanoSPECT imaging (30min, temporal resolution;10 sec) at 4 hrs post injection. Kidneys were harvested, sliced, and subjected to autoradiography. 4) Validity of the method was assessed by comparing wild type mice and a model of congenital nephron deficiency Os/+ mice.

Results: FITC-Dextran500 was deposited in glomerular mesangium up to 36 hrs post injection, while it was eliminated from plasma within 6 hrs. In SPECT imaging, renal cortex was strongly labeled with [^{99m}Tc]Dextran-500, yet its binding to other organs was highly limited. The renogram time-activity curves (TACs) displayed a maximum uptake of the radiotracer within the first 2 min followed by some secretion and plateauing of the binding. Glomerular deposition of [^{99m}Tc]Dextran-500 was confirmed by renal autoradiograms. In contrast to wild type, kidneys in Os/+ mice were only weakly labeled with [^{99m}Tc]Dextran-500.

Conclusions: We have developed a SPECT imaging method using [^{99m}Tc] Dextran-500, which could allow non-invasive assessment of glomerular numbers in living subjects.

Funding: NIDDK Support

TH-PO171

Positron Emission Tomography and Immunofluorescence Microscopy of Angiotensin II Subtype 1 Receptor in Renal Ischemia Ali Gholamrezanezhad, Jinsong Xia, Majid Chalian, Kelvin Hong, Zsolt Szabo. *Nuclear Medicine/ Radiology, Johns Hopkins Medical institutions, Baltimore, MD.*

Background: Recent *in vivo* evidence from Positron Emission Tomography (PET) has demonstrated increased cortical radioligand binding at the Angiotensin II Subtype 1 Receptor (AT1R) in animal models of renal ischemia. However, the histopathological distribution and cellular components showing upregulation of AT1R remain elusive. We investigated tissue distribution of AT1R expression in experimental models of pig renal ischemia, in order to clarify the significance of AT1R PET as a renal injury imaging biomarker.

Methods: Using domestic pigs, two animal models were developed: 1) chronic renal artery stenosis; and 2) renal artery reperfusion-revascularization by stent treatment. *In vivo* PET imaging of the AT1R was performed with [^{11}C]KR31173. Renal perfusion was estimated with [^{15}O]water PET. Animals were then euthanized and kidneys were removed. The tissues were frozen, and were cut at 10-μm thickness. Tissue level distribution of AT1R, podocin and NGAL (Neutrophil Gelatinase-Associated Lipocalin) were imaged by indirect immunofluorescent staining and laser scanning confocal microscopy using. Cell nuclei were counterstained with 4-6 diamidino-2-phenylindole dihydrochloride (DAPI). *In vitro* AT1R binding was quantified by [I-125]-[Sar1Ile8]angiotensin II autoradiography.

Results: Both *in vivo* and *in vitro* cortical AT1R binding were increased in ischemia but not in normal perfusion. The finding was further supported by immunofluorescence staining of the chronic stenotic kidney tissue, which revealed increased AT1R expression in some glomeruli. There was also a correlation between the distribution of AT1R and NGAL expression.

Conclusions: Increased AT1R binding measured with PET and autoradiography correlates with increased glomerular AT1R in the renal cortex. Thus, Immunofluorescence imaging corroborates well with the PET imaging results and supports the role of AT1R PET as an imaging biomarker of renal ischemic injury.

Funding: NIDDK Support, Other NIH Support - Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (Grant Number R01 DK50183) and shared instrumentation grant (NIH S10RR022528).

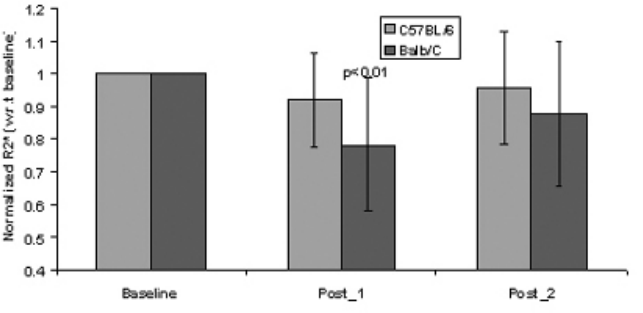
TH-PO172

Blood Oxygenation Level Dependent MRI in Reversible Unilateral Ureteral Obstruction Model Tipu S. Puri,[1] Muhammad Haque,[2] Tammy Franklin,[2] Liby Mathew,[1] Pottumarthi V. Prasad.[2] *[1]Medicine/Nephrology, University of Chicago, IL; [2]Radiology, Northshore University HealthSystem, Evanston, IL, Ukraine.*

Background: Reversible unilateral ureteral obstruction (rUUO) provides a useful model of chronic kidney disease (CKD) amenable for longitudinal monitoring of structural and functional changes during development of CKD (Puri T, AJP Renal Physiol 2010). We have reported strain-dependent susceptibility to development of CKD after rUUO in C57Bl/6 (susceptible) and Balb/C (resistant) mice. In this preliminary study, renal BOLD MRI was used to detect any early changes in oxygenation status in this model.

Methods: A total of 31 mice (19 C57Bl/6 and 12 Balb/C) underwent rUUO. Blood samples were obtained via retro-orbital bleeding for BUN measurements at three time points (baseline, ~2days and ~28 days post release of UUO). BOLD MRI was performed on a 4.7 T Bruker Biospec scanner using a 35 mm mouse body coil at the same time points.

Results: BUN showed enhanced values in both strains at day 28 but to a significantly higher value in C57Bl/6 mice compared to baseline (25.6±5.0 to 48.6±13.0 mg/dL, $p<0.01$ in C57Bl/6 and 27.5±3.2 to 36.4±6.0 mg/dL, $p<0.01$ in Balb/C). As shown in figure below, BOLD MRI demonstrated differential response at day 2 probably related to the difference in susceptibility to developing fibrosis.



Conclusions: BUN measurements were consistent with previous findings in this model. BOLD MRI measurements at ~2day time point in Balb/C are consistent with previous reports in kidneys with ureteral obstruction in humans [Thoeny H, Radiology 2008]. While it is not yet clear why C57Bl/6 do not exhibit similar reduction in R2*, it may be suggestive of increased level of hypoxia which in turn can explain higher functional deficit. Future studies should evaluate water content changes at this time point in order to better characterize renal oxygenation changes.

Funding: Other NIH Support - Clinical and Translational Science Award, Private Foundation Support

TH-PO173

The Utility of Electronic Screening for Enrollment in Clinical Studies: A Descriptive Study Kianoush Banaei-Kashani,[1 and 2] Laura N. Hanson,[3] Man Li,[2] Vitaly Herasevich.[2] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Pulmonary and Critical Care, Mayo Clinic, Rochester, MN; [3]Mayo Validation Support Services, Mayo Clinic, Rochester, MN.*

Background: Enrollment of patients to clinical studies with time sensitive conditions in the critical care field consumes significant portions of study coordinators' time. They need to continuously evaluate patient's medical records for eligibility or rely on the notification from bedside providers.

Methods: METRIC (Multidisciplinary Epidemiology and Translational Research in Intensive Care) Datamart which is a Microsoft SQL-based integrative near-real time (15min to 1 hour delay) database was served as the main data source for rule based alert utility (sniffer). The sniffer engine periodically scans the data under the datamart and sends the alerts to the study coordinator by pager whenever a patient meet predefined alert criteria. Alert was triggered if patient had two consecutive measurements of systolic blood pressure less 90 mmHg or an order for vasoactive agents. The alerts were screened for eligibility criteria by the study coordinators during the work hours. If patient met inclusion criteria, she obtained informed consent and collected time sensitive urine and plasma samples for measurement of acute kidney injury biomarkers. Our institutional review board approved the study procedures

Results: After excluding patients who declined research authorization the number of admited patients between October 2008 and February 2009 was 3779. During the same time period the alert system sent 1044 notifications for patients who met shock state criteria. The distribution of the alerts between work hours (7am- 5pm, Mon-Fri) and off-hours were 345 and 699, respectively. After evaluation for eligibility criteria 121 patients were recruited. Coordinators needed to screen only 27% of total ICU admissions (1044/3779) by using shock state sniffer.

Conclusions: Electronic screening ("shock state sniffer") facilitated the enrollment into clinical study of AKI and potentially saves significant amount of study coordinator's time for electronic medical records screening.

Funding: Private Foundation Support

TH-PO174

Delivery of Recombinant Erythropoietin (rEPO) in Conjunction with Renal Cell Therapy during Continuous Peritoneal Dialysis P. Smith,[1] L. Charles,[1] K. Johnston,[1] D. Buffington,[1] David Humes.[2] *[1]Innovative BioTherapies, Inc., Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI.*

Background: Anemia is a common side effect of chronic kidney disease (CKD). It is postulated that impaired kidney function leads to hypo-responsive EPO release, resulting in reduced red blood cell maturation. Erythropoietic stimulating agents (ESA), including rEPO, and intravenous injections of polysaccharide-conjugated iron have been the corner stone for treating anemia associated with CKD by raising the hemoglobin concentration to 11-13 g/dl. To attempt to improve the treatment of anemia in CKD, a method to deliver rEPO during continuous ambulatory peritoneal dialysis (CAPD) is being tested. As a proof of concept, HEK-293 cells were stably transfected with a human EPO expression cassette and seeded as the surrogate cellular component of a BRECS. Once confluent, the BRECS unit was circulated in 250 ml of serum-containing media for 24 hours, prior to the infusion of this conditioned media into the dry peritoneum of a uremic sheep. Serum was monitored for human EPO every two hours for a 24 hour period using a human-specific EPO ELISA. The EPO concentration peaked between 4 and 8 hours, well within the expected range found in the human population (3.3-16.6 mIU/ml), at >12 mIU/ml, and decayed in a near-linear fashion to 2.5 mIU/ml at 24 hours. As a comparison, a similar experiment was constructed, with the exception that 250 ml of conditioned BRECS media was infused into the peritoneum in the presence of 2 liters of peritoneal dialysis fluid, prior to monitoring serum for the presence of human EPO. In this instance, the serum concentration of EPO peaked between 4 and 8 hours at >4.5 mIU/ml, and decayed in a near-linear fashion to a concentration of 2 mIU/ml at 24 hours. These experiments demonstrate that this approach has the potential to provide hormonal stimulation for erythropoiesis in a manner much more consistent with normal physiology while providing a method that may lead to cost savings over the current treatment regimens.

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TH-PO175

Evaluation of Adult Human Renal Epithelial Progenitor Cells in Cell Therapy Devices A. Westover,[1] D. Buffington,[1] David Humes.[1,2] *[1]Innovative BioTherapies, Inc., Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI.*

Background: Renal epithelial cell (REC) therapy promises to improve the morbidity of patients with renal disease. To this end**,** therapeutic devices addressing the neglected biologic component of renal replacement therapy are being advanced. Because human donor tissue is limited, an enhanced propagation method to expand REC progenitors from available adult human kidney transplant discards has been established to provide the biomass for REC-based therapeutic products.

Methods: REC progenitor cell isolation has been successful using kidneys from donors including those previously considered suboptimal (n=19). Donor profiles include long-term diabetes, hypertension, greater than 70 years old, donation post cardiac death with prolonged warm ischemia, and one donor with end stage renal disease (ESRD) on dialysis for >2 years.

Results: Cell yields are consistently higher than 10^{11} cells/gram cortex allowing for the manufacture of over 100,000 devices per donor kidney, indicating that autologous therapy may be possible using cells derived from a wedge biopsy taken early in the renal failure cascade. Kidneys from healthy donors (obtained due to anatomical flaw or procedural error) yielded over 10^{12} cells/gram cortex or potentially over 1,000,000 devices per donor kidney. Greater than 10^{9} progenitor cells/gram cortex were isolated from the ESRD kidney indicating that proliferative potential remains and would allow for the manufacture of >100 autologous devices. A cryopreservation procedure, necessary to allow for processing the large cell yields has been advanced using a potentially FDA compliant procedure and cell death from apoptosis post thaw minimized. Additionally, the therapeutic potential of progenitor derived REC was conserved as evaluated using a surrogate panel for efficacy including LPS stimulated IL8 secretion, γGT and 25-hydroxyvitamin D-1-alpha hydroxylase enzyme activities.

Conclusions: Differentiated REC progenitors, integrated into trabeculated carbon disk based bioartificial REC systems (BRECS), continue to be evaluated in previously established large animal models of ovine ESRD and porcine septic shock.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO176

Immuno-Modulatory Effect of the Bioartificial Renal Epithelial Cell System (BRECS) in an Ovine Model of Uremia D. Buffington,[1] A. Westover,[1] L. Lou,[1] L. Charles,[1] P. Smith,[1] K. Johnston,[1] C. Pino,[1] J. Liu,[3] David Humes.[2] *[1]Innovative BioTherapies, Inc.; [2]Univ. of Mi.; [3]Huashan Hospital.*

Background: Studies were performed to assess BRECS impact on the pro-inflammatory state that develops during chronic uremia. The BRECS is a compact, cryopreservable renal epithelial cell therapy device under development for treatment of renal and inflammatory disease states. The ovine uremic model employs a continuous-flow peritoneal dialysis (CFPD) circuit for uremic control and recycling PD from the host sheep through the BRECS.

Methods: Sequential nephrectomies were performed at the time of PD catheter placement and one day before initiation of CFPD. BRECS containing 10^{8} cells were incorporated into the PD circuits. BRECS O_2 consumption and glutathione (GSH) degradation rates were assessed to monitor cell viability and functionality. Inflammatory indices were measured to assess BRECS impact to modulate the pro-inflamed uremic state. CD11b expression on the neutrophil (NE) cell surface was used as an indicator of the "activated state" of the NE population. Oxidative burst, as a measure of leukocyte function, was monitored and compared to acellular sham devices. Systemic NE apoptotic potential was assessed via Annexin V staining assay. BRECS therapy continued up to seven days, followed by an additional post-therapy evaluation period of 48 hours

Results: BRECS remained viable with detectable O_2 consumption and GSH degradation rates for the entire therapeutic time course. CD11b mean fluorescent intensity was lower beginning after 5 days of BRECS therapy when compared to acellular control sheep. Enhancement in oxidative burst was also seen in the BRECS treatment group. Importantly, these two affects continued during the 48 hour post-BRECS therapy period. BRECS therapy caused a shift in the percent NEs that maintain apoptotic progression, indicating a return to steady state with respect to NE activation and function, as seen in normal, non-uremic sheep.

Conclusions: BRECS therapy positively modulates the inflammatory state of the uremic sheep; indicated by a decrease in the circulating NE activated state, increase in oxidative burst and improved NE apoptotic potential.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO177

Modeling Ultrasound (US)-Induced Heating of Hemodialysis Grafts Yan-Ting E. Shiu,[1,2] Mark R. Brinton,[3] Alfred K. Cheung,[1,4] Russell Stewart Stewart,[2] Douglas Christensen.[2,3] *[1]Medicine, Univ. of Utah, SLC, UT; [2]Bioengineering, Univ. of Utah, SLC, UT; [3]Electrical and Computer Engineering, Univ. of Utah, SLC, UT; [4]Medical Service, VASLCHCS, SLC, UT.*

Background: Expanded polytetrafluoroethylene (ePTFE) grafts fail at high rates due to stenosis caused by neointimal hyperplasia (NH). Currently there is no effective method to prevent or treat NH. We explored a novel approach to reduce NH in ePTFE grafts using focused US-induced mild hyperthermia. We have previously reported a higher sensitivity to hyperthermia-induced death for cells cultured on ePTFE than on a surrogate tissue surface. We identified the temperature range (45-47°C) necessary to induce significant apoptosis among cells cultured on ePTFE but not on surrogate tissues. The present study addresses the feasibility of selectively heating ePTFE to the optimal temperature by US.

Methods: The acoustic finite-difference time-domain method was used to calculate the beam propagations from 1.5- and 3.2-MHz transducers through a simplified 3-dimensional model containing fat, muscle, blood flow and ePTFE graft. Next, the spatial pattern of the heat derived from the US power was simulated using the COMSOL Multiphysics heat-transfer module. The simulation included skin surface cooling to 20°C, laminar blood flow through the graft lumen, and the effect of blood perfusion on heat transfer in the tissue regions. Cyclic 30-sec heating and cooling periods were used to mimic a pulsed ultrasound cycle.

Results: The greater US attenuation by ePTFE than by tissues caused about 5 times more power deposited in ePTFE than in adjacent tissues. The transducer with a higher frequency produced a more confined power deposition pattern than the transducer with lower frequency. Importantly, high temperatures (48-49°C) were found only on ePTFE, while blood and most tissues remained at 37°C.

Conclusions: Our approach exploits the differential acoustic properties between ePTFE and tissues, and their differential sensitivity to hyperthermia-induced cell death. Our results show promise for the use of focused US as a safe and effective strategy to prevent and treat NH on the ePTFE surface through hyperthermia-induced apoptosis.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support, Private Foundation Support

TH-PO178

ER-Stress Induced Mega-Cluster microRNAs in Mouse Models of Diabetic Nephropathy Mitsuo Kato, Mei Wang, Zhuo Chen, Xiwei Wu, Sumanth Putta, Linda L. Lanting, Rama Natarajan. *Diabetes, Beckman Research Institute of City of Hope, Duarte, CA.*

Background: microRNAs (miRNAs) have been shown to play major roles in renal diseases. However, the comprehensive study of miRNAs has not completed in diabetic nephropathy.

Methods: In this study, we profiled miRNAs in RNA extracted from glomeruli from control and streptozotocin (STZ)-injected type1 diabetic mice by high-throughput sequencing (solexa, Illumina Inc). Expression levels of candidate miRNAs were validated by qPCRs.

Results: Some of confirmed miRNAs are miR-34 family (miR-34a, miR-34b, miR-34c & miR-34c*), miR-486 and miR-379. miR-34 family is regulated by p53 and possibly involved in renal hypertrophy. miR-486 is also involved in renal hypertrophy by targeting Pten and activating Akt kinase under diabetic conditions. miR-379 is a member of miR-495 mega cluster miRNAs (~40 miRNAs). Other members (miR-495, miR-380, miR-376b, miR-382 and miR-379) in this mega cluster were also confirmed to be upregulated in glomeruli from STZ-injected type1 diabetic mice. Interestingly, miRNAs in this cluster were regulated by ER stress inducible transcription factor CHOP upregulated by high glucose conditions (HG) or TGF-b in kidney cells. The potential targets of this mega cluster are Tnrc6b (15 sites by 12 miRs), Cugbp2 (14 target sites by 12 miRs) and Pten (13 sites by 10 miRs). Decrease of these potential targets was confirmed in glomeruli from diabetic mice and in kidney cells treated with HG or TGF-b. Tnrc6b is a mammalian homologue of GW182 which is located in P-bodies and regulates protein translation. Cugbp2 is a regulator of insulin RNA splicing but also a negative translational regulator of Cox2 which is increased in diabetic glomeruli. Pten inhibition by the mega cluster may activate Akt to enhance protein synthesis and hypertrophy.

Conclusions: These results demonstrate that diabetic condition induces renal hypertrophy through down regulation of negative translational regulators targeted by the mega cluster miRNAs upregulated by ER-stress induced CHOP. ER-stress induced mega cluster miRNAs may be therapeutic targets of diabetic nephropathy.

Funding: NIDDK Support

TH-PO179

Basement Membrane Remodeling and Endothelial Differentiation When Mouse Stem Cells Are Seeded into Acellular Rat Kidney Scaffolds

Edward A. Ross,[1] Dale R. Abrahamson,[2] Matthew James Williams,[1] Gary W. Ellison,[1] Patricia St. John,[2] Chris Batich.[1] *[1]University of Florida; [2]Kansas University Medical Center.*

Background: Due to transplant organ shortage we have pursued tissue regeneration using decellularized kidneys seeded with pluripotent precursor cells. We hypothesize that these scaffolds retain matrix signals that can induce progenitor cells to differentiate and recapitulate native structures: matrix-to-cell signaling followed by cell-to-cell and then cell-to-matrix interactions that would gradually remodel and replace the original scaffold matrix. This would reduce scaffold antigenicity and enable xeno-allografts.

Methods: We previously showed that when arterially seeded into acellular rat whole kidney scaffolds murine embryonic stem cells (mESCs) attach, multiply and demonstrate morphologic, immunohistochemical and gene expression evidence (Pax2, Ksp-cadherin) for differentiation. We now tested for more specific evidence of differentiation into endothelial lineage and remodeling of the matrix basement membranes from rat to mouse ("murinization"). mESCs were infused arterially into rat kidney scaffolds and incubated for 10 days. Endothelialization of vascular cells was tested by endothelial specific BsLB4 lectin and anti-VEGFR2 (Flk1) antibodies. Matrix murinization was assessed by a monoclonal antibody specific for mouse laminin β1 chain, which does not cross react with rat.

Results: As evidenced by co-localized staining with DAPI nuclear label, cells in arterial vessels and glomeruli were positive for both BsLB4 lectin and VEGFR2. Labeling became progressively intense with longer incubations, from 4 to 10 days. Rat scaffold's basement membrane (co-localized with cell growth) demonstrated IgG-horseradish peroxidase immunolabeling with monoclonal rat anti-mouse laminin β1, again in a time dependent manner.

Conclusions: We have provided new evidence for matrix-to-cell signaling in acellular whole organ scaffolds that induces differentiation of pluripotent precursor cells to endothelial lineage. Production of mouse basement membrane supports remodeling of host (rat)-derived scaffolds and thereby warrants further investigation as a promising approach for xenotransplantation.

TH-PO180

Bio-Mechanical Properties of the Mammalian Urothelium

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Background: Successful bladder filling, urine storage and voiding requires an intact urothelium because urothelial surface umbrella cells (UC's) maintain an exceptionally tight barrier between urine and blood. This barrier remains intact despite profound urothelial stretching and subsequent relaxation, during the processes of bladder filling and voiding respectively. Specialized proteins called uroplakins form plaques on the surface of umbrella cell and cover up to 90% of the urothelial surface, and is considered to be rigid. It is not clear how a rigid surface can be flexible as the bladder undergoes stretch and relaxation cycles. The biomechanical properties of UC's, including deformability and spontaneous dynamic remodeling, likely play a critical role in both barrier function and mechanotransduction.

Methods: To develop an understand of the mechanical properties of the urothelial surface we attached magnetic beads coated with polylysine to the apical membrane (AM) surface of intact UC's in intact urothelia and measured their stiffness G, in response to oscillating magnetic fields (optical magnetic tracking cytometry, OMCT) as we varied the frequency of oscillation between 0.1 to 1000 Hz.

Results: Control UC's exhibited G values which tracked closely with highly deformable red blood cells. Removal of surface UC's with protamine exposed underlying intermediate cells, which exhibited G values 12-15 fold higher than UC's, and similar to cultured MDCK cells. Fixation of UC's with formaldehyde raised G by two orders of magnitude. Because submembrane actin often plays a critical role in reducing deformability, we performed confocal microscopy on control UC's and found very little subapical actin. In UC's basolateral actin was abundant, and actin was also abundant under apical and basolateral membranes of intermediate cells.

Conclusions: We conclude that UC's are strikingly deformable, despite the dense array of uroplakins in their apical membranes. Whether this deformability results from the physical effect of uroplakins or the relative lack of subapical actin will be determined using uroplakin knockout mice.

Funding: NIDDK Support

TH-PO181

Nephrology Blogs as an Educational Tool: A Survey of Global Blog Readers

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Background: An increasing number of providers author medical blogs. However, little data exists about reader's perceptions. This information is especially important for nephrology educators who wish to use social media to attract younger students to nephrology careers. Our investigation uncovers the perception of a nephrology-specific blog by its global readership.

Methods: Nephrology On-Demand is a comprehensive educational website that provides multimedia information. We measured the usage & appeal of the blog format.11 blogs from national/international and local meetings were published online, detailing the key learning points of selected seminars. We used Google Analytics to measure usage data for each blog during the first 90 days after publication. Access to any blogs could be achieved by completing a short, Qualtrics-hosted survey.

Results: A total of 828 visitors and 1465 pageviews were recorded. The average number of visitors & pageviews to blogs of local meetings were 32 and 76, respectively. These numbers increased to 112 and 181 for the national/international meeting blogs. International readers contributed between 25-45% of visits to the blogs of local meetings. Of 254 surveys, 94% were completed. More than 80% of non-first-time readers viewed the blogs as accurate (mean 1.44, SD 0.73), current (1.49, 0.79), objective (1.53, 0.81) and useful (1.5, 0.79). These findings were similarly observed at all training levels.

Conclusions: Our investigation has 2 key points. First, local meetings generally attract local providers, but blogs of such meetings can attract a global audience. Albeit a small number of visits, blogs allow presenters of local scientific meetings to showcase their material worldwide. To date there are no nephrology blogs that have published such data. Second, knowledge that blogs are viewed in a positive manner may help attract a younger generation of learners to nephrology. Further data collection is underway to determine if blogging of scientific meetings can increase the awareness and interest of nephrology with students and residents.

TH-PO182

A Knowledge Base Compiling Data from Literature, To Access, At-a-Glance, Gene- and Protein-Expression across Kidney Segments and Kidney Diseases

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Background: Kidney research continues to produce large genomic and proteomic data sets from different part of the kidney and across different diseases. However, not only this information is scattered across hundreds of publications, it is also hidden in figures and supplementary data. To facilitate data exploration and analysis, and to help generate new hypothesis in kidney research, a supporting database related to the kidney and urinary pathways (KUP), compiling existing knowledge from databases and scientific literature, would be of great help.

Methods: We have developed a KUP Knowledge Base (KUPKB) that integrates KUP related gene and protein expression data. A specialized KUP Ontology (KUPO) provides a common schema for annotating the data.

Results: Currently the KUPKB consists of 163 gene and protein expression datasets extracted manually from 66 publications (figures, tables, and supplementary data), from the Gene Expression Omnibus (20 microarrays experiments), or from other databases such as Euregene, the Human Glomerulus Protein Database or the Urinary Exosome Protein Database. Experiments include mRNA, protein and phosphoprotein data, in specific kidney cells (e.g. podocyte), kidney segments (e.g. glomerulus) or urine, and across different kidney diseases or animal models (e.g diabetic nephropathy, interstitial fibrosis and tubular atrophy) and different in vitro models (e.g. TGFbeta, high glucose).

Conclusions: The user can search using a publicly available interface called the iKUP browser for a gene or a list of genes of interest. The result of the query is returned in a tabular format to see at a glance: where are a these genes expressed? In which diseases/models are they expressed? Are they present, up/down-regulated in these conditions? We will present the results of typical queries using the iKUP browser and compare the results of these queries with the classical way of data interrogation to demonstrate the added value of the KUPKB.

The KUPKB and the iKUP browser are available at: http://www.kupkb.org

Funding: Government Support - Non-U.S.

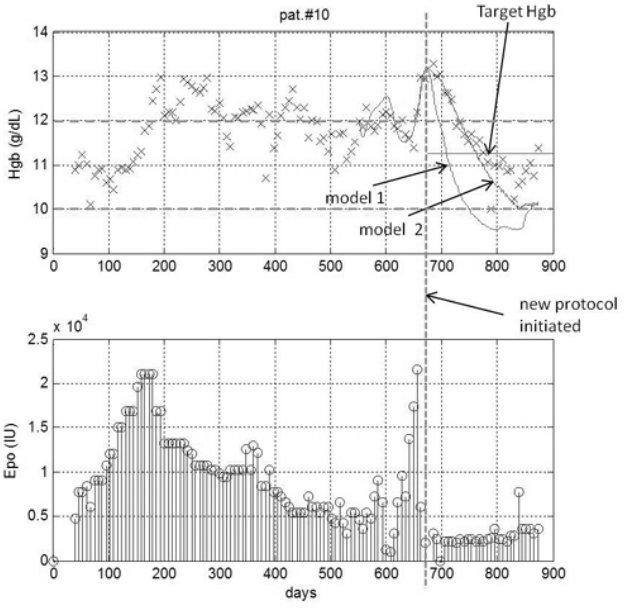
TH-PO183

A Novel Anemia Management Protocol (AMP) Yossi Chait,[1] Michael J. Germain,[2] Joseph Horowitz,[1] Christopher V. Hollot,[1] Rajiv P. Shrestha.[1] *[1]University of Massachusetts; [2]Western New England Renal & Transplant Associates.*

Background: We report observations from a pilot clinical study on the feasibility of AMP designs based on mathematical modeling of erythropoiesis and on feedback control. This is in contrast to current AMPs, which are largely rule-based, expert systems formulated on the basis of experience and clinical studies.

Methods: A pharmacokinetic/pharmacodynamic (PK/PD) erythropoiesis model was employed and a new AMP was designed using robust control principles, based on retrospective 3x/week hemoglobin (Hgb) data. The dosing protocols for four ESRD patients were switched to the new AMP designs.

Results: For parsimonious parameter identification, we modeled the erythropoiesis stages using an Epo-stimulated saturable production function with a time delay to model the maturation of the progenitor cells into red blood cells (RBCs). Epo PK was modeled using linear plus nonlinear clearances. The RBC pool was modeled with a 2nd-order gamma distribution describing mean RBC lifespan. Quantitative Feedback Theory was used to design the AMP which used past and current Epo doses and Hgb data to compute the upcoming week's Epo dose. Patient-specific model parameters and corresponding algorithm parameters were periodically updated based on newer Hgb data. Preliminary results support the feasibility of the new AMP in maintaining Hgb within the desired range (10-12 g/dL); with reduced variability and decreased Epo usage; see Figure 1. We also deliberate the hypothesis that variability in Hgb due to iron kinetics or fluid volume cannot be eliminated with an AMP based only on Hgb data.



Hemoglobin (top) and IV Epo doses (bottom) traces of pat. #10 (starting 1.1.2009). Prior to day 675, different AMPs have been used; the new protocol was initiated on day 675 with a target of 11.25 g/dL; model 1 and model 2 correspond to predicted responses using two different sets of estimated parameters, indicating intrapatient variability.

Conclusions: Patient-specific AMPs based on PK/PD models and robust control principles can offer improved Hgb regulation.

Funding: Other U.S. Government Support

TH-PO184

Development of a Clinical Terminology Index by the Spanish Society of Nephrology: Standardization Process Katia Lopez-Revuelta,[1] Elena Corchete,[1] Alberto Ortiz.[2] *[1]Nephrology, Hospital Universitario Fundación Alcorcón, Madrid, Spain; [2]Nephrology, Fundación Jiménez Díaz, Madrid, Spain.*

Background: Achieving a common medical terminology is a main concern for the implementation of electronic medical record. The use of standardized clinical terminology facilitates electronic data collection for multiple purposes: clinical, research, registries and health policy planning. Coding systems, fully implemented in hospitalization are lacking in outpatient care. PURPOSE: To develop a standardized clinical terminology index validated by the Spanish Society of Nephrology, pre-codified by CIE9-MC system to apply in Clinical Nephrology Outpatient Care.

Methods: A systematic review of pathologies was carried out by the research team dealing with terms covering referral findings and diagnosis. Classified terms have been reviewed by groups of experts in different Nephrology fields. The worked up terminology index has been independently codified by two different health coding technicians following CIE9-MC system. Concordance analysis was assessed by Kappa test.

Results: The final version of the terminology index has a total of 1104 items grouped in 14 pathologic categories: Symptoms, Signs and Syndromes referral, Water and Electrolyte Disorders, Inherited Kidney Diseases, Glomerular Disorders, Multisystem Diseases, Diabetic Nephropathy, Infectious Diseases, Vascular Kidney Diseases, Acquired Chronic Tubulointerstitial Nephritis, Urinary Tract Infection, Nephrolithiasis, Hypertension, Renal Neoplasia and Acute Renal Failure.

Each item has its corresponding CIE-9 MC pre-attached code. The global agreement observed between both coding technicians was 95%, 100% in most pathologies except 94% in Water and Electrolyte Disorders and 74% in Vasculitis.

Conclusions: A Standardized Terminology Index by the Spanish Society of Nephrology with CIE9-MC system pre-codified will be a useful tool for Clinical Nephrology Outpatient care that will be available at the Society web site. A preliminary implementation study is needed to validate this classification. It would be desirable to complete the list with dialysis and transplantation terms.

Funding: Private Foundation Support

TH-PO185

Computer Simulations in the Design and Evaluation of Anemia Management Protocols (AMPs) Yossi Chait,[1] Joseph Horowitz,[1] Michael J. Germain,[4] Rajiv P. Shrestha,[1] Christopher V. Hollot,[1] Adam E. Gaweda.[5] *[1]University of Massachusetts; [3]Western New England Renal & Transplant Associates, PC; [4]University of Louisville.*

Background: Current AMPs rely exclusively on clinical data and physicians' experience. We use computer simulations to compare the performance of two AMPs: a current AMP used at our dialysis facility, and a new one designed on the basis of mathematical modeling and feedback control techniques. A hyper responsive and intermediate responsive patient models are used in the comparison.

Methods: The erythropoiesis model comprises a PK compartment with linear+nonlinear clearance, a PD compartment with a saturable time-delay RBC production function, and a 2nd-order gamma lifespan distribution describing the RBC pool dynamic. Model parameters were estimated to match the each patient's clinical profile.

Results: To study protocol performance under intrapatient variability, we introduced random variations in the response to Epo stimulation and in the mean reticulocyte hemoglobin content. The current AMP is population-oriented and consists of a set of rules based on hemoglobin (Hgb) trending and Hgb ranges whereas the new AMP is patient-specific. Both protocols assume weekly Epo dose adjustments with 3x/week administration. Hgb variability, defined as the sum of absolute consecutive Hgb changes divided by the number of data, is an accurate measure of how 'fast' a variable varies.

type (protocol)	Hgb mean (SD)	Hgb Variation	Hgb In Range [10-12] (%)	Weekly Epo Mean(SD)
Hyper-responsive (new)	10.98(0.52)	6.01	99	2794(1692)
Hyper-responsive (current)	11.13(0.91)	10.29	65	4097(4182)
Intermediate-responsive (new)	11.24(0.42)	7.43	93	14067(1408)
Intermediate-responsive (current)	10.77(109)	33.00	52	11871(8726)

Conclusions: Population-oriented AMP consisting of a single set of rules may not be able to achieve acceptable maintenance for the entire spectrum of hyper-, intermediate-, and hypo-responsive patients. AMPs that can be tuned to individual patients through mathematical modeling, robust control principles, and parameter identification, may offer improved Hgb behavior. In conjunction with clinical trials, simulations could become an essential tool in the design and evaluation of AMPs.

Funding: Pharmaceutical Company Support

TH-PO186

Overweight and Adiposity in Chronic Kidney Disease Patients Rachel Bregman, Barbara Vale, Carla C.S. Lemos, Laura Carvalho Kawakami, Maria Ines Barreto Silva. *Nutrition, Nephrology, State University of Rio de Janeiro, Brazil.*

Background: Central adiposity (CAd) and inflammation are pointed as risk factors for cardiovascular disease (CVD) in chronic kidney disease (CKD) patients. A new index for evaluation of CAd was proposed to CKD patients, named waist-to-height ratio (WheiR).

Methods: We evaluated 134 CKD patients under treatment with a multidisciplinary team. Nutritional status and body composition were determined by body mass index (BMI), serum albumin, waist-to-hip ratio(WHR) and DEXA-dual X-ray absorptiometry. Obesity index WheiR was calculated as waist circumference (WC;cm)/ body height (cm). Glomerular filtration rate was estimated (eGFR) by MDRD. Multiplexed analysis of pro- and anti-inflammatory markers (high sensitive C reactive protein (CRP), interferon gamma (IFN-γ), leptin, intercellular adhesion molecule-1 (ICAM-1), insulin resistance index (homeostasis model assessment: HOMA-IR), high molecular weight (HMW) adiponectin and interleukin-10 (IL-10), were performed. Statistical analysis using StataCorp 8.2.

Results: Data: mean±SD, patients were under treatment for 3±2 years, eGFR: 29±13 ml/min; age: 65±12 years; 56% males, BMI: 26±4 kg/m^2. Patients were divided according to WheiR: Group 1: WheiR<0.55; n=63; Group 2: WheiR>0.55; n=71. Gender, age, albumin and eGFR were not different among groups. Group 2 showed higher WheiR, BMI, WC, WHR, DEXA total and central body fat ($p<0.0001$). WheiR index presented a better correlation with DEXA trunk fat ($p<0.0001$) than with DEXA total fat and WHR. The CRP, leptin, and HOMA-IR were higher in group 2, but HMW adiponectin was lower. The Spearman's coefficient was significant between WheiR and IFN-γ and IL-10. In the stepwise multiple regression analysis, WheiR was positively associated with HOMA-IR and leptin and, negatively associated with HMW adiponectin.

Conclusions: WheiR is very easy to be measured, presented a significant correlation with DEXA, and can be used to evaluate CAd. In this CKD population, high CAd was followed by inflammation. In conclusion present data suggest that body adiposity is an independent risk factor for CVD in patients with CKD and should be a target in the nutritional treatment of this population.

TH-PO187

Longitudinal Relationship between Blood Pressure and Left Ventricular Mass in Children with Chronic Kidney Disease Juan C. Kupferman,[1,2] Derek Ng,[2] Joseph T. Flynn,[2] Susan L. Furth,[2] Bradley A. Warady,[2] Mark Mitsnefes.[2] *[1]Maimonides Medical Center, Brooklyn, NY; [2]CKiD investigators.*

Background: The longitudinal association between casual blood pressure (BP) and left ventricular mass index (LVMI) has not been previously studied in children with chronic kidney disease (CKD).

Methods: In a prospective cohort study, BP was assessed annually. Initial echocardiogram was performed 1 year after study entry (V2) and 2 years later (V4). A linear mixed model with a random subject effect assessed the effect of change in BP on LVMI. The main exposure was average BP z-score, defined as the average of 2 BPs spaced 1-year apart prior to each LVMI measurement. Models for systolic and diastolic BP (SBP, DBP) were adjusted for age, sex, race, height, CKD diagnosis, and years with CKD. Each model included a time effect, allowing LVMI to be different at V2-V4. We also allowed the effect (slope) of average BP to be different.

Results: There were 260 subjects with complete follow-up data; age (median [IQR]) 11 [8, 15], 61% male; 21% black; median iohexol GFR 42 ml/min/1.73m^2. Over 2 year follow up, median SBP z-score decreased from 0.34 to 0.07 while DBP z-score decreased from 0.47 to 0.23; median LVMI decreased from 33.8 to 31.8 g/m$^{2.7}$.

Effects of BP and Time on LVMI

	Covariate	Estimated change (95% CI)	p-value
Model 1	Slope of SBPz (V2)	3.9% (1.7%, 6.2%)	<.01
	Slope of SBPz (V4)	6.5% (3.7%, 9.4%)	<.01
	Effect of Time (2 yrs)	-32% (-44%, -17%)	<.01
Model 2	Slope of DBPz (V2)	3.4% (0.8%, 6.1%)	0.01
	Slope of DBPz (V4)	5.8% (2.5%, 9.3%)	<.01
	Effect of Time (2 yrs)	-29% (-41%, -14%)	<.01

LVMI had a 2-year average decrease of 32% when adjusting for SBP and confounders. For every one standard deviation increase in SBP at V2, LVMI increased by 3.9% and this effect increased to 6.5% at V4. The linear association between DBP z-score and LVMI was significant and the effect was higher for V4 compared to V2 (5.8% vs. 3.4%).

Conclusions: BP and LVMI improved in children with CKD over time. Elevated SBP and DBP are associated with increased LVMI. While the effect of BP on LVMI was not significantly modified by time, these results suggest a clinically meaningful effect of long-term elevated BP on LVMI.

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

TH-PO188

Elevated Albuminuria Increases the Risk of Recurrent Venous Thromboembolism: Results from a Population Based Cohort Study Inge van Schouwenburg,[1] B. Khan Mahmoodi,[1,2] Nic Veeger,[1,3] Hanneke C. Kluin-Nelemans,[1] Ron T. Gansevoort,[2] Karina Meijer.[1] *[1]Division of Hemostasis and Thrombosis, Department of Hematology, University Medical Center Groningen (UMCG); [2]Department of Nephrology, UMCG; [3]Department of Epidemiology, UMCG, Netherlands.*

Background: Microalbuminuria has been identified as risk factor for first venous thromboembolism (VTE). Whether it is also a risk factor for recurrence is unknown. Knowledge on this issue is relevant because it may help in assessing the duration of anticoagulant therapy after a first VTE. We therefore investigated the risk of recurrent VTE in patients with elevated albuminuria.

Methods: Data of a prospective population based cohort study (PREVEND), that started in 1997 and included 40.856 subjects aged 28-75 yrs, were used. In all participants, albuminuria was measured and VTE occurrence was monitored. Patients with first VTE between study entry and January 2009 were identified by database record linkage with the national registries of hospital discharge diagnoses and death certificates and the regional anticoagulation clinic. Of identified patients medical records were utilized for verification and for obtaining additional information.

Results: Of 351 subjects with first venous thromboembolism (49% male; median age at first event 64 years, unprovoked 43% and provoked [external risk factor present] 48%), 37 subjects developed a recurrence during a median follow-up period of 3.3 (interquartile range, 1.1-6.4) years. Annual incidence of recurrence in subjects with elevated albuminuria (≥20 mg/L) was 5.00 per 100 person years (95% confidence interval [CI]; 2.16-9.85), compared to 2.38 (95%CI; 1.59-3.41) in subjects with normal albuminuria (<20 mg/L). Hazard ratio for recurrence was 1.95 (95%CI; 0.89-4.30) after adjustment for age and sex. This hazard ratio was 3.35 (95%CI; 1.18-9.47) in patients with first unprovoked, and 1.12 (95%CI; 0.25-5.01) in those with a first provoked event.

Conclusions: Subjects with elevated albuminuria who experience an unprovoked VTE are at an increased risk of recurrence, independent of age and sex. These results implicate that such patients may benefit from long-term anticoagulant therapy.

TH-PO189

Novel Risk Factors for Incident Peripheral Arterial Disease among Patients with Chronic Kidney Disease: A Prospective Analysis from the CRIC Study Jing Chen, Dawei Xie, Michael Shlipak, Raymond R. Townsend, Lawrence J. Appel, Lisa C. Nessel, Dominic S. Raj, Akinlolu O. Ojo, Martin J. Schreiber, Xin Wang, Louise Frances Strauss, Claudia M. Lora, Mahboob Rahman, L. Lee Hamm, Jiang He. *Tulane University.*

Background: Patients with chronic kidney disease (CKD) have an increased risk of developing peripheral arterial disease (PAD) compared to those with normal kidney function. The underlying etiology for this increased risk is not fully understood.

Methods: We studied the effects of novel risk factors on the development of PAD among 3,090 participants in the Chronic Renal Insufficiency Cohort (CRIC) study. Patients aged 21 to 74 years old with an estimated glomerular filtration rate (eGFR) of 20-70 mL/min/1.73 m^2 were recruited from 7 clinical centers in the US. Incident PAD was defined as a new onset ankle-brachial index (ABI) of <0.9 or clinical PAD confirmed by an endpoint assessment committee during the follow-up period among participants with ABI >0.9 and no PAD history at baseline examination.

Results: After adjustment for age, race, gender, and clinical sites, the following novel risk factors were associated with an increased risk of incident PAD (hazard ratio and 95% confidence interval for a one standard deviation higher level): HOMA-insulin resistance (IR) (1.12; 1.05-1.19; p<0.001), hemoglobin (Hb) A1C (1.25; 1.13-1.39; p<0.001), total parathyroid hormone (1.12; 1.01-1.24; p=0.03), C-reactive protein (CRP) (1.11; 1.05-1.18; p<0.001), white blood cell count (1.18; 1.06-1.31; p=0.003), fibrinogen (1.29, 1.15-1.45, p<0.001), alkaline phosphatase (1.18; 1.09-1.29; p<0.001), cystatin C (1.19; 1.06-1.34; p=0.003), and 24-hour urine albumin (1.16; 1.04-1.29; p=0.006). After further adjustment for traditional cardiovascular risk factors including eGFR, the following remained significantly associated with increased risk of PAD: HOMA-IR, HbA1C, CRP, fibrinogen, alkaline phosphatase, and cystatin C.

Conclusions: These data indicate that insulin resistance, inflammation, prothrombotic state, bone metabolism and kidney function are associated with risk of PAD independent from traditional risk factors among patients with CKD.

Funding: NIDDK Support

TH-PO190

Increasing Levels of Novel Kidney Function Markers Predict Outcomes in the General Population: The Atherosclerosis Risk in Communities (ARIC) Study Brad C. Astor,[1] Tariq Shafi,[1] Ron C. Hoogeveen,[2] Christie Ballantyne,[2] Josef Coresh.[1] *[1]Johns Hopkins University; [2]Baylor College of Medicine.*

Background: Decreasing kidney function is associated with higher risk of death, CVD and ESRD. Several novel markers of kidney function have been proposed as adjuncts to serum creatinine (SCr). The risk of outcomes associated with changes in these markers is unknown.

Methods: We examined the association between a ≥25% increase over 6 years in SCr, cystatin C (CysC), beta trace protein (BTP) and beta-2 microglobulin (B2M) and subsequent outcomes (death, coronary heart disease [CHD], hospitalized heart failure [HF], acute kidney injury [AKI] and ESRD) over the following 11 years among 2,186 selected participants in the ARIC Study with an estimated GFR (eGFR) ≥60 mL/min/1.73m^2 at baseline.

Results: An increase in 1 or more markers was associated with death and heart failure after adjustment. Increases in ≥3 markers was strongly associated with all outcomes. Results were similar among participants without a ≥25% increase in SCr (n=1,821) or CysC (n=1,621).

	Adjusted* Incidence Rate Ratio (95% CI)				
# Markers with ≥25% increase	Death (n=536)	CHD (n=459)	Heart Failure† (n= 410)	AKI (n=124)	ESRD (n=115)
0	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
1	1.4 (1.1, 1.7)	0.9 (0.6, 1.2)	1.5 (1.0, 2.0)	1.3 (0.7, 2.7)	0.9 (0.3, 2.8)
2	1.7 (1.2, 2.3)	1.2 (0.8, 1.8)	2.0 (1.3, 3.0)	2.3 (1.0, 5.3)	2.1 (0.7, 6.4)
3	2.0 (1.5, 2.9)	2.0 (1.3, 3.1)	2.4 (1.5, 3.7)	3.1 (1.4, 6.8)	2.9 (0.9, 9.2)
4	2.6 (1.6, 4.0)	1.8 (1.0, 3.4)	2.1 (1.1, 3.8)	4.7 (1.8, 12.0)	9.0 (2.7, 30.2)
p-trend [Overall]	<0.001	0.002	<0.001	<0.001	<0.001
p-trend [eGFR≥60 mL/min/1.73m^2 at 6 year visit (n=1,835)]	<0.001	0.009	<0.001	0.001	0.01

* Adjusted for age, sex, race, eGFR, prevalent CHD, diabetes, blood pressure, anytihypertensive medication, smoking, LDL, HDL, triglycerides. †106 participants with prevalent heart failure excluded.

Conclusions: Increases in multiple markers of kidney function strongly predict subsequent outcomes, even among individuals without a significant rise in SCR or CysC or a decrease in eGFR to <60 mL/min/1.73m^2. These results suggest that serial measurements of multiple markers may add significant prognostic information to that obtained from a single marker.

Funding: NIDDK Support

TH-PO191

Endogenous Anti-Atherosclerotic Property Is Decreased in Patients with Renal Dysfunction: Evaluation Form the Circulating Soluble fms-Like Tyrosine Kinase-1 (sFlt-1) Level after Heparin Injection Masaru Matsui, Shiro Uemura, Yukiji Takeda, Takaki Matsumoto, Ayako Senou, Kimihiko Nakatani, Yasuhiro Akai, Masayuki Iwano, Yoshihiko Saito. *First Department of Internal Medicine, Nara Medical University, Kashihara, Nara, Japan.*

Background: CKD is usually associated with atherosclerosis. Placental growth factor (PlGF), a family of VEGF, plays important roles in the development of atherosclerosis, which is antagonized by sFlt-1, a soluble form of PlGF receptor. sFlt-1 is circulating and is also bound to extracellular matrices of vascular bed by its heparin binding motifs. Given that heparin injection increases plasma sFlt-1 levels, we hypothesized that the heparin-induced increment of plasma sFlt-1 level would indicate the antagonizing capacity, but baseline circulating sFlt-1 level wouldn't. Here we verify the hypothesis and investigate significance of PlGF/sFlt-1 system in CKD-related atherosclerosis.

Results: Five minutes after bolus injection of heparin (0.4unit/kg), plasma sFlt-1 levels were increased from 111.8±52.5pg/ml to 291.6±145.7pg/ml ($p<0.001$) in 251 CKD patients. The increment was increased with elevating eGFR ($r=0.746$, $p<0.001$). Consequently, baseline sFlt-1 levels were negatively correlated with eGFR ($r=-0.456$, $p<0.001$), but those after heparin injection were positively correlated ($r=0.549$, $p<0.001$). Plasma PlGF levels were not changed by heparin, and the ratio of PlGF to sFlt-1 was more strongly correlated with eGFR ($r=0.688$, $p<0.001$). The ratio was also significantly associated with severe coronary artery disease in 329 CKD patients, who underwent coronary angiography with (0.4-0.5 unit/kg) heparin injection ($p<0.001$). In mice with 5/6 nephrectomy, sFlt-1 mRNA expression in organs examined (kidney, lung, and heart) was lower by about 20-50% than that in normal mice. In the pharmacokinetic analysis of PlGF, plasma level of human PlGF immediately after injection of recombinant human PlGF (3.0mg/rabbit) was higher in rabbits with 3/4 nephrectomy than in normal rabbits. These findings support the reduction of antagonizing capacity of sFlt-1 for PlGF in CKD.

Conclusions: PlGF and sFlt-1 at least partly play roles in development of atherosclerosis in CKD.

TH-PO192

Atheroprotective Function of High Density Lipoprotein (HDL) Is Defective in End Stage Renal Disease Patients on Hemodialysis (ESRD-HD) Suguru Yamamoto,[1] Patricia G. Yancey,[2] Talat Alp Ikizler,[2] W. Gray Jerome,[4] Ichiei Narita,[3] Macrae F. Linton,[2] Sergio Fazio,[2] Valentina Kon.[1] *[1]Pediatric Nephrology, Vanderbilt University, Nashville, TN; [2]Department of Medicine, Vanderbilt University, Nashville, TN; [3]Department of Medicine, Niigata University, Niigata, Japan; [4]Department of Pathology, Vanderbilt University, Nashville, TN.*

Background: Traditional risk factors do not account for increased cardiovascular disease (CVD) in CKD, particularly in ESRD-HD, who are also resistant to traditional lipid lowering therapies. Since HDL provides important anti-atherogenic benefits through cholesterol efflux and anti-inflammatory capacities, we examined HDL functionality in ESRD-HD.

Methods: Cellular cholesterol efflux and inflammation were assessed in human macrophage THP-1 cells exposed to HDL isolated from ESRD-HD (n=29) and matched normal controls (n=28). HDL was isolated by sequential density ultracentrifugation, and cellular lipid content assessed by gas chromatography measured cholesterol efflux. Inflammatory markers were determined by cytokine mRNA.

Results: There were no differences in plasma lipids, whereas hsCRP was elevated in ESRD-HD. HDL from ESRD-HD was dramatically impaired in facilitating efflux from cholesterol-loaded THP-1 ($p=0.0001$). Efflux impairment was also observed in subgroups of diabetic ESRD-HD vs diabetic controls (8.1 1.6 vs 13.6 1.1%, $p=0.04$) and was not improved in subgroups on statin therapy. HDL of ESRD-HD had less effective anti-chemotactic activity and greater macrophage inflammation for TNF-α, IL-6, IL-1β which was quelled in statin-takers. Notably, there was no correlation between individual HDL impairment in cholesterol efflux and circulating hsCRP or cellular inflammation.

Conclusions: HDL of ESRD-HD has profoundly impaired cholesterol acceptor function and heightened inflammatory capacity. Statins do not improve cholesterol efflux but quell inflammatory response. Findings predict increased foam cell formation and suggest that dysregulated cellular lipid metabolism is a key driver for excess CVD that may explain dissociation of CVD with plasma lipids and resistance to lipid lowering therapies in ESRD-HD.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO193

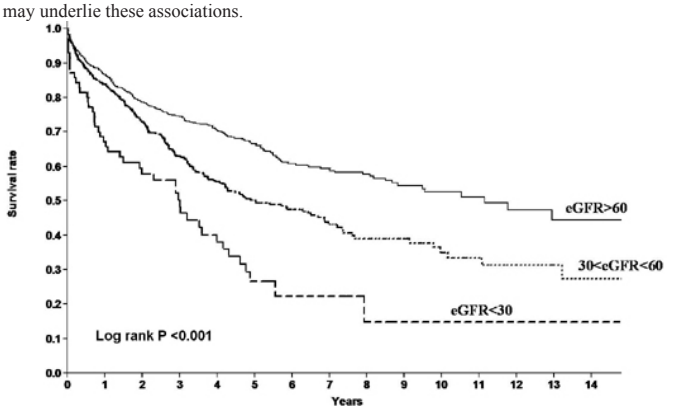
Chronic Kidney Disease, Pulmonary Hypertension and All-Cause Mortality Edgard I. Wehbe,[1] Sankar D. Navaneethan,[1] Jesse D. Schold,[1] Joseph V. Nally,[1] Martin J. Schreiber,[1] Raed A. Dweik.[2] *[1]Nephrology and Hypertension, Glickman Urological and Kidney Institute; [2]Pulmonary and Critical Care, Cleveland Clinic, Cleveland.*

Background: Pulmonary hypertension is associated with high mortality rates. Chronic kidney disease (CKD) is widely prevalent in patients with pulmonary hypertension. We examined the associations of non-dialysis dependent CKD and all-cause mortality in patients with pulmonary hypertension (PH).

Methods: Study population included 1328 patients who underwent right heart catheterization for confirmation of PH (1980-2010). Patients with end stage renal disease (n=15) or who lack serum creatinine levels (n=35) were excluded. We examined the risk factors associated with CKD as well as the association between different stages of CKD and all cause mortality in PH.

Results: Out of 1278 patients, 482 (37.7%) had stage 3 CKD (eGFR 30-59 ml/min/1.73m2) and 70 (5.4%) had stage 4 CKD (eGFR <30 ml/min/1.73 m^2). On multivariable analysis, older age, presence of hypertension, higher pulmonary artery systolic pressure and pulmonary capillary wedge pressure were independently associated with CKD. Kaplan-Meier survival plots indicated significant differences in all-cause mortality for patients with and without CKD (log rank $p<0.001$) (Figure 1). After adjusting for relevant covariates, presence of stage 4 CKD was associated with all-cause mortality (Hazard ratio[HR] 1.59, 95% CI 1.02, 2.40), while stage 3 CKD was not associated with a statistically significant increased hazard for death (HR 1.19, 95% CI 0.94, 1.52). When GFR was examined as a continuous measure, every 5 ml/min decline in GFR was associated with a 3% (95% CI 1.02-1.06) increased hazard for death.

Conclusions: CKD is common in patients with PH. Stage 4 CKD is associated with all-cause mortality in patients with PH. Future studies should explore the mechanisms that may underlie these associations.



TH-PO194

Identification of Differentially Expressed Genes in Arteries from Patients with Renal Failure Jane Stubbe,[1] Vibe Skov,[2] Helle C. Thiesson,[2] Karl-Egon Larsen,[2] Maria Lyck Hansen,[2] Boye Jensen,[1] Bente Jespersen,[3] Lars Rasmussen.[1] *[1]University of Southern Denmark, Denmark; [2]Odense University Hospital, Denmark; [3]Aarhus University Hospital, Denmark.*

Background: The molecular pathology behind arterial disease in uremia is only partially known. Our aim was to identify differentially expressed genes in human arteries from uremic patients by microarray and pathway analysis.

Methods: Tissue samples were obtained from 16 iliac arteries from uremic patients undergoing kidney transplantation and from 19 renal arteries from living kidney donors. In addition, mammary artery samples from 10 patients undergoing coronary by-pass operations were used (5 patients with plasma creatinine above 140 µM and 5 age- and gender-matched individuals with no known kidney disease). Gene expression profiles of these samples were generated using Affymetrix HG-U133A 2.0 microarrays.

Results: We found that 17 gene transcripts differed significantly in iliac samples from patients with uremia with a false discovery rate (FDR)<0.003, $p<1.0*10-5$. Using GenMAPP, we found that 13 pathways were significantly regulated. Both the apoptosis pathway and the TNF-alpha-NFκB pathway were up-regulated, while the smooth muscle contraction- and glycogen metabolism pathways were down-regulated. Verhoeff-van Gieson stained artery samples showed no obvious compositional differences between the 2 groups. To compensate for bias because of different artery location between the uremic and non-uremic groups, we extended our findings with microarray data obtained from the mammary artery samples from the coronary by-pass patients. We then found that 23 gene transcripts were congruently downregulated and 8 gene transcripts upregulated in both comparisons when the setting FDR <0.05 in the iliac/renal artery study and the p-value <0.2 in the mammary artery study were used. This gene transcript group contains vimentin, CD9, hypoxia inducible factor 3A, matrix molecules, and smooth muscle differentiation factors.

Conclusions: The identified genes are likely to be associated with the development of cardiovascular disease in patients with renal failure.

Funding: Private Foundation Support

TH-PO195

Brain White Matter Abnormalities in Children wth Chronic Kidney Disease
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Background: There is increasing evidence that children with chronic kidney disease (CKD) have neurocognitive deficits. We hypothesized that these deficits result from subtle brain injury and abnormal brain white matter microstructure. Our objectives were: (1) to determine the prevalence of brain injury or abnormalities and (2) to compare white matter integrity in children with CKD relative to healthy controls.

Methods: Magnetic resonance imaging (MRI) studies were obtained in a prospective cohort of 29 children with CKD (mean age of 14.4 ± 2.7 years (range 9-18)), and 20 healthy age matched controls (mean age of 13.6 ± 2.9 years (range 9 – 18)) at two pediatric renal centres. Conventional MRIs were reviewed by a single blinded neuroradiologist to determine the prevalence of brain injury. Fractional anisotropy (FA) maps calculated from diffusion tensor imaging scans were generated to compare white matter microstructure in both groups using tract-based spatial statistics .

Results: Focal or multifocal white matter injury was seen on conventional MRI in 6 children with CKD (21%) and in 1 control. Relative to controls, reduced white matter FA was seen in the anterior limb of the internal capsule of the CKD subjects, a region that contains white matter pathways involved in cognitive functions. The FA reduction in CKD children was greater in the non-transplant CKD group relative to the transplant group.

Conclusions: In this preliminary study, white matter injuries are observed in children with CKD. Abnormal white matter microstructure, reflected in reduced FA, is also observed in children with CKD. The potential difference observed between children with renal transplant vs. CKD requires further investigation.

Funding: NIDDK Support, Private Foundation Support

TH-PO196

Polyclonal Immunoglobulin Free Light Chain Concentrations and Survival in Patients with Renal Disease
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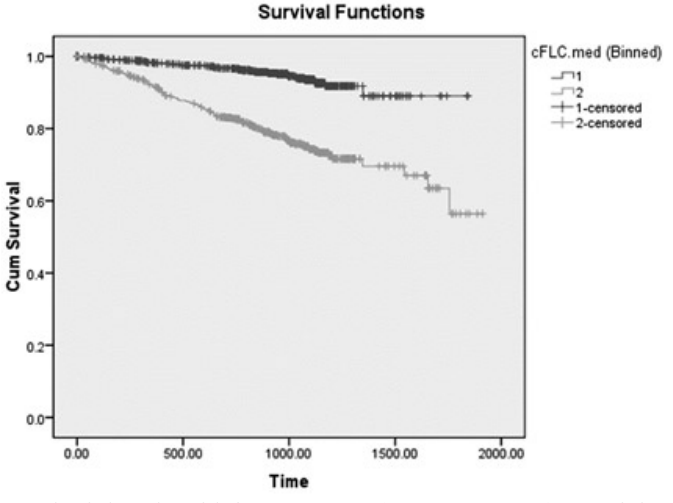
Background: Chronic kidney disease (CKD) affects around 10% of adults, with associated high morbidity and mortality. Enhanced risk stratification to identify those with CKD at highest risk of poor outcomes is required to optimise the management of CKD. Polyclonal immunoglobulin free light chains (FLC) are both markers of kidney function and of systemic inflammation. The purpose of this study was to evaluate the utility of polyclonal serum FLCs for mortality risk in people with CKD.

Methods: Serum concentrations of polyclonal FLCs were measured by immunoassay in 1275 individuals with CKD, these comprised: (i) 848 with CKD stages 1-5; (ii) 382 renal transplant recipients; and (iii) 45 dialysis patients (CKD 5d). The cohort was prospectively followed up for a median of 45 months. Cox regression analysis was undertaken to determine variables associated with survival.

Results: Serum FLC concentrations closely correlated with kidney function. The co-linearity between the two FLC isotypes (κ and λ) and total FLC levels were high, therefore in the survival analysis total levels were used. In the overall cohort, high serum polyclonal FLC levels were an independent risk factor for death: HR 2.06 (1.55-2.75), P<0.001.



Other independent risk factors were age (HR 1.79, 1.54-2.09), pre-existing cardiovascular disease (HR 1.73, 1.2-2.48) and hs-CRP (1.22, 1.09-1.38), all P<0.003. Kidney function was not an independent risk factors for death. On subgroup analysis FLC concentrations were independently associated with death in the CKD and ESRD subgroups.

Conclusions: Serum concentrations of polyclonal FLCs independently predicted survival in patients with CKD and ESRF but not renal transplant recipients. Strategies now need to be devised to apply this observation to clinical practice.

TH-PO197

Rapide Decline of eGFR Predicts Long Term Improvement of Renal Function after Revascularization of Renal Artery Stenosis
Antonio Mannarino, Giuseppe Lucian Spatoliatore. *Nephrology, San Giovanni di Dio, Firenze, Italy.*

Background: to determine if decline of renal function prior to the time of stenting impacts on renal outcome after stenting in a cohort of patients with Atherosclerotic Renal Artery Stenosis (ARAS)

Methods: 30 patients with CKD stages 3 to 4 and ARAS underwent renal stenting. Mean follow up : 33 (SD 21) months ; the change of eGFR before and after stenting was expressed as negative or positive value in mL/month (ΔGFR).

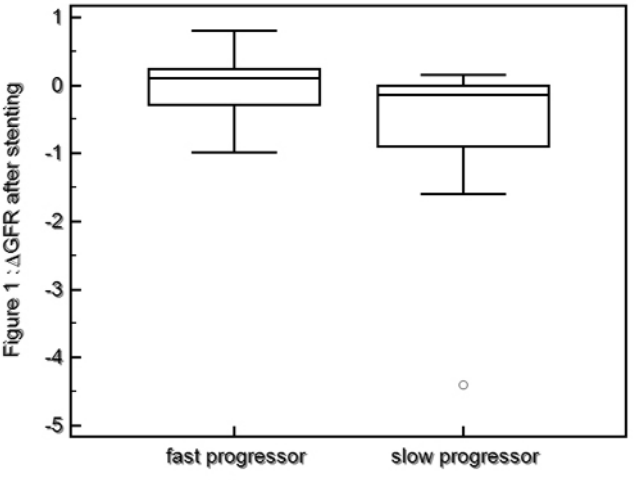
We indentified 2 types of subgroups : the first on the basis of declining of renal function (cut point -0,25 mL/mo) by assessing ΔGFR in a prestenting period of 10 months (Slow or Fast Progressor) N=24 and the second on the basis of stenosis type : 1 (unilateral) N= 13 ; 2 (7 bilateral ; 2 single kidney ; 8 prevalent kidney) N= 17. No difference at baseline was noted between subgroups for the following variables : age, eGFR, renal lenght, resistive index, proteinuria, diabetes and vascular diseases.

Results: 37 stents were placed successfully. No periprocedural death occurred. At latest follow up renal function improved in 14 patients (46,6%) , stabilized in 6 patients (20%) and worsened in 10 patients (33%). 7 of these 10 patients reached ESRD which required hemodialysis.

Being in Fast Progressor subgroup was associated with improved renal function after stenting (8 of 13 patients ; p=0,013; Fisher's test).

Belonging to the subgroup 2 was predictor for improvement of renal function (11 of 17 patients ; p=0,032; Fisher's test)

After stenting median ΔGFR was significantly greater in the Fast Progressor subgroup compared to the Slow Progressor (0,10 vs -0,14 ; p=0,04; Mann Whitney test)



Conclusions: Predictable benefit from renal stenting may be most likely in patients presenting with a rapid decline of GFR associated with ARAS affecting the whole renal mass that is both kidneys or single functioning kidney.

TH-PO198

Lipoprotein Abnormalities, Monocytosis, and Accelerated Atherosclerosis in Patients with CKD
Anjali Ganda,[1] Laurent Yvan-Charvet,[1] Rupali Avasare,[1] Andrew Murphy,[1] Nora Bijl,[1] Bibhas Chakraborty,[2] Linda Vernocchi,[1] Alan Tall.[1] *[1]Medicine, College of Physicians&Surgeons, Columbia University, New York, NY; [2]Biostatistics, Mailman School of Public Health, Columbia University, New York, NY.*

Background: In mouse models, HDL-mediated cholesterol efflux results in decreased expression of the common beta subunit (IL-3Rβ) of the interleukin-3 (IL-3) and granulocyte macrophage colony-stimulating factor (GM-CSF) receptors on the surface of hematopoietic stem cells and monocytes, and decreased IL-3/GM-CSF mediated proliferative responses. Pro-atherogenic CD14++CD16+ monocytes predict CV events in CKD. We hypothesized that monocytosis in CKD may be IL-3/GM-CSF mediated, reflecting HDL dysfunction.

Methods: EDTA-treated blood from adult CKD patients (eGFR<30 ml/min/1.73m²) and matched controls (eGFR≥60 ml/min/1.73m²) was subjected to red blood cell lysis followed by flow cytometry. Monocytes were HLA-DR+, B-cell, T-cell, NK-cell, and granulocyte marker negative, classified into 3 subsets by CD14 and CD16. IL-3Rβ mean fluorescence intensity (MFI) was measured.

Results: CKD patients vs. controls: eGFR/MDRD 19.4±1.0 vs. 81.2±2.7 ml/min/1.73m² p<0.00001, triglycerides 173.4±17.7 vs. 117.4±12.4 mg/dL p=0.02, no difference in sex, % diabetic, systolic BP, HDL, LDL, total cholesterol, total peripheral white blood cell.

Monocytosis in CKD and increase in cell surface IL-3Rβ

Monocytes	Control (n=30) % of leukocytes	CKD (n=30) % of leukocytes	P-value	Control (n=30) IL-3RβMFI	CKD (n=30) IL-3Rβ MFI	P-value
Total	4.41±0.34	6.43 ±0.66	0.008	271.03 ±14.73	341.43±21.04	0.008
CD14++CD16-	3.07±0.24	4.52 ±0.48	0.009	264.50 ± 17.80	329.10±22.71	0.03
CD14dimCD16+	0.96±0.11	1.37±0.19	0.06	322.58 ±18.77	400.40 ±28.14	0.03
CD14++CD16+	0.30±0.04	0.45 ±0.05	0.04	348.97±24.31	437.77±31.76	0.03

Among CKD patients, monocyte counts were strongly correlated with cell surface IL-3Rβ (r=0.7, P<0.0001).

Conclusions: We found monocytosis involving all major subsets in CKD, likely contributing to CVD risk. Monocytosis may reflect increased proliferation secondary to increase in IL-3Rβ. Although HDL levels were unchanged, this abnormality suggests underlying dysfunction of HDL-mediated cholesterol efflux pathways affecting monocytes or their progenitors.

Funding: Private Foundation Support

TH-PO199

Effects of Fibrates in CKD: A Systematic Review and Meta-Analysis Min Jun, Alan Cass, Vlado Perkovic. *George Institute for Global Health, Uni of Sydney, Australia.*

Background: Individuals with chronic kidney disease (CKD) often have high triglyceride and low HDL cholesterol levels, but there is limited evidence about the effects of fibrate therapy in this population. A systematic review and meta-analysis was undertaken to synthesize the available evidence.

Methods: MEDLINE, EMBASE, and the Cochrane Library were systematically searched for trials (1950-May 2011). Prospective RCTs assessing the effects of fibrate therapy in CKD and/or on kidney-related outcomes compared with placebo were included. Summary RRs and 95% CIs were calculated using a random effects model. Outcomes included change in kidney function or albuminuria, end-stage kidney disease (ESKD), renal death, and in patients with kidney disease: cardiovascular (CV) events, all-cause death, CV death, and adverse events.

Results: We identified 11 studies (13488 participants) of which 4 were subgroup analyses of larger trials in the general population, while the others were generally small and of suboptimal quality. Fibrate therapy increased the likelihood of albuminuria regression by 19% (95% CI: 8-32%, p<0.0001). There was significant heterogeneity observed in its effect on the progression of albuminuria (RR 0.66, 0.35-1.26; p=0.210; I^2=75.6%, p for hetero=0.043). Due to differences in reporting methodologies, it was not possible to pool effects on change in kidney function. Five studies reported no effect and one showed a significantly delayed GFR loss in the fibrate group. There was limited data regarding the effect of fibrate therapy on ESKD or renal death with no clear effect on either outcome. One study reported a reduced incidence of CV events using gemfibrozil in patients with early CKD (RR 0.76, 0.61-0.94, p=0.010). Most studies reported significantly increased serum creatinine in the fibrate group compared to placebo but also reported normalization or improvement after end of therapy.

Conclusions: Fibrate therapy improves the rate of albuminuria regression in diabetic patients with early stage CKD, but there is limited evidence regarding the effect of fibrate therapy on long-term kidney function. Trials with sufficient power to detect a difference in hard renal outcomes are needed.

TH-PO200

Renal Impairment Profoundly Impairs the Efficacy of Thrombolytic Therapy in Acute Ischemic Stroke Albert J. Power, Daniel Epstein, Diane Ames, Arindam Kar, Dima Dahdaleh, Neill Duncan, David Taube. *Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Thrombolysis for acute ischemic stroke is the current standard of care. However there is a lack of comprehensive US data on the safety and efficacy of this treatment in patients with chronic kidney disease [CKD] who are at highest risk for stroke.

Methods: We examined all patients treated with alteplase [0.9mg/kg] for acute ischemic stroke at our center [1st December 2009 – 14th January 2010]. Stroke severity was recorded using the NIH Stroke Scale [NIHSS] at 0, 2 & 24hrs and at 7 days. Time to treatment was the interval between symptom onset and alteplase dosing. Risk factors for stroke were coded on admission, eGFR was calculated using the 4-variable MDRD equation.

Results: 100 patients were treated [mean age 71.5±11.9yrs, 61% male, 29% diabetic, median eGFR 73ml/min. 32% had an eGFR<60ml/min, one was on hemodialysis. Diabetes was more prevalent in patients with eGFR<60ml/min [44% vs 20%, p=0.005], no other significant differences in major comorbidities between the groups.

Median NIHSS score at presentation was 10 [IQR 7-15], median time to treatment 2.5hrs [IQR 2.0-3.3hrs] with no difference between groups [p=0.7 & p=0.8 respectively]. Thrombolysis improved median NIHSS score by 3 at 2hrs, 6 at 24hrs and by 7 at one week [n=82].

Patients with eGFR<60ml/min had on average 32% less improvement in NIHSS score at 24hrs compared to those with eGFR>60ml/min on univariate analysis [p=0.03] and multivariate analysis adjusting for stroke severity [38% less, p=0.02]. Age, gender, ethnicity, time to treatment and comorbidities had no significant effect on outcome. There was no difference in hemorrhagic complications between groups [n=5, 1 with CKD, p=0.9]. 7-day mortality was 4% and not influenced by renal impairment.

Conclusions: In the largest European study of its kind to date we found significant attenuation of neurological outcome following thrombolysis for acute stroke in patients with renal impairment but no excess in hemorrhagic complications. This may relate to greater subclinical cerebrovascular burden and uremic endothelial dysfunction and requires comprehensive study.

TH-PO201

Serum Phosphate Qualify as Atherosclerotic Risk Factor, Particularly in the Setting of Older Chronic Disease Patients Marisa Martin Conde,[1] Victor Lorenzo,[2] Angels Betriu,[1] Adriana S. Dusso,[4] Jose M. Valdivielso,[4] Elvira Fernandez.[1] *[1]Department of Nephrology, Arnau de Vilanova Hospital, Lleida, Spain; [2]Department of Nephrology, Canary Island University Hospital, La Laguna, Tenerife, Spain; [3]Department of Nephrology, Arnau de Vilanova Hospital, Lleida, Spain; [4]Experimental Nephrology, IRB Lleida, Lleida, Spain; [5]Experimental Nephrology, IRB Lleida, Lleida, Spain; [6]Department of Nephrology, Arnau de Vilanova Hospital, Lleida, Spain.*

Background: Translational studies demonstrate that hyperphosphatemia promotes atherosclerotic vascular calcification. However, the association of serum phosphorus (P) with parameters of atherosclerosis (AT) remains controversial in Chronic Kidney Disease (CKD) patients

Methods: This multicenter observational study included 1279 CKD patients from the spanish multicenter study NEFRONA (54, 33 and 14% at CKD stages III, IV and V, respectively), 60±12 years, 63% males, 28% diabetics. We applied a standardized protocol including carotid intima–media thickness, ankle-brachial index and presence/abscense of carotid plaques to determine the AT score (Coll B et al, NDT 2010). The influence of study variables was evaluated applying uni- and multivariate logistic regression model, and using AT score as the dependent variable.

Results: Patients were classified in 2 groups: 1) no or mild AT (44%); 2) moderate to severe AT (56%). The presence and severity of AT was significantly associated with older age (HR 1.087 [CI95% 1.073-1.102], p<0.001), male sex (1.361 [1.007-1.841], p=0.045), higher P (1.354 [1.135-1.616], p=0.001), systolic blood pressure (1.010 [1.004-1.016], p=0.002), smoking (2.056 [1.535-2.754], p=0.001), and diabetes (1.554 [1.148-2.102], p=0.004) in the multivariate model. The level of renal failure, serum calcium, PTH, and dislipidemia were not associated with AT score. In subsequent analysis, the risk for moderate to severe AT is multiplied by a factor of 2.8 for patients >63 years with P>4.2 mg/dl (2.761 [1.639-4.649], p<0.001).

Conclusions: We verified that high P represent a risk for AT in CKD patients with GFR<60 ml/min. This association is more consistent in older patients even with P levels within or close to normal range

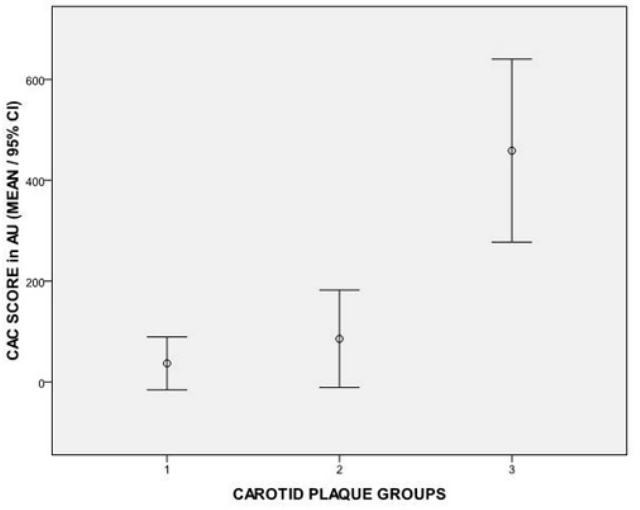
TH-PO202

Carotid Plaques Predict Presence and Severity of Coronary Artery Calcium in Chronic Kidney Disease Luis Gerardo D'Marco,[1] Cristina Karohl,[2] Andrew Smith,[3] Emir Veledar,[3] Paolo Raggi.[3] *[1]Nephrology, Ruiz y Paez University Hospital, Bolivar, Venezuela; [2]Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; [3]Division of Cardiology and Department of Medicine, Emory University, Atlanta, GA.*

Background: Coronary artery calcium (CAC) measured by multi slice computer tomography (MSCT) is predictive of mortality in CKD. Due to the high cost, radiation exposure and limited availability of MSCT non-radiation based methods such as carotid ultrasound imaging (CUI) may be preferable. We assessed the association of plaque on CUI and CAC in CKD-5D patients.

Methods: Ninety-one CKD-5D patients (mean vintage 31±24 months, 51% women, 71% Black, 68% diabetic) underwent CT for CAC imaging. CAC scoring was performed with the Agatston method and stratified as < 10 (minimal), 10-99 (mild), 100-399 (moderate), and ≥ 400 (severe). Patients were also grouped according to presence of carotid plaques and classified as follows: group 1 (no plaques), group 2 (unilateral plaque/s), and group 3 (bilateral plaque/s).

Results: Carotid plaques were visualized in 74 (81%) patients. CAC score was <10 in 35 (38%), mild in 18 (20%) and moderate to severe in 38 (42%) patients. The mean CAC was 55.6 ± 103 for group 1, 114 ± 150 for group 2 and 586 ± 951 for group 3 (*P= 0.02 for trend*).



The presence of carotid plaques was significantly correlated with CAC in univariate analysis (*r=.277; P=0.008*) and after adjustment for age, gender, race, CKD etiology, and dialysis vintage (*P=0.025)*.

Conclusions: We showed an association between presence of carotid plaques and severity of CAC in CKD-5D patients. These results suggest that CUI may be used to predict the presence of coronary vasculopathy without need to perform radiation based imaging.

TH-PO203

Lipid Profile and Opposing Changes in Intima Media Thickness and Atheromatous Plaque in the Course of Chronic Kidney Disease Angels Betriu,[2] Adriana S. Dusso,[2] M. Vittoria Arcidiacono,[2] Montserrat Martinez-Alonso,[2] Jose M. Valdivielso,[2] Elvira Fernandez.[1] *[1]Hospital Arnau Vilanova; [2]IRBLleida, Lleida, Spain.*

Background: Arterial intima-media thickness (IMT) is an early marker of atheromatous disease, highly influenced by lipid metabolism in normal individuals, but its accuracy to predict atheromatosis in chronic kidney disease (CKD) is poorly understood.

Methods: Cross sectional study examining 1785 patients from the Spanish Multicenter Study NEFRONA, average age 58 years; 62% males; 54% smokers; CKD stages (%) 3:33; 4-5:32; and 5D:35%. IMT was mesured by bilateral carotid ultrasound; values represent mean IMT from common, bulb and internal carotid.

Results: IMT increases with age at all stages of CKD, in both genders.It is higher in men and in patients with atheromatous plaque (0.78 vs 0.67, p<0.001); and lower in CKD4-5 and 5D than CKD3 (0.70, 0.72 vs 0.77; p<0.001) while the % of patients with plaque increases in the course of CKD (3: 55; 4-5: 56; 5D: 62). In the course of CKD, in patients (%) not receiving statins (3:35; 4-5: 29; 5D:47), average LDL and HDL levels decrease (LDL;HDL: 3:117;51; 4-5:108;50; 5D:91;47) and both correlate positively with albumin and BMI, and negatively with ferritin. Also, the % of patients with LDL>145 decrease (3: 11; 4-5: 11; 5D: 6*) and those with LDL<100 increases (3:37; 4-5: 50; 5D: 62*). The % of patients with HDL>60 also decreases (3:22; 4-5:19; 5D:16*), while the % of patients with HDL<40 increases (3:22; 4-5:30;5D: 33*) (*p<0.001). In multivariant analysis, IMT associated positively with age**, smoking**, diabetes**, BMI (p=0.01), pulse pressure** and negatively with female gender**, CKD4-5**, and HDL>40 mg/dl (p=0.02); ** p<0.0001.

Conclusions: In the course of CKD: 1.IMT does not increase in spite of increases in plaque formation. Thus, ITM measurements underestimate the risk of atheromatosis; 2.LDL and HDL decrease in all patients. Thus, LDL levels do not contribute to increase IMT but HDL levels maintain vascular protection; 3.IMT associates with traditional atheromatosis risk factors and their control should slow disease progression; 4.The opposing impact of the lipid profile on IMT and plaque suggests a distinct pathogenesis.

Funding: Pharmaceutical Company Support

TH-PO204

Atorvastatin Slows the Progression of Chronic Kidney Disease in Patients with Inflammation Robert G. Fassett,[1] Iain Robertson,[2] Madeline J. Ball,[2] Dominic P. Geraghty,[2] Jeff S. Coombes.[3] *[1]Renal Medicine, University of Queensland, Brisbane, Queensland, Australia; [2]Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; [3]Human Movement Studies, University of Queensland, Brisbane, Queensland, Australia.*

Background: Inflammation may be present in patients with CKD and associated with its progression. The Lipid lowering and Onset of Renal Disease (LORD) trial was a three-year randomised, double-blind, placebo-controlled trial investigating the effects of atorvastatin on kidney function in CKD patients. The aim of this sub-study was to assess the effects of atorvastatin on inflammation and kidney function in chronic kidney disease (CKD) patients in the LORD trial.

Methods: Eighty-six patients with serum creatinine >120μmol/l were randomized to receive atorvastatin 10 mg/day (47) or placebo (39) and followed for a mean of 2.9 years. Thirty three individuals with normal kidney function were controls. Inflammatory markers measured included plasma pentraxin-3 (PTX3), TNFα, hs-CRP, IL-6, IL-8 and IL-10 and kidney function was assessed using MDRD eGFR. The association (Odds Ratio) between inflammatory markers and eGFR were estimated using repeated measures ordinal logistic regression. An OR >1.00 indicates a positive association, and an OR <1.00 indicates a negative association. General linear modelling was used to determine relationships with the change in eGFR.

Results: At baseline, compared with controls, CKD patients had significantly increased levels of PTX3 (mean, 1.07 vs 0.58 ng/ml; difference, 0.50; 95% CI 0.08-0.92; P=0.02), TNFα (p=0.02) and IL-8 (p=0.01). Baseline plasma PTX3 was associated with eGFR at all time points (OR 0.6, 95% CI 0.5-0.7, P<0.001) but not (P>0.05) the change in eGFR. However, atorvastatin-treated patients with baseline plasma PTX3 levels >1.5 ng/ml had less eGFR decline than placebo-treated patients (atorvastatin, n=8, -0.4; vs placebo n=5, -1.6 ml/min/1.73m²/yr; difference=1.2 95% CI 0-2.4; P=0.058).

Conclusions: Patients had increased levels of inflammatory biomarkers and those with plasma PTX3 >1.5 ng/ml at baseline had less eGFR decline when treated with atorvastatin. Atorvastatin may slow eGFR decline in CKD patients with inflammation.

Funding: Pharmaceutical Company Support

TH-PO205

Serum High Sensitivity Cardiac Troponin T Is a Significant Biomarker of Left Ventricular Diastolic Dysfunction in Patients with Chronic Kidney Disease Masashi Kitagawa, Hitoshi Sugiyama, Hiroshi Morinaga, Tatsuyuki Inoue, Keiichi Takiue, Yoko Kikumoto, Ayu Ogawa, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Chronic kidney disease (CKD) is associated with left ventricular diastolic dysfunction (LVDD), which progresses to diastolic heart failure with a poor prognosis. Recently, high sensitivity cardiac troponin T (hs-cTnT) has been utilized in the clinical settings.

Methods: In 132 patients with CKD, the relationships among the results of echocardiography, hs-cTnT, B-type natriuretic peptide (BNP), and renal function were evaluated. The LV mass index (LVMI), peak early diastolic mitral filling velocity (E), peak early diastolic mitral annular velocity (E'), and E/E' were recorded.

Results: Based on our findings, E' significantly decreased, while E/E', BNP, and hs-cTnT significantly increased in association with the progression of CKD stages. The natural logarithm of hs-cTnT negatively correlated with E', whereas it was positively associated with proteinuria, cystatin C, BNP, mean arterial pressure and LVMI. The CKD patients with LVDD who had E' < 5 cm/sec had a significantly higher hs-cTnT level, as well as a significantly higher BNP level, compared to those with E' ≥ 5 cm/sec. The areas under the receiver-operating characteristic curves for hs-cTnT and BNP to detect E' < 5 cm/sec were 0.847 (p = 0.0032) and 0.770 (p = 0.0125), respectively. A hs-cTnT value of 9 pg/mL had a sensitivity of 100%, and a specificity of 67%. A BNP value of 20.3 pg/mL had a sensitivity of 88%, and a specificity of 59%. The univariate logistic regression analyses of the data corroborated the association between E' < 5 cm/sec and the serum hs-cTnT levels per 10 pg/mL increase (odds ratio [OR] 1.48 [95% confidence interval (CI) 1.14-1.96]; p = 0.0004), and the association between the E' < 5 cm/sec and plasma BNP levels per 30 pg/mL increase (OR 1.45 [1.09-1.99]; p = 0.0129).

Conclusions: These data strongly suggest that the newly established parameter, serum hs-cTnT, may be associated with LVDD in CKD patients.

TH-PO206

Progression of Coronary Artery Calcification in CKD: Risk Factors and Association with Mortality Khaled Nashar,[1] Bryan R. Kestenbaum,[2] Mark J. Sarnak,[3] Robert Boudreau,[1] Michael Shlipak,[4] Daniel Edmundowicz,[1] Ali Yazdanyar,[5] Anne B. Newman,[1] Linda F. Fried.[1,6] *[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Univ. of Washington, Seattle, WA; [3]Tufts New England Medical Center, Boston, MA; [4]San Francisco VA and UCSF, San Francisco, CA; [5]St Luke's Hospital, Bethlehem, PA; [6]VAPHS, Pittsburgh, PA.*

Background: Progression of coronary artery calcification (CAC) has been used as a surrogate outcome in dialysis patients. Risk factors for CAC progression and the association with mortality are not known in moderate-severe CKD.

Methods: We assessed change in CAC in community dwelling older individuals in Pittsburgh participating in the Cardiovascular Health Study. Baseline EBCT was obtained 1998-2000; a follow-up scan was performed a mean of 3.4 years later. The median follow-up after the second EBCT was 5.3 yrs. CAC was assessed using Agatston method. CKD was defined as eGFR < 60 ml/min/1.73m² using the CKD-EPI formula. A clinically relevant change in CAC in individuals with baseline score >0 was defined as absolute change >100 or annualized change > 20%/yr. Logistic regression was used for rapid progression and proportional hazards model for mortality after the second scan.

Results: Of 319 patients with an initial scan > 0, median baseline CAC score was 332, 58% were females 21% were African American, 32% had CKD. Median change in CAC was 111 (IQR 33, 343); 100 died, 191 individuals had change 100/year, 120 had > 20%/y change. Neither CKD nor eGFR were significant predictors of change in CAC (p >0.1). In age, race, gender, baseline CAC adjusted analyses, change > 100 (HR 2.4 (1.1, 5.2)) or 20% change (2.4 (1.2, 4.8) predicted mortality in CKD, but not in those without CKD

(1.0 (0.6,1.7) and 0.9 (0.5, 1.7) respectively. However, the p-values for interaction terms (CKD*change) were not significant (p= 0.36 for change >100 and 0.07 for 20% change). The association of the CAC score at second scan w/o change in model was of borderline significance for mortality (per 100 higher score, 1.03 (0.999, 1.07) and 1.02 (0.997,1.05) in CKD and non-CKD respectively.

Conclusions: CKD did not predict CAC progression in older individuals. However, a CAC change predicted mortality in individuals with CKD.

Funding: Other NIH Support - NHLBI, NIA

TH-PO207

Hyperleptinemia Is Associated with the Presence and Severity of Myocardial Ischemia in Non-Dialysis Dependent Chronic Kidney Disease

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Background: Experimental data suggest that leptin receptors are expressed in coronary arteries and that hyperleptinemia causes significant coronary endothelial dysfunction. It is presently unknown if uremic hyperleptinemia is linked to myocardial ischemia (MI) in CKD patients (pts).

Methods: We cross-sectionally evaluated 118 non-dialysis dependent CKD stages 3-5 pts (median age: 59 years [52– 67], 80 men). MI (presence and extension) was screened by 99mTc-sestamibi myocardial perfusion scintigraphy (MPS). All analyses were performed by the same, blinded, physician.

Results: Fifty five pts (33 men) presented an ischemic MPS. MI extension was positively correlated with BMI (rho = 0.25; P <0.01); leptin (rho = 0.26; P < 0.01) and leptin/BMI (rho=0.24; P=0.01). Pts were divided into three groups (no-MI [n=63; 64% men], MI < 10% [n=28; 68% men], and MI ≥ 10% of cardiac area [n=27; 78% men]). Across these groups, and as MI became more severe, BMI (27.8 ± 5.7 vs. 31.7 ± 6.6 vs. 30.8 ± 5.6 Kg/m^2; P <0.01), Leptin (11.9 [4.0 – 25.4] vs. 19.1 [4.9 – 44.2] vs. 26.0 [10.2 – 42.0] ng/mL; P = 0.02), as well as the Leptin/BMI (0.40 [0.17 – 0.85] vs. 0.64 [0.18 – 1.15] vs. 0.74 [0.48 – 1.36]; P = 0.03) were incrementally higher. In crude and adjusted multinomial logistic regressions (considering pts without MI as the reference category), leptin/BMI was associated with higher odds of increased severity in MI **(Table 1)**

Table 1

Model	Covariates	Odds-ratio (95% Confidence Interval)	
		Ischemia <10%	Ischemia ≥ 10%
1	Leptin/BMI	1.84 (0.94 – 3.63)	2.01 (1.03 – 3.95)
2	1 + Age > 60 and gender	2.27 (1.06 – 4.86)	2.89 (1.31 – 6.35)
3	2 + Diabetes and 24h Creatinine Clearance	2.37 (1.10 – 5.15)	2.89 (1.30 – 6.39)
4	3 + Inflammation (CRP >10 mg/L)	2.27 (1.05 – 4.89)	3.33 (1.46 – 7.62)

Conclusions: Excess leptinemia (as depicted by an increased Leptin/BMI) is associated to the presence and severity of MI in non-dialysis dependent CKD pts. Our data suggests further links between hyperleptinemia and cardiovascular risk in CKD.

Funding: Government Support - Non-U.S.

TH-PO208

Cardiovascular Injury in Patients with Diabetic Nephropathy

Yuxi Yang, Songmin Huang. *Division of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: Cardiovascular diseases become the primary death cause of patients with diabetic nephropathy(DN). We investigated the relationship between Urine protein and cardiovascular risk factors in 1046 cases of patients with DN.

Methods: A sample of 1046 adults with DN from West China Hospital of Sichuan University participated in the study. The patients' general situation (gender, age, blood pressure) were recorded. Fasting blood glucose, HbA1c, urinary protein, estimated glomerular filtration rate, serum creatinine, triglyceride, cholesterol, peripheral arterial ultrasound, echocardiography and chest X-ray were observed. The ways of treatments were noted.

Results: The prevalence of DN was higher among male (67.7%) than among female. The prevalence of DN increased with increasing age, which was most common among people 58-70 years old. 70.3% DN patients' fasting blood glucose were below 7mmol/L, but only 22.1% DN patients' blood pressure reached the standard(<130/80mmHg). 26.1% of DN patients had TG<1.7mmol/L and/or CHOL<4.5mmol/L.Only 28.4% of patients had a HbA1c test and 44.4% of them were below 6%. Majority were already in chronic renal failure (82.5% of DN patients'Serum creatinine were above 450umol/L and 86.6% of DN patients' eGFR below 30 ml/min/1.73 m2) when they first visited doctor. 44.5% of DN patients showed abnormal peripheral arterial ultrasound (Atherosclerotic plaque and/or arterial intimal thickening). While 51.4% of DN patients had abnormal echocardiography, including the growing heart and functional damage. Meanwhile 13% of DN patients' chest X-ray showed artery calcification. About half of the DN patients' conditions kept stable by medication, and one third of them chose hemodialysis. Meanwhile less than 20% chose peritoneal dialysis. The survival of patients with hemodialysis and peritoneal dialysis were 96.3% and 99% respectively, while the mortality were 3.7% and 1.0%. The urinary protein was positively correlated with triglyceride and cholesterol(r=0.811, p<0.01;r=0.803, p<0.01). The HbA1c was positively correlated with triglyceride and cholesterol(r=0.896, p<0.01;r=0.908, p<0.01).

Conclusions: The results indicate that DN was positively correlated with Cardiovascular damage.

TH-PO209

Time on Kidney Transplant Waiting List Leads to Progression of Echocardiographic Abnormalities Associated with Adverse Outcomes

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Background: Echocardiographic (ECHO) abnormalities are well described in chronic kidney disease and are associated with increased cardiovascular (CV) events and total mortality. Little is known regarding progression of ECHO abnormalities in patients awaiting kidney transplantation (KT).

Methods: This study assessed the progression of ECHO abnormalities in patients awaiting KT between Jan 2004 and Dec 2010 with a repeat ECHO at least 1 year after baseline ECHO. All patients were followed for cardiac events and mortality over 51±20 mo.

Results: We assessed 63 patients (60% male; age 63±10 years; 30% diabetes; 51% predialysis). On baseline ECHO, left ventricular (LV) hypertrophy [left ventricular mass index (LVMI) ≥110 in F, ≥125 g/m in M] using Penn formula and indexed for height was present in 54% of patients, left atrial (LA) enlargement (LA≥4 cm) in 42% and systolic dysfunction [fractional shortening (FS) ≤30%] in 22%.

On repeat ECHO (31±19 mo from baseline) LVMI increased (133±43 to 146±50 g/m, *P*=0.02). The thicknesses of intraventricular septum (1.05±0.26 *v.* 1.12±0.30 cm, *P*=0.01) and LV posterior wall increased (1.01±0.22 *v.* 1.09±0.28 cm; *P*=0.003) and LV end-diastolic diameter did not change (4.8±0.6 *v*. 4.8±0.6, *P*=0.9). Left atrial diameter (LAD) increased (3.8±0.6 to 4.1±0.8 cm; *P*=0.02), whilst FS did not change (37±7 *v.* 36±8%, *P*=0.7). Age, gender, diabetes, baseline SBP and hemoglobin were not predictors of LVMI increase.

During follow up very few deaths and CV events were observed (n=4, 6.4%). Interestingly, patients with adverse outcomes had higher baseline LAD (4.8±0.9 *v.* 3.8±0.6 cm, *P*=0.01), higher LVMI (184±19 *v.* 129±42 g/m, *P*=0.012) and lower FS (26±8 *v*. 37±7%, *P*=0.05). On Cox analysis, in a model adjusted for traditional CV risk factors, LAD (*P*=0.03) and FS (*P*=0.02) were predictors of adverse events.

Conclusions: Echocardiographic abnormalities are prominent in patients awaiting KT. Time on waiting list is associated with increases in LAD and LVMI. Both of these are markers of adverse outcome. This might have implications for timing of KT and ECHO screening.

TH-PO210

Advanced Glycation End Products Are Independently Associated with Arterial Stiffness in Patients with Progressive Chronic Kidney Disease

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Background: The RIISC (Renal Impairment In Secondary Care) study aims to elucidate the risk factors for progression of CKD and define the bio-clinical characteristics of patients with progressive CKD. Here we report early cross-sectional data with a focus on non-invasive markers of macrovascular (central BP and PWV) and microvascular (AGE reader (skin autofluorescence)) health.

Methods: Patients fulfilled the following criteria for enrolment: (i) progressive CKD and/or (ii) an ACR >70 mg/mmol on 2 consecutive occasions, and CKD 3-5. Patients underwent a detailed CV assessment which included a detailed history, blood pressure by BpTRU (equivalent to daytime ABPM), pulse wave velocity (PWV) by Vicorder, and measurement of AGEs using an AGE reader. These were performed in a temperature controlled room that was maintained between 22-24°C.

Results: To date 100 patients have been recruited. The mean age was 61.3 years (SD 18.1) and 61% were men. 70% were of white ethnicity, 15% each South Asian and Black. The mean eGFR at enrolment was 24ml/min/1.73m^2 (SD 8.9). 29% had a previous diagnosis of CV disease (ischaemic heart disease, cerebrovascular disease and peripheral vascular disease combined) and 37% of the cohort patients had diabetes mellitus. The mean PWV was 9.7 m/s (SD 2.5); the mean AGE reading was 3.1 AU (SD 0.8). In univariate analysis AGEs was significantly associated with PWV (r=0.414; p <0.01). This association persisted after correction by linear regression for renal function, age, BMI, blood pressure and smoking and diabetic status (β=0.393; p=0.001). Similar results were obtained when non-diabetic subjects were analysed separately.

Conclusions: We have found that in both diabetic and non-diabetic patients with progressive CKD, AGEs are significantly associated with arterial stiffness. This raises the possibility that the measurement of AGEs could form part of risk stratification for patients with progressive CKD.

Funding: Private Foundation Support

TH-PO211

Glomerular Filtration Rate and Cardiovascular Outcomes after Acute Myocardial Infarction: Results from Korea Acute Myocardial Infarction Registry Eun Hui Bae,[1] Sang Yup Lim,[2] Joon Seok Choi,[1] Chang Seong Kim,[1] Jeong-Woo Park,[1] Seong Kwon Ma,[1] Myung Ho Jeong,[1] Soo Wan Kim.[1] *[1]Department of Internal Medicine, Chonnam National University Medical School, Gwnagju, Korea; [2]Department of Internal Medicine, Korea University, Ansan, Korea.*

Background: Renal dysfunction is associated with one of the highest risks, but relation between chronic kidney disease (CKD) stage and cardiovascular outcomes after acute myocardial infarction (AMI) is not well defined.

Methods: As a part of the Korea Acute Myocardial Infarction Registry (KAMIR), we identified 12,636 patients with acute Myocardial infacrtion between November 2005 and July 2008. The glomerular filatration rate (GFR) was estimated by means of the four-component Modification of Diet in Renal Disease equation, and the patients were grouped according to GFR. Primary end points were death and complication in hospital courses. Secondary end points were major adverse cardiac event (MACE) during follow-up.

Results: The mean age was 64 ± 13 years, and 70.4 percent of the group were men. A graded association was observed between GFR and clinical outcomes. The group III, IV and V independently predicted short-term and long-term MACE (hazard ratio, 1.954, 2.764, 2.722; p<0.001; 1.538, 2.194, 2.491; p<0.001). After adjustment with confounders, decreasing GFR was important risk predictors of short-term and long-term MACE. Age, Killip class >1, percutaneous coronary intervention and high-sensetive C-reactive protein also were risk predictors of short-term and long-term MACE. Use of beta-blocker, angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and statin was associated with reduced risk for MACE.

Conclusions: Renal dysfunction was an independent risk factor for the mortality and complications of AMI, while degree of GFR did not affect the short-term MACE in patients with GFR ≤ 30 mL/min/1.73 m^2. The use of beta-blockers, ACEIs or ARB and statin reduced risk for short-term and long-term MACE

TH-PO212

Relationship between Use of AST-120 and Abdominal Aortic Calcification in Chronic Kidney Disease Patients Shunsuke Goto, Ken Kitamura, Keiji Kono, Kentaro Nakai, Hideki Fujii, Shinichi Nishi. *Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan.*

Background: Although several experimental papers have reported indoxyl sulfate has progressed vascular calcification, little is known about the association between indoxyl sulfate and vascular calcification in human. The aim of the current study was to investigate the relationship between abdominal aortic calcification and use of AST-120, which has adsorption ability for indoxyl sulfate, in pre-dialysis chronic kidney disease (CKD) patients.

Methods: We conducted a retrospective analysis for all the pre-dialysis CKD patients (stage 4 and 5) who underwent abdominal plain computed tomography in our institution between 2005 and 2010. Abdominal aortic calcification was assessed by aortic calcification index (ACI).We divided these patients into two groups by whether AST-120 was taken for at least 6 months or not and compared their ACI between the two groups.

Results: Two hundred patients were enrolled in the present study. ACI was significantly lower in patients taking AST-120 (12.2 [2.5-30.3] vs. 25.8 [13.5-45.3] %, p < 0.05). By multiple regression analysis, use of AST-120 was independently and significantly associated with ACI after adjustment for age, sex, diabetes, hypertension, total cholesterol, estimated glomerular filtration rate, hemoglobin, albumin, calcium, phosphorus, smoking, and use of statin.

Conclusions: It is suggested that AST-120 suppresses the progression of aortic calcification in pre-dialysis CKD patients.

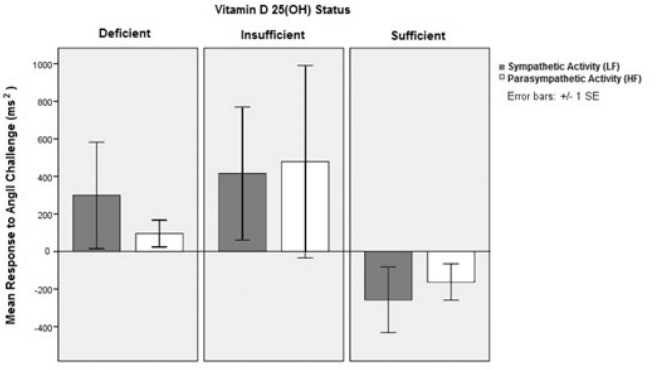
TH-PO213

Vitamin D Status and the Cardiac Autonomic Response to Angiotensin II in Healthy Humans Michelle C. Mann,[1] Brenda Hemmelgarn,[1,2] Derek Exner,[1,3] Darlene Y. Sola,[1] Tanvir Chowdhury Turin,[1] Sofia B. Ahmed.[1,2] *[1]University of Calgary, AB, Canada; [2]Alberta Kidney Disease Network, AB, Canada; [3]Libin Cardiovascular Institute, Calgary, AB, Canada.*

Background: Cardiovascular disease (CVD) remains the leading cause of death in chronic kidney disease (CKD) patients. Increased cardiac sympathetic activity [low-frequency (LF) heart rate variability (HRV)] and vagal (high frequency (HF) HRV) withdrawal, are novel risk factors for CVD in CKD. CKD patients also exhibit upregulation of the renin-angiotensin system (RAS) and often low vitamin D (VD) levels, both of which are associated with CVD, thus we sought to evaluate the role of VD status in modulating HRV in response to angiotensin (Ang II) in healthy humans.

Methods: 36 subjects (21 women, 15 men, age 38±2 yrs) were studied in high-salt balance. Subjects were categorized according to serum 25OH VD (nmol/L): deficient (<50, n=9), insufficient (50-79, n=14) and sufficient (>80, n=13). HRV, calculated by spectral power analysis (LF, HF, and LF:HF), was recorded at baseline and in response to AngII (3ng/kg/min x30min, 6ng/kg/min x30min). The primary outcome was the association between VD status and HRV response at 60 minutes.

Results: Baseline cardiac autonomic function differed according to VD status. In response to AngII, increasing VD status was associated with a blunting of an unfavourable increase in sympathetic activity (LF p=0.17 for trend), decrease in vagal activity (HF p=0.23 for trend) and overall shift in sympathovagal balance (LF:HF, p=0.75 for trend).



Conclusions: Poor VD status may be associated with unfavourable cardiac autonomic function. In response to AngII, VD sufficient healthy adults largely maintain sympathovagal balance. This cardioprotective response appears to be impaired in VD insufficient/deficient humans, suggesting that low VD status coupled with RAS activation may augment the risk of CVD in CKD.

TH-PO214

Effect of Elevated Blood Pressure on Quality of Life in Children with Chronic Kidney Disease Cynthia Wong,[1] Matthew Matheson,[2] Juan C. Kupferman,[2] Joseph T. Flynn,[2] Susan L. Furth,[2] Bradley A. Warady.[2] *[1]Pediatrics, Stanford University, Stanford, CA; [2]CKiD Investigator.*

Background: Hypertension (HTN), which is common in children with chronic kidney disease (CKD), has known adverse impact on health-related quality of life (HRQoL) in adults, yet similar pediatric data are lacking.

Methods: Study design and Participants: Cross-sectional and longitudinal analysis of blood pressure (BP), antihypertensive medication use, and HRQoL of children with CKD enrolled in the prospective, multicenter Chronic Kidney Disease in Children Prospective Study (CKiD). BP was measured standardly by auscultation with aneroid sphygmomanometer at annual study visits and HRQoL was assessed using the PedsQL questionnaire. Data analysis: Cross-sectional analysis of BP and PedsQL domain scores at baseline, and longitudinal analysis using logistic regression to assess whether higher baseline BP or use of antihypertensive medications was associated with decline in QOL scores over time.

Results: Univariate baseline comparisons of PedsQL scores across BP groups showed a significant difference (p=0.02) in the child reported physical PedsQL median score for children with diastolic HTN (median score: 78) compared to those with normal diastolic BP (median score: 84). Logistic regression analysis showed children who were taking one or more antihypertensive medication were twice as likely to have decline in physical functioning QOL over time regardless of blood pressure, OR 2.043 (p=0.0042) in model adjusted for systolic BP, and OR 2.069 (p=0.0039) in model adjusted for diastolic BP.

Conclusions: Children with diastolic HTN have significantly lower median physical PedsQL scores at baseline as compared to normotensive children. In this analysis, elevated BP was not associated with a decline in HRQoL over time. However, children on antihypertensive medication are twice as likely to show decline in self-reported physical QOL, regardless of BP status.

Funding: NIDDK Support, Other NIH Support - Division of Kidney, Urologic, and Hematologic Diseases of the NIDDK, National Institute of Neurological Disorders and Stroke, National Institute of Child Health and Human Development, and National Heart, Lung, and Blood Institute

TH-PO215

The Association between Arterial Stiffness and Mildly Kidney Damage among a Chinese Population-Based Population Fukun Niu,[1,2] Luxia Zhang,[1] Xingyu Wang,[3] Lisheng Liu,[3] Haiyan Wang.[1] *[1]Institute of Nephrology and Division of Nephrology, Peking University First Hospital, Beijing, China; [2]Nephrology and Rheumatology, First Affiliated Hospital of Zhengzhou University, Zheng Zhou, Henan, China; [3]Beijing Hypertension League, Beijing, China.*

Background: Previous study revealed increased arterial stiffness among patients with advanced chronic kidney disease (CKD), while studies among patients with mildly kidney damage were limited.

Methods: Eight hundred and eight-two community-based participants in Beijing, China were included in the present study. Arterial stiffness was assessed by brachial-ankle Pulse Wave Velocity (PWV). Indicators of kidney damage included estimated glomerular filtration rate (eGFR) and urinary albumin-creatinine-ratio (ACR) were tested. All participants had an estimated glomerular filtration rate (eGFR) >30mL/min/1.73m2, and none of them had ACR meet the definition of macroalbuminuria. Multivariable linear regression models were used to evaluate the associations between kidney damage and PWV.

Results: The average age was 65.4±9.2 years and 45.1% were males. Altogether 105 (11.9%) participants had microalbunminuria, and 28 (3.2%) had eGFR <60 ml/min/1.73m2. PWV was significantly higher among participants with microalbuminuria (1995.4±575.1 cm/s vs 1738.7±357.1 cm/s,P<0.001) and among participants with eGFR

<60 ml/min/1.73m2 (1950.1±416.6 cm/s vs 1763.4±396.0 cm/s,P=0.01). After adjusting for potential confounders,microalbuminuria, but not eGFR, was still positively associated with PWV (β=122.7;p<0.001)

Conclusions: Microalbuminuria was independently associated with arterial stiffness.

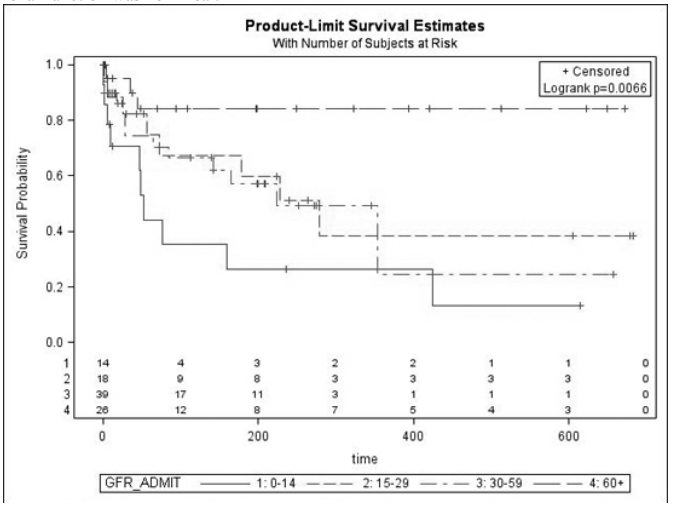
TH-PO216

Outcome of Hyperkalemia in the Emergency Department: Impact of Hyperkalemic Severity, Renal Function and CHF on Survival Venu Velagapudi,[1] Bruce Barton,[2] Jeffrey S. Stoff.[1] *[1]Renal Medicine, UMass SOM; [2]Quant Health Sciences, UMass SOM.*

Background: Hyperkalemia is common and lethal electrolyte disorder with little known long-term consequences. This was retrospective, observational study of hospitalized patients with initial serum K > 5.3 mEq/L. 143 consecutive episodes of hyperkalemia were analyzed in 133 patients. Survival was analyzed by parameters of renal dysfunction (admit eGFR), CHF, admit K and EKG abnormalities.

Methods: Hazard ratios (HR) for mortality were computed by Cox proportional hazards multivariate regression. Primary end point, all-cause mortality determined by Social Security Death Index and medical record review.

Results: Admit eGFR was the most powerful predictor of mortality. The effect of renal function was nonlinear.



Highest mortality is eGFR group of 15-59 HR 6.92. More severe renal impairment with eGFR <15 HR 4.10 and AKI requiring hemodialysis (HD) HR 3.67. ESRD had lower mortality HR 1.33.

Hyperkalemic severity had a modest effect. Compared to patients Admit K 5.3-5.9 mEq/L, patients with K 6-7, HR 2.21 (p=0.0210) and K >7.0, HR 2.62 (p=0.0521). History of CHF, increased mortality by univariate analysis (p<0.0001) but CHF had no independent effect in HD patients. In non-HD patients, CHF had an independent effect when both admit eGFR and K were added to the model. Patients with EKG abnormalities had higher K (p<0.003), but these changes did not impact mortality (p=0.126).

Conclusions: Survival in hyperkalemic patients is predicted by lower admit eGFR in a non-linear fashion. ESRD patients exhibited lower mortality perhaps reflecting adaptation to chronic hyperkalemia. CHF has an additive effect on mortality in non HD patients. We emphasize that 86% of the mortality was after discharge. This extraordinary mortality necessitates the need to develop risk stratification strategies in the long-term care of the hyperkalemic patients.

Funding: Private Foundation Support, Clinical Revenue Support

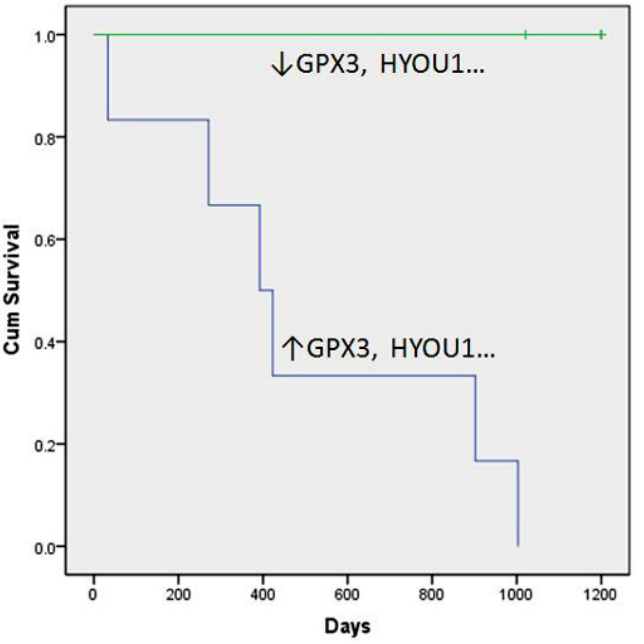
TH-PO217

An Unbiased Proteomic Screen Is Linked to Clinical Outcomes Elucidating the Mechanism of Cardiovascular Disease in Patents with Kidney Disease Andrew M. Siedlecki. *Internal Medicine, Washington University in St. Louis, St. Louis, MO.*

Background: Biomarker discovery is a major goal of proteomic analysis. We hypothesized that certain serum proteins contribute to cardiac disease in patients with chronic kidney disease which can be targeted for therapeutics.

Methods: Each patient was followed prospectively over a 42 month period from October 2007 – March 2011. Participants were monitored for either heart failure exacerbation requiring hospitalization or death. Proteomic analysis was performed on each blood specimen. Relative levels of oligopeptides in each sample were derived from Fourier transformed luminescent intensity. Intensity was normalized to the mean intrasample intensity and to the corresponding unique peptide fragments identified in each sample.

Results: 20 patients were enrolled in the study. Eight were diagnosed with end-stage renal disease and received maintenance hemodialysis for renal replacement therapy at study outset. 7 patients had a cardiac event, 3 of which were diagnosed with chronic kidney disease that did not require dialysis (GFR 20.3 ± 5 mL/min) and 3 of which received intermittent hemodialysis. 182 proteins were identified in each patient. 7 unique proteins were elevated in the cohort of patients that achieved the primary endpoint of heart failure or death.



Unique proteins include glutathione peroxidase 3 precursor (GPX3), hypoxia up-regulated protein 1 precursor (HYOU1), cell surface glycoprotein MUC18 (MUC18), lipopolysaccharide binding protein (LBP), Vitamin K-dependent protein S (PROS), platelet factor 4 variant (PF4V), and retinol-binding protein 4 precursor (RBP4).

Conclusions: Proteomic analysis can identify novel biomarkers that contribute to the renal-cardiac syndrome. The veracity of these markers is confirmed by outcomes analysis which increases the likelihood of clinical impact in diagnosis and therapy .

TH-PO218

Influence of Variations of Inflammatory Markers in the Prediction of Cardiovascular Events from CKD Non-Dialysis Patients Borja Quiroga, Marian Goicoechea, Soledad Garcia de Vinuesa, Ursula Verdalles, Claudia Yuste, Daniel Barraca, Nayara Panizo, Jose Luno. *Hospital General Universitario Gregorio Marañón.*

Background: In CKD patients on dialysis, plasma IL-6 levels have been shown to better predict death than IL-1β, TNF-α and CRP levels. However, only one study has demostrated the predictive role of IL-6 in non-dialysis CKD patients. Besides, the impact of intra-individual changes of inflammation markers on cardiovascular events in CKD patients is unknown.

Methods: The aim of this study was to analyze what is the best inflammatory marker of cardiovascular outcome in non-dialysis CKD patients and to examine whether the simple determination of an inflammatory marker is a better predictor than the variability of the serum levels of the marker over a 6-month period.

Ninety patients (mean age: 68.5±12.8 years) at different stages (1-4) of CKD were evaluated. Serum CRP, IL-6, IL-1β and TNF-α were measured basally and after six months. Three patterns were defined for each inflammatory marker: baseline measurement, mean of two measurements and variation: increase or decrease after six month.

Results: During the follow-up period of the study (mean time of 72.7±19.8 months), 14 patients died, 11 patients were included on dialysis program and 29 patients suffered a cardiovascular event. Patients with persistently elevated IL-6 values had higher risk of cardiovascular events (OR: 1,21 (1,11-1,32), p=0,000). Mean of two measurements of IL-6 was a significantly better predictor than CRP, TNF-α and IL-1β. Patients with a mean IL-6 above 6 pg/mL and patients with previous peripheral vascular disease had an increase risk of having cardiovascular events (2,34 (1,05-5,22), p=0,037 and 2,95 (1,27-6,93) p=0,011, respectively).

Conclusions: IL-6 is better inflammatory marker than PCR, TNF-α and IL1β to predict cardiovascular events in CKD non-dialysis patients. Mean of two measurements is better than simple determinations to predict cardiovascular outcome. Moreover, IL-6 values persistently elevated predicted cardiovascular events in CKD patients.

TH-PO219

Predicting Significant Coronary Angiographic Disease in Patients with Chronic Kidney Disease Using Nuclear SPECT Myocardial Perfusion Imaging Milagros Zegarra,[1] Rabab Moshin,[1] Matthew Whitbeck,[1] Bruce T. Kuo,[1] Claude S. Elayi,[1] Debabrata Mukherjee.[2] *[1]Medicine, University of Kentucky, Lexington, KY; [2]Medicine, Texas Tech University, El Paso, TX.*

Background: Chronic Kidney Disease(CKD) is considered an independent risk factor for coronary artery disease (CAD). The gold standard test to diagnose CAD is coronary angiography. Patients with a Glomerular Filtration Rate (GFR) less than 60 cc/min/1.73m2 are at an increased risk of worsening renal failure and bleeding during this

procedure. Nuclear SPECT MPI remains an attractive modality to evaluate ischemia in this population. Perfusion defects in this population have been associated with an increased risk in cardiac death, but not with significant angiographic disease. Our objective was to determine predictors of significant angiographic disease in patients with CKD using Nuclear SPECT MPI.

Methods: We retrieved from our patient population all patients with a GFR < 60 cc/min/1.73m2, a nuclear SPECT MPI stress test and coronary angiography within 2 years of the test over the last 4 years. Statistical analysis was performed using bivariate analysis and multivariate regression models.

Results: A total of 65 patients were identified among 400 patients. Abnormal myocardial perfusion was considered significant in 46 of them. Of those patients 34 had significant >50% CAD seen on angiography. Significant patient characteristics that predicted angiographic disease were history of hyperlipidemia (p=0.0268) and hypertension(p value =0.0405). The only stress test predictor for significant disease was abnormal wall motion on gated stress imaging (HR 4.24 ; 95% CI 1.39-12.9, p value=0.0109). Usual high risk predictors, such as sum stress, rest and difference score or transient ischemic dilation failed to predict significant angiographic disease(p value=0.14-0.98).

Conclusions: In patients with CKD the presence of abnormal wall motion during stress was the only reliable predictor of significant CAD.

TH-PO220

Cornell Product Is the Best Electrocardiographic Surrogate of Left Ventricular Mass in Non-Dialysis Dependent Chronic Kidney Disease

Antonio C. Cordeiro,[1,2] Márcio G. Sousa,[1] Juan J. Carrero,[2] Gabriel J. Nunes,[1] Marcus R.O. Santana,[1] Waldyr Grimaldi,[1] Celso Amodeo,[1] Fernanda C. Amparo,[1] Amanda G.M.R. Sousa,[1] Bengt Lindholm.[2] *[1]Dante Pazzanese Insitute of Cardiology, Brazil; [2]Baxter Novum and Renal Medicine, Karolinska Institute, Sweden.*

Background: Left ventricular mass indexed to body surface area (LVM/BSA) by echocardiography (ECO) is the most efficient method for left ventricular hypertrophy diagnosis. However, electrocardiographic (ECG) criteria may also be useful. We sought to determine associations between ECG criteria and LVM/BSA in CKD patients (pts).

Methods: In 151 non-dialysis dependent CKD stages 3-5 pts (median age: 59 years [52-67], 96 men), ECO was performed to assess LVM/BSA and ECG was used to assess Cornell and Sokolow-Lyon voltages [respectively CV and SLV] and products [respectively CP and SLP]). Analyses were done by the same, blinded, physician.

Results: All ECG criteria were associated with LVM/BSA in sex adjusted linear regressions (r^2 = 0.15 for SLV, 0.23 for SLP, 0.31 for CV and 0.35 for CP; P < 0.01 for all); and CP showed the strongest association. Pts were divided according to the CP sex specific tertiles (low [n=51], middle [n=52] and high [n=48]). Across the tertiles there were differences in hemoglobin (11.2 ± 2.3 vs. 12.2 ± 1.9 vs. 13.0 ± 5.6 g/dL; P=0.03) and BNP levels (56.2 [27.2- 144.0] vs. 48.8 [22.9 - 129.0] vs. 142.6 [65.1 - 391.3] pg/mL; P <0.01), as well as in LVM/BSA (122 [101-142] vs. 144 [123-166] vs. 175 [147-201] g/m^2; P < 0.01). In a logistic regression analysis (using the lowest CP tertile as the reference category) CP was associated with a higher odds for LVM/BSA (depicted as LVM/BSA higher than the sex adjusted median) in the crude analysis (OR: 3.85 [1.59-9.31] and 11.39 [4.46-29.08]; respectively middle and higher CP tertiles) and even after the adjustment for age, mean blood pressure, hemoglobin, BNP and CKD stages (2.89 [1.17-7.10] vs. 6.13 [2.35-15.99]; respectively middle and higher CP tertiles).

Conclusions: Cornell product associates with LVM/BSA stronger than other electrocardiographic criteria. Our results reinforce the usefulness of ECG, especially assessing CP, as a simple and inexpensive diagnostic method for LVH in CKD.

Funding: Government Support - Non-U.S.

TH-PO221

Vitamin-K Epoxide Reductase Complex Subunit 1 Genetic Polymorphisms Predict Arterial Stiffness and Serum MGP Levels in Chronic Kidney Disease Patients

Yasmin Yasmin,[1] David Hsing-Yu Chen,[1] Ben Caplin,[2] Cees Vermeer,[3] Leon J. Schurgers,[3] Michael B. Rubens,[4] Carmel M. McEniery,[1] John Cockcroft,[5] Ian Wilkinson,[1] John Cunningham,[2] David C. Wheeler,[2] Kevin O'Shaughnessy.[1] *[1]Clinical Pharmacology Unit, University of Cambridge, United Kingdom; [2]UCL Medical School, London, United Kingdom; [3]University of Maastricht, Netherlands; [4]Royal Brompton Hospital, London, United Kingdom; [5]Cardiff University, United Kingdom.*

Background: Arterial stiffness is an independent predictor of cardiovascular risk in chronic kidney disease (CKD). Calcium deposition is a major determinant of arterial stiffness and common polymorphisms in the Vitamin-K epoxide reductase complex subunit 1(VKORC1) predict aortic calcification scores. We investigated the influence of VKORC1 polymorphisms (-1639G>A, +1173C>T, +1542G>C, +2255C>T, +3730G>A) on arterial stiffness in 280 stage 3-5 CKD patients who are mostly Caucasians.

Methods: Blood pressure (BP), body mass index (BMI), pulse wave velocity (PWV), coronary artery calcification (CAC) and serum uncarboxylated matrix Gla protein (t-ucMGP) were determined, and genotyping performed.

Results: The +1542G>C and +3730G>A polymorphisms showed genotype-specific higher PWV and lower t-ucMGP (P<0.05). The combined recessive allele model showed a significant step-wise reduction in PWV (P<0.005); subjects homozygous for both risk alleles had the highest PWV as compared to those who had one or none. In a regression model after adjustment for age, gender, mean BP, BMI and racial group, each +1542G allele was independently associated with a 0.8 m/s (95% CI 0.09 to 1.57) higher PWV and each +3730A allele was associated with 1.0 m/s (95% CI 0.14 to 1.98) higher PWV. Although in this cohort serum t- ucMGP levels correlated inversely with CAC score (P<0.001), VKORC1 genotypes did not.

Conclusions: We have demonstrated for the first time that VKORC1 polymorphisms (+1542G>C and +3730G>A) influence aortic stiffness and serum t-ucMGP levels in CKD patients. Although needing replication in an independent cohort, these findings suggest that Vitamin-K dependent processes may be important in arterial stiffness through previously un-characterized VKORC1 related mechanisms.

TH-PO222

Fibroblast Growth Factor 23 Predicts Left Ventricular Mass and Induces Cell Adhesion Molecule Formation

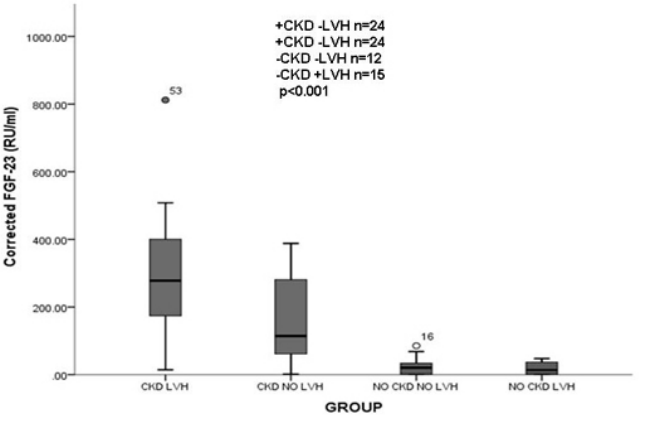
Kathryn K. Stevens,[1,2] Emily P. McQuarrie,[1,2] Dianne Z. Hillyard,[1] Rajan Kantilal Patel,[1,2] Patrick Barry Mark,[1,2] Alan G. Jardine.[1,2] *[1]Renal Research Group, ICAMS, University of Glasgow, United Kingdom; [2]Renal Unit, Western Infirmary, Glasgow, United Kingdom.*

Background: Elevated FGF-23 is associated with left ventricular hypertrophy (measured by echocardiography) in CKD. It may be a biomarker or a direct toxin. We studied FGF-23 and LVH in CKD using CMRI, the gold standard measure of LVH and the effect of phosphate, FGF-23 and Klotho on E-selectin and VCAM production in endothelial cells (HUVECs).

Methods: Patients with CKD stages 3/4 (n=48) or hypertension in the absence of CKD (n=27) were recruited. FGF-23 concentrations were measured by ELISA. Non-contrast CMRI was performed with a 1.5-Tesla Siemens scanner. HUVECs were grown in normal (0.5mM) or high (3mM) phosphate concentration medium and treated with FGF-23/Klotho/both. Cell based ELISA for E-Selectin and VCAM was performed and results normalized to GAPDH.

Results: 50% of CKD and 56% of 'control' patients had LVH. LVMI was similar between the two groups. Patients with CKD had significantly higher phosphate, parathyroid hormone and FGF-23 concentration (237.9RU/ml (109.6-393.3) v 12.5RU/ml (1.5-35.9), p<0.001). Those with CKD and LVH had higher FGF-23 concentrations than those with CKD without LVH (283.4RU/ml v 118.4RU/ml).

Figure 1: FGF-23 concentration stratified according to the presence or absence of CKD and LVH



Multivariate analysis revealed systolic blood pressure, FGF-23 and proteinuria to be independent predictors of LVH in CKD. E-selectin and VCAM production is elevated in HUVECs cultured in high phosphate media with FGF-23 and Klotho (p=0.005 and 0.035).

Conclusions: FGF-23 independently predicts LVH. Our preliminary data support that FGF-23 may be toxic causing activation of the vascular endothelium but do not prove causality with LVH. Whether lowering levels of FGF-23 translates to improved outcomes remains to be seen.

TH-PO223

Ferric Carboxymaltose-Mediated Effects on Platelet Count in Cardio-Renal Patients: Results from the FAIR-HF Trial

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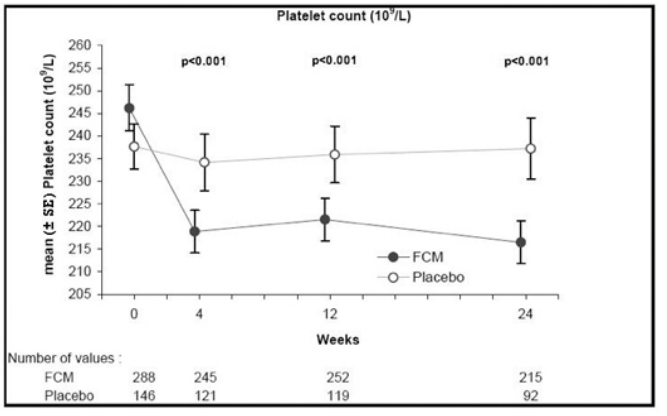
Background: Chronic heart failure (CHF) is a hypercoagulable state with an increased incidence of thromboembolic events. Iron deficiency (ID) and treatment with ESAs, particularly high doses, are risk factors for the development of thrombocytosis. Patients (pts) with CHF often suffer from CKD. Both ID and the use of ESAs are common in these cardio-renal pts. Using data from FAIR-HF, we analyzed the effects of intravenous (IV) iron as ferric carboxymaltose (FCM) on platelet count (plt ct) in CHF pts with ID and mild to moderate CKD.

Methods: FAIR-HF was a randomized, double-blind, placebo-controlled trial of IV

iron as FCM in 459 pts with CHF of NYHA class II or III, LVEF ≤40% (NYHA II) or ≤45% (NYHA III), ID (ferritin <100 µg/L or 100–299 µg/L, if TSAT <20%), and Hb 95 to 135 g/L. Baseline characteristics: age 68±11y, NYHA III 82%, LVEF 32±6%, Hb 119±13 g/L, eGFR 64±23 ml/min (42% <60ml/min).

Results: Baseline plt cts were available for 434 (94.6%) pts, 89% of which were within the normal range (140-370 x10^9/L), with 5% below and 6% above this range. Mean plt ct at baseline was 246±86 in the FCM and 238±60 x10^9/L in the placebo group (p=NS). The mean changes in baseline plt ct in the FCM vs placebo group were -27±54 vs -4±48 (4 wk), -24±59 vs -4±46 (12 wk) and -25±56 vs 0±53 (24 wk).



Conclusions: FCM effectively treats ID and reduces the risk of thrombocytosis by decreasing the mean plt ct by 9-10%. This reduction is seen as early as 4 wk after treatment initiation and remains stable over time. This effect may improve potentially detrimental rheological features that are present in pts with cardio-renal syndrome and hence improve their outcomes.

Funding: Pharmaceutical Company Support

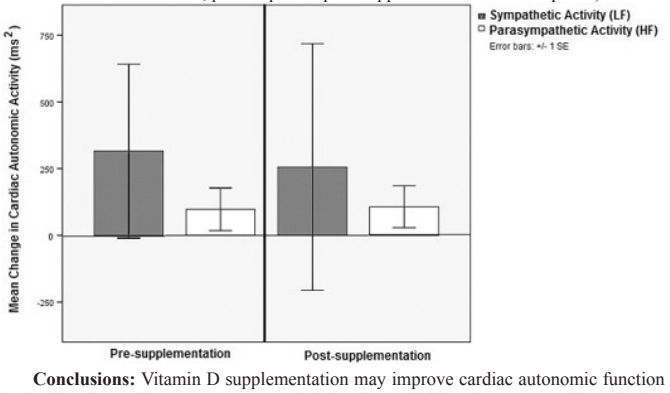
TH-PO224

Vitamin D Supplementation and the Cardiac Autonomic Response to Angiotensin II in Vitamin D Deficient Humans Michelle C. Mann,[1] Brenda Hemmelgarn,[1,2] Derek Exner,[1,3] Darlene Y. Sola,[1,2] Tanvir Chowdhury Turin,[1] Sofia B. Ahmed.[1,2] *[1]University of Calgary, AB, Canada; [2]Alberta Kidney Disease Network, AB, Canada; [3]Libin Cardiovascular Institute, Calgary, AB, Canada.*

Background: Cardiovascular disease (CVD) is the leading cause of death in chronic kidney disease (CKD) patients. Vitamin D (VD) deficiency is a risk factor associated with unfavourable cardiac autonomic function (increased sympathetic activity [low-frequency (LF) heart rate variability (HRV)] and vagal [high frequency (HF) HRV] withdrawal) observed in the CKD population, a group that also exhibits upregulation of the renin-angiotensin system (RAS). We sought to determine the effect of VD supplementation on HRV, both at baseline and in response to angiotensin (AngII) in VD-deficient healthy humans.

Methods: 9 VD-deficient subjects (4 men, 5 women) were studied in high-salt balance. HRV (LF, HF, LF:HF), was recorded at baseline and in response to AngII (3ng/kg/min x30min, 6ng/kg/min x30min). Subjects repeated the study following 28 days of oral VD supplementation (5000 IU/day). The primary outcome was the HRV response to AngII at 60 minutes.

Results: Mean 25OH VD levels increased significantly post-supplementation (44±2 vs 82 ±15nmol/L, p=0.03). Post-supplementation, there was a slight improvement in baseline HRV (LF 746±195 vs 1327±522ms^2, p=0.3; HF 375±102 vs 935±608ms^2, p=0.4; LF:HF 2.1±0.4 vs 2.6±0.5, p=0.5 pre- vs. post-supplementation) and a blunting of the unfavourable autonomic response to AngII (LF 315±325 vs 255±462, p=0.6; HF 98±80 vs 107±78, p=0.9; LF:HF 1.9±0.6 vs -0.2±1.0, p=0.09 pre vs. post-supplementation delta response).



Conclusions: Vitamin D supplementation may improve cardiac autonomic function both at baseline and in response to AngII infusion in VD-deficient humans. Studies to further evaluate the association between VD and cardiac autonomic function are required.

TH-PO225

Left Ventricular Mass Index and the Erythropoiesis Stimulating Agent/ Hemoglobin Level Index in Chronic Hemodialysis Patients Ana Cabrita, Anabela Malho, Ana Pinho, Ana Paula Silva, Idalecio Bernardo, Pedro Neves. *Serviço de Nefrologia, Hospital de Faro, EPE, Faro, Portugal.*

Background: Cardiovascular disease and anemia are frequent in chronic kidney disease patients. Left ventricular hypertrophy is an independent predictor of morbidity and mortality in dialysis populations. Recently, the erythropoiesis stimulating agent/ hemoglobin level (ESA/Hb) index emerged as a new factor also associated with increased morbidity and mortality in this population.

Methods: The aim of this study was to evaluate the association between left ventricular mass index (LVMI) and the (ESA/Hb) index in a population of patients on chronic hemodialysis.

Results: We did an observational study with 95 patients (female=31, male=64) on chronic hemodialysis. Clinical and laboratorial data were collected and we calculated the average weekly darbepoetin dose, as well as the average hemoglobin level. The weekly darbepoietin dose/Kg was first multiplied by 200 and after, this figure was divided by the Hb level, to calculate the ESA/Hb index. To evaluate the LVMI we used the Deveraux method. In the statistical analyse we used a linear regression model.

The mean age of the patients was 61.5 ± 16.9 years, the mean time on hemodialysis was 50.9 months, the mean LVMI was 157 .9 g/m2 and the mean weekly dose of erythropoietin was 132 UI/Kg. Forty four patients were medicated with anti-hypertensive drugs (12 were on angiotensin II receptor blockers and 19 were on angiotensin converting enzyme inhibitors). Using a linear regression model, we found a statistically positive correlation between the LVMI and the ESA/Hb index (r = 0.226, p=0.034).

Conclusions: In this study with chronic hemodialysis patients, we found a positive association between the LVMI and the ESA/Hb index, and may explain at least in part, why the ESA/Hb index is a predictor of morbidity and mortality.

TH-PO226

Effect of AST-120 on Aortic and Mitral Valve Calcification in Chronic Kidney Disease Patients at Dialysis Initiation Kentaro Nakai, Hideki Fujii, Keiji Kono, Shunsuke Goto, Shinichi Nishi. *Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan.*

Background: Cardiovascular disease is a major cause of morbidity and mortality in chronic kidney disease (CKD) patients. In CKD patients, valvular calcification is frequently observed as a consequence of aging, lipid abnormalities, and accumulated uremic toxins. AST-120 is an oral charcoal adsorbent that removes uremic toxins, and recently, we demonstrated that AST-120 prevents progression of cardiac damage in a CKD rat model through suppression of oxidative stress. The aim of this study is to investigate the association between AST-120 and valvular calcification in CKD patients at dialysis initiation.

Methods: All the study patients were admitted to our hospital and underwent echocardiography between April 2008 and May 2011. A total of 78 patients at the initiation of maintenance dialysis treatment were included in this study. We divide these patients into two groups based on whether AST-120 had been taken for more than 3 months or not (AST-120 group: n=34, Contorl group: n=44). And we evaluated the relationship of the number of calcified valves (0, no calcification; 1, calcification of the aortic or mitral valve; 2, calcification of both valves) with clinical characteristics and laboratory parameters.

Results: Age, sex, blood pressure, use of rennin-angiotensin system inhibitors, statins, hemoglobin levels, calcium levels, brain natriuretic peptide levels, and estimated glomerular filtration rate did not significantly differ between the AST-120 and control group. Serum phosphorus levels were tended to be lower in the AST-120 group. Despite comparable clinical characteristics and laboratory parameters, the number of calcified valves was significantly lower in the AST-120 group than in the control group. Age, diabetes mellitus, diastolic blood pressure, and the use of AST-120 were significantly associated with the number of calcified valves. Furthermore, multivariate analysis indicated that use of AST-120 tended to be negatively associated with the number of calcified valves.

Conclusions: It is suggested that AST-120 was associated with prevention of aortic and mitral valve calcification.

TH-PO227

Cardiac Dysautonomia in Patients with End Stage Renal Disease on Hemodialysis Hafiz I. Ahmad,[1] Arif Asif,[3] Syed Rizwan Bokhari,[1] Muhammad Zaman Khan Assir,[2] Muhammad Awais,[1] Sabin Nasir.[1] *[1]Department of Nephrology, Allama Iqbal Medical College/Jinnah Hospital, Lahore, Pakistan; [2]Department of Medicine, Allama Iqbal Medical College/Jinnah Hospital, Lahore, Pakistan; [3]Division of Nephrology, University of Miami School of Medicine, FL.*

Background: Sudden cardiac death is common in chronic hemodialysis patients. Cardiac dysautonomia (dysfunction of cardiac autonomic nervous system) has been shown to be associated with increased frequency of cardiac arrhythmias and may be the cause of higher incidence of sudden cardiac death.

Methods: In this observational analysis, we studied the frequency of cardiac dysautonomia in our dialysis population. Seventy hemodialysis patients were enrolled in this study. Three tests to assess the status of cardiac autonomic dysfunction (1] heart rate response to deep breathing, 2] Valsalva ratio, 3] 30:15 ratio of R-R interval measured at beats 15 and 30 after an upright position) were performed. Twenty patients were excluded

because of the inability to perform Valsalva maneuver. Based on previous studies, presence of at least 2 abnormal tests was considered to be positive for cardiac dysautonomia.

Results: Fifty patients completed the tests successfully. 26 were male (52%), 32% were diabetic and the mean age was 43.9 years. Forty four (88%) were found to have cardiac dysautonomia. Abnormal heart rate response to deep breathing (E:I ratio less than 1.17) was found in 47 patients (94%), abnormal Valsalva ratio (longest to shortest R-R ratio less than 1.2) in 45 patients (90%), abnormal 30:15 ratio (30th to 15th R-R ratio on standing less than 1.03) in 34 patients (68%). We found 3 abnormal tests in 32 patients (64%), 2 abnormal tests in 12 patients (24%), 1 abnormal test in 5 patients (10%) and no abnormal test in 1 patient (2%). Overall, 44 (88%) of the patients had cardiac dysautonomia.

Conclusions: This single center study finds a high incidence of cardiac dysautonomia in chronic hemodialysis patients. Future studies should investigate the association between cardiac dysautonomia and sudden cardiac death.

TH-PO228

Optimising the Accuracy of Blood Pressure Monitoring in Chronic Kidney Disease: The Utility of BpTRU in Kidney Disease Shona Brothwell,[2] Mary Dutton,[1] Charles Ferro,[1,2] Stephanie J. Stringer,[1,2] Paul Cockwell.[1,2] *[1]Renal Institute of Birmingham, Department of Nephrology, University Hospitals Birmingham, Birmingham, United Kingdom; [2]University of Birmingham, Birmingham, United Kingdom.*

Background: Accurate blood pressure (BP) monitoring is critical in CKD. However, management is often based on a single BP reading performed in an uncontrolled setting; this will over-diagnose hypertension in up to 30% and miss around one third who are truly hypertensive. Therefore, we compared BP obtained by the routine clinical pathway with BP obtained by BpTRU, which carries out multiple BP measurements to derive a fixed reading and has been validated in a non-CKD setting as being equivalent to (daytime) 24 hour ambulatory BP monitoring (ABPM).

Methods: Patients (n=45) attending renal outpatient clinics had standard BP measurements with a calibrated DINAMAP PRO400 in a clinical assessment room. The patients then underwent repeat assessment with BpTRU.

Results: The clinic mean (± SD) systolic BP (149.7 ± 18.5 (range 117 – 209) mmHg) was significantly higher than the BpTRU reading (122.0 ± 13.9 (96 – 150) mmHg; $P < 0.001$). In a subgroup (n=24), the clinic mean systolic (143.8 ± 15.5 mmHg) was significantly higher than a repeat standard BP in a quiet room (129.9 ± 19.9 mmHg; $P < 0.001$), but the BpTRU reading was significantly lower than both (117.3 ± 14.1 mmHg; $P < 0.001$). The clinic mean diastolic (82.4 ± 11.2 (49 – 100) mmHg) was significantly higher than the BpTRU reading (78.4 ± 10.0 (53 - 97) mmHg; $P < 0.001$; n = 45). Out of 29 patients with a clinic BP above the threshold for altering antihypertensive medication (> 130/80 mm Hg), only 9 were hypertensive with BpTRU. Finally, clinic BpTRU measurements in this setting (CKD) were not significantly different to the day-time mean of 24 hour ABPM.

Conclusions: Standard BP measurements are significantly higher than measurements using a BpTRU machine in a quiet room, which accurately reflects the day-time mean of 24 hour ambulatory BP monitoring in CKD. Adjusting clinic protocols to utilise the most accurate BP device available is a simple manoeuvre that could deliver major improvements in clinical practice.

TH-PO229

Early Detection of Atheromatous Disease at All Stages of Chronic Kidney Disease Improves with Simultaneous Femoral and Carotid Ultrasound Angels Betriu,[1] Adriana S. Dusso,[2] M. Vittoria Arcidiacono,[2] Montserrat Martinez-Alonso,[3] Jose M. Valdivielso,[2] Elvira Fernandez.[4] *[1]UDETMA., Arnau Vilanova Hospital, Lleida, Spain; [2]Experimental Nephrology, IRB Lleida, Lleida, Spain; [3]Estadistic Department, University Lleida, Lleida, Spain; [4]Nephrology Service, Arnau Vilanova Hospital, Lleida, Spain.*

Background: Atheromatous disease occurs early and at high frequency in the course of chronic kidney disease (CKD). The location of the atheromatous lesion in the vascular territory determines the type of adverse cardiovascular events and mortality rates.

Methods: Cross-sectional study to examine whether femoral (common and superficial) ultrasound could help enhance the detection of atheromatous disease achieved with carotid (common, bulb and internal) ultrasound. Left and right carotid and femoral ultrasounds were obtained and analyzed by the same operators from 1785 patients, CKD stages 3 (38%), 4&5 (32%), and stage 5 undergoing dialysis (30%), average age: 57 years±13; 62.4% men; 25% diabetics, from the Spanish Multicenter Study NEFRONA.

Results: The % of patients with arterial plaques is shown in the table below, stratified by gender and stage of CKD.

	stage 3		stages 4&5		stage 5D	
	men	women	men	women	men	women
carotid only	25	40*	23	38*	29	31
femoral only	20	19	18	19	17	15
carotid & femoral	55	41	59	45*	54	51
total carotid	80	81	82	82	83	82
total femoral	75	60*	77	63*	71	66

*p<0.01 compared to men with the same stage of CKD

Conclusions: These results demonstrate that femoral plaques are more prevalent in men at all CKD stages prior to renal replacement. Thus, exclusive carotid ultrasound underestimates atheromatous disease in men. Also, simultaneous femoral and carotid ultrasound increased the detection of atheromatous plaques by 15-20% in both genders at all stages of CKD. Thus, the screening for an early detection of atheromatous disease should include both carotid and femoral ultrasound.

Funding: Pharmaceutical Company Support

TH-PO230

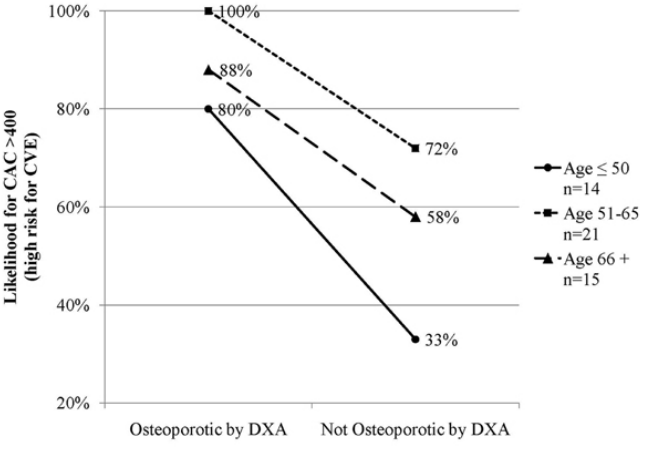
Relationship between Coronary Calcifications and Osteoporosis in Patients with CKD-5 Hartmut H. Malluche,[1] James N. Wise,[2] John Nathan Simmons,[2] Stephanie Fugate,[1] Kimberly McLaughlin,[1] Daniel Davenport,[3] Marie-Claude M. Faugere.[1] *[1]Division of Nephrology, University of Kentucky; [2]Department of Radiology, University of Kentucky; [3]Department of Surgery, University of Kentucky.*

Background: Coronary artery calcifications (CAC) scores were assessed by Agatston scores (Ags) obtained by MSCT in 81 patients (pts) with CKD-5D. In addition, bone mass of the spine and hip was assessed by dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT).

Methods: There were 42 men (age: 52.2 ± 2.18 yrs) and 39 women (age 56.0 ± 2.25 yrs). Dialysis duration was 59.9 ± 6.54 mos. 36 % of the pts were diabetics. Traditional and nontraditional risk factors for cardiovascular events (CVE) were also recorded.

Results: 21 pts had CAC score of 0, 10 pts had CAC scores between 1-30, 16 pts had CAC scores between 30-400 (low to medium risk for CVE), and 34 pts had CAC scores above 400 (high risk for CVE).

Osteoporosis (OP) diagnosed by DXA and QCT was more frequently found in pts with high CVE risk by Ags than in pts with low to medium CVE risk (DXA 44% vs. 12.5%; QCT 32% vs. 12.5%). There were no differences between the CVE risk groups with respect to age, sex, dialysis vintage, BMI, HTN, and lipids. CAC scores correlated negatively with DXA and QCT results of the hip and spine. With adjustment for age, diagnosis of OP by DXA was a significant predictor of high risk CAC levels (odds ratio 8.92, 95% confidence interval 1.49-53.29). Age group 51-65 had higher incidence of high risk calcification than the younger and older age groups (odds ratio relative to <50 years 5.54; 95% C.I. 1.09-28.2). (See below)



Conclusions: In conclusion, with adjustments for age when non-invasive tests for bone mass demonstrate presence of OP, there is high probability of coexistence of CAC scores known to have high risk for CVE. Other tested risk factors did not influence this relationship.

Funding: NIDDK Support

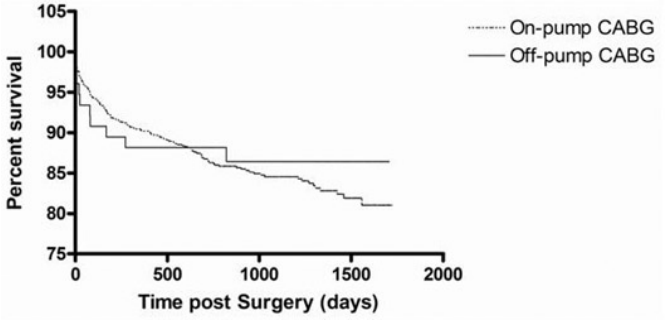
TH-PO231

Is Off-Pump Coronary Artery Bypass Grafting Preferable in Patients with Renal Impairment? Sean Gallagher, Matt Lovell, Dan A. Jones, Zia Buckhoree, Andrew Wragg, Akhil Kapur, Rakesh Uppal, Magdi Yaqoob. *Cardiac and Renal, Barts and the London NHS Trust, United Kingdom.*

Background: Patients with renal impairment undergoing conventional 'on-pump' coronary artery bypass grafting (CABG) have a significant risk of postoperative complications. 'Off-pump' coronary CABG has been reported to be beneficial within this cohort of patients, but this remains a controversial area.

Methods: Analysis of prospectively collected data upon 874 patients with renal impairment (eGFR <60mls/min) undergoing CABG at a tertiary Cardiac centre between 2003 and 2007 was undertaken. We have compared outcomes of 76 patients that had 'off-pump' CABG with 798 that had 'on-pump' All-cause mortality was determined via Office of National Statistics.

Results: Patients selected for 'off-pump' CABG had less previous MIs (27.6vs48.1%, p=0.0209), and more single-vessel coronary disease (14.4vs0.88%, p=<0.0001). Post operatively no difference in incidence of acute kidney injury (AKI) (defined as 50% increase in serum creatinine) (21.1vs20.1%, p=0.8392), peri-procedural stroke (1.3vs1.1%, p=0.5993) or in-hospital mortality (5.2%vs3.1%, p=0.3083) was seen between the groups. At 5 years there was no difference in survival between the groups (86.8%vs84.2%, p=0.5585).



Indeed, the use of 'off-pump' surgery was not independently associated with long term mortality. Only Euroscore (a model use to estimate risk of death following cardiac surgery) HR 1.14 (CI 1.07 to 1.22) and the development of AKI HR 2.38 (CI 1.67 to 3.38) were independently associated with long term mortality.

Conclusions: The use of 'off-pump' surgical techniques do not seem to be associated with improved prognosis within our cohort of patients with renal impairment. The time has come for an adequately powered randomised control trial to definitely determine the value of 'off-pump CABG surgery in patients with renal impairment.

TH-PO232

Heart Rate and Blood Pressure Variability in Children with Chronic Kidney Disease: Report from CKiD Study Gina-Marie Barletta,[1] Joseph T. Flynn,[2] Mark Mitsnefes,[3] Derek Ng,[4] Bradley A. Warady,[5] Joshua A. Samuels,[6] Tim Poffenbarger,[6] Susan L. Furth.[7] *[1]Phoenix Children's Hospital; [2]Children's Hospital and Regional Medical Center; [3]Cincinnati Children's Hospital; [4]Johns Hopkins School of Public Health; [5]Children's Mercy Hospital; [6]University of Texas, Houston; [7]Children's Hospital of Philadelphia.*

Background: Alterations in autonomic nervous system function, including decreased heart rate (HR) variability & differences in blood pressure (BP) variability, are predictors of future cardiovascular events in adults, particularly in those with underlying chronic kidney disease (CKD).

Methods: We examined the degree of HR & BP variability in children with CKD. HR & BP variability were evaluated by 24 hour ambulatory BP monitoring in 104 participants not receiving antihypertensive medications enrolled in CKiD, an observational cohort study of children (1-16 yrs) with Schwartz estimated GFR 30-90 mL/min/1.73 m2. Variability was assessed by coefficient of variation (CV) for HR, systolic (SBP) & diastolic (DBP), and compared across categories of GFR and hypertensive (HTN) status, with adjustment for age, gender & race.

Results: Median age was 11 years (interquartile range: 7, 14), 37% female, 20% black, 58% were HTN, median iohexol GFR: 48 mL/min/1.73 m2 (36, 59); 22% had GFR>60 mL/min/1.73m2, 60 % between 30-60, and 18 % GFR<30.

HR variability was significantly lower in HTN vs. normotensive children when adjusting for age, gender & race, and was independent of activity state and GFR level. Other comparisons are outlined in Table.

Dependent variable Independent variables	HR CV Relative % difference (95%CI)	SBP CV Relative % difference (95%CI)	DBP CV Relative % difference (95%CI)
HTN vs Normotensive	**-16%(-24,-8)**	-3%(-12,+6)	**-13%(-21,-4)**
GFR<30 vs GFR>60	+4%(-10,+19)	+9%(-5,+24)	**+16%(+1,+34)**
GFR 30-60 vs GFR>60	**+14%(+2,+27)**	+3%(-8,+14)	+10%(-1,+24)

Bold indicates p<0.05

Conclusions: The degree of variability in both HR and BP is different among HTN and normotensive children with CKD. These findings are similar to that encountered in adults with CKD and may emphasize the childhood origin and progression of adverse cardiovascular outcomes.

Funding: NIDDK Support

TH-PO233

Suprarenal Aortic Clamp in Open AAA Repair Decreases Postoperative Renal Function Satoshi Unuma,[1] Masaomi Nangaku,[1] Katsuyuki Hoshina,[2] Tetsuro Miyata,[2] Toshiro Fujita,[1] Takamoto Ohse.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Japan; [2]Vascular Surgery, University of Tokyo, Japan.*

Background: Open repair of abdominal aortic aneurysm (AAA) requires infrarenal (IR) or suprarenal (SR) aortic clamping. Since SR leads to renal ischemia, open AAA repair with SR may cause the decrease in renal function. The detail statistical analysis in the change of renal function was performed in this study to clarify the risk factor of AAA repair.

Methods: Retrospective analysis was performed with 178 patients undergoing open AAA repair in the University of Tokyo Hospital between January 2004 and December 2008. After the application of the criteria, 134 patients were enrolled into the 2 weeks study (15±5 days after the operation) and 99 patients were into the 1 year study (360±180 days). We evaluated the change of estimated glomerular filtration rate (eGFR) from the preoperative points to these two points. Furthermore, we conducted multivariate regression analysis with six factors (age, gender, preoperative eGFR, site of clamping, operative duration, diameter of aneurysm) as variables.

Results: The mean age of the patients was 74.1 in the 2 weeks study and 72.9 in the 1 year study. Among the patients in the 2 weeks study and the 1 year study, SR accounted for 14.5%, 13.1% and the mean preoperative eGFR was 58.2mL/min/m², 60.9mL/min/m², respectively.

Statistical analysis revealed the significant difference in the change of eGFR between IR and SR at 2 weeks (+10.3% vs -3.0%, respectively, p<0.05). Also, the significant difference was observed between IR and SR at 1 year (-4.1% vs -15.3%, respectively, p<0.05).

The multivariate regression analysis was performed to find the formula best describing the post-operative eGFR at 1 year with multiple variables. Finally, preoperative eGFR, site of clamping, age were detected as the important factors.

Conclusions: Our statistical analysis revealed eGFR was significantly decreased in SR group when compared to IR group both at 2 weeks and 1 year. Postoperative eGFR was associated with preoperative function, site of clamping and age. These results may suggest the renal ischemia during operation has a high impact on the postoperative renal function.

TH-PO234

Admission Renal Function Has Little Value in Predicting Mortality in Stroke Patients Abhiramy Rudran,[1] Momina Hameed,[1] Abdelgalil Abdelrahman Ali,[1] Velaitham Umachandran,[2] Sumith C. Abeygunasekara,[1] Kakit Chan.[1] *[1]Renal Department, Broomfield Hospital, Chelmsford, Essex, United Kingdom; [2]Stroke Unit, Broomfield Hospital, Chelmsford, Essex, United Kingdom.*

Background: The prevalence of chronic kidney disease [CKD] in elderly patients is unclear, although it is a common finding in those presenting with cerebrovascular accident [CVA]. The impact of CKD on mortality following CVA is poorly described in the literature. In this study, we examine the age-adjusted risk of CKD and lower estimated glomerular filtration rate [eGFR] on mortality in patients with stroke in our unit.

Methods: 406 consecutive patients who presented with CVA in 2009 were included in this study [205f, 201m; mean age 73.9+13.5 yrs; mean eGFR 55.5+18.5 ml/min/1.73m²; 85.5% ischaemic CVA, 4.68% ischaemic with haemorrhagic transformation, 9.85% haemorrhagic]. Of these, 263 [64.8%] patients have CKD stage 3 or above. 46.7% of patients in the older age group [above 84] have eGFR<43.7 ml/min/1.73m2, compared to 8.3% in the younger age group [below 66].

Results: 107 died within the 2 year follow up. Overall patient survival was 80.0%, 76.6% and 72.7% at 6, 12 and 24 months after CVA.

Unadjusted analysis found that haemorrhagic CVA [HR 2.1, 95%CI 1.3, 3.5; p=0.006], older age [Age 66-77.5: HR 3.5; Age 77.5-84: HR 6.1 & Above 84: HR 14.9; p<0.001] and low eGFR [<43.7 ml/min/1.73m²: HR 2.5, 95%CI 1.4, 4.2; p=0.001] are associated with adverse outcome. Albeit statistically significant, these results were confounded by the effect of age and time.

Time and age adjusted survival model was used to correct for confounders. Independent of age, haemorrhagic CVA is associated with 2.4 times risk of death [95%CI 1.4, 4.1; p=0.001]. However, the effect of renal function on survival has diminished; [eGFR< 43.7 ml/min/1.73m2: HR 0.9, 95%CI 0.5,1.6, p=0.747; CKD stage 3: HR 0.4, 95%CI 0.2,1.2; p=0.091; CKD stage 4: HR 1.0, 95%CI 0.3,3.2, p=0.999].

Conclusions: This study demonstrated CKD is common in patients with CVA, and more so in the older age group. Haemorrhagic CVA is an independent risk factor for mortality. Renal function on admission has little impact on survival after adjusting for age.

Funding: Clinical Revenue Support

TH-PO235

Plasma S100A12 Levels and Peripheral Arterial Disease in End-Stage Renal Disease Yasukiyo Mori,[1] Yayoi Shiotsu,[1] Eiko Matsuoka,[1] Atsushi Kosaki.[2] *[1]Department of Cardiology and Nephrology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Department of Medicine II, Kansai Medical University, Hirakata, Japan.*

Background: S100A12, formerly called EN-RAGE, is an endogenous ligand for the receptor for advanced glycation end products (RAGE). Engagement of S100A12 with RAGE promotes the underlying microinflammation. Our working hypothesis is that S100A12 protein might contribute to the development of atherosclerosis, leading to cardiovascular disease (CVD) such as coronary artery disease, stroke and peripheral arterial disease (PAD). The recent clinical study from us showed that the level of plasma S100A12 was an independent factor associated with the prevalence of cardiovascular disease in hemodialysis patient (Shiotsu Y and Mori Y et al. Clin J Am Soc Nephrol 2011; 6: 718–723). In this study, we focus on the relationship between plasma S100A12 levels and PAD.

Methods: A total of 133 subjects who had the medical record of ankle–brachial index (ABI), were selected from 550 HD patients in our affiliated hospital. We investigated the history of PAD and quantified plasma S100A12 levels.

Results: The present study showed that 1) plasma S100A12 levels in HD patients with PAD (n = 26; 21.9 [13.6–33.4] ng/ml) were significantly higher than those in HD patients without PAD (n = 107; 10.7 [7.2–16.7] ng/ml; P < 0.001), 2) in multivariate logistic regression analysis, the plasma S100A12 levels (odds ratio [OR], 7.81; 95% confidence interval [CI], 1.32–46.2; P = 0.023) as well as the presence of diabetes mellitus (OR, 3.77; 95% CI, 1.04–13.6; P = 0.043), high-sensitivity CRP levels (OR, 3.02; 95% CI, 1.01–9.04; P = 0.048), and ABI (OR, 0.53; 95% CI, 0.38–0.74; P < 0.001) were identified as an independent factor associated with PAD prevalence, 3) in another multiple logistic analysis using categorized clinical factors, the higher S100A12 level (≥12.4ng/mL) was associated with PAD prevalence (OR, 5.15; 95% CI, 1.43–18.6; P = 0.012).

Conclusions: These findings indicate that plasma S100A12 is strongly associated with the predicting PAD in ESRD patients.

Funding: Other U.S. Government Support

TH-PO236

Occupational Risk and Chronic Kidney Disease: A Population-Based Study in US Adult Population Sofia Rubinstein, Chengwei Wang. *Medicine, Division of Nephrology, Nassau University Medical Center, East Meadow, NY.*

Background: Previous studies on occupational risk for CKD have been limited in the range of occupations with a focus on nephrotoxins. There has not been a population-based study conducted on a nationally representative sample of the US adult population that investigates the association between a comprehensive list of occupations and risk of CKD.

Methods: This population-based complex survey study uses the National Health Interview Survey data from 2004 to 2008 that randomly sampled non-institutionalized adults in the United States. Main outcome measure was self-reported CKD defined as having weakening/failing kidneys in the past 12 months as diagnosed by a physician. Occupation categories were based on US census bureau coding of Standard Occupation Classification. Univariate analysis was used to identify high risk occupations, multivariate logistic regression applying weights necessary to make accurate population prevalence estimates was used to assess the relative risk.

Results: 91,340 adults completed the question of both CKD status and occupation. CKD was reported in 1,197 (1.3%) participants. After applying appropriate weights, controlling for age, gender, hypertension status and education, and with physical and social science occupation as a reference group, respondents working in Building, Grounds Cleaning and Maintenance Occupations were 4.3 times (95% CI: 1.1-17.7) more likely to develop CKD, while the likelihood of developing CKD were 4.4 times higher in Healthcare Practitioners and Technical Occupations (95%CI: 1.1-18.2), 4.7 times higher in Transportation and Material Moving Occupations (95% CI: 1.2-19.0), 4.7 times higher in Computer and Mathematical Occupations (95% CI: 1.1-20.7), 4.8 times higher in Production Occupations (95% CI: 1.2-19.7), 5.3 times higher in Food Preparation and Serving Related Occupations (95% CI: 1.3-20.8), 6.1 times higher in Healthcare Support Occupations (95% CI: 1.5-25.3), and 6.1 times higher (95% CI: 1.2-30.3) in Legal Occupations.

Conclusions: This study compares the prevalence of CKD among US adults in different occupations. Research on characteristics of high risk occupations is needed for guiding prevention in US job settings.

TH-PO237

Joint Associations of Gout and Serum Uric Acid on Mortality among Subjects with Reduced Kidney Function in the General Population Austin G. Stack,[1] Bhamidipati V.R. Murthy,[2] Arif I.F. Mutwali,[1] Hoang Thanh Nguyen.[1] *[1]Regional Kidney Centre, Letterkenny General Hospital, Letterkenny, Donegal, Ireland; [2]Division of Renal Disease and Hypertension, University of Texas Houston, TX.*

Background: Gout is a chronic inflammatory disorder of uric acid metabolism and is associated with increased cardiovascular (CV) risk. Whether gout and hyperuricaemia contribute independently and synergistically to total and CV mortality has not been previously studied.

Methods: A cohort of 15,773 subjects age >= 20, and representative of the U.S. population, was identified from the Third National Health and Nutrition Examination Survey (1988-1994). Vital status was obtained through linkage with the National Death Index through to 2006. Serum uric acid and physician-diagnosed gout was modeled with total and CV mortality according to estimated glomerular filtration rate (eGFR) (< 60, 60-90, >90 ml/min). Cox regression modeled mortality relationships.

Results: In an average of 10 years of follow-up, total mortality was 9.7 % of which 4.4 % were cardiovascular. Adjusting for confounding, the multivariable relative risks (RR) for subjects with gout were 1.30 (95% Confidence Interval [CI] 1.03-1.65) for total mortality and 1.50 (95% CI 1.08-2.07) for CV mortality. The relative mortality risks per 1 mg/dl increase in serum uric acid were 1.12 (95% CI 1.07-1.17) for total mortality and were 1.10 (95% CI 1.06-1.15) for CV mortality. These relationships were significant for subjects with eGFR < 60 and 60-90 ml/min but not > 90 ml/min. In the conjoint analysis, the multivariable mortality risks for subjects with gout and increasing quartile of serum uric acid were significantly greater than for subjects without gout.

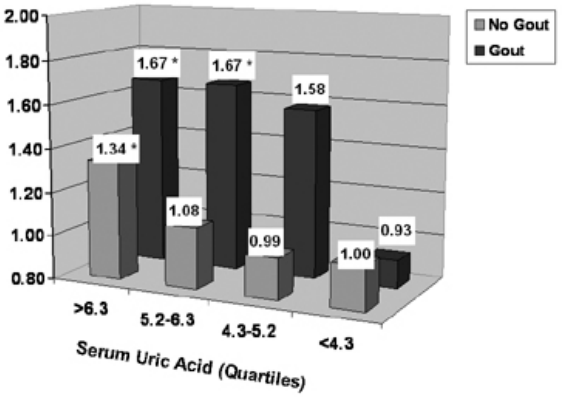


Figure 1. P<0.001 for group comparisons

Conclusions: Both gout and hyperuricaemia independently predicted total and CV mortality. The mortality risks were greatest for those with gout and the highest uric acid concentrations.

TH-PO238

Hypoalbuminemia Is Prevalent and Predictive in Appalachian Patients with Chronic Kidney Disease (CKD) Rebecca J. Schmidt, Bethany S. Pellegrino. *Section of Nephrology, West Virginia University School of Medicine, Morgantown, WV.*

Background: Chronic kidney disease (CKD) has reached epidemic proportions worldwide and in the United States, minorities of race and ethnicity reach end stage renal disease (ESRD) at a disproportionate rate. A rural state in the heart of Appalachia, West Virginia leads the nation in rates of incident ESRD, despite its predominantly Caucasian population.

Methods: Clinical characteristics and demographics of 4258 patients seen between January 1, 2001 and July 31, 2010 by a university-based nephrology group across north central West Virginia were retrospectively analyzed for their impact on progression to dialysis or death. Socioeconomic information for state and county was obtained from census data. Main outcome measures were time from first clinic visit to death or start of dialysis.

Results: Patients were predominantly Caucasian (94.3%), with a mean age of 60.1±16.7, a 39% prevalence of diabetes; 39% presented with an albumin level ≤ 3.5 gm/dl. Patients with higher presenting albumin levels had better survival and less progression to dialysis than those with lower albumin levels ($p<0.0001$). Hypoalbuminemia, hypocalcemia, hyperparathyroidism and anemia all independently correlated with reduced survival and more rapid progression to ESRD ($p<0.0001$). Compared to those from more affluent counties, patients from counties of low socioeconomic status had lower albumin levels (3.36±0.014 vs 3.68±0.079 gm/dl; $p<0.04$) and higher rates of progression to dialysis or death ($p<0.016$).

Conclusions: In this north central West Virginia region of Appalachia, CKD patients were predominantly white, often hypoalbuminemic and most hailed from counties of low socioeconomic status. Hypoalbuminemia and residence in a socioeconomically disadvantaged county independently predicted overall survival and progression to dialysis, suggesting that poverty and culture, irrespective of race, warrant further study for their impact on outcomes in patients with CKD.

TH-PO239

Short Sleep Duration as a Novel Predictor of Proteinuria Ryohei Yamamoto,[1] Yasuyuki Nagasawa,[1] Hirotsugu Iwatani,[1] Yoshitsugu Obi,[1] Maki Shinzawa,[1] Junya Teranishi,[1] Toshihiro Ishigami,[1] Hiromi Rakugi,[1] Yoshitaka Isaka,[1] Toshiki Moriyama.[2] *[1]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Osaka University Healthcare Center, Toyonaka, Osaka, Japan.*

Background: Although multiple studies revealed that sleep duration was a predictor of cardiovascular diseases and also mortality, few studies reported an association between sleep duration and chronic kidney disease charactrized by impaired renal function and proteinuria.

Methods: The prensent retrospective cohort study included 6020 employees of Osaka University aged 19 - 55 years, who visited Osaka University Healthcare Center for their mandatory annual health examination between May 2006 and September 2010. Questionnaires about their life style, including sleep duration, and blood and urine examinations at the first examination during study period were retrospectively collected as the baseline date. The outcome of interest was time to development of proteinuria defined as ≥1+ by dipstick test after the baseline examination.An association between the baseline sleep duration and the outcome was assessed using conventional multivariate Cox proportional hazards model, propensity score-based models, and disease risk score-based models.

Results: Self-reported baseline sleep duration was 6.0 ± 0.9 hours, which reflected the mean sleep duration during median 2.0 (interquartile range 1.2 to 3.0) years of observational period. After the baseline examinations, proteinuria was observed in 498 employees (8.3%). A multivariate Cox proportional hazards model clarified that shorter sleep duration was associated with proteinuria in a stepwise fashion, even after adjustment for clinically relevant factors (vs. 7 hours; ≤3 hours, hazard ratio 2.76 [95% CI 0.86 to 8.87], P = 0.09; 4 hours, 1.65 [1.08 to 2.54], P = 0.02; 5 hours 1.30 [1.01 to 1.68], P = 0.05; 6 hours, 1.06 [0.84 to 1.34], P = 0.6; ≥8 hours, 0.96 [0.51 to 1.79], P = 0.9). Propensity score-based models and disease risk score-based models also ascertained ≤5 hours of sleep duration were significantly associated with proteinuria.

Conclusions: Short sleep duration, especially ≤5 hours, was a novel predictor of proteinuria.

TH-PO240

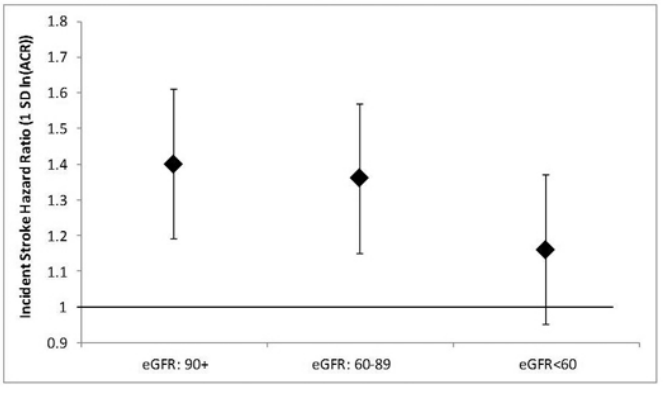
Albuminuria, Kidney Function and Risk of Stroke in the REGARDS Cohort

Orlando M. Gutierrez,[1] Suzanne E. Judd,[1] Dana Rizk,[1] Paul Muntner,[1] William M. McClellan,[2] David G. Warnock.[1] *[1]UAB; [2]Emory; [3]Vermont.*

Background: Chronic kidney disease associates with stroke risk but the independent contributions of higher urinary albumin/creatinine ratio (ACR) and lower estimated glomerular filtration rate (eGFR) are unclear.

Methods: Associations of ACR and eGFR with incident stroke were examined in the REasons for Geographic and Racial Differences in Stroke (REGARDS), a national prospective cohort of 30,239 black and white adults. After excluding those with prevalent stroke, end stage renal disease, or missing data, 24,777 participants were analyzed. Cox regression was used to examine associations of eGFR and ACR at baseline with subsequent, physician-verified incident stroke. Adjustment was made for baseline age, sex, race, age-race interaction, medication use, blood pressure, smoking, atrial fibrillation, diabetes, heart disease, income and education.

Results: In fully-adjusted models, there was no association of eGFR with incident stroke (eGFR 90-120 ref; 60–89, HR 0.92, 95%CI 0.8,1.1; 45-59, HR 0.85, 95%CI 0.6,1.2; <45, HR 1.21, 95%CI 0.8, 1.8). In contrast, there was a step-wise increase in HR of stroke with increasing ACR (<10 mg/g ref; 10–29, HR 1.36, 95%CI 1.1,1.7; 30-300, HR 1.68, 95%CI 1.3,2.1; >300, HR 1.85, 95%CI 1.2,2.8). When examined on a continuous scale, the association of logACR with HR of stroke was modified by eGFR (P for interaction 0.05). In fully adjusted models, each SD increase in logACR was associated with higher HR of stroke in participants with eGFR≥60 but not <60.



Conclusions: Higher ACR was independently associated with higher stroke risk only in those with eGF R≥60. eGFR was not associated with stroke risk after accounting for ACR and other risk factors. These findings suggest that ACR might be helpful to assess stroke risk in adults with eGFR≥60.

Funding: Other NIH Support - NINDS, Pharmaceutical Company Support

TH-PO241

Bone Mineral Density and Fracture Risk in Older Individuals with Chronic Kidney Disease: The Health, Aging and Body Composition Study

Robert H. Yenchek,[1] Joachim H. Ix,[2] Michael Shlipak,[3,4] Doug Bauer,[4] Nahid J. Rianon,[5] Stephen B. Kritchevsky,[6] Tamara Harris,[7] Anne B. Newman,[1] Jane Cauley,[1] Linda F. Fried.[1,8] *[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]University of California, San Diego, CA; [3]San Francisco VA, San Francisco, CA; [4]UCSF, San Francisco, CA; [5]University of Texas Health Sciences Center, Houston, TX; [6]Wake Forest School of Medicine, Wake Forest, NC; [7]NIA, Bethesda, MD; [8]VAPHS, Pittsburgh, PA.*

Background: KDIGO guidelines recommend against bone mineral density (BMD) screening in patients with CKD with mineral bone disease, due to a lack of association of BMD with fractures in cross-sectional studies in CKD patients.

Methods: We assessed whether BMD predicted fracture in participants with and without CKD in the Health, Aging and Body Composition study, a prospective cohort study of well-functioning older individuals. BMD was measured by dual energy x-ray absorptiometry. CKD was categorized as eGFR < 60 ml/min/1.73m^2 using the MDRD formula. Adjudicated incident total non-spine fractures to study year 11 were analyzed using Cox proportional hazards analysis. Interaction terms were tested to assess whether the association of BMD with fracture differed in those with and without CKD.

Results: Of the 2,754 with BMD and laboratory values, mean age was 73.6 ±2.9, 51% were women, 40% were Black, 60% were white, 21% had CKD (96% with stage 3). There were 465 incident non-spine fractures. Femoral neck BMD (FNBMD) and Total Hip BMD (THBMD) were associated with non-spine fracture, regardless of CKD status. After adjustment for age, race, gender, BMI, high PTH ( )65 pg/ml), and low 25-vitamin D (< 20ng/ml) the hazard ratio per standard deviation higher FNBMD was 0.40 (0.30, 0.54) and 0.52 (0.44, 0.60) for those with and without CKD, respectively (p for interaction 0.93). Likewise, the hazard ratio for THBMD for fracture was 0.42 (0.31, 0.57) and 0.49 (0.42, 0.58) for those with and without CKD (p for interaction 0.66).

Conclusions: BMD measurement provides information on a patient's risk for fracture in individuals with or without moderate CKD.

Funding: Other NIH Support - National Institutes of Aging

TH-PO242

The Structure of Health-Related QOL Varies According to Underlying Diseases of CKD: Results from the CKD-JAC Study

Yasuo Ohashi,[1] Satoshi Iimuro,[1] Tadao Akizawa,[2] Enyu Imai,[2] Seiichi Matsuo,[2] Tsuyoshi Watanabe,[2] Kosaku Nitta,[2] Hirofumi Makino,[2] Akira Hishida.[2] *[1]Univ Tokyo, Japan; [2]CKD-JAC Study Group.*

Background: CKD-JAC was established to prospectively study the renal and cardiovascular outcomes in 2,977 Japanese patients with CKD stages 3-5. CKD is caused by various underlying diseases, and the breakdown of Health Related(HR)-QOL is expected to vary depending on these diseases.

Methods: Data at registration and HR-QOL data were analyzed. In addition, canonical discriminant analysis(CDA) was performed for multidimensional analysis.

Results: Of the 2,526 patients analyzed, 36% had diabetes. The patients were divided into four major groups by disease. As the CKD stages progressed, almost all of the SF-36 domains worsened, especially in GH. PSQI and BDI also worsened. As for disease groups, SF-36, PSQI, and BDI were worse in those with diabetes.

CDA was performed about CKD stages [1] or the disease groups [2] as the criterion variables.

[1]One significant canonical discriminant function was obtained. The standardized discriminant coefficients of GH, RE, and PSQI were large and the mean values of the discriminant function for each stage were 0.14(Stage 3), -0.03(Stage 4), and -0.32(Stage 5).

[2]Two significant discriminant functions were obtained. The first one determined the presence of diabetes, and the second determined the presence of GN or DN(Table1).

Table 1
Canonical discriminant analysis

standardized discriminant coefficient			
var		axis1	axis2
SF-36	Physical functioning(PF)	0.883	0.085
	Role physical(RP)	0.011	-0.199
	Bodily pain(BP)	0.297	-0.658
	General health(GH)	0.026	0.799
	Vitality(VT)	-0.465	0.450
	Social functioning(SF)	-0.157	0.244
	Role emotional(RE)	0.069	-0.004
	Mental health(MH)	-0.077	-0.560
Pittsburgh Sleep Quality Index (PSQI)		0.202	0.304
Beck Depression Inventory (BDI)		0.239	-0.008
mean of discriminant function			
no DM / no GN		0.040	0.155
no DM / GN		0.268	-0.121
DM / no DN		-0.166	0.130
DM / DN		-0.418	-0.166

DM: Diabetis Mellitus, GN: Glomerular Nephritis, DN: Diabetic Nephropathy

Conclusions: As the CKD stage progresses, HR-QOL were found to worsen. A strong correlation was also observed with the underlying disease, and components of HR-QOL that strongly correlated varied with the type of disease.

Funding: Pharmaceutical Company Support

TH-PO243

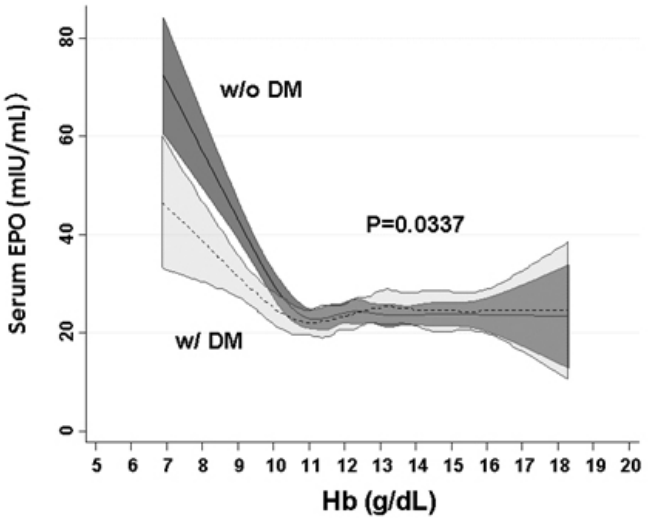
Erythropoietin Responses in Chronic Kidney Disease: A Cross-Sectional Analysis from the CKD-JAC Study

Tadao Akizawa,[1] Takayuki Hamano,[2] Enyu Imai,[2] Seiichi Matsuo,[2] Hirofumi Makino,[2] Tsuyoshi Watanabe,[2] Kosaku Nitta,[2] Yasuo Ohashi,[2] Akira Hishida.[2] *[1]Syowa Univ, Japan; [2]CKD-JAC Study Group.*

Background: Erythropoietin(EPO) production by renal cells is driven by low oxygen levels in the renal tissue. Aim is to elucidate the factors to determine serum EPO in Chronic Kidney Disease(CKD) not on dialysis.

Methods: EPO levels in 1796 ESA therapy-naive patients with CKD stages 3-5 were measured at 1 year after enrollment. We explored associations of EPO levels with various parameters including hemoglobin(Hb), mean corpuscular volume(MCV), eGFR, urinary and serum albumin, C-reactive protein(CRP), and prior cardiovascular disease(CVD) using multiple regression. Restricted cubic spline regression was used for parameters having non-linear relationships with serum EPO.

Results: A significantly non-linear negative relationship between EPO and Hb levels was observed. The threshold of Hb when EPO levels plateaued was 11g/dL on average; this threshold decreased with the advance in CKD stages. The slope between EPO levels and Hb below the threshold was significantly steeper in patients without diabetes(DM), suggesting impaired EPO response to anemia in DM.



There was a U-shaped relationship between MCV and EPO levels with the bottom in the normal ranges of MCV. Albuminuria and eGFR were independent negative and positive determinants of EPO levels, respectively. Low serum albumin, high CRP levels, and prior CVD were associated with high EPO levels.

Conclusions: Given that EPO production is driven by hypoxia, our findings might suggest that targeting Hb levels greater than 11 g/dL does not further improve oxygen status in the kidney. DM was an effect modifier of the association between EPO and Hb. Malnutrition, inflammation, and atherosclerosis were associated with higher EPO levels, possibly because oxygen delivery to renal tissues is disturbed due to microvascular damage.

Funding: Pharmaceutical Company Support

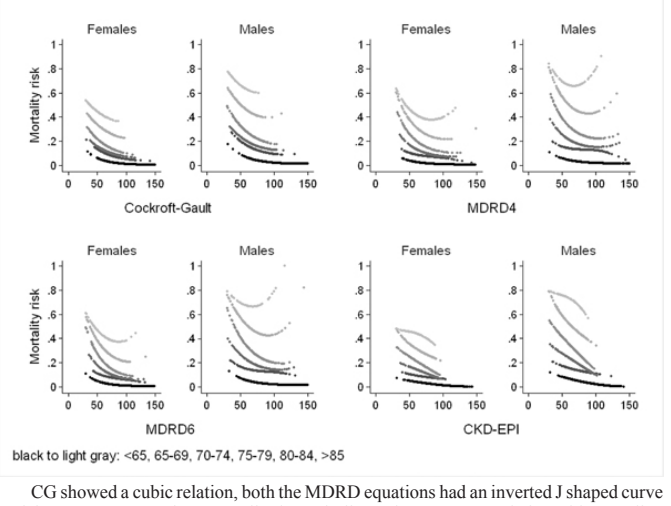
TH-PO244

The Role of eGFR, Age and Gender in Mortality Risk Jan A.J.G. van den Brand,[1] Martin Den Heijer,[2] Lambertus Kiemeney,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Epidemiology, Biostatistic and HTA, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: The effect of eGFR on mortality is modified by age and sex. Our goal was to explore the dose-response relationship of these risk factors and compare GFR estimation equations.

Methods: We obtained data for 2816 male and 3269 female Caucasian participants of the Nijmegen Biomedical Study, a population based, age and sex stratified sample of inhabitants of Nijmegen. Serum creatinine values were determined with the Jaffé method calibrated against mass spectrometry and were used to calculate eGFR with the Cockroft-Gault (CG), MDRD4, MDRD6 and CKD-EPI equations. Demographics, health status and medication use were obtained by postal questionnaire. We used a poisson regression model weighted for sampling fractions to estimate mortality risk for eGFR by age, sex and comorbidities and used fractional polynomials to evaluate non-linear relations.

Results: During 39,855 person-years of follow-up 316 people died. Figure 1 shows the unadjusted association for eGFR and mortality by age and sexe.



CG showed a cubic relation, both the MDRD equations had an inverted J shaped curve and the CKD-EPI equation generally showed a linear dose response relation with mortality. The latter formula thus allowed use of a linear model to describe the association between eGFR and mortality. The adjusted relative risk (RR) of mortality increased with decreasing eGFR. However, the effect of eGFR was attenuated in people >75 years.

Adjusted RR of mortality

	Age			
eGFR-CKD-EPI	<75	75-79	80-84	≥85
90	1 (ref)	1 (ref)	1 (ref)	1 (ref)
60	3.0	1.3	1.2	1.1
30	5.3	1.5	1.3	1.1

Indexed to 90 ml/min/1.73m² for each age category separately

Conclusions: The CKD-EPI equation allows linear modeling of mortality risk. Lower eGFR-CKD-EPI is an important risk factor for mortality, but not in people aged >75 years.

Funding: Private Foundation Support

TH-PO245

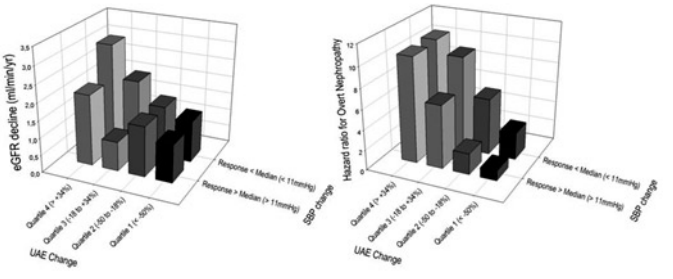
Albuminuria Response to Angiotensin Receptor Blockade Is a Determinant of Renal Protection in Patients with Type 2 Diabetes and Microalbuminuria in the IRMA-2 Trial Merel E. Hellemons,[1] Frederik I. Persson,[2] Peter Rossing,[2] Stephan J.L. Bakker,[1] Dick de Zeeuw,[1] Hans-Henrik Parving,[3] Hiddo Jan Lambers Heerspink.[1] *[1]UMCG; [2]STENO Diabetes Center; [3]University Hospital of Copenhagen.*

Background: Albuminuria (UAE) and systolic blood pressure (SBP) are both renal risk markers in patients with type 2 diabetes (T2DM). ARBs reduce SBP and UAE and are renoprotective. However, UAE and SBP reduction varies and can be discordant within an individual. We tested the SBP and UAE response to ARB and effect on renal function decline in T2DM patients.

Methods: Data from the IRMA-2 trial were used. In this post-hoc analysis we assessed the extent of variability and discordance in SBP and UAE response (0-6 months) in 531 subjects. We analyzed the effect of month 6 SBP and UAE change on renal outcome, looking at glomerular filtration rate (eGFR) changes as well as development of overt nephropathy during 2 years follow-up.

Results: On Irbesartan treatment, 85 (24%) patients had a reduction in UAE but not in SBP at month 6. Conversely, 67 (19%) had a reduction in SBP but not in UAE. A larger reduction in UAE (p=0.0037) but not SBP (p=0.087) was independently associated with a slower rate of eGFR decline. The risk reductions for overt nephropathy per 50% reduction in UAE and 5 mmHg SBP reduction were 44% (95%CI 39-59%; P<0.001) and 9% (95% CI 20 to +2%; P=0.098) resp. Renal function decline according to combined SBP and UAE change demonstrated that across both SBP categories (defined by the median), a progressively lower eGFR decline and lower risk for overt nephropathy was observed with a larger UAE reduction. (figure 1)

Conclusions: SBP and UAE response to ARB therapy may be discordant. The UAE response individually determined renal outcome. The results suggest that in T2DM patients with microalbuminuria both SBP and UAE should be separate targets for renoprotection.



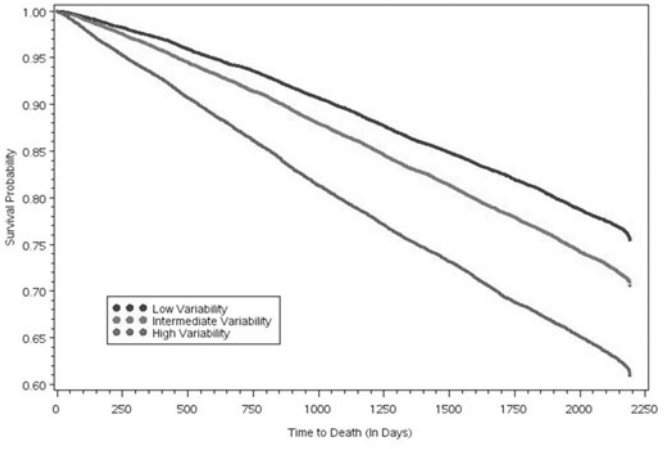
TH-PO246

Variability in Kidney Function and the Risk of Death Ziyad Al-Aly,[1] Tarek M. El-Achkar,[1] Ann M. O'Hare.[2] *[1]Department of Medicine, Division of Nephrology, Saint Louis Veterans Affairs Medical Center, Saint Louis, MO; [2]Department of Medicine, Division of Nephrology, Veterans Affairs Puget Sound Healthcare System, Seattle, WA.*

Background: Intra-individual variability in kidney function is a commonly observed phenomenon. However, predictors of kidney function variability and its prognostic implications are not known.

Methods: We used national data from the Department of Veterans Affairs to assemble a cohort of 60,641 patients with least 3 outpatient serum creatinine measurements between October 1, 1999 and September 30, 2002. Variability in kidney function was defined for each patient as the coefficient of variation of the regression line coefficient fitted to all outpatient measures of estimated glomerular filtration rate (eGFR) during this time frame. We built logistic regression models to examine predictors of variability and Cox survival models to examine the association between kidney function variability and the risk of death.

Results: Black race, female gender, diabetes, cardiovascular disease, peripheral artery disease, chronic lung disease, hepatitis C, dementia, hospitalizations, and the use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers and diuretics were predictors of high variability in eGFR. After a median follow-up of 5.9 years, there were 4,563 (22.57%), 5,552 (27.47%), and 7,493 (36.80%) deaths among patients in low, intermediate, and high tertiles of eGFR variability; respectively. In adjusted analyses, patients in the highest tertile of eGFR variability had an increased risk of death (Hazard Ratio=1.33, 95% Confidence Interval=1.28-1.38) compared with the referent of those in the lowest tertile.



Conclusions: Our results demonstrate that greater variability in kidney function is independently associated with increased risk of death.

Funding: Veterans Administration Support

TH-PO247

Age and Baseline Kidney Function Modify the Association between Rate of Kidney Function Decline and the Risk of Death Ziyad Al-Aly,[1] Ann M. O'Hare,[2] Tarek M. El-Achkar,[1] Michael I. Rauchman.[1] *[1]Division of Nephrology, Saint Louis Veterans Affairs Medical Center, Saint Louis, MO; [2]Department of Medicine, Division of Nephrology, Veterans Affairs Puget Sound Healthcare System, Seattle, WA; [3]Division of Nephrology, Walter Reed Army Medical Center, Washington, DC.*

Background: The relationship between rate of kidney function decline and mortality has not been examined in different age groups and in patients with kidney disease.

Methods: Using the Department of Veterans Affairs national databases, we built a cohort of 22,568 patients and categorized them into 4 groups: those who experienced no decline (rate of eGFR change greater than 0 ml/min/year) and those with mild, moderate and severe decline defined as eGFR loss of 0-1, 1-4, and greater than 4 ml/min/year; respectively. We built Cox survival models to examine the relationship between rate of kidney function decline and the risk of death in patients according to age groups (less than 60, 60-75, and greater than 75 years old) and eGFR categories (baseline eGFR greater than 90, 60-89, 45-59, 30-44, and 15-29 ml/min).

Results: After a median follow up of 5.9 years, the risk of death in patients with severe decline in eGFR was strongest in the youngest patients (Hazard Ratio (HR) 1.6, Confidence Interval (CI) 1.2-2.1 for <60 years old) and was gradually attenuated across groups of increasing age (HR=1.2, CI=1.0-1.3 for greater than 75 years old, P for interaction=0.0307). In groups according to baseline eGFR, the risk was significant but weak in patients with baseline eGFR greater than 90 ml/min (HR=1.30, CI=1.13-1.50) and became increasingly more pronounced in groups with decreasing baseline eGFR (HR=2.8, CI=1.8-4.2 for 15-29 ml/min, P for interaction <0.001).

Conclusions: Our results show that the independent association between the rate of decline of kidney function and mortality is attenuated with increasing age and may be more pronounced in patients with advanced baseline kidney disease.

Funding: Veterans Administration Support

TH-PO248

Clinical Outcomes of Clostridium difficile Infection in Patients with Chronic Kidney Disease Mira T. Keddis,[1] Sahil Khanna,[2] Larry M. Baddour,[3] Darell S. Pardi,[2] Qi Qian.[1] *[1]Nephrology, Mayo Clinic, Rochester, MN; [2]Gastroenterology, Mayo Clinic, Rochester, MN; [3]Infectious Diseases, Mayo Clinic, Rochester, MN.*

Background: The outcomes of *Clostridium difficile* infection (CDI) in chronic kidney disease (CKD) patients have not been previously described. The objective of this study is to examine clinical outcomes of hospitalized patients with CKD and CDI using the National Hospital Discharge Survey (NHDS) database.

Methods: We analyzed NHDS data for 2005-2009; the database contains demographic information, ICD-9 diagnosis and procedure codes, and discharge information for patients admitted to non-Federal short-stay United States hospitals. Clinical information of patients with CKD and CDI was abstracted and analyzed using SAS version 9.2 and JMP version 9.0.1.

Results: 59,715 hospitalized patients with CKD were identified with mean age 68.5 ±15.8 years, 52.4% male sex. 41.9% of patients had unspecified CKD severity, 37.7% with CKD stage V, 18.6% CKD III and IV, and 1.8% CKD I and II. Of them, 17,189 (28.8%) patients required dialysis during their hospitalization. CDI occurred in 918 (1.5%) patients. CKD patients with CDI were significantly older (72 ±0.5 vs. 68.4 ±0.07 years), required longer length of stay (10 ±0.2 vs. 6.2 ±0.02 days), and were more likely to be dismissed to a care facility (50.5 vs. 25.7%) compared to CKD patients without CDI (all p<0.001). CKD patients with CDI had a higher in-hospital mortality rate (8.50 vs. 3.95%,OR 2.25 [95% CI 1.78-2.85]) and likelihood of requiring colectomy (OR 3.33 [95% CI 1.7-6.54], p<0.001). After adjusting for age, the association of CDI with these clinical outcomes remained significant.

Conclusions: In hospitalized CKD patients, CDI was associated with prolonged hospitalization, and an increased likelihood of both undergoing colectomy and suffering in-hospital mortality. Prevention, early recognition and treatment of CDI in CKD patients are necessary to decrease CDI associated morbidity and mortality in this population.

TH-PO249

Troponin T and B-Type Natriuretic Peptide Are Associated with Cardiovascular Outcome Despite Their Cross-Sectional Association with Chronic Kidney Disease Lieneke Scheven,[1] Paul E. de Jong,[1] Hiddo Jan Lambers Heerspink,[2] Lucas Joost Van Pelt,[3] Jenny E. Kootstra-Ros,[3] Stephan J.L. Bakker,[1] Ron T. Gansevoort.[1] *[1]Nephrology; [2]Pharmacology; [3]Clinical Chemistry, University Medical Centre Groningen, Netherlands.*

Background: It has been suggested that troponins and natriuretic peptides are falsely elevated in chronic kidney disease (CKD) because of decreased renal clearance. The value of these biomarkers to predict cardiovascular (CV) outcome in subjects with CKD has therefore been debated. We investigated this issue in a population based cohort study.

Methods: For the present study 8,121 subjects who participated in the PREVEND Study of whom at baseline high sensitive Troponin T (hsTnT) and N-terminal pro-B-type Natriuretic Peptide (NT-pro-BNP) were available, were included. HsTnT ≥0.01 µg/L and NT-pro-BNP ≥125 ng/L were defined as elevated.

Results: Of our cohort, 6.7% had an elevated hsTnT and 12.2% an elevated NT-pro-BNP. eGFR and albuminuria were significantly associated with hsTnT and NT-pro-BNP in linear regression analyses. After adjustment for age, gender and CV risk factors these associations remained significant. Both hsTnT and NT-pro-BNP appeared associated with CV events during follow-up (both p<0.001). These associations remained significant after adjustment for eGFR, albuminuria, age, gender and CV risk factors (both p<0.001). No interaction was found between eGFR and hsTnT or NT-pro-BNP in predicting CV outcome. Moreover, the value of an increased hsTnT or NT-pro-BNP to predict CV outcome was similar in subjects with or without CKD (in both, for both hsTnT and NT-pro-BNP p<0.001).

Conclusions: These data indicate that a finding of an increased hsTnT or NT-pro-BNP in a subject with CKD should be taken seriously as a prognostic marker heralding an unfavourable CV outcome and not be discarded as merely a reflection of decreased renal clearance.

TH-PO250

Isolated Microalbuminuria Heralds a Poor Medical Prognosis Lieneke Scheven,[1] Paul E. de Jong,[1] Hiddo Jan Lambers Heerspink,[2] Stephan J.L. Bakker,[1] Ron T. Gansevoort.[1] *[1]Nephrology; [2]Pharmacology, University Medical Centre, Groningen, Netherlands.*

Background: Microalbuminuria (MA) is often regarded as a sign of end-organ damage due to diabetes and/or hypertension and to be associated with an increased risk for cardiovascular (CV) events. It has been questioned whether isolated microalbuminuria (IMA) - i.e. microalbuminuria in absence of diabetes, hypertension and/or CV morbidity - has clinical relevance.

Methods: For this study data were used of 8,592 subjects who participated in the first 4 screening rounds of the PREVEND study, a prospective, community-based cohort study with serial measurements during follow-up. IMA was defined as albuminuria 30-300 mg/24hr and a fasting glucose <7.0 mmol/L (no glucose lowering drugs), blood pressure <140/90 mmHg (no antihypertensive drugs) and a negative CV disease history. Logistic regression analyses was used to determine the risk for developing diabetes (ADA criteria) and hypertension (JNC-7 criteria), and Cox regression analysis to determine the risk for CV events.

Results: A total of 300 subjects met the definition of IMA. In subjects with IMA the incidence rates of diabetes, hypertension and CV events were 8.5, 27.0 and 15.3 per 1,000 patient years follow-up, respectively. Subjects with IMA had an increased risk for developing diabetes (Odds Ratio (OR) 4.69 (2.92-7.51); p<0.001), hypertension (OR 1.95 (1.47-2.59); p<0.001) and CV events (Hazard Ratio 2.23 (1.63-3.07); p<0.001), compared to subjects without albuminuria, diabetes, hypertension and CV history. This increased risk remained significant after adjustment for age and gender. The risk held by IMA was similar to the risk held by microalbuminuria in subjects that do have diabetes, hypertension and/or a CV disease history.

Conclusions: Microalbuminuria, even in the absence of diabetes, hypertension and a CV disease history heralds a poor prognosis and warrants medical attention.

TH-PO251

Predictors of Progression in Albuminuria in the General Population Lieneke Scheven, Nynke Halbesma, Paul E. de Jong, Ron T. Gansevoort. *Nephrology, University Medical Centre Groningen, Netherlands.*

Background: Progression of CKD is usually defined as loss of renal function. However, also an increase in albuminuria (UAE) is associated with a poor prognosis, both with respect to CV as well as renal outcome. We investigated which factors predict progressive UAE in the general population using data of subjects participating in a prospective, community-based cohort study with serial follow-up (PREVEND).

Methods: After exclusion of subjects with known renal disease or macroalbuminuria at baseline, or missing follow-up data on UAE, 5,825 subjects were eligible. Subject were defined as having progressive UAE if they belonged to the quintile with the strongest annual increase in UAE, and UAE ≥150 mg/24h during follow-up. Multivariable regression models were built using stepwise backward selection to identify risk factors for progressive UAE.

Results: During a median follow-up of 9.3 years 132 subjects met our definition of progressive UAE (median value at baseline 67.4 versus 252.7 mg/24h at end of follow-up). Univariably associated with progressive UAE were: male gender, history of cardiovascular disease, lower eGFR, higher age, BMI, SBP, glucose and baseline UAE, and use of antihypertensive and lipid lowering medication (all p<0.001). Variables that contributed significantly to the multivariable model are shown in the table. Importantly, most predictors for progressive UAE were, although statistically significant, of limited value when compared to baseline UAE, as indicated by the Wald statistics (table) and a clinical score chart based on the Beta-values of the multivariable model.

Variable	Odds Ratio	p-value	Wald statistic
Male gender	2.26	<0.001	12.2
Age (yrs)	1.03	<0.001	13.1
BMI (kg/m2)	1.06	0.01	6.0
UAE (ln mg/24h)	5.78	<0.001	275.3
* Change in SBP (mmHg)	1.02	0.006	7.4

* Change in systolic blood pressure during follow up

Conclusions: A high baseline UAE is by far the most important predictor of progressive UAE. Thus, screening for baseline UAE will be more important than screening for cardiovascular risk factors in order to identify subjects at risk for progressive UAE.

TH-PO252

Glomerular and Tubular Damage Markers in Subjects with Progressive Albuminuria Ferdau L. Nauta,[1] Lieneke Scheven,[1] Stephan J.L. Bakker,[1] Willem Van Oeveren,[2] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]Nephrology, UMCG; [2]Haemoscan, Groningen, Netherlands.*

Background: Albuminuria is associated with risk for renal and cardiovascular disease. It is difficult to predict which subjects will progress in albuminuria. We investigated whether assessment of urinary markers representing damage to different parts of the nephron help to identify subjects that will progress in albuminuria.

Methods: Subjects were selected from a prospective community-based cohort study with serial follow-up (PREVEND, n=8592) and defined as progressor if they were in the quintile with most rapid annual increase in albuminuria and reached albuminuria 150 mg/d during follow-up. Subjects with known renal disease were excluded. Progressors were 2:1 matched to control subjects based on age, sex and baseline albuminuria. IgG was measured as glomerular marker, KIM-1, NAG, β-2-microglobulin, cystatin C as proximal tubular damage markers and NGAL and MCP-1 as inflammatory markers.

Results: After a follow-up of 9.3 yr 183 subjects met our criteria for progressive albuminuria. Baseline clinical characteristics between progressors and controls were comparable. However, both urinary excretion and fractional excretion of total IgG were significantly higher in progressors (p<0.001), whereas in these subjects urinary and fractional excretion of all tubular markers except cystatin C were lower (all p<0.01).

Urinary excretions

	Progressors	Controls
Albumin (mg/d)	58 (33-106)	52 (31-88)
IgG (µg/d)	1285 (926-2181)	1118 (696-2039)**
KIM-1 (µg/d)	3.9 (1.9-7.0)	7.4 (3.2-15.0)**
CysC (µ/d)	0.011 (0.005-0.030)	0.015 (0.005-0.041)
B2MG (µg/d)	103 (39-395)	169 (58-641)**
NAG (µg/d)	0.011 (0.000-0.255)	0.694 (0.000-1.863)**
NGAL (µg/d)	5.5 (2.7-9.6)	6.7 (3.9-11.4)*
MCP-1 (ng/d)	467 (261-825)	741 (522-911)**

Medians (IQR) * p<0.05, ** p<0.01

Conclusions: These data suggest that albuminuria associated with glomerular damage is more likely to progress, whereas albuminuria associated with tubulointerstitial damage is more likely to remain stable.

TH-PO253

The Effect of Frozen Storage on Urinary Renal Damage Markers Ferdau L. Nauta,[1] Stephan J.L. Bakker,[1] Hiddo Jan Lambers Heerspink,[2] Willem Van Oeveren,[3] Dick de Zeeuw,[2] Henk Bilo,[4] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]Nephrology; [2]Pharmacology; [3]Haemoscan, Groningen; [4]Diabetes Center, Isala Clinics, Zwolle, Netherlands.*

Background: Epidemiological studies that investigate the value of renal damage markers to predict outcome often use urine samples that have been stored frozen for prolonged time. Little is known about the effect of frozen storage on urinary concentrations and variability of renal tubular damage markers. We therefore investigated the effect of storage at -20 and -80 °C.

Methods: Urine samples were collected in 95 patients with diabetes mellitus. In each sample we measured IgG, IgG-4, KIM-1, NGAL, NAG, cystatin C and H-FABP fresh and after 1 week, 6 months and 1 year of frozen storage at -80 C. Furthermore, the effect of 1 year frozen storage at -20 C and the effect of various specific storage protocols was investigated.

Results: Average marker concentrations showed a gradual decrease and an increase in variability after frozen storage at -80 °C.

	Fresh	1 week	6 months	1 year
IgG total (µg/ml)	6.4 (2.1-28.9)	5.7 (1.9-10.7)*	5.2 (3.4-11.0)	5.0 (2.2-15.3)
IgG-4 (µg/ml)	1.2 (0.9-2.6)	0.8 (0.3-2.5)	0.6 (0.6-0.7)**	0.7 (0.3-2.9)
KIM-1 (ng/ml)	1.3(0.7-2.2)	1.0 (0.4-1.5)**	1.0 (0.6-1.7)	0.7 (0.4-1.0)**
NAG (U/L)	8.5 (5.4-13.3)	7.8 (5.4-14.1)	2.0 (1.2-3.6)**	0.3 (0.2-0.3)
Cystatin C (mg/L)	0.03 (0.01-0.05)	0.03 (0.02-0.05)	0.03 (0.01-0.05)	0.04 (0.02-0.06)**
NGAL (ng/ml)	34.2 (13.3-98.3)	32.4 (7.8-89.6)	18.3 (3.7-93.6)	18.3 (7.3-77.5)*
H-FABP (ng/ml)	1.6 (0.4-7.6)	1.5 (0.4-4.3)*	1.0 (0.4-4.6)	1.0 (0.4-4.1)

Medians (IQR). *p<0.05, ** p<0.001 vs fresh

Specific storage protocols (adding protease inhibitors, and alkalizing before and after storage) could not prevent these effects. One year frozen storage at -20 °C yielded essentially similar results. Importantly, after frozen storage all marker concentrations remained significantly associated with the values measured in fresh urine samples (all p<0.001).

Conclusions: Renal damage markers should preferably be measured in fresh urine samples. Marker studies using frozen urine samples should be interpreted with caution.

TH-PO254

The Association of Circulating Fetuin-A with Incident Cardiovascular Disease Differs by Diabetes Status in Community-Living Older Persons: The Cardiovascular Health Study Majken K. Jensen,[1] Traci M. Bartz,[2] Kenneth J. Mukamal,[3] Luc Djousse,[4] Jorge R. Kizer,[5] Russel Tracy,[6] Eric B. Rimm,[1] David Siscovick,[2] Michael Shlipak,[7] Joachim H. Ix.[8] *[1]HSPH, MA; [2]University of Washington, WA; [3]Beth Israel Deaconess Medical Center, MA; [4]Brigham and Women's Hospital, MA; [5]Cornell University, NY; [6]University of Vermont, VT; [7]UCSF, CA; [8]UCSD, CA.*

Background: Fetuin-A inhibits arterial calcification and insulin activity. High fetuin-A levels associate with diabetes, whereas low levels are associated with CVD in ESRD patients. The association with incident cardiovascular disease (CVD), and the potential dependence on prevalent diabetes in non-ESRD is less certain.

Methods: 3,718 participants aged >65 years, free of CVD in 1992, followed for incident CVD (MI, stroke, or CVD death) through 07/2008.

Results: Mean age was 76 years, and mean eGFR was 74 ml/min/1.73m². 1,415 participants had a CVD event. The association of fetuin-A with CVD was modified by diabetes (p interaction=0.02). In participants without diabetes the highest risk was observed for the lowest fetuin-A levels. The adjusted hazard ratio (HR) per each SD higher fetuin-A (0.1 g/L) was 0.94 (0.89-1.01). In contrast, fetuin-A was not associated with CVD in those with diabetes (table).

Table. Association of Fetuin-A Quartiles with Incident Cardiovascular Disease Events in Older Participants without ESRD, Stratified by Diabetes: The Cardiovascular Health Study

Without diabetes, n=3224	Q1	Q2	Q3	Q4	Per SD
HR's*	1 (ref)	0.88 (0.75,1.03)	0.90 (0.76,1.06)	0.91 (0.77,1.08)	0.94 (0.89,1.01)
With diabetes, n=494					
HR's*	1 (ref)	1.37 (0.91,2.05)	1.16 (0.75,1.79)	1.34 (0.88,2.03)	1.06 (0.92,1.22)

*Adjusted for: age, sex, race, center, physical activity, smoking status, use of hormone therapy (women), alcohol, HTN, eGFR, CRP, BMI, triglycerides, HDL, LDL

Among persons without diabetes, similar effect modification was observed by obesity (p=0.2) and in those with HOMA below the median(p=0.02), where fetuin-A was associated with CVD in the non-obese or low HOMA subgroup (HR's per SD=0.92 [0.86-0.98] and 0.87 [0.79-0.96]).

Conclusions: Insulin resistance/diabetes modifies the association of fetuin-A with risk of CVD in older individuals.

Funding: Other NIH Support - The National Heart Lung and Blood Institute (NHLBI) R01 HL094555. The Cardiovascular Health Study was supported by contract numbers N01 HC-85079 through N01HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01 HC-75150, N01 HC-54133, N01-HC-80007 and grant number U01 HL080295 from the NHLBI, with additional contributions from the National Institute of Neurological Disorders and Stroke.

TH-PO255

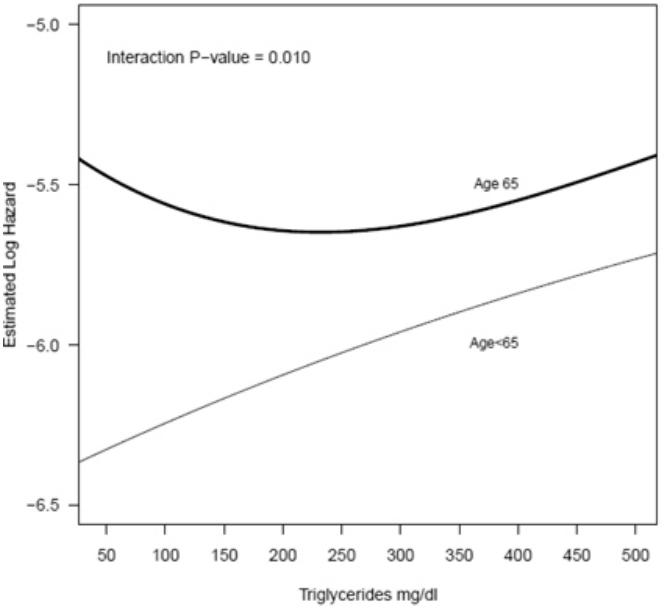
Triglycerides and All-Cause Mortality in Non-Dialysis Dependent Chronic Kidney Disease Sankar D. Navaneethan,[1] Jesse D. Schold,[1,2] Susana Arrigain,[2] Stacey Jolly,[3] John W. Sharp,[2] Anil K. Jain,[3] James F. Simon,[1] Emilio D. Poggio,[1] Titte Srinivas,[1] Martin J. Schreiber,[1] Joseph V. Nally.[1] *[1]Nephrology & Hypertension, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]Medicine, Cleveland Clinic.*

Background: Elevated triglyceride (TG) level is associated with cardiovascular and all-cause mortality in the general population. The association between TG levels and all-cause mortality among chronic kidney disease (CKD) patients is unclear.

Methods: Patients with stage 3 and stage 4 CKD patients who had TG levels measured after the diagnosis of CKD in our health system were included. We examined the associations of TG levels as categorical and continuous variables with all-cause mortality among CKD patients using logistic regression, Cox-proportional hazard models and Kaplan-Meier survival curves.

Results: Out of 23,592 CKD patients, 37.1% (n=8735) had TG levels ≥150 mg/dl. Presence of diabetes, hypertension, obesity and lower eGFR were associated with TG levels ≥150 mg/dl while increasing age, male gender (odds ratio [OR] 0.87, 95% CI 0.82, 0.92), and African American race (OR 0.40, 95% CI 0.37, 0.44) were associated with

lesser risk of ≥150 mg/dl. Kaplan-Meier survival plot did not show significant differences in all-cause mortality in the different TG groups. However, after covariate adjustment, each unit in log transformed TG levels was associated with a 10% increased risk (95% CI 1.01, 1.20) for death. Age modified the association between TG levels and mortality with patients <65 years having 24% higher risk of death (95% CI 1.07, 1.44) and ≥65 years with no increased risk for death.



Conclusions: Elevated TG may be a modifiable risk factor for all-cause mortality in patients <65 years but not in older CKD patients. Future studies should explore the associations of TG with cause-specific mortality in younger CKD patients with high triglyceride levels.

Funding: Other NIH Support - National Institutes of Health, the National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990.

TH-PO256

Chronic Kidney Disease and Fracture Risk in Children and Young Adults: A Population-Based Study Using the Health Improvement Network Database

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Background: Chronic kidney disease (CKD) may impair bone accrual and is associated with increased fracture risk in older adults. Fracture risk in children and young adults with CKD has not been assessed. The objective was to determine if CKD is associated with greater risk of fracture in this population.

Methods: In a retrospective cohort study using The Health Improvement Network (THIN) Database, subjects with CKD stages 3-5, diagnosed at ≤ 30 years, were identified using serum creatinine values or previously validated diagnosis codes. All sex- and age-matched subjects from a practice with a CKD subject were included for comparison. Cox regression analysis was used to estimate the hazard ratio (HR) for first fracture.

Results: 2,649 subjects with CKD (51% female) and 1,055,056 subjects without CKD (52% female) were identified. 185 first fractures (15/1,000 person-years) occurred in CKD subjects CKD vs 66,982 (9/1,000 pyears) in non-CKD subjects over a median follow-up time of 4 and 5 years, respectively. Overall, the HR for fracture in males vs females was 2.0 (1.97, 2.03), and the effect of CKD differed significantly by sex (interaction p<0.001).

HR for Fracture CKD vs Non-CKD

Age	Male	Female
0-9	1.21 (0.60, 2.41)	1.92 (0.96, 3.85)
10-14	0.70 (0.35, 1.40)	1.25 (0.52, 3.01)
15-19	0.88 (0.49, 1.59)	2.30 (1.09, 4.83)
20-29	1.00 (0.67, 1.48)	3.17 (2.30, 4.38)
30+	1.68 (1.22, 2.32)	2.47 (1.81, 3.39)

Forearm/wrist/hand fractures were the most common site in both groups, but the distribution of site differed in CKD vs non-CKD subjects. Hip fractures comprised a greater proportion of fractures in CKD (3.8% vs 0.9%, p=0.002), while skull and facial fractures represented a greater proportion in non-CKD (1.6% vs 6.7%, p=0.003).

Conclusions: Early onset CKD was associated with significantly increased risk of fracture in adolescent and young adult women. In males, early onset CKD was associated with increased risk of fracture only after age 30.

Funding: NIDDK Support, Other NIH Support - Center for Pediatric Clinical Effectiveness, The Children's Hospital of Philadelphia

TH-PO257

Longitudinal Classification of Chronic Kidney Disease Biomarkers and Their Association with Coronary Artery Calcium: The Spokane Heart Study

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Background: Kidney function decline, marked by decreasing levels of estimated glomerular filtration rate (eGFR) and increasing levels of serum phosphorus, is associated with coronary artery calcification (CAC), but the dynamic nature of this relationship is unclear. The study aim was to examine distinct trajectory classes of serum phosphorus levels, controlling for eGFR, that predict CAC.

Methods: The Spokane Heart Study is a longitudinal prospective cohort study of community-dwelling adults (n=721). Phosphorus and eGFR were classified as a combined biomarker variable via finite growth mixture modeling. Pre-specified variables included: high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, smoking status, systolic and diastolic blood pressure, body mass index, diabetes status, age, and gender. The primary outcome was CAC as both binary (i.e., onset) and continuous (i.e., accumulation) variables. 2-part latent growth curve modeling was the main statistical method used.

Results: Finite growth mixture modeling identified 4 distinct classes of phosphorus trajectories (low to high). Membership in 1 phosphorus trajectory class versus the next highest level predicted an increase in the probability of a positive CAC scan (β=0.26; 95% CI: 0.14-0.38, p<.001) and the amount of CAC burden (β=0.50; 95% CI: 0.38-0.61, p<.001) while controlling for other covariates. The magnitude of this finding is similar to those of major risk factors for cardiovascular disease (CVD), including effects of age (β=0.28; 95% CI: 0.16-0.40, p<.001) and gender on CAC onset (β=-0.34; 95% CI:-0.45- -0.25, p<.001), and gender's impact on CAC accumulation (β=-0.33; 95% CI:-0.44- -0.23, p<.001).

Conclusions: Classification of serum phosphorus into time-dependent trajectories, even within the normative range, indicates a graded association with CAC. Identification of such high-risk individuals could be useful for participant selection in clinical trials assessing effects of phosphorus lowering on CVD.

Funding: Private Foundation Support

TH-PO258

Risk of Retinopathy among Individuals with Evidence of Kidney Damage or Dysfunction

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Background: Both chronic kidney disease (CKD) and retinopathy involve pathogenic changes in microvasculature and are strongly associated with diabetes and hypertension. We examined whether albuminuria and kidney dysfunction are associated with excess risk of retinopathy, beyond that conferred by shared risk factors, in the adult U.S. population.

Methods: Participants ≥40 years (*n*=5119) from the 2005-2008 National Health and Nutrition Examination Survey with complete retinal imaging data and information on diabetes (self-report or glycohemoglobin≥6.5%); hypertension (self-report or measured blood pressure ≥140/≥90 mmHg, in the absence of diabetes); albuminuria [urinary albumin:creatinine ratio 30-299 (microalbuminuria) or ≥300 (macroalbuminuria) mg/g]; and kidney dysfunction [estimated GFR <60 (reduced kidney function) or >120 (hyperfiltration) ml/min/m^2] were examined. Multivariable logistic regression with stratification by diabetes and hypertension was used to assess the independent association of albuminuria and kidney dysfunction with retinopathy (defined as any type of retinopathy, scored by two independent graders, in either eye).

Results: Retinopathy was common, with a prevalence of 9.6% overall and 14.5% in those with either albuminuria or kidney dysfunction. Macroalbuminuria, but not microalbuminuria or kidney dysfunction, was associated with retinopathy among persons with diabetes (OR=2.94, 95% CI 1.51-5.69), after adjustment for demographics, glycohemoglobin, blood pressure, and smoking. Among those with hypertension alone, the association was similar but not statistically significant (OR=2.98, 95% CI 0.55-16.2). Neither albuminuria (*P*=0.79) nor reduced kidney dysfunction (*P*=0.93) was associated with retinopathy among those without diabetes or hypertension.

Conclusions: Further research is needed to determine whether routine retinopathy screening based on the presence of chronic kidney disease markers alone is warranted in the general population.

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Clinico-Histological Examination of Elderly Nephropathy Patients over 65 Years Old

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Background: Even in Japan where the percentage of elderly patients requiring dialysis is increasing, the primary diseases causing nephropathy are often unclear. To examine the primary disease of elderly nephropathy patients over 65 years old would be useful for improving treatment.

Methods: A total of 503 patients aged over 65 year old who underwent renal biopsy between 1985 and 2010 at our institution were analyzed retrospectively, including 297 men and 206 women.

Results: Renal biopsy was performed for proteinuria of less than 3.5 grams daily (44.5%), nephrotic-range proteinuria over 3.5 grams daily (29.3%), acute kidney damage including rapidly progressive renal failure (19.9%), and chronic-progressive kidney disease (5.8%). Membranous nephropathy (MN) was the most frequent type of nephropathy (19.0%), followed by diabetic nephropathy (DN) (16.7%), IgA nephropathy (IgAN) (13.1%), hypertensive nephrosclerosis (9.1%), crescentic glomerulonephritis (CG) (7.6%), minimal change nephritic syndrome (5.7%), amyloidosis (5.6%), and focal segmental glomerulosclerosis (5.6%). In the 65 - 69 years old group, DM, IgAN, and MN were predominant, accounting for 18.0%, 17.0%, and 14.0%, respectively. In the 70 - 79 years old group, MN, DN and IgAN were predominant (22.4%, 14.2%, and 7.6%, respectively). In the over 80 years old group, CN was the most common diagnosis (20%). Nephrotic-range proteinuria was caused by MN (47.8%), MCNS (21.7%), and DN (8.7%).

Conclusions: MN and DN were the most frequent diagnoses in each group. Renal biopsy is useful for evaluation and treatment of elderly nephropathy patients.

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Depressive Symptoms, Cardiovascular Disease (CVD) Risk Factors, and Subclinical CVD among Individuals in the Chronic Renal Insufficiency Cohort (CRIC) Study Michael J. Fischer,[1] Neil Jordan,[3] Dawei Xie,[3] Virginia Ford,[3] Marie Krousel-Wood,[3] Manjula Kurella Tamura,[3] John W. Kusek,[2] Leigh Rosen,[3] Louise Frances Strauss,[3] Valerie L. Teal,[3] Kristine Yaffe,[3] Willem Johan Kop,[3] Neil R. Powe,[3] James P. Lash.[1] *[1]Jesse Brown VA/U.Illinois; [2]NIH/NIDDK; [3]CRIC Study Group.*

Background: Depression is associated with prevalent cardiovascular disease (CVD) and an increased risk of CVD-related events in adults with chronic kidney disease (CKD). However, the relationship between depression, CVD risk factors, and subclinical CVD is not known.

Methods: We conducted a cross-sectional analysis of depressive symptoms (DS) in adults at entry into CRIC Study. DS were assessed by the Beck Depression Inventory (BDI) and defined by a BDI score ≥11. CVD risk factors were ascertained by questionnaires and lab studies. In addition to self-reported CVD, subclinical CVD measures included left ventricular hypertrophy (LVH), coronary artery calcification (CAC) score >400, and ankle-brachial index (ABI) < 0.9. Logistic regression was used to assess the relation between DS and CVD.

Results: Among 3863 CRIC participants, 28.5% had DS. Self-reported CVD was found in 33.3% and subclinical CVD as follows: 53.6% LVH, 21.6% CAC >400, and 16.1% ABI < 0.9. Adults with DS had a greater burden of traditional CVD risk factors compared to those without DS, including a higher prevalence of diabetes, hypertension, hyperlipidemia, smoking, low eGFR, and elevated urine protein (all $p<0.05$). Control of blood pressure (<130/80 mmHg) and diabetes (HbA1c<7%) was less common in those with DS (both $p<0.05$). DS were also associated with the presence of non-traditional CVD risk factors such as lower serum hemoglobin and albumin, and higher serum phosphorus, parathyroid hormone, and c-reactive protein (all $p<0.001$). In analyses adjusted for sociodemographic and traditional CVD risk factors, DS were associated with self-reported CVD (OR 1.58; 95% CI: 1.32-1.90) and LVH (OR 1.24; 95% CI: 1.00-1.54) but not CAC >400 or ABI < 0.9.

Conclusions: In a large diverse CKD cohort, DS were associated with an adverse profile of traditional and non-traditional modifiable CVD risk factors, and increased odds of self-reported CVD and LVH.

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Low Ankle Brachial Index Is Associated with Rapid Glomerular Filtration Rate Decline Meredith C. Foster,[1] Nimrta Ghuman,[2] Shih-Jen Hwang,[1] Joanne Murabito,[1,2] Caroline S. Fox.[1,3] *[1]Framingham Heart Study, Framingham, MA; [2]Boston University School of Medicine, Boston, MA; [3]Harvard Medical School, Boston, MA.*

Background: A low ankle brachial index (ABI) is associated with increases in serum creatinine. We sought to investigate the association of ABI with the development of rapid kidney function decline or stage 3 chronic kidney disease (CKD).

Methods: Participants (n=2592, mean age 57 years, 54% women) attended Framingham Offspring Exams 6 (1995-98) and 8 (2005-08). Baseline ABI was classified into 3 groups: normal (>1.1 to <1.4; n=1719), low-normal (>0.9 to 1.1; n=822), and low (≤0.9; n=51). Glomerular filtration rate was estimated (eGFR) using the MDRD Study equation. Rapid eGFR decline was defined as eGFR decrease of ≥3mL/min/1.73m²/year. Incident stage 3 CKD was defined as eGFR<60mL/min/1.73m² at Exam 8 among those free of stage 3 CKD at baseline. Rapid eGFR decline and stage 3 CKD were modeled as functions of ABI using logistic regression, with multivariable (MV) adjustment for age, sex, eGFR, and standard CKD risk factors.

Results: Over 9.5 years of follow-up, 11.9% (n=309) experienced rapid eGFR decline. Compared to participants with a normal ABI, those with a low ABI had a 5.8-fold increased odds of rapid eGFR decline ($p<0.0001$), which persisted with further MV adjustment (Table, $p=0.0007$). Among those free of baseline stage 3 CKD, 9.0% (n=219) developed stage 3 CKD. After adjusting for age, sex and baseline eGFR, participants with a low ABI had a 2.9-fold increased odds of stage 3 CKD ($p=0.006$) when compared to those with a normal ABI. We observed some attenuation upon MV adjustment, although low ABI remained associated with a 2.1-fold increased odds developing stage 3 CKD (Table, $p=0.07$). Low-normal ABI was not associated with rapid eGFR decline or incident stage 3 CKD.

Table: Odds ratios (95% Confidence Intervals) of rapid eGFR decline & CKD

	Rapid eGFR Decline		Stage 3 CKD	
Covariates	Low-normal ABI	Low ABI	Low-normal ABI	Low ABI
Age, sex, baseline eGFR	1.3 (1.0-1.9)	5.8 (2.7-12.7)	1.3 (0.9-1.8)	2.9 (1.4-6.3)
Multivariable	1.3 (0.9-1.8)	4.3 (1.9-9.9)	1.2 (0.9-1.7)	2.1 (0.9-4.7)

Referent = Normal ABI

Conclusions: Low ABI is associated with an increased risk of rapid eGFR decline.

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Multiple Markers of Kidney Function Predict Mortality and End-Stage Renal Disease in the General Population: The Atherosclerosis Risk in Communities (ARIC) Study Brad C. Astor,[1] Tariq Shafi,[1] Ron C. Hoogeveen,[2] Christie Ballantyne,[2] Josef Coresh.[1] *[1]Johns Hopkins University; [2]Baylor College of Medicine.*

Background: Lower estimated GFR based on serum creatinine ($eGFR_{CKD-Epi}$) or cystatin C ($eGFR_{Cys}$) is associated with higher risk of death and ESRD. Beta-trace protein (BTP) and beta-2 microglobulin (B2M), two novel markers of kidney function, have also been shown to predict events. The prognostic utility of multiple markers of kidney function is undetermined.

Methods: We used data from 10,091 participants in the ARIC Study, a population-based cohort study of 4 US communities, to examine the prospective association between the number of markers indicating kidney dysfunction and death and ESRD over 11 years of follow-up. Kidney dysfunction was defined as $eGFR_{CKD-Epi}$ (n=684) or $eGFR_{Cys}$ (n=1,320) <60mL/min/1.73m² or the upper decile of BTP (n=1,001) or B2M (n=1,006).

Results: The number of markers indicating kidney dysfunction was strongly associated with risk of death and ESRD, even among those with $eGFR_{CKD-Epi}$ and $eGFR_{Cys}$ ≥60mL/min/1.73m². Results were similar after further adjustment for urinary albumin:creatinine.

Adjusted* Incidence Rate Ratio (95% CI) of Death and ESRD

	Death (n=1,675)			ESRD (n=168)		
Number of Markers	**Overall**	**$eGFR_{CKD-Epi}$≥60 mL/min/1.73m²**	**$eGFR_{CKD-Epi}$ and $eGFR_{Cys}$≥60 mL/min/1.73m²**	**Overall**	**$eGFR_{CKD-Epi}$≥60 mL/min/1.73m²**	**$eGFR_{CKD-Epi}$ and $eGFR_{Cys}$≥60 mL/min/1.73m²**
0	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0(Reference)	1.0(Reference)	1.0(Reference)
1	1.2 (1.0-1.4)	1.2 (1.0-1.4)	1.2 (1.0-1.5)	2.2 (1.2-4.2)	2.3 (1.2-4.6)	2.4 (1.1-5.3)
2	1.7 (1.4-2.0)	1.84 (1.5-2.3)	1.5 (0.9-2.5)	6.1 (3.3-11.4)	6.3 (3.0-13.3)	11.2 (3.8-32.9)
3	2.4 (2.0-3.0)	2.7 (2.1-3.4)	X	8.4 (4.4-16.0)	7.4 (3.2-17.1)	X
4	2.9 (2.4-3.5)	X	X	49.9 (32.5-73.8)	X	X
p-trend	<0.001	<0.001	0.01	<0.001	<0.001	<0.001

* Adjusted for age, sex, race, prevalent coronary heart disease, diabetes, systolic blood pressure, antihypertensivie medication use, current smoking, LDL and HDL cholesterol, and triglycerides

Conclusions: Using multiple markers of kidney function may provide the most accurate prognostic information in the general population. Studies should explore the optimal method for combining information from multiple markers.

Funding: NIDDK Support

TH-PO263

A Study of the Association between Cinacalcet Adherence and Biochemical Outcomes Andrew Lee,[1] Vasily Belozeroff,[1] Richard Mutell,[2] T. Christopher Bond,[2] William G. Goodman.[1] *[1]Amgen Inc; [2]DaVita Clinical Research.*

Background: Cinacalcet is used to treat secondary hyperparathyroidism in patients receiving dialysis. As with other oral medications, non-adherence may prevent patients from experiencing the full benefits of therapy.

Methods: This retrospective cohort study of prevalent hemodialysis patients (> 120 d since initial dialysis) in the DaVita Rx and Clinical Data Warehouse assessed the association of cinacalcet adherence with control of serum parathyroid hormone (PTH). The analysis included adult (≥ 18 years old) patients with at least 3 months of data prior to and 12 months (follow up period) after their initial cinacalcet prescription was filled by DaVita Rx between Jan 1 and Dec 31 2009. Patients who had clinically appropriate reasons for discontinuation (eg hypocalcemia, parathyroidectomy, successful kidney transplant) were excluded. Medication possession ratio (MPR) during the follow up period was used to categorize patients by cinacalcet adherence profile as non-adherence (NA): refill gap (RG) ≥ 180 days, low adherence (LA): RG < 180 days and MPR < 0.8, or high adherence (HA): RG < 180 days and MPR ≥ 0.8. Controlled PTH was defined as PTH < 600 pg/ml. We calculated percent of follow up period months with controlled PTH (dependent variable) by adherence category and ran a generalized linear model (GLM) to examine the association while controlling for patient characteristics and co-morbidities.

Results: A total of 2367 patients met the study criteria: 847 NA, 320 LA, and 1200 HA patients. Baseline demographic and clinical characteristics were similar across categories. Percent of months with controlled PTH were higher for HA patients (78.6; SD 28.3) compared to LA (77.1; SD 27.8) and NA (75.3; SD 29.8) patients. Adherence category was statistically significant ($p < 0.001$) in the GLM. When controlled PTH was defined as < 300 or 150-300 pg/ml instead of < 600 pg/ml, the observed patterns persisted but were smaller in magnitude.

Conclusions: Our study suggests that increased adherence to cinacalcet may be associated with improved control of PTH. This is important to consider in light of prior studies linking controlled biomarkers over time with improved survival.

Funding: Pharmaceutical Company Support

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Comparison of CVD Prevalence between Japanese and American CKD Patients (Collaboration between CKD-JAC and CRIC) Takayuki Hamano,[1] Enyu Imai,[2] Kosaku Nitta,[2] Hirofumi Makino,[2] Akira Hishida,[2] Lisa C. Nessel,[1] Elsayed Z. Soliman,[3] Ana C. Ricardo,[3] Martin J. Schreiber,[3] Dawei Xie,[1] Harold I. Feldman.[1] *[1]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA; [2]CKD-JAC Investigators, Japan; [3]CRIC Investigators.*

Background: The DOPPS revealed that the prevalence of cardiovascular disease (CVD) and mortality are much higher in American hemodialysis patients compared to Japanese hemodialysis patients. However, international comparative studies of CVD have not been conducted in predialysis patients with chronic kidney disease (CKD).

Methods: We compared the baseline prevalence of CVD combining the CRIC (Chronic Renal Insufficiency Cohort) and CKD-JAC (CKD-Japanese Cohort) studies, which consisted of 3939 and 2977 patients, respectively.

Results: After adjustment for age, sex, diabetes, and eGFR, the odds ratio (OR)(95% CI) for having prevalent CHF, MI, or stroke for Japanese, compared to American CKD patients, was 0.30(0.24-0.38), 0.40(0.35-0.47), and 1.02(0.85-1.23), respectively. Since the ascertainment processes for CVD at study entry were different between the studies (patient questionnaire in CRIC and medical chart in JAC), we studied the consistency between patient questionnaire and medical record-derived data using a sample of patients in one of the clinical centers of CRIC. Based on these data, we performed multiple imputation for those in CRIC with missing chart-based prevalent CVD as a sensitivity analysis. The ORs for CHF, MI, and stroke comparing Japanese to American study participants were 0.41(0.19-0.90), 0.55(0.40-0.75), and 1.21(0.75-1.94), respectively. With regard to CHF, additional adjustment for HbA_1C or CRP levels eliminated the significance of lower OR for Japanese. However, even extensive adjustment for laboratory data did not eradicate the significantly lower rate of MI in Japanese.

Conclusions: American CKD patients have significantly higher prevalence of CHF and MI compared to their Japanese counterparts and a comparable prevalence of stroke after adjustment for age, sex, diabetes, and eGFR. These data will be the basis for future longitudinal comparisons of incident CVD.

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Differential Response in Serum Potassium, Albuminuria and Blood Pressure to ACEi or ARB Therapy in Individual Type 2 Diabetic Patients Sara S. Roscioni,[1] Dick de Zeeuw,[1] Giuseppe Remuzzi,[2] Philippe J. Vanhille,[3] Hans-Henrik Parving,[4] Hiddo Jan Lambers Heerspink.[1] *[1]Kidney Centre, University Medical Centre Groningen, Netherlands; [2]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [3]Dept of Nephrology, Hospital Valenciennes, Valenciennes, France; [4]Dept of Endocrinology, Rigshospitalet, Copenhagen, Denmark.*

Background: Treatment with angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB) reduces blood pressure (BP) and albuminuria (UACR) and increases serum potassium. The BP and UACR reduction affects the renal and cardiovascular (CV) outcome positively while the increase in potassium affects the renal and CV outcome negatively. Determining the net response to these offsetting effects in individual patients may offer unique predictive value for their ultimate renal/CV outcome.

Methods: We conducted a post-hoc analysis in four randomized controlled trials enrolling patients with type 2 diabetes at early (BENEDICT and IRMA-2) and late (RENAAL and IDNT) stage of renal disease. Regression analysis was performed between responses in serum potassium and responses in BP or UACR after 3 months of ACEi or ARB treatment.

Results: ACEi/ARB-induced responses in potassium did not correlate with responses in BP or UACR both in diabetic patients at early and late stage of renal disease (table1). Furthermore, the achieved month 3 potassium levels did not correlate with the respective achieved BP or UACR levels within the same patients (table1). The percentage of patients who developed hyperkalemia was independent of the response in BP or UACR.

Correlations (presented with R^2) between changes in (Δ) potassium and BP/UACR				
	Early stage reanl disease		Late stage renal disease	
	BENEDICT	IRMA-2	RENAAL	IDNT
ΔBP	0.012	0.032	0.000	0.003
ΔUACR	0.002	0.013	0.005	0.004
Correlations between achieved potassium and BP/UACR				
BP	0.001	0.008	0.000	0.003
UACR	0.002	0.014	0.000	0.001

Conclusions: The response in serum potassium to ACEi or ARB therapy does not correlate with the response in BP or UACR. As the response in each of these parameters may affect long-term renal/CV outcome, our data support the need to monitor and optimize the patient response in each parameter to improve long-term renal and CV protection.

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Age Modifies the Prognostic Role of Main Risk Factors for ESRD and Death in Non-Dialysis CKD Patients under Nephrology Care. The TABLE Prospective Cohort Study Luca De Nicola,[1] Paolo Chiodini,[2] Silvio Borrelli,[1] Ciro Gallo,[2] Giuseppe Conte,[1] Roberto Minutolo.[1] *[1]Nephrology, Second University, Naples, Italy; [2]Medical Statistics, Second University, Naples, Italy.*

Background: Prevalence of elderly CKD patients in nephrology clinics is growing. However, how age might affect the prognostic role of the commonly reported risk factors in CKD is undefined.

Methods: We prospectively followed, from 2003 to death or May 2011, 1248 adult patients with CKD stage 3-5 attending 25 Italian outpatient nephrology clinics for ≥12 months before enrolment. Primary endpoints were ESRD and death. We estimated rates of ESRD and death (per 100 patient-year) by age, and tested interactions between age and a number of baseline risk factors (gender, BMI, diabetes [DM], cardiovascular disease [CVD], smoking, systolic blood pressure, hemoglobin, phosphate, cholesterol, uric acid, GFR, 24h proteinuria [Uprot]) in Cox models stratified by center. ESRD was entered as time-dependent covariate when death was assessed.

Results: 481 (38%) patients were less than 65 yrs old, 410 (33%) patients were between 65 and 75 yrs and 357 (29%) patients were over 75 yrs. Within each class, GFR values were equal to 31±15 mL/min/1.73m^2, 32±14, and 29±13, respectively. Rates of ESRD and death were respectively 9.0 (95% confidence interval [CI] 7.8-10.4) and 1.74 (95% CI 1.3-2.3) in <65 yrs, 7.3 (6.1-8.8) and 6.1 (5.1-7.4) in 65-75 yrs, and 7.9 (6.4-9.8) and 14.5 (12.6-16.7) in over 75 yrs. In the final models, age interacted significantly with CVD (P=0.032), GFR (P=0.011), and Uprot (P<0.001) for ESRD risk, and with CVD (P=0.025) and DM (P=0.004) for death risk. Prognostic effect of CVD and DM decreased with older age, while lower GFR and higher Uprot increased the risk of ESRD with advancing age. Male gender, lower BMI, and higher phosphate increased the risk of ESRD independently of age, whereas lower GFR was an independent risk factor for death (P<0.005 for all).

Conclusions: In CKD patients regularly followed in nephrology clinics, age not only is a strong modifier of prognosis but it also selectively changes the prognostic role of other main risk factors. These results may help in identifying the elderly patients at higher risk.

TH-PO267

Prevalent Use of Dietary Supplements Potentially Harmful in Chronic Kidney Disease in the United States Vanessa Grubbs,[1,2] Laura C. Plantinga,[1] Delphine S. Tuot,[1] Elizabeth Hedgeman,[3] Rajiv Saran,[3] Sharon Saydah,[4] Deborah Rolka,[4] Neil R. Powe.[1,2] *[1]University of California, San Francisco; [2]San Francisco General Hospital; [3]University of Michigan; [4]Centers for Disease Control and Prevention.*

Background: The National Kidney Foundation (NKF) identifies 39 herbs that may be harmful in the setting of chronic kidney disease (CKD) (http://www.kidney.org/atoz/content/herbalsupp.cfm), but the prevalent use of such herbs in the U.S. by CKD status is unknown.

Methods: Using 1999-2008 National Health and Nutrition Examination Survey data, we examined the reported use of dietary supplements in the past 30 days among 21,169 non-pregnant adults (age 20+ years). CKD stage 1/2 was defined by urinary albumin:creatinine ratio of ≥30 mg/g with eGFR ≥60 ml/min/1.73 m2 and CKD stage 3/4 by eGFR 15-59 ml/min/1.73 m2. Any dietary supplement containing at least one NKF-identified herb was defined as potentially harmful. The prevalence and odds of taking a potentially harmful supplement by CKD status were estimated via multivariable logistic regression weighted to the U.S. population.

Results: While an estimated 52.4% of participants reported taking any dietary supplement, the supplement was potentially harmful among 15.3%. The crude estimated prevalence of those taking a any dietary supplement increased with greater CKD severity (no CKD 51.4%; CKD stage 1/2 49.1%; CKD stage 3/4 65.8%, p<0.001), but decreased among those taking a potentially harmful supplement (16.1%, 13.0%, and 10.0%, respectively, p<0.001). However, after adjustment for demographics, co-morbid disease, and healthcare visits, CKD status was not a significant determinant of taking any supplement (stage 1/2 0.95, 0.82-1.10; stage 3/4 OR 1.07, 0.93-1.23, vs. no CKD) or a potentially harmful supplement (stage 1/2: OR 0.97, 0.73-1.28; stage 3/4: OR 0.90, 0.67-1.21, vs. no CKD).

Conclusions: The use of dietary supplements potentially harmful in CKD is common and, as use is not statistically different by CKD status, patients with CKD may be unaware of risk. Health care providers, too, may be unaware of potentially harmful supplements and that patients with CKD are taking them. Further research and education are warranted.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO268

Risk Assessment of CKD-EPI Equation Compared with That Based on MDRD Study Equation in the Japanese General Population Masaki Ohsawa. *Department of Hygine and Preventive Medicine, Iwate Medical University, Morioka, Japan.*

Background: Estimated glomerular filtration rate (eGFR ml/min/1.73m^2) calculated by CKD-EPI equation corresponds to the actual measured value more accurately than those estimated by MDRD equation. However, comparative studies between those equations have not been examined based on longtudinal studies in the Japanese general population.

Methods: A prospective cohort study of 25,354 participants was carried out. Participants were divided into ten groups according to eGFR and urinary albumine creatinine ratio (UACR). Sex- and age-adjusted mortality rates (/1000 pys) were calculated using Poisson regression model (see table).

Results: The results are shown in the table.

Numbers of death (crude mortality rates) and sex- and age-adjusted mortality rates (95% CI)

groups stratified by eGFR		eGFR≥90	60≤eGFR<90	45≤eGFR<60	30≤eGFR<45	eGFR<30
estimated by CKD-EPI equation						
subjects (n)		2570	21246	1329	168	41
No. of death (crude mortality)		27 (1.88)	754 (6.34)	112 (15.5)	29 (32.3)	10 (43.3)
adjusted mortality (95%CI)	all subjects	6.92 (4.25-9.58)	3.06 (2.69-3.43)	3.38 (2.57-4.18)	6.18 (3.72-8.64)	9.42 (3.38-15.5)
	ACR<30	4.76 (2.31-7.21)	2.89 (2.51-3.26)	2.96 (2.06-3.87)	4.48 (1.08-7.88)	0.00
	ACR≥30	13.2 (5.38-21.1)	3.74 (3.11-4.38)	4.44 (2.96-5.92)	8.31 (4.49-12.1)	11.7 (4.17-19.2)
estimated by MDRD equation						
subjects (n)		4581	17653	2827	250	43
No. of death (crude mortality)		116 (4.48)	620 (6.28)	151 (9.72)	34 (25.3)	11 (46.6)
adjusted mortality (95%CI)	all subjects	4.14 (3.36-4.92)	3.09 (2.70-3.47)	3.12 (2.49-3.75)	5.91 (3.73-8.09)	10.6 (4.12-17.1)
	ACR<30	3.59 (2.70-4.48)	2.92 (2.53-3.32)	2.74 (2.04-3.43)	4.61 (1.67-7.55)	0.00
	ACR≥30	5.61 (3.98-7.24)	3.72 (3.05-4.39)	4.05 (2.86-5.23)	7.77 (4.30-11.2)	13.0 (5.04-21.0)

Mortality rates are expressed as /1000 person-years.

Conclusions: Classifications by eGFR alone showed undistinguished elevated risks for death among participants with mildly to moderately reduced eGFR (30≤GFR<60) either in estimation by CKD-EPI or estimation by MDRD equation. Combined classification of eGFR and UACR predicted high risks for death among participants with elevated eGFR (GFR≥90) and mildly reduced eGFR (45≤GFR<60), especially in estimation by CKD-EPI equation.

Funding: Government Support - Non-U.S.

TH-PO269

The Relationship between Minnesota Code Fndings and CKD: Result from the CKD-JAC Study Satoshi Iimuro,[1] Takeo Okada,[2] Enyu Imai,[2] Kosaku Nitta,[2] Tsuyoshi Watanabe,[2] Seiichi Matsuo,[2] Tadao Akizawa,[2] Hirofumi Makino,[2] Yasuo Ohashi,[1] Akira Hishida.[2] *[1]University of Tokyo, Japan; [2]CKD-JAC Study Group.*

Background: The Japan CKD cohort study (CKD-JAC) was established in September 2007 to prospectively study the renal and cardiovascular outcomes in 2,977 Japanese patients with CKD stage 3-5 visiting 17 outpatient clinics in Japan. To evaluate the electrocardiogram (ECG) findings at enrollment in the study, ECG waveforms obtained from 1,536 patients were analyzed according to the Minnesota Code.

Methods: Two experts independently coded the waveforms using the Minnesota code. In the cases where the two had different opinion in coding a waveform, another expert joined to consult and decide which code to apply. Only the major classification codes and lead information were subjected to the analysis.

Results: Patients' characteristics were: mean age, 61.9 (±11.2) years; diabetes, 41.7%; hypertension, 84.6%; CKD stage 3, 41.8%; CKD stage 4, 41.3% and CKD stage 5, 16.9%. Patients without ECG abnormalities (Code1-0-0) accounted only for 20.2%. The common findings were High Amplitude R waves (16.7%), ST abnormalities (16.5%), and T-wave abnormalities (26.4%). When stratified by CKD stage, these wave abnormalities increased as the CKD stage advanced. When stratified by diabetes, the ratio of ST or T-wave abnormalities increased in patients with diabetes but that of High Amplitude R waves didn't increase. Regarding number of leads, only 1-lead abnormality was most common in patients with High Amplitude R waves (84%). ST or T-wave abnormalities were found more commonly in I, aVL, V5 and V6 leads than in II, III, or aVF lead. Number of leads with abnormal findings tended to increase as CKD stage advanced. On the other hand, the number of leads classified to St or T-wave abnormalities decreased in patients without diabetes but with glomerulonephritis.

Conclusions: In this report, we studied the Minnesota code findings in CKD patients. The findings well reflected CKD stage and patients' characteristics, which suggests that ECGs contain a host of clinical information. This report will be valuable basic data for epidemiologic study.

Funding: Pharmaceutical Company Support

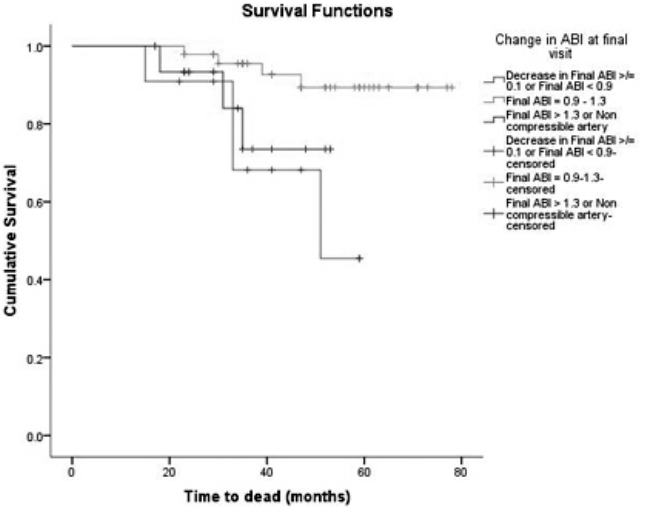
TH-PO270

Change in Ankle-Brachial Indices over Time and Mortality in Diabetics with Proteinuria Sirin Jiwakanon,[1,2] Sharon G. Adler,[1] Rajnish Mehrotra.[1] *[1]Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA; [2]Medicine, Hatyai Hospital, Hatyai, Songkhla, Thailand.*

Background: Peripheral vascular disease is common in diabetic chronic kidney disease (CKD) and is characterized either by abnormally low or high ankle-brachial index (ABI). Whether low or high ABI and the direction of change over time carry similar prognostic value is currently unknown.

Methods: The association of ABI with all-cause mortality over 40 ± 21 months (mean ± SD) was tested in a prospective cohort of 167 individuals with diabetic CKD (age, 57 ± 7 years; median urine protein-creatinine, 2.5 g/g; estimated glomerular filtration rate, 58 ± 23 ml/min/1.73 m2). Association of change in ABI with all-cause mortality was determined in the sub-group of 75 subjects with normal ABI at baseline.

Results: At baseline, 41% had an abnormal ABI:< 0.9, 18%; > 1.3 or non-compressible arteries, 23%. Upon follow-up, 43 subjects died: 0.9-1.3, 18%; < 0.9, 40%; and > 1.3 or non-compressible, 34%. Only individuals with low ABI had a significantly higher risk for all-cause mortality (hazards ratio (95% confidence interval), HR: 2.23 (1.07, 4.65)). In subjects with initially normal ABI, vascular disease worsened over 23 ± 6 months in 39%; 17% had a decrease in ABI by ≥ 0.1 or a final ABI < 0.9, and 21% had a final ABI > 1.3 or non-compressible arteries. Previous cardiovascular disease was the only significant predictor of decline in ABI. Over the subsequent 21 ± 16 months, 15% died. However, only individuals who had a declining had a significantly higher risk for death (adjusted HR, 7.41 (1.63, 33.65)).



Conclusions: Peripheral vascular disease is common and progresses rapidly in proteinuric diabetics. Low or declining ABI is a strong predictor of all-cause mortality. Routine measurement of ABI is a simple bed-side procedure that can permit easy risk-stratification in diabetic CKD patients.

Funding: Other NIH Support - NCRR

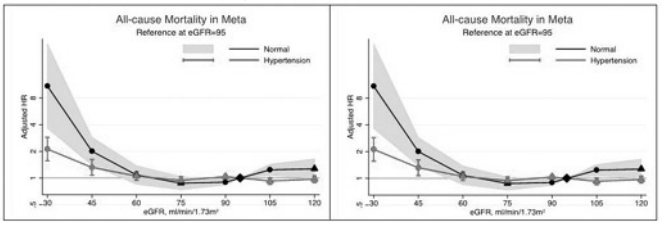
TH-PO271

Assessing Interaction on GFR-Risk Association in Meta-Analyses: An Example of Hypertension in the CKD Prognosis Consortium B. Khan Mahmoodi, Kunihiro Matsushita, Yingying Sang, Morgan E. Grams, Brad C. Astor, Mark Woodward, Ron T. Gansevoort, Josef Coresh. *Dep. Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Background: Meta-analysis methods for evaluating interactions are not well described. Assessing interactions with eGFR is particularly challenging due to its non-linear association with risk. We describe methods to evaluate point-wise and overall interactions using an example of hypertension–eGFR interaction on mortality.

Methods: To test the applicability of our methods, we selected nine of 46 cohorts joining CKD Prognosis Consortium. In each study eGFR linear splines (knots at each 15 from 15 to 105 ml/min/1.73m2) and their product terms with hypertension were fitted, providing hazard ratios (HRs) for eGFR (vs. eGFR 95) in both hypertensive and non-hypertensive groups. From this model, the interaction was evaluated as the relative HR (rHR) in hypertension vs. non-hypertension per 1 increment of eGFR from 15 to 120 (point-wise interaction). HRs and rHRs for each eGFR value in each cohort were pooled using random effects models. The overall interaction was assessed as the inverse-variance average of difference in all spline coefficients between hypertensive vs. non-hypertensive groups.

Results: There were 47,893 participants and 7,468 deaths. Low eGFR was associated with increased mortality in the hypertensive (gray line) and non-hypertensive (black line) groups (figure). As depicted by significant rHRs (dashed line), significant interactions by hypertension status were observed at eGFR ranges of 30-40, 85-90, and 100-115. However, the overall interaction was not significant (P=0.44).



Conclusions: We demonstrated meta-analytic methods to assess point-wise and overall interactions in non-linear regression models. Interactions should be tested using both methods and interpreted based on biological and clinical relevance.

Funding: Private Foundation Support

TH-PO272

Dietary Protein Intake in Chronic Kidney Disease from the National Health and Nutrition Examination Survey Linda W. Moore,[1] Laura Byham-Gray,[2] James S. Parrott,[2] Diane Radler,[2] Stephen L. Jones,[1] Sreedhar A. Mandayam,[3] William E. Mitch,[3] A. Osama Gaber.[1] *[1]Surgery, The Methodist Hospital, Houston, TX; [2]SHRP-Nutrition, UMDNJ, Newark, NJ; [3]Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Dietary protein intake (DPI) is important for metabolic balance and tissue maintenance. Dietary guidelines from the Institute of Medicine (IOM) recommend a DPI that is adequate for 97-98% of the healthy adult population. This amount, 0.8g/kg/day and the estimated average requirement (EAR) 0.66g/kg/day, fulfill their criteria. The Kidney Disease Outcomes Quality Initiative (KDOQI) for Nutrition recommends 0.6-0.75g/kg/day for stage 4 CKD. We evaluated the DPI of people across CKD stages for differences from IOM and KDOQI Guidelines and from DPI of adults without CKD.

Methods: Data from NHANES 2001-2008 were analyzed for the presence of CKD (MDRD formula, staged according to NKF guidelines). DPI was assessed from 24-hr recall, systematically collected by trained interviewers using the Automated Multiple Pass Method. Records from each of the 7 days/week were seasonally obtained. Complex survey analyses were used to report population estimates of CKD, and DPI at each stage of CKD and those who did not have evidence of CKD (NoCKD, no proteinuria, eGFR ≥60).

Results: Of 41,658 NHANES participants, 16,872 (40.5%) were ≥20 years of age and a CKD stage was established. 70% of NoCKD and 60% of stages 1-3 had DPI above the estimated average, while DPI of 50% of those in stages 4&5 (not receiving dialysis) exceeded the KDOQI guidelines. Using DPI of NoCKD as the comparator (mean±SE, 1.34±0.01g/kg/d), lower DPI was reported by adults at stage 2 CKD (1.27±0.03, p=0.0008), stage 3 CKD (1.14±0.2, p<0.0001), and stages 4&5 CKD (not receiving dialysis; 1.04±0.05; p<0.0001). Results from 10% of NoCKD and 20% of stages 2-5 (not yet receiving dialysis) had DPI below IOM or KDOQI Guidelines.

Conclusions: DPI of adults with CKD differed significantly from NoCKD. From stage 2-5 CKD, DPI decreased, but at each stage, the level exceeded IOM or KDOQI Guidelines; only 20% were at risk of eating an inadequate DPI. The majority of adults with or without CKD consume excess protein.

TH-PO273

Screening for Early Impairment of Glomerular Filtration Rate by Means of Urinary Beta-Trace Protein Carlo Donadio, Danika Tognotti, Angeliki Kanaki, Elena Donadio. *Internal Medicine, Nephrology, University of Pisa, Italy.*

Background: The screening for chronic kidney diseases (CKD) patients with impaired GFR needs the measurement of serum creatinine (SCr) or cystatin C (SCys).

Methods: Aim of this study was to evaluate the possibility to screen patients with a GFR < 90 mL/min/1.73 m², by means of serum levels and urinary excretion of different low molecular weight proteins (LMWP), cystatin C (Cys), β2-microglobulin (β2M), retinol-binding protein (RBP), beta-trace protein (BTP), and by means of the derived prediction equations for GFR.

Results: Two hundred ninthy-five adult CKD patients, with various degree of impairment of renal function (SCr 0.40-12.1 mg/dL), were examined.

GFR was measured, with a radio-isotopic method, as the renal clearance of ^{99m}Tc-DTPA.

Creatinine, beta-trace protein, cystatin C, β2-microglobulin, retinol-binding protein, and of urinary albumin were measured with standard laboratory methods.

In the 295 CKD patients (females 137), at all CKD stages, a very high correlation was found between GFR (^{99m}Tc-DTPA), and serum Cr, Cys, β2M, and BTP. All these serum markers showed a similar accuracy as indicators of different GFR impairments. RBP had the lowest correlation with GFR, and was also significantly less accurate. The different prediction formulas derived from gender, anthropometric data and SCr or S-LMWP had a diagnostic accuracy similar to that of serum Cr, Cys, β2M, and BTP. Urinary albumin was inadequate as indicator of any level of GFR impairment. Urinary excretion of Cys and β2M increased significantly only in patients with GFR<30 ml/min/1.73m², while urinary BTP increased already at GFR<90 ml/min/1.73m². In this selected group of CKD patients, the positive predictive value of urinary BTP for GFR<90 ml/min/1.73m² was 85%, indicating that, in CKD patients, a urine-based test can predict a slight GFR impairment.

Conclusions: In conclusion, serum levels of LMWP are not more sensitive or accurate than serum creatinine as indicators of GFR impairment. Urinary BTP had a positive predictive value of 85% for GFR<90 ml/min/1.73m², indicating that, in CKD patients, a urine-based test can predict even a slight GFR impairment.

Funding: Government Support - Non-U.S.

TH-PO274

Daily Salt Intake Is Associated with Proteinuria and the Systolic Blood Pressure in Japanese Female Patients with Chronic Kidney Disease Hiroshi Morinaga, Hitoshi Sugiyama, Masashi Kitagawa, Keiichi Takiue, Yoko Kikumoto, Ayu Ogawa, Tatsuyuki Inoue, Shinji Kitamura, Shigeru Akagi, Yohei Maeshima, Hirofumi Makino. *Okayama University Graduate School, Okayama, Japan.*

Background: There is a considerable body of literature linking a higher salt intake with higher blood pressure (BP) and increased cardiovascular risk. However, the effects of dietary salt intake on proteinuria, BP and the progression of chronic kidney disease (CKD) are still uncertain. Moreover, few studies have based their assessments on 24 hour urinary sodium excretion, which is the most accurate method for assessing salt intake.

Methods: A total of 159 Japanese outpatients with CKD (mean age: 54 years, males: 50.3%, chronic glomerulonephritis: 60.4%) who underwent 24 hour urine collection in 2007 were enrolled and retrospectively followed-up until 2010. Their baseline salt intake was estimated by the measurement of urinary sodium excretion in 24 hour pooled urine. The predictors for either the doubling of serum creatinine or the introduction of renal replacement therapy were determined.

Results: The median follow-up period was 36 months, during which time, 16 patients reached the renal composite endpoint (10.1%). A significant correlation between the baseline systolic BP and estimated daily salt intake was detected in both all individuals (r=0.260; P<0.01) and in female subjects (r=0.331; P<0.01). A similar correlation was found between common logarithm of the baseline urine protein excretion and daily salt intake in the total (r=0.184; P<0.05) and in the female (r=0.277; P<0.05) subjects. In the Cox regression analysis, the daily salt intake was independently associated with the endpoint, but the risk was not independent of the urinary protein excretion.

Conclusions: In patients with CKD, salt intake was found to be significantly associated with higher systolic BP and proteinuria, especially in female subjects, and it was also associated with renal dysfunction. These findings indicate that salt restriction may have a beneficial effect on BP, proteinuria and the renal outcome in CKD patients. Prospective trials should be performed to confirm the effect of sodium restriction on such patients.

TH-PO275

Initial Reduction in Albuminuria Should Be Sustained over Time To Achieve Optimal Renoprotection Frank Holtkamp,[1] Tineke M. Kats,[1] Zhongxin Zhang,[2] Shahnaz Shahinfar,[3] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]University Medical Center, Groningen, Netherlands; [2]J&J Pharmaceutical R&D, NJ; [3]Children Hospital Philadelphia, PA.*

Background: Previous studies have shown that the initial reduction in albuminuria during Angiotensin Receptor Blocker therapy partly explains long-term renal protection. However, part of the long-term renal risk remains unexplained, possibly because initial reduction in albuminuria is not always sustained over time. We aimed to assess whether total exposure to albuminuria over time is a better determinant of renal risk.

Methods: A post-hoc analysis in the RENAAL trial was performed. Albuminuria (albumin:creatinine ratio) exposure from month 6 to end of follow-up was assessed by area under the albuminuria curve (AUC) for each subject using the trapezoidal rule. C-statistics compared the predictive performance for End Stage Renal Disease (ESRD) of month 6 albuminuria and total albuminuria exposure from month 6. In addition, subjects who had a reduction in albuminuria ≥ 30% from baseline to month 6 were divided in those in whom reduction was sustained until the end of follow-up and those in whom the reduction was not sustained. Multivariate adjusted Cox regression analysis was applied to compare the risk for ESRD between these two groups.

Results: Subjects who showed a sustained reduction in albuminuria ≥ 30% from month 6 until the end of follow-up had a 55% (95%CI 65 to 14; p=0.010) lower risk for ESRD compared to subjects without a sustained reduction. These data suggest that total albuminuria exposure may be a better indicator of long-term renal risk. Indeed, the C-statistic of the albuminuria AUC from month 6 until the end of treatment was significantly higher (0.84 (0.81-0.87)) than month 6 albuminuria (0.81 (0.78-0.84; p<0.001)).

Conclusions: The overall reduction in albuminuria over time is a better indicator of long-term renal risk than the initial reduction in albuminuria alone. These data imply that one should strive to sustain the initial albuminuria reduction over time in order to optimize long-term renal protection.

TH-PO276

Metabolic Phenotypes Associate with Kidney Function and Chronic Kidney Disease in the General Population Oemer Necmi Goek,[1] Jerzy Adamski,[2] Christian Gieger,[3] Margit Heier,[3] Thomas Illig,[3] Wolfgang Koenig,[4] Timothy D. Spector,[5] Karsten Suhre,[6] Guangju Zhai,[5,7] Anna Kottgen,[1] Christa Meisinger.[3] *[1]University of Freiburg; [2]Genome Analysis Center, Helmholtz Zentrum München; [3]Helmholtz Zentrum München; [4]University of Ulm; [5]King's College London; [6]Weill Cornell Medical College in Qatar, Doha; [7]Faculty of Medicine, Memorial University of Newfoundland.*

Background: Metabolites such as creatinine are established kidney function markers. High-throughput metabolic profiling in large general population studies spanning normal kidney function and chronic kidney disease (CKD) has not been undertaken.

Methods: 151 metabolites were quantified by mass spectrometry in serum from 3,011 KORA F4 study participants. Glomerular filtration rate (eGFR) was estimated separately from cystatin C and creatinine using CKD-EPI equations and related to metabolic phenotypes (metabolites and their 22,650 ratios) by multivariable-adjusted linear regression and corrected for multiple testing. Phenotypes associated with eGFR (p<3.3*10-4 for metabolites and p<2.2*10-6 for ratios) were validated and meta-analyzed with an independent general-population sample, the TwinsUK study (n=984). Replicated phenotypes were related to CKD in KORA F4 (eGFR <60 ml/min/1.73m²; n=172).

Results: Reproducible associations with eGFR were observed for 24 metabolites and 431 ratios. P-values ranged from 1.8*10-3 to 9.6*10-64 for replicated metabolites: acylcarnitines such as glutarylcarnitine were inversely associated with eGFR (-3.4 ml/min/1.73m² per standard deviation (SD), p=9.6*10-64). For replicated ratios, p-values ranged from 1.0*10-6 to 4.6*10-69; many contained amino acids such as serine. Over 98% of replicated phenotypes associated with CKD (p<0.05): per SD increment, odds ratios ranged from 0.35 (phosphatidylcholine diacyl C38-3/glutarylcarnitine, p=1.4*10-14) to 1.90 (decenoylcarnitine/serine, p=8.9*10-16).

Conclusions: Distinct metabolic phenotypes are reproducibly associated with eGFR and CKD, reflecting altered metabolite clearance, synthesis or conversion. Longitudinal studies should clarify whether changes in metabolic phenotypes precede or result from kidney function impairment.

Funding: Government Support - Non-U.S.

TH-PO277

CKD Progression Depends upon Degree of Proteinuria but Not Blood Pressure or Serum Bicarbonate Levels in Stage 3 and 4 CKD Patients

Jennifer L. Ennis,[1] Elaine M. Worcester.[2] *[1]Litholink Corporation, Chicago, IL; [2]Section of Nephrology, University of Chicago, IL.*

Background: In several large prospective studies, proteinuria, blood pressure (BP), and serum bicarbonate (CO2) were associated with a more rapid decline in renal function in CKD patients. We sought to confirm these relationships in a cohort of unselected CKD patients from a variety of US primary care and nephrology practices.

Methods: We performed a retrospective analysis of longitudinal data collected on 1721 stage 3 (n=1194) and stage 4 (n=527) US CKD patients enrolled in the Litholink CKD program, a national laboratory based CKD management service. Presence of proteinuria (n=790) was defined as albumin:creatinine ratio >30 µg/mg or protein:creatinine ratio >200 mg/g at enrollment. We excluded patients with a follow-up period of 50 days or less.

Results: In a mean follow-up period of 184 and 182 days, respectively, patients with proteinuria had a mean fall in eGFR of 1.2 ± 0.3 ml/min/1.73m^2, while patients without proteinuria had a mean rise in eGFR of 1.7 ± 0.3 ml/min/1.73m^2. Within stage, significant differences between proteinurics and non-proteinurics remained. For both groups, patients in stage 3 had mean decline in eGFR of 1.1 ml/min//1.73m, while stage 4 patients had mean increase in eGFR of 1.5 ml/min/1.73m^2 In a general linear model with change in eGFR as the dependent variable, and presence of proteinuria, CKD stage, and the cross product of proteinuria with CKD stage as independent variables, presence of proteinuria and CKD stage were both significant predictors of change in eGFR ($p<0.001$). The cross product was not significant. Initial CO2, initial BP, final BP, and gender did not enter the model.

Conclusions: In this cohort of unselected, US stage 3 and 4 CKD patients, eGFR fell more rapidly in patients with proteinuria than in those without proteinuria. BP and CO2 levels were not predictive factors for eGFR decline in this cohort during this short follow-up period. For both groups, stage 4 patients had slight improvement in mean eGFR during the follow up period when compared to stage 3 patients. Reasons for this are presently unclear and warrant further study.

TH-PO278

Nationwide Survey of Familial Juvenile Hyperuricemic Nephropathy (FJHN) Caused by UMOD Mutations

Peter Kotanko,[1] Sian Piret,[2] Gere Sunder-Plassmann,[3] Rajesh V. Thakker,[2] Karl Lhotta.[4, 5] *[1]Renal Research Institute, New York, NY; [2]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, United Kingdom; [3]Div. of Nephrology & Dialysis, Medical Univ. Vienna, Vienna, Austria; [4]Dept Nephrology & Dialysis, Academic Teaching Hospital, Feldkirch, Austria; [5]Vorarlberg Inst. for Vascular Investigation & Treatment, Feldkirch, Austria.*

Background: FJHN is an autosomal dominant (AD) disease, caused by uromodulin (UMOD) mutations in 40% of patients. FJHN leads to end-stage renal disease (ESRD). This study examines FJHN epidemiology on a nationwide basis.

Methods: All Austrian dialysis and transplant patients are documented in a nationwide database. Following a database query in 2001, patients with unclear renal diagnoses or genetic diseases were asked whether they had (a) family members with kidney disease, and (b) gout. A detailed family history was obtained in patients who provided 2 affirmative answers; if this was suggestive for FJHN, UMOD was genotyped. In addition, data from all Austrian FJHN families known in 2010 were collected. Cox analysis was employed to test for a relationship between UMOD genotype and progression to ESRD.

Results: Based on the database query in 2001, 541 out of 6210 patients were asked to participate in the questionnaire; 19 out of 353 responders gave 2 affirmative answers. A nephropathy compatible with FJHN was present in 7 of them; in 1 patient an UMOD mutation was identified. Independent of the screening project, 4 families were diagnosed on clinical grounds with FJHN due to UMOD mutations. In 2010, 17 FJHN patients from 5 families with UMOD mutations lived in Austria (2.0 per million population), 6 of them require renal replacement therapy (0.73 per 1000 patients).

Progression to ESRD was significantly different for patients with different UMOD mutations.

Conclusions: This first nationwide survey of FJHN caused by UMOD mutations demonstrates its infrequency. FJHN patients in the ESRD population can be identified by a simple questionnaire and a subsequent focused family history. Our data indicate that UMOD genotype may modify renal disease progression.

TH-PO279

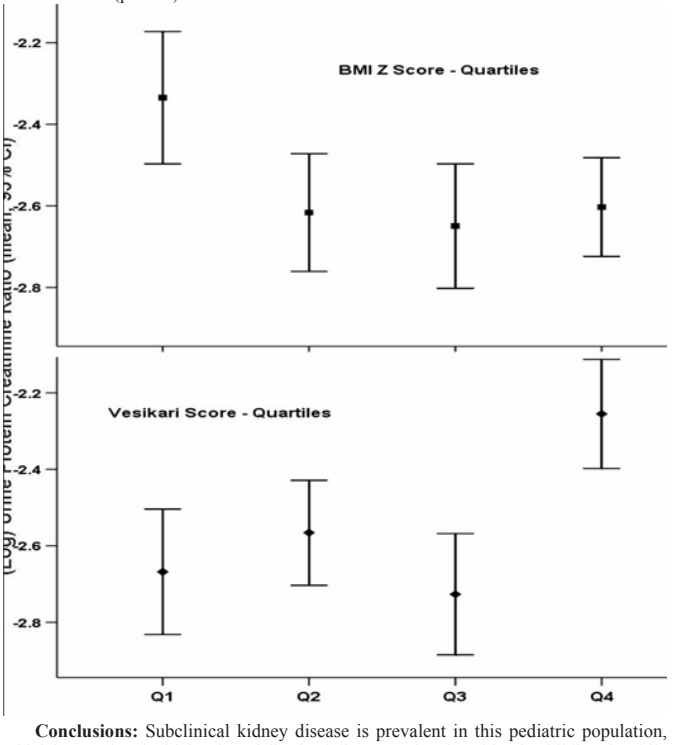
Enteric Infections Are a Risk Factor for Subclinical Proteinuria in Childhood

Lucy E. Horton,[1] Gagandeep Gagandeep Kang,[2] Madhumathi Rao.[3] *[1]Tufts University School of Medicine, Boston, MA; [2]Department of Gastrointestinal Sciences, Christian Medical College, Vellore, Tamil Nadu, India; [3]Division of Nephrology, Tufts Medical Center, Boston, MA.*

Background: India is currently facing an epidemic of chronic kidney disease (CKD), that affects a younger demographic than in the West. The 'hygiene hypothesis' suggests that chronic antigenic stimulation from the environment could generate a low grade nephritogenic immune complex response and increase risk for CKD. We hypothesize that recurrent diarrheal infections and gut antigen exposure in infancy, together with malnutrition, may be a risk factor for CKD in later life.

Methods: We studied a birth cohort of 305 children from an urban slum in South India for enteric infections and nutritional status, from birth (2002) until age 3. At age 7-8 years (2010) we measured anthropometry, blood pressure (BP) and urinary protein creatinine ratio (UPCR). Linear regression was used to determine the association of UPCR with BMI Z-score and severity of diarrhea (measured by Vesikari score).

Results: At age 7-8 years, 30% were underweight, 9% stunted and 92.5% had experienced one or more episodes of diarrhea in infancy (median, IQR 4, 2-7episodes). Proteinuria (UPCR >200) was seen in 8%, and 13% had elevated BP (>90th% systolic BP for age-and-height). Severity of diarrhea, current malnutrition (BMI Z-score) and decreased growth velocity from age 3-7 (change in BMI Z-score) were independent predictors of current UPCR ($p<0.05$).



Conclusions: Subclinical kidney disease is prevalent in this pediatric population, which is associated with burden of diarrheal disease and malnutrition in childhood. While a causal relationship cannot be inferred, public health interventions to reduce the risk of childhood diarrhea may help mitigate adult CKD burden.

Funding: Other NIH Support - This work was supported by the National Institutes of Health Office of the Director, Fogarty International Center, Office of AIDS Research, National Cancer Center, National Eye Institute, National Heart, Blood, and Lung Institute, National Institute of Dental & Craniofacial Research, National Institute On Drug Abuse, National Institute of Mental Health, National Institute of Allergy and Infectious Diseases, and NIH Office of Women's Health and Research through the International Clinical Research Scholars and Fellows Program at Vanderbilt University (R24 TW007988) and the American Relief and Recovery Act., Private Foundation Support

TH-PO280

Cystatin C Predicts End-Stage Renal Disease Better Than Iothalamate GFR in Patients with Macroalbuminuria and Type 2 Diabetes

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Background: We compared the predictive values of cystatin C (cysC), serum creatinine (SCr), and iothalamate GFR (iGFR) for diabetic end-stage renal disease (ESRD) in patients with type 2 diabetes and macroalbuminuria.

Methods: Individuals were followed from their first diabetic examination with macroalbuminuria (ACR ≥300 mg/g) until December 2010, onset of ESRD, or death,

whichever came first. Incidence rate ratios (IRR) adjusted for age, sex, and diabetes duration were computed by Mantel-Haenszel stratification. The ability of these variables to predict ESRD was compared with receiver operating characteristic (ROC) curves calculated from Cox regression models that included age, sex, diabetes duration, height, weight, HbA1c, ACR and the relevant markers.

Results: 142 Pima Indians with a median age of 44.9 years (range 25.5-67.8 years) were followed for a median of 8.9 years (range 1.5-21.0 years). ESRD developed in 63 subjects. ESRD incidence was significantly higher among patients in the lowest vs. highest tertiles of 1/cysC and iGFR (IRR for 1/cysC=3.5, 95%CI 1.7-7.4; IRR for iGFR=2.4, 95%CI 1.2-4.9), but did not differ significantly for 1/SCr (IRR=1.5, 95%CI 0.8-2.6). The model including all three filtration markers had the highest AUC, but its AUC was indistinguishable from the model with 1/cysC alone. By contrast, models with 1/SCr or iGFR alone had significantly lower AUCs than either the full model or the model with 1/cysC alone.

Conclusions: CysC was a better predictor of diabetic ESRD than either SCr or iGFR in Pima Indians with type 2 diabetes and macroalbuminuria.

Funding: Other U.S. Government Support

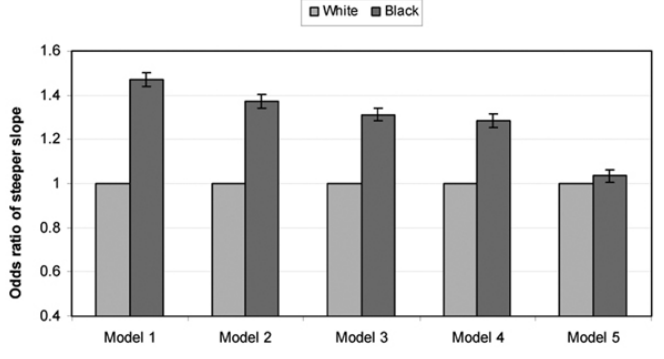
TH-PO281

Black Race Is Associated with Significantly Steeper Decline in Glomerular Filtration Rate (GFR) in a Large Cohort of US Veterans with Non-Dialysis Dependent CKD Csaba P. Kovesdy,[1,2] Evan H. Lott,[3] Jun Ling Lu,[4] Sandra M. Malakauskas,[1,2] Jennie Z. Ma,[2] Mark D. Okusa,[2] Kamyar Kalantar-Zadeh.[5] *[1]Salem VA Medical Center; [2]University of Virginia; [3]VA Informatics and Computing Infrastructure; [4]Salem Research Institute; [5]Harbor UCLA.*

Background: The high prevalence of blacks among patients with ESRD could be due to race-specific differences in the progression of CKD. Causes of faster progression of CKD in blacks with CKD need to be better characterized.

Methods: We compared slopes of eGFR in 48,692 blacks and 422,074 whites in a nationally representative cohort of US veterans with CKD stages 1-4 in 2005-2006. Slopes were calculated using a median (interquartile range) of 8 (5-13) eGFR values over up to 5 years. Unadjusted associations (Model 1) of black race with the odds of steeper slopes (defined as slopes <-4 ml/min/1.73m^2/year) were examined in logistic regression models. The effects of sociodemographic characteristics (Model 2), comorbidities (Model 3), blood pressure (Model 4) and laboratory variables (Model 5) on racial differences in the slopes of eGFR were explored in multivariable models.

Results: Blacks were younger, more likely to have diabetes, and had higher blood pressure, lower bicarbonate and hemoglobin, and higher cholesterol. The median (IQR) of eGFR slopes in blacks was -1.38 ml/min/1.73m^2/year (-4.32, 1.14) and in whites was -0.88 (-3.30, 1.27), p<0.001. Black race was associated with steeper slopes (crude odds ratio (95%CI): 1.47 (1.44-1.50), p<0.001). Adjustments lead to substantial attenuation in this association (Figure).



Conclusions: Black race is associated with steeper slopes of eGFR in patients with CKD. Most of this difference is explained by identifiable risk factors of progressive CKD. The effect of race-specific interventions to slow CKD progression in blacks needs to be tested in clinical trials.

Funding: NIDDK Support, Veterans Administration Support

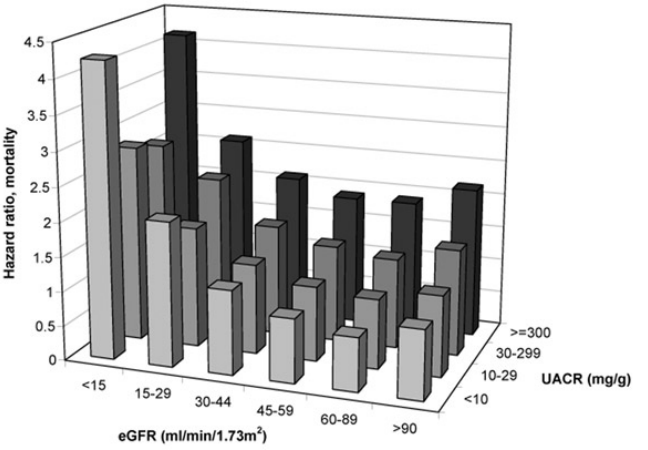
TH-PO282

CKD Stage Modifies the Association of Urine Microalbumin-Creatinine Ratio (UACR) with Mortality in a Large Cohort of US Veterans Csaba P. Kovesdy,[1,2] Evan H. Lott,[3] Jun Ling Lu,[4] Sandra M. Malakauskas,[1,2] Jennie Z. Ma,[2] Mark D. Okusa,[2] Kamyar Kalantar-Zadeh.[5] *[1]Salem VA Medical Center; [2]University of Virginia; [3]VA Informatics and Computing Infrastructure; [4]Salem Research Institute; [5]Harbor-UCLA.*

Background: The association of albuminuria with mortality in patients with various stages of CKD is not well characterized.

Methods: We examined the association of UACR with all-cause mortality in a nationally representative cohort of 298,779 US veterans with eGFR >15 ml/min/1.73m^2 in 2005-2006. Associations of UACR with all-cause mortality overall and stratified by CKD stages were examined in Cox models. Models were adjusted for sociodemographics, comorbidities, blood pressure and laboratory variables.

Results: Patients were 65.4±11.5 years old, 97% were males and 64% were white. Patients with higher UACR were older and had a higher prevalence of diabetes and cardiovascular disease. During a median follow-up of 5.0 years a total of 49,802 patients died (mortality rate, 95% confidence interval [CI]: 35.5/1000 patient-years, 35.2-35.8). A 1-unit increment in UACR on natural-log scale was associated with a crude mortality hazard ratio (95%CI) of 1.31 (1.30-1.32) p<0.001, which remained significant after multivariable adjustments (1.09, 1.08-1.10 p<0.001). The association of higher UACR with mortality was linearly incremental in patients with eGFR≥30 ml/min/1.73m^2, whereas in patients with eGFR<30 ml/min/1.73m^2 this association appeared U-shaped (Figure).



Conclusions: Higher UACR is independently associated with increased all-cause mortality. This association incremental in patients with eGFR≥30 and U-shaped in patients with eGFR <30. Interventions targeting proteinuria-lowering as a means to improve survival in patients with CKD should consider CKD stage as a potential effect modifier.

Funding: NIDDK Support, Veterans Administration Support

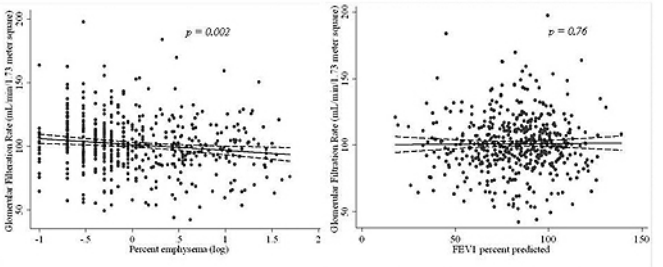
TH-PO283

The Relationship between Pulmonary Emphysema and Kidney Function in Smokers Divay Chandra, Jason Stamm, Yingze Zhang, Sheldon Bastacky, Mark T. Gladwin, Paul M. Palevsky, Frank Sciurba. *University of Pittsburgh Medical Center.*

Background: Kidney disease is prevalent in patients with COPD. We hypothesized that kidney dysfunction would associate with emphysema rather than with airway obstruction in patients with COPD, and the association would be independent of common risk factors for kidney disease.

Methods: 508 participants with >10 pack year smoking history completed a chest CT scan, pulmonary function tests, and measurement of serum creatinine. Glomerular filtration rates (eGFR) were estimated using the method of the Chronic Kidney Disease Epidemiology Collaboration. Severity of emphysema was determined by density mask examination the chest CT.

Results: Mean age of the 508 participants was 66 ± 7 years and mean eGFR 101 ± 22 ml/min/1.73m^2. Percent emphysema was a significant univariate predictor of eGFR: each 10% increase in emphysema was associated with 3.7 mL/min/1.73 meter^2 decline in eGFR (p=0.002), unlike FEV_1 which was not a significant predictor.



The association between emphysema and eGFR was also present on multivariate analysis: each 10% increase in emphysema was associated with a 4.4 ml/min/1.73 meter^2 decline in eGFR (p = 0.007) independent of airflow obstruction (FEV_1), age, gender, diabetes mellitus, hypertension, coronary artery disease, patient reported dyspnea, and pack-years of smoking.

Conclusions: Worsening emphysema, rather than airflow obstruction, predicts kidney dysfunction in patients with COPD, independent of common risk factors for kidney disease. In smokers with kidney dysfunction undetected emphysema may be contributing to diminished exercise capacity and quality of life. This is the first description of a possible emphysema – kidney injury phenotype; further investigation of shared molecular and mechanistic links between emphysema and kidney dysfunction is needed.

Funding: Other NIH Support - 1P50 HL084948, P50-CA90440, R01 HL085096, and UL1 RR024153

TH-PO284

Apolipoprotein L1 (*APOL1*) Nephropathy Risk Variants Associate with HDL Subfraction Concentration in African Americans Barry I. Freedman,[1] Carl D. Langefeld,[2] Mariana Murea,[1] Lijun Ma,[1] James D. Otvos,[5] Jolyn Turner,[1] Peter A. Antinozzi,[3] Michael V. Rocco,[1] John S. Parks.[3,4] *[1]Department of Internal Medicine-Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Division of Public Health Sciences, Wake Forest School of Medicine; [3]Department of Biochemistry, Wake Forest School of Medicine; [4]Department of Pathology-Lipid Sciences, Wake Forest School of Medicine; [5]Liposcience, Ltd, Raleigh, NC.*

Background: Coding variants in the *APOL1* gene (G1: rs73885319, rs60910145 and G2: rs71785313) are strongly associated with non-diabetic etiologies of nephropathy in African Americans. ApoL1 proteins associate with HDL particles in the circulation.

Methods: We compared plasma HDL particle subclass concentrations in 73 African Americans based on their *APOL1* genotypes to detect differences that could potentially contribute to kidney disease. These individuals were first-degree relatives of patients with non-diabetic end-stage renal disease (ESRD). HDL subclass concentrations were measured using nuclear magnetic resonance spectroscopy. Participants had estimated glomerular filtration rates (eGFR) > 80 ml/min and lacked albuminuria. Additive effects of the number of *APOL1* risk variants on natural logarithm transformed HDL subclass concentrations were computed. Analyses were performed unadjusted and after adjustment for log serum triglyceride concentration.

Results: Participants were 58.9% female with mean ± SD age 47.2 ± 13.3 years and eGFR 92.4 ± 18.8 ml/min. The numbers with 2, 1, and 0 *APOL1* nephropathy risk variants, respectively, were 36, 17, and 20. Mean ± SD medium-sized HDL concentrations were significantly lower for each additional *APOL1* risk allele (2 vs.1 vs. 0 risk alleles: 9.0 ± 5.6 vs. 10.1 ± 5.5 vs.13.1 ± 8.2 µmol/L, respectively; p=0.0192 unadjusted; p=0.0190 triglyceride-adjusted).

Conclusions: Lower medium-sized HDL subclass concentrations are present in African Americans based on increasing numbers of *APOL1* nephropathy risk alleles. Mechanistic roles for altered medium HDL concentrations on susceptibility to *APOL1*-associated renal microvascular diseases should be evaluated.

Funding: NIDDK Support

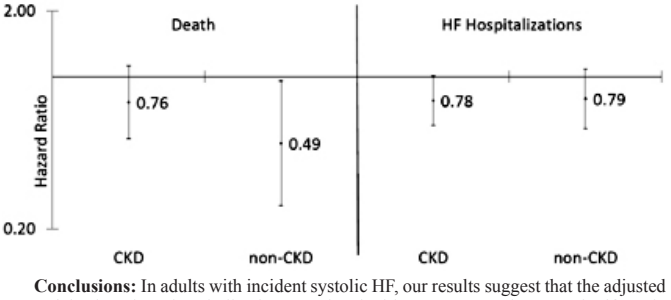
TH-PO285

β-Blockers and Outcomes in Systolic Heart Failure with Chronic Kidney Disease Tara I. Chang,[1] Jingrong Yang,[2] James Freeman,[1] Alan S. Go.[2] *[1]Stanford University; [2]Kaiser Permanente.*

Background: There are few data regarding the effectiveness and safety of oral β-blockers (BBs) in patients with systolic heart failure (HF) complicated by chronic kidney disease (CKD).

Methods: We identified 1241 adults in Kaiser Permanente of Northern California with incident clinical HF and reduced left ventricular (LV) function between 2006-2008. We excluded patients with baseline BB use or prior dialysis. Using outpatient serum creatinine values during the year before or on the index date, we classified patients as having CKD if estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m2 (n=627). Patients were followed for incident BB use and the outcomes of all-cause death and HF hospitalization through 8/31/10 identified from health plan databases. We analyzed the association of time-varying new BB use with outcomes using extended Cox regression, with adjustment for age, sex, race, smoking, body mass index, systolic blood pressure, LV function, diabetes, cardiovascular disease and other comorbidities, medications, and hemoglobin.

Results: Over a median follow-up of 2.4 years, 75% of patients had new BB use. Compared to patients without CKD, patients with CKD had higher annual rates (per 100 person-years) of death (7.5 vs. 17.1) and HF hospitalization (19.3 vs. 31.7). The Figure shows adjusted hazard ratios (95% confidence intervals) associated with BB use in patients with and without CKD in the setting of systolic HF.



Conclusions: In adults with incident systolic HF, our results suggest that the adjusted rates of death and HF hospitalization associated with new BB use does not significantly vary by CKD status. Given their higher event rates, patients with CKD would gain greater absolute benefits from medications that reduce their risks. Overall, our analysis supports the use of BBs in eligible patients with systolic HF and CKD.

Funding: Private Foundation Support

TH-PO286

Rural and Micropolitan Disparities in the Quality of Care Prior to Initiation of Renal Replacement Therapy Saugar Maripuri, Talat Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt University Medical Center, Nashville, TN.*

Background: Pre-ESRD care is associated with improved outcomes on dialysis. It is unknown how rural or micropolitan residence affects the achievement of recommended pre-ESRD care.

Methods: A cross-sectional study was performed utilizing data from the US Renal Data System (USRDS). Patients >18 years of age who initiated renal replacement therapy (RRT) between 1/2006 and 12/2006 were included and classified as rural, micropolitan, or urban based on ZIP code specific rural urban commuting codes. Outcomes of interest included the following prior to initiation of RRT: nephrology referral >12 months prior, permanent vascular access placement (AVF or AVG) in patients choosing hemodialysis, erythropoietin use in moderate to severe anemia (hemoglobin <9.0 g/L), dietary education, and pre-emptive transplant listing. The likelihood of achieving each outcome was estimated using Poisson multivariate regression with robust variances while adjusting for demographic factors, socioeconomic status, medical co-morbidities, and other potential confounders.

Results: Of 102,700 patients included in analysis, 10.2% were micropolitan and 9.9% were rural. Rural and micropolitan patients were older, less racially diverse, had more medical co-morbidities, and more likely to choose peritoneal dialysis compared to urban patients. After adjustment, rural patients were modestly more likely to have early referral to a nephrologist (RR 1.04 CI 1.01-1.08) compared to urban patients. Despite this, both micropolitan and rural patients were significantly less likely to have received dietary education (RR 0.85 CI 0.79-0.91 and RR 0.85 CI 0.80-0.91, respectively) and erythropoietin in the setting of moderate to severe anemia (RR 0.89 CI 0.82-0.97 and RR 0.91 CI 0.83-0.98, respectively). There were no significant differences in the likelihood of permanent vascular access placement or pre-emptive transplant listing.

Conclusions: Non-urban residence is associated with less erythropoietin use in anemic patients and lower likelihood of dietary education prior to the initiation of RRT, despite timely nephrology referral. Non-urban living may be associated with worse pre-ESRD care.

Funding: NIDDK Support

TH-PO287

Rural and Micropolitan Disparities in Dialysis Mortality and Likelihood of Kidney Transplantation Saugar Maripuri, Talat Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt University Medical Center, Nashville, TN.*

Background: Rural and micropolitan dialysis patients face unique challenges when initiating renal replacement therapy (RRT), including barriers to healthcare access. It is not known if such barriers increase risk for mortality or decrease likelihood of kidney transplantation among rural and micropolitan patients.

Methods: A retrospective cohort study was performed utilizing data from the US Renal Data System (USRDS). Patients >18 years of age who initiated RRT between 1/2006 and 12/2006 were included and classified as rural, micropolitan, or urban based on ZIP code specific rural urban commuting codes. Outcomes of interest included time to death >90 days after initiation, time to wait-listing for kidney transplant, and time to kidney transplantation. Censoring was determined at loss to follow-up or 10/1/2009. Cox proportional hazard regression models were created for each outcome while adjusting for demographic factors, socioeconomic status, medical co-morbidities, eGFR at initiation, dialysis modality, achieved pre-ESRD care, and other potential confounders.

Results: Of 93,148 patients included for analysis, 10.1% were micropolitan and 9.8% were rural. During follow-up, 44.5% and 6.8% of patients either died or where transplanted. An interaction between dialysis modality and degree of rurality (p value 0.001) was observed. Rural and micropolitan peritoneal dialysis (PD) patients were more likely to die on dialysis (HR 1.16 CI 1.02-1.32 and HR 1.29 CI 1.12-1.49, respectively) compared to urban PD patients. Mortality for hemodialysis patients was similar across geographic strata. There was no difference in the likelihood of wait-listing for transplant, but rural and micropolitan patients were more likely to be transplanted (LR 1.21 CI 1.09-1.34 and LR 1.12 CI 1.01-1.23, respectively) compared to urban patients.

Conclusions: For patients who initiate RRT, non-urban residence is associated with increased risk for mortality among patients on PD. However, non-urban patients appear more likely to undergo kidney transplantation. Observed relationships persisted after adjustment for achieved pre-ESRD care.

Funding: NIDDK Support

TH-PO288

Achieved Pre-ESRD Care Is Associated with Greater Likelihood of Kidney Transplantation Saugar Maripuri, Talat Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt University Medical Center, Nashville, TN.*

Background: Small studies have shown that planning for the initiation of renal replacement therapy (RRT), also known as pre-ESRD care, is associated with decreased mortality on maintenance dialysis. Little is known of the impact of pre-ESRD care on the likelihood of kidney transplantation.

Methods: A retrospective cohort study was performed utilizing data from the US Renal Data System (USRDS). Patients >18 years of age who initiated RRT between 1/2006 and 12/2006 were included. Patients deemed unsuitable for transplant were excluded. Each subject was assessed for the following prior to initiation of RRT: nephrology referral >12 months prior, permanent vascular access placement (AVF or AVG), dietician referral, and pre-emptive transplant listing. Outcomes of interest included incidence of wait-listing

and kidney transplantation. Censoring was determined at loss to follow-up or 10/1/2009. Poisson regression was performed with adjustment for demographic factors, socioeconomic status, medical co-morbidities, kidney function (eGFR) at initiation, and other potential confounders.

Results: 87,294 patients were included for analysis. The overall transplantation rate was 3.91 per 100 ESRD patient-years. After adjustment, early nephrology referral (IRR 1.23 CI 1.18-1.29), permanent access placement (IRR 1.20 CI 1.14-1.26), and dietary referral (IRR 1.19 CI 1.13-1.26) were associated with increased likelihood of wait-listing. Early nephrology referral (IRR 1.27 CI 1.20-1.34), dietary referral (IRR 1.20 CI 1.12-1.29), and pre-emptive listing (3.92 CI 3.65-4.20) were associated with greater likelihood of kidney transplantation. Patients who were listed for transplant within 90 days of RRT initiation were significantly less likely to be transplanted (IRR 0.91 0.82-0.99) compared to those who were pre-emptively listed prior to ESRD.

Conclusions: Achieved pre-ESRD care is associated with increased likelihood of kidney transplantation after initiation of RRT. Listing prior to onset of ESRD significantly increases the likelihood of transplantation, even when compared to patients listed shortly after initiation of RRT.

Funding: NIDDK Support

TH-PO289

Life Expectancy of Chinese Patients with Chronic Kidney Disease without Dialysis Chi-Bon Leung, Cheuk-Chun Szeto, Bonnie Kwan, Kai Ming Chow, Philip K.T. Li. *Department of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong.*

Background: Long term dialysis is life-saving for patients with end stage renal disease (ESRD). However, in ESRD patients with multiple comorbid conditions, dialysis may actually be futile, and conservative management is advisable. We studied the life expectancy of Chinese ESRD patients treated conservatively.

Methods: We reviewed 63 consecutive ESRD patients who were treated conservatively in our center. Duration of survival was computed from the date of initial assessment for dialysis, as well as the expected date of needing dialysis based on previous trend of renal function decline.

Results: At the end of the observation period, 55 patients died. Twelve patients died before the expected date of needing dialysis because of unrelated reasons, while 36 deaths were directly attributed to uremia. The median overall survival after initial assessment for dialysis was 41.3 months (95% confidence interval [CI], 33.2 to 49.4 months). The median overall survival was 4.43 months (inter-quartile range, -4.80 to 8.85 months) from the expected date of needing dialysis. The survival from the expected date of needing dialysis did not correlate with patient age, sex, diabetic status, or baseline renal function.

Conclusions: In Chinese ESRD patients treated conservatively, the median survival is around 4 months after the expected date of needing dialysis. Our result provides an important piece of information for the decision of dialysis and patient counseling.

TH-PO290

Different Impacts of Pulse Pressure on Proteinuria or Low eGFR between Diabetic and Non-Diabetic Populations Yuji Sato,[1] Yuichiro Yano,[1] Shouichi Fujimoto,[2] Tsuneo Konta,[2] Tsuyoshi Watanabe.[2] *[1]Internal Medicine, University of Miyazaki, Japan; [2]Steering Committee for the Examination of the Positioning of CKD in Specific Health Check and Guidance, Japan.*

Background: Systolic blood pressure (SBP) is an established risk factor for proteinuria (PRU), while the risk of pulse pressure (PP) remains controversial. Since autoregulation of renal microcirculation is impaired in diabetes (DM) subjects, we hypothesize that PP may be more harmful to DM than non-DM subjects. We examined whether there is a significant association between PP and PRU or a low eGFR.

Methods: This study was a cross-sectional analysis using Japanese nationwide data on subjects aged 20-88 years, who were recruited for a special health checkup system in 2008.

Results: DM was identified by fasting glucose ≥126 mg/dl or HbA1c ≥6.1% or using anti-DM drugs. PRU was defined as at least 1+ positive for the dipstick test, and low eGFR group was defined as <60 mL/min/1.73m2. High PP was defined as the highest quartile of PP (>60mmHg, n=8,903).

Adjusted odds ratios (OR) were calculated using multivariable logistic regression analysis, adjusted for ever reported covariates included SBP.

A total of 228,778 participants were analyzed (mean age: 63.2±8.9 years, 39.3% men). Compared with non-DM subjects, DM subjects had a higher prevalence of PRU (4.4 vs. 11.3%) and low eGFR (13 vs. 14.8%, both P<0.001).

Among DM subjects, those with PRU or low eGFR had a significantly higher level of SBP (140 vs. 133 mmHg or 135 vs. 133 mmHg) and PP (60 vs. 56 mmHg or 58 vs. 56 mmHg; all P<0.001) than those without, and

High PP (>60mmHg) was significantly associated with PRU independently of significant covariates including SBP level (OR: 1.18, P=0.001), an association which was not observed in non-DM subjects (OR: 0.98,P=0.60). In contrast, high PP was significantly and positively associated with eGFR in subjects without DM (OR: 0.93,P<0.001), an association which was not observed in DM subjects (OR: 0.99, P=0.83).

Conclusions: High PP was an independent risk factor of PRU from the SBP level in DM subjects, but not in subjects without DM. In contrast, high PP was associated with high eGFR in subjects without, but not with DM.

Funding: Government Support - Non-U.S.

TH-PO291

Effects of Change in Serum Uric Acid on Cardiovascular Outcome: A Post-Hoc Analysis of the RENAAL and IDNT Trials Paul Smink,[1] Stephan J.L. Bakker,[1] Gozewijn Dirk Laverman,[1] Tomas Berl,[2] Mark E. Cooper,[3] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Kidney Center, University Medical Center, Groningen, Netherlands; [2]University of Colorado Medical School, Denver, CO; [3]Baker Heart Research Institute, Melbourne, Australia.*

Background: Emerging data suggest that increased serum uric acid (SUA) is an independent risk marker for cardiovascular (CV) complications. Treatment with the angiotensin-receptor-blocker (ARB) losartan lowers SUA in contrast to other ARBs. Whether reductions in SUA during losartan therapy are associated with CV protection is unclear. We aimed to test this hypothesis.

Methods: In a posthoc analysis of the Reduction of Endpoints in non insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy (IDNT) trials, we determined the relationship of a short-term change in SUA with long-term CV outcome by means of Cox regression.

Results: Compared to placebo, losartan significantly lowered SUA (0.16 mg/dl (95%CI 0.01 to 0.30; p=0.031) whereas irbesartan did not (0.09 mg/dl (95%CI -0.9 to 0.28; p=0.30). Each 0.5 mg/dL decrement in SUA during losartan treatment in the first 6 months resulted in a reduction of long term CV outcome of 5.3% (95%CI 0.9 to 9.9; p=0.017). Adjustment of the CV treatment effect of losartan for month 6 change in SUA attenuated the effect from 9.2% (95%CI -7.9 to 23.6) to 4.6% (95% CI -16.2 to 22.0), suggesting that part of the protective CV effect of losartan is attributable to its effect on SUA.

Conclusions: Losartan but not irbesartan significantly lowers SUA compared to placebo in patients with type 2 diabetes and nephropathy. The degree of reduction in SUA explains part of the CV effect of losartan. This supports the postulate that in type 2 diabetics with nephropathy SUA may be a modifiable risk factor for CV disease.

Funding: Government Support - Non-U.S.

TH-PO292

Epidemiology and Outcomes of Acute Kidney Injury after Cardiac Surgery Robert I. Griffiths,[1] Michelle Gleeson,[1] Viken Paragamian,[2] Mark D. Danese,[1] Robert M. Brenner.[2] *[1]Outcomes Insights, Westlake Village, CA; [2]AlloCure, Burlington, MA.*

Background: While there is substantial literature on associations between various definitions of acute kidney injury (AKI) and outcomes, we sought to examine the relationships between the development of AKI at 24 hours or at 48 hours after cardiac surgery and hospital length of stay, receipt of dialysis, kidney recovery, and mortality.

Methods: Using data from Cerner Health Facts, we identified 6,967 patients across 53 hospitals in the United States who had cardiac surgery between 01/2000 and 06/2010, and also had an increase from baseline serum creatinine ([SCr] 1-14 days before surgery) to ≥ 0.3 mg/dL, ≥ 0.5 mg/dL, ≥ 50%, or ≥ 100% at 24 or at 48 hours after surgery (8 non-mutually exclusive groups). Patients were followed for changes in SCr until hospital discharge or death. We used Kaplan-Meier analysis to describe the time to resolution of AKI, defined as the first SCr ≤ the baseline SCr. Also, we described hospital length of stay, dialysis use (any), and discharge status (alive/dead) in the 8 groups.

Results: AKI was detected in 3,594 (52%) patients at 24 hours and in 3,373 (48%) patients at 48 hours after surgery. The average age in the cohort was 68 years, 67% were male, 85% had ≥ 4 SCr values after surgery, and the mean baseline SCr was 1.37 mg/dL. Overall, development of AKI at 24 hours was associated with greater risk of dialysis (3.5%) and hospital mortality (10.3%), and extended time to kidney recovery, compared to development of AKI at 48 hours (dialysis use 2.2%; mortality 5.3%). Patients in the SCr ≥ 100% group had the highest risk of mortality (31.5% for AKI at 24 hours; 11.5% for AKI at 48 hours). However, there were only 405 (5.8%) patients in this group. The 0.5 mg/dL group had a greater risk of dialysis than did the 50% and 100% groups, likely indicative of the fact that the 50% and 100% groups included patients with very low baseline SCr values.

Conclusions: Earlier development of AKI, at 24 hours after surgery, was associated with worse clinical outcomes. Also, compared to a SCr increase of ≥ 0.3 mg/dL, an increase of ≥ 0.5 mg/dL was associated with higher mortality and dialysis use in this population.

Funding: Pharmaceutical Company Support

TH-PO293

Prescription of and Adherence to Cardioprotective Drug Therapy in Chronic Kidney Disease Patients: Impact of Referral to Pre-Dialysis Clinics Lorraine Fradette,[1] Marc Dorais,[2] Heloise Cardinal,[3] Stephan Troyanov,[1,3] Jacques Lelorier,[3] Francois Madore.[1,3] *[1]Hopital du Sacre-Coeur de Montreal; [2]StatSciences Inc., , [3]Department of Medicine, Universite de Montreal, Montreal, Canada.*

Background: Cardiovascular disease is highly prevalent in chronic kidney disease (CKD) emphasizing the importance of appropriate cardioprotective drug therapy. However, the impact of referral to pre-dialysis clinics on the prescription of cardiovascular drug therapy and on the adherence to prescribed medications is still unknown. The objectives of this study were to evaluate: a) the prescription of and b) the adherence to lipid-lowering agents (LLA) and antihypertensive agents (AHA) in the year following referral to pre-dialysis clinics compared to that observed in the year preceding the first visit to the clinics.

Methods: Data were collected using medical records and health insurance databases that contain information on drug dispensation. Adherence to drug therapy was defined as

the percentage of days, during a pre-defined observation period, in which patients have on-hand supply of their prescribed medications.

Results: This cohort study included 385 CKD patients (stage 4 and 5). Mean glomerular filtration rate at the time of referral was 15.2/ml/min/1.73m^2. Mean age was 63 ± 13 years and 59% of patients were males. At the time of the first visit in the pre-dialysis clinic, 39% of patients were prescribed LLA (including statins and fibrates). This proportion increased to 47% (McNemar p<0.01) during follow-up. Similarly, there was a significant increase in the prescription of ACE inhibitors (from 34 to 39%), Angiotensin II receptor blockers (from 11 to 14%), β-blockers (from 40 to 51%), and diuretics (from 66 to 78%) (all p-values <0.01 on McNemar). Mean adherence to LLA and AHA did not change significantly in the year following the first visit to the pre-dialysis clinics compared with the preceding year (before: 91% and 95%; after: 84% and 95%; Mann-Whitney p>0.05).

Conclusions: In summary, these results suggest that referral to pre-dialysis clinics may increase the prescription of cardioprotective drug therapy in CKD patients but does not appear to improve the adherence to these therapies.

Funding: Government Support - Non-U.S.

TH-PO294

Low Income and Albuminuria among Reasons for Geographic and Racial Differences in Stroke Study Participants Deidra C. Crews,[1] William M. McClellan,[2] David A. Shoham,[3] Liyan Gao,[4] David G. Warnock,[4] Suzanne E. Judd,[4] Paul Muntner,[4] Brian D. Bradbury,[5] Edgar R. Miller,[1] Neil R. Powe.[6] *[1]Johns Hopkins University, Baltimore, MD; [2]Emory University, Atlanta, GA; [3]Loyola University Chicago, Maywood, IL; [4]University of Alabama at Birmingham, AL; [5]Amgen Corporation, Thousand Oaks, CA; [6]San Francisco General Hospital and University of California-San Francisco, San Francisco, CA.*

Background: Socioeconomic factors are believed to contribute to racial disparities in chronic kidney disease. Albuminuria is an important clinical risk factor for progressive chronic kidney disease (CKD), and is more prevalent among blacks than whites. Our objectives were to determine whether an association between low income and albuminuria exists, and whether this association varies by race.

Methods: In the population-based Reasons for Geographic and Racial Differences in Stroke (REGARDS) study of 22,828 U.S. adults aged 45 years and older, multivariable logistic regression was used to examine the race-stratified association between low income (annual household income < $20,000) and albuminuria and between level of income and albuminuria.

Results: Low income was present for 19.7% of participants; 29.8% of blacks and 12.9% of whites. The geometric mean urinary albumin:creatinine ratio (ACR) for blacks (11.8 mg/g) was higher than for whites (9.3 mg/g), p <0.01. With and without adjustment for demographics, lifestyle factors, comorbid illnesses and kidney function, low income was associated with ACR >30mg/g to a similar degree among blacks [crude odds ratio (OR) 1.5, 95% confidence interval (CI) 1.4-1.7; adjusted 1.2, CI 1.1-1.4] and whites [crude OR 1.8, CI 1.6-2.1; adjusted 1.3, CI 1.1-1.5]. When extremes of income were examined for their association with ACR >300mg/g, low income was statistically significantly associated with albuminuria among blacks (OR 3.4, CI 1.9-6.0, comparing income <$20,000 to >=$75,000), but not among whites (OR 1.5, CI 0.9-2.4).

Conclusions: Black race and low income are independently associated with an increased prevalence of albuminuria, and may interact in determining disparities in significant albuminuria.

Funding: Other NIH Support - National Institute of Neurological Disorders and Stroke, Private Foundation Support

TH-PO295

Decline in Kidney Function and Coronary Artery Calcium in Young Adults: Results from the CARDIA Study Nisha Bansal,[1] Eric Vittinghoff,[1] Carmen A. Peralta,[1] Chi-Yuan Hsu,[1] Michael Shlipak,[1] David R. Jacobs,[2] David Siscovick,[3] Michael Steffes,[2] John Jeffrey Carr,[4] Kirsten Bibbins-Domingo.[1] *[1]University of California, San Francisco; [2]University of Minnesota; [3]University of Washington; [4]Wake Forest School of Medicine.*

Background: Presence of coronary artery calcium (CAC) is associated with future cardiovascular events. Whether kidney function decline is associated with subsequent CAC among young adults with preserved estimated glomerular filtration rate ($eGFR_{CYS}$) is unknown.

Methods: The Coronary Artery Risk Development in Young Adults (CARDIA) study is a large biracial cohort of 5,115 young adults (ages 18-30 at enrollment) with over 20 years of follow-up. We included participants who had at least one cystatin C measurement at Years 10, 15, or 20 and CAC measurements at Years 15 or 20. Those with $eGFR_{CYS}$ < 60 ml/min/1.73 m^2 were excluded. We estimated annualized change in $eGFR_{CYS}$ using mixed models, then used a log-link continuation-ratio model with robust standard errors to estimate the association between $eGFR_{CYS}$ decline and detectable CAC (yes/no), adjusting first for age, race, sex, and time-varying body mass index, diabetes, systolic blood pressure, and then also for microalbuminuria (defined as ≥ 30 mg/g of creatinine in spot urine collections).

Results: Among 3,620 participants, (47% black, 55% women), mean age was 35 years and mean $eGFR_{CYS}$ was 109 m/min/1.73m^2 at the Year 10 examination. Nine percent (273) had detectable CAC at Year 15 and an additional 13% (352) developed detectable CAC by Year 20. Each 1% annual decline in $eGFR_{CYS}$ over the 10 years from Year 10 to Year 20 was associated with a 25% increased risk of CAC (95% CI 9%-42%, p<0.001) in models adjusted for age, race, sex, body mass index, diabetes and systolic blood pressure. After additional adjustment for microalbuminuria, the risk of detectable CAC was 17%(95% CI 2%-34%, p=0.02).

Conclusions: Even small decrements in $eGFR_{CYS}$ among young adults less than 50 years of age with $eGFR_{CYS}$ > 60 ml/min/1.73 m^2 are associated with detectable CAC, potentially signaling future cardiovascular risk.

Funding: NIDDK Support

TH-PO296

Family Income and Type of Renal Replacement Therapy (RRT) in Children with Chronic Kidney Disease (CKD). Results from the CKiD Study Guillermo Hidalgo,[1] Derek Ng,[2] Bradley A. Warady,[4] Marva M. Moxey-Mims,[5] Susan L. Furth.[3] *[1]Pediatrics, East Carolina University, Greenville, NC; [2]School of Public Health, John Hopkins University, Baltimore, MD; [3]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA; [4]Pediatrics, Mercy Children's Hospital, Kansas City, MO; [5]NIDDK, NIH, Bethesda, MD.*

Background: Low socioeconomic status (SES) has been associated with CKD severity and worst outcomes in adults. We sought to describe the association between SES, determined by family income, and the cumulative type of RRT in the CKiD cohort.

Methods: Percentages were used to describe the cumulative incidence of RRT (dialysis or transplant), by income category and CKD diagnosis (glomerular vs. non-glomerular) over time since entry in the CKiD cohort. Fisher's exact test was used to determine differences across income categories.

Results: There were 572 subjects at enrollment with complete family income data and a median follow up of 3.9 years. The cummulative incidence of RRT was the same across income groups (approximately 20%), indicating hogeneity in disease severity. However the type of RRT was significantly different across income. Those with high income were more likely to receive a transplant (11%) than those with low income (4%). The difference was significant after stratification for cause of CKD specially among those with a non-glomerular cause.

Percentage of income category experiencing study events.

RRT	Income <$30K	Income $30K - 75K	Income > 75K	p value
All CKD causes	n=202	n=212	n=158	
Transplant	4%	9%	11%	0.03
Dialysis	15%	9%	8%	0.13
Transplant or dialysis	19%	19%	20%	0.53
CKD. Non-glomerular	n=155	n=166	n=128	
Transplant	4%	8%	12%	0.04
Dialysis	9%	5%	6%	0.34
Transplant or dialysis	40%	41%	29%	0.54
CKD. Glomerular	n=47	n=46	n=30	
Transplant	6%	13%	10%	0.55
Dialysis	34%	28%	19%	0.41
Transplant or dialysis	40%	41%	29%	0.53

Conclusions: Among children enrolled in the CKiD study, the cumulative incidence of RRT was the same across income groups. However those with higher income were more likely to receive transplants while lower income was associated with dialysis. Particulary, those with non-glomerular cause of CKD.

Funding: NIDDK Support

TH-PO297

Determining National Priorities: Healthy People 2020 Chronic Kidney Objectives Asel Ryskulova,[1] Lawrence Agodoa,[2] Paul W. Eggers.[3] *[1]Centers for Disease Control and Prevention, Hyattsville, MD; [2]National Institutes of Health, Bethesda, MD; [3]National Institutes of Health, Bethesda, MD.*

Background: An estimated 11.5% of adults ages 20 or older had physiological evidence of chronic kidney disease (CKD) determined from data collected through the 1999-06 National Health and Nutrition Examination Survey (NHANES). Each year in the United States, more than 100,000 people are diagnosed with end stage renal disease (ESRD), the final stage of CKD.

Methods: Reflecting the importance of CKD, fourteen CKD objectives area were included as a new topic area in the Healthy People 2010 national health goals to reduce new cases of CKD and its progression, complications, disability, death, and economic costs. The Healthy People 2020 initiative was launched in December 2010.

Results: After the revision of the objectives by the Healthy People 2020 work group, all Healthy People 2010 objectives were retained with modifications and 6 new Healthy People 2020 objectives were added. New objectives are focused on monitoring and tracking: improvements in cardiovascular care in patients with CKD; increases in proportion of patients with CKD and diabetes who received recommended evaluation and treatment; reductions in death rate and percentage of U.S. population with CKD; and increasing CKD awareness in persons with impaired renal function. In addition to the US Renal Data System, National Health and Nutrition Examination Survey and the National Death Index have been analyzed to provide baselines and tracking data for the HP 2020 objectives.

Conclusions: The presentation will include a review of key Healthy People 2010 final results for CKD objectives, the Healthy People 2020 CKD objectives and targets, and development process used to identify the Healthy People 2020 CKD objectives.

TH-PO298

Contribution of Genetic and Environmental Factors to the Association between Preeclampsia and Later End-Stage Renal Disease Bjorn Egil Vikse,[1,2] Lorentz M. Irgens,[3] S. Ananth Karumanchi,[4] Rolv Skjaerven.[3] *[1]Department of Medicine, Haukeland University Hospital, Bergen, Norway; [2]Institute of Medicine, University of Bergen, Bergen, Norway; [3]Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway; [4]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston.*

Background: Women with preeclampsia have increased risk of later developing end-stage renal disease (ESRD). To investigate the association further, we have in the present study investigated contribution of genetic and environmental factors.

Methods: The Norwegian Population Registry has registered data on parents and siblings for most inhabitants born since the 1950's. The Norwegian Medical Birth Registry has registered data on all births since 1967 and the Norwegian Renal Registry has registered data on all patients who have developed ESRD since 1980. We linked these registries and investigated whether preeclampsia in first pregnancy of siblings, children or partner were associated with risk of ESRD. Cox regression statistics were used.

Results: In the analyses of siblings' risk, we included 605,231 women of who 312 developed ESRD. As compared to women without preeclampsia who had not had siblings with preeclampsia, women without preeclampsia who had siblings with preeclampsia had a relative risk of ESRD of 0.93 (95% CI 0.58-1.5), women with preeclampsia who had not had siblings with preeclampsia had a relative risk of 5.6 (4.1-7.6) and women with preeclampsia who had siblings with preeclampsia had a relative risk of 2.6 (0.83-8.1). These results were unchanged if the analyses for siblings were performed separately for brothers and sisters. In the analysis of parents' risk, we included 322,974 women of who 450 developed ESRD, similar findings as in the analyses of siblings' risk were made. Male partners of women with preeclampsia had a relative risk of 1.6 (1.03-2.4), 1.5 (0.94-2.5) after adjustment for educational status.

Conclusions: Risk of ESRD is not increased if siblings or children have preeclampsia but partners of women with preeclampsia have a slightly increased risk.

Funding: Government Support - Non-U.S.

TH-PO299

Effect of a Multifactorial Intervention with the Aid of Nurse Practitioners on Renal Outcome in Patients with Chronic Kidney Disease: Results of the MASTERPLAN Study Mieke J. Peeters,[1] Arjan D. Van Zuilen,[2] Jan A.J.G. van den Brand,[1] Peter J. Blankestijn,[2] Jack F. Wetzels.[1] *[1]Radboud University Nijmegen Medical Centre, Nijmegen; [2]University Medical Centre Utrecht, Utrecht, Netherlands.*

Background: Chronic kidney disease (CKD) is a major public health problem worldwide. CKD patients are at risk of progression to end stage renal disease (ESRD). We showed that strict implementation of current guidelines directed at multiple risk factors with the aid of nurse practitioners did not reduce cardiovascular events in patients with prevalent CKD. In the current analysis we evaluated renal endpoints.

Methods: In MASTERPLAN (Multifactorial Approach and Superior Treatment Efficacy in Renal Patients with the Aid of Nurse practitioners) 788 patients with mild to moderate CKD were randomized to receive nurse practitioner support added to physician care (intervention group) or physician care alone (control group). Patients were followed for a median of 4.4 years. Several renal end points were defined.

Results: 395 patients were randomized to the intervention group, 393 to the control group. Baseline variables were balanced. During follow up, mean blood pressure was significantly lower in the intervention group (-3/-2 mmHg). Significant differences were also found for LDL cholesterol (-0.11 mmol/L), phosphate (-0.03 mmol/L), hemoglobin (+0.1 mmol/L), and proteinuria (-0.12 g/24u). No differences were found for smoking, BMI, sodium intake, physical activity level, PTH, and HbA1c.

The intervention did not significantly reduce the incidence of ESRD (hazard ratio 0.86; 95% CI 0.63-1.16). Also, there were no significant differences when using a 50% increase of serum creatinine or the change in eGFR as endpoint. ΔeGFR was -1.52 in the intervention group and -1.74 ml/min/1.73m²/year in the control group, p=0.48.

Conclusions: Additional support by nurse practitioners in CKD patients improved some risk factor levels, but did not significantly attenuate the decline of kidney function or reduce the rate of ESRD.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-PO300

A Meta-Analysis on 2100 Non-Dialysis- and Dialysis-Patients Exposed to Once Monthly C.E.R.A. Demonstrates Effective and Safe Treatment Danilo Fliser,[1] Thomas Weinreich,[2] Stefan N. Heidenreich,[3] Fuat Bozkurt,[4] Christoph Wanner.[5] *[1]University of Saarland, Homburg, Germany; [2]Dialysis Center, Vill-Schwenn, Germany; [3]Dialysis Center, Aachen, Germany; [4]Dialysis Center, Daun, Germany; [5]University of Würzburg, Würzburg, Germany.*

Background: In Germany the prospective studies MIRACEL, SESAM, SUPRA, NASA and MERCUR had been initialized in chronic kidney disease (CKD) patients (pts), stages IV and V.

Methods: Data from all five interventional and non- interventional studies were pooled in a single database and analyzed with respect to individual hemoglobin (Hb)-fluctuation and Hb in target range during the evaluation period, and dose adaptations within the individual observation phase. Further analyses were performed for the following subgroups:

- Route of administration (iv, sc)
- Gender (male, female)
- ESA pre-treatment (Darbepoetin, Epoetin, C.E.R.A)
- Diabetes (yes, no)

Additionally, the dialysis and the non dialysis CKD population was compared.

Results: The study population consisted of 1581 dialysis (D) and 519 non-dialysis (ND) pts. The age of the dialysis and non-dialysis pts was 65,1 ±14,4 and 68,4 ±14,5 years, respectively, with 42,8% (D) and 48,7% (ND) being female. 38,7% (n=612) of the D and 44,5% (n= 44,5) of the ND pts suffered from diabetes.

	Deviation from the individual Hb mean ≤ 1g/dl (%)	Pat in Hb target (%)	Dose adaptations (mean)
	D/ND	D/ND	D/ND
Total	84.1/84.7	66.4/67.6	2.1/1.7
Admin route iv	85.1/50	67.3/75	2.0/0.2
Admin route sc	82.3/86	68/67.9	2.2/1.7
Female	82.7/85.4	63.4/63.7	2.1/1.8
Male	85.1/84	68.6/71.6	2.1/1.6
ESA pre Darbepoetin	82.6/76.9	67.1/68.6	2.0/1.6
ESA pre Epoetin	85/92.9	66.4/69	2.0/1.3
ESA pre C.E.R.A.	85.2/100	67.6/50	2.7/0.8
Diabetes no	83.7/84.4	65.4/69.1	2.1/1.6
Diabetes yes	84.7/85.1	68/65.7	2.1/1.7

A comparable safety-profil to other ESA had been observed in all 5 C.E.R.A. studies.

Conclusions: Once monthly C.E.R.A. maintains stable Hb-levels in patients both on D and ND with only minimal Hb fluctuations. These results were confirmed for various subgroups, thus demonstrating that once monthly C.E.R.A. is a suitable treatment for all pts suffering from CKD related anemia with only few dose-changes needed.

Funding: Pharmaceutical Company Support

TH-PO301

Progression of Chronic Kidney Disease in a National Sample of United States Veterans Ahmad Mahallati,[1] Elizabeth Hedgeman,[1] Brenda W. Gillespie,[1] Meda E. Pavkov,[2] Nilka Rios Burrows,[2] Laura C. Plantinga,[3] Neil R. Powe,[3] Rajiv Saran.[1] *[1]University of Michigan, Ann Arbor, MI; [2]Centers for Disease Control and Prevention, Atlanta, GA; [3]University of California, San Francisco, CA.*

Background: Patterns and clinical indicators of kidney function decline were examined in a population with baseline chronic kidney disease (CKD).

Methods: We identified a cohort of national Veterans Affairs (VA) patients with CKD (eGFR <60ml/min/1.73m² by the MDRD equation) and at least two serum creatinine measurements over 2 to 5 years. Each patient was assigned a best-fit slope of annual eGFR change using linear regression. Those with negative slopes were equally split into slow progression or fast progression groups. Multivariable logistic regression was used to assess clinically important factors associated with fast vs. slow progression of CKD (age, race/ethnicity, gender, diabetes, baseline eGFR, albuminuria, hemoglobin, and serum albumin).

Results: Of the 13,634 patients identified with CKD, 45% (n= 6153) experienced no decline in kidney function and were not included in the multivariable analysis; of those patients experiencing a decline in kidney function, 50% were classified as 'slow' (n= 3,738; slope 0 to -2.18 ml/min/1.73m² per year) and 50% as 'fast' (n= 3,743; slope -2.19 to -25.72 ml/min/1.73m² per year) progressors. Progressors were predominantly male (97.0%), with a mean age of 72.5 years and baseline eGFR of 48.0 ml/min/1.73m². The odds of fast progression were higher with increasing baseline eGFR (OR =1.03, 95% CI 1.02-1.05), and urine albumin-to-creatinine ratio ≥300 mg/g (OR=2.39, 95% CI 1.67-3.42), but lower with increasing serum hemoglobin (g/dl) concentration (OR=0.89, 95% CI 0.83-0.94).

Conclusions: In this U.S. veteran cohort, the overall mean progression of all-cause CKD was relatively slow (-0.08 ml/min/1.72m² per year), with faster progressors experiencing an average decline of -4.67 ml/min/1.72m² per year and nearly half of the population showing no reduction in eGFR over 2 to 5 years. Future studies to link outcomes with these and other risk factors are warranted.

Funding: Other NIH Support - Centers for Disease Control and Prevention

TH-PO302

Plant Protein Intake Is Associated with Fibroblast Growth Factor 23 and Serum Bicarbonate in Patients with CKD: The Chronic Renal Insufficiency Cohort Study Julia J. Scialla,[1] Lawrence J. Appel,[1] Myles S. Wolf,[2] Wei Yang,[3] Xiaoming Zhang,[3] Edgar R. Miller,[1] Stephen M. Sozio,[1] Lydia Bazzano,[4] Magdalena M. Cuevas,[3] Melanie Glenn,[3] Radhakrishna Reddy Kallem,[3] Eva Lustigova,[4] Anna C. Porter,[5] Raymond R. Townsend,[2] Matthew R. Weir,[6] Cheryl A. Anderson.[1] *[1]Johns Hopkins University; [2]University of Miami; [3]University of Pennsylvania; [4]Tulane University; [5]University of Illinois at Chicago; [6]University of Maryland.*

Background: Plant-based proteins have lower bioavailability of phosphorus and lower acid load than animal-based proteins and may be preferred in CKD. Adverse effects due to inhibition of iron absorption and potassium loading are also possible.

Methods: We calculated the percentage of protein intake from plant sources (% plant protein) in 2938 CRIC participants by scoring individual food items from a food frequency questionnaire. Using linear regression we modeled associations between % plant protein and serum phosphate, log fibroblast growth factor 23 (FGF23), log parathyroid hormone (PTH), serum bicarbonate (HCO_3), serum potassium, serum albumin and hemoglobin.

Models were adjusted for age, sex, race, diabetes, body mass index, eGFR, income, smoking, total energy intake, total protein intake, 24 hour urinary sodium, use of ACE inhibitors/ ARBs and use of diuretics.

Results: Adjusted means of FGF23 and HCO_3 across levels of % plant protein are presented in the table.

	<24%	24-29%	30-35%	36-44%	>44%	p-trend
FGF23 (RU/mL)	157.4	154.8	152.8	150.6	146.9	0.05
HCO3 (mEq/L)	24.4	24.5	24.6	24.7	24.8	0.01

Higher % plant protein was associated with lower FGF23 (p=0.05) and higher HCO_3 (p=0.01), but not with serum phosphate (p=0.9) or PTH (p=0.5). There was no association of higher % plant protein with serum potassium (p=0.2), serum albumin (p=0.2) or hemoglobin (p=0.3). The associations of % plant protein with FGF23 and HCO_3 did not differ by diabetes status, sex, race, CKD stage (2/3 vs. 4/5) or total dietary protein intake (≤ 0.8 g/kg/d vs. >0.8 g/kg/d) (p-interaction > 0.10 for each).

Conclusions: In conclusion, consumption of a higher percentage of protein from plant sources may lead to a more favorable metabolic profile in patients with CKD.

Funding: NIDDK Support, Other NIH Support - through CTSA awards, Private Foundation Support

TH-PO303

Determinants of sRAGE in a Community-Based Population: The Atherosclerosis Risk in Communities (ARIC) Study

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Background: Advanced glycation endproducts (AGEs) and their receptors are implicated in the development of diabetic complications and kidney disease. When stimulated by AGEs, the receptor for AGEs (RAGE) induces inflammation and may fuel progression of disease through NF-kB mediated signaling. Soluble circulating RAGE (sRAGE) may counteract the effects of RAGE.

Methods: We identified determinants of low levels (lowest quartile) of sRAGE (ELISA, R&D Systems, CV<3%) from stored plasma in a random sample of 1185 participants in the ARIC Study (ages 47-68) with $eGFR_{CKD-EPI} \geq 60$ mL/min/1.73m^2.

Results: sRAGE was inversely correlated with BMI (Spearman's r=-0.28), HbA1c (r=-0.22), CRP (r=-0.25), $eGFR_{Cystatin}$ (r=-0.17) and $eGFR_{CKD-EPI}$ (r=-0.11). sRAGE was lower among men vs. women (958.5 vs 1100.6 pg/mL, p<0.001), blacks vs. whites (756.6 vs 1118.8 pg/mL, p<0.001), and diabetic vs. nondiabetic adults (901.8 vs 1050.0 pg/mL, p=0.01). Only male sex, black race, smoking, CRP, and eGFR remained associated with low sRAGE after adjustment. $eGFR_{Cystatin}$ was more strongly associated with sRAGE than was $eGFR_{CKD-EPI}$.

Adjusted* ORs (95% CI) for lowest quartile of sRAGE (<708.8 pg/mL) N=1,185

	OR (95%CI)
Age >55 (vs <55)	1.1 (0.8, 1.5)
Male	1.7 (1.2, 2.4)†
Black	4.6 (3.3, 6.5)†
A1c 5.7-6.4% (vs <5.7%)	1.2 (0.7, 2.0)
A1c>6.5% or diabetes (vs <5.7%)	0.9 (0.5, 1.5)
Former smoker (vs current)	1.6 (1.1, 2.5)†
Never Smoker (vs current)	1.0 (0.6, 1.5)
BMI >30 kg/m2 (vs <25kg/m2)	1.5 (1.0, 2.2)
BMI 25-30 kg/m2 (vs <25 kg/m2)	3.0 (1.9, 4.6)†
CRP 1-3 mg/L (vs <1mg/L)	1.7 (1.1, 2.6)†
CRP >3 mg/L (vs <1mg/L)	3.0 (1.9, 4.6)
History of coronary heart disease	1.4 (0.7, 2.6)
eGFR cystatin C (per 10mL/min/1.73 m2)	1.3 (1.1, 1.4)†
	†p-value <0.05

*Adjusted for all variables listed

Conclusions: Low levels of sRAGE may indicate susceptibility to inflammatory processes or a state of increased oxidant stress. The lack of association of sRAGE with diabetes is surprising and suggests that sRAGE may be a generalized measure of ill health and not as specific to diabetes as previously thought. The robust racial difference in sRAGE deserves further examination.

Funding: NIDDK Support

TH-PO304

Development of Chronic Kidney Disease in Rheumatoid Arthritis

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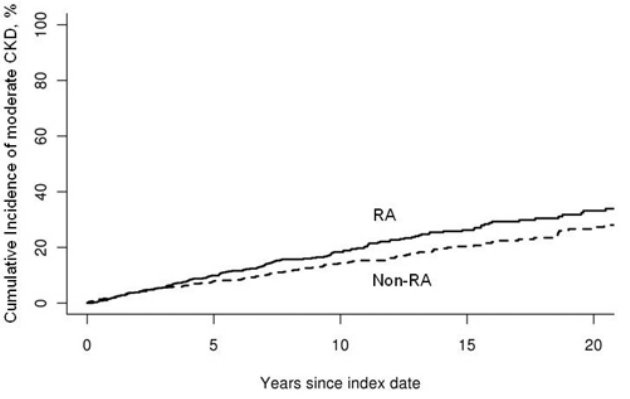
Background: Rheumatoid arthritis (RA) is associated with a variety of kidney disorders. However, it is unclear whether the development of chronic kidney disease (CKD) is higher in patients with RA compared to the general population.

Objective: To elucidate the occurrence of CKD in patients with RA.

Methods: Retrospective review of incident adult onset RA cases from a defined geographic population base from 1980-2007 and a comparison cohort of non-RA patients. CKD was defined by estimated glomerular filtration rate (eGFR) <60 and moderate CKD was eGFR <45 ml/min/1.73m^2. The cumulative incidence of CKD was estimated adjusting for the competing risk of death.

Results: 813 RA patients and 813 non-RA patients were assembled (mean age 55.9, 68% female). There was no difference in the presence of CKD at time of RA development, p=0.84. Among RA patients, 38% had an eGFR<60 and 11% had eGFR<45, similar to non-RA patients (38% and 9%) observed at the RA incidence reference date. The 10 year cumulative incidence of CKD was higher in RA patients compared to non-RA (43% vs 32%, p=0.008). This difference occurred primarily in the first year. The 10 year cumulative incidence of moderate CKD was also higher in patients with RA compared to non-RA (18% vs 14%, p=0.037). This difference became apparent 3 years after incident RA (figure). The development of CKD was associated with erythrocyte sedimentation rate (HR 1.1 per 10 mm/hr, CI 1.03-1.20, p=0.008) and cardiovascular disease (HR 2.1, CI 1.4-3.2, p<0.001).



Conclusions: RA patients were more likely to develop CKD over time. Inflammation and cardiovascular disease appear to play a role in this development. Further studies are needed to improve understanding of these relationships.

TH-PO305

Coexistence of High Serum Hepcidin-25 Level and Proteinuria Has Strong Impact on Short-Term Mortality in Non-Hodgkin's Lymphoma Patients

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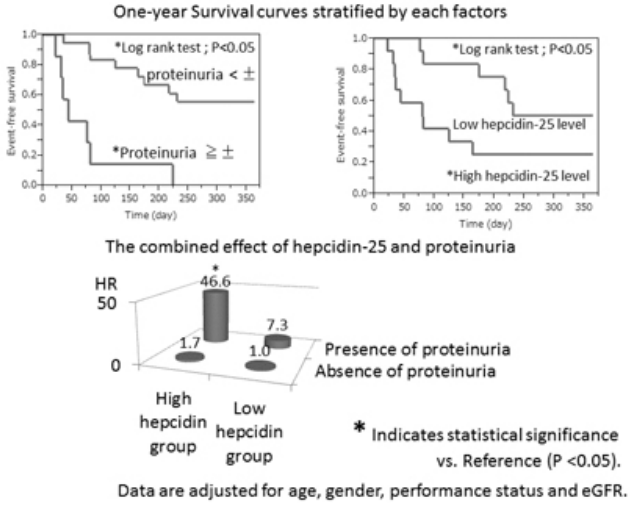
Background: The excessive hepatic production of hepcidin-25 due to chronic disorders including cancer is responsible for anemia with underutilization of iron. This study addressed the combined impact of hepcidin-25 and proteinuria on short-term mortality in lymphoma patients.

Methods: One-year prospective cohort study was conducted in a total of 24 patients with non-Hodgkin's lymphoma. Serum hepcidin-25 level was measured by liquid chromatography-mass spectrometry. Proteinuria was defined as a dipstick test ≥±. Survival curve was drawn by the Kaplan Meier method, which was stratified into 2 groups by either median value of serum hepcidin-25 (43.1 ng/ml) or presence of proteinuria. In addition, each group which was stratified by hepcidin-25 was divided into 2 subgroups according to presence of proteinuria. Multivariate Cox proportional hazards analysis, adjusted for age, gender, performance status and estimated GFR, was used to calculate mortality HR for the combined impact of hepcidin-25 and proteinuria.

Results: Mean hepcidin-25 level was 53.9±48.9 ng/ml, which was approximately 2-fold greater than the reference value (22.2±12.3 ng/ml). The cumulative survival rate was significantly lower in the high hepcidin-25 group or in the proteinuria (+) group than in each opposite. The mortality HR (95% CI) was 46.6 (6.33-442.54) for patients with both proteinuria and high hepcidin-25; 7.3 (0.80-57.80) for those with proteinuria and low hepcidin-25; and 1.7 (0.30-9.28) for those with no proteinuria and high hepcidin-25, as compared to the reference with neither of them.



Conclusions: Non-Hodgkin's lymphoma patients with both high serum hepcidin-25 level and coexsiting proteinuria are likely at high risk for early mortality.

TH-PO306

Early Invasive Management of Acute Coronary Syndrome and Renal Outcomes Matthew T. James,[1] Marcello Tonelli,[2] Neesh I. Pannu,[2] Scott Klarenbach,[2] Braden J. Manns,[1] Brenda Hemmelgarn.[1] *[1]Medicine, University of Calgary, Canada; [2]Medicine, University of Alberta, Canada.*

Background: Acute kidney injury following coronary angiography is associated with adverse outcomes. The risk of acute kidney injury and other renal complications associated with invasive versus conservative management of acute coronary syndrome is unclear. We examined the relationships between early invasive management and acute kidney injury, dialysis, end-stage renal disease, and survival.

Methods: We performed a retrospective cohort study including all Alberta residents aged 18 year or older with a primary admission diagnosis of non-ST elevation acute coronary syndrome from six acute care hospitals in Alberta, Canada between 1 January 2004 and 31 October 2009. We developed a propensity score for early invasive management (coronary angiography within 2 days of hospital admission). Treatment groups were matched 1:1 on propensity scores, and followed to determine the risks of acute kidney injury (>0.3 mg/dl or 50% increase in serum creatinine concentration in hospital), acute kidney injury requiring dialysis, end-stage renal disease, and mortality.

Results: Of 10,538 included patients, 4,281 (40.6%) received early invasive management. After propensity score matching of conservative management patients with characteristics similar to those who received early invasive therapy (n=7,430), early invasive management was associated with an increased risk of acute kidney injury (8.8 versus 5.6%, risk ratio [RR] 1.52, [95% CI 1.29-1.80]; P<0.001). However, no significant differences were observed between the matched groups in the risks of acute kidney injury requiring dialysis during hospitalization (0.3 vs 0.2%, RR 1.33 [0.56-3.16]; p=0.514) or end-stage renal disease (hazard ratio [HR] 0.81, [0.68-0.96]; p=0.019). Early invasive management was associated with improved long-term survival (HR 0.71, [0.68-0.96]; p=0.019).

Conclusions: Early invasive management of acute coronary syndrome is associated with a modest increase in risk of acute kidney injury but not dialysis or end-stage renal disease. These findings suggest that the risk of acute kidney injury should not preclude early invasive management of acute coronary syndromes

Funding: Government Support - Non-U.S.

TH-PO307

Low Taste Acuity for Salt Indicates Fast Decline of Renal Function in Patients with CKD Stages 4 and 5 Yoshie Kanazawa,[1,2] Toshiyuki Nakao.[1] *[1]Department of Nephrology, Tokyo Medical University, Tokyo, Japan; [2]Tokyo Kasei Gakuin University, Tokyo, Japan.*

Background: Salt restriction is a fundamental issue in the management of chronic kidney disease (CKD). At present, there are few data on taste acuity for salt (TAS) and its association with the decline of renal function in CKD patients.

Methods: Among 248 consecutive CKD patients who were evaluated for TAS in August 2007, 84 patients with CKD stages 4 and 5 who were being assessed for renal function throughout the period of more than 6 months were recruited in this study. All patients were instructed to restrict salt intake. TAS was assessed by determining the threshold of response to a set of test papers with 7 kinds of concentrations of salt crystal (i.e., 0.6% to 1.8%). The evaluated patients were classified into 3 groups according to their thresholds of response to salt concentration: 0.6 and 0.8 as sensitive (S); 1.0, 1.2 and 1.4 as intermediate (I); and 1.6 and 1.8 as insensitive (U).

Results: A total of 38 patients were classified into group S, 25 into group I and 21 into group U. The mean observational period was 21.2 ± 5.1 months, with no significant differences between the groups. The estimated glomerular filtration rate (eGFR: ml/min), serum creatinine level (Cr: mg/dl) and urea nitrogen level (UN: mg/dl) at the start of the study were 14.4 ± 5.8, 3.87 ± 1.55 and 48.4 ± 5.2 in group S, 10.4 ± 5.1, 4.03 ± 1.78 and 53.3 ± 21.2 in group I, and 13.9 ± 5.6, 4.29 ± 2.57 and 50.5 ± 20.4 in group U, respectively. The eGFR, Cr and UN at the end of the study were 10.5 ± 5.9, 5.72 ± 2.81 and 60.8 ± 23.4 in group S, 13.5 ± 6.3, 5.20 ± 2.49 and 60.6 ± 34.5 in group I, and 7.4 ± 4.4, 8.21 ± 4.11 and 67.0 ± 29.2 in group U, respectively. The decline in eGFR (ml/min/month) was -0.20 ± 0.18 in group S, -0.14 ± 0.11 in group I and -0.34 ± 0.28 in group U, with group U showing faster decline than in groups S and I. The elevation rates of Cr (mg/dl/month) were 0.10 ± 0.11 in group S, 0.07 ± 0.10 in group I and 0.25 ± 0.35 in group U, and with group U showing faster elevation rate than groups S and group I.

Conclusions: Low TAS indicates fast decline of renal function in patients with CKD stages 4 and 5.

TH-PO308

Detection of Chronic Kidney Disease and Proactive Referral in the General Population – Results of Three Years Follow-Up of a Community Based Intervention Program Shlomo Vinker, Eran Rotman. *Medical Director Office, Clalit Health Services, Central District, Rishon Lezion, Israel.*

Background: CKD is common, especially among patients with co-morbidities as diabetes, hypertension or cardiovascular disease (CVD). In the early stages of CKD family physicians' awareness is usually low. We aimed to evaluate early detection of CKD and three years nephrologists' follow-up.

Methods: We reviewed all laboratory results of our members in 2007 (about 500000) for the last calculated eGFR using the abbreviated MDRD equation. Patients with eGFR 20-50 ml/min were eligible for the intervention program. We included all patients with eGFR 20-30 ml/min and patients with eGFR >30 ml/min having at least one of the following: diabetes, hypertension or CVD. Patients that visited a nephrologist in 2007 had been excluded.Main outcome: Changes in eGFR and CVD risk factors.

Results: 418 patients with CKD-4 (age 74.0±10.2, eGFR 26.3±2.7 ml/min) and 2067 patients with CKD-3 (age 66.0±7.3) met the inclusion criteria and had been invited to a nephrologist. 201 CKD-4 patients visited a nephrologist. After 3 years, 31 patients died and 53 developed end stage kidney disease (ESKD) and 27 lost from follow-up the rest had stable eGFR (27.6±8.1), Blood pressure (BP)was lower (129.1±14.5/72.1±8.7 vs. 137.7±21.5/77.7±12.5, p<0.05) lower LDL cholesterol (LDL-c) (93.2±28.6 vs. 101.3±32.1, p<0.05), and stable Hemoglobin level. The percent of patients treated with erythropoietin and analogues increased significantly from 11.9% to 36.6%. 630 CKD-3 patients visited a nephrologist. After 3 years, 43 patients died and 27 developed ESKD and 71 lost from follow-up the rest had a decline in eGFR (39.7±11.5 vs. 42.3±5.6), BP was lower (129.3±15.6/73.5±8.8 vs. 138.1±19.3/78.3±10.2, p<0.05) lower LDL-c(93.3±30.5 vs. 101.3±34.2, p<0.05), and stable Hemoglobin level. The percent of patients treated with erythropoietins increased significantly from 1.7% to 8.1%.

Conclusions: A proactive program to detect CKD 4 and high risk CKD 3 patients and the implementation of nephrologists' follow-up service improves treatment. More efforts to recruit all the high risk patients are needed. A longer follow up is needed to evaluate if this will affect prognosis.

TH-PO309

The Association between Serum Uric Acid and Renal Damages in the Japanese Population: The Takahata Study Kazuko Suzuki, Tsuneo Konta, Kazunobu Ichikawa, Yusuke Mashima, Ami Ikeda, Isao Kubota. *Yamagata University School of Medicine, Yamagata, Japan.*

Background: The increase in serum uric acid is frequently observed in the subjects with renal insufficiency.

Methods: However, the association between serum uric acid and renal damages in general population is largely unknown. To clarify this point, we conducted a community-based study, using urinary albumin and beta 2-microglobulin as markers of glomerular and tubular damages.

The subjects studied were 3341 Japanese subjects (1497 men, 1844 women) without history of kidney disease. The urinary albumin-creatinine ratio (UACR) and beta 2-microglobulin-creatinine ratio (UBCR) were assessed in morning spot urine samples.

Results: In this population, the mean values of serum uric acid (mg/dl) were 5.8 ± 1.3 in men and 4.5 ±1.1 in women, respectively. The prevalence of albuminuria (UACR >30 mg/g) were significantly increased along with the increase in uric acid both in men and women (P <0.05). In contrast, the prevalence of high UBCR (>300 μg/g) were deceased along with the increase in uric acid both in men (P <0.01) and women (P =0.056). Multivariate analysis showed that albuminuria was independently related with increased uric acid (>7 mg/dl for men, >6 mg/dl for women, vs. 5.0-5.9 mg/dl) and high UBCR was not associated with uric acid neither in men nor women, after adjustment for possible confounders. The one-year longitudinal analysis in 1517 subjects showed the positive relationship between baseline uric acid and UACR changes across the tertiles of uric acid (P for trend <0.05).

Conclusions: This study showed that uric acid was an independent risk factor for albuminuria, but not high UBCR, suggesting that uric acid might induce glomerular damage, but not tubular damage in general population.

TH-PO310

Etiology, Comorbidity and Factors Associated with Renal Function Decline in Chinese Chronic Kidney Disease Patients Guangyan Cai, Xiang-Mei Chen. *Department of Nephrology, State Key Laboratory of Kidney Disease, PLA Genaral Hospital.*

Background: For the lack of nation-wide investigations in China, features of CKD etiology, comorbidity and factors associated with renal function decline is not clear. The Chinese Society of Nephrology initiated a nation-wide investigation of inpatients with CKD in China.

Methods: A cross sectional study was conducted on inpatients with CKD from 61 big hospitals among 31 provinces in Mainland China. Data on demographic characteristics, lifestyle risk factors, medical records, and laboratory tests results were obtained using a standard questionnaire administered by trained physicians. Estimated GFR was calculated with CKD-EPI equation. Binary logistic regression was used to analyze factors associated with renal function decline.

Results: 10728 patients were included in the analysis, in which male-female ratio was 1.18:1. The mean age was 48.14 ± 17.98 years (15ys-97ys). The distribution in CKD stages was as follows: stage one 28.2%, stage two 15.0%, stage three 12.4%, stage four 8.6% and stage five 35.7%. Primary glomerulopathies accounted for 52.3% diagnosis of the whole inpatients with CKD, in which 18.8% was IgA nephropathy. Diabetic kidney disease accounted for 11.9%, lupus nephritis 6%, hypertensive nephropathy 4.8%, cystic kidney disease 2.4%. The common comorbidities were mineral and bone disorder (66.8%), anaemia (64.4%), hypertension (62.2%), hyperlipemia (56.9%), hypoalbuminemia (49.2%), hyperuricemia (46.2%), diabetes (23.8%) cardiovascular disease (19.1%), cerebrovascular disease (6.1%) and so on. By multivariable logistic regression analysis, renal function decline was significantly associated with age (OR 1.612), male (OR 1.777), hypertension (OR 3.562), diabetes (OR 1.517) , anemia (OR 7.983) and hyperuricemia (OR 3.154).

Conclusions: This investigation described the clinical features of inpatients with CKD in China for the first time. Presently, primary glomerulopathy is the leading cause

of CKD in China. In Chinese CKD patients, the prevalence of hypertension, diabetes, cardiovascular diseases and cerebrovascular diseases were lower, but the prevalence of anemia and hypoalbuminemia are higher than those from developed countries.

TH-PO311

Educational Hospitalization Effectively Delays Progression of CKD Shotaro Naito, Soichiro Iimori, Kayoko Eto, Eisei Sohara, Tomokazu Okado, Yumi Noda, Tatemitsu Rai, Shinichi Uchida, Sei Sasaki. *Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

Background: In Japan, over thirteen million people are diagnosed as chronic kidney disease (CKD). Appropriate diet modification and control of blood pressure are essential for prevention of progression of CKD. However, it is difficult to achieve these goals in the outpatient clinic. In our hospital, we have hospitalized CKD patients as 'Educational Hospitalization' (EH) to provide them with the knowledge of diet and medication, and to control their blood pressure and fluid volume balance. We analyzed the effect of EH on the progression of CKD.

Methods: During the period from January 2007 to December 2010, a total of 712 CKD patients visited our nephrology department. Among them, 206 patients were hospitalized for EH. 199 EH patients, whose estimated GFR (eGFR) were under 60 ml / min / 1.73 m^2, were retrospectively analyzed. The effect of EH was evaluated by the change in eGFR during 6 months (ΔeGFR (ml / min / 1.73 m^2 / year)) before and after hospitalization.

Results: Compared with patients who were not hospitalized, the EH patients had significantly higher male gender, higher diabetes prevalence, older age, lower hemoglobin, lower serum albumin, lower eGFR, lower serum total cholesterol and higher urinary protein ($P < 0.05$). We compared patient status at the point of hospitalization and after 6 months in the EH group. Hemoglobin (10.7 → 10.9 g/dl) and serum albumin (3.7 → 3.8 mg/dl) significantly increased, and eGFR decreased during this period. Changes of serum total cholesterol and urinary protein were not significant. ΔeGFR after hospitalization was improved (-7.5 → -3.0 ml/min/year). When stratified by CKD stage, ΔeGFR in stage 4 and 5 patients were significantly improved but not in stage 3. Moreover the degree of improvement was largest in stage 5.

ΔeGFR before and after EH

	ΔeGFR (before EH)	ΔeGFR (after EH)	P-value
CKD stage 3	-2.5 ± 10.8	-3.3 ± 11.7	0.76
CKD stage 4	-8.6 ± 9.6	-3.5 ± 10.7	0.004
CKD stage 5	-9.3 ± 7.9	-2.4 ± 6.4	< 0.0001

Data are given as the mean ± SD

Conclusions: Educational hospitalization is an effective method to delay progression of CKD, especially in advanced stages.

TH-PO312

The Role of ARB in Treatment of Left Ventricular Hypertrophy in Dialysis Patients: A Meta-Analysis Liya Yang, Ge Xiao, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: Left ventricular hypertrophy (LVH) is a major cardiovascular complication and an important predictor of mortality in diaysis patients.Studies have suggested that angiotensin-receptor blockers (ARBs) could ameliorate LVH, but results are controversial. The purpose of this study was to evaluate the effect of ARBs versus placebo or alternative treatments on LVH in dialysis patients.

Methods: We performed systematic searches of PubMed, Embase, Cochrane Central Register of Controlled Trials, reference lists and expert contacts according to a standardized protocol, through November, 2010.

Results: Six studies involving 177 participants reported results of 4 comparisons with hemodialysis patients and 2 comparisons with peritoneal dialysis patients. ARBs ameliorated LVMI compared with non-ARB in dialysis patients (WMD -26.34g/m^2, 95% CI-37.77~-14.90, $p<0.01$). However, there was no significant difference between the ARB group and the non-ARB group (WMD 1 g/m^2, 95% CI -0.56 – 2.56, $p=0.21$) in the study of EF. ARBs treatment induced a greater reduction in LVMI when compared with non-ARB group (WMD -22.4g/m^2, 95% CI -35.37-- -9.11,$p=0.0009$) in heamodialysis patients. Interestingly, ARBs did not work as well as in peritoneal dialysis patients. In alternative treatment groups, the result suggested that losartan were superior than enalapril in the regression of LVMI (WMD -16.23 g/m^2, 95% CI -21.79 – -10.67, $p <0.01$). For left ventricular function, enalapril showed no inferior than losartan reflecting by the parameter of EF (WMD -0.36 g/m^2, 95% CI -2.92 – 2.21, $p =0.79$). One article reported the combination of ARBs and ACE inhibitors further reduced LVH more than either agent alone.

Conclusions: This meta-analysis suggests that ARBs are effective in treatment of left ventricular hypertrophy in patients with hemodialysis.

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TH-PO313

Patient Referral to Active Supportive Care: A Retrospective, Observational Study of Changes in Demographics and Outcome Thalakunte M. Muniraju,[1] Frances E. Marr,[1] Therese Wood,[1] Christopher John Chisholm,[1] Alison Brown,[1] Neil S. Sheerin.[1,2] *[1]Renal Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom; [2]Newcastle University, Newcastle upon Tyne, United Kingdom.*

Background: The majority of patients with advanced chronic kidney disease (CKD) have a significant number of comorbidities and high mortality rate. Renal replacement therapy may not be the ideal or preferred treatment for all patients with advanced kidney disease.

Methods: Active supportive (conservative) care (ASC), is well recognised as one of the management options in patients with advanced CKD and significant comorbidity. This study was undertaken to look at the demographics and survival of patients referred for ASC.

A retrospective, observational study of all patients who were referred for ASC from March 2002 to December 2010 at Newcastle Freeman Hospital Renal Unit (United Kingdom) was undertaken. Patients were identified and demographic data obtained from ASC database. Patients other demographics and estimated Glomerular Filtration Rate (eGFR) were collected using the hospital electronic record.

Results: A total of 387 (186 male and 201 female) patients were referred for ASC during the study period. The number of patients referred more than doubled from 2002 to 2003 and has remained consistent for the 8 years thereafter. The average age of the patients referred was 81.46 years, and it has been gradually increasing since 2006. Average eGFR was 17.00 mls/min/1.73m^2, with no significant difference between years. Out of 387 patients, 286 are deceased and 89 remain under follow up. 12 patients changed modality. The average time on ASC for the 286 deceased patients was 14.02 months.

Conclusions: This study demonstrates that number of patients referred for ASC has increased over time with increasing awareness, patient education and preference. There is no significant difference in eGFR at the time of referral over time, however, the age of referral has gradually increased since 2006. The average time spent on ASC is just over a year. This could represent more effective low clearance care. These findings demonstrate significant service implications to the trust and may reflect a shift in attitude.

TH-PO314

Multiple-Intervention Model May Preserve Better the Renal Function of Patients with Type 2 Diabetes Mellitus and Early Nephropathy Compared with the Standar Care Model Héctor R. Martínez Ramírez, Alfonso M. Cueto-Manzano. *Unidad de Investigación Medica en Enfermedades Renales, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

Background: Renal function (RF), can be preserved by trained family physicians (FP), in DM2 and early nephropathy (EN), but negative lifestyle (LS) habits weren't modify. Multiple-Intervention Model (MIM), could be an option. Aim: To determine the effect of two health-care models on RF in DM2/ EN patients.

Methods: One unit received MIM, other standar care model (SCM). All FP were trained on EN. Patients in MIM received 2h/week an educative intervention by a multidisciplinary team for 4 wks in small self-help groups: emotional (social worker); nutritional (dietitian); exercise (physical trainer); and health-related problems (FP); evaluated at 0 and 6 months by LS questionnaires, clinical and biochemical (Mx. score is 100=better SL)

Results: Of 113 patients included; 44 in MIM and 48 SCM ended 6 moths of follow up.

Differences in lifestyle questionnaire habits	MIN (N 44)	SCM (N 48)
Knowledge	2.6 ± 3.0†*	0.8 ± 3.0
Adherence	2.9 ± 11.7	0.6 ± 4.9
Emotion	2.5 ± 3.8†*	0.30 ± 3.3
Exercise	1.6 ± 3.8†*	-0.08 ± 3.5
Tobacco Consumption	0.32 ± 1.3	0.13 ± 1.1
Alcohol Consumption	0.81 ± 2.1*	0.51 ± 2.0
Diet	3.4 ± 5.0*	2.5 ± 5.5*
Total	11.7 ± 12†*	5.6 ± 12*
Differences in clinical and biochemical variables		
Body mass index (Kg/m2)	-0.77 ± 1.3†*	-0.26± 1.07
Waist circumference (cm)	-2.1 ± 3.5†*	-0.14 ± 5.0
Systolic BP (mmHg)	-10.4 ± 20*†*	-14.2 ± 20
Diastolic BP (mmHg)	-3.9 ± 12 *	-5.2 ± 11*
Hb A1C, (%)	-0.84 ± 2.1†*	-0.21 ± 1.9*
Cholesterol (mg/dl)	-6.5 ± 32	-0.47 ± 47
Triglycerides (mg/dl)	-19.3 ± 32†	-0.31 ± 11
HDL-cholesterol (mg/dl)	1.18 ± 9.5	2.5 ± 11
LDL-cholesterol (mg/dl)	-3.5 ± 26	-0.5 ± 30
Creatinine (mg/dl)	0.04 ± 0.19	0.03 ± 0.16
eGFR (ml/min/1.73m2)	-2.2 ± 14.6	-3.19 ± 15.3
Albumin/creatinine ratio (mg/g)	-32.5 ± 104†	51.0 ± 246

* $p < 0.05$ vs baseline the same model; † vs conventional health-care model

Conclusions: A MIM could helpful to improve the LS of patients with DM2 and EN, and preserve better their renal function

TH-PO315

Epidemiology of Acute Kidney Injury in Critically Ill Patients: A Multicenter-Multinational Study Nattachai Srisawat, Florentina E. Sileanu, John A. Kellum. *For the AKI-6 Investigators, The CRISMA center, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: Estimates of the ICU period prevalence of acute kidney injury (AKI) have varied from 20% to 70% across different centers. Estimates of hospital mortality and dialysis rates have also varied. Variation could be due to actual differences in disease incidence and clinical course or it could simply be a function of data ascertainment and application of diagnostic criteria.

Methods: We conducted a retrospective study of ICU patients treated at 6 academic hospitals, 2 each in North America, Europe and Australia. Our aim was to determine and compare the occurrence and outcomes of AKI in the ICU using standard methods across all centers.

Results: Of 15,567 critically ill patients, 435 were excluded due to history of chronic kidney disease or insufficient data. Reference creatinine was defined by the baseline creatinine if available or the lower of the ICU admission creatinine or the creatinine derived from MDRD formula if no baseline was available. We used the modified RIFLE criteria as proposed by the AKI Network (AKIN). Since urine output was not available from some centers, we examined the variation in AKI rates and outcomes using creatinine criteria alone. The ICU period prevalence of AKI (creatinine data only) varied from 14.6% to 43.8% (P<0.001). Hospital mortality rates of AKI patients by center varied from 20.4% to 35.9% (P<0.001). Even after removing the center with the largest difference in AKI event rate compared to the rest and after excluding another center with the largest difference in hospital mortality there was still significant variation in both ICU period prevalence for AKI (P<0.001) and associated hospital mortality (P<0.001).

Conclusions: Variation in occurrence of AKI and in associated hospital mortality is not explained on the basis of varied application of AKI criteria. Differences in epidemiology of AKI across centers may be due to difference in case mix or to secular trends in therapy. Multicenter studies of AKI epidemiology may lead to improved understanding of risk factors and prevention and treatment strategies for AKI.

TH-PO316

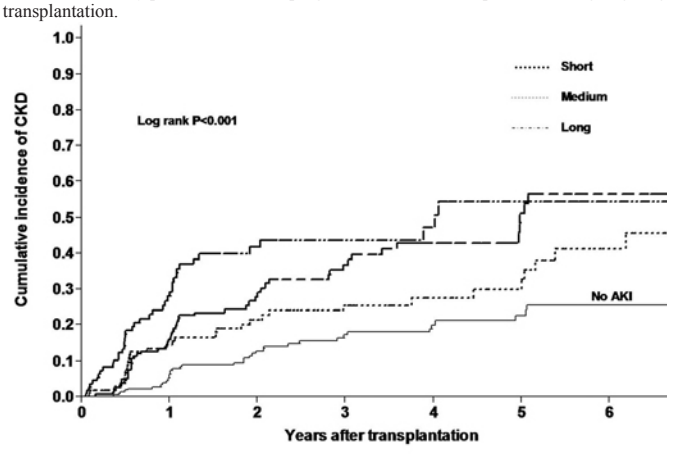
The Duration of Postoperative Acute Kidney Injury and Cumulative Incidence of Chronic Kidney Disease after Lung Kransplantation Edgard I. Wehbe,[1] Martin J. Schreiber,[1] Marie M. Budev,[2] Rachel Lauren Brock,[1] Sevag Demirjian,[1] Brian R. Stephany.[1] *[1]Nephrology and Hypertension, Glickman Urological and Kidney Institute; [2]Pulmonary and Critical care, Cleveland Clinic.*

Background: To determine if the duration of perioperative acute kidney injury (AKI) after lung transplantation predicts chronic kidney disease (CKD)

Methods: We retrospectively evaluated data on 657 patients who underwent lung transplantation from 1997 to 2009. AKI was defined by absolute rise in creatinine by >= 0.3 mg/dl and categorized into three stages by the magnitude rise in creatinine according to the AKIN classification and by the duration from baseline to nadir of creatinine (short (less than 5 days), medium (5–10days) or long (10 days or more)). Outcomes analyzed were the cumulative incidence of CKD stage 4 or 5 (eGFR <30 ml/min/1.73 m2) on long term follow-up

Results: We identified 424 patients (65 %) who had at least one AKI event in the first 2 weeks after transplantation. 115 (17.5%), 184(28%) and 125(19%) experienced short, medium and long duration AKI respectively. At one year the cumulative incidence of CKD was 15%, 16% and 27% in the short, medium and long duration AKI respectively. Figure 1 shows the competing risk analysis for the cumulative incidence of CKD stratified per duration of AKI. After adjusting for appropriate covariates (age, gender, sex, type of lung transplant, post-transplant HTN and DM, baseline creatinine, primary diagnosis leading to transplant), the hazard ratio for CKD was 2.0 (95% CI 1.2-3.3), 2.7(95% CI 1.7-4.2) and 4.4 (95% CI 2.7-7.0) for short, medium and long duration AKI respectively.

Conclusions: This study showed that the duration of AKI is independently associated with CKD and may provide additional prognostic information in patients undergoing lung transplantation.



TH-PO317

Rapid Progression of Chronic Kidney Disease Is Associated with Cardiovascular Mortality in the Japanese Population: The Gonryo Study Tae Yamamoto,[1] Masaaki Nakayama,[3] Mariko Miyazaki,[1] Hiroshi Sato,[5] Toshinobu Sato,[4] Sadayoshi Ito.[2] *[1]Division of Blood Purification, Tohoku University Hospital, Sendai, Japan; [2]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [3]Division of Nephrology, Endocrinology Vascular Medicine and Diabetology, Fukushima Medical University, Fukushima, Japan; [4]Nephrology, Sendai Shakaihoken Hospital, Sendai, Japan; [5]Clinical Pharmacology, Tohoku University Graduate School of Pharmacology, Sendai, Japan.*

Background: Chronic kidney disease (CKD) is a public health problem due to a high risk for cardiovascular disease (CVD). Currently, decreased estimate glomerular filtration rate (eGFR) and proteinuria are accepted as independent risk factors. However, the reliability of the decline rate of eGFR for predicting cardiovascular mortality is not fully settled.

Methods: The study comprised 2,694 CKD patients recruited from 11 outpatient nephrology clinics, and they classified by eGFR in six CKD stages including stage 3A and 3B. The progression of CKD was defined as the decrease of eGFR with increasing CKD stages during two years. Risks of CVD, all-cause mortality and initiating dialysis were examined.

Results: During the observation time, 192 cases initiated to chronic dialysis therapy, and CVD events or death occurred in 135 patients. The frequencies of the CKD progression were 10% in stage 1, 30% in stage 2, 22% in stage 3A, 16% in stage 3B and 14% in stage 4, and it did not differ significantly by underlying renal diseases. Not only dialysis induction (6%, 1%, p<.001) but also CVD mortality (8%, 3%, p<.001) increased significantly in the CKD progression group compared to the non-progression group, and the odds ratio for CVD mortality was 2.78 (95% confidence interval, 1.77-4.35) by logistic regression analysis. When we focused for stage3, the CVD mortalities differed significantly between groups of the CKD progress in stage 3B, while it did not in stage 3A.<<

Conclusions: The progression of CKD by eGFR increased risk of CVD events and death. The subdivision of CKD stage 3 is useful to classify the higher risk people in early CKD.

Funding: Government Support - Non-U.S.

TH-PO318

Natriuretic Peptides as Predictors of All-Cause Mortality in Outpatients with Chronic Heart Failure and Renal Dysfunction Bård Waldum,[1] Arne S. Westheim,[2] Ingrid Os.[1] *[1]Department of Nephrology, Oslo University Hospital, Ullevål, Oslo, Norway; [2]Department of Cardiology, Oslo University Hospital, Ullevål, Oslo, Norway.*

Background: Brain natriuretic peptide (BNP) is released from the heart muscle cells in response to wall distension, and is a valuable diagnostic marker of heart failure (HF) and an independent predictor of prognosis in patients with chronic HF. Renal dysfunction is considered a confounding factor in the evaluation of BNP in HF patients, and since renal failure is common in HF patients, the interpretation of BNP levels is often limited in clinical practice. The aim was to investigate the prognostic utility of BNP in outpatients with chronic HF and renal dysfunction.

Methods: Valid data on BNP were available in 1930 outpatients with chronic HF included in the Norwegian heart failure registry from October 2000 to March 2010. Since analyzes were performed locally with various methods, quartiles of BNP at the last visit were defined at each center among patients with normal renal function (MDRD eGFR ≥ 60 ml/min). Patients with renal dysfunction (eGFR < 60 ml/min) were then stratified to four BNP stages according to the same quartile limits. Multivariate Cox regression models were used to investigate the prognostic utility of BNP with respect to all-cause mortality.

Results: Renal dysfunction was present in 734 patients (38 % of the total population). Median age was 76 years and 69 % were men. 58.7 % (431 patients) had BNP levels in the highest stage defined by quartiles in the patients without renal dysfunction. BNP stage independently predicted all-cause mortality (HR 1.47, 95 % CI 1.22-1.76, p< 0.001) together with lower systolic blood pressure, higher NYHA class, age and lower GFR. Two year survival of patients in the highest BNP stage was 61 % compared to 88 % in lowest BNP stage (p<0.001). No interaction between the prognostic information of BNP levels and renal dysfunction were found.

Conclusions: BNP level in outpatients with HF is a strong independent predictor of all-cause mortality and provide important prognostic information also among patients with renal dysfunction even though they are at greater risk of having increased levels of natriuretic peptides.

TH-PO319

How Well Do Family Practitioners Record Chronic Kidney Disease in 6 Million Patients? Poorva Jain,[1] Paul Cockwell.[2] *[1]Primary Care Clinical Sciences, University of Birmingham, Birmingham, United Kingdom; [2]Department of Nephrology, Queen Elizabeth Medical Centre, Birmingham, United Kingdom.*

Background: Chronic Kidney Disease (CKD) prevalence was reported as 10% in a primary care based cohort in the UK. However national estimates are much less at 4.3% but are derived from whether the practices diagnose the patient as having CKD using specific 'codes' introduced in 2007. We investigated the prevalence of CKD and whether these patients were coded as having CKD.

Methods: The Health improvement Network (THIN) is an anonymised database of 6.7 million patients from 426 primary care centres in the UK. THIN contains the patient's demographic information, consultations, laboratory results and prescriptions. We collected the demographics, laboratory creatinines and estimated and the 'codes' for CKD. The age standardised prevalence of CKD stages 3-5 was calculated between 2005-2009 using 1)A single creatinine transformed into MDRD GFR (Note IDMS calibrated creatinine MDRD equation was used for 2009 as only by 50% of UK laboratories) 2)A single laboratory eGFR 3) Codes for CKD.

Results: The mean age of CKD stage 2-5 (77.58±12.94) versus those with a GFR >60 (53.56±16.55) . The majority of patients with CKD were female except in CKD stage 5.The prevalence of CKD stages 3-5 were in years 2005, 2006, 2007, 2008 and 2009, using a single creatinine, 7.32%, 7.70 . 7.81 %, 7.36% and 7.88 respectively. The reduction in prevalence in 2008 estimates was likely due to the change to IDMS creatinine.

In 2009 CKD 3a is the most prevalent in the cohort (5.12%,and CKD was more common in women compared to men The prevalence was lower using lab reported eGFR.

CKD prevalence

%	2005	2006	2007	2008	2009
Using eGFR*	7.32	7.70	7.81	7.36	7.88
Using lab eGFR"	1.09	2.08	5.04	5.73	5.87
Practice Diagnosis of CKD	0.46	1.17	4.05	4.82	5.11

* single creatinine converted to MDRD eGFR "lab reported MDRD eGFR

The coded prevalence was again even further reduced and prevalence of CKD 3-5 was 5.11% in 2009.

Conclusions: The prevalence of CKD is much lower than previously reported studies, especially using laboratory eGFR, but family practice clinicians may not be aware that their patients have CKD.

TH-PO320

A Longitudinal Study of the Effect of Smoking on Progression and Survival in CKD Darren Green, Philip A. Kalra. *Vascular Research Group, Manchester Academic Health Sciences Centre, Salford Royal Hospital, United Kingdom.*

Background: Smoking affects vascular integrity, blood pressure and inflammation. Smoking-related lung disease is associated with polycythemia and loss of acid-base regulation. The theoretical effect of smoking on patients with CKD may therefore be to affect survival, progression of disease or even ESA and oral bicarbonate. However, evidence to support this and to assess the effect of smoking cessation is lacking.

Methods: This was a longitudinal study of the effect of smoking on CKD. 868 patients with CKD stages 3-5 not on dialysis were recruited over 5 years. Patients were classified as current smokers if they had smoked within 12 months, ex-smokers or non-smokers, and were followed up for up to 5 years. End points were death or progression of CKD of eGFR of >3mL/min/1.73m2/year or reaching ESKD. Concomitant clinical data were obtained at baseline and annual intervals.

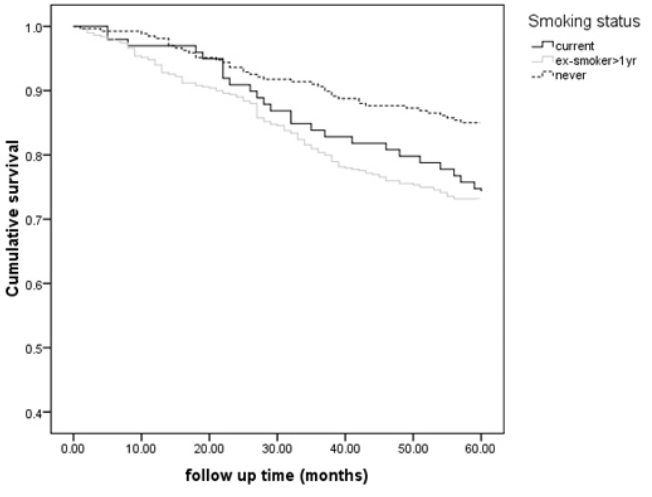
Results: Baseline characteristics are summarized in table 1.

Table 1. Baseline characteristics.

	Current	Ex	Never
N	99	502	267
Age	59	68	62
eGFR	36	34	34
%male	62	70	49
BMI (kg/m2)	26.7	28.4	28.3
Systolic BP	141	136	135
Pack years	30	25	0
CAD%	24	35	19

Current smokers were more likely to develop progressive CKD than ex- or non-smokers. Multivariate logistic regression indicates that this may be due to worse blood pressure control in current smokers. There was no difference in survival between current and ex-smokers but non-smokers showed significantly better outcome.

Figure 1. Survival in CKD according to smoking status



In this model, survival was dependent on pack years of smoking and not whether the patient continued to smoke. COPD did not affect a patient's ESA or oral bicarbonate requirements, nor progression of CKD.

Conclusions: Pack year history and not current smoking predicts survival in CKD. Progression of renal disease in current smokers correlates with poorer blood pressure control. A diagnosis of COPD does not affect patient maedication.

Methods: TH-PO321

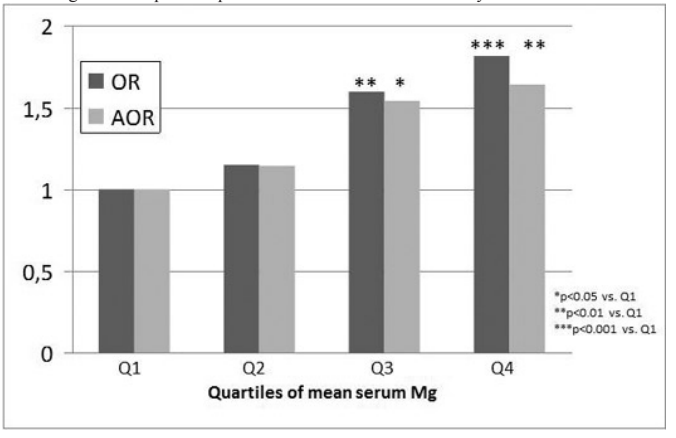
Serum Magnesium as a Predictor of Decline of Kidney Function in Chronic Kidney Disease Steven Van Laecke, Francis Verbeke, Evi V. Nagler, Wim Van Biesen, Raymond C. Vanholder. *Renal Division, University Hospital Ghent, Ghent, Belgium.*

Background: Hypomagnesemia is correlated with endothelial dysfunction, insulin resistance, and hypertension and as such might predict deteriorating kidney function. Analyses on small cohorts of diabetics or renal transplant recipients demonstrated that lower magnesium (Mg) levels are associated with faster decline of estimated glomerular filtration rate (eGFR). The aim was to confirm this association in a larger cohort of prevalent patients with chronic kidney disease (CKD).

Methods: Retrospective single tertiary center analysis of patients with CKD with at least 12 months of follow-up and excluding renal transplant recipients. Adjusted logistic regression analysis to examine the association between mean serum Mg levels and according to quartiles, averaged over the first year of follow-up and stable kidney function based on mean eGFR (CKD-EPI) slope, calculated by linear regression of individual slopes over time. Stable kidney function was defined as a mean eGFR decrease less than -1/ml/min per year during follow-up.

Results: In this cohort of 1670 patients, aged 57.4±17.3 years, 56% male; 97.7% Caucasian and 31.2 percent diabetic, 33.6% had a stable kidney function during a follow-up of 55.±29.9 months. On average, the eGFR slope decreased with -2.42 (IQR 5.44) ml/min/year. Mg was associated with decline in eGFR in univariate analysis and remained so when adjusted for age, diabetes, gender, serum protein, uric acid, C-reactive protein, calcium, phosphorus and hemoglobin (AOR of 1.08 with CI 1.03-1.13; p=0.003 per increase of 0,1mg/dl).The adjusted odds for stable kidney function were 1.66 times higher for the highest vs. the lowest Mg quartile (CI 1.20-2.30; p=0.002).

Conclusions: In a large cohort of diabetic and non-diabetic patients with CKD, low serum Mg is an independent predictor of faster decline of kidney function.



TH-PO322

Blood Pressure and Kidney Failure: A Systematic Review and Meta-Analysis in 2.7 Million People Celine Foote,[1] John Kent Lin,[2] Goodarz Danaei,[2] Majid Ezzati,[3] Mark Woodward,[1,4] Toshiharu Ninomiya,[5] Alan Cass,[1] Josette M. Eris,[6] Vlado Perkovic.[1] *[1]The George Institute for Global Health, AUS; [2]Harvard School of Public Health, USA; [3]School of Public Health, Imperial College London, UK; [4]Johns Hopkins Uni, USA; [5]Kyushu Uni, Japan; [6]Royal Prince Alfred Hosp, AUS.*

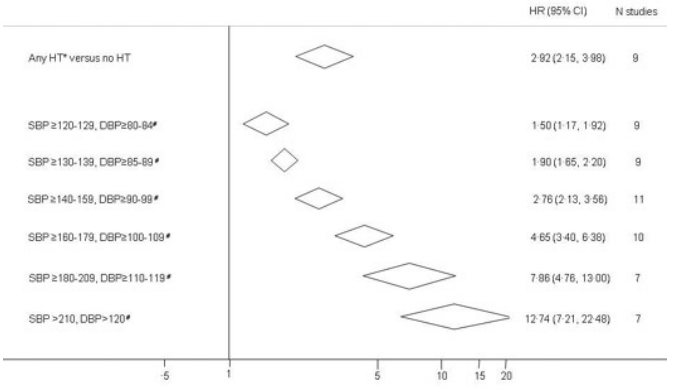
Background: The relative importance of blood pressure (BP) as a risk factor for kidney failure is still not clearly defined. A systematic review and meta-analysis was undertaken to understand the nature and magnitude of the relationship between BP and kidney outcomes.

Methods: We systematically searched MEDLINE and EMBASE for studies published before July 2009. Observational studies assessing the association between BP and kidney failure were included. Summary risk estimates and 95% confidence intervals (CI) were calculated using random effects models. The primary outcome was kidney failure defined as one or both of death due to kidney disease or treated end stage kidney disease (ESKD).

Results: We identified 106 cohorts that included 2.7 million individuals, among whom 7691 cases of kidney failure were observed. A 10mmHg higher systolic BP was associated with a 27% higher risk of kidney failure (95% CI 1.21-1.34, p<0.001) overall, including a 24% higher risk of death due to kidney disease (95% CI 1.16-1.33, p<0.001) and a 38% higher risk of treated ESKD (95% CI 1.32-1.44, p<0.001). The relationship was similar for systolic and diastolic BP, and across participant subgroups.

Conclusions: Association between BP (overall and JNC7 categories) and subsequent kidney failure

P for trend by JNC7 categories<0.001



*SBP≥140mmHg

Reference "optimal BP: SBP <120mmHg, DBP<80mmHg"

Conclusions: BP levels are consistently and directly associated with the risk of kidney failure. The strength of this relationship indicates that lowering BP could lead to a substantial reduction in the burden of kidney failure.

TH-PO323

Pain Management in a Nephrology Department: First Results of the PAIN Study Sarah Zimner-Rapuch,[1] Elisabeth Collin,[2] Nicolas Janus,[1] Sabine Amet,[1] Maud Grimault,[3] Elfie Bruce,[1] Laurence Rouillon,[1] Gilbert Deray,[3] Corinne Isnard-Bagnis,[3] Vincent Launay-Vacher.[1] *[1]Service ICAR Nephrology, Pitie Salpetriere Hospital, Paris, France; [2]Pain Management and Treatment department, Pitie Salpetriere Hospital, Paris, France; [3]Nephrology Department, Pitie Salpetriere Hospital, Paris, France.*

Background: Pain (P) remains a symptom that is underevaluated and not treated as it should be. In renal insufficiency patients (RI), dealing with pain killers is complicated due to dosage adjustment which could frighten the clinician and lead to undertreating patients. The PAIN study (Protocole Antalgie by ICAR in a Nephrology department) aims to evaluate P, its specifics, its management and its consequences on the emotional status of RI patients admitted in our department of nephrology.

Methods: PAIN is a prospective study of all patients who were admitted in our nephrology department. Data collected were: sex, age, weight, height, creatininemia, all the treatments received and if the patient was dialysed or transplanted. For each patient, four forms commonly used in P management were filled to evaluate nociceptive P, neuropathic P, care related P and emotional status respectively.

Results: During 3 months, 34 patients have been included, 59% males; median age 60 years. Eight patients were hemodialyzed and 4 had received a renal graft. Median estimated glomerular filtration rate was 39 ml/min/1.73 m2. 44% of patients received a pain killer. 20% had neuropathic P, among whom 6% were not treated. 76% of patients suffered from care related P and only 18% received a preventive treatment. Anxiety and depression occurred in 27% and 12 % respectively. Only one patient received an antidepressant drug. A dosage adjustment was necessary for 100% of the prescribed treatments and 50 % of drugs were not well renally adjusted. A week before the P evaluation, the mean score was 5 out of 10 in a numeric P scale, and on the day of evaluation, the score was 1.4.

Conclusions: Patients included are not well treated and their emotional status is barely taken into account. Therefore, it is crucial to relieve these patients, with appropriate drugs renally adapted. Relief seems to start with the medical staff being concerned.

TH-PO324

The German Chronic Kidney Disease (GCKD) Study: Design and Methods Kai-Uwe Eckardt, The GCKD Study Group. *Nephrology and Hypertension, University Clinic Erlangen, Erlangen, Germany.*

Background: Chronic kidney disease (CKD) is being recognized as a global health problem, but the conditions leading to CKD, the health impact of CKD and the prognosis of patients affected by CKD differ markedly. In particular renal failure and cardiovascular mortality are competing risks. Opportunities of targeted intervention are very limited so far and require an improved understanding of the natural course of CKD, of risk factors associated with various comorbidities and outcomes of the underlying pathomechanisms.

Methods: The German Chronic Kidney Disease (GCKD) - Study is a prospective observational national cohort study, aiming to enrol 5000 patients with CKD of various aetiology referred to nephrologists. At the time of enrolment patients have an eGFR of 30-60 ml/min x 1.73 m² or overt proteinuria in the presence of an eGFR above 60 ml/min x 1.73 m². A core set of lab parameters is determined in a central lab. Standardized collection of biomaterials, including DNA, serum, plasma and urine will allow identification and validation of biomarkers associated with CKD, CKD progression and related complications using hypothesis-driven and hypothesis-free approaches. Recruitment and patient-follow is organized through a network of academic nephrology centres collaborating with practising nephrologists throughout the country.

Results: Close to 3000 patients have already been successfully enrolled by the beginning of June. Study procedures have proved effective and biomaterials of all patients have been frozen shortly after collection and transferred to the central biobank on dry ice. Patient interview audits and other quality measures indicate good data quality.

Conclusions: The GCKD Study will establish one of the largest cohorts to date with CKD patients not requiring renal replacement therapy within central Europe. Similarities in its design with other observational CKD studies, including cohorts that have already been established in the US and Japan will allow comparative and pooled analyses to identify important regional differences and increase the statistical power.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO325

Kidney Function and Age-Related Macular Degeneration Gareth J. McKay,[1] Chris C. Patterson,[1] Gerard Savage,[1] Orla McNally,[1] A.J. McKnight,[1] Damian G. Fogarty,[1] Giuliana Silvestri,[2] Alexander P. Maxwell.[1] *[1]Centre for Public Health; [2]Vision and Vascular Science, Queen's Universtity Belfast, United Kingdom.*

Background: Age-related macular degeneration (AMD) shares risk factors and pathways with several diseases affecting the kidney most notably those relating to complement activation and premature cardiovascular disease. Previous reports demonstrate association between reduced kidney function and AMD. Our primary analysis sought to assess modulation of AMD risk as a consequence of kidney function using glomerular filtration rate (eGFR) in an AMD case-control cohort, with a secondary analysis to assess genetic variation in key alternative complement pathway genes and their effect on eGFR.

Methods: eGFR was estimated by CKD-EPI equations on serum creatinine levels measured in 307 AMD cases (219 late AMD; 88 early AMD) and 172 no disease controls. Binary logistic regression analysis estimated effect size of eGFR on AMD risk after adjustment for sex and age. Further adjustment was made for additional covariates such as genetic risk in key complement genes, Factor H, Factor B, Component 2 and Component 3 and also smoking status. Ordinal regression was used to evaluate the effect of complement genetic variants on eGFR in a secondary analysis.

Results: In the primary analysis, a non-significant decreased risk for all types of AMD (early and late) was estimated for moderate CKD (eGFR <60ml/min/1.73m²) OR=0.62 (95% CI: 0.24-1.63; P=0.38) after adjustment for age and sex. Subset analysis for late AMD only, showed similar effect. Adjustment for complement genetic covariates and also smoking status failed to further modify risk. Secondary analysis assessing genetic variation in key complement regulatory genes, failed to detect any association with eGFR in an ordinal regression analysis.

Conclusions: Although several independent studies have demonstrated increased risk of AMD associated with impaired renal function, we were unable to replicate these findings in our cohort. The cross-sectional nature of our sample with potential confounding may have limited our findings. Secondary analysis assessing genetic variation in complement-related genes also failed to demonstrate association with eGFR.

TH-PO326

Impaired Kidney Function at Hospital Discharge Has a Major Impact on Long-Term Survival of Critically Ill Patients Recovered from Renal Failure Susanne Stads,[2] Gijs Fortrie,[1] Jasper Van Bommel,[2] Robert Zietse,[1] Michiel G.H. Betjes.[1] *[1]Nephrology, Erasmus MC, Rotterdam, Netherlands; [2]Intensive Care, Erasmus MC, Rotterdam, Netherlands.*

Background: Acute renal failure (ARF) in patients on the intensive care unit (ICU) necessitating renal replacement therapy (RRT) is associated with high mortality. However, little is known about renal recovery in surviving patients and the effect of impaired kidney function on long-term survival after hospital discharge.

Methods: A retrospective cohort study was performed including all patients older than 18 years (n=1218) admitted to the intensive care unit (ICU) of a tertiary care center who received continuous RRT. Data were collected for the period 1994 to 2010.

Results: In-hospital mortality was 54.9%. After hospital discharge, the overall mortality was 75.3 % after a median follow up of 8.5 years (range 1-17 years). Univariate analysis showed that age, surgical or non-surgical patients and kidney function at discharge were associated with survival. Multivariate Cox regression analysis of the association of kidney function and patient survival was performed, adjusting for the variables: age, sex, surgical or non-surgical patients and cause of ARF on the ICU. eGFR at hospital discharge was independently associated with long-term survival (p <0.001). Only 87 (15.8%) patients were discharged with an eGFR > 90 ml/min (using the MDRD formula). In this group 5 and 10-years survival were respectively 77.6% and 66.7%. Mortality risk increased for every increase in stage of CKD (hazard ratio 1.25, p<0.001). Patients discharged with an eGFR <30 ml/min (CKD 4-5, 37.3% of patients at hospital discharge) had a 5 and 10 years survival of only 42.5% and 28.5%. After exclusion of the patients known with CKD before admittance to the ICU, the eGFR at hospital discharge remained strongly negatively associated with long-term survival.

Conclusions: The majority of critically ill patients who received CRRT on the ICU have an impaired kidney function at hospital discharge, which has a major negative impact on their long-term survival. These results stress the importance of preserving kidney function in ICU patients and the need for long-term nephrological follow-up.

TH-PO327

Presence of Diabetes in CKD Is Associated with Higher Rates of Comorbidities and Medication Use: Results from a Large US National Healthcare Claims Database Justo Sierra-Johnson, Trong Le, Kecia Godbey, James R. Voelker. *Eli Lilly and Company, Indianapolis, IN.*

Background: To describe the pharmacological treatment pattern and comorbidities among US patients diagnosed with chronic kidney disease (CKD) with and without diabetes (DM).

Methods: Employing a retrospective cohort study design on a large US national healthcare claims database (MarketScan), patients aged 18+ with a diagnosis for CKD (ICD-9 code: 585.x) between January 1, 2007, and December 31, 2008 were identified and the first CKD diagnosis was the index date. All patients selected were required to have 12 months of continuous pharmaceutical and medical benefit enrollment. Descriptive statistics were summarized for patient demographics, comorbid medical conditions, and medication use. Chi-square and Wilcoxon tests were applied.

Results: There were 116,512 patients that met inclusion criteria with a mean age of 65 years and 56% male. The prevalence of diabetes in CKD patients was 45%. Significantly higher proportion of CKD w/DM had a diagnosis code for cardiometabolic risk factors (100% vs. 71%, p<0.0001), anemia (46% vs. 35%, p<0.0001), cardiovascular disease (39% vs. 25%, p<0.0001), nephritis & nephrosis (34% vs. 27%, p<0.0001), other renovascular disease (27% vs. 22%, p<0.0001), disorders of fluid electrolyte & acid-base balance (20% vs. 14%, p=0.0083) and cerebrovascular disease (17% vs. 12%, p<0.0001) than CKD w/o DM . These rates increased as CKD stages increased. CKD w/DM also had significantly higher rates of medication use for hypertension, lipid disorders, diabetes and anemia compared to CKD w/o DM. Furthermore, CKD patients w/DM incurred higher overall costs (total medical and drug costs) compared to CKD w/o DM.

Conclusions: In CKD, patients with DM have higher rates of cardio- and cerebral-vascular diseases, cardiometabolic conditions, anemia, and renal disorders (nephritis, nephrosis, renovascular, fluid, electrolyte & acid-base) than patients without DM. Medication use and medical costs were also higher in the DM cohort. Thus, CKD patients with DM represent a particularly high risk group for associated comorbidities that require extensive medical resources.

Funding: Pharmaceutical Company Support

TH-PO328

Serum Bicarbonate, Anion Gap, and Cardiorespiratory Fitness in US Adults Matthew K. Abramowitz, Thomas H. Hostetter, Michal L. Melamed. *Department of Medicine, Albert Einstein College of Medicine, Bronx, NY.*

Background: Chronic metabolic acidosis as a manifestation of chronic kidney disease is believed to contribute to skeletal muscle protein breakdown and insulin resistance. In the general population, levels of serum bicarbonate and anion gap (AG) have been associated with reduced muscle strength, insulin resistance, and hypertension. Whether these associations extend to other cardiovascular disease risk factors is unknown.

Methods: We examined the association of serum bicarbonate and AG with cardiorespiratory fitness in adult participants aged 20-49 years in the National Health and Nutrition Examination Survey 1999-2004.Fitness was determined by submaximal exercise testing and categorized based on age- and sex-specific cutpoints.

Results: The mean serum bicarbonate was 24.6 mEq/L (SE 0.1) and the mean AG was 10.26 mEq/L (SE 0.18). After multivariable adjustment, sex, fasting length, soda consumption, systolic blood pressure, serum phosphate, and hemoglobin were independently associated with both serum bicarbonate and AG. For each, the direction of association differed for serum bicarbonate and AG. Low fitness was most prevalent among those in the lowest quartile of serum bicarbonate or highest quartile of AG (18.9% (95% confidence interval (CI) 15.6-22.2) and 20.7% (95% CI 16.8-24.6), respectively). After multivariable adjustment, a 1 SD higher serum bicarbonate or AG was associated with an odds ratio for low fitness of 0.80 (95% CI 0.70-0.91) and 1.30 (95% CI 1.15-1.48), respectively. The associations of bicarbonate with fitness may have been mediated by differences in percent lean body mass (%LBM), which was directly associated with serum bicarbonate. Adjustment for %LBM rendered associations of serum bicarbonate with fitness non-significant.

Conclusions: In conclusion, lower levels of serum bicarbonate and higher levels of AG are associated with lower cardiorespiratory fitness in adults aged 20-49 years in the general US population. Further studies are needed to elucidate the determinants of serum bicarbonate and AG in persons without overt kidney disease and to prospectively examine associations with fitness and other CVD risk factors.

Funding: NIDDK Support, Other NIH Support - CTSA grants UL1RR025750, KL2RR025749 and TL1RR025748

TH-PO329

Benefits of Revascularization for ARVD Patients with Flash Pulmonary Edema James Ritchie, Constantina Chrysochou, Philip A. Kalra. *Vascular Research Group, Manchester Academic Health Sciences Centre, University of Manchester, Salford Royal Hospital, United Kingdom.*

Background: Though there are case series suggesting a benefit of renal artery revascularization in the context of flash pulmonary edema (FPE) and atherosclerotic renovascular disease (ARVD), randomized data is lacking. By analyzing data collected prospectively since 1995 in our local ARVD patient cohort we aimed to quantify any benefits of intervention in patients presenting with ARVD and FPE.

Methods: Clinical data for all patients (n=819) were reviewed for evidence of presentation with FPE (acute decompensated heart failure with preserved ventricular function) prior to diagnosis of ARVD. Annual follow up data (range 1-12 years, mean 5 years) were reviewed for endpoints of death, cardiovascular and dialysis events. Comparisons were drawn between: 1) an interventional group - FPE patients revascularized (n=15) vs. FPE patients not revascularized (n=44) and 2) a medically managed group - FPE patients not revascularized (n=44) vs. non-revascularized ARVD patients without FPE (n=624). Cox proportional hazards corrected for age, sex, BP, eGFR, vessel patency, proteinuria and angiotensin blockade was performed.

Results: When patients with FPE who underwent revascularization were compared to those not revascularized, there was a dramatic and statistically significant reduction in hazard ratio for death (HR 0.08, p=0.015). For the same group, a reduction in cardiovascular events approached statistical significance (HR 0.05, p=0.059). There were insufficient dialysis events in the revascularized group for meaningful analysis.

Comparison of outcomes of non-revascularized patients with and without FPE showed statistically significant increases in HR for death (HR 2.37, p=0.011) and cardiovascular events (HR 2.4, p=0.031) for those with FPE.

	INTERVENTION		MEDICAL	
ENDPOINT	HR (95% ci)	p	HR (95% ci)	p
Death	0.08 (0.01-0.61)	0.015	2.37 (1.22-4.6)	0.011
Cardiovascular	0.05 (0.01-1.12)	0.059	2.4 (1.09-5.32)	0.031
Dialysis			2.66 (0.91-7.8)	0.075

Conclusions: Our observational data confirms that in the context of FPE, renal artery revascularization for ARVD appears to offer significant benefits over medical management.

TH-PO330

Serum Skeletal Alkaline Phosphatase (SAP) Levels Do Not Explain the Associations of Serum Total Alkaline Phosphatase (TAP) with ↑ Serum C-reactive Protein (CRP): National Health and Nutrition Examination Survey (NHANES) Rebecca Filipowicz,[2] Guo Wei,[2] Tom H. Greene,[1,2] Alfred K. Cheung,[1,2] Kalani L. Raphael,[1,2] Srinivasan Beddhu.[1,2] *[1]VA; [2]Univ Utah.*

Background: Higher serum TAP levels are associated with ↑ serum CRP levels in the general and CKD populations. It is unclear to what extent this association is related to bone disease. Therefore, we examined the associations of serum SAP levels with ↑ serum CRP (> 3 mg/L) in 8,515 adult participants in the 1999-2002 NHANES.

Methods: Details of NHANES data and sample collection have been published elsewhere. TAP, SAP, and CRP were measured in central labs following standardized protocols. The component of TAP not explained by SAP was represented from the residuals of a regression of log transformed TAP on log transformed SAP. Stata XI was used to conduct analyses.

Results: The mean (± SD) age was 46.3 ± 14 years, 48.5% were men, and 10% African-Americans. The median serum TAP levels and SAP levels were 73.8 IU/L and 13.1 µg/L, respectively. The median eGFR was 86.1 mL/min/1.73 m². 37% of the cohort had ↑ serum CRP.

Associations of serum TAP and SAP with odds ratios (OR) for ↑ serum CRP

	Entire cohort: OR (95% CI)	Non-CKD: OR (95% CI)	CKD: OR (95% CI)
Model 1*			
OR per doubling of serum TAP	1.78 (1.56, 2.02)	1.75 (1.53, 2.01)	2.08 (1.27, 3.43)
Model 2*			
OR per doubling of serum SAP	0.95 (0.86, 1.06)	0.89 (0.80, 1.01)	1.07 (0.74, 1.52)
OR per doubling of residuals**	3.82 (3.20, 4.57)	3.70 (3.09, 4.41)	5.24 (3.24, 8.47)

**Logistic regression models adjusted for demographics, MI, CHF, stroke, DM, SBP, DBP, waist circumference, eGFR, serum Ca, P, AST, ALT, bilirubin and GGT. **OR per doubling of ratio of observed TAP to the component of TAP accounted for by SAP

Conclusions: Bone disease and liver disease are unlikely explanations for the associations of serum TAP with inflammation. Further studies are needed to unravel the mechanisms of these associations.

Funding: NIDDK Support, Other NIH Support - National Center for Research Resources

TH-PO331

Association between Aortic Pulse Wave Velocity (aPWV) and Kidney Function Decline: The Health ABC Study Magdalena Madero,[1] Carmen A. Peralta,[2] Ronit Katz,[3] Robert B. Canada,[4] Linda F. Fried,[5] Samer S. Najjar,[6] Eleanor Marie Simonsick,[8] Kushang Patel,[9] Kim Sutton-Tyrrell,[7] Michael Shlipak,[2] Edward G. Lakatta,[8] Mark J. Sarnak.[10] *[1]Instituto Nacional de Cardiologia; [2]UCSF; [3]Wash U; [4]Tenesse U; [5]VA Pitt; [6]Medstar; [7]Pitt U; [8]NIA; [9]NIH; [10]Tufts.*

Background: Large vessel arterial stiffness is associated kidney disease in cross sectional studies.The association of large artery stiffness, measured by aPWV, and kidney function decline is less well established.

Methods: 2129 older adults with baseline measurement of aPWV and at least two measurements of cystatin C (year 3 or year 10 of follow-up). Linear regression was used to study the association between log transformed aPWV with kidney function decline (ml/min/1.73 m²/year). Logistic regression was used to evaluate associations with "rapid decline" (defined as $eGFR_{cysC}$ loss of > 3ml/min/1.73m² per year). Models were adjusted for demographics and cardiovascular (CVD) risk factors

Methods: Higher aPWV was associated with faster rates of decline in demographic adjusted models. Adjustment for blood pressure did not attenuate this association. Additional adjustment for CVD risk factors attenuated this association to a small extent.

Association between aPWV and Kidney Function Decline

aPWV	N	Demo adjusted* β(95% CI)	BP adjusted**β(95% CI)	Fully adjusted***β(95% CI)
Continuos (log transformed)	2129	-0.26 (-0.51, -0.02)‡	-0.26 (-0.50, -0.02)‡	-0.22 (-0.47, 0.02)
Quartiles				
<642	546	0 (ref)	0 (ref)	0 (ref)
642-808	546	-0.07 (-0.33, 0.19)	-0.07 (-0.33, 0.19)	-0.06 (-0.33, 0.20)
809-1052	518	-0.30 (-0.57, -0.02)‡	-0.30 (-0.57, -0.02)‡	-0.31 (-0.59, -0.03)‡
>1052	519	-0.27 (-0.54, -0.01)‡	-0.27 (-0.53, -0.0001)	-0.22 (-0.49, 0.04)

per 1SD increase in aPWV, ‡ p<0.05*adjusted for demographics**Model 1 plus SBP, DBP & HTN meds,***Model 2 plus CVD risk factors

Results: Higher aPWV was also associated with "rapid decline" in demographic adjusted models (OR per 1SD higher log aPWV, 95% CI, 1.33, 1.03, 1.72). The relationship was attenuated after adjustment for blood pressure

Conclusions: Large artery stiffness is associated with decline in kidney function among older adults. These relationships are partly attenuated by cardiovascular risk factors and blood pressure

TH-PO332

Vascular Disease-Associated Mortality during CKD Progression: The MDRD Study Morgan E. Grams,[1] Hocine Tighiouart,[2] Josef Coresh,[1] Mark J. Sarnak.[2] *[1]Johns Hopkins University; [2]Tufts University.*

Background: Patients with vascular disease are at high risk for both progression of CKD as well as mortality prior to ESRD. The goal of this analysis was to characterize the competing risks of ESRD and all-cause mortality associated with vascular disease at various levels of eGFR.

Methods: Data from the Modification of Diet in Renal Disease (MDRD) study (N=1,795, including 840 randomized participants) were used to evaluate the risk of ESRD and pre-ESRD death associated with vascular disease, defined as coronary artery, cerebrovascular, or peripheral vascular disease. ESRD outcomes were obtained from USRDS and mortality from NDI. Cox proportional hazards analyses were adjusted for age, race, sex, blood pressure, cholesterol, current smoking, cause of ESRD, log-24-hour-proteinuria, and eGFR, and censored at ESRD, death, or end of study (12/31/2007), whichever came first. Interactions between vascular disease and eGFR were tested.

Results: Of the 1,722 participants with complete data, 224 (13.0%) had vascular disease. Mean GFR was 35 with a range of 5 to 117 ml/min/1.73 m2. Overall, 64% reached ESRD, and 14% died prior to ESRD. Participants with vascular disease were older, more often white, male, and diabetic, with higher systolic blood pressures, lower diastolic blood pressures, and lower HDL levels. The unadjusted incidence of ESRD increased with decreasing GFR (p for trend: <0.001); rates were similar between participants with and without vascular disease (p=0.6). In contrast, unadjusted mortality rates prior to ESRD were significantly higher among participants with vascular disease. In adjusted analyses, vascular disease remained significantly associated with death prior to ESRD (aHR, 1.66, 95% CI: 1.23-2.24, p<0.001) but not ESRD itself (aHR, 1.11, 95%: 0.91-1.35, p=0.3). There was no significant interaction between vascular disease and eGFR for either outcome (p>=0.4 for both interaction terms).

Conclusions: In the MDRD Study, participants with vascular disease were 66% more likely to die prior to ESRD; however, there was no modification of vascular disease-associated mortality risk by eGFR. In contrast, patients with vascular disease were not more likely to reach ESRD.

Funding: NIDDK Support

TH-PO333

The Associations of Serum Total Alkaline Phosphatase (TAP) with ↑ Mortality Is Not Explained by Serum Levels of Skeletal Alkaline Phosphatase (SAP): National Health and Nutrition Examination Survey (NHANES) Rebecca Filipowicz,[1] Guo Wei,[1] Tom H. Greene,[1,2] Alfred K. Cheung,[1,2] Kalani L. Raphael,[1,2] Srinivasan Beddhu.[1,2] *[1]Univ Utah; [2]VA.*

Background: Higher serum TAP levels are associated with ↑ mortality in the general and chronic kidney disease (CKD) populations. It is unclear to what extent this association is related to bone disease.

Methods: Therefore, we examined the associations of serum SAP levels with mortality in the 1999-2002 NHANES adults with estimated eGFR < 150 ml/min/1.73 m^2 (n=8,515). Details of NHANES data and sample collection have been published elsewhere. The component of TAP not explained by SAP was represented from the residuals of a regression of log transformed TAP on log transformed SAP. Mortality data were obtained from the NHANES Linked Mortality File through December 31, 2006. Stata XI was used for statistical analysis.

Results: The mean (± SD) age was 46.3 ± 14 years, 48.5% were men, and 10% African-Americans. The median serum TAP levels and SAP levels were 73.8 IU/L and 13.1 µg/L, respectively. The median eGFR was 86.1 mL/min/1.73 m^2.There were 705 deaths over a total of 2420 years of follow-up. There were 276 deaths over 864 years in the non-CKD and 429 deaths over 556 years in the CKD sub-populations.

Associations* of serum TAP and SAP levels with mortality

	Enitre cohort HR (95% CI)	Non-CKD population HR(95% CI)	CKD population HR (95% CI)
Model 1			
HR per doubling of serum TAP	1.33 (1.10, 1.60)	1.63 (1.24, 2.13)	1.17 (0.82, 1.67)
Model 2			
HR per doubling of serum SAP	1.05 (0.91, 1.22)	1.08 (0.89, 1.30)	1.15 (0.83, 1.59)
HR per doubling of residuals**	1.68 (1.16, 2.42)	2.11 (1.41, 3.17)	0.92 (0.51, 1.66)

*All Models are adjusted for demographics, MI, CHF, stroke, DM, SBP, DBP, waist circumference, eGFR, serum Ca, P, AST, ALT, bilirubin and GGT. **HR per doubling of ratio of observed TAP to the component of TAP accounted for by SAP

Conclusions: The association of serum TAP with ↑ mortality is not explained by SAP. Further studies are warranted to determine the mechanisms by which increased mortality risk is associated with ↑ levels of serum TAP.

Funding: NIDDK Support, Other NIH Support - National Center for Research Resources

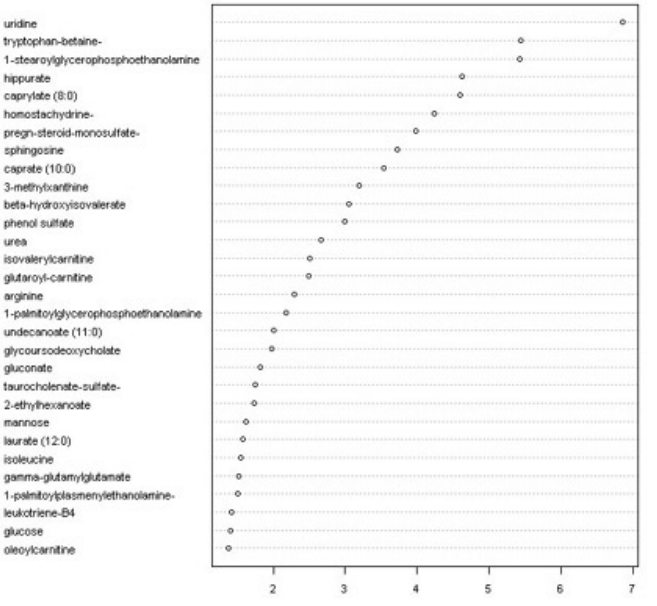
TH-PO334

Plasma Metabolic Signature and Renal Function Decline in Subjects with Type 1 Diabetes and Proteinuria Monika A. Niewczas,[1] Jan Skupien,[1] Elizabeth Kensicki,[2] Jacob Wulff,[2] William Walker,[1] Adam Smiles,[1] Robert P. Mohney,[2] James Warram,[1] Andrzej S. Krolewski.[1] *[1]Research Division, Joslin Diabetes Center, Boston, MA; [2]Metabolon, Inc., Durham, NC.*

Background: Diabetes is characterized by significant metabolic disturbances; however, impact of specific metabolites on the diabetic nephropathy progression has not been studied. This study aimed to determine a plasma global metabolic profile and its association with renal function decline in subjects with type 1 diabetes (T1D) and proteinuria.

Methods: This nested case-control study consisted of 60 subjects with T1D, proteinuria and estimated glomerular filtration rate (eGFR) > 60 ml/min/1.73m2 followed for 10 years. Subjects (n=30) with annual eGFR decline greater than -3.5 ml/min/1.73m2 were defined as decliners. The Metabolon mass spectrometry-based platform (UHPLC-MS/MS, GC-MS) was used to measure biochemicals by a non-targeted approach in baseline plasma samples.

Results: Among 335 biochemicals measured, 31 (9.25%) were significantly different (P<0.05) between decliners and non-decliners. Random Forest analysis identified biochemicals involved in the following pathways as important to the classification scheme: arginine metabolism (arginine, creatine), branched-chain amino acids (valine, isoleucine), medium-chain fatty acids (caprylate), and inflammation (prostaglandin E2, leukotriene B4).



The Importance Plot ranks metabolites according to their contribution to the distinction between decliners and non-decliners. Metabolites are listed on the y-axis in order of decreasing importance.

Conclusions: In this global metabolic profiling study, we identified key biochemicals that were altered at baseline in subjects with T1D, proteinuria and subsequent renal function decline. This study provides important insights into markers of metabolic dysfunction as predictors of diabetic nephropathy progression.

Funding: NIDDK Support, Other NIH Support - Child Health Services Research Training Program

TH-PO335

Long Term Decrimant in eGFR Following Leptospirosis Associated Acute Kidney Injury in the South of Ireland Donal John Sexton, Marek J. Mazur, Joseph A. Eustace. *Department of Renal Medicine, Cork University Hospital, Cork, Ireland.*

Background: Leptospirosis induced acute kidney injury (AKI) is a relatively uncommon disorder. Current data suggests that in the majority of cases eGFR returns promptly to premorbid baseline levels following treatment. There is relatively little data on the rate of renal recovery following AKI in a European population.

Methods: We conducted a cross sectional study of eGFR in all patients with previous acute kidney injury (AKI) due to confirmed acute Leptospirosis (n=27) in the southwest of Ireland over the prior ten years. The association, significance and independance of the relationship between baseline variables at the time of acute kidney injury and subsequent decline in eGFR were assessed by linear regression using SPSS v18 with a type one error rate of 0.05. The descriptive statistics are expressed as mean (sd) unless otherwise stated. eGFR was calculated using the CKD-EPI formula.

Results: Mean (sd); age 52(16)yrs, eGFR prior to AKI 91(18)ml/min/1.72m^2, eGFR post AKI 73(25)ml/min/1.73m^2. Median (IQR); follow up 1(0.5-3)yrs, peak serum creatinine during AKI 562(110-1000)µmol/L with 66.7% (18/27) of patients developing AKIN3, 7.4% (2/27) with AKIN2, 25.9% (7/27) with AKIN1, peak Bilirubin 62 (15-450) µmol/L. 88.9% (24/27) of patients were male. 40.7% (11/27) required renal replacement therapy, the Median (IQR) number of hemodialysis treaments in this group was 8 (3-16) treatments. 70.4% (19/27) of cases of AKI had sterile pyuria at presentation. Serovar type was available in 40.7% (11/27) and consisted of icterohaemorrhagica 25.9%, Hardjo 11.1% and Negat 3.7% of cases.On separate univariate linear regression models the β (95% CI) for the association with decrimant in eGFR at follow up were sex 0.02 (9.9, 26), nadir platelet count -0.51 (-0.48, -0.5) and peak serum bilirubin 0.35 (3.1, 22.6). On multivariate modeling using backward stepwise regression the final model β (95% CI) was sex -0.35 (-0.29, -0.04) and nadir platelet count -0.5 (-0.46, -0.06).

Conclusions: Despite recovery from AKI due to leptospirosis, many patients have significant decrimants in renal function which warrant long term follow up and may possibly be predicted by the nadir platelet count.

TH-PO336

Effect of Extended-Release Niacin/Laropiprant on Lipoprotein(a), Apolipoprotein A1, and Apolipoprotein B Levels in Dyslipidemic Stage 3 Chronic Kidney Disease Patients Andrew G. Bostom,[1] Jinyu Zhang,[2] Joachim H. Ix,[3] Nathan Spence,[4] Diane Tipping,[5] Andrew Tershakovec.[6] *[1]Division of Kidney Diseases, Rhode Island Hospital, Providence, RI; [2]Brown Medical School, Providence, RI; [3]Division of Nephrology, UCSD, San Diego, CA; [4]Division of Medicine, Rhode Island Hospital, Providence, RI; [5]Tipping LLC, Green Lane, PA.*

Background: Given the paucity of data regarding niacin's impact on experimental measures of dyslipidemia in chronic kidney disease (CKD), we examined the drug's effect on apolipoprotein (apo) A1 and B, and lipoprotein(a) [Lp(a)] levels among patients with stage 3 CKD.

Methods: Subjects (n=261) with a baseline estimated glomerular filtration rate (eGFR) of 30 to < 60 ml/min/1.73 m^2 were drawn from two completed trials of extended-release niacin/laropiprant (ERN-L, laropiprant being an inhibitor of niacin-induced flushing; ERN-L at 2g/d, n=177; n=84 placebo). Apo A1, apo B, and Lp(a) were measured serially over 24-weeks.

Results: The ERN-L and placebo groups were comparable with respect to baseline mean age, eGFR, apo A1, apo B, and Lp(a) levels, as well as the distribution of gender, concurrent statin use, and diabetes. Repeated measures analyses revealed that ERN-L, relative to placebo, caused a decrease (95% confidence interval) of -20.5% (-25.1.0%, -16.0%) in mean apo B levels, and – 27.8% (-38.4%, -23.2%) in median Lp(a) levels, as well as an increase of +11.1% (+14.7%, +7.4%) in mean apo A1 levels. Stratified analyses revealed that these effects on apo B, apo A1, and Lp(a) were entirely consistent with what was observed among dyslipidemic patients whose eGFR was ≥ 60 ml/min/1.73 m^2.

Conclusions: Niacin treatment results in robust lowering effects on apo B, and Lp(a) levels, as well as substantial increases in apo A1 levels, among patients with stage 3 CKD. Till now, stage 3-4 CKD patients have been significantly under-represented in secondary cardiovascular disease prevention trials. We conclude that a niacin treatment arm merits serious consideration within any future clinical trials targeting stage 3-4 CKD patients for the potential reduction of cardiorenal outcomes.

TH-PO337

Effect of Extended-Release Niacin on Serum Phosphorus and Fibroblast Growth Factor 23 Levels in Stage 2-3 Chronic Kidney Disease Andrew G. Bostom,[1] Andreea Poenariu,[1] Joachim H. Ix,[2] William Hanlon,[3] Darbie Maccubbin,[3] Michael Steffes.[4] *[1]Division of Kidney Diseases, Rhode Island Hospital, Providence, RI; [2]Division of Nephrology, University of California-San Diego, CA; [3]Merck Sharp & Dohme Corp, Rahway, NJ; [4]Merck Sharp & Dohme Corp, Rahway, NJ; [5]Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN.*

Background: The hypophosphatemic effect of niacin is now increasingly recognized. We examined whether niacin-induced phosphorus-lowering among dyslipidemic stage 2-3 chronic kidney disease (CKD) patients was accompanied by any impact on their levels of the phosphatonin fibroblast growth factor (FGF)-23.

Methods: Intact serum FGF23 and phosphorus (phos) levels were determined in n=327 patients with a baseline estimated glomerular filtration rate (eGFR) of 30 to 74 ml/min/1.73 m^2 randomized to active extended-release niacin (N) 2g/once daily; with or without the prostaglandin-mediated flushing inhibitor laropiprant), or placebo (n=260 N; n=67 on placebo [P]), at weeks 0 (baseline) and 24.

Results: Mean (± SD) age [N= 61 ± 11; P= 61 ± 9 years], eGFR [N=62.8 ± 8.6; P= 62.2 ± 9.4 ml/min/1.73 m^2], as well as both baseline serum FGF23 [N=44.0 ± 18.9; P= 43.2 ± 18.6 pg/ml] and phos levels [N= 3.33 ± 0.48; P=3.39 ± 0.51 mg/dl] were comparable between the groups. While the 24-week change (mean ± SE) in serum phos among patients on active N (-0.45 ± 0.03; -13.5%) relative to P treatment (+0.03 ± 0.06) was highly significant (p <0.001), N treatment (-0.9 ± 1.0) had no effect compared to P (+1.8 ± 2.0) on serum intact FGF23 levels (p=0.238). Our findings confirm the sustained (i.e., 24-week) and relatively robust (-13.5%) hypophosphatemic effect of N in patients with stage 2-3 CKD. Conversely, such ERN treatment has no apparent impact on serum FGF23 levels at these stages of CKD.

Conclusions: Thus use of FGF-23 levels as a surrogate for phos-lowering effects, or as specific entry criteria for trials ostensibly designed to test the hypothesis that hypophosphatemic therapy will reduce cardiorenal outcomes in stage 3-4 CKD, may not be warranted.

Funding: Pharmaceutical Company Support

TH-PO338

Phosphate, CKD Progression and ACE-Inhibition – *A Post-Hoc Analysis of the REIN Trial* Carmine Zoccali,[1] Piero Ruggenenti,[2] Annalisa Perna,[2] Daniela Leonardis,[1] Rocco Tripepi,[1] Giovanni Tripepi,[1] Francesca Mallamaci,[1] Giuseppe Remuzzi.[2] *[1]CNR-IBIM, Clin. Epid. and Physiopath. of Renal Dis. and Hypert., Reggio Calabria, Italy; [2]Mario Negri Institute, Bergamo, Italy.*

Background: Phosphate has been suggested to play a role in the onset and progression of chronic nephropathies.

Methods: We evaluated the relationships between baseline serum phosphate levels, disease progression and response to angiotensin converting enzyme (ACE) inhibition in 331 patients with proteinuric nephropathies prospectively monitored by gold standard procedures in the setting of the Ramipril Efficacy In Nephropathy (REIN) trial.

Results: Independent of treatment, progression to end stage renal disease (ESRD) alone or in combination with doubling serum creatinine was significantly faster in patients with phosphate levels in the higher 3rd and the 4th quartiles than in those with lower phosphate (P<0.001). Similar findings were observed in crude and adjusted analyses considering phosphate as a continuous variable (P≤0.004). The ramipril renoprotective effect progressively decreased for increasing serum phosphate with a significant (P≤0.008) effect modification by phosphate levels on both outcomes and such an interaction was unmodified after adjustments for potential confounders, such as GFR and urinary protein.

Conclusions: In patients with proteinuric chronic nephropathies, phosphate is an independent risk factor for renal disease progression and may limit or even blunt the renoprotective effect of ACE inhibitor therapy. Trials are needed to test whether reducing phosphate exposure may improve renal outcomes and optimize the renoprotective effect of ACE inhibition in this population.

*On behalf of REIN working group.

Funding: Government Support - Non-U.S.

TH-PO339

Abstract Withdrawn .

TH-PO340

Impact of a Chronic Kidney Disease Registry and Provider Education on Guideline Adherence – A Cluster Randomized Controlled Trial Paul E. Drawz,[1,2] Brook Watts,[2] Simran Singh,[2] Elizabeth Frazier Owen Kern,[1] R. Tyler Miller.[1,2] *[1]Nephrology & Hypertension, Case Western Reserve University, Cleveland, OH; [2]Medicine, Louis Stokes Cleveland VAMC, Cleveland, OH.*

Background: Low adherence to KDOQI guidelines may be due to unrecognized CKD and lack of guideline awareness on the part of providers. The goal of this study was to evaluate the impact of provider education and a CKD registry on guideline adherence.

Methods: We conducted a cluster randomized controlled trial at the Louis Stokes Cleveland VAMC. One of two primary care clinics was randomized to intervention. Providers from both clinics received a lecture on CKD guidelines at study initiation. Providers in the intervention clinic were given access to and shown how to use a CKD registry, which identifies patients with CKD and is automatically updated daily. Eligible patients had at least one primary care visit in the last year, had CKD based on eGFR, and had not received renal replacement therapy. The outcome was the percent of patients with at least one PTH measured in the past year and was evaluated at baseline and after the 12 month intervention. Secondary outcomes were percent at goal BP and percent prescribed ACEI/ARB.

Results: At baseline, 387 patients in the control clinic and 357 patients in the intervention clinic were eligible. The percent of patients with a PTH in the past year increased in both clinics from baseline to 12 months (14.7% to 22.7% in the control clinic; 15.4% to 28.4% in the intervention clinic). However, there was no difference between clinics at 12 months (P=0.07). After 12 months, there was no difference in the percent of subjects at goal BP (40.7% vs 44.1%) or the percent of subjects on an ACEI/ARB (74.9% vs 78.2%). Only 5 of the 37 providers in the intervention clinic accessed the registry.

Conclusions: An intervention that included education on CKD guidelines and access to a CKD patient registry did not improve guideline adherence over education alone. Adherence to the primary process measure improved in both clinics, but no improvement was seen in intermediate clinical outcomes. Improving the care of patients with CKD will likely require a multifaceted approach including system redesign.

Funding: NIDDK Support

TH-PO341

Does Recognition of Chronic Kidney Disease in the Electronic Health Record Problem List Improve Care? Stacey Jolly, Sankar D. Navaneethan, Jesse D. Schold, Susana Arrigain, John W. Sharp, Anil K. Jain, Martin J. Schreiber, James F. Simon, Joseph V. Nally. *Cleveland Clinic, Cleveland, OH.*

Background: CKD recognition in the clinical setting is low. Whether CKD recognition in a problem list for a patient improves care is unclear.

Methods: Patients entered an electronic health record-based CKD registry if two outpatient eGFRs were <60 ml/min/1.73 m^2 at least 90 days apart from Jan 2005 to Apr 2011. CKD recognition was defined by having CKD in the problem list (ICD-9 codes for CKD, diabetic kidney disease, and hypertensive kidney disease) within 1 yr after entry. We calculated proportion of CKD patients with >1 yr follow-up whose CKD was recognized and examined differences by patient demographics, eGFR, nephrology visit, labs, and metformin prescription among diabetics.

Results: Among 45176 eligible patients, only 3356 (7%) had CKD noted in their problem list which differed markedly by race, age, gender, CKD stage, diabetic status and care by nephrologists (Table 1). Labs (iPTH, Vitamin D and LDL) were significantly more likely checked among patients with CKD in their problem list and inappropriate medication prescription was common among those without CKD in their problem list.

Table 1

Factor	No CKD recognition (n=41820)	CKD recognition (n=3356)	p-value
Age, mean (sd)	70 (12)	66 (14)	<0.01
follow-up, years, mean (sd)	3.4 (1.4)	3.3 (1.6)	<0.01
Male, N (%)	17287 (41)	2017 (60)	<0.01
White, N (%)	36749 (88)	2495 (74)	<0.01
Black, N (%)	4256 (10)	788 (24)	
eGFR at entry, mean (sd)	49 (10)	34 (15)	<0.01
CKD Stage at entry, N (%)			
Stage 3a (45-59)	30007 (72)	916 (27)	<0.01
Stage 3b (30-44)	9328 (22)	1164 (35)	
Stage 4 (15-29)	1996 (5)	790 (24)	
Stage 5 (<15)	489 (1)	486 (14)	
Diabetes, N (%)	8105 (19)	1380 (41)	<0.01
among diabetics, Metformin prescribed, N (%)	1407 (17)	117 (8)	<0.01
HTN, N (%)	33884 (81)	3072 (92)	<0.01
Nephrology visit within 1 yr, N (%)	1229 (3)	1152 (34)	<0.01
labs checked			
iPTH, N (%)	2455 (6)	1059 (32)	<0.01
Vitamin D, N (%)	6209 (15)	796 (24)	<0.01
LDL, N (%)	17393 (42)	1836 (55)	<0.01

Conclusions: There was low CKD recognition which translated into less CKD appropriate care. Targeted awareness along with increased use of the EHR problem list may improve CKD quality of care and outcomes in the clinical setting.

Funding: Other NIH Support - NHLBI Diversity Supplement Grant U01HL064244-10S1

TH-PO342

Racial Disparity between African Americans (AA) and Caucasian Americans (CA) in Pre-ESRD Care: A Multi-Level National Study Guofen Yan,[1] Jennie Z. Ma,[1] Alfred K. Cheung,[2] Tom H. Greene,[2] Mohammed Norman Oliver,[1] Wei Yu.[1] *[1]University of Virginia; [2]University of Utah.*

Background: Pre-ESRD nephrology care is an important predictor for ESRD outcome. Racial disparity in pre-ESRD care is well known, but the cause is multifactorial, including geography. Using USRDS, this study employed a multilevel modeling approach to systematically examine to what extent regional characteristics contribute to the disparity.

Methods: The analysis included all AA or CA incident dialysis patients who completed the new Medical Evidence form in 2005-09. The care indicator examined was whether a patient had seen a nephrologist for at least 6 months prior to ESRD therapy. The three-level logistic regression models corresponding to patients, states and regional divisions were used to estimate the probability of seeing a nephrologist and its variability across states and divisions, and examine patient- and state-level predictors. We compared results of AA and CA by age >65 (1AA: n=36,868; 1CA: n=132,187) and age ≤65yrs (2AA: n=70,444; 2CA: n=109,384).

Results: The estimated probability of seeing a nephrologist was 55.8% for 1AA, 55.6% for 1CA, 48.5% for 2AA and 52.8% for 2CA, suggesting that at the national level the racial disparity (AA vs. CA) was in the younger population, but not in older. The estimated variability across states was 0.46 for 1AA, 0.33 for 1CA, 0.41 for 2AA and 0.35 for 2CA (SD on logit), indicating substantial inter-state differences in all groups (e.g., 60-71% probability in New England states vs. 38-53% in East South Central states). The disparity within state (AA minus CA in probability) varied substantially across states, from -13% to 3% in younger population, and -9% to 12% in older. Strong patient predictors included patient's insurance coverage, working status and comorbid conditions. Strong state predictors for state-to-state variability included state's poverty, racial composition and education level.

Conclusions: Pre-ESRD care depends highly on which state the patient lives in; AA more so than CA. The disparity among states is greater than that within states. Efforts to reduce racial disparity will be more effective by intervention at state level and targeting younger population.

Funding: NIDDK Support

TH-PO343

Blood Pressure Lowering Agents for the Primary Prevention of Diabetic Kidney Disease: A Systematic Review and Meta-Analysis Jicheng Lv,[1,2] Vlado Perkovic,[1] Jonathan C. Craig,[3] Celine Foote,[1] Haiyan Wang,[2] Alan Cass,[1] Min Jun,[1] Hiddo Jan Lambers Heerspink,[4] John P. Chalmers,[1] Johannes F. Mann,[5] Giovanni F.M. Strippoli.[3,6,7] *[1]The George Institute for International Health, The University of Sydney, Australia; [2]Renal division, Peking University First Hospital, Institute of Nephrology, Peking University, Beijing, China; [3]Centre for Kidney Research, The Children's Hospital at Westmead, School of Public Health, The University of Sydney, Sydney, Australia; [4]Department of Clinical Pharmacology, University Medical Center Groningen, University of Groningen, The Netherlands; [5]Schwabing General Hospital, and KfH Kidney Centre, Ludwig Maximilians University Munchen, Germany; [6]Department of Pharmacology and Epidemiology, Mario Negri Sud Consortium, S Maria Imbaro, Chieti, Italy; [7]Diaverum Medical Scientific Office, Lund, Sweden.*

Background: We undertook a systematic review and analysis to quantify the benefits and harms of blood pressure lowering agents in normoalbuminuric individuals with diabetes.

Methods: We systematically searched MEDLINE, Embase, Cochrane Library from 1966 to January 2011. We included randomized controlled trials in normoalbuminuric participants with diabetes. Meta-analysis was done with a random effects model.

Results: We identified 28 trials involving 40,688 patients. ACE inhibitors reduced the risk of new onset of micro and/or macroalbuminuria compared with placebo (RR 0.71; 95% CI, 0.56-0.89), and compared to calcium channel blockers (RR 0.60; 95% CI, 0.42-0.85). ACE inhibitors also reduced the risk of death compared with placebo (RR 0.84, 95% CI 0.73-0.97). No effect was observed for ARBs for new onset nephropathy (RR 0.91; 95% CI 0.76-1.09) or death (RR 1.09, 95% CI, 0.89-1.34), compared with placebo, however meta-regression suggested possible benefits of ARBs for the prevention of kidney disease in high risk patients.

Conclusions: ACE inhibitors prevent new onset nephropathy and death in normoalbuminuric people with diabetes, and should therefore be used preferentially in this population. More data are needed to clarify the role of ARBs and other drug classes on the prevention of diabetic kidney disease.

TH-PO344

Assessment of Quality Incentive Program (QIP) Preparedness in Hospital-Based Dialysis Centers (HBDCs) and Independent Dialysis Organizations (IDOs) in 2010 George N. Coritsidis,[3] Gregory A. Maglinte,[1] Chun-Lan Chang,[2] Anjali Acharya,[3] Nathan Thompson,[3] Jerrold W. Hill,[2] Ze Cong,[1] Akhtar Ashfaq,[1] Jeffrey Petersen.[1] *[1]Amgen; [2]SDI Heath; [3]NYC Health and Hospitals Corporation.*

Background: QIP was implemented by Centers for Medicare and Medicaid Services (CMS) in 2011 to ensure that Prospective Payment System changes would not adversely impact ESRD dialysis programs. We assessed preparedness of HBDCs and IDOs for QIP in 2010 compared to 2008 national averages, per CMS methodology.

Methods: We conducted a retrospective analysis of an electronic medical record database from 6348 hemodialysis patients in 52 HBDCs and 31 IDOs in the US (15 states and all payer types) from Jan 1-Dec 31 2010. Analytic cohorts based on QIP criteria each comprised patients ≥18 years of age at first dialysis, and administered erythropoiesis stimulating agents ≥90 days after first dialysis with ≥4 hemoglobin (Hb) measures 5-20 g/dL (Hb cohort), or dialysis <5 times/week with ≥4 urea reduction ratios (URRs) ≥183 days after first dialysis (URR cohort). The proportion of facilities meeting 2008 national performance rates (≤2% of patients with mean Hb <10 g/dL, ≤26% of patients with Hb >12 g/dL, and ≥96% of patients with mean URR ≥65%) was assessed.

Results: Demographics among the cohorts were comparable: 55% men, mean age 60 years, >40% diabetes, >60% hypertension, mean Hb 11.3, and mean URR 73.4%. In 2010, the proportion of patients with mean Hb<10 g/dL, mean Hb>12 g/dL, and mean URR≥65% were similar in HBDCs and IDOs (Table 1). Although 93% of facilities in 2010 met criteria for limiting high Hb, 66% of facilities failed to meet criteria for low Hb and 60% failed to meet URR criteria.

Table 1. Performance Trends of HBDCs and IDOs in 2010 using CMS Methodology for QIP

	HBDCs				IDOs			
	Patient level		Facility level		Patient level		Facility level	
	% of patients[a]	n	% of facilities[b]	n	% of patients[a]	n	% of facilities[b]	n
mean Hb value < 10 g/dL	4.2%	3966	38.5%	52	5.2%	2382	25.8%	31
mean Hb value > 12 g/dL	12.8%	3966	94.2%	52	12.0%	2382	90.3%	31
mean URR ≥ 65%	93.8%	3231	46.0%	50	92.8%	2026	30.0%	30

[a]Proportion of patients with annual mean Hb or URR values as indicated.
[b]Percent of facilities achieving performance rates based on 2008 national averages; ≤2% of patients with mean Hb <10 g/dL, ≤26% of patients with Hb >12 g/dL, and ≥96% of patients with mean URR ≥65%

Conclusions: Improved performance is needed, as only 16.0% of HBDCs and 10.0% of IDOs would have achieved 100% QIP performance.

Funding: Pharmaceutical Company Support

TH-PO345

Community Based Participatory Approach to Interrelated Epidemics of Obesity Diabetes and Kidney Disease in the Zuni Pueblo Vallabh O. Shah, Donica M. Ghahate, Jeanette Bobelu, Phillip Sandy, Mick T. Romancito, Arlene Bobelu, Philip Zager. *University of New Mexico Health Sciences Center, Albuquerque, NM.*

Background: The Zuni Pueblo has a relatively endogamous population of 6,300. The socio-economically disadvantaged Pueblo faces a public health challenge from interrelated epidemics of obesity, diabetes and kidney disease. Decreasing the impact of these epidemics is complicated by historical, economic and cultural barriers, which limit health care utilization. The Zuni Health Initiative (ZHI) is a chronic care model that accounts for the demographics, family structure, culture and traditions in the pueblo.

Methods: Prior to implementing the ZHI, we conducted a series of 1 hour focus groups with community members of different ages, healthcare workers and public school employees to identify perceived barriers to health care utilization. Community health representatives (CHR) led the sessions and presented a series of questions, developed from a literature review and designed to elicit information on perceived barriers to health care. These included: (1) Are your health care needs being met; if not, why not? (2) What help do you need? (3) What barriers do you encounter? (4) How does your diet and level of physical activity impact your risk for diabetes and kidney disease? Probes and follow-up questions were used to explore dominant themes. Audiotapes were translated and transcribed by bilingual ZHI staff. We reduced text to thematic categories, and constructed a coding dictionary and inserted these into Atlas-TI program.

Results: We identified the following perceived barriers to the participation of the community members in their care: (1) inadequate transportation infrastructure in the pueblo; (2) inadequate transportation between the pueblo and secondary and tertiary care sites; (3) inadequate staffing; (4) lack of staff continuity among Zuni Indian Health Service providers; and (5) lack of awareness of existing health promoting programs in the community.

Conclusions: Development of a CCM that addresses these perceived barriers will improve community access to health care and decrease the impact of interrelated epidemics of obesity, diabetes and kidney disease.

Funding: Other NIH Support - NCRR supported New Mexico INBRE

TH-PO346

Assessment of Body Composition by Dry Mass Index and a Ratio of Total Body Water to Estimated Volume Based on Bioimpedance Analysis in Chronic Kidney Disease Patients Yasushi Ohashi, Takatoshi Otani, Reibin Tai, Ken Sakai, Sonoo Mizuiri, Atsushi Aikawa. *Nephrology, Toho University School of Medicine, Tokyo, Japan.*

Background: Adequate assessment of fluid and nutritional status is of major importance in monitoring Chronic Kidney Disease (CKD) patients; Body Mass Index and Bioimpedance analysis (BIA) have been proposed as acceptable markers. There remains uncertainty, however, regarding the impact of fluid status in CKD on BMI and the BIA measurements. Dry Mass Index (DMI), which removes TBW from weight, might be able to more adequate assessment tool. The aim of this study was, first, to assess agreement between TBW and estimated Volume (eV) by the Watson formula and second, to assess the reliability of the DMI as a marker of nutritional status.

Methods: A total of 45 CKD patients randomly were measured TBW, Intracellular Water (ICW), Extracellular Water (ECW) and a ratio of ECW to TBW as edematous index using the BIA. The participants were surveyed for age, gender, underlying disease, BMI, blood pressure (BP), serum albumin (Alb), eGFR, and proteinuria. The DMI was calculated by the formula [(weight - TBW)/height^2], and the correlation between DMI and BMI was assessed. As a marker of fluid status, we also evaluated a ratio of TBW to eV. The range of euvolemic status was defined from 0.970 to 1.029.

Results: The formula indicating relationship between BMI and DMI is obtained as [DMI = 0.5595 × BMI 2.091] by the linear regression analysis. Based on BMI, DMI and TBV/eV, the numbers of the participants were categorized among the following: BMI ≥ 25 with hypovolemia: 6, with euvolemia: 10 or with hypervolemia: 3; 18.5 ≤ BMI < 25 with hypovolemia: 11, with euvolemia: 9 or with hypervolemia: 5; and BMI < 18.5 with euvolemia: one patient. The TBW/eV was positively-correlated BP (p=0.02), %ICW (p<0.01) and %ECW in BW (p<0.01), was negatively-correlated age (p=0.01) and Alb (p=0.03). In contrast, the increase in ECW/TBW was not correlated ECW volume excess, but ICW deficit with aging (p<0.01).

Conclusions: Combing DMI to BMI could assess body composition in changes of body weight. The ratio of TBW to eV could be a suitable tool to assess fluid volume status in CKD patients.

TH-PO347

Prevention of Acute Kidney Injury (AKI) in Elective Abdominal Surgery Ana Serrano, Javier Villacorta, Javier Zamora, Beatriz Prada, Alfonso Muriel, Maria Teresa Tenorio, Susana Iglesias, Fernando Liano. *Nephrology, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain.*

Background: AKI after abdominal surgery is not uncommon and is associated with high morbi-mortality.

Methods: The primary objective was to evaluate the effect of isotonic saline solution (ISS) administration in preventing AKI in patients undergoing elective non-laparoscopic abdominal surgery (Eab-surg) Secondary objective was to determine the incidence of AKI in this context.

Study design: A single center, prospective, randomized, controlled,open label, phase III trial. The study population included adult patients undergoing Eab-surg(ClinicalGovTrial NCT 00953940). Minor surgery procedures, patients with heart failure, GFR <60 ml/min and ASA classification ends scores (1 and 5) were excluded. The sample population necessary to demonstrate a 5% reduction of AKI (a=0.05 and 80% power) was estimated at 300 patients per arm. Intravenous ISS was administered in the treatment group at a dose of 1.5 ml/kg/h 12h before surgery. The control group did not received prior hydration. Serum creatinine and cystatin C were mesured before and 24, 48 and 72h after surgery. These results are from an interim analysis performed with the first 300 patients enrolled.

Results: Both groups were similar in demographics, comorbidity, type of surgery, baseline renal function (median and range) [Crs treated group 0.92 (0.58 to 1.86) SCr untreated 0.92 (0.49 - 1.57)] (similar data for cystatin C) and need for intraoperative volume or ICU requirement. Using the RIFLE classification, 1 patient with ISS was R at 24 hours and 2 patients without prophylaxis were R and F respectively at 48 and 72 hours after surgery. The AKIN classification identified 7 patients (3 in the ISS group) with functional impairment at 24 hours, 3 patients treated with ISS and 4 controls with AKI at 72h.

Conclusions: In non-laparoscopic elective abdominal surgery preoperative hydration in patients with normal renal function does not provide any protection against AKI. In this setting the rate of decline in renal function estimated by AKIN classification was low (2.33%).

Funding: Government Support - Non-U.S.

TH-PO348

Coronary Angiography with Online Hemodialysis To Prevent Contrast-Induced Nephropathy in CKD Patients Georg Schlieper,[1] Thilo Krueger,[1] Malte Kelm,[2] Jurgen Floege,[1] Ralf Westenfeld.[2] *[1]Nephrology, RWTH University Hospital, Aachen, NRW, Germany; [2]Cardiology, Heinrich-Heine University Hospital, Düsseldorf, NRW, Germany.*

Background: Optimal therapy to prevent contrast-induced nephropathy (CIN) in high risk patients with CKD remains a challenge. Hemodialysis after exposure to radiocontrast agents seems to have no benefit for the prevention of CIN but may be too late. In this study we analyzed the effect of online hemodialysis performed during coronary angiography on kidney function.

Methods: In late 2007 we changed our policy for the routine care of CKD 3-5 patients during coronary angiography from supportive care only (hydration with NaCl 0.9%, ACC; group STD) to supportive care plus online hemodialysis (Genius® dialysis via femoral vein access; group STD+HD) whilst performing the coronary angiography. Glomerular filtration rate (GFR) was estimated (MDRD formula) one and three months after angiography and at any other time point available after angiography. Moreover, the need for hemodialysis was determined.

Results: In group STD+HD 22 patients underwent coronary angiography. They were compared to a historical control group of 57 patients with coronary angiography (group STD) of the years 2006 and 2007. Baseline parameters (gender, prevalence of diabetes, body mass index, baseline GFR [30±9 vs. 32±13 ml/min; STD+HD vs. STD], amount of contrast media [93±59 vs. 110±63 ml]) were not significantly (ns) different between the two groups except for age (69 vs. 78 years, p<0.001). After coronary angiography the GFR in STD+HD patients did not differ significantly from that of STD patients (1 month: 25 vs. 31 ml/min; 3 months: 22 vs. 29 ml/min; any time point: 24 vs. 29 ml/min; all ns). The percentage of patients requiring hemodialysis was also not different between the 2 groups during 24 months of follow-up (32% vs. 23%, ns). There were no treatment-related major complications in the STD+HD group.

Conclusions: In conclusion, online hemodialysis during coronary angiography appears to be safe, however it is not superior to coronary angiography with standard supportive therapy. Thus, in high risk patients with CKD stage 3-5 online dialysis during angiography does not reduce the risk for CIN.

TH-PO349

Lifetime Costs of Hemodialysis and Peritoneal Dialysis Patients in Taiwan
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Background: This study compared the lifetime costs between hemodialysis(HD) and peritoneal dialysis(PD) patients in Taiwan.

Methods: The data base of a cohort of patients who had received maintenance dialysis and were registered under the National Health Insurance (NHI) from July, 1997 to December, 2005 was utilized. Patients who were under 18 years old, were diagnosed with cancer or had received both PD and HD were excluded. PD patients were then matched with HD patients on age, sex and diabetic status, and followed up until Dec 31th, 2006. Patients who had stopped using the subsidies of the NHI for at least 3 months were considered dead. Life expectancy, expected years of life lost(EYLL), total lifetime costs(lifetime costs of ambulatory and inpatient care), and costs per year of both HD and PD patients were then compared using the ISQoL software.

Results: There were 3062 pairs of matched HD and PD patients (mean age, 53.2±15.4 years). Life expectancies and EYLL were not different between HD and PD patients. Diabetic patients who underwent HD had a slightly longer life expectancy than those who underwent PD (p=0.0061), while there was no difference in life expectancy between non-diabetic HD and PD patients. For diabetic patients, the total lifetime costs of those treated with HD were higher than those treated with PD, no matter the discount rate was 3% or 5% (P<0.001). For patient without DM, the total lifetime costs of those treated with HD were also higher than those treated with PD, whether the discount rate was 3%(P=0.0186) or 5%(P=0.0018). The costs per year for HD patients were higher than that of PD patients. Lifetime costs of ambulatory care were significantly higher for HD patients than for PD patients no matter the patients had DM or not and the discount rate was 3% or 5%. When lifetime costs of inpatient care were compared, there was no statistical difference between HD and PD. When adjusted by survival, patients undergoing HD had higher monthly health costs than PD patients.

Conclusions: Both the total lifetime costs, lifetime costs of ambulatory care and costs per year paid by the NHI were higher for HD than were for PD in Taiwan.

TH-PO350

Serum gamma- Glutamyltransferase Levels Are Inversely Related to Endothelial Dysfunction in Chronic Kidney Disease
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Background: Gamma- glutamyltransferase (GGT) is an enzyme responsible for the extracellular catabolism of the antioxidant glutathione and recently implicated in the pathogenesis of atherosclerosis. Endothelial dysfunction is a prodomal feature of atherogenesis. Since oxidative stress is highly present in uremia and a causally linked to endothelial dysfunction, we hypothesized that GGT may be a factor implicated in this process.

Methods: We investigated the relationship between flow-mediated vasodilatation (FMD) and circulating GGT in a series of 214 nondiabetic nondialyzed patients with stage 3-5 CKD. Exclusion criteria contemplated, among others, alcohol consumption, manifest cirrhosis and established atherosclerotic complication.

Results: Serum GGT levels were negatively associated with FMD (r= -0.41, p<0.001) and eGFR (r= -0.34, p<0.001) in univariate analysis. Multvariate regression analysis showed that the association between GGT and FMD persisted after adjustment for age, sex, smoking, LDL-cholesterol, GFR C-reactive protein, systolic blood pressure, proteinuria, and homeostatic model assessment (HOMA) index.

Conclusions: Circulating GGT levels significantly associate with endothelial dysfunction, an important early feature of the atherogenic process. GGT might be an early marker of oxidative or other cellular stress that it is possibly directly related to the pathogenesis of endothelial dysfunction.

Funding: Clinical Revenue Support

TH-PO351

Patient Reported Outcomes Measurement Information System (PROMIS): Pediatric Chronic Kidney Disease
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Background: The PROMIS study goal was to create patient reported outcome evaluation tools. 233 children, ages 8-17, with CKD (stages I-V or kidney transplant) were enrolled from MWPNC sites in this cross-sectional study designed to validate the PROMIS instrument in children with CKD.

Methods: Stepwise regression analyses were conducted to assess association of child characteristics with 8 domain scores: peer relationships, depression, anxiety, pain, fatigue, upper extremity dexterity and mobility; regression coefficients must be significant at p < 0.15 to be selected in the model. Significant (p<0.5) results of multivariate analysis are reported (β(SE)).

Results: Patients with recent hospitalizations and with edema were associated with increased domain scores for depression, anxiety, pain, and low energy. Hospitalization and active edema were associated with decreased mobility scores. Co-morbidities negatively affected all domains except for pain. There were not significant changes in domain scores in response to different stages of CKD. Patients did not view ESRD negatively affecting their social relationships.

PROMIS Domain Scores for CKD (β(SE))

Covariate	Depression	Anxiety	Social-Peer Relationships	Pain	Low Energy	Upper Extremity Dexterity	Mobility
Hospitalizations		4.8(1.6)					-2.7(1.2)
Edema	5.9(2.2)	5.2(2.1)		7.4(2.5)	6.7(2.8)		-3.8(1.6)
Co-Morbidities Number	1.7(0.6)	1.8(0.6)	-2.5(0.6)		3.4(0.9)	-1(0.5)	-1.6(0.5)
CKD (stage 1-4)							
ESRD			6.9(2.1)				

Conclusions: The PROMIS domains are sensitive to disease severity indicators in children with CKD. Longitudinal studies documenting the responsiveness to change in disease activity over time will strengthen the validation and future utility of this tool.

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TH-PO352

Changes in Serum Creatinine after Bariatric Surgery
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Background: Weight loss after bariatric surgery is thought to improve serum creatinine in patients with and without chronic kidney disease (CKD). These changes in serum creatinine may be mediated through loss of muscle mass rather than improvement in renal function. Therefore, we examined the potential factors influencing the changes in serum creatinine among CKD patients who underwent bariatric surgery.

Methods: We conducted a retrospective study of stage 2-3 CKD patients (eGFR 30-89 ml/min/1.73m^2 and with ICD-9 code diagnosis for various kidney diseases) who underwent both restrictive and malabsorptive types of bariatric surgery. Renal parameters (serum creatinine and eGFR using CKD-EPI equation), body mass index (BMI), and systolic blood pressure (SBP) data were collected at baseline and at 6 months after surgery to analyze changes arising from weight loss. Factors predicting change in serum creatinine were examined using linear regression.

Results: Seventy-eight patients with mean serum creatinine of 1.3 mg/dl and mean eGFR of 52.8 ml/min/1.73m^2 were included. At the end of 6 months, BMI decreased from 44.6 kg/m^2 to 35.0 kg/m^2, SBP decreased from 134 +/- 21 to 130 +/- 17 mm Hg and serum creatinine decreased to 1.09 mg/dl with an improvement in eGFR to 66.5 ml/min/1.73m^2 (all paired t-test p<0.001). Number of antihypertensive and oral hypoglycemic agents decreased after surgery. The decrease in serum creatinine was significantly correlated with the decrease in BMI (r = 0.20) and SBP (r = 0.28). In the regression model, females had a 0.12 mg/dl lesser decline in serum creatinine than males (p=0.01), and Caucasians had a 0.11 mg/dl lesser decline in serum creatinine than African Americans (p=0.04) after surgery.

Conclusions: Serum creatinine decreases with weight loss after bariatric surgery. The decline in serum creatinine is less among females and Caucasians, populations who usually have lower muscle mass compared to males and African Americans. Future studies need to examine how weight loss after bariatric surgery produces a change in renal function, and how these changes in renal function are linked with changes in body composition.

Funding: Other NIH Support - National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990

TH-PO353

Diet, Physical Activity, Obesity and Intentional Weight Loss in Chronic Kidney Disease: NHANES 1999-2006

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Background: Previous reports have shown that the chronic kidney disease (CKD) population has a higher body mass index (BMI) and waist circumference (WC) than non-CKD. We aimed to examine a) the lifestyle (dietary intake and physical activity) and behavioral factors (desire to weigh less) that might explain these differences and b) factors related to pursuing weight loss among a nationally representative sample of adults.

Methods: Cross-sectional analysis of 15,993 adult participants in the National Health and Nutrition Examination Surveys conducted between1999-2006. CKD was defined as patients with estimated glomerular filtration rate < 60 ml/min/1.73m^2 or urine albumin creatinine ratio >30 mg/g. Differences in lifestyle and behavioral factors between CKD and non-CKD participants were compared with sas survey procedures. Factors associated with pursuing weight loss were examined using survey logistic regression.

Results: Participants with CKD (n=2,812) had higher WC (100.2 cm vs. 95.3 cm, p<0.001) and a higher proportion had a BMI >30 kg/m^2 (37% vs. 29%, p<0.001). CKD and non-CKD populations did not differ based on percentage of energy derived from carbohydrate, fat, saturated fat and protein. However, 68% of the CKD population did not meet the minimum recommended leisure time physical activity goals (<450 METS/min/week) compared to 55% among non-CKD (p<0.001). Proportions of CKD and non-CKD participants who expressed desire to weigh less were not different. CKD participants had lower odds (0.84, 95% CI 0.71, 0.98) of pursuing a weight loss program, and so did African Americans (0.67, 95% CI 0.58, 0.76) and elderly adults.

Conclusions: Dietary composition and the desire to weigh less are not different for CKD and non-CKD populations. However, the CKD population is less active and pursues weight loss less often than non-CKD populations. These results suggest the need for studies to examine the underlying reasons for these differences, and studies aimed at developing targeted weight loss programs for CKD patients.

Funding: Other NIH Support - National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990

TH-PO354

Albuminuria Is Strongly Associated with Low BMI and Only Weekly to Cardiovascular Risk Factors in Apparently Healthy Subjects Younger Than Forty

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Background: Albuminuria reflects increased cardiovascular risk in high risk groups. However, albuminuria occurs also frequently in absence of cardiovascular risk factors. This study evaluated which risk factors are associated with albuminuria depending on age.

Methods: The unreferred renal insufficiency (URI) study is a cross sectional study on the prevalence of metabolic risk factors in Belgian workers. Subjects with abnormal urinary sediment or missing data of interest were excluded, leaving a cohort of 1,361 apparently healthy workers. A single urine sample was used to measure albumin to creatinine ratio (ACR).

Results: As expected blood pressure (p<0.001), heart rate (p<0.001), plasma glucose (p<0.001), serum cholesterol (p<0.001), and BMI (p<0.001) were higher in those ≥40 vs. <40 years (median of age), but in spite of this higher prevalence of traditional cardiovascular risk factors, ln ACR was identical (0.78±1.13 vs. 0.78±1.17, p=0.97). In subjects under 40 years of age (n=668), ACR was strongly associated with BMI < 20 kg/m^2 (p<0.001) whereas it associated only borderline with cholesterol (p=0.03) and with antihypertensive treatment (p=0.01). In contrast, in subjects over forty (n=693), ACR was positively associated with BMI >30 kg/m^2 (p<0.001), heart rate (p<0.001), systolic blood pressure (p<0.001), diabetes (p<0.001), CRP (p=0.001) and smoking (p=0.001)

Conclusions: Screening for cardiovascular risk by using urinary albumin in apparently healthy subjects under 40 years of age was unexpectedly associated with low BMI and with virtually no traditional cardiovascular risk factors. Only in subjects over forty, albuminuria follows the traditional cardiovascular risk pattern.

Trial registration 2006 NCT00365911

Funding: Pharmaceutical Company Support

TH-PO355

Corticosteroid Therapy in IgA Nephropathy: A Systematic Review and Meta-Analysis

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Background: Although several trials have evaluated steroids in IgA nephropathy(IgAN), there is still much uncertainty on such a treatment in IgAN. In this study we did a systematic review and meta-analysis to assess the efficacy and safety of corticosteroids in IgAN.

Methods: We systematically searched Medline, Embase, and the Cochrane Library for randomized controlled trials of corticosteroids therapy in IgA nephropathy published between 1966 and 2011.We evaluated the effects of corticosteroid on renal function and proteinuria , as well as adverse events. Meta-analysis was done with a random effects model.

Results: We identified 9 relevant trials with 536 patients with IgAN, the quality of which was suboptimal. Overall steroids therapy was associated with a lower risk of progression to kidney failure (double serum creatinine or ESKD) (RR:0.29, 95%CI: 0.13-0.65, p=0.02, I^2=0.0%, p for heterogeneity =0.47) and proteinuria reduction (WMD:-0.46g/d, 95%CI: -0.63- 0.29).

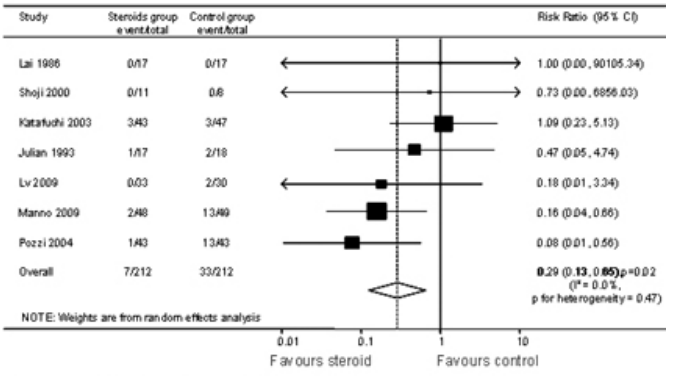

Figure 1 . Effect of corticosteroid on renal function

Subgroup analysis revealed that the effect of steroids is modified by the dose of steroids (p for heterogeneity =0.032) or the risk of kidney failures (p for heterogeneity =0.048). Full dose of steroids produced significant renal protection (RR 0.14, 95%CI: 0.05 - 0.39) while not in low dose steroids (RR 0.84, 95%CI 0.23 - 3.04). Steroids didn't increase the overall risk of adverse events (RR: 1.62, 95%CI: 0.83-3.16, p=0.15).

Conclusions: Corticosteroid produces renal protection in patients with IgAN without increase the risk of overall adverse events. However the low quality of included studies limited the study conclusions.

TH-PO356

Short-Term Effects of a Keto/Amino Acid Supplemented Low Protein Diet on the Delay of Progressive Renal Failure in Chronic Kidney Disease

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Background: A protein-restricted diet with keto/amino acids (KA) supplement showed favorable effects on uremia, and delayed renal replacement therapy in patients with chronic kidney disease.

Methods: We conducted an open, prospective, randomized, and multi-center study for 6 months. A total of 67 patients were randomly assigned into two groups. The LPD+KA group was advised to take less than 0.6 g/kg/day of protein with KA. The LPD group was advised to consume less than 0.6 g/kg/day protein. Clinical and biochemical parameters were evaluated at baseline, 3 and 6 months.

Results: Calcium and phosphorous (Ca×P) product level measured at 3 months was significantly lower in the LPD + KA group than in the LPD group (LPD + KA group: 33.5 ± 5.0 mg^2/dL^2 vs. LPD group: 36.9 ± 7.9 mg^2/dL^2, $p < 0.05$), while those at 6 months were not different. The slope of the glomerular filtration rate (GFR slope) and the percentage of the GFR slope (GFR slope %) at 3 months were more preserved in the LPD + KA group than in the LPD group. The GFR slope and GFR slope % at 6 months were not significantly different. In the entire subjects, the GFR slope was negatively correlated with Ca×P product levels at 3 months, total cholesterol at baseline, and urine protein-creatinine ratio at baseline and 6 months (r = -0.255, r = -0.296, r = -0.412, r = -0.371, $p < 0.05$). A multiple regression analysis revealed Ca×P product at 3 months was the only independent factor affecting the GFR slope at 3 months.

Conclusions: The present study suggests that a low protein diet supplemented with keto/amino acids had a beneficial effect on preserving renal function and improving calcium and phosphorus disturbances in patients with chronic kidney disease.

TH-PO357

Baseline Observations from a Randomized Crossover Trial of Lowering Sodium Intake in Chronic Kidney Disease (CKD)-LOSALT Study Rajiv Saran,[1] Robin L. Sands,[1] Brenda W. Gillespie,[1] Michael Heung,[1] Scott L. Hummel,[3] Vimal K. Derebail,[4] Nathan W. Levin,[2] Fansan Zhu,[2] Peter Kotanko,[2] Philip J. Klemmer.[4] *[1]UM-KECC, Univ. of MI, Ann Arbor, MI; [2]Renal Research Institute, New York, NY; [3]Dept. of CV Medicine, Univ. of MI, Ann Arbor, MI; [4]Dept of Medicine, Univ. of NC, Chapel Hill, NC.*

Background: Many patients with stage 3-5 CKD may be overhydrated. We are conducting a randomized crossover trial of sodium (Na) restriction to primarily assess change in hydration status as assessed by bioelectrical impedance (BIA; lower=overhydration). Baseline data were used to explore the relationship between hydration status and GFR.

Methods: Adult patients with Stage 3-4 CKD with stable clinical course were randomized in phase I to a low salt diet (<2g of Na/day) *vs.* 'usual diet' for 4 weeks, with the alternate treatment given in phase II. Weekly dietary monitoring was provided and 24-hour urine Na measurments assessed compliance. A modified spectrum bioimpedance device and a switch box (Xitron 4200) automatically calculated ten measurements for each segment, arm, trunk, leg, calf, and whole body while the patient was in the supine position.

Results: Baseline data prior to randomization are presented in subjects enrolled so far (n=60; target=66). Mean age was 62±13, 56% male, 71% white, 39% diabetic, 87% hypertensive, mean BMI 32±5 g/m^2, eGFR 39±11ml/min/1.73m^2, and 24-hr urine Na 160±148 mEq. The table shows some significant differences in BIA measurements across CKD stages ($p<0.10$), baseline 24-hr urine Na correlated significantly with Calf (ECV/ICV, ECV/TBW, ECV/WT) and Whole Body (ECV/WT) BIA readings.

Conclusions: Baseline measurements in this clinical trial of Na restriction in CKD Stages 3-4 suggest greater degree of hydration across CKD stages 3-4 that may be amenable to Na restriction and/or specific pharmacotherapy.

Baseline BioImpedance Measurements (n=60)

BIA Measurement	Overall	CKD Stage			p-value*
		Stage 3a (45 < eGFR < 60)	Stage 3b (45 < eGFR ≤ 30)	Stage 4 (30 < eGFR ≤ 15)	
Calf					
Extracellular volume (L) [ECV]	0.18 ± 0.06	**0.20 ± 0.08**	**0.16 ± 0.04**	**0.17 ± 0.04**	**0.0574**
Intracellular volume (L) [ICV]	0.50 ± 0.16	0.55 ± 0.15	0.46 ± 0.14	0.49 ± 0.19	0.2305
ECV/ICV	0.38 ± 0.13	0.38 ± 0.14	0.37 ± 0.13	0.39 ± 0.13	0.8601
ECV/Total Body Water [ECV/TBW]	0.27 ± 0.06	0.27 ± 0.07	0.26 ± 0.06	0.27 ± 0.07	0.8614
ECV/Body Weight [ECV/Wt]	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.0005	0.002 ± 0.0004	0.1339
Standardized Rho (Wt·m^2/kg)	0.16 ± 0.09	0.14 ± 0.04	0.18 ± 0.13	0.15 ± 0.03	0.3115
Sum of Segments					
ECV (L)	21.85 ± 5.00	**22.24 ± 5.34**	**20.06 ± 4.18**	**23.57 ± 4.94**	**0.0634**
ICV (L)	25.74 ± 9.69	**28.91 ± 14.42**	**22.02 ± 6.08**	**27.75 ± 6.70**	**0.0434**
ECV/ICV	0.89 ± 0.17	**0.82 ± 0.16**	**0.94 ± 0.19**	**0.87 ± 0.13**	**0.0728**
ECV/TBW	0.47 ± 0.05	**0.45 ± 0.05**	**0.48 ± 0.05**	**0.46 ± 0.04**	**0.0853**
ECV/Wt	0.24 ± 0.03	**0.23 ± 0.03**	**0.22 ± 0.03**	**0.25 ± 0.04**	**0.0303**
Whole Body					
ECV (L)	19.82 ± 4.76	20.55 ± 4.40	18.30 ± 4.45	20.69 ± 5.22	0.1774
ICV (L)	25.58 ± 7.78	**28.53 ± 9.40**	**22.76 ± 6.55**	**26.14 ± 6.84**	**0.0539**
ECV/ICV	0.79± 0.12	0.75 ± 0.12	0.82 ± 0.12	0.80 ± 0.09	0.1278
ECV/TBW	0.44 ± 0.04	0.42 ± 0.04	0.45 ± 0.04	0.44 ± 0.03	0.1186
ECV/Wt	0.21 ± 0.03	0.22 ± 0.02	0.20 ± 0.02	0.22 ± 0.03	0.1458

*Comparisons between the CKD stage categories were made using t-tests

Funding: Private Foundation Support

TH-PO358

First Human Exposure Suggests a Unique Metabolic Profile with Multiple Active Species for CTP-499, a Novel Agent for Treatment of Chronic Kidney Disease Dolly A. Parasrampuria, Virginia Braman, Changfu Cheng, Brett Grotbeck, David J. Turnquist, Kristine Hogan, Ara Aslanian, Lijun Wu, Robert Zelle, Philip B. Graham, James Shipley, Roger Tung. *Concert Pharmaceuticals Inc, Lexington, MA.*

Background: CTP-499 is a deuterated analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine, the active metabolite of pentoxifylline (PTX), a compound reported in multiple clinical trials to have beneficial effects in chronic kidney disease.

Methods: Single 400 mg doses of three different extended-release (ER) formulations of CTP-499 and ER pentoxifylline were administered to 16 healthy subjects in a randomized, crossover study. Blood samples were collected over a 48 hour period for the quantitation of CTP-499, pentoxifylline and their metabolites. Safety measurements included adverse event monitoring, clinical laboratories, 12-lead ECGs, and vital signs.

Results: All treatments were well tolerated. Only 9 adverse events were reported. All were mild in severity and did not require any action or treatment. CTP-499 was absorbed rapidly and metabolized extensively to five metabolites, nominally described as M1-M5. The medium-release formulation had the optimum pharmacokinetics profile based on minimizing peak-to-trough fluctuation and achieving complete drug-release. All metabolites were structurally identical (4 metabolites remained deuterated) to species resulting from ER pentoxifylline. The plasma AUCs for M1, M2 & M5 from CTP-499 dosing were each greater than 10% of parent drug. Whereas CTP-499 and M1 were comparable, M5 was lower and M2 was double the total exposure versus the equivalent non-deuterated moiety from PTX dosing. In whole blood, CTP-499, M1 & M2 demonstrated anti-inflammatory and anti-oxidative burst activity, inhibiting LPS-induced TNF-alpha and anti-CD3-induced INF-gamma secretion, as well as fMLP-induced oxidative burst in human neutrophils.

Conclusions: All treatments were well tolerated. The medium-release formulation was selected for development. The metabolism profile of CTP-499 is unique and may result in favorable biologic activity as an anti-inflammatory, anti-oxidant and anti-fibrotic agent in chronic kidney disease.

Funding: Pharmaceutical Company Support

TH-PO359

Single Ascending Dose Pharmacokinetics, Safety and Tolerability of CTP-499, a Novel Agent Being Investigated for Treatment of Chronic Kidney Disease Dolly A. Parasrampuria, Virginia Braman, Changfu Cheng, James Shipley, Brett Grotbeck, David J. Turnquist, Philip B. Graham, Roger Tung. *Concert Pharmaceuticals Inc, Lexington, MA.*

Background: CTP-499 is a deuterated analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine, the active metabolite of pentoxifylline, a compound reported in multiple clinical trials to have beneficial effects in chronic kidney disease.

Methods: This was a two part study. Part A was randomized, double-blind, parallel group evaluation of single ascending doses (6 active, 2 placebo for each dose) of an extended-release (ER) formulation of CTP-499 in healthy adult subjects at doses of 600, 1200, 1800, and 2400 mg. Part B involved open-label administration of a single dose of 400 mg immediate-release (IR) powder in capsule to 6 subjects. Blood & urine samples were collected over 32 hours for the quantitation of CTP-499 & its metabolites. Safety measurements included adverse events, clinical labs, 12-lead ECGs, and vital signs.

Results: A total of 38 subjects participated in the study; none discontinued. Two subjects dosed with 400 mg IR and 4 subjects dosed with 2400 mg ER formulation had emesis, which was thus dose and formulation related, but was not necessarily concentration related. All other adverse events were mild. CTP-499 was rapidly absorbed and extensively metabolized. With increasing doses, exposures increased more or less linearly, although nonlinearity cannot be ruled out. The dose levels were achieved by the use of multiples of 200 mg ER tablets, a factor to be considered in interpreting the data. Five metabolites were quantified in plasma, nominally described as M1-M5. Metabolites M1, M2 & M5 were present at greater than 10% of parent drug. In urine, M5 was the major metabolite, followed by M2 & CTP-499; M1, M3, M4 were negligible. The metabolism profiles were different for IR and ER, with more of the active moieties (M1, M2) formed with ER formulation.

Conclusions: CTP-499 dosed as an ER formulation was well tolerated at single doses of 1800 mg and less. Pharmacokinetic parameters indicate the potential for once daily dosing. Based on these data, further clinical evaluation in patients with chronic kidney disease is planned.

Funding: Pharmaceutical Company Support

TH-PO360

Prevalence of Advance Directives in Patients with Kidney Disease or Other General Medical Diagnoses Frieder Keller, Florian Driehorst. *Medical Department, Division of Nephrology, University Hospital, Ulm, Germany.*

Background: After the law on living will was released in 2009, an increasing use of advance directives by patients was to observe in Germany. Since kidney patients are facing the potential or real need for dialysis, our hypothesis was that kidney patients less frequently state their living will than patients with other diseases.

Methods: An interview of all patients on our ward was performed who could give signed consent. Excluded were patients unable to talk and patients only admitted for a time period of less than 48 hours. Data are given as percentages or arithmetic means +/- standard deviation. Differences between kidney and other patients were tested for significance by the Student t-test or chi-squared test. Odds ratios with the 95 % confidence interval (CI) were calculated for the prevalence of living will by multiple logistical regression analysis of the main independent variables namely, age, sex and diagnosis.

Results: Among 505 admitted patients, 211 were included (42 %) and 200 patients completed the study. Of them, 75 were female and 125 male, 121 had a renal diagnosis and 79 another diseases. Overall, advance directives were prevalent in 52 cases (26 %). Of them, significantly more patients did forego resuscitation than did dialysis in their living will (31 % vs 8 %, $p = 0.001$). Patients having fixed an advance directive were significantly older (72 +/- 13 vs 59 +/- 16 years, $p = 0.001$) and significantly more women (36 % vs 20 %, $p = 0.01$). Overall, the prevalence of an advance directive was significantly less in kidney patients than in those with other diagnoses (20 % vs 35 %, $p = 0.015$). However, kidney patients were significantly younger than those with other diagnoses (60 +/- 16 vs 67 +/- 17 years, $p = 0.004$). Upon multiple logistical regression analysis the odds ratio for an advance directive was significantly only influenced by age (1.06 CI 1.0 - 1.1, $p = 0.0001$), but no longer by the sex (1.5 CI 0.7 - 3.1, $p = 0.228$) or by the kidney diagnosis (1.5 CI 0.7 - 3.0, $p = 0.287$).

Conclusions: Age was found the more impacting variable than the kidney diagnosis on the prevalence of advance directives. In their living will only a minority stated to forego dialysis (8 %).

Funding: Pharmaceutical Company Support

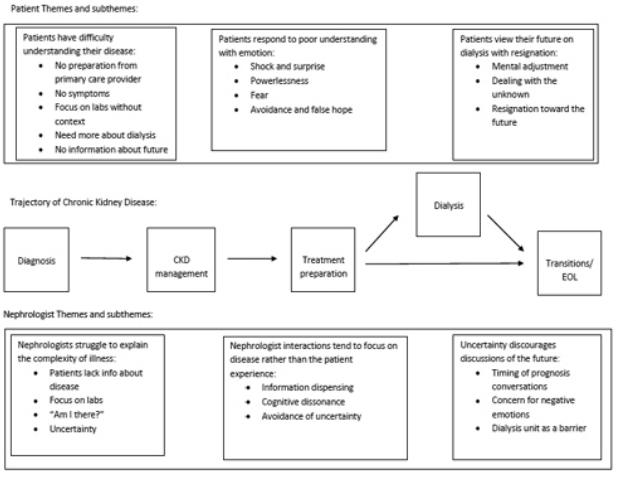
TH-PO361

A Qualitative Study: How Elderly Patients and Nephrologists Discuss the Kidney Disease Course Jane O. Schell,[1] Uptal D. Patel,[1] James A. Tulsky.[2] *[1]Nephrology, Duke University, Durham, NC; [2]Duke Center for Palliative Care, Duke University, Durham, NC.*

Background: Elderly patients with advanced kidney disease often suffer considerable disability, morbidity and mortality. In preparing for this disease trajectory, the impact of the physician-patient interaction toward understanding is not known. We sought to describe how nephrologists and older patients discuss and understand the course of kidney disease and its prognosis.

Methods: We conducted focus groups and semi-structured interviews with 11 nephrologists and 29 patients over age 65 with advanced CKD or receiving dialysis. All interviews were audio-recorded and transcribed. We used qualitative analytic methods to identify common and recurrent themes related to the primary research question.

Results: Prognosis was rarely discussed and the disease course was often followed through test results with less emphasis on the patient experience. We identified 6 themes which describe the challenges and experience of understanding the trajectory of kidney disease: 1) patients have difficulty understanding their disease; 2) patients respond to poor understanding with emotion; 3) patients tend to view their future on dialysis with resignation; 4) nephrologists struggle to explain the complexity of illness; 4) nephrologist interactions tend to focus on the disease rather than the patient experience; 5) uncertainty discourages discussions of the future.



Conclusions: We identify important challenges among patients and nephrologists in understanding and p**Conclusions:** reparing for the kidney disease course. While nephrologists recognize the special needs of elderly patients, communication barriers exist that prevent discussions which address the patient experience. Communication interventions that respond to patient emotion and address uncertainty may impact understanding and preparation.

Funding: Other NIH Support - Loan Repayment Program

TH-PO362

Mortality and Functional Status in Elderly Dialysis Patients Residing in Assisted Living Care Facilities Keren Mandelzweig, Paul Komenda, Claudio Rigatto, Lisa M. Miller, Joe A. Bueti, Clara Bohm, Manish M. Sood. *Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: As the elderly ESRD population grows, a larger proportion will require living in an assisted care facility such as a nursing home. We set out to investigate mortality and functional status in elderly ESRD patients residing in assisted living care facilities compared to age and co-morbidity matched ESRD controls who reside at home.

Methods: We developed a 3:1 age and Charlson co-morbidity score matched cohort of 192 elderly ESRD patients residing at home to 64 residing in an assisted care facility. Patients were identified during an acute hospitalization and with follow up beginning upon discharge. Mortality was examined by Kaplan Meier and multivariate adjusted Cox proportional hazards models with p value<0.05 considered significant.

Results: Individuals residing in assisted living facilities were more likely to have a history of stroke and dementia, have more illness acuity and a longer length of hospital stay compared to the matched controls. Severity and presence of impairments in all 6 domains of the ADL score (bathing, dressing, toileting, feeding, incontinence and transferring) were more likely in the assisted care group. The majority in assisted living (75%) had multiple areas (>1) of severe impairment requiring full care. Survival time was shorter in those residing in assisted living (Median 216 (84-347) vs. 891 days (651-1130), p<0.0001) and this persisted after adjustment for sex, APACHE score, admission diagnosis, history of stroke and dementia and hospital length of stay (HR 1.74, 95%CI 1.12-2.70). The hazard ratio attenuated after addition of ADL score into the model (HR 1.36 95%CI 0.87-2.13).

Conclusions: Elderly ESRD patients discharged to an assisted care facility have a significantly increased mortality compared with age and co-morbidity matched controls. The discrepancy in long-term survival can be predicted by a simple measure of functional status upon admission to hospital.

TH-PO363

Safety, Pharmacokinetics and Efficacy of AP214, a Novel Melanocortin Receptor Agonist, in Patients Undergoing Cardiac Surgery on Cardiopulmonary Bypass Daniel Steinbrüchel,[1] Thomas E.N. Jonassen,[2] Jorgen Frokiaer,[4] Soren Nielsen,[5] Peter A. Pallesen.[6] *[1]Department of Thoracic Surgery, University of Copenhagen, Denmark; [2]Institute of Biomedicine, University of Copenhagen, Denmark; [3]Department of Thoracic Surgery, University of Copenhagen, Denmark; [4]Water and Salt Research Center, University of Aarhus, Aarhus, Denmark; [5]Water and Salt Research Center, University of Aarhus, Aarhus, Denmark; [6]Department of Thoracic Surgery, Odense University Hospital, Odense, Denmark.*

Background: Development of Acute Kidney Injury (AKI) after cardiac surgery is associated with increased morbidity and mortality. Melanocortin receptor (MCr) agonists have marked immune modulating and organ protective effects in sepsis and surgery induced AKI animal models. AP214 is a novel pan-MCr agonist under development for prevention of AKI and organ failure after cardiac surgery.

Methods: Objective: Assessment of safety, pharmacokinetics and efficacy of AP214 in cardiac surgery patients with increased risk for post-OP AKI.

Double-blind placebo controlled randomized clinical trial comparing AP214, administered at three different dose levels (10, 50 or 200 ug/kg, respectively) given as bolus iv infusions, pre- peri- and 6 hours PO to patients (N=42) on cardiopulmonary bypass.

Results: AP214 at 200 ug/kg was safe and well tolerated with a similar side effect profile compared to placebo. Estimated half-life of AP214 was 12-14 min with no signs of accumulation. There was a good relationship between dose and exposure and no dose dependency in any PK parameter observed. In the placebo group 6/13 patients developed AKI compared to 3/12 patients in the AP214 200ug/kg group. Estimated GFR, serum creatinine and additional surrogate markers indicated a high degree of kidney function preservation after AP214 treatment, with no post surgical changes in any of the parameters.

Conclusions: AP214 is safe and well tolerated. Efficacy data suggest that AP214 might be a valuable pharmacological approach to reduce the severity and frequency of post-operative AKI in patients undergoing cardiac surgery on cardiopulmonary bypass.

Funding: Pharmaceutical Company Support

TH-PO364

FG-4592 Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor Corrects Anemia in Nondialysis CKD Patients without IV Iron Anatole Besarab,[1] Robert Provenzano,[2] Steven Fishbane,[3] Chao H. Sun,[6] Diogo S. Belo,[4] Thomas B. Neff,[5] Tyson T. Lee,[5] Marietta Franco,[5] Robert Leong,[5] Kin-Hung Peony Yu.[5] *[1]Henry Ford Hosp., Detroit, MI; [2]St. Clair Specialty Physicians, Detroit, MI; [3]Winthrop Dialysis Ctr., Mineola, NY; [4]California Inst. of Renal Research, Chula Vista, CA; [5]FibroGen, Inc., San Francisco, CA; [6]Apex Research, Riverside, CA.*

Background: Anemia is largely undertreated in nondialysis CKD. We report data from an ongoing phase 2b, open-label study of FG-4592 to correct anemia in adult nondialysis CKD patients (pts). Ninety-six pts (Hb ≤10.5 g/dL at baseline [BL]) who had not received ESAs for the prior 12 weeks (wks) were randomized to 4 equal cohorts. Cohorts A and B: 16 wks initial tiered, weight-adjusted doses of 60-140 mg FG-4592 thrice weekly (TIW); Cohorts C and D: 24 wks initial fixed doses of 50 and 100 mg FG-4592 TIW, respectively. Cohort-dependent dose adjustment was allowed every 4 wks. IV iron was not permitted. BL demographics were similar across cohorts. For 95 efficacy-evaluable pts, overall mean±SD BL Hb was 9.7±0.7 g/dL. FG-4592 treatment led to increases from BL Hb in all cohorts (Table). After 16 wks of treatment, all A and B pts treated >5 wks (n=46) had a Hb response (≥11 g/dL and ≥1 g/dL from BL), except 3 pts with BL Hb ≤8.5 g/dL who achieved mean Hb 10.4 g/dL. At wk 16, mean doses were 91 and 113 mg in A and B, respectively. B pts who had a Hb response were converted to twice weekly dosing. Responses were observed whether BL ferritin or TSAT was normal or subnormal. After 8 wks of treatment, there was a dose-dependent increase from BL Hb; and for 33 pts with data available, mean hepcidin decreased 36% from BL (p<0.0001). FG-4592 was well tolerated. No treatment-related serious adverse events were reported to date. Treatment with initial weight-adjusted or fixed oral FG-4592 doses corrected anemia in CKD pts in the absence of IV iron repletion.

			Mean±SD ΔHb (g/dL)		
			Wks of Treatment		
Cohort	Starting Dose FG-4592 (mg) TIW	n	4	8	16
A	60-140	24	1.6±1.1	2.4±1.2	2.3±1.2
B	60-140	24	1.1±0.9	1.8±1.0	2.3±1.0
C	50	23	0.6±0.7	1.0±0.9	NA
D	100	24	1.5±1.0	2.1±1.1	NA

LOCF method used to impute missing data. NA=data not yet available.

Funding: Pharmaceutical Company Support

TH-PO365

Adherence to Treatment among Children and Adolescents with Chronic Kidney Disease Rita Rosicker,[1] Sudha Chennasamudram,[2] Tetyana L. Vasylyeva.[2] *[1]Department of Family Medicine, Texas Tech University Health Sciences Center, Amarillo, TX; [2]Department of Pediatrics, Texas Tech Unviersity Health Sciences Center, Amarillo, TX.*

Background: Adherence to a prescribed treatment regimen reduces morbidity and mortality among patients with chronic renal diseases (CKD).Children often fail to adhere adequately to their medication plans.This report addresses behavioral functioning and child self-reporting of medication adherence among 10-21 year old patients with CKD.

Methods: The objective of this study was to examine patient-perceived factors that impact adherence to treatment using a qualitative descriptive individual interview approach.The questionnaire included 20 questions, answered by total of 12 children and was administered anonymously.

Results: Children admitted that they skipped their medications an average 1.6 days/ week. One third of the patients "did not like all of their medications." One of the least "favorite" medications was prednisone. Two-thirds of the patients felt either "upset" or "sometimes upset" by taking medications. Although 91.6% were frustrated by having their condition, only 25% stated that taking medications interferes with their daily lives, while 50% stated that taking medications did NOT interfere with their daily lives, and the other 25% were not sure. Most adolescents (66.6%) did not care what their friends think of them having a condition requiring medication. Interestingly, we discovered that the biggest problem in taking medications existed at home, not at school. Thus, 75% forgot to take medications at home, and just 16.6% forgot to take medications at school. The patients surveyed also were more likely to forget to take medications during the week (42%) versus the weekend (33%). As for ideas to help adolescents remember to take their medications, the most popular idea was a pill box with 66.6% saying it would help, while only 33.3% said that a reminder alarm would help, and 25% thought that a better tasting medicine would help.

Conclusions: Adherence to medications among pre-adolescents and adolescents with CKD is a serious medical problem which affects treatments and quality of life and requires developing a systematic approach.

TH-PO366

Ph II Study of Eculizumab (ECU) in Patients (PTS) with Atypical Hemolytic Uremic Syndrome (aHUS) Receiving Chronic Plasma Exchange/Iinfusion (PE/PI) Christoph Licht,[1] Petra Muus,[2] Christophe M. Legendre,[3] Kenneth Douglas,[4] Maryvonne Hourmant,[5] Yahsou Delmas,[6] Maria Herthelius,[7] Antonella Trivelli,[8] Timothy Goodship,[9] Camille Bedrosian,[10] Chantal Loriat.[11] *[1]Hosp for Sick Children; [2]Radboud Univ Nijmegen Med Ctr; [3]Hop Necker; [4]Beaston W Scotland Cancer Ctr; [5]CHU Hotel Dieu-Nantes; [6]CHU Pellegrin; [7]Karolinskia Univ Hosp; [8]Inst G. Gaslini; [9]Newcastle Univ; [10]Alexion Pharm; [11]Hop Debre.*

Background: Permanent uncontrolled complement activation drives systemic thrombotic microangiopathy (TMA) and life threatening complications in aHUS. Despite PE/PI, >50% of pts develop ESRD/die within 1yr of diagnosis. We report final 26wk and follow-up data for pts with aHUS receiving ECU, a terminal complement inhibitor.

Methods: Pts ≥12 yrs with aHUS, receiving PE/PI enrolled in a controlled, open-label, single-arm, PhII trial (pts enrolled 2009-2010). Prim endpt: TMA event-free status (≥12 consecutive wks stable platelet count, no PE/PI, no new dialysis). Sec endpts included TMA intervention rate (# plasma and new dialysis events/pt/day), renal function safety. We report the final 26wk and follow-up results as of data cut-off (10/10).

Results: 20pts received ECU thru wk26. 19pts continued into an extension. Median time from diagnosis to screening=48mths (range:.66-286). ECU median treatment (Tx) duration =40wks (26-52) at time of analysis. Median age=28yrs. Prim & sec endpts were achieved with high clin & stat. significance.

Key Endpoints	Wk 26	Follow-up (ECU=40 wks)
PRIMARY TMA event-free status, n (%)	16* (80)	16* (80)
SECONDARY Median TMA intervention rate (pre-Tx [0.23])	Post-Tx: 0 (*P*<.0001)	Post-Tx: 0 (*P*<.0001)
CKD improvements ≥1 stage, n (%)	7 (35)	7 (35)
Point estimate change in QoL	0.11 (*P*<.0001)	0.12 (P=.001)
Pts achieving a minimally important difference (MID)	8/11 (73%)	8/11 (73%)

*The 4 pts who did not achieve TMA-event free status had normal platelet count at study entry and maintained platelet count ≥150x10^9/L throughout. However, at certain time points, plt count in these pts was >25% change from baseline.
MID= change of at least 0.08 on the US Time Trade-off Value

ECU was similarly effective in pts w/wo identified complement mutations and was well tolerated; only 6pts had AEs deemed related to drug.

Conclusions: Continued ECU Tx resulted in sustained TMA suppression and led to permanent discontinuation of chronic PE/PI. Sustained ECU Tx resulted in stabilized/ improved renal function, was well tolerated and demonstrates potential as the new SOC for aHUS.

TH-PO367

Continued Improvements in Renal Function with Sustained Eculizumab (ECU) in Patients (PTS) with Atypical Hemolytic Uremic Syndrome (aHUS) Resistant to Plasma Exchange/Infusion (PE/PI) Laurence A. Greenbaum,[1] Sunil Babu,[2] Richard Furman,[3] Neil Sheerin,[4] David J. Cohen,[5] A. Osama Gaber,[6] Frank Eitner,[7] Yahsou Delmas,[8] Chantal Loriat,[9] Camille Bedrosian,[10] Christophe M. Legendre.[11] *[1]Emory Univer; [2]Fort Wayne Med; [3]Weill Cornell Med Coll; [4]Newcastle Univ; [5]Columbia Univ Med Center; [6]Methodist Hosp; [7]Univ Aachen; [8]CHU Pellegrin-Bordeaux; [9]Hopital Robert Debre; [10]Alexion Pharmacetuicals, Inc; [11]Universite Paris Descartes & Hopital Necker.*

Background: aHUS is a genetic, devastating, systemic disease, caused by permanently uncontrolled complement activation, resulting in thrombotic microangiopathy(TMA). Despite PE/PI, >50% of pts develop ESRD/die within 1 yr of diagnosis. We report longer follow-up data from a phase II trial of ECU, a terminal complement inhibitor.

Methods: Pts ≥12 yrs with aHUS and persistent TMA despite ≥4 PE/PI sessions 1 wk before screening were enrolled in a 26-wk, controlled, open-label, single-arm phase II trial (2009-2010). Prim. Endpoint: change in platelet (plt) count (a measure of TMA) (73x109/L; p=0.0001). Sec. endpoint: 15/17 pts (88%) achieved TMA event-free status (≥12 wks of stable plt count, no PE/PI and no new dialysis). 4/5 pts on dialysis permanently discontinued dialysis. ECU was well tolerated (Legendre. ASN 2010). We report follow-up results as of data cut-off (10/2010).

Results: 17 pts enrolled (2 discontinued; SLE and an AE unrelated to ECU, respectively). Median time from diagnosis to screening =10mo (range:<1-236). Median age=28 yrs. ECU median duration~38 wks (range: 26-64 wks) at time of analysis. 13 pts entered the extension study. 13 pts with low platelets at baseline had plt normalization at week 26 and continued to maintain normal levels at data cut-off. Renal function improved (≥1 CKD stage=10 pts and ≥25% decrease in creatinine from baseline=11 pts). ECU was well tolerated; 10 pts with adverse events deemed related to ECU (generally mild/moderate). Longer follow-up (>1 yr) will be presented.

Conclusions: In this early intervention study, sustained treatment with ECU prevented TMA and improved renal function. These data further strengthen the evidence for ECU as standard of care for aHUS.

Funding: Pharmaceutical Company Support

TH-PO368

Neutrophil Gelatinase-Associated Lipocalcin (NGAL) and Alternate Pathway of Complement (APC) Factors as Biomarkers of Response to Treatment in Patients with Focal Segmental Glomerulosclerosis (FSGS) Howard Trachtman,[1] Prasad Devarajan,[2] Michael R. Bennett,[2] Joshua M. Thurman,[3] Milena Radeva,[4] Debbie S. Gipson,[5] Frederick J. Kaskel,[6] Aaron L. Friedman,[7] Marva M. Moxey-Mims,[8] Suzanne M. Vento.[1] *[1]Pediatrics, Cohen Children's Medical Center, New Hyde Park, NY; [2]Pediatrics, Cincinnati Children's Medical Center, Cincinnati, OH; [3]Medicine, University of Colorado Denver School of Medicine, Aurora, CO; [4]Quantitative Health Sciences, Cleveland Clinic Foundation, Cleveland, OH; [5]Pediatrics, University of Michigan, Ann Arbor, MI; [6]Pediatrics, Albert Einstein College of Medicine, Bronx, NY; [7]Pediatrics, University of Minnesota, Minneapolis, MN; [8]NIDDK, KUH, Bethesda, MD.*

Background: The FSGS-Clinical Trial evaluated a 12-month course of cyclosporine (CSA) *versus* combination dexamethasone (DEX) and mycophenolate mofetil (MMF) in 138 patients with steroid-resistant FSGS. This ancillary study evaluated the use of NGAL and APC factors as biomarkers of response to treatment.

Methods: Urine and plasma were obtained at weeks 0, 26, 52, and 78. NGAL and APC factors -- Ba and Bb, and soluble C5b-9 -- were determined by ELISA. Outcomes were defined by a 6-ordinal scale (1, complete remission, 6 no reduction in Up/c). Results are provided as mean±SEM.

Results: 19 patients (10M:9F) were included, 7 received CSA and 12 DEX/MMF. There were no differences between these two groups. There was a decline in plasma sC5b-9 in response to DEX/MMF (P\<0.001); however, there were no therapy-related differences in urinary NGAL or APC levels (ng/mg creatinine). Baseline urinary NGAL excretion correlated with primary outcome at 52 wk (P=0.08). When pooled by outcome, 1-3 *versus* 4-6, baseline urinary NGAL (54±17 vs 227±190) and sC5b-9 (168±36 vs 450±181) levels were numerically lower in those with a favorable response. Urine Ba excretion at the end of treatment correlated with outcome (P<0.01).

Conclusions: Urinary excretion of NGAL and select APC factors may be useful indices for patient stratification to predict outcome and to assess adequacy of response to treatment in patients with primary steroid resistant FSGS.

Funding: NIDDK Support

TH-PO369

The Specificity of Urinary Aquaporin-1 and Adipophilin To Diagnose Renal Cell Carcinoma Jeremiah J. Morrissey, Evan D. Kharasch. *Anesthesiology, the Siteman Cancer Center, Biochemistry and Molecular Biophysics, Washington University in St. Louis, St. Louis, MO.*

Background: Renal cancer accounts for about three percent of adult malignancies and is most frequently asymptomatic until late stages of disease. This investigation tested whether aquaporin-1 (AQP-1) and adipophilin (ADFP), sensitive biomarkers of kidney cancer, are specific biomarkers to detect asymptomatic kidney cancer in comparison to patients with common forms of kidney disease.

Methods: Urine samples were obtained from 32 patients with clear cell or papillary kidney cancer undergoing partial or radical nephrectomy, 15 control patients undergoing surgery for non-kidney related reasons, 44 patients with documented urinary tract infection, 24 patients diagnosed with diabetic nephropathy, and 18 patients diagnosed with glomerulonephritis. Samples for the last two patient cohorts were obtained from the Kidney Translational Research Core of the Washington University George M. O'Brien Center for Kidney Disease Research. Urinary concentrations of AQP-1 and ADFP were determined by a sensitive and specific Western blot procedure and corrected for urinary creatinine excretion

Results: Overall, there was a 23- to 46-fold increase in AQP-1 and a 51- to 77-fold increase in ADFP in the median concentration of these two biomarkers in the urine of patients with kidney cancer compared to the median concentrations found in the urine of patients with these 3 common kidney diseases or the urine of the surgical control patients with AROC values of 0.99 to 1.00 ($P<0.001$).

Conclusions: Our study shows that measuring urinary AQP-1 and ADFP concentrations provide a means of screening patients to identify those with kidney cancer without interference from underlying common kidney diseases

Funding: Clinical Revenue Support

TH-PO370

Hydronephrosis Predicts the Presence of Severe Vesicoureteral Reflux Husam A. Abdulnour, Eduardo H. Garin. *Pediatrics, University of Florida, Gainesville, FL.*

Background: The finding of hydronephrosis by renal ultrasound has been suggested to predict the presence of Vesicoureteral Reflux (VUR). A review of published data has shown conflicting results. We hypothesized that in patients with VUR grade IV or V, hydronephrosis will be found, if the patient does have a full bladder during the renal ultrasound examination.

Methods: This retrospective study included 837 patients, median age 1.3 years with a range of 0-18.7 year, 569 female and 268 male patients. Each patient underwent at least one voiding cystourethrogram (VCUG) and one renal ultrasound examination. On renal ultrasound, particular attention was paid to the presence of hydronephrosis and bladder filling status in patients with VUR grade IV or V.

Results: Sensitivity and specificity for the renal ultrasound showing hydronephrosis to detect the presence of VUR grades IV and V were 60 % and 92 %, respectively. Positive predictive value (PPV) and negative predictive value (NPV) were 74 % and 87 %, respectively. The Breslow-Day test for the homogeneity of the odds ratios supports the hypothesis that odds ratios differ by VUR grade , with more severe VUR associated with a stronger hydronephrosis/full bladder relationship ($p=0.045$). It also supports the hypothesis that odds ratios differ by bladder status, with a full bladder associated with stronger hydronephrosis and severe VUR relationship ($p=0.046$)

Conclusions: Hydronephrosis will be likely observed on a renal ultrasound examination in the presence of a full bladder, in patient with VUR grade IV or V.

Funding: Clinical Revenue Support

TH-PO371

Inpatient Healthcare Utilization for Hemolytic Uremic Syndrome in the U.S.A. Hyunjung Stella Shin,[1] Brian Becknell,[1] John D. Mahan,[2] David S. Hains.[2] *[1]Pediatrics, Nationwide Children's Hospital, Columbus, OH; [2]Pediatrics, Division of Nephrology, Nationwide Children's Hospital, Columbus, OH.*

Background: Hemolytic uremic syndrome (HUS) is a leading cause of pediatric acute renal failure. In this study, we evaluated the demographics and economic impact of inpatient hospitalizations for HUS in the U.S.A, and determined the specific effect of extrarenal comorbidities and procedures on length of stay (LOS).

Methods: We analyzed 2276 pediatric HUS admissions, using the Kids' Inpatient Database of the Healthcare Cost and Utilization Project, from 1997, 2000, 2003, and 2006. Admissions with a primary diagnosis of HUS were identified by ICD-9 code. Patient sex, age, LOS, hospital charges, comorbidities and procedures were evaluated.

Results: 60.7% (1326) of admissions were for children 1-4 years old, and 56.7% (1284) of admissions were for female patients. Mean LOS was unchanged over the period of study (10 ± 0.7 days), and hospital charges increased 2.1-fold, from $29,605 ± $3,460 to $62,960 ± $5,957. Among extrarenal comorbidities, pancreatitis notably occurred in 10.5% of admissions and was associated with increased LOS (20.4 ± 2.8 days). Increased LOS was also noted with gastrointestinal hemorrhage (15 ± 3.5 days) and respiratory insufficiency (26.1 ± 3.7 days). Among procedures, admissions requiring dialysis (19%) were associated with increased LOS (18.4 ± 1.5 days). Red blood cell (RBC) transfusion was associated with decreased LOS (6.4 ± 0.4 days).

Conclusions: Our results demonstrate multiple novel findings: (1) We identify sex-skewing among pediatric HUS admissions, with a female predominance. (2) There has been no reduction in LOS for HUS over the study period, despite rising hospital charges. (3) Extrarenal comorbidities – particularly pancreatitis and respiratory insufficiency – significantly impact LOS. This highlights the need to aggressively identify and manage extrarenal comorbidities in patients with HUS. (4) The potential association between RBC transfusion and decreased LOS is consistent with studies recommending isotonic fluid resuscitation in HUS patients and merits further evaluation.

TH-PO372

Low Utilization of Growth Hormone Therapy in Children with Short Stature in the CKiD Cohort Amira Al-Uzri,[1] Hilary M. Hotchkiss,[2] Ora Yadin,[2] Michael F. Schneider,[2] Laurence A. Greenbaum,[2] Frederick J. Kaskel,[2] Susan L. Furth,[2] Bradley A. Warady.[2] *[1]Oregon Health & Science University, Portland, OR; [2]CKiD Study Group.*

Background: Growth hormone (GH) improves growth velocity in children with chronic kidney disease (CKD), but multiple studies have demonstrated underutilization of GH in children with CKD and short stature (SS). We describe the rate of utilization of GH and risk factors for underutilization in the multicenter, Chronic Kidney Disease in Children (CKiD) cohort study.

Methods: This is a cross sectional baseline analysis of 135 participants at enrollment. SS was defined as height SDS <-1.88 for age and gender. 63 subjects had SS and were not on GH and 72 were on GH (24 also had SS). Demographic data and iohexol GFR were obtained on all subjects. The two groups were compared using X^2 tests for categorical variables or Wilcoxon rank-sum tests for continuous variables.

Results:

Characteristics *	SS and no GH use (N=63)	GH use (N=72)	p-value
Age, y			0.418
Age1-<6 y	24% (15)	29% (21)	
Age 6- ≤ 10 y	25% (16)	26% (19)	
Age > 10 y	51% (51)	44% (32)	
Male	51% (32)	63% (45)	0.170
White Race	65% (41)	75% (54)	0.208
Age-gender specific Height SDS	-2.31 (-2.85, -2.03)	-1.51 (-2.28, -0.59)	<0.001
Glomerular etiology of CKD	17% (11)	8% (6)	0.111
Tanner Stage II-V	34% (21)	25% (17)	0.221
Iohexol GFR , ml/min/1.73m²	39.6 (30.1 - 50.0)	33.7 (28.3 to 40.3)	0.009
Family income > $75,000/y	27% (16)	28% (19)	0.872
Maternal education, high school or higher	45% (27)	60% (42)	0.088
Private Health Insurance	44% (27)	52% (36)	0.368

* Median (inter-quartile range) or % (n)

Conclusions: SS in children with CKD is frequently not treated with GH. This is not due to advanced pubertal maturation since most untreated children are Tanner stage 1. The association of GH usage with lower GFR and with higher maternal education may reflect an increased focus on growth as GFR declines and an increased access to resources or more concern about SS, respectively. Longitudinal analysis of this cohort may yield better understanding of the risk factors for GH underutilization, and the medical and psychosocial impact of low GH utilization.

TH-PO373

Long-Term Maintenance of Efficacy with Losartan in Children with Proteinuria: Results of a Randomized Controlled Trial Nicholas J. Webb,[1] Shahnaz Shahinfar,[2] Thomas G. Wells,[3] Rachid Massaad,[4] Gilbert Gleim,[5] Emanuela P. Santoro,[5] Christine McCrary Sisk,[5] Chun Lam.[5] *[1]Royal Manchester Children's Hospital, Manchester, United Kingdom; [2]S. Shahinfar Consulting, Inc., Newton Square, PA; [3]University of Arkansas for Medical Sciences, Little Rock, AR; [4]MSD, Brussels, Belgium; [5]Merck, Whitehouse Station, NJ.*

Background: Reducing proteinuria is a key treatment goal in children with CKD. In a 12-week, double-blind, multinational study, losartan significantly lowered proteinuria compared with placebo and amlodipine and was well tolerated in children aged 1-17 y. The aim of the present open-label, extension phase of this study was to assess the long-term efficacy and tolerability of losartan versus enalapril after 3 y of treatment.

Methods: Patients who had completed the 12-week core study were re-randomized to remain on losartan or start enalapril, and followed for proteinuria and renal function. The extension study continued until 100 patients completed 3 y of follow up.

Results: A total of 268 patients were randomized in a 1:1 ratio to losartan (0.30 to 4.42 mg/kg/d) or enalapril (0.02 to 1.13 mg/kg/d). After 3 y of treatment, the LS mean percent reduction from baseline in the urinary protein/creatinine ratio was 30.3% in the losartan group vs. 41.7% in the enalapril group [geometric mean ratio (95% CI) = 1.2 (0.9, 1.6)] and the LS mean change from baseline in GFR was 3.3 ml/min/1.73m² in the losartan group versus 7.0 ml/min/1.73m² in the enalapril group [treatment difference (95% CI) = -3.8 ml/min/1.73m² (-15.2, 7.6)]. The adverse event (AE) incidence was relatively low, including that of hyperkalemia (3.0% on losartan vs. 6.7% on enalapril) and renal dysfunction (8.2% on losartan vs. 6.7% on enalapril), and comparable in both treatment groups. Fewer drug-related AEs occurred in the losartan group than in the enalapril group (6.0% vs. 11.2%, respectively).

Conclusions: In children aged 1-17 y with proteinuria, losartan and enalapril significantly reduced proteinuria, an effect that was maintained for the entire 3-y study period. Both losartan and enalapril were generally well tolerated.

Funding: Pharmaceutical Company Support

TH-PO374

Vesico-Ureteral Reflux as a Risk Factor for Acute Pyelonephritis and Renal Damage in Children with UTI: Systematic Review and Meta-Analysis

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Background: Urinary tract infection (UTI) is one of the most common bacterial infections in childhood. UTI may result in renal scarring (RS), and vesicoureteral reflux (VUR) may be a risk factor for both acute pyelonephritis (APN) and RS that predisposes to long-term complications. However, which imaging should be performed after UTI is strongly debated, and part of the debate is due to the remained question of the true relationship between VUR, and both APN and RS. Recently, two meta-analyses found conflicting results, because of important missing studies, and pooling together APN and RS. We aim to update the meta-analysis and study if VUR is a risk factor for APN, and RS.

Methods: A systematic review and meta-analysis was performed according to the CDR guidelines. All studies of children with UTI, DMSA scan and cystography, were identified by a systematic electronic search in MedLine, and Embase, until 2011. Pooled estimates were calculated using DerSimonian and Laird random-effects model.

Results: From the 1558 potentially relevant articles, 80 were included, representing 11,410 children; children had a first UTI in 53 (66%) studies; 48 (60%) studies were prospective, and in 15 (19%) children underwent both early and late DMSA scan. At all, 6,681 children with an early scan were included, and 5,879 with a late scan. All-grade VUR was significantly associated to both APN (OR=2.0; 95%CI: 1.8-2.3) and RS (OR=4.8; 95%CI: 4.3-5.5). High-grade (≥3) was also significantly related to APN (2.4; 95%CI: 1.9-3.1) and RS (OR=5.7; 95%CI: 4.5-7.3). Pooled estimates were found with heterogeneity, partly explained by the delay between UTI and late DMSA scan, and by the number of UTI.

Conclusions: Children with VUR had a higher risk of APN and RS. These data suggest that identification of VUR can be a practical method of identifying children who are at risk for renal scarring.

Funding: Institut Pasteur

TH-PO375

End Stage Renal Disease Health-Related Quality of Life in Korean Children

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Background: Health-related quality of life (HRQOL) is very important issue in children with end stage renal disease (ESRD) and their family. Moreover, this problem can maintain all their life long. We performed the cross-sectional investigation of HRQOL in Korean children undergoing renal replacement therapies such as dialysis, renal transplantation due to ESRD.

Methods: We used the Korean version of PedsQL 4.0 Generic Core Scales and PedsQL 3.0 ESRD module simultaneously and compared differences of HRQOL score between various clinical characteristics including gender, age, dialysis modality, socioeconomic status of patient's parents. Ninety two pediatric patients with ESRD aged 2-18 year old were enrolled in four Korean university hospitals.

Results: Male: Female ratio was 44:48 and most common cause of ESRD was chronic glomerulonephritis. Fifth five children have been treated by dialysis (mean age: 12.47 years-old) and thirty seven children received renal transplantation (mean age: 11.57 years-old). On parent proxy-report, three scales including About My Kidney Disease Scale, Treatment Problems Scale, and Perceived Physical Appearance Scale showed significantly higher HRQOL in children with a renal transplant than children receiving dialysis. However, on child self-report, there was no statistically significant difference of HRQOL score between treatment modalities. There was also no significant difference of HRQOL score according to age and gender. Child self-report showed significantly lower HRQOL in three scales including General Fatigue Scale, Family and Peer Interaction Scale, Worry Scale than that of parent proxy-report. Children on peritoneal dialysis self reported significantly higher QOL than children on hemodialysis.

Conclusions: QOL should be evaluated in all children with ESRD to support more favorable growth and development as well as disease problem. The PedsQL 3.0 ESRD Module may be useful as an ESRD-specific instrument to evaluate HRQOL in Korean children and larger, longitudinal prospective study is needed.

TH-PO376

ERA-EDTA Registry and European Alport Registry: Prognosis on Renal Replacement Therapy and Improvement of Life-Expectancy in Alport Patients as Well as in Heterozygous Carriers

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Background: The hereditary renal disease Alport Syndrome (AS) is caused by mutations of type IV collagen genes. AS leads to an impaired function of the glomerular basement membrane progressing to end stage renal disease (ESRD). Heterozygous carriers also have a relatively high risk to develop ESRD.

Methods: We used data from 11 European countries providing data to the ERA-EDTA Registry to compare Alport males on renal replacement therapy (RRT, dialysis as well as transplanted patients) with age-matched males with other renal diseases. Furthermore, we used data from the European Alport Registry to analyze the effects of a preemptive RAAS-blockade (ACE-inhibitor or ARB) regarding age at start of RRT and life expectancy in Alport patients and in heterozygous carriers.

Results: ERA-EDTA registry data showed better patient survival for Alport males on RRT when compared to age-matched males with other diseases; furthermore, kidney graft survival was found to be better in transplanted Alport patients . The age at start of RRT of Alport patients tended to increase somewhat sharper after the year 2000 compared with the 1990s. Alport registry data show a better life-expectancy in Alport patients who received RAAS-blockade before onset of RRT. Both results (better life-expectancy and delayed start of dialysis) also apply for heterozygous Alport carriers in the Alport registry.

Conclusions: a. Data from two different independent registries support our hypothesis that pre-emptive RAAS-blockade may delay ESRD in Alport patients . b. Alport carriers also seem to profit from RAAS-blockade. This should increase alertness for oligosymptomatic patients with microhematuria as possible heterozygous carriers for autosomal Alport mutations (1% of total population). c. Life expectancy of Alport patients with ESRD might be better than that of patients with other renal diseases.

TH-PO377

Performance of Two Strategies for Urgent ANCA and Anti-GBM Analysis in the Diagnosis of Suspected Severe Small Vessel Vasculitis

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Background: In acute situations, patients suspected of small vessel vasculitis (SVV) may benefit from rapid and reliable testing for ANCA and anti-GBM antibodies. We analysed the diagnostic performance of two rapid methods for assessing the presence of ANCA and anti-GBM in a cohort of severly ill patients with suspected SVV and compared the results with capture ANCA ELISA

Methods: Between Jan 2003 and Nov 2010, we received 260 requests for urgent analysis of ANCA and anti-GBM. These samples were analyzed by qualitative Dotblot (Biomedical Diagnostics) for PR3-, MPO-ANCA and anti-GBM. Routine IIF and ELISA were performed afterwards. We retrospectively analyzed these samples with the novel high sensitive automated Phadia EliA $PR3^s$ and MPO^s and anti-GBM EliA. Results were related to the final clinical diagnosis and compared with our routine ELISA.

Results: In 74 of 260 patients (28%) a final diagnosis of AAV (n=62) or anti-GBM disease was made (n=12).

Both Dotblot and EliA detected all 12 cases of anti-GBM disease (sensitivity 100%, negative predictive value (NPV) 100%). Both methods found one false positive (specificity 99%) resulting in a positive predictive value (PPV) of 93%.

Dotblot detected anti-PR3 or anti-MPO in 55 of 62 AAV-patients (sens 89%, NPV 96%), but showed 6 false positives (spec 97%, PPV 90%). The Phadia EliA $PR3^s$ or MPO^s was positive in 58 of 62 AAV- patients (sens 94%), and had 9 false positives (spec 95%), resulting in a PPV of 87% and a NPV of 97%. The false positives in the Dotblot or ELIA were diagnosed with f.e TIN, sarcoidosis and IgA-nephropathy. Our standard ELISA was equally accurate as EliA and slightly more accurate than dotblot due to less false positives (sens of 94 %, spec of 97%, PPV of 91%, and NPV of 98%).

Conclusions: The Dotblot and Phadia EliA on GBM^s ,$PR3^s$ and MPO^s performed excellent in our cohort of patients clinically suspected of AAV or anti-GBM disease in whom urgent testing was ordered. As results are almost identical to ELISA, we concluded that both tests are very powerful tools for a rapid serological diagnosis.

TH-PO378

Bleeding Complications of Native Kidney Biopsy: A Systematic Review and Meta-Analysis

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Background: Kidney biopsy provides important information for nephrologists but the risk of complications has not been systematically described. We studied the rate of macroscopic hematuria and erythrocyte transfusion following native kidney biopsy with real-time ultrasound guidance and a spring-loaded biopsy device using meta-analysis.

Methods: A PubMed search was conducted using MeSH terms "biopsy" and "kidney" with subheadings "complications" and "adverse effects" from January, 1980 through December, 2010. Retrieved abstracts were reviewed for inclusion using pre-

specified criteria. Random effects meta-analysis was used to determine the complication rates following kidney biopsy. Subgroup analysis was used to identify predictors of complications.

Results: 2327 studies were reviewed with 32 selected for analysis. The overall rate of macroscopic hematuria was 3.9% (95% CI, 2.8-5.4%) and transfusion was 1.5 % (1.0-2.2%). Significantly higher rates of transfusion were seen with the following factors: 14 gauge needles when compared with smaller needles (3.5% vs. 1.1%, p<0.001), studies with ≥ 50% women (3.4% vs. 1.1%, p=0.003), and with ≥ 10% of biopsies for acute kidney injury (1.5% vs. 0.4%, p=0.005). Higher transfusion rates were also observed in studies with mean creatinine ≥ 2.0 mg/dl (2.4% vs. 0.9%, p=0.054) and mean age ≥ 40 years (1.7% vs. 0.6%, p = 0.067). Similar but non-significant relationships were noted in the macroscopic hematuria rate with the same predictors. Other factors such as number of needle passes and coagulation and platelet parameters were not associated with either outcome.

Conclusions: Native kidney biopsy using spring-loaded device and real-time ultrasound is associated with a small risk of macroscopic hematuria and transfusion requirement. Using a smaller gauge needle may lower complication rates. Patient selection may affect outcome as studies with more women, older patients, higher creatinine, or acute kidney injury had higher complication rates. Future studies are required to further evaluate the risk factors for complications.

TH-PO379

Development of an Outpatient Native Kidney Biopsy Service Gearoid M. McMahon,[1] Molly McGovern,[1] Vanesa Bijol,[2] Julie Lin.[1] *[1]Division of Nephrology, Brigham and Women's Hospital, Boston, MA; [2]Department of Pathology, Brigham and Women's Hospital, Boston, MA.*

Background: In the U.S., native kidney biopsies are usually inpatient procedures. We developed an outpatient biopsy protocol for low-risk pts and assessed its safety and efficacy.

Methods: Pts referred for biopsy were reviewed by the nephrology service director and deferred if they had SBP>140 mmHg, BMI >35 kg/m² or took anticoagulants, ASA or NSAIDS in the preceding 7 days. Under real-time ultrasound guidance, biopsies were performed using a Boston Scientific 16G spring-loaded needle that was changed to a Bard 14G needle in Oct 2009. Vital signs and urine voids were monitored in an outpatient observation unit; CBC was sent 4 hrs after the procedure. Pts were discharged after 5 hrs of strict bedrest if there were no signs of bleeding, and complications were tracked carefully.

Results: Between Nov 2008 and Apr 2011, 105 pts (56 male) underwent outpatient biopsies. Mean age was 49±16 yrs. A 16G needle was used in 43 pts (Group A) and a 14G was used in 62 (Group B). A median of 25 (4-64) glomeruli were obtained in Group A vs. 39 (10-107) in Group B (p<0.001). One pt in each group had insufficient tissue for diagnosis. Complications requiring admission were noted in 7 pts (16%) in Group A vs. 5 pts (8%) in Group B (p=0.22). Most were minor (gross hematuria, perinephric hematoma, fall in Hb) and did not require any intervention. One pt in Group B had clinically significant bleeding in the setting of persistent hypertension that was treated with embolization. Of the 12 pts with procedural complications, 8 experienced these during the 5-hour observation period while 4 pts presented >48 hours after biopsy. The mean fall in Hb was 0.5 g/L in Group A vs. 0.6 g/L in Group B (p=0.29).

Conclusions: In a carefully selected population of pts, outpatient renal biopsy is safe with low complication rates. The majority (75%) of complications occurred during the observation period. The remainder occurred after 48 hours and thus an overnight admission would not have altered management. Importantly, the routine use of a 14G biopsy needle resulted in a greater yield of glomeruli compared to a 16G needle without increased complications.

TH-PO380

Non-Invasive Evaluation of Kidney Hypoxia and Fibrosis Using MRI Tsutomu Inoue,[1] Eito Kozawa,[2] Hirokazu Okada,[1] Tsuneo Takenaka,[1] Hiromichi Suzuki.[1] *[1]Nephrology, Saitama Medical University, Iruma-gun, Saitama, Japan; [2]Diagnostic Radiology, Saitama Medical University International Medical Center, Hidaka, Saitama, Japan.*

Background: Interstitial fibrosis and hypoxia are 2 major complications that accelerate the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether 2 magnetic resonance imaging (MRI) techniques—diffusion-weighted (DW) MRI and blood oxygen level-dependent (BOLD) MRI—could be used to determine kidney fibrosis and hypoxia. Last year, we reported that a decreased eGFR was accompanied by reduced apparent diffusion coefficient (ADC) values, as observed on DW MRI. Additionally, BOLD MRI indicated hypoxia, as determined from the decreased T2* values, in advanced renal dysfunction patients with non-diabetic nephropathy but not in patients with diabetic nephropathy.

Methods: In this study, we examined (1) the reproducibility of DW and BOLD MRI techniques, (2) the effect of kidney size on ADC and T2* values, and (3) the relationship between the ADC values and the degree of interstitial fibrosis in renal biopsy specimens.

Results: We found that (1) the degree of variability for both T2* and ADC values was approximately 4% in the same subject (T2*: 4.08 ± 3.33%, ADC: 4.18 ± 2.75%); (2) the kidney size positively correlated with the eGFR, ADC, and T2* values (r = 0.57, 0.29, and 0.52, respectively) in non-diabetic CKD patients, whereas in patients with diabetic nephropathy, the renal size weakly correlation with the ADC values (r = 0.31), and the T2* values were completely independent of the renal size (r = 0.24); and (3) the percentage of fibrotic area in the renal biopsy specimens significantly correlated with not only the ADC, but also the T2* values (r = 0.69 and 0.48, respectively).

Conclusions: Thus, we conclude that the renal size reflects the degree of interstitial fibrosis, which can be evaluated by measuring the ADC values by using DW MRI. Smaller kidneys have more severe interstitial fibrosis, lower ADC values, and decreased eGFR. The functional MRI values showed a good correlation with the pathological and physiological characteristics of the kidney.

Funding: Government Support - Non-U.S.

TH-PO381

Reference Ranges and Biological Variability of Kidney Injury Biomarkers Sushrut S. Waikar,[1] Venkata Sabbisetti,[1] Thanh Thu T. Ngo,[1] Julie A. Berkley,[1] Mark Radlinski,[1] Joseph V. Bonventre,[1] Rebecca A. Betensky, Philip L. De Jager. *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Biostatistics, Harvard School of Public Health, Boston, MA.*

Background: Urinary biomarkers of tubular injury may improve early diagnosis and aid clinical management of individuals with acute and chronic kidney diseases. In order for biomarkers to be used clinically it is important to define normal clinical reference levels and biological variability.

Methods: We collected urine samples from 90 community-dwelling healthy volunteers aged 18 to 52y (median 33.5y, 55% female). Twenty percent were black, 21% Asian or other, and 58% white. Fifty-one of the volunteers provided one or two repeat subsequent urine samples within one year. All urine samples were non-fasting, second-void (or later) morning samples. Urine samples were immediately refrigerated at 4C and then within 4h centrifuged at 3200 rpm for 5 min after which aliquots of the supernatant were stored at -80C and tested within 12 months (mean, 6 months). We measured urinary levels of kidney injury molecule-1 (KIM-1), N-actetyl-β-D-glucosaminidase (NAG), and neutrophil gelatinase-associated lipocalin (NGAL), each normalized to the urinary creatinine concentration. Coefficients of variation were < 15% for each biomarker. We examined associations between biomarkers and age, race, sex, body mass index, and blood pressure. Statistical techniques included random effects models with a random effect for each subject and the Spearman correlation coefficient.

Results: The median (range) concentrations were: KIM-1, 0.23 ng/mg creatinine (0.02 – 0.98); NGAL, 7.5 ng/mg (0.06 – 53.50); NAG, 1.39 U/g (0.45 – 4.90).

KIM-1 levels were lower in blacks by 0.17 ng/mg (P=0.005). NAG levels correlated positively with age (R = 0.41, P < 0.0001) and were higher in Asians by 0.13 U/g. NGAL levels were higher in females by 0.33 ng/mg. The intra-class correlation coefficients were: KIM-1, 0.76; NAG, 0.58; NGAL, 0.19.

Conclusions: The biological and clinical significance of biomarker variability according to age and race merits further study. Intra-individual variability of NGAL was high, which may have implications for epidemiologic studies.

Funding: NIDDK Support

TH-PO382

Diagnostic Value of Serum κ/λ Free Light Chain Assay for Monoclonal Gammopathy in Renal Insufficiency Patients Jeong-Woo Park, Yeo Kyeoung Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Internal Medicine, Chonnam National University Medical School, Gwangju, Korea.*

Background: Patients with plasma cell disorder (PCD) frequently showed reduced renal function, hence, the differential diagnosis should include PCD in these patients. Serum free light chain (sFLC) κ/λ ratio (rFLC, conventional reference range: 0.26-1.65) was generally used as a screening test for the presence of PCD. Recently, an extended renal reference range of rFLC (0.37-3.17) was proposed for renal failure patients, which seems to have more validated in clinical setting.

Methods: Patients who visited the nephrologists due to renal insufficiency were enrolled (n=538). Nephelometric sFLC quantification in combination with serum (s-PEP), urine protein electrophoresis (u-PEP), and pathologic diagnosis were performed to determine the diagnostic usefulness of sFLC testing in these patients.

Results: Median serum Cr and eGFR were 1.9 mg/dL (ranges; 1.4-21.8) and 31 mL/min/1.73 m² (2-60) (56.9% CKD, 43.1% AKI). Of total 538 patients, 23.4% were diagnosed as PCD. The renal range of rFLC increased sensitivity (87.3% vs. 86.5%) and specificity (95.8% vs. 91.4%) for diagnosis of PCD compared to those with conventional one. The area under the ROC curve in renal range increased from 0.89 (conventional range, 95% CI: 0.85-0.93) to 0.92 (0.88-0.95). rFLC results using renal range were more sensitive to find PCD compared to those of PEP (sensitivity: s-PEP 79.7%, u-PEP 67.8%). Combining renal range rFLC and s-PEP resulted in 97.4% of sensitivity for detecting PCD. In twenty-nine patients with light-chain multiple myeloma (LCMM), rFLC assessment (100% positive) showed higher accuracy in diagnosis compared than those of s- and u-PEP (58.6% and 69.0%).

Conclusions: In summary, sFLC immunoassay is sensitive and specific tool to detect PCD in renal failure patients, especially for LCMM. Extended renal reference range is more sensitive and specific than conventional one. For the screening of PCD in patients with renal impairment, physicians should try to integrate the s-PEP and rFLC results to find the underlying cause of renal insufficiency.

TH-PO383

Impact of Multiple Myeloma and Treatment with G-CSF on U and P-HNL/NGAL Jan Melin, Karin Edberg, Kristina E. Carlson, Bengt C. Fellstrom, Per Venge. *Medical Sciences, Uppsala University, Uppsala, Sweden.*

Background: Renal failure is an important complication of multiple myeloma. In myeloma malignant plasma cells produce monoclonal immunoglobulin (M-component). With treatment the M-component can disappear. The M-component can form casts that obstruct tubular flow, be deposited in the renal tissue and cause amyloidosis. Myeloma can also cause renal injury by secondary mechanisms such as hypercalcemia and dehydration. G-CSF is used for autologous stem cell mobilization in myeloma. HNL/NGAL is a promising biomarker of acute kidney injury. The aim of this study was to investigate whether U or P-HNL/NGAL levels were affected by myeloma.

Methods: We examined 50 patients with myeloma who were either on a scheduled visit to the hematology outpatient unit or were admitted to the hospital for treatment. U and P-HNL/NGAL was measured by a sandwich ELISA. Active disease was defined as significantly rising M-component. Comparisons between groups were made by the non-parametric Mann-Whitney U-test.

Results: P-HNL/NGAL, but not U-HNL/NGAL, was significantly increased in patients on treatment with G-CSF. Bence-Jones proteinuria, M-component or active disease were not associated with elevated HNL/NGAL-levels. As expected P-HNL/NGAL correlated with creatinine levels in plasma.

U and P-HNL/NGAL in myeloma patients

	U-HNL/NGALµg/L			P-HNL/NGALµg/L		
	Yes	No	P	Yes	No	P
G-CSF	55.2±35.3 N=6	34.8±10.9 N=44	NS	192.7±54.2 N=6	41.5±3.6 N=44	<0.001
Bence-Jones proteinuria	48.7±29.5 N=14	32.9±9,0 N=36	NS	51.7±8.8 N=14	62.8±13.2 N=36	NS
M-component in serum	39.7±13.7 N=34	32.1±14.9 N=16	NS	53.4±8.3 N=34	73.1±25.2 N=16	NS
Active disease	57.6±26.8 N=17	26.8±7.4 N=33	NS	47.1±7.7 N=17	66.1±14.2 N=33	NS

Values are means±SEM

Conclusions: We did not find any evidence that myeloma per se affects the levels of U and P-HNL/NGAL. However, G-CSF-treatment was associated with a significant increase in P-HNL/NGAL. These findings make it unlikely that the myeloma itself interferes with the interpretation of U and P-HNL/NGAL in patients with multiple myeloma.

Funding: Government Support - Non-U.S.

TH-PO384

Effect of Pegloticase (PGL) on Renal Function in Patients with Chronic Kidney Disease (CKD) Faith Debra Ottery,[1] Robert A. Yood,[2] Marsha Wolfson.[1] *[1]Savient Pharmaceuticals, Inc., E. Brunswick, NJ; [2]Fallon Clinic, Worcester, MA.*

Background: CKD is common in gout patients. PGL is FDA-approved for refractory chronic gout. In 2 replicate Phase 3 clinical trials (n=212) conducted to assess efficacy and safety of PGL, 49% had stage 3-4 CKD, defined by the National Kidney Foundation. Using pooled data, we evaluated the impact of up to 6 months of PGL on renal function in these patients.

Methods: Data for 103 subjects were available for analysis: PGL 8mg q2wk (n=42) or q4wk (41) or placebo (20). Renal function was assessed by estimated glomerular filtration rate (eGFR, mL/min/1.73^2) using the 4-variable MDRD formula at screening (Week 0) and Wks 7, 13, 19 and 25 post-randomization. Linear mixed effects (random intercept) model was used to analyze eGFR. Treatment, time, treatment X time, age, sex and race were included as fixed effects; subject was included as a random effect. The random intercept model assumes missing data are random for valid treatment inferences. Basis for discontinuation was reviewed to assess bias effects.

Results: Baseline characteristics: mean age 62±12 years, 67% White and 71% males. Table 1 summarizes mean eGFR over time by randomized treatment group and overall. eGFR at Wk 0 was numerically but not statistically higher in the placebo group. Results of the model suggest that change in eGFR was not differentially affected by treatment (treatment X time interaction: p=0.28), independent of age, sex or race. No discontinuation pattern was observed. Absolute and relative changes from baseline in eGFR for individuals were consistent with overall findings. The most common adverse events: gout flare and infusion reaction.

Conclusions: Patients with refractory chronic gout and stage 3-4 CKD had no adverse renal effects with up to 6 months of pegloticase therapy.

Table 1. Mean (±SD) eGFR over time

Time Point	Pegloticase q2wks(n)	Pegloticase q4wks(n)	Placebo (n)	Overall (n)
Wk 0	40±12(42)	40±13(41)	43±13(20)	41±12(103)
Wk 7	43±14(36)	44±19(36)	43±16(20)	44±16(92)
Wk 13	41±13(36)	40±15(33)	46±16(19)	42±14(88)
Wk 19	44±15(33)	41±15(31)	45±15(19)	43±15(83)
Wk 25	42±11(31)	41±15(30)	47±13(18)	43±13(79)

Funding: Pharmaceutical Company Support

TH-PO385

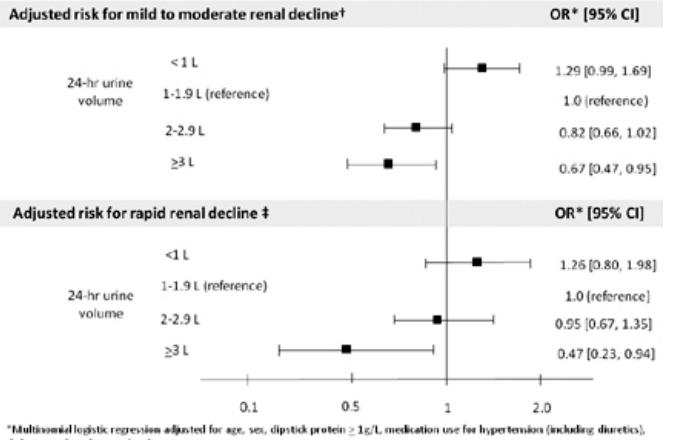
Urine Volume and Renal Decline: Results from a Community-Based Cohort Study William F. Clark,[1,2] Jessica M. Sontrop,[1,2] Jennifer J. Macnab,[1] Rita Suri,[1,2] Louise M. Moist,[1,2] Marina I. Salvadori,[1,2] Amit X. Garg.[1,2] *[1]University of Western Ontario, Canada; [2]London Health Sciences Centre, Canada.*

Background: The effect of increased fluid intake on kidney function is unclear. This study evaluates the relationship between urine volume and change in estimated glomerular filtration rate (eGFR) over six years in a large, community-based cohort.

Methods: Prospective cohort study, Canada (2002-2008). We obtained 24-hr urine samples from adult participants with eGFR≥60 ml/min/1.73m^2 at study entry. Percentage annual change in eGFR from baseline was categorized as average decline <1% per year; between 1% and 4.9% (mild-to-moderate decline) or ≥5% (rapid decline).

Results: A total of 2148 participants provided valid 24-hour urine samples, grouped as <1 L/day (14.5%); 1-1.9 L/day (51.5%); 2-2.9 L/day (26.3%); and ≥3L/day (7.7%). Baseline eGFR for each category of urine volume was 90, 88, 84 and 87 ml/min/1.73m^2, respectively. Overall, eGFR declined by 1% per year, with 10% demonstrating rapid decline and 58% demonstrating mild-to-moderate decline. An inverse, graded relationship was evident between urine volume and renal decline: for each increasing category of 24-hour urine volume, percentage annual eGFR decline was progressively slower from 1.3%, 1.0%, 0.8% to 0.5%, respectively; p=0.02. Compared to those with urine volume 1-1.9 L/day, those with urine volume >3 L/day were significantly less likely to demonstrate mild-to-moderate decline (adjusted odds ratio 0.67; 95% confidence interval: 0.47-0.95) or rapid decline (adjusted odds ratio 0.47; 95% confidence interval: 0.23-0.94); adjusted for age, sex, medication use for hypertension (including diuretics), proteinuria and cardiovascular disease.

Conclusions: In this community-based cohort, decline in kidney function was significantly slower in those with higher vs. lower urine volume.



*Multinomial logistic regression adjusted for age, sex, dipstick protein ≥ 1g/L, medication use for hypertension (including diuretics), diabetes and cardiovascular disease.
†Mild to moderate renal decline: eGFR decline from baseline between 1% and 4.9%.
‡ Rapid renal decline: eGFR decline from baseline ≥5%.

Funding: Government Support - Non-U.S.

TH-PO386

The Effects of Rubber Band Exercise Training with Low Protein Diet on Muscle Mass in Chronic Kidney Disease Patients Burin Laohawatana,[1] Yupa Chanwikrai,[2] Bancha Satirapoj,[1] Ouppatham Supasyndh.[1,2] *[1]Division of Nephrology, Phramongkutkalo Hospital; [2]Biomedical Research and Development Center, Phramongkutklao Hospital, Bangkok, Thailand.*

Background: Chronic kidney disease (CKD) leads to muscle wasting, which may be aggravated by low-protein diets prescribed to delay disease progression. Resistance training is effective to maintain or recover muscle mass and strength. However, it is often difficult to motivate many such patients to undergo exercise training chronically. The objective of the study is to demonstrate the effect of 12 weeks resistance exercise by using rubber band on muscle mass in CKD patients stage 3-5 receiving low-protein diet

Methods: The randomized control trial was conducted in CKD clinic at Phramongkutklao hospital during June to December 2010. The eligible CKD stage 3-5 participants were asked to restrict low protein diet (0.6-0.8 g/kg/day) and randomly assigned to do only gentle exercise (control group) or plus resistance exercise by using rubber band (treatment group) for 12 weeks.The exercise training was supervised and strictly monitored by sport scientist every four weeks. Dual energy absorptiometry was performed to determine lean body mass. The maximum repetition of rubber band exercise was recorded to demonstrate the muscle strength. Daily energy and protein intake were guided using 3-day food record and normalized protein nitrogen appearance.

Results: There were 28 patients (mean age 67.64±9.67 years) in control group and 26 patients (mean age 65.04±12.68 years) in study group. The average dietary protein intake between control and study group was 0.68±0.17 vs. 0.72±0.26 g/kg body weight and daily energy intake was 20.09±5.81 vs. 21.31±6.62 kcal/kg body weight, respectively. There was statistically significant in mean change of total lean body mass in study group (2.45±3.30 vs. 10.79±3.6 g/kg BW, p = 0.006).

Conclusions: Resistance exercise with rubber band is effective in increasing muscle mass in CKD patients with dietary protein restriction.

TH-PO387

Fenoldopam Increases Renal Artery and Parenchimal Blood Flow and Reduces Renal Resistive Index in Hypertensive Patients with Chronic Kidney Disease Roberto Palumbo,[1] Michele Ferrannini,[1] Annalisa Noce,[2] Simone Manca di Villahermosa,[2] Nicola Di Daniele.[2] *[1]Nephrology and Dialysis Department, S.Eugenio Hospital, Rome, Italy; [2]Nephrology and Dialysis Unit, Tor Vergata University, Rome, Italy.*

Background: Fenoldopam mesilate (FM), a short-acting agonist of post-synaptic dopaminergic receptor DA1, is a powerful hypotensive agent reducing systemic arterial resistances at an infusion rate >0.1mcg/kg/min. At infusion rates <0.1mcg/kg/min it seems show renoprotective properties without effect on blood pressure (BP). It could be due to reduced renal vascular resistance (VR) and increased blood flow (BF), as observed in experimental models but not yet in humans. To investigate the effect of <0.1mcg/kg/min FM infusion on renal vascular and parenchimal resistances, renal BF and systemic BP in hypertensive patients with chronic kidney disease.

Methods: Sixty hypertensive patients aged 65,20±14,54 years with CKD (stage I-IV NKDOQI) were enrolled. FM was e.v. infused 0.1mcg/kg/min for 60 minutes. Renal resistive index (RI) and Systolic and Diastolic flow velocity (SF, DF)were recorded by Eco-(color)-Doppler ultrasound, at renal artery origin, mid portion and at renal hylum, before and during FM infusion. BP and heart rate were monitored.

Results: During infusion, both SF and DF were significantly higher than baseline, either at renal artery origin, mid portion and at renal hylum (SF 48,9±11,3 vs. 55,9±15,3cm/sec, p=0,0135; 52,8±9,4 vs. 55,2±12,4cm/sec, p=0,0001; 44,2±9,9 vs. 49,4±10,9cm/sec, p=0,00193; DF 13,84±1,96 vs. 27,69±1,10cm/sec, p<0,001, 14,37±2,67 vs. 27,39±1,53cm/sec, p<0,001, 13,63±… vs. 25,03±5,53cm/sec, p=0,0001, respectively). RI were significantly lower than baseline (0,73±0,05 vs. 0,65±0,06, p<0,0001). BP and heart rate was not significantly affected by infusion. These results were observed in all CKD stages.

Conclusions: Our preliminary data suggest that 0.1mcg/kg/min of FM e.v. infusion increases renal blood flow and reduces RI with no effect on BP and heart rate.

TH-PO388

Long-Term Risks of Hospital Readmission and Death in Patients with Kidney Disease Kenn B. Daratha,[1,2] Robert Short,[2] Katherine R. Tuttle.[2] *[1]College of Nursing, Washington State University, Spokane, WA; [2]Providence Medical Research Center, Spokane, WA.*

Background: Patients with kidney disease are at high risk of hospitalization and death. However, long-term risks of hospital readmission and associated death are unclear. The primary objective of this study was to determine risks of hospital readmission and death in a full-spectrum of patients with early stages of chronic kidney disease (CKD) to end-stage renal disease treated by dialysis or kidney transplant.

Methods: This longitudinal cohort study included persons hospitalized in the State of Washington from Oct 2005 through Dec 2008 (WA-CHARS). Patients who survived their index hospital hospitalization (n=579,381) were classified into 4 cohorts defined by ICD9 diagnostic codes for CKD (n=27,647), dialysis (n=4,884), kidney transplant (n=867), and reference (non-kidney-disease diagnoses, n= 545,983). Primary outcomes were time to hospital readmission and hospital readmission resulting in death. Cox Proportional Hazard models controlling for age, gender, and primary diagnosis were conducted for time-to-event analyses. Analyses were internally validated with bootstrapping techniques.

Results: Risks for hospital readmission significantly increased among patients in the CKD (HR 1.35, 99% CI: 1.32-1.39, p<0.001), dialysis (HR 2.25, 99% CI: 2.15-2.36, p<0.001), and kidney transplant (HR 1.85, 99% CI: 1.65-2.07, p<0.001) cohorts independent of other index hospitalization diagnoses for up to 39 months. Risks for hospital readmission resulting in death were also independently increased for those in the CKD (HR 1.72, 99% CI; 1.63-1.83, p<0.001), dialysis (HR 4.17, 99% CI: 3.80-4.59, p<0.001), and kidney transplant (HR 1.95, 99% CI: 1.37-2.79, p<0.001) cohorts. Risks of readmission and death increased in a graded manner by CKD stage. Internal validation demonstrated ~99 % coverage and unbiased estimates.

Conclusions: Long-term risks of hospital readmission and death for patients with diagnoses of CKD, dialysis, and kidney transplant were among the highest observed in a large, statewide population. Patients with the full-spectrum of kidney disease diagnoses should be a major focus of efforts to reduce hospital readmissions and death.

TH-PO389

Chronic Endothelin-A Receptor Antagonism Modifies Novel Cardiovascular Risk Factors in CKD Neeraj Dhaun. *University of Edinburgh.*

Background: CKD patients have an increased risk of cardiovascular disease (CVD) that is not explained by conventional CVD risk factors alone. Arterial stiffness (AS) and endothelial dysfunction (ED) are features of CKD and may contribute to the CVD with which it is associated. Impairment of the nitric oxide (NO) system may lead to ED/AS. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO production that promotes vasoconstriction. ADMA concentrations are increased in CKD. Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor that is also upregulated in CKD. Hyperuricemia is another feature of CKD that contributes to ED/AS and so CVD risk. ETA receptor antagonism is a promising novel therapy for proteinuric CKD. Its effects on ADMA, the renal ET system and urate in CKD are not known.

Methods: In a randomized double-blind, 3-way crossover study, 27 subjects with proteinuric CKD received 6 weeks treatment with placebo, sitaxentan 100mg, an ETA antagonist, and nifedipine 30mg. (Dhaun et al Hypertension 2011). Serum urate, ADMA, plasma ET-1 and urine ET-1/creat, as a reflection of renal ET-1 production, were measured at baseline and week 6 of each treatment period alongside the primary endpoints of proteinuria, blood pressure (BP), and AS.

Results: Whereas placebo and nifedipine did not affect urate, ADMA or urine ET-1/creat, sitaxentan reduced all three (baseline vs. week 6 ± SEM – urate: 506±21 vs. 451±22µmol/l, p<0.01; ADMA: 0.52±0.01 vs. 0.48±0.01µmol/l, p<0.0001; urine ET-1/creat: 783±84 vs. 613±81ag/mmol, p<0.01). Plasma ET-1 was unaffected by all treatments. In multivariate analysis, the reduction in proteinuria and BP following sitaxentan treatment was independently predicted by the change in urine ET-1/creat, and the reduction in pulse wave velocity (as a measure of AS) by the change in ADMA.

Conclusions: In addition to currently recognised effects on proteinuria, BP and AS, ETA antagonism may modify novel CVD risk factors and so have broader cardioprotective effects in CKD. Larger and longer-term trials with these specific endpoints are now warranted.

Funding: Pharmaceutical Company Support

TH-PO390

Effect of Extended-Release Niacin/Laropiprant on Lipid Profiles in Dyslipidemic Stage 3 Chronic Kidney Disease Patients Andrew G. Bostom,[1] Jinyu Zhang,[2] Peter Shorter,[1] Joachim H. Ix,[3] Andrew Tershakovec,[4] Diane Tipping.[5] *[1]Division of Kidney Diseases, Rhode Island Hospital, Providence, RI; [2]Brown Medical School, San Diego, Providence; [3]Division of Nephrology, University of California-San Diego, CA; [4]Merck and Company, Rahway, NJ; [5]; [6]Tipping Consulting LLC, Green Lane, PA.*

Background: Among dyslipidemic patients free of chronic kidney disease (CKD), extended-release niacin (ERN) at 2 g/d causes beneficial lipoprotein-modifying effects. Given the paucity of data regarding niacin's impact on dyslipidemia in CKD, we examined the drug's effect on the standard lipid profiles of patients with stage 3 CKD.

Methods: Subjects (n=261) with a baseline estimated glomerular filtration rate (eGFR) of 30 to < 60 ml/min/1.73 m^2 were drawn from two completed trials of extended-release niacin/laropiprant (ERN-L, laropiprant being an inhibitor of ERN-mediated flushing; ERN-L at 2g/d, n=177; n=84 placebo [P]). Lipid profiles were measured serially over 24-weeks.

Results: The ERN-L and P groups were comparable with respect to baseline mean age, eGFR, total cholesterol (TC), low density lipoprotein-C (LDL), high density lipoprotein-C (HDL), and triglyceride (TG) levels, as well as the distribution of gender, concurrent statin use, and diabetes. Repeated measures analyses revealed that ERN-L, relative to P, caused a decrease (95% CI) of -22.5% (-16.0%, -29.1%) in mean LDL, - 9.1% (-12.7%, -5.5%) in mean TC, and – 28.0% (-43.5%, -21.8%) in median TG, as well as an increase of +28.7% (+23.7%, +33.7%) in mean HDL levels. Stratified analyses revealed that these effects were entirely consistent with what was observed among dyslipidemic patients whose eGFR was ≥ 60 ml/min/1.73 m^2.

Conclusions: ERN-L results in very favorable lipid changes among patients with stage 3 CKD. Till now, stage 3-4 CKD patients have been significantly under-represented in secondary cardiovascular disease prevention trials. We conclude that a niacin treatment arm merits serious consideration within any future clinical trials targeting stage 3-4 CKD patients for the potential reduction of cardiorenal outcomes.

TH-PO391

The Association of Cardiac Concentricity with Kidney Function Decline: Data from the Multi-Ethnic Study of Atherosclerosis (MESA) Meyeon Park,[1] Michael Shlipak,[1,2] Ronit Katz,[3] Subhashish Agarwal,[4] Joachim H. Ix,[5] Chi-Yuan Hsu,[1] Carmen A. Peralta.[1] *[1]UCSF, San Francisco, CA; [2]SF VA Medical Center, CA; [3]Univ. of Washington, Seattle, WA; [4]Oakwood Hospital, Dearborn, MI; [5]UCSD, San Diego, CA.*

Background: Chronic kidney disease (CKD) is strongly associated with heart failure (HF), and HF is associated with faster kidney function decline. Whether subclinical abnormalities of cardiac structure are associated with kidney function decline is not known.

Methods: We included 3866 individuals in MESA (39% white, 25% black, 23% Hispanic, 13% Chinese), free of known cardiac disease, with estimated glomerular filtration rate (eGFR)>60 ml/min/1.73m^2 at baseline and at least 2 measures of kidney function over 5 years of follow-up. Concentricity was assessed by magnetic resonance imaging and calculated as left ventricular (LV) mass over LV end diastolic volume. eGFR was assessed by cystatin C (eGFRcys) and creatinine (eGFRcr). Using multivariate linear mixed models, we investigated the association of concentricity with kidney function decline; covariates included demographics, systolic blood pressure, diabetes, inflammatory markers (HDL, IL-6, CRP), and albuminuria.

Results: Mean (SD) age was 60 (10) years; mean eGFRcys at baseline 97 (16) ml/min/1.73m^2; mean decline per year -1.11 (4.26) by eGFRcys and -1.53 (2.86) by eGFRcr. Higher concentricity was independently associated with faster rates of eGFR decline, based on cystatin C or creatinine.

Decline in eGFR among MESA participants without CKD

Quartiles of Concentricity (g/ml)	N	eGFRcys*	eGFRcr*
		β (95% CI)	β (95%CI)
<0.95(F);<1.06(M)	966	Ref.	Ref.
0.95-1.06(F);1.06-1.17(M)	967	-0.17 (-0.38, 0.05)	-0.19 (-0.33, -0.04)
1.07-1.20(F);1.18-1.35(M)	967	-0.11 (-0.33, 0.11)	-0.22 (-0.37, -0.07)
>1.20(F);>1.35(M)	966	-0.52 (-0.74, -0.29)	-0.57 (-0.73, -0.41)
Per SD change concentricity (SD 0.24)	3866	-0.17 (-0.25, -0.09)	-0.20 (-0.25, -0.14)

*fully adjusted

Conclusions: Subclinical alterations in cardiac structure are associated with kidney function decline independent of the effects of hypertension and other comorbidities. Future studies should focus on elucidating mechanisms to explain these associations.

Funding: Other NIH Support - NHLBI

TH-PO392

Angiopoietin-Like Protein 2 Is Associated with Chronic Kidney Disease in a General Japanese Population: The Hisayama Study Tomoko Usui,[1,2] Toshiharu Ninomiya,[1,2] Masaharu Nagata,[1,2] Takanari Kitazono,[2] Yuichi Oike,[3] Yutaka Kiyohara.[1] *[1]Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [3]Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.*

Background: Angiopoietin-like protein 2 (Angptl2) is an adipocytokine which promotes inflammation and endothelial dysfunction of the vessels. People with chronic kidney disease (CKD) have a greater risk of atherosclerotic disease. The aim of this study is to assess the relationship between serum Angptl2 levels and CKD.

Methods: A total of 3,163 community-dwelling subjects (1,368 men, 1,795 women), aged ≥40 years, were divided into quintiles by Angptl2 levels. The estimated filtration rate (eGFR) was calculated using the new Japanese equation for eGFR from serum creatinine. CKD was defined as having either eGFR <60 ml/min/1.73m^2 or urinary albumin to creatinine ratio ≥30 mg/g. The odds ratio for the presence of CKD was calculated using logistic regression model.

Results: The median value (interquartile range) of Angptl2 was 2.72 (2.15-3.47) ng/mL. The prevalence of CKD was 37.4%. The age- and sex-adjusted prevalence of CKD increased linearly for Angptl2 levels of <2.01, 2.01-2.48, 2.49-2.99, 3.00-3.65, and ≥3.66 ng/mL, being 26.0%, 35.4%, 39.4%, 41.3%, and 44.2%, respectively (p for trend <0.001). Every 1 ng/mL increment in Angptl2 levels was associated with 1.09-fold (95% confidence interval 1.01-1.18) greater likelihood of CKD, even after adjusting for age, sex, systolic blood pressure, antihypertensive drug use, hemoglobin A1c, total cholesterol, high-density lipoprotein cholesterol, triglycerides, lipid-modifying drug use, body mass index, high-sensitivity C-reactive protein, electrocardiogram abnormality, history of cardiovascular disease, current smoking, and drinking. In regard to CKD stages, similar associations were observed for stages 1-2 and stages 3-5 with albuminuria, but not for stages 3-5 without albuminuria.

Conclusions: Elevated Angptl2 levels were associated with the likelihood of CKD in the general population.

Funding: Private Foundation Support

TH-PO393

Modulation of Erythropoietin by Prolyl Hydroxylase Inhibitor in a Repeat Dose, Randomized Placebo Controlled Trial Richard A. Brigandi,[1] Steven F. Russ,[2] Coreen Oei,[2] Mark E. Westerman,[3] Gordana Olbina,[3] Peter Hodsman,[4] Sanjay Kumar.[1] *[1]GlaxoSmithKline, King of Prussia, PA; [2]GlaxoSmithKline, Research Triangle Park, NC; [3]Intrinsic LifeSciences, La Jolla, CA; [4]Nucleus Network, Melbourne, VIC, Australia.*

Background: Prolyl hydroxylases are enzymes that hydroxylate hypoxia inducible factor (HIF) leading to degradation. GSK1278863A is a novel small molecule that has demonstrated *in vitro* and *in vivo* inhibition of the prolyl hydroxylases resulting in HIF stabilization and HIF-mediated gene expression including erythropoietin (EPO).

Methods: Study PHI112842 was a single-blind, randomized, placebo controlled, dose-rising, 14-day repeat dose, sequential parallel group study to investigate the safety, tolerability, PK and PD of GSK1278863A in healthy adult subjects. Subjects were dosed once daily for 14 days, sequentially, in four ascending cohorts ranging from 15 to 100 mg with randomization to receive GSK1278863A or placebo in a ratio of 2:1. Twice daily dosing for 14 days was also evaluated in one cohort of subjects.

Results: Oral administration of GSK1278863A was generally well tolerated. Drug exposure generally increased with increasing dose up to 75 mg. Exposure was similar between 75 and 100 mg likely due to intersubject variability in absorption. Mean t_{max} ranged from 1.5-2.8 hours post dose, and actual $t_{1/2}$ ranged from 0.8-4.1 hours. There was no drug accumulation and no statistical difference in $AUC_{0\text{-}24h}$ between 25 mg bid and 50 mg daily. Changes were observed across hematologic and iron metabolism biomarkers in a dose dependent manner. Dose-dependent increases in EPO, reticulocyte count, and hemoglobin increases were observed. Subjects receiving greater than 15 mg daily had an average hemoglobin increase of ~1 g/dL by day 14. A decrease from baseline in Hepcidin, a biomarker of iron utilization and inflammation was also observed in a dose dependent manner. Compared to placebo subjects, an increase in VEGF concentration was noted in subjects receiving higher GSK1278863A doses (75 and 100 mg).

Conclusions: These data indicate that GSK1278863A has the potential to induce erythropoiesis in patients with anemia.

Funding: Pharmaceutical Company Support

TH-PO394

Modulation of Erythropoietin by Prolyl Hydroxylase Inhibitor in Renal Impaired Patients in a Single Dose Cross-Over Study Richard A. Brigandi,[1] Steven F. Russ,[2] Coreen Oei,[2] Mark E. Westerman,[3] Gordana Olbina,[3] Richard Austin Robson,[4] Sanjay Kumar.[1] *[1]GlaxoSmithKline, King of Prussia, PA; [2]GlaxoSmithKline, Research Triangle Park, NC; [3]Intrinsic LifeSciences, La Jolla, CA; [4]Christchurch Hospital, Christchurch, New Zealand.*

Background: Prolyl hydroxylases are enzymes that hydroxylate hypoxia inducible factor (HIF) leading to degradation. GSK1278863A is a novel small molecule inhibitor of HIF prolyl hydroxylases resulting in HIF stabilization and HIF-mediated gene expression including erythropoietin (EPO).

Methods: Study PHI112843 was a two part study. Part 1 was a single dose, 2-period crossover, randomized sequence, single blind to sequence and dose study in Stage 3 and 4 CKD patients and matched healthy subjects. Patients were administered a single dose of 50 mg and 150 mg GSK127863A across the two periods. Part 2 was a single dose, 2-period, fixed sequence, open label study in Stage 5 hemodialysis dependent (HDD) patients. Patients were administered a single dose of 150 mg GSK127863A across the two periods. Period 1 dosing was on a hemodialysis (HD) day, 1 hour prior to HD start. Period 2 was on a day without HD.

Results: Oral administration of GSK1278863A was generally well tolerated. There were no marked differences in drug exposure across CKD patients, matched healthy subjects and HDD patients on a non-HD session day. Plasma concentrations were elevated in subjects actively receiving HD. Rapid, dose-dependent increases were observed in mean EPO concentrations in all cohorts following dosing in Period 1, with the greatest increases observed in CKD and HDD patients. Mean reticulocyte and RBC counts also increased. Mean hemoglobin concentrations were observed to increase slightly in healthy subjects in both dosing periods and over time in CKD patients. In HDD patients small increases in mean Hgb concentrations were noted at 24 hours following dosing on an HD day, but returned to baseline at the follow-up assessment. A decrease from baseline in Hepcidin, a biomarker of iron utilization and inflammation, and an increase in VEGF was observed after dosing in all populations.

Conclusions: These data indicate that GSK1278863A has the potential to induce erythropoiesis in patients with anemia.

Funding: Pharmaceutical Company Support

TH-PO395

The Pathogenesis and Clinical Significances of Renal Insulin Resistance Syndrome Hitoshi Minakuchi, Shu Wakino, Koichi Hayashi. *School of Medicine, Keio University, Shinanomachi 35 Shinjuku, Tokyo, Japan.*

Background: The association between chronic kidney disease (CKD) and insulin resistance (IR) has been recognized as "renal insulin resistance syndrome (RIRs)", To delineate the mechanism and significance of RIRs, we conducted a clinical study.

Methods: The cross-sectional study was performed among 186 patients with CKD. Insulin sensitivity was evaluated with the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Glomerular filtration rate (GFR) was estimated with modified MDRD-equation adapted for a Japanese population and evaluated in estimated GFR (eGFR). The intervention with mineralocorticoid receptor (MR) antagonist spironolactone was performed with some patients with RIRs. In prospective analysis, we evaluated the 3-year changes in various parameters of renal function and analysed the correlation between HOMA-IR and these parameters.

Results: Of CKD patients, 5 were in stage 1, 89 were in stage 2, 78 were in stage 3, 6 were in stage 4, and 8 were in stage 5. A negative correlation was observed between HOMA-IR and eGFR. Plasma aldosterone level was correlated negatively with eGFR and positively with the levels of fasting blood sugar, insulin and HOMA-IR. By multiple regression analysis, the aldosterone level was identified as an independent risk factor for RIRs. This analysis also revealed that the markers for urinary tubular damages, a1-microglobulin were also correlated with HOMA-IR . The treatment with spironolactone ameliorated the IR status in CKD. In addition, prospective analysis revealed that the decline in eGFR was significantly correlated with HOMA-IR, and multiple regression analysis indicated that HOMA-IR was the independent risk for CKD progression.

Conclusions: These studies demonstrate that plasma aldosterone plays a relevant role in the pathogenesis of RIRs. MR antagonists can be a therapeutic strategy for the prevention against RIRs and its complications including cardiovascular disease. The presence of RIRs have some roles in the progression of CKD probably through the damages of urinary tubular damages. Our study demonstrated for the first time the clinical relevances of RIRs, and RIRs can be the new target for CKD treatment.

Funding: Government Support - Non-U.S.

TH-PO396

The Effects of 1,25-Dihydroxyvitamin D3 on the Risk of Death in a Japanese General Population: The Hisayama Study Toshiharu Ninomiya,[1] Masaharu Nagata,[2] Tomoko Usui,[2] Yutaka Kiyohara,[2] Takanari Kitazono.[1] *[1]Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Japan; [2]Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Japan.*

Background: Recent evidence emerged to imply that lack of 1,25-dihydroxyvitamin D3 (VD3) increased the risk of death and cardiovascular disease. However, this issue has not been fully evaluated. We assessed whether lower serum VD3 levels were associated with higher risk of death.

Methods: We followed a total of 3,091 community-dwelling Japanese individuals aged 40 years or older, without history of cardiovascular disease and kidney failure, and with available VD3 data, for 7 years, and examined the relationship between serum VD3 levels and the risk of death by using the Cox proportional hazards model.

Results: The mean value of serum VD3 levels in the study population was 66.6 pg/ml (standard deviation 17.4). During the follow-up, 270 subjects died in total. The mortality rate from any causes increased gradually with lower VD3 levels: the risk of death from any causes increased significantly with VD3 levels of <54.8, 54.9-65.8, 65.9-78.5, and ≥78.6 pg/ml, being 18.2, 15.1, 9.9, and 10.3 per 1,000 person-years, respectively (p for trend <0.001). After adjusting for potential confounders: namely, age, sex, systolic blood pressure, use of anti-hypertensive agents, diabetes, total cholesterol, use of lipid-modifying agents, body mass index, estimated glomerular filtration ratio (eGFR), calcium-phosphate product, smoking habits, and alcohol intake, every 10 pg/ml decrement in VD3 levels was associated with a 1.19-fold (95% confidence interval, 1.11-1.28) greater risk of death from any causes. With regard to the causes of death, the risk of death from cardiovascular disease and infectious disease tended toward increasing with lower VD3 levels. There was no evidence of heterogeneity in the association between subjects with eGFR below and above 60 ml/min/1.73 m^2 (p for heterogeneity=0.58).

Conclusions: Our findings suggest that lower serum VD3 level is associated with a greater risk of death in the general Japanese population, regardless of kidney function.

Funding: Private Foundation Support

TH-PO397

Bone-Specific Alkaline Phosphatase and Coronary Artery Calcification and Mortality in Diabetic Chronic Kidney Disease Magdalena A. Sarna,[1] Sirin Jiwakanon,[2] Rajnish Mehrotra.[2] *[1]Department of Medicine, University of Calgary, Calgary, AB, Canada; [2]Department of Medicine, Los Angeles Biomedical Research Institute, Torrance, CA.*

Background: Higher levels of alkaline phosphatase (ALP) have been previously shown to be associated with increased mortality in patients with chronic kidney disease (CKD) and hemodialysis. However, most studies have used serum ALP rather than bone-specific alkaline phosphatase (BALP), a marker of bone turnover and mineralization. The aim of our study was to evaluate the role of BALP in predicting vascular calcification, using coronary artery calcification (CAC) scores, as well as mortality and progression to end-stage renal disease (ESRD) in type 2 diabetic patients with CKD.

Methods: A total of 166 participants from two prospective cohort studies of CAC in non-dialysis-dependent type 2 diabetics with nephropathy were included. An immunoenzymetric assay was used for quantifying measurement of BALP. Baseline patient characteristics were further stratified according to BALP tertiles. CAC was measured by electron beam computer tomography. Cox regression model was used for mortality and ESRD analysis.

Results: The baseline patient demographics included a mean age of 57 years, estimated glomerular filtration rate (eGFR) of 57 ml/min/1.73 m^2 and a median urine protein-creatinine ratio of 2.6. The median BALP level was 13.8 (10.9–18.2) µg/L. There was no difference in CAC scores according to BALP tertiles (p=0.12). Multivariate analysis for baseline BALP as outcome showed age, parathyroid hormone and triglyceride, but not CAC levels, to be predictors in the model. As expected, a relationship was found between all-cause mortality and higher CAC scores (p= 0.018) but not ESRD (p= 0.99). BALP was not found to be predictive for all-cause mortality (p= 0.69) or ESRD (p= 0.998).

Conclusions: BALP levels do not appear to be correlated with greater vascular calcification. In this study, baseline BALP levels were not predictive of all-cause mortality or progression to ESRD. Further studies are needed to validate whether BALP, a marker of bone turnover, is predictive of cardiovascular risk in patients with CKD.

Funding: Other NIH Support - NCRR

TH-PO398

Predictive Value of a Multi-Organ Cardiovascular Disease Target Organ Score for Mortality and Cardiovascular Events in a Low Risk Population Branko Braam,[1] Lutgarde Thijs,[2] Tatiana Kouznetsova,[2] Jasjeet K. Minhas Sandhu,[3] Dean Eurich,[3] Jan A. Staessen,[2] Carlo A. Gaillard.[4] *[1]Medicine/Nephrology, University of Alberta, Edmonton, AB, Canada; [2]Department of Molecular and Cardiovascular Research, University of Leuven, Belgium; [3]Department of Public Health Sciences, University of Alberta, Edmonton, AB, Canada; [4]Department of Nephrology, VU University Medical Center, Amsterdam, Netherlands.*

Background: An intrinsic limitation of cardiovascular risk prediction models is that they rely on risk factors, not on individual target organ damage (TOD). The current pilot study tested whether application of a multi-organ TOD scoring system had independent value above standard risk models to predict death and cardiovascular events in a low risk cohort.

Methods: To this purpose, a population-based prospective cohort of subjects age >30 yrs, was analyzed for cardiovascular risk factors to populate the SCORE and Framingham equations and TOD using a new framework, addressing TOD severity in arteries, brain, cardiac and kidney (ABCK).

Results: Of 1970 subjects, sufficient data were available to obtain baseline risk and TOD scores. During a follow up of 10 years, 163 (8%) subjects died, 49 (2%) had an AMI, 45 (2%) had a stroke, 46 (2%)developed end-stage renal disease and 14 (0.7%) developed severe peripheral artery disease. The ABCK TOD score was related to overall and cardiovascular mortality and to cardiovascular events. When compared to the SCORE and Framingham risk models, the ABCK score was independently associated with (fatal and non-fatal) cardiovascular outcome in medium and high-risk individuals over 10 years of follow up. For the intermediate TOD ABCK score the HR were 2.6 and 3.0, for severe TOD ABCK score the HR were 10.7 and 11.4 independent from SCORE and Framingham respectively.

Conclusions: Of interest, scoring renal damage (based on proteinuria and GFR) most strongly added to the prediction by the SCORE and Framingham. This pilot study indicates that addition of a relatively simple to use TOD score can improve the prediction of cardiovascular events.

Funding: Government Support - Non-U.S.

TH-PO399

Comparison of Sexual Dysfunction between Renal Replacement Therapies: A Systematic Review Temitope Olufade, Priscilla Auguste, Deidra C. Crews, Julio Lamprea, Tanjala S. Purnell, Raquel Greer, Patti Ephraim, Johanna Sheu, Neil R. Powe, Hamid Rabb, L. Ebony Boulware. *Johns Hopkins Medical Institutions.*

Background: Sexual dysfunction is common in end stage renal disease (ESRD). However, the strength of evidence regarding differences in sexual dysfunction among patients on different renal replacement therapies (RRTs) is unknown.

Methods: We performed a systematic review to identify published studies describing rates of sexual dysfunction among patients on different RRTs (hemodialysis-HD, peritoneal dialysis-PD and renal transplant- TX). We searched PubMed (English language, after 1987) and hand-searched bibliographies to identify relevant studies. Independent reviewers assessed study quality (internal and external validity). We calculated standardized effect sizes (Cohen's D) to compare the direction and magnitude of associations between RRT and several sexual function outcomes.

Results: Of 130 potentially eligible studies identified, 18 described sexual dysfunction among men (7), women (1), or both genders (10). Studies compared 12 sexual dysfunction outcomes using observational designs (2 longitudinal cohort, 11 cross-sectional, 5 pre-post). Most studies (9 of 10) reported no differences in outcomes among patients on HD versus PD. In 13 studies, transplant was the most consistently favored RRT (no difference to a great amount less sexual dysfunction) compared to other modalities. Study quality varied (low to moderate; no high quality), with few studies adequately accounting for potentially confounding factors such as comorbidity.

Table 1: Comparison of Sexual Dysfunction Outcomes by Renal Replacement Therapy

	HD vs. PD			HD vs. TX			PD vs. TX			Dialysis vs. TX		
Domain (Number of Studies)	Favors HD	Favors Neither	Favors PD	Favors HD	Favors Neither	Favors TX	Favors PD	Favors Neither	Favors TX	Favors Dialysis	Favors Neither	Favors TX
Men												
Sexual Desire (4)		2				3			2		1	
Erectile Function (6)		3			1	3		1	1			1
Premature Ejaculation (2)		2			1	1		2				
Dyspareunia (2)		2				2		2				
Orgasmic Function (2)		2			1	1		2				
Frequency of Intercourse (2)		0				1					1	
Sexual Satisfaction (1)		1				1			1			
Women												
Sexual Desire (2)		2			1	1		1	1			
Sexual Arousal (1)		1				1		1				
Dyspareunia (2)		2			2			1	1		1	
Orgasm Attainment (2)		2			2			2				
Lubrication (1)		1			1			1				
Both Gender												
Frequency of Intercourse (1)		1				1			1			
Sexual Satisfaction (4)	1	2			1	1		1	1			1
Quality of Sex Life (4)		3			1			1				

Conclusions: Limited but variable quality evidence suggests transplant is associated with less sexual dysfunction among patients with ESRD. Rigorously designed prospective studies are needed to better inform patients and providers about the impact of RRTs on this important patient reported outcome.

Funding: Other U.S. Government Support

TH-PO400

Relation between Glomerular Filtration Rate (GFR) and Medical Expense among the Screened Subjects of the Japan Health Insurance Association Kunitoshi Iseki,[1] Tsuyoshi Watanabe.[2] *[1]Dialysis Unit, University Hospital of the Ryukyus, Nishihara, Okinawa, Japan; [2]Division of Nephrology, Fukushima Medical School, Fukushima, Japan.*

Background: Few studies examined the medical expenses according to the baseline GFR.

Methods: We combined registries both health check and report of medical expenses (receipts). The health check was done from April 2008 to March 2009, and all the eligible subjects were covered by the Okinawa Branch of the Japan Health Insurance Association. Every monthly report of medical expense such as physical examination, laboratory tests, surgical procedures and other related expenses were reviewed during April 2008 to March 2010 (24 months). Serum creatinine was measured by the enzymatic method and the estimated GFR (eGFR, ml/min/1.73m^2) was calculated by the Japanese Society of Nephrology. By using the ID number, we could obtain the information of the receipts after the screening. After obtaining written contract with the association, we were provided anonymously coded data.

Results: A total of 74305 subjects, 38.2% females and the mean age of 48.1 years (35 to 74 years) has participated the health check. The total number of receipt was 773,276 during the study period. . The average receipt point per month, 1 point=10 Yen, was extraordinary high as 29700 in subjects with eGFR<15. The relationship between eGFR and medical expense was U-shaped and the expense was lowest at eGFR 90-104. It was 2369 in eGFR 15-29, 2120 in eGFR 30-44, 1022 in 45-59, 689 in eGFR 60-74, 614 in eGFR 75-89, 606 in eGFR 90-104, 622 in eGFR 105-119, 883 in eGFR 120 and over, respectively.Similarly, it was 637 in subjects with proteinuria (-), 695 in proteinuria (+/-), 1001 in proteinuria (1+), 2210 in proteinuria (2+ and over), respectively. Medical cost increased with ageing.

However, in each age-group medical cost was higher in CKD (eGFR<60), in particular younger generation.

Conclusions: The medical expenses increased as lower the eGFR and higher the degree of proteinuria. Reasons for higher cost among subjects with higher eGFR, 120 and over, remained to be investigated.

Funding: Government Support - Non-U.S.

TH-PO401

Health-Related Quality of Life, Functional Status, and Survival of Poor Prognosis Endstage Renal Disease Patients Ying-Ying Seow,[1] Chung Pheng, Alethea Yee,[2] Limin Qu,[2] Sze Huey Tan,[3] Kok-Seng Wong,[4] Cynthia R. Goh.[5] *[1]Renal Medicine, Khoo Teck Phuat Hospital, Singapore; [2]Palliative Care, National Cancer Centre, Singapore; [3]Clinical Trials and Epidemiological Sciences, National Cancer Centre, Singapore; [4]Renal Medicine, Singapore General Hospital, Singapore; [5]Lien Centre for Palliative Care, Duke - NUS Graduate Medical School, Singapore.*

Background: ESRD patients who are very elderly (≥75 years old) or with high comorbidity burden,have poor survival even on RRT.Symptoms and functional status do not always improve with initiation of RRT in these poor prognosis groups.We studied a group of extremely poor prognosis ESRD patients (median life expectancy <24months) to monitor change in QoL and functional status,whether on RRT or conservatively managed(CM).

Methods: This was a prospective longitudinal study of ESRD patients at the Singapore General Hospital.101 patients with eGFR 8-12ml/min,and either ≥75 years old or with Charlson Comorbidity Index≥8 were followed up for 24months.At enrollment and at fixed time intervals (0,3,6,9,12,18,24months) KDQoL,Karnofsky Performance Score and eGFR were recorded.Baseline demographic data was also recorded.

Results: 38 patients started RRT and 63 were CM.At 24m,33/38(86.8%) RRT and 24/63(38.1%) CM patients were alive.Median survival for CM was 14.6m and GFR at time of death was 6ml/min.GFR at initiation of RRT was 6.3ml/min.For CM,there was a decline over time for Social Support,PF,GH,SF and PCS ($p<0.05$) but an improvement in Burden of Kidney Disease,Work Status and RE.Among RRT,comparing KDQoL and KPS scores closest to initiating RRT and first recorded scores≥3m after initiation,only BKD was improved but symptoms,sleep,overall health,Energy,RP,PCS and MCS were all worse ($p<0.05$).KPS was comparable pre and post-RRT.

Conclusions: Asian RRT populations have been shown to have good survival and QoL.Our study showed that the worst prognosis ESRD patients survived longer on RRT compared to CM.However, their QoL did not improve,on the contrary, this deteriorated on RRT.Functional status remained unchanged.For CM,there was a gradual decline in some HRQoL domains but improvement in others.Scores in general were good.

Funding: Government Support - Non-U.S.

TH-PO402

Colonic Necrosis Is Not Associated with the Use of Sodium Polystyrene Sulfonate (SPS) Potassium Binding Resins Maura A. Watson, Annie Nguyen, David K. Oliver, Kevin C. Abbott, Christina M. Yuan. *Nephrology, Walter Reed Army Medical Center, Washington, DC.*

Background: The FDA in 2009 recommended against the "concomitant use of sorbitol" with SPS powder due to associated cases of colonic necrosis (CN), a rare (but potentially fatal) event. Little data exists to suggest that oral SPS formulations cause CN.

Methods: This retrospective cohort examined all 121,812 Walter Reed Army Medical Center inpatient subjects between 9/1/2001 and 10/31/2010. Pharmacy data was queried for inpatient SPS/sorbitol (SPS) prescriptions (CHCS/Essentris). CoPath™ anatomic pathology database was used to identify all cases of CN during that time period based on tissue diagnosis. The primary outcome was CN in subjects who were exposed to SPS within one month prior to diagnosis (a plausible temporal association) and associated clinical features in index cases.

Results: SPS was prescribed to 2,195 subjects (0.02%). 83 CN cases were identified (0.07%). Three received oral SPS (1gm/4ml) up to one month prior to CN diagnosis (3.2% of CN cases). Nine-year cumulative CN incidence was 0.14% in those exposed to SPS. SPS exposure was not significantly associated with CN (unadjusted relative risk = 2.08; 95% CI 0.65-6.57; P = 0.20). The number needed to harm was 1411. Exposure to SPS was not significantly associated with CN when adjusted for age>65, eGFR<30, ICU admission, or post surgical status. Sample size analysis showed it would require a population of 4974 patients age>65 treated with SPS and a comparison group ten times as large to perform rigorous multivariate analysis of the risk of SPS for CN.

Conclusions: Inpatient use of SPS was not significantly associated with the development of CN by unadjusted analysis in this cohort of hospitalized subjects at a single tertiary care medical center over 9 years. CN is an extremely rare outcome therefore a single medical center may not have sufficient cases for robust statistical analysis, and analysis of a coordinated national system would be required.

Disclaimer: The views expressed in this paper are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense, or the United States government.

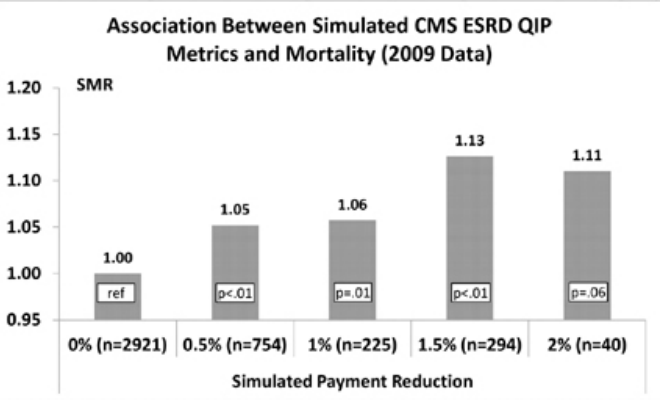
TH-PO403

Simulated CMS ESRD Quality Incentive Program and Association with Mortality Robert A. Wolfe,[1] Emily E. Messersmith,[1] Regina S. De los Santos,[1] Erik Roys,[2] Jeffrey Pearson,[1] Alissa Kapke,[1] Matthew Paul,[2] Bruce M. Robinson,[1] Joseph M. Messana.[2] *[1]Arbor Research Collaborative for Health; [2]University of Michigan Kidney Epidemiology and Cost Center.*

Background: As part of the Medicare Improvements for Patients and Providers Act of 2008, the Centers for Medicare & Medicaid Services (CMS) were mandated to implement a Quality Incentive Program (QIP) for payments to dialysis facilities in 2012. The QIP will use 3 measures of dialysis facility performance: the percent of pts with hemoglobin <10 g/dL, hemoglobin >12 g/dL, and urea reduction ratio ≥65%. Each dialysis facility's performance in 2010 will be compared with their facility-specific historical performance in 2007 or national performance in 2008; facilities not meeting one of these standards on any of the 3 measures may face payment reductions of up to 2% in 2012.

Methods: Using CMS data, we simulated the QIP with 2009 facility performance (N=4,718 facilities). After calculating the quality measures, we calculated the total performance score (0-30 points) and categorized facilities by payment reductions (none, 0.5%, 1.0%, 1.5%, or 2%). To assess the validity of the QIP, we related these facility categorizations to a 2009 facility standardized mortality ratio (SMR) using Poisson regression. The SMR accounts for patient case-mix and general population state death rates. Facilities without sufficient data to calculate performance scores were excluded from the analysis.

Results: Facilities receiving payment reductions had higher mortality than facilities with no reductions (see figure).



484 facilities did not have 2007 baseline data; the QIP does not penalize these facilities and we found no evidence for a mortality difference from high performing facilities (p=0.59).

Conclusions: The QIP score categorization has construct validity through its correlation with patient mortality at the facility level.

Funding: Other U.S. Government Support

TH-PO404

Human Resource Allocation to Intensive Home Hemodialysis (HHD) Programs in Canada Robert P. Pauly,[1] Deborah Lynn Zimmerman,[2] Paul Komenda.[3] *[1]University of Alberta, Canada; [2]University of Manitoba, Canada; [3]University of Ottawa, Canada.*

Background: There is growing interest in HHD though there are almost no published data on the human resource requirements to maintain a home program. The purpose of this study was to leverage Canadian expertise in HHD delivery and characterize human resource allocation in our programs.

Methods: Between Jul and Dec 2010, 19 dialysis programs (14 university-based centers and 5 community centres known to provide all options of home dialysis therapies) were surveyed to describe their staffing complement and the roles of various disciplines.

Results: Seventeen of 19 (89%) programs responded. The median number (and range) of patients cared for by 1 full-time equivalent (FTE) nurse and dialysis technologist is 14.4 (10.0-24.3) and 22.7 (12.5-42.0) respectively; the median ratio of patients to clerical support FTE is 54:1. Twelve of 17 (71%) units have a dedicated modality educator/coordinator associated with their renal program. Eleven of 17 (65%) programs have a designated dietician, 9/17 (53%) have a social worker, and 7/17 (41%) have a pharmacist; 81% of these positions are part-time FTEs indicating these resources are shared broadly in most renal programs. A single nephrologist cares for all HHD patients in 3/17 (18%) programs, a specialized group of HHD physicians attends in 10/17 (58%) units, and all nephrologists share caring for HHD patients in only 4/17 (24%) units. Technical support for HHD is completely outsourced to a third party vendor in 5/17 (29%) programs, shared with conventional HD in 6/17 (35%) programs and designated solely to HHD in 5/17 (29%) programs. A unique activity in the HHD unit includes the technical assessment of patients' homes which is performed by program technologists (7/17 – 41%), third party vendors (5/17 – 29%), and a combination of a technologist and a vendor (5/17 – 29%).

Conclusions: Significant variability exists in staffing HHD programs in Canada. This may relate to the local practice environment and/or the experience of the centre; this requires further study. Understanding the staffing complement among established HHD programs may be valuable for new programs in considering resource allocation.

TH-PO405

Tacrolimus and Breastfeeding: How Safe Is it? Kate Bramham,[1] Gary Chusney,[2] Janet Lee,[2] Alexander Paul Wardle,[2] Catherine Nelson-Piercy,[3] Liz Lightstone.[2] *[1]Maternal and Fetal Research Unit, King's College London; [2]Renal and Transplant Centre, Imperial College NHS Healthcare Trust; [3]Obstetric Medicine, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom.*

Background: Women have been advised not to breastfeed if taking tacrolimus (Tac), due to theoretical risks of neonatal immunosuppression & assumed secretion into breastmilk. Our aim was to assess neonatal exposure during breastfeeding.

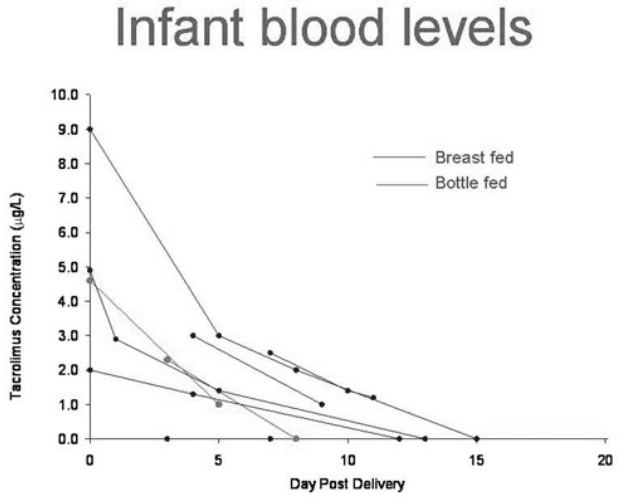
Methods: Maternal, cord & infant blood and breastmilk samples were collected postpartum.Tac levels were analysed by liquid chromatography tandem mass-spectrometry.

Results: Eleven women, taking Tac during pregnancy and lactation, & their 12 infants, 10 of whom were subsequently breastfed, were assessed. Maternal blood (n=12), cord blood (n=6), infant blood (n=21, 1-3/infant), & breastmilk (n=18 samples/8 women, including 1 bottle fed infant) were collected at days 1-72 post delivery. Infant details & breastmilk analysis are shown.

Table

Infant characteristics	
Median Gestation at Delivery (wks)	36, IQR 34.5-36.5
Birth Weight (g)	2432, IQR 1862-2496
Breastmilk Tacrolimus levels	
Median Breastmilk level	0µg/l, IQR 0-0.65
Median Breastmilk : Maternal Blood ratio	0.045, IQR 0-0.13

By 14 days postpartum, Tac levels were undetectable regardless of feeding method.



Estimated infant dose is 0.78µg/day, equivalent to 0.32% of maternal dose (based on highest breastmilk concentration 2.1µg/l, assumed maternal & infant weights 60 & 2.5kg respectively, maternal Tac dose 6mg/day & breastmilk ingestion 0.15l/kg/day).

Conclusions: Ingestion of Tac by infants via breastmilk is negligible. Breastfeeding does not appear to slow the decline of infant Tac levels from high levels present in cord blood. Women taking Tac should not be discouraged from breastfeeding if monitoring of infant Tac levels is available.

Funding: Government Support - Non-U.S.

TH-PO406

Differential Effects of Renal Replacement Therapy on Plasma Inflammatory and Apoptotic Biomarkers in Acute Kidney Injury Xiao-Yan Wen,[1] Raghavan Murugan,[1] Lan Kong,[1,3] Nilesh Shah,[1,3] MinJae Lee,[1,3] Melinda J. Carter,[1] Michele M. Elder,[1] Paul M. Palevsky,[2,4] Mark L. Unruh,[2] John A. Kellum.[1,2] *[1]Critical Care Medicine, Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), University of Pittsburgh, Pittsburgh, PA; [2]Department of Medicine, University of Pittsburgh, PA; [3]Department of Biostatistics, University of Pittsburgh Graduate School of Public Health, PA; [4]VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: Higher concentrations of inflammatory and apoptotic biomarkers are strongly associated with increased risk of death and non-recovery following acute kidney injury (AKI). Whether intensity of renal replacement therapy (RRT) influences circulating biomarker concentrations in patients is unknown.

Methods: Using a subset of 819 patients enrolled in the Acute Renal Failure Trial Network study, we measured 11 circulating inflammatory and apoptotic plasma biomarkers before (day 1) and after (day 8) RRT initiation. We examined whether higher intensity RRT was associated with lower circulating biomarker concentrations.

Results: With the exception of interleukin (IL)-8, we found no differences in any biomarker levels between intensive and less-intensive RRT arms on days 1 and 8. However, compared to less-intensive treatment, intensive treatment was associated with lower day 8 concentrations when day 1 levels were high. In contrast, intensive RRT was associated with higher biomarker concentrations on day 8 when day 1 levels were lower. Differences reached statistical significance for death receptor (DR)-5, IL-6, macrophage migration inhibitory factor (MIF) and tumor necrosis factor receptor (TNFr)-1, but similar trends were seen for all 11 markers.

Conclusions: Intensity of RRT did not impact plasma biomarker concentrations overall. However, higher-intensity RRT had differential effects on day 8 marker concentrations relative to lower-intensity RRT depending on baseline (day 1) levels. Thus, high intensity RRT may be immune modulating in individuals with very high or very low baseline inflammation. Further study is needed to understand the clinical significance of this finding.

Funding: NIDDK SupportBiological Markers of Recovery for the Kidney (BioMaRK) R01DK070910

TH-PO407

Frequency of Antibiotic Underdosing in Critically Ill Patients Receiving Renal Replacement Therapy Suneel M. Udani, Ling Chen, Mitchell J. Daley, Ishaq Lat, Jay L. Koyner. *Medicine, University of Chicago, IL.*

Background: Achieving target serum antibiotic concentrations in infected ICU patients on renal replacement therapy (RRT) is complex but essential for supportive care. Rates of adherence to antibiotic dosing guidelines for patients receiving RRT is not well studied and given the increased volume of distribution in critically ill patients underdosing antibiotics may have unintended adverse outcomes.

Methods: We conducted a retrospective cohort study evaluating rates of antibiotic underdosing in ICU patients receiving RRT between July 2007 and June 2009 at a single academic medical center. Appropriate dosing was determined by comparison of dose administered with established guidelines and denoted as accurate if adjustment occurred prior to the third administered dose, or, if appropriate dosing is every 12 hours or more, within 24 hours.

Rates of underdosing were compared (to normal and over-dosing) according to modality of RRT (Intermittent Hemodialysis (IHD) vs. Continuous RRT (CRRT)), indication for RRT (Acute Kidney Injury (AKI) vs. End Stage Renal Disease (ESRD)) and the presence of a clinical pharmacist on ICU rounds.

Results: 546 of 723 patients receiving RRT during the study period received antibiotics and had charts available for review. 1761 individual antibiotic administrations were available for analysis. Under-dosing occurred more frequently in antibiotics requiring dose adjustment (22.8% vs. 1.5%, $p<0.001$). Dosing errors were more common in the group receiving CRRT vs. IHD (38.4% vs. 6.5%, $p<0.001$) and in those ICUs without a clinical pharmacist on rounds (31.3% vs. 18.7%, $p<0.001$). Frequency of dosing errors did not differ between those receiving RRT for AKI vs. ESRD (23.4% vs. 21.1%, $p=0.477$). There was no difference of in-hospital mortality in underdosed individuals, (52.3%) compared to those not under-dosed (51.6%), $p=0.92$.

Conclusions: Antibiotic underdosing occurs frequently in ICU patients receiving RRT. Increased awareness of dose adjustment guidelines for CRRT and having a Clinical Pharmacist participate directly in rounds may improve rates of underdosing.

Funding: Other NIH Support - 5T32DK007510-23

TH-PO408

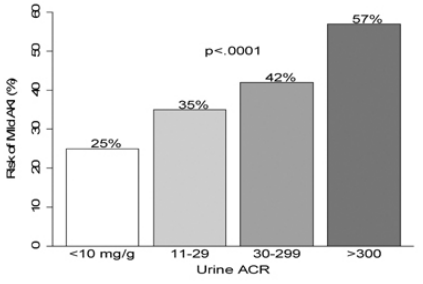
Pre-Operative Proteinuria Predicts Acute Kidney Injury in Patients Undergoing Cardiac Surgery Steven G. Coca, Divakar Jammalamadaka, Kyaw Sint, Heather Thiessen Philbrook, Michael Shlipak, Michael Zappitelli, Prasad Devarajan, Amit X. Garg, Chirag R. Parikh. *TRIBE-AKI Consortium.*

Background: To examine the utility of using proteinuria in pre-operative risk stratification for acute kidney injury (AKI). AKI is a common and important complication for patients undergoing cardiac surgery. Proteinuria, which reflects structural damage to the glomeruli or renal tubules, may aid the prediction of AKI.

Methods: The ratio of urine albumin to creatinine (UACR) and dipstick proteinuria concentration were prospectively measured in 1300 adults and children undergoing cardiac surgery. The cohort was organized into four clinical risk categories based on the pre-operative UACR: UACR ≤ 10 mg/g (≤ 1.1 mg/mmol), 11-29 mg/g (1.2-3.3 mg/mmol), 30-299 mg/g (3.4-33.8 mg/mmol), and ≥ 300 mg/g (≥ 33.9 mg/mmol). The primary outcome was post-operative AKI, defined by the AKIN stage I criterion (serum creatinine rise by ≥50% or ≥ 0.3 mg/dL (26.5 µmol/L)).

Results: An increase in the incidence of AKI was noted across the UACR categories (25%, 35%, 42%, 57%, respectively.

Figure: Incidence of Stage I AKI across the UACR categories



Stage I AKI is defined as serum creatinine rise by 50% or 0.3 mg/dL (26.5 µmol/L). The incidence of AKI increases across the UACR categories in a step-wise manner (p < 0.001 for trend).

Adding UACR to the clinical model to predict AKI improved the AUC from 0.61 to 0.66 ($p < 0.001$) and the continuous net reclassification improvement (NRI) was 32% ($p < 0.001$). UACR categories were also independently associated with risk of in hospital death or dialysis ($p = 0.04$), and ICU and hospital length of stay ($p = 0.005$), ($p = 0.008$), respectively. Surgery status and pre-operative GFR were effect modifiers; the association was stronger amongst those undergoing elective surgery ($p < 0.001$) and those with eGFR 45 mL/min per 1.73 m^2($p = 0.003$).

Conclusions: Pre-operative proteinuria provides graded stratification risk for AKI and is an independent predictor of other outcomes in adults and children undergoing cardiac surgery.

Funding: NHLBI

TH-PO409

Renal Artery Calcium and Mortality among Community-Living Adults Dena E. Rifkin,[1,2] Joachim H. Ix,[1,2] Christina Wassel,[1] Michael H. Criqui,[1] Matthew Allison.[1] *[1]UC San Diego; [2]VA Healthcare System, San Diego.*

Background: The associations of calcification in the renal vasculature, independent of calcification in other vascular beds, with subsequent mortality risk in the general population are unknown.

Methods: We assessed renal artery calcification (RAC) by abdominal CT scan and used Cox-proportional hazards models to examine the association of RAC with mortality in 4,469 community living adults without known cardiovascular disease (CVD) who presented for preventive screening examinations.

Results: The mean age of the study sample was 56.7, and 42.6% were women. RAC was present in 629 of 4,469 (14%) participants. Over a median follow-up of 8.2 years, 177 individuals died. After adjustment for age, gender, diabetes, smoking, cholesterol and family history of CVD, the presence of RAC conferred a more than 70% increased hazard for all-cause mortality (HR 1.71, 95% CI 1.22-2.39, p = 0.002). Adjustment for calcification in other vascular beds attenuated this effect, although findings remained significant (HR 1.47, 95% CI 1.04-2.06, p= 0.027). Additional adjustment for blood pressure, a potential mediator of the association, did not substantially change the results (HR 1.44, 95% CI, 1.03-2.03, p = 0.036).

Conclusions: We found that RAC is associated with an increased risk of subsequent mortality in individuals without known CVD, independent of traditional CVD risk factors. The risk was attenuated somewhat by adjustment for vascular calcification in other vascular beds, suggesting an effect of systemic calcified atherosclerosis, and was not mediated by hypertension.

Hazard of all-cause mortality at different levels of RAC

	Age-sex adjusted	risk adjusted*	fully adjusted**	putative mediator adjusted***
RAC (any vs. none)	1.86(1.33,2.59)	1.71(1.22,2.39)	1.47(1.04, 2.07)	1.44(1.03,2.03)
p for HR	p < 0.001	p = 0.002	p = 0.027	p = 0.036
RAC (per log change)	1.16 (1.08, 1.25)	1.14 (1.06, 1.23)	1.10 (1.02, 1.19)	1.10 (1.02, 1.19)
p for HR	p < 0.001	p < 0.001	p = 0.01	p = 0.02

* adjusted for smoking, hypercholesterolemia, diabetes, and family history of CVD; ** additionally adjusted for # of other beds with vascular calcification; *** additionally adjusted for hypertension (yes/no)

Funding: Other NIH Support - NHLBI, Veterans Administration Support

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The Use of Palliative Care Services amongst End Stage Kidney Disease Patients in an Irish Tertiary Referral Centre Lynn Redahan,[1] Bernadette Brady,[2] Andrew Smyth,[1] Stephen Higgins,[2] Catherine A. Wall.[1] *[1]Department of Nephrology, AMNCH Tallaght, Dublin, Ireland; [2]Department of Palliative Medicine, AMNCH Tallaght, Dublin, Ireland.*

Background: It is well accepted that patients with end stage kidney disease (ESKD) have a shortened life expectancy. Despite this, it has been recognised that end of life care is suboptimal in this patient population. The aim of this study was to review the utilisation of palliative care services amongst ESKD patients in a tertiary referral centre.

Methods: We conducted a retrospective chart review of patients with ESKD who died between January 2005 and October 2009. Eligible patients were identified using the renal database. We included patients who had undergone renal replacement therapy for a minimum of 3 months. We recorded palliative care referrals, modality of renal replacement therapy, age at death and place of death.

Results: 128 patients were included in the study. The final modality of renal replacement therapy was haemodialysis in 100 patients (78.1%) and peritoneal dialysis in 28 patients (21.9%). The average age at death was 65 years. Forty five patients (35.2%) were referred to the palliative care services. The palliative care team were involved in the patients' management for a median of 11 days before death. 102 patients (79.7%) died in an acute hospital setting, nine patients (7.0%) died at home, two patients (1.6%) died in an inpatient hospice and the place of death was unknown for fifteen patients (11.7%). Dialysis was withdrawn prior to death in forty eight patients (37.5%).

Conclusions: The palliative care services were involved in the antemortem care of approximately one third of our patients and the majority of referrals were sent at a late stage. A decision to withdraw dialysis was made in a high proportion of cases studied. Given the short timeframe until death once dialysis is withdrawn, it is imperative that appropriate end of life care is instituted for these patients. We have identified an underutilisation of palliative care services for ESKD patients in our hospital. Improved integration of palliative care and nephrology services may allow us to optimise end of life care for these patients.

TH-PO411

Cer1 Is Involved in the Control of Ureteric Bud Branching by Antagonizing Bmp4-Mediated Inhibition of the Gdnf/Wnt11 Positive Feedback Loop during Kidney Development Seppo J. Vainio. *Medical Biochemistry and Molecular Biology, Biomedicine, Oulu, Finland.*

Background: Ureteric bud branching is critical for mammalian kidney development since the process generates the urine transporting collecting duct system and the ureter and the ureteric bud tips trigger also nephrogenesis.

Results: We show here by using gain and loss of function approaches that a secreted Bmp antagonist Cerberus homologue (Cer1) regulates kidney organogenesis. A gain of Cer1 function in the ureteric bud promotes kidney size and enhances formation of trifid and lateral types of ureteric bud branches rather than bifid ones during early stages of organogenesis as revealed by time-lapse image capture in organ culture. These changes are likely behind the Cer1-induced alterations in the 3D structure of the ureteric tree in the advanced kidney and the changes in several 3D ureteric tree parameters as revealed by the optical projection tomography (OPT) in comparison to controls. Cer1 functions by binding and antagonizing signalling of Bmp2/4 that both act as inhibitory signals for ureteric bud development. In specific by binding Bmp4 Cer1 relieves the inhibitory effect of Bmp4 on Gdnf and Wnt11 expression and promotes by this way their co-operated positive influence on ureteric growth control. Consistent with these notions genetic reduction of Wnt11 or excess Bmp4 inhibit the genetically enhanced, Cer1-stimulated ureteric bud branching process.

Conclusions: The results point that Cer1 functions as part of the genetic programme that coordinates ureteric bud branching and Cer1 mediates this by regulating the activity of Wnt11 and Gdnf via the control of availability and out put of Bmp4 signalling.

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TH-PO412

Kidney Disease Genes in Pre-Diabetics: Epigenomic Approach Vallabh O. Shah,[1] Marilee Morgan,[3] Quynh-Anh Bui,[1] Joan Goldsworthy,[1] Thomas A. Vander Jagt,[1] Julie S. Broyles,[1] Gretchen M. Ray,[1] Donald W. Bowden.[2] *[1]Uni of New Mexico; [2]WFU; [3]MRN.*

Background: T2DM affects more than 27 million in US. About 79 million adults have pre-diabetes (preDM), but only 10% are aware of being preDM. Recent studies suggest that gene-environment interactions relevant for T2DM are at least partly regulated by epigenomic mechanisms.

Methods: We performed DNA methylation study in bisulphite converted DNA from Pre-DM (n=12) at baseline and at their transition to T2DM using Illumina Infinium® HumanMethylation27 BeadChip, that enables the query of 27,578 individual cytosines at CpG loci throughout the genome, which are focused on the promoter regions of 14,495 genes.

Results: Our initial analysis indicated that very significant components of DNA methylation variations (hypo- and hyper-) correlated with PreDM progressing to T2DM. With a false discovery rate of 0.05, we observed about 694 CpG sites hypomethylated and about 174 CpG sites hypermethylated from preDM to T2DM progression representing many genes. Many of the genes identified as hypomethylated have been reported to be involved in glucose, fructose transporter, and inflammation, oxidative and mitochondrial stress and fatty acid metabolism.

Interestingly, CpG loci methylation changes associated with the progression from normal baseline expression patterns to preDM were evident in 21 identified gene loci. These changes in CpG methylation in DNA of preDM remained persistent during the progression to T2DM in these patients. Among the 21 identified genes that were hyper or hypomethylated, the CpG loci associated with carnosinase was hypomethylated in PreDM and remained hypomethylated during the progression to T2DM. This gene has been suggested, from previous GWAS studies, to be involved in genetic disposition to diabetes as well as diabetic nephropathy. SLC22A12, a gene in the family of GLUT9 which is a uric acid and fructose transporter identified in kidney, became hypomethylated after the transition into diabetes.

Conclusions: These data suggest that some epigenomic changes that may be involved in the progression of diabetes and/or the development of complications may be apparent at the preDM state or during the transition to diabetes.

Funding: Other NIH Support - NCRR supported CTSA pilot project grant from University of New Mexico health Sciences Center

TH-PO413

Alterations in Renal MicroRNA Expression in a Mouse Model of Congenital Obstructive Nephropathy Susan E. Ingraham,[1] Kirk M. McHugh.[2] *[1]Division of Nephrology, Nationwide Children's Hospital, Columbus, OH; [2]Center for Molecular and Human Genetics, Nationwide Children's Hospital, Columbus, OH.*

Background: Congenital obstructive nephropathy (CON) is the most common cause of chronic renal failure in children. The megabladder (mgb) mouse, a unique genetic model of CON, allows exploration the molecular changes of CON, potentially facilitating identification of novel biomarkers or therapeutic targets for this devastating condition. MicroRNAs (miRs), a class of small non-coding RNA, are important gene regulators controlling key cellular functions. A number of miRs are highly and differentially expressed in the kidney, and have been implicated in key physiological or pathological renal processes. This project explores changes in miR expression associated with early stages of renal involvement in the mgb mouse.

Methods: Four week old mgb mice were stratified based on general health and ultrasound findings as previously described (Ingraham et al., 2010). Males classified as having moderate renal involvement were selected, along with age- and gender-matched controls. Total kidney RNA was used for comparative miR microarray analysis. miRs that exhibited ≥1.5-fold alteration in expression by microarray were further investigated by quantitative reverse transcription-coupled PCR (RT-qPCR).

Results: Comparative microarray analysis yielded a panel of 22 miRs whose expression levels were altered at least 1.5-fold in mgb kidneys compared to normal controls. RT-qPCR analyses of these miRs confirmed that eight were significantly increased in expression in mgb kidneys ($p<0.05$). Five miRs showed a trend toward increased expression but did not reach statistical significance. Of three miRNAs with decreased expression by microarray, two showed a downward trend by qPCR but did not reach statistical significance. Two miRs showed no difference in expression between mgb and control, and five could not be tested by qPCR due to low sequence complexity.

Conclusions: Alterations in miR expression are associated with early stages of renal involvement in the mgb mouse model of CON. Further characterization of miRs in this animal model may yield new insights into this complex disease process.

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TH-PO414

Genome-Wide Association Study (GWAS) of Novel Kidney Function Biomarkers in 6744 European Americans Adrienne Tin,[1] Brad C. Astor,[1] Eric Boerwinkle,[2] Josef Coresh,[1] Wen Hong Linda Kao.[1] *[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Human Genetics Center, University of Texas School of Public Health, Houston, TX.*

Background: Serum beta trace protein (BTP) and beta-2 microglobulin (B2M) have potential as novel biomarkers for renal function. Little is known about the genes influencing either their levels alone or through glomerular filtration rate estimated from serum creatinine ($eGFR_{Cr}$) or cystatin C ($eGFR_{cysC}$).

Methods: We conducted a GWAS of log-transformed serum BTP and B2M levels and further examined their association with known eGFR loci in 6744 European Americans from the Atherosclerosis Risk in Communities (ARIC) study.

Results: The GWAS of BTP identified a genome-wide significant locus upstream of *PTGDS,* the gene that encode BTP (rs7040970, beta=0.04, $p=3.3x10^{-17}$, RSqr=0.8%), which was not associated with either $eGFR_{cr}$ or $eGFR_{cysC}$ ($p>0.05$). The GWAS of B2M identified 2 genome-wide significant loci. One was in the HLA region on chromosome 6 that spans 7 Mb with over 1000 SNPs attaining genome-wide significance (lowest p-value=$1.8x10^{-23}$ for rs9264638, betas=0.02 to 0.07). In this locus, 7 signals with low linkage equilibrium accounted for 3.5% of log(B2M) variance but were not associated with $eGFR_{cr}$ or $eGFR_{cysC}$. The other locus of B2M was on chromosome 12 (rs3184504 in *SH2B3,* beta=0.02, $p=3.1x10^{-8}$, RSqr=0.4%), which was previously implicated as a renal locus. Of the 16 $eGFR_{cr}$ loci previously identified (Kottgen et. al 2010), within our sample $eGFR_{cr}$ was associated with 7, $eGFR_{cysC}$ with 4, BTP with 3, and B2M with 2 (all $p\leq0.05/16$).

Conclusions: Genetic analysis of multiple filtration markers can identify both the genetic effects that influence only one marker (Cr, CysC, BTP, or B2M) and those that influence multiple markers, consistent with an effect on GFR itself. Extending previous work on Cr and CysC to BTP and B2M, we found novel marker-specific loci and confirmed the association of several loci of eGFRcr with BTP and B2M. These findings add to our understanding of the metabolism and future clinical use of BTP and B2M as filtration markers as well as improve genetic research of GFR determinants.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO415

Next Generation Sequencing (NGS) of Targeted Exons in the UK Steroid Resistant Nephrotic Syndrome in Childhood Cohort: A Pilot Study Hugh J. McCarthy, Agnieszka Bierzynska, Matthew Wherlock, Moin Saleem. *Academic Renal Unit, University of Bristol, Bristol, United Kingdom.*

Background: Approximately 350 children in the UK have Steroid Resistant Nephrotic Syndrome (SRNS). This condition displays considerable genetic heterogeneity with an estimated 25% of all patients having a mutation in one of 10 'nephrotic' genes. The prevalence is not known within the UK population where clinical testing for the majority of these genes is not available.

Methods: Utilising the newly formed Rare Renal Disease Registry (renalradar.org), a national cohort of children with SRNS has been developed, with detailed and ongoing clinical phenotyping. This pilot study looked at the effectiveness of undertaking targeted exon capture with sequencing in 12 of these patients for exons in 478 genes. 5 of these patients had previously undergone Sanger sequencing for exons in 3 'nephrotic' genes.

The genes were chosen for reported or potential biological involvement in this condition. Using a Roche Nimblegen Sequence Capture (12x135K) Custom Array, the exons of these genes of interest were targeted. Paired-end sequencing was undertaken using an Illumina GAIIx. Data analysis was performed with CLC Genomics Workbench software.

Results: From all reads 98.2% were mapped uniquely to the reference sequence. 82% of reads were mapped to capture targets. All variants previously detected using Sanger sequencing were identified using NGS. A total of 37511 different variants were identified with 887 causing an amino acid change with an average depth coverage of 31. One homozygous and 2 heterozygous single nucleotide changes were identified in three patients in 'nephrotic' genes which are not previously described in dbSNP (build 132). 13 homozygous and 123 heterozygous variants not described in dbSNP were found in the remaining genes. 27 different deletion - insertion polymorphisms causing an amino acid change were detected, although none in the 10 'nephrotic' genes. (All specific changes described had coverage >10).

Conclusions: This pilot study demonstrated the potential of NGS to provide valid data. In this well phenotyped cohort it allows for exploration of disease modifying variations in a huge number of biologically relevant genes.

TH-PO416

Effects of Lovastatin Treatment on the Metabolic Distributions in the ADPKD Rat Kidney Jelena Klawitter, Jost Klawitter, Uwe Christians, Charles L. Edelstein. *Anesthesiology; Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: We have previously demonstrated that lovastatin decreases kidney and cyst volume and improves kidney function in the Han:SPRD (Cy/+) rat model of PKD. As statins are known to affect energy metabolism, we used NMR-based metabolomic profiling to investigate the metabolic pathways that might be responsible for the protective effect of lovastatin in PKD.

Methods: Cy/+ and normal littermate controls (+/+) were treated with either lovastatin (4mg/kg/day) or vehicle (ethanol) from 3-8 weeks of age. ^{1}H-NMR analysis was performed on water-soluble kidney fractions following a perchloric acid extraction (n=4-5).

Results: PKD in Cy/+ rats was accompanied by changes in several metabolites as compared to the +/+ rats. The concentrations of Krebs cycle intermediates citrate and succinate significantly decreased from 1366.9±296.8 and 3353.4±278.2 nmol/g to 919.3±150.7 ($p<0.05$) and 2219.6±557.0 nmol/g ($p<0.01$), respectively. Their decrease was accompanied by a decrease of kidney glucose concentration. The observed impairment of the glucose import could possibly be a result of a feedback action induced by the decreased Krebs cycle activity. A product of uric acid oxidation and a marker of kidney injury, allantoin, increased almost 3-fold in the Cy/+ rat kidneys ($p<0.001$).

Statin treatment reversed the metabolic changes induced by the PKD in the Cy/+ rats. Citrate concentration increased to 1419.2±92.2 nmol/g ($p<0.005$) and so did the lactate concentration, suggesting a recovery of energy producing Krebs cycle and glycolysis pathways. The increased glucose consumption of the lovastatin treated kidneys further lowered the glucose concentration. Allantoin levels significantly decreased as well, but still remained almost 2-fold higher as in the +/+ controls.

Conclusions: We describe for the first time changes in energy metabolism in PKD kidneys. Lovastatin increased the activity of energy producing metabolic pathways is association with improvement in PKD. NMR-based metabolomics may be a tool to study the relationship between metabolic changes and cellular signaling in PKD and to develop urine biomarkers for the diagnosis of PKD.

TH-PO417

Transcriptome and ChIP-Seq Analyses of JunD Levels Reveal the Basis of *Jund*-Mediated Activation Networks Associated with Crescentic Glomerulonephritis (Crgn) in Rat Macrophages Richard P. Hull,[1] Prashant K. Srivastava,[1] Zelpha D'souza,[1] Santosh S. Atanur,[1] Jennifer Smith,[3] Charles D. Pusey,[3] Jacques Behmoaras,[2,3] H. Terence Cook,[2,3] Timothy J. Aitman.[1] *[1]MRC CSC; [2]CCIR; [3]Renal Medicine, Imperial College, London.*

Background: We previously investigated the genetic basis for the unique susceptibility of the Wistar-Kyoto (WKY) rat to Crgn and identified the AP-1 transcription factor JunD as a primary determinant of macrophage activation. JunD is markedly overexpressed in WKY macrophages compared to the Crgn-resistant Lewis strain and this over-expression, controlled in *cis*, results in increased activity of these cells.

Methods: We studied how differences in *Jund* expression alter the macrophage transcriptome and its cistrome, the genome wide set of JunD-DNA binding sites, following (LPS) stimulation by two approaches: 1. siRNA knockdown of *Jund* in WKY bone marrow derived macrophages (BMDMs) followed by genome-wide expression analysis 2. Genome-wide expression combined with ChIP-Seq analyses in WKY and *Jund* congenic BMDMs where the *Jund* locus from a Lewis rat was introgressed onto a WKY background (WKY.L*Crgn2).*

Results: We identified 1672 genes in basal and 1476 genes in LPS stimulated BMDMs that were differentially expressed following siRNA knockdown of *Jund* and 830 differentially expressed genes between WKY and WKY.L*Crgn2* over a LPS stimulation timecourse. Gene ontology analysis enriched for processes including responses to

stimulation, cell activation and protein kinase cascades in all 3 datasets. Overexpression of JunD in WKY BMDMs results in significantly increased numbers of binding sites in basal (27124 peaks) and LPS stimulated BMDMs (36687 peaks) compared to WKY.L*Crgn2* (16593 peaks basal, 8689 peaks LPS stimulation) which were also associated with genes involved in stimulus responses, protein kinase cascades and cell activation.

Conclusions: This work shows that *Jund* expression levels have modulatory effects on macrophage gene expression as a result of alterations in the JunD cistrome and provides the basis for understanding how JunD mediates macrophage overactivity in the WKY rat enabling the identification of novel targets with which to modulate macrophage function.

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TH-PO418

Analysis of RNA from Urinary Exosomes/Microvesicles to Non-Invasively Diagnose Renal Cancer and Diabetes Related Renal Dysfunction Leileata M. Russo,[1] Kendall Bate,[1] Neda Sadeghi,[2] Guillermo Salazar,[1] Wayne Comper,[1] Siham Accacha,[3] John K. Maesaka,[3] James McKiernan.[2] *[1]Exosome Diagnostics., Inc., New York, NY; [2]Columbia University Medical Center, New York, NY; [3]Winthrop University Hospital, Mineola, NY.*

Background: Microvesicles, including exosomes, are small lipid bilayer vesicles released from all cells into bodily fluids. These vesicles harbor nucleic acids (in particular RNA) from their parent cell and can be used to interrogate the transcriptional profile of cells in vivo in a non-invasive manner. Using our rapid microvesicle isolation technique we have now demonstrated our ability to non-invasively detect mutational status in a 200 patient prostate cancer cohort demonstrating detection of oncogene fusion status (TMPRSS2:ERG) as well as qPCR analysis of 20 genes previously documented as being associated with prostate cancer progression. Using this experience as a foundation, we now turn our attention to the non-invasive detection of two important renal related diseases i) renal cell carcinoma (RCC) ii) diabetic nephropathy (DN).

Methods: Urine samples were collected from Columbia University (RCC patients) and Winthrop University Hospital (DN patients) under approved IRB. Urinary exosomes/microvesicles were isolated and high integrity RNA was extracted as analyzed via the Agilent Bioanalyzed Pico chip. RNA was subjected to various forms of analysis including array analysis, qPCR and next-generation sequencing (NGS).

Results: We demonstrate that a wide array of genes are detectable within urinary microvesicles using array and NGS analysis. This included CA9 in RCC and various markers of the renin angiotensin system and beyond in the DN patients including angiotensin II receptor, angiotensinogen and angiotensin converting enzyme mRNA expression.

Conclusions: Urinary exosomes/microvesicles offer a novel method to non-invasively monitor renal transcription in various renal related diseases. Array and NGS analysis of urinary exosomes/microvesicles may enable the selection of a diagnostic panel of markers to enable patient stratification and early detection of renal disease.

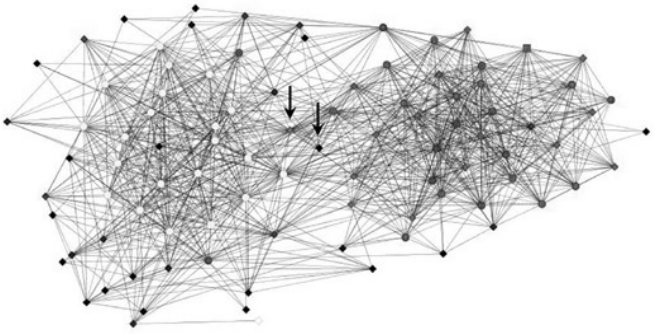
TH-PO419

Transcriptional Pathways identified by Genotype-Phenotype Integration in CKD Sebastian Martini,[1] Viji Nair,[1] Felix H. Eichinger,[1] Katherine Gurdziel,[1] Carsten A. Böger,[4] Caroline S. Fox,[3] Clemens D. Cohen,[2] Matthias Kretzler.[1] *[1]University of Michigan; [2]University of Zürich; [3]National Institutes of Health; [4]University of Regensburg.*

Background: Integrating genome-wide association studies (GWAS) with gene expression data sets will help to define interactions of chronic kidney disease (CKD) associated molecular pathways with transcriptional regulators.

Methods: Candidate genes within 60kb of loci associated with CKD in GWAS reported by CKDGen were investigated for correlation of their renal mRNA levels with eGFR in the independent ERCB cohort. Correlated mRNAs were interrogated in genome wide renal gene expression profiles of 177 CKD patients for biological functionality by pathway mapping. An interaction network of individual pathways connected by genes 1) occurring in two or more pathways, and 2) co-regulated with one or more CKD candidate genes, was analyzed.

Results: Renal mRNA levels of 17 out of 29 expressed CKDGen candidate genes correlated with eGFR in an independent cohort. To define functional context of CKDGen associated genes, co-regulated transcripts were identified in the ERCB expression data set. These co-regulated transcripts were significantly enriched in 148 pathways. CKD associated pathways were displayed as pathway network using genes shared between pathways as edges. The network displays two major subclusters: 1. containing metabolism pathways (yellow in Fig. 1, i.e. Fatty acid metabolism, Glycerolipid metabolism) 2. inflammation-related pathways (red in Fig. 1, i.e. NFkappaB-, TNF-signaling). NRF2-mediated Oxidative Stress Response and PPARgamma-signaling (arrows in Fig. 1) are key hubs connecting the two main subclusters.



Conclusions: CKDGen transcripts and their co-regulated mRNAs identify the interplay of inflammation and metabolism pathways in CKD. The CKD pathway network can link specific molecules in their larger context.

TH-PO420

LC-MS/MS and Antibody-Array Based Urinary Proteome Analysis of Obstructive Nephropathy Caubet Cécile,[1] Chrystelle Lacroix,[2] Flavio Bandin,[1,3] Anne Gonzalez de Peredo,[2] Odile Burlet-Schiltz,[2] Jean-Loup Bascands,[1] Stéphane Decramer,[1,3] Joost Schanstra.[1] *[1]Inserm U1048, Toulouse, France; [2]CNRS - IPBS, Toulouse, France; [3]CHU Toulouse, Hôpital des Enfants, Service de Néphrologie, Toulouse, France.*

Background: We have previously shown that urinary peptidome analysis allows early prediction of obstructive nephropathy (ON) in newborns. Although these urinary peptidome biomarkers are of great potential clinical value, they are less informative on the pathophysiology of the disease. Therefore we have focussed on the changes in the urinary proteome of ON patients using label-free LC-MS/MS and antibody–array analyses.

Methods: Urine samples (n=5/group) were divided into 4 groups: bladder urine from controls (healthy), mild ON (NoOp), severe ON (Op) and pelvis urine from severe ON (Pelvis). For antibody array analysis, 90 µg of Cy5-tagged urinary proteins were applied onto antibody arrays (XP725 Sigma). Fluorescence intensity was normalized by the mean intensity of each array. For LC-MS/MS analysis, 30 µg of the protein was digested by trypsin and analysed by online capillary LC-MS/MS. Proteins were identified and quantified as described (Mouton-Barbosa et al., MCP, 2010).

Results: The number of differentially secreted proteins between the different groups (p value <0.05) is shown in the table.

Number of over-secreted proteins
Number of under-secreted proteins

	Healthy/Mild	Healthy/ Bladder-Surgery	Healthy/ Pelvis-Surgery	Mild/ Bladder-Surgery	Mild/ Pelvis-Surgery	Bladder-Surgery/ Pelvis-Surgery
LC MS/MS	23	64	213	13	161	149
	48 pBH<0.05				*1 pBH<0.05*	
	6 | 17	35 | 29	77 | 136	11 | 2	92 | 69	51 | 98
AB ARRAY	22	43	28	7	3	7
	17 | 5	18 | 25	23 | 5	1 | 6	2 | 1	6 | 1

pBH : pvalue after Benjamini-Hochberg adjustment

pBH indicates the number of differentially secreted proteins after Benjamini-Hochberg adjustment. Only the LC-MS/MS comparison of the control group versus pelvic urine yielded 48 proteins that survived correction for multiple testing. Based on these comparisons we established a list of proteins that are specific for the obstructed or controlateral kidney.

Conclusions: We are validating a subset of these proteins on new samples. Two different approaches for validation are used: antibody-based validation and multiple reaction monitoring on small (max 1.5 ml) urine samples.

Funding: Government Support - Non-U.S.

TH-PO421

Alterations in Cellular Proteome of Macrophage Induced by Calcium Oxalate Monohydrate Crystals Are Associated with Phagocytosis Visith Thongboonkerd, Nilubon Singhto, Kitisak Sintiprungrat. *Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

Background: The presence of macrophage in renal tubulointerstitium is the key feature of progressive renal inflammation in kidney stone disease. Macrophage can engulf and eliminate foreign body or microbe, and subsequently produces various inflammatory mediators. However, molecular responses of macrophage to calcium oxalate monohydrate (COM) crystals, the major crystalline composition of kidney stone, remained unclear. Therefore, this study aimed to investigate alterations in cellular proteome of macrophage induced by COM crystals using a proteomics approach.

Methods: Macrophages were derived from U937 human monocytic cells by treatment with 100 ng/ml phorbol myristate acetate for 48 h. Macrophages were then incubated with or without 100 µg/ml COM crystals for 24 h. Thereafter, proteins derived from whole cell lysates of COM-treated and controlled cells were resolved by 2-DE (n=5 gels/group; each was from independent culture) and stained with Deep Purple fluorescent dye. Differentially

expressed protein spots were then identified by Q-TOF MS and MS/MS analyses. Functional significance of these proteins was addressed by global protein network analysis using STRING version 8.3 and double immunofluorescence staining.

Results: Spot matching and quantitative intensity analysis revealed 18 differentially expressed protein spots. These proteins were successfully identified by Q-TOF MS and MS/MS analyses, including those involved in cellular structure, carbohydrate metabolism, DNA/RNA processing, protein metabolism and stress response. The altered levels of α-tubulin, β-actin and ezrin were validated by Western blot analysis. Double immunofluorescence staining revealed a phagosome-like structure outlined with co-localized F-actin and HSP90 and contained COM crystal inside.

Conclusions: Proteomic analysis revealed altered cellular proteome profile of macrophage induced by COM crystals. These findings also showed that the altered proteins were also involved in enhanced phagocytic activity of the COM-exposed macrophage. Our data may help understanding of important role of macrophage in kidney stone disease.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO422

Renal Mendelian Genes Based Pathway Analysis Afshin Parsa, Young J. Kim. *Medicine, University of Maryland School of medicine, Baltimore.*

Background: The development of complex diseases such as chronic kidney disease (CKD) and end-stage renal disease (ESRD) often require the interaction of various genes and environmental factors within individual or interacting pathways. Similarly, injury along multiple points of interacting pathways can results in similar disease phenotypes. As such, we hypothesized that exploring protein to protein interactions (PPI) networks related to Mendelian genes associated with renal phenotypes could help better elucidate pathways critical to the development of renal pathology.

Methods: Starting with over 600 OMIM listings of Mendelian diseases with renal phenotypes, we identified 339 unique entries which were manually curated to 227 unique disease entries with at least one identified autosomal gene and identifiable renal phenotypes. These were classified within 3 broad categories: 1) Developmental and glomerular 2) Tubular, and 3) Secondary renal disease (e.g., deposition of protein within the kidney). Using the Pathway Studio tool, we systematically explored protein interaction network related to our selected Mendelian genes and identified overrepresented biologic pathways within our 3 broad categories of renal disease types.

Results: We identified numerous pathways that were significantly over represented within our Mendelian gene based PPI networks, including FGFR, leptin, PECAM, and guanylate cyclase. These pathways relate, at least in part, to angiogenesis, branching morphogenesis, adipose activation of sympathetic activity, endothelial cell adhesion and nitric oxide signaling. We also found significant overrepresentation of expression targets related to many of our genes, including SHH, PAX2, ACE, LMX1b, ALB, SGK1, AVP, FOXI1, SCNN1G, WNK4, FGF23, PLAU, FOXM1, ERG, RAG2 and PPARG.

Conclusions: By mining genes associated with renal pathology, we have identified numerous first order protein based interactions converging from diverse genetically mediated renal diseases onto definable pathways. These overrepresented pathways provide promising logical targets for further study in both fundamental mechanism and treatment interventions for CKD and ESRD.

Funding: Other NIH Support - K12

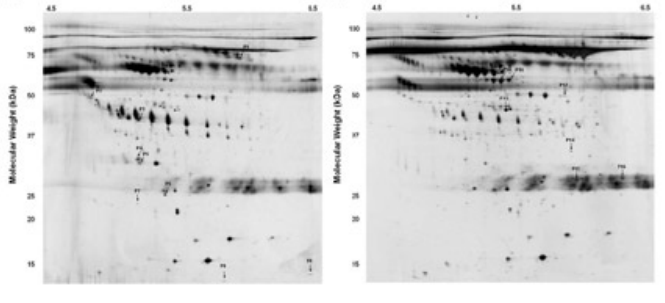
TH-PO423

Impact of Uremic Environment on Peritoneum: A Proteomic View Hsien-Yi Wang, Wei-Chih Kan, Chih-Chiang Chien. *Nephrology, Chi-Mei Medical Center, Tainan, Taiwan.*

Background: Morphology and function of peritoneal membrane are abnormal in patients with uremia, but the contributing pathophysiology is unclear. Here we attempted to characterize the differential protein targets that may be related to peritoneal membrane changes in patients with uremic condition and haven't exposed to peritoneal dialysis fluid

Methods: Protein profiles of peritoneal fluids collected from patients with uremia and patients with normal renal function receiving laparoscopic cholecystectomy were displayed by two-dimensional gel electrophoresis (2-DE). Altered protein spots were excised and subjected to in-gel tryptic digestion followed by liquid chromatography- tandem mass spectrometry (LC-MS/MS) analysis

Results: Sixteen 2-DE protein spots were altered between two groups of samples.



Western blots analysis confirmed that kininogen-1, apoptosis inhibitor 2, cat eye syndrome critical region protein 1, and apolipoprotein A-I had higher expression levels in the uremic samples. In contrast, synaptic vesicle 2-related protein, glial fibrillary acidic protein, and envelope glycoprotein (C2-V5 region) showed lower levels [figure2]

Conclusions: The increased expression of differential proteins may result from changes in the permeability of the peritoneal membrane to middle-sized proteins or peritoneal inflammation with proteins sloughing off. All the identified proteins may provide a novel understanding of peritoneal membrane changes injured by uremic toxins and may manifest as predictive biomarkers of peritoneal function or therapeutic targets during the regular peritoneal dialysis in the future

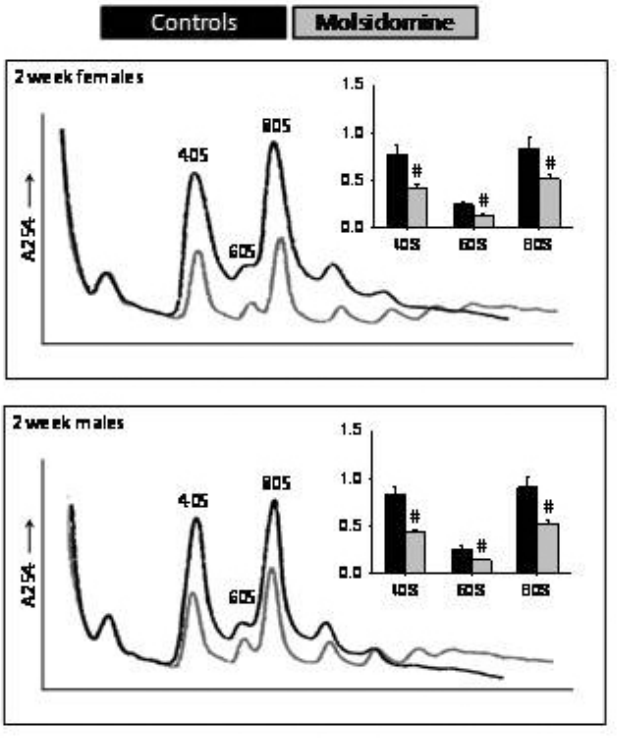
TH-PO424

Perinatal Exogenous Nitric Oxide in Fawn-Hooded Hypertensive Rats Reduces Renal Ribosomal Biogenesis in Early Life Sebastiaan Wesseling,[1] Paul B.M. Essers,[4] Maarten P. Koeners,[1] Tamara Pereboom,[4] Branko Braam,[3] Ernst van Faassen,[2] Alyson W. Macinnes,[4] Jaap A. Joles.[1] *[1]Nephrology, UMCU, Utrecht; [2]Nephrology, LUMC, Leiden; [3]Nephrology and Physiology, Univ Alberta, Edmonton; [4]Hubrecht Institute, Utrecht, Netherlands.*

Background: Nitric oxide (NO) is known to depress ribosome biogenesis in vitro. We analyzed the influence of exogenous NO on ribosome biogenesis in vivo using a proven antihypertensive and renoprotective model of perinatal NO administration in genetically hypertensive rats (Am J Physiol 2008;294:R1847-55).

Methods: Fawn-hooded hypertensive rat (FHH) dams were supplied with the NO donor molsidomine in drinking water from 2 weeks before to 4 weeks after birth. Kidneys were collected from 2d, 2wk and adult offspring.

Results: Although the NO donor increased maternal NO metabolite excretion, renal NO status at 2wk was unchanged as assayed by EPR spectroscopy of NO trapped with iron-dithiocarbamate. Nevertheless, microarray analysis revealed marked differential down-regulation of ribosomal protein genes in 2wk old males. These changes in 2wk males were confirmed by polysome profiling, which also showed down-regulation of ribosomes in 2wk females. Polysome profiles were not affected at 2d or in adults.



Kidneys of 2wk old control and molsidomine treated FHH (n=8/group) were profiled to measure assembled ribosome structures. # P<0.01 vs. controls of the same peak.

Conclusions: Marked decreased postnatal expression of renal ribosomal genes and proteins at 2wk of age in the absence of a change in renal NO status suggest that these alterations are epigenetically programmed by NO in the foetus. In conjunction with prolonged antihypertensive and renoprotective effects of perinatal NO administration in FHH, these data provide a salient example of drug-induced reduction of renal ribosome biogenesis accompanied by beneficial long-term effects in both males and females.

Funding: Private Foundation Support

TH-PO425

Identifying Urinary Peptidomic Biomarkers for Metabolic Syndrome with Early Renal Injury Bixia Gao,[1] Mingxi Li,[1] Xuejiao Liu,[1] Jianfang Cai,[1] Xiaohong Fan,[1] Xiaolin Yang,[2] Xuemei Li,[1] Xue-Wang Li.[1] *[1]Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China; [2]Biomedical Engineering, CAMS, Beijing, China.*

Background: Metabolic syndrome (MS) is associated with chronic kidney disease. Our study aims to establish peptide models and identify urinary biomarkers of MS with early renal injury.

Methods: Samples of overnight (8hr) urine were collected from subjects participating an epidemiologic study of MS with CKD. MS was diagnosed by ATP III criteria, while MS with early renal injury was defined as 20μg/min≤UAE<200μg/min and eGFR≥60ml/min·1.73m^2. Participants were grouped into healthy (G I, n=65), MS with normoalbuminuria(G II, n=54) and MS with MAU(G III, n=46). The urine was fractionated by magnetic bead based weak cation exchange chromatography and subsequently analyzed with MALDI-TOF-MS. The peptide models were respectively established using statistical tests combined GA (ClinProTools2.1) and RF algorithm combined SVM (Matlab7.10.0). The performance of diagnostic models was assessed using 10-fold cross validation and ROC curve. Differential peptide peaks between groups were identified by LC-MS/MS (LTQ Orbitrap Velos).

Results: (1) G I vs G III: GA model showed 100% sensitivity, 92% specificity and 96% accuracy in training set in identifying MS with early renal injury, and it revealed 76% sensitivity, 80% specificity and 78% accuracy in testing set; Correspondingly, SVM model reported 82% sensitivity, 91% specificity and 87% accuracy, AUC value of ROC curve was 0.924. (2)G II vs G III: GA model showed 100% sensitivity, 88% specificity and 93% accuracy in training set, and it revealed 71% sensitivity, 73% specificity and 72% accuracy in testing set; SVM model reported 89% sensitivity, 81% specificity and 86% accuracy, AUC value was 0.911. (3)Three peptide peaks identified were all peptide fragments of fibrinogen α chain (Fgα), m/z 2562.67 was up-regulated in G II and G III, m/z 1884.33 and 2661.41 were up-regulated in G II.

Conclusions: Peptide fragments of Fgα might be urinary peptide biomarkers of MS with early renal injury and Fgα might involve in the pathogenesis of MS and MS with early renal injury.

Funding: Government Support - Non-U.S.

TH-PO426

CD36 Polymorphisms Associated with Measures of Renal Disease in Zuni Indians Latisha Love-Gregory,[1] Venkata Saroja Voruganti,[2] Sandra L. Laston,[2] Karin Haack,[2] Jean W. Maccluer,[2] Shelley A. Cole,[2] Vallabh O. Shah,[3] Jeanette Bobelu,[3] Arlene Bobelu,[4] Donica M. Ghahate,[3] Antonia M. Harford,[3] S. Paine,[3,4] Philip Zager,[3,4] Anthony Comuzzie,[2] Nada A. Abumrad,[1] Aldi T. Kraja.[1] *[1]Washington University School of Medicine, St. Louis, MO; [2]Texas Biomedical Research Institute, San Antonio, TX; [3]University of New Mexico School of Medicine, Albuquerque, NM; [4]Dialysis Clinic, Inc., Albuquerque, NM.*

Background: The multi-ligand lipid receptor CD36 is implicated in renal pathophysiology in rodents but its potential role in kidney disease (KD) in humans is unknown. We examined whether common (minor allele frequency, maf >0.05) SNPs in the *CD36* gene associate with measures of renal function in the Genetics of Kidney Disease in Zuni Indians study(n=878) who have a disproportionately high prevalence of diabetic and non-diabetic renal disease (RD).

Methods: Linear mixed effect models were applied in a family-based association test to examine the influence of *CD36* SNPs on renal quantitative measures. Minor allele frequencies and linkage disequilibrium structure were determined using 168 *CD36* SNPs and 66 representative tag SNPs (maf >0.05) were selected for association analyses adjusted for age and gender.

Results: Four putative regulatory SNPs (rs3211820, rs3211822, rs3211834, rs3211816) mapping to a region of strong linkage disequilibrium in intron 3 (p=0.006-0.01) and an additional SNP in intron 1 (rs4545029, p=0.02) associated with HDL levels independent of BMI, hypertension and glycated hemoglobin. Two intragenic SNPs inversely associated with MDRD glomerular filtration rate (GFR) (p=0.002-0.017) and one (rs2906199, p=0.007) with KD status (n=202 with KD vs n=662 without KD). Six promoter SNPs and 3 intronic SNPs associated with Cystatin C estimated GFR in a subset of subjects (n=246), (p=0.00068 for SNP rs10249397). Multivariate factor analysis revealed a correlation between urine protein/creatinine and blood pressure at a common promoter variant (rs4545029, p=0.0097).

Conclusions: Our results suggest a potential influence for *CD36* SNPs in RD. In particular the findings regarding Cystatin C will need to be investigated further.

Funding: NIDDK Support, Clinical Revenue Support

TH-PO427

The Updated Database of Glomerulus-Enriched Genes by Combining with the Glomerulus Proteome Database for Novel Biomarker Discovery Hidehiko Fujinaka,[1] Tadashi Yamamoto.[2] *[1]Institute for Clinical Research, NIigata National Hospital, Kashiwazaki, Niigata, Japan; [2]Structural Pathology, Institute of Nephrology, Niigata University, Niigata, Japan.*

Background: A comparative study of the 5 different glomerulus-enriched (Gl-E) gene databases (DBs) using different techniques has revealed only 7 genes, out of 1,407 genes identified by at least one DB, were identified as Gl-E genes in all 5 DBs (He et al., *J Am Soc Nephrol* 2008). We constructed a human glomerular cDNA microarray DB (MAd-761), in which 761 genes of Gl-E (intensity ratio of Cy5 (glomerulus)/Cy3 (cortex) >2.0) were ranked in order of glomerular abundance. The present study aimed to update MAd-761 by comparing it with another Gl-E gene DB from Stanford (StDB) and with human glomerulus proteome DB.

Methods: We constructed a human glomerulus proteome DB by separating glomerular proteins with electrophoresis coupled with the shotgun analysis by LC-MS/MS (2DGE-LS-MS/MS, http://www.hkupp.org/). The 6,686 glomerular proteins (representing 2,966 distinct genes) were ranked in order of glomerular abundance (spectra numbers). Kidney immunohistochemistry of each listed protein was checked in the Human Protein Atlas (HPA, http://www.proteinatlas.org/). Gl-E protein was defined the staining intensity in glomerulus was strong (weak or negative in cortex) or moderate (negative in cortex).

Results: In 761 genes in MAd-761, 203 genes were listed in StDB as Gl-E genes. And among the top 1,000 glomerular proteins in the 2DGE-LC-MS/MS, 83 proteins (31 distinct genes) were shown Gl-E protein by HPA. In 761 genes in MAd-761, 26 genes were shown Gl-E protein. And in 203 Gl-E genes in MAd-761 with StDB, 13 genes were demonstrated Gl-E protein; PODXL, PTPRO, SPARC, NES, PCOLCE2, ARHGDIB, CD34, ANXA1, PDGFRB, WT1, ARRB1, TJP1, ITGA8. The first 10 of 13 were included in the top 200 glomerulus-abundant genes in MAd-761. Among these, increased urinary ARHGDIB was demonstrated only in some patients of glomerulonephritis, but in none of normal humans by real-time PCR.

Conclusions: The Gl-E gene DB has been updated by combining with the glomerulus proteome DB, which can be useful for novel biomarker discovery in human glomerular diseases.

Funding: Private Foundation Support

TH-PO428

Gene Expression Profiling of Oxalate Nephrotoxicity Hari K. Koul, Sweaty Koul. *Urosciences Program-Urology/Surgery, University of Colorado School of Medicine, Aurora, CO.*

Background: Oxalate interactions with the renal cells results in a plethora of changes, including cell growth, death and altered gene expression as shown in previous studies. This study has been designed to identify specific gene ontology groups and new signaling pathways that undergo changes as a result of oxalate exposure.

Methods: We utilized microarray analysis using Affymetrix HG_U133_plus2 gene chip. Data analysis was performed suing Data Mining Tool (DMT 3.1, Affymetrix) and GeneSpring 7.2 (Silicon Genetics). Differentially expressed genes were classified according to the Gene Ontology functional category (GenMAPP v2) and functional significance of differentially expressed genes was determined using Ingenuity Pathways Analysis Software (Ingenuity® Systems, www.ingenuity.com). Cluster and Heatmap images were generated using BRB-Array tools30. Changes in gene expression were further validated by relative quantitative RTPCR.

Results: Novel signaling pathways and specific genes involved in promoting cell damage and death, nephrosis and tubular damage are highlighted by this study for the first time. We show that CDK2, CDK6 and CDC2 responsible for G1 to S phase transition are highly down-regulated by oxalate. On the other hand,Retinoic acid Receptor and CBX7 that are involved in maintaining the repressive state of many genes involved in cell growth are highly up-regulated. Cluster analysis of genes that are known members of MAPK pathways, identified up-regulation of many genes that are know activators of the p38 MAPK pathway, like Ras oncogene, MKK3 and ASK1 consistent with our previous demonstration of p38 MAP kinase activation. On the other hand Epidermal Growth factor (EGF) signaling is down regulated. We also show that many genes that are potentially involved in nephrosis and injury to the kidney were identified to be differentially expressed in HK-2 cells upon exposure to oxalate for as little as 4 hours.

Conclusions: The results of this study also provide first direct demonstration of activation of distinct pathways in a comprehensive genomic response to oxalate and identifies the potential of gene chip technology in identification of novel targeted pathways in an unbiased fashion.

Funding: NIDDK SupportNIH-RO1-54084 to HK;AEF and Chair commitment Funds, The Department of Surgery, Division of Urology, School of Medicine.

TH-PO429

Metabolomic Profiling of the Autosomal Dominant Polycystic Kidney Disease Rat Model Takafumi Toyohara,[1] Takehiro Suzuki,[1] Yasutoshi Akiyama,[1] Daisuke Yoshihara,[2] Eikan Mishima,[1] Yoichi Takeuchi,[1] Sadayoshi Ito,[1] Shizuko Nagao,[2] Tomoyoshi Soga,[3] Takaaki Abe.[1,4] *[1]Divison of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University, Sendai, Japan; [2]Education and Research Center of Animal Models for Human Diseases, Fujita Health University, Toyoake, Japan; [3]Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan; [4]Department of Clinical Biology and Hormonal Regulation, Division of Medical Science, Tohoku University, Sendai, Japan.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is an inherited systemic disease characterized by renal cyst expansion, resulting in renal failure. With the progression of renal damage, the accumulation of uremic compounds is recently reported to subsequently cause further renal damage and hypertension. The aim of this study was to analyze the profile of uremic retention solutes of ADPKD by capillary electrophoresis–mass spectrometry (CE-MS) using the Han:SPRD rat model.

Methods: Two hundred and ninety-seven cations and 190 anions were comprehensively analyzed by CE-MS in Han:SPRD rats and control rats.

Results: We found 21 cations and 19 anions that accumulated significantly in the heterozygous (Cy/+) ADPKD rat model compared with control rats. Among the compounds, increases in 5-methyl-2'-deoxycytidine, glucosamine,ectoine, allantoate, α-hydroxybenzoate, phenaceturate and 3-phenylpropionate and decreases in 2-deoxycytidine, decanoate and 10-hydroxydecanoate were newly identified in the ADPKD Cy/+ rats. We also compared these compounds with human or rat uremic solutes reported in privious studies. As a results, 3 cations and 4 anions were specific uremic solutes in ADPKD rat and could be a new marker(s) for detecting ADPKD.

Conclusions: We comprehensively identified new uremic solutes in the serum from ADPKD Cy/+ rats and revealed differences in uremic solutes among human CKD patients, the rat renal failure model and Cy/+ rats. We also found new biomarker candidates for detecting renal damage in ADPKD. The results are useful for further studies to elucidate mechanisms accumulating uremic toxins.

TH-PO430

Development of a Scanning Electron Microscopy (SEM) Based Assay for Exosome-Like Vesicle (ELV)-Cilium Interactions Jason L. Bakeberg, Marie C. Hogan, Peter C. Harris, Christopher James Ward. *Mayo Clinic, Rochester, MN.*

Background: Exosome-like vesicles (ELVs) are 50-200nm membrane bound vesicles found in urine and bile that are too small to be resolved by light microscopy. In the kidney, polycystin-1 (PC1) positive ELVs (PKD-ELVs) can interact with primary cilia (Hogan et al 2009). The early assays for this interaction were cumbersome, involving surface biotinylation of human ELVs and multiple purification steps to obtain PC1+ ELVs, and were not suited for high throughput analysis. Furthermore, to visualize the streptavidin gold conjugates on primary cilia we used 22nm carbon to coat the cells, which obscured morphology although it gave good backscatter contrast between the carbon and gold.

Methods: Here we describe a new assay based on the *Pkhd1*$^{Pk(+)/Pk(+)}$ mouse that has an epitope tagged *Pkhd1* gene, with two SV5-Pk tags inserted immediately C-terminal to the signal peptide, so that the tags are at the N-terminus of this large type-1 membrane protein. The *Pkhd1*$^{Pk(+)/Pk(+)}$ mouse is normal and secretes SV5-Pk tagged ELVs in its urine and bile. Wildtype primary mouse kidney epithelial cells were cultured and allowed to polarize and make cilia. *Pkhd1*$^{Pk(+)/Pk(+)}$ urine was then applied for 1-10 mins, the cells are fixed in 4% paraformaldehyde and ELVs detected using the SV5-Pk1 antibody and 15nm anti-mouse IgG gold post-fixed in OsO_4 and critical-point dried. To obtain optimum morphology the cells are then coated with a 1nm layer of osmium metal using plasma deposition. Using this technique we are able to detect ELVs interacting with primary cilia.

Results: Non treated cilia had no gold positive ELVs associated with them. However, cells treated with *Pkhd1*$^{Pk(+)/Pk(+)}$ urine for 1, 2, 5 and 10 minutes have: 1.5, sd+/-1.5; 1.7, sd+/-0.7; 0.55, sd+/-0.68 and 0.36, sd+/-0.67 ELVs with 1 or more gold particles on them, respectively.

Conclusions: The ELV primary cilium assay described above will be used to assess the ability of various recombinant domains derived from the extracellular part of PC1 and fibrocystin to block or stall the interaction and will help define the functional domains required for interaction.

Funding: NIDDK Support

TH-PO431

Podocin a Novel Substrate for Chaperone Mediated Autophagy Substrate Alejandro Quiroga,[1] Ana Maria Cuervo.[2] *[1]Pediatric Nephrology, Montefiore Childrens Hospital, New York, NY; [2]Department of Development and Molecular Biology, Albert Einstein College of Medicine, New York, NY.*

Background: Podocin is a component of the glomerular slit diaphragm and genetic mutations of this protein lead to steroid resistant nephrotic syndrome (SRNS). Altered levels of podocin have been reported in the podocytes of the affected patients, but the contribution of changes in the intracellular turnover of podocin to these altered levels remains unknown. In this study, we have explored the mechanisms that normally contribute to podocin turnover and analyzed changes in this degradation in an experimental model of nephrotic syndrome.

Methods: Endoplasmic reticulum, mitochondria, lysosomes (CMA positive and CMA negative) and cytosol were obtained using whole kidney sub-cellular fractionation from Wistar male rats. Podocin was tagged by green fluorescent protein and transfected into fibroblasts. Lysosome-associated membrane protein 2A (LAMP2A, CMA receptor) was knocked down by shRNA in mouse fibroblasts. Puromycin aminoglicoside (PAN) was used as a model of nephrotic syndrome in cultured human podocytes.

Results: We have found that podocin associates with and is actively degraded in lysosomes. Lysosomal degradation of podocin is maximally activated during nutritional deprivation, however, macroautophagy, one of the starvation-induced forms of lysosomal degradation, does not significantly contribute to the normal turnover of this protein. Instead, we have found that podocin bears in its amino acid sequence a conserved pentapeptide motif used for targeting of proteins for lysosomal degradation via chaperone-mediated autophagy (CMA).

Conclusions: We have confirmed that podocin is preferentially degraded in lysosomes with high CMA activity and that blockage of CMA abrogates this degradation. Lysosomal delivery of podocin via CMA is enhanced in response to PAN treatment, but we have identified a compromise in its degradation in this compartment under these conditions. Our results support that CMA contributes to the normal turnover of podocin in podocytes and that this degradation may be upregulated as a first line of defense in response to damaging agents of the slit diaphragm.

Funding: Other NIH Support - Hirsh/Weill-Caulier Career Scientist Award (to AMC)

TH-PO432

Gene Screening Identified Early Growth Response 1 as a Regulator of Tubulogenesis in Diabetic Renal Embryopathy Ching-Yuang Lin. *China Medical University and Hospital, Taichung, Taiwan.*

Background: Maternal hyperglycemia can inhibit morphogenesis of ureteric bud branching, with glial cell line-derived neurotrophilic factor (GDNF) as a key regulator of its initiation. Early growth response gene-1 (EGR-1) is one immediate early gene. Our preliminary study found EGR-1 consisting of expression with GDNF in hyperglycemic environment.

Methods: To evaluate the potential relationship of hyperglycemia-GDNF-EGR-1 pathway, *in vitro* human renal proximal tubular epithelial (HRPTE) cells as target and *in vivo* streptozotocin-induced mouse model were used.

Results: *In vivo* microarray, real time-PCR and confocal morphological observation confirmed apoptosis in hyperglycemia-induced fetal nephropathy via activation of the GDNF/MAPK/EGR-1 pathway. *In vitro* evidence indicated high glucose suppressing HRPTE cell migration, and enhanced GDNF-EGR-1 pathway induced apoptosis. Knockdown of EGR-1 by siRNA negated hyperglycemic suppressed GDNF-induced HRPTE cell migration. Also, EGR-1 siRNA alleviated GDNF/EGR-1-induced cRaf/MEK/ERK phosphorylation by 80%.

Conclusions: Our findings demonstrated EGR-1's crucial role in HRPTE cell apoptosis and fetal hyperglycemic nephropathy.

TH-PO433

Stem Cell Microvesicles Rescue Cystinosis In Vitro Diana Iglesias,[1] Reyhan El-Kares,[1] Francesco Emma,[2] Elena N. Levtchenko,[3] Nicoletta Eliopoulos,[4] Paul R. Goodyer.[1] *[1]McGill University, Montreal, Canada; [2]Ospedale Pediatrico Bambino Gesù, Rome, Italy; [3]University Hospital Leuven, Leuven, Belgium; [4]Lady Davis Institute, Montreal, Canada.*

Background: In 2009, Syres *et al* reported that infusion of bone marrow stem cells into a cystinotic mouse dramatically reduces tissue cystine and improves organ function. Since transdifferentiation of stem cells was rare, the benefit seems to involve a paracrine effect. Recent studies suggest that, by shedding microvesicles, cancer stem cells re-program the biology of their neighbors. We hypothesized that normal stem cells release microvesicles (MV) that reduce cystine accumulation in CTNS (-/-) cells.

Methods: Normal bone-marrow (bmMSC) and amniotic fluid (amMSC) mesenchymal stem cells were used as MV donors. Fibroblasts and proximal tubular epithelial cells (PTEC) isolated from cystinotic patients with homozygous CTNS deletions were used as target cells. MV were isolated from culture medium by ultracentrifugation. Cystine was measured by HPLC.

Results: When MSC were co-cultured with cystinotic fibroblasts (1:4), cystine levels decreased by 60%, an effect which could not be explained by cell dilution. We then cultured various donor cells separately and harvested MV shed into the culture medium. When MV (100-300nm) were added to CTNS (-/-) target cell monolayers, cystine content was dramatically reduced in dose-dependent manner after 24 hours. The effect was blocked by MV pre-treatment with annexin V. To address whether MV transfer wildtype CTNS protein to the mutant target cells, we transfected amMSC cells with wildtype CTNS/Red fusion protein. In co-culture, we observed direct transfer of the label to intracellular lysosomes of adjacent mutant fibroblasts. We also identified CTNS mRNA in amMSC MV and demonstrated its transfer to CTNS (-/-) targets. By introducing a CTNS siRNA, we showed that the MV rescue effect is primarily due to transfer of CTNS protein. A rescue effect was not seen when MV were prepared from mutant amMSC or PTEC cells.

Conclusions: We propose a model in which uptake of MV from stem cells containing wildtype CTNS protein can reduce pathologic accumulation of cystine in adjacent mutant cells.

Funding: Government Support - Non-U.S.

TH-PO434

Existence of Neural-Derived Cells in Nephron and Mesangial Cells Akiko Oguchi, Jin Nakamura, Nariaki Asada, Motoko Yanagita. *Graduate School of Medicine, Kyoto University, Kyoto, Japan.*

Background: Recent lineage tracing method demonstrated that almost all cells in the nephron are derived from Six2+ progenitor cell in metanephric mesenchyme during development, and contribution of other cells types is considered less likely, whereas the origin of mesangial cells and glomerular endothelial cells remains controversial. Here we demonstrated the existence of neural-derived cells in the nephron, glomerular endothelial cells and mesangial cells.

Methods: To test the contribution of neural cells in kidney development, we utilized Nestin-CreERT2 mice, in which inducible Cre is expressed under the control of Nestin promoter, and is activated only after the administration of tamoxifen at desired time points. Nestin is a well-known marker for neural stem cells. We bred Nestin-CreERT2 mice to R26ECFP indicator mice, administered tamoxifen at various time points during embryogenesis as well as in adult life, and permanently labeled Nestin+ cells with the expression of ECFP.

Results: When we administered tamoxifen during embryogenesis, ECFP+ cells were detected in tubular epithelial cells, collecting duct, glomerular endothelial cells and mesangial cells, whereas none of Six2+ progenitor cells were positive for ECFP after the administration of tamoxifen. When we administered tamoxifen 1 month after birth, ECFP+ cells were detected only in the inner medulla and papilla.

Conclusions: Taken together, we demonstrated that some cells in the nephron are not derived from Six2+ progenitor cells, but of neural origin. In addition, some mesangial cells and glomerular endothelial cells were derived from Nestin+ cells during embryogenesis. Kidney papilla has attracted attention as a potential niche for kidney stem cells, and lineage tracing of ECFP+ cells in the papilla might give us some insight in the behavior and contribution of these cells during kidney regeneration.

TH-PO435

Morphometric Analysis of Urinary Tract Development in Mice Ashley R. Carpenter,[1] Brian Becknell,[2] Carlton M. Bates,[3] David S. Hains,[2] Kirk M. McHugh.[1] *[1]Molecular & Human Genetics, Nationwide Children's Hospital, Columbus, OH; [2]Pediatric Nephrology, Nationwide Children's Hospital, Columbus, OH; [3]Pediatric Nephrology, Chidren's Hospital of Pittsburgh, Pittsburgh, PA.*

Background: Disorders of the urinary tract affect both sexes of all ages and represent a major cause of morbidity and impaired quality of life.

Methods: To understand the morphological events responsible for normal urinary tract development, we performed 3-D reconstructive analysis of the developing mouse urinary tract from embryonic day (E)13 to E17 and postnatal day (P)1. These results were compared to a similar analysis of urinary tract development in the megabladder mouse (mgb-/-) and Fgfr2Mes-/- murine model of vesicoureteral reflux (VUR).

Results: Morphometric analysis showed that detrusor smooth muscle differentiation initiates in the bladder dome and progressively moves caudal, with the leading edge of differentiation extending down the right posterior surface of the bladder. The developing bladder becomes completely invested with smooth muscle by E15, after which it thickens creating a distinct octahedral morphology. Total bladder trigone area and perimeter increased at distinct linear rates during development. From E13 through E16, the bladder trigone progressed from an isosceles triangle with a long base between the ureters to an equilateral triangle due to preferential lengthening of the distance between the ureters and urethra. Morphometric analysis confirmed that the primary defect in mgb-/- bladders was a reduction in the percentage of detrusor smooth muscle throughout development. Morphometric comparison of the developing bladder trigone in Fgfr2Mes-/- mice versus control revealed that deviation from normal bladder trigone patterning was the most consistent predictor of VUR. Direct comparison of the right refluxing versus left non-refluxing ureters indicated that intravesicular tunnel length was a poor predictor of VUR.

Conclusions: In conclusion, our findings have significant implications for bladder morphogenesis as well as the ontogeny of VUR and indicate the 3-D morphometric analysis is a powerful tool to assess both normal and pathologic development of the urinary tract.

Funding: NIDDK Support

TH-PO436

Pax2 Maintains Self-Renewing Nephron Progenitors during Mammalian Kidney Development Akio Kobayashi. *Renal Division, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.*

Background: The functional unit of the kidney, the nephron, is repetitively generated during mammalian kidney development. Previously, our fate map analysis revealed that the Six2+ cap mesenchyme represents a multipotent, self-renewing nephron progenitor population throughout kidney development (Kobayashi et al., 2008, Cell Stem Cell). We further found that the nephron and interstitium form distinct compartments with a strict lineage boundary during kidney organogenesis. Currently, it is not well known how the nephron progenitor population is maintained during kidney organogenesis.

Methods: The paired-domain transcription factor Pax2 is expressed in multiple urogenital tissues including the cap mesenchyme. However, Pax2 function in the cap mesenchyme has not been examined in vivo. Therefore, we investigated Pax2 function in the cap mesenchyme using mouse genetic approaches, including tissue-specific inactivation and mosaic analysis.

Results: In this study, we found that the Pax2 mutant mice fail to maintain the cap mesenchyme in the developing kidney. Surprisingly, fate map analysis in the mutants showed that cap mesenchyme-derived cells lacking Pax2 activity are not lost, but persist throughout kidney development. Detailed molecular marker analysis indicated that these cap mesenchyme-derived cells can trans-differentiate into medullary interstitial cells. Our mosaic analysis reveled a cell-autonomous requirement of Pax2 activity for cap mesenchyme cells.

Conclusions: Taken together, our observations suggest that Pax2 maintains a self-renewing nephron progenitor population by repressing interstitial cell fates. Thus, Pax2 activity establishes a lineage boundary between the nephron and non-nephron compartments during kidney organogenesis.

Funding: Private Foundation Support

TH-PO437

A Novel Iron Transporter Controls Organogenesis Andong Qiu, Jonathan M. Barasch. *Medicine, Columbia.*

Background: It is thought that iron crosses the plasma and endosomal membranes by a Fe^{2+}: H^+ transporter known as the Divalent Metal Transporter 1 (DMT1), but transport is induced by a substantial H^+ gradient that is not typical of the transferrin endosome. In addition, the DMT1 knockout did not inhibit organogenesis. Hence, mechanisms that transfer transferrin-bound iron from circulation to developing organs are currently unknown.

Methods: In order to discover transmembrane transporters of iron we preformed an extensive informatics search of proteins distantly related to yeast and mammalian iron transporters. We performed expression cloning in oocytes and 50 candidate genes were evaluated for their ability of iron transport.

Results: We identified a novel transporter that was specific for Fe^{2+} and active at neutral and mildly acidic pH (7.0-6.5). It had a low micromolar Km for iron conductance.Tagged constructs expressed in cell lines demonstrated that the protein localized in endosomes that contained serum transferrin. There was no overlap in localization with DMT1 which appeared to inhabit a separate endosomal-lysosomal compartment. Application of the transferrin to cells overexpressing the novel gene resulted in increased iron content of the cytosol, and knockdown of the gene had the opposite effect. The knockout of the gene *in vivo* did not inhibit the formation of the embryonic body plan; however, organogenesis throughout the embryo was inhibited including eye, blood, liver, and kidney at E14.5. There was an increase in endosomal oxidized damage induced by accumulated iron in this intracellular compartment of KO cells.

Conclusions: The mechanisms of iron delivery in the embryo are unkown. We have identified a novel transporter sufficient and necessary for iron traffic from the serum iron donor transferrin to developing organs. The is one of the few iron transporters ever identified.

Funding: NIDDK Support, Private Foundation Support

TH-PO438

The Proto-Oncogene, Mdm2, Is Indispensable for Maintenance of the Progenitor Cell Population in the Cap Mesenchyme Sylvia Hilliard, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Background: The cap mesenchyme (CM) can be demarcated by its elevated expression of the transcription factor, Six2, and represents a self-renewing population of cells that provides the kidney with its total endowment of nephron precursors. The dorsally located CM cells with high levels of Six2 respond to canonical Wnt signals to maintain their proliferative status while those ventral to the branching ureteric bud (UB) have lowers levels of Six2 and respond to Wnt signals by committing to a nephron fate. *Mdm2*, a negative regulator of p53 stability and activity, is expressed abundantly in the metanephric mesenchyme. Accordingly, we examined the requirement for Mdm2 in the Six2 population by intercrossing homozygous *Mdm2* floxed mice to *Six2-GFP-Cre* mice.

Results: $CM^{Mdm2-/-}$ mice die at birth and show severely dysplastic kidneys with few isolated glomeruli and depletion of the nephrogenic zone. The proximal tubules that do form are superficially located at the kidney periphery. $CM^{Mdm2+/-}$ kidneys are both intermediate in size and in the degree of renal defects sustained when compared to those of $CM^{Mdm2+/+}$ and $CM^{Mdm2-/-}$ mice. Six2, Pax2 and GFP immunostaining revealed marked thinning of the CM in the kidneys of $CM^{Mdm2-/-}$ mice. Consequently, the Meis1- expressing stroma expands to compensate for the loss of CM cells in $CM^{Mdm2-/-}$ kidneys. TUNEL staining revealed that the apoptotic foci were far more numerous in the CM of the null mutant kidneys. Also the fraction of proliferating cells (pH3-positive) in the Six2 staining CM is much reduced in the $CM^{Mdm2-/-}$ kidneys. The mosaic nature of Six2-GFP-Cre transgene allows the survival of enough wild type CM cells to prevent ectopic/dorsal specification of renal vesicles in these mice. The Lhx1 expressing nephron precursors are identifiable in $CM^{Mdm2-/-}$ kidneys at E14.5 but are largely lost by E16.5. Ex-vivo cultures of $CM^{Mdm2-/-}$ kidneys reveals secondary defects in UB branching characterized by thick trunks with poor bifurcation of the UB tips.

Conclusions: We conclude that Mdm2-p53 signaling is required to strike a balance between progenitor cell expansion and differentiation during normal metanephric development.

Funding: NIDDK Support

TH-PO439

Renal Cystic Hypodysplasia in Mice Lacking HDAC1/HDAC2 Genes in the Ureteric Epithelium Shaowei Chen, Stacy Lyn Rosenberg, Xiao Yao, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Background: HDAC1 and HDAC2, two highly homologous histone deacetylases, have redundant as well as distinct functions in global and tissue-specific gene expression. HDAC1 and HDAC2 are expressed in the nephric epithelial and mesenchyme cell lineages in an overlapping manner, suggesting a potential role in regulation of kidney development. Here, we examined the functional requirement of HDAC1 and HDAC2 in the ureteric bud (UB) lineage.

Methods: To circumvent the early embryonic lethality associated with global gene deletion of *HDAC1* or *HDAC2*, mice bearing conditional null alleles were crossed to Hoxb7-creEGFP transgenic mice to delete *HDAC1* and *HDAC2* genes, singly or in combination, specifically in the UB epithelium

Results: UB-specific deletion of either HDAC1 or HDAC2 led to no overt phenotype, whereas concurrent deletion of both HDAC1 and HDAC2 resulted in early postnatal lethality. At birth, HDAC1 and HDAC2 double knockout (DKO) mice have bilateral renal cystic hypodysplasia, including absent nephrogenic zone, lack of cortico-medullary patterning, decreased nephron number, and multiple cysts in both cortical and medullary zones. Immunostaining of Six2, Pax2, two markers of renal progenitor cells, and phosphorylated histone H3, an indicator of cell proliferation, demonstrated that the DKO mice completely lost renal progenitor cells. The renal cysts originate from glomeruli (WT1), proximal tubules (LTA and angiotensinogen), and collecting ducts (cytokeratin and AQP2). Ex vivo real-time monitoring of GFP fluorescence revealed that DKO mice

exhibited aberrant UB branching pattern as early as E12.5, followed by degeneration of UB tissue over 2-3 days in culture. At E12.5, there was no difference in Six2 and Pax2 expression between DKO and wild type kidneys. By E13.5-14.5, hypoplasia due to defective UB branching was clearly evident and accompanied by reduced number of glomeruli and dysmorphic proximal tubules.

Conclusions: We conclude that HDAC1 and HDAC2 perform redundant yet essential functions in the UB lineage. Double deletion of HDAC1 and HDAC2 disrupts UB branching morphogenesis and differentiation causing a cystic hypodysplastic renal phenotype.

Funding: NIDDK Support

TH-PO440

Therapeutic Effects of Mesenchymal Stem Cells in Wistar-Kyoto Rats with Anti-Glomerular Basement Membrane Glomerulonephritis Kei Matsumoto, Masayuki Iyoda, Takanori Shibata, Yuki Hirai, Yoshihiro Kuno, Tadao Akizawa. *Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan.*

Background: Multipotent mesenchymal stem cells (MSC) have become a popular and promising therapeutic approach in many clinical conditions. In this study, we tested the hypothesis that MSC can provide a potential therapy for human anti-glomerular basement membrane (GBM) glomerulonephritis (GN).

Methods: Nephrotoxic serum nephritis was induced in Wistar-Kyoto (WKY) rats on day 0. Groups of animals were given either human MSC (3 x 106) or vehicle by intravenous injection on day 4; all rats were sacrificed at either day 7 or day 13. Vehicle-treated groups received an equal volume of Hank's balanced salt solution (HBSS). For each group, 10 to 15 rats were analyzed. Proteinuria (U-P), serum creatinine (Cr), and body weight (BW) were measured periodically. Renal morphological investigations were performed at sacrifice.

Results: BW was comparable between the two treatment groups throughout the study. Serum Cr level was significantly lower in the MSC-treated rats than in the HBSS-treated rats on day 13 (WKY-HBSS vs. WKY-MSC, Day 7: NS, Day 13: $p < 0.05$). When compared to vehicle treatment, MSC-treated rats had reduced U-P on day 7 and 13 (Day 7: $p < 0.05$; Day 13: $p < 0.01$). MSC treatment also decreased kidney weight (Day 7: $p < 0.05$; Day 13: $p < 0.01$) and glomerular tuft area (Day 7: $p < 0.05$; Day 13: $p < 0.05$). The percentage of crescentic glomeruli was identical between the two treatment groups. ED1-positive macrophages ($p < 0.05$), CD8-positive cells ($p < 0.05$), and apoptotic cells ($p < 0.001$), assessed by TUNEL staining, in glomeruli were significantly reduced by MSC treatment on day 7. Renal cortical mRNA for TNF-α ($p < 0.0001$), IL-1β ($p < 0.001$), and IL-17 ($p < 0.01$) was decreased, whereas IL-4 ($p < 0.05$) and Foxp3 ($p < 0.01$) was increased in the MSC-treated group on day 7. Collagen type I ($p < 0.01$), type III ($p < 0.01$), and TGF-β ($p < 0.05$) mRNA were significantly decreased by MSC treatment on day 13.

Conclusions: MSC treatment attenuates the progression of renal injury in experimental anti-GBM GN via anti-inflammatory and immunomodulatory effects.

TH-PO441

Critical Role of the Stroma in Mediating Vascular Development of the Kidney Sunder Sims-Lucas, Jose Paredes, George K. Gittes, Carlton M. Bates. *Children's Hospital of Pittsburgh, Pittsburgh, PA.*

Background: Kidney structural abnormalities are the leading cause of pediatric chronic kidney disease, producing significant mortality. Understanding the interactions of different renal lineages is critical for impacting structural renal disease. While many kidney development studies focus on ureteric and nephrogenic lineages, very few examine vascular network formation and patterns of fetal blood flow. The purpose of this study is to elucidate development of the vasculature and blood flow in the kidney and to understand how perturbations in ureteric and stromal lineages affects vascular development.

Methods: Wildtype or *Hoxb7creFgfr2*$^{lox/lox}$ (*Fgfr2*$^{UB-/-}$) mice from Embryonic day (E) 13.5-17.5 were subjected to in utero cardiac microinjection of tomato lectin (which adheres to the endothelial cells) followed by immunostaining and confocal imaging to visualize blood flow through the renal vasculature.

Results: By E13.5, normal kidneys had integrated vascular beds and a moderate number of vessels containing blood flow. Several glomeruli and collecting ducts stained for tomato lectin implying glomerular filtration (challenging current thoughts that GFR begins at E15.5). In older embryos, the amount of perfused vessels and lectin-stained glomeruli increased. Co-labeling of the renal stroma (Foxd1) with vascular markers (PECAM) revealed not only that the vasculature was deeply embedded in the renal stroma but also that stromal-derived cells may be giving rise to some of the renal vascular endothelium. Utilizing a mouse model with defective ureteric branching and cortical stromal thickening (*Fgfr2*$^{UB-/-}$) we visualized regions of significantly thickened blood vessels with aberrant networks.

Conclusions: This study reveals that the robust vascular development, blood flow, and possibly GFR occurs earlier than previously thought. Furthermore, alterations in ureteric and/or stromal lineages had a profound impact on vascular development and blood flow in kidney development. The effects of structural kidney disease on the developing renal vasculature may have a major role in how fetal reprogramming results in postnatal diseases such as hypertension and progressive kidney disease.

TH-PO442

Critical Role of Fgfr2 Adaptor Binding Protein Frs2α in Regulating Ureteric Morphogenesis, Cyst Formation and Cilia Length Valeria E. Di Giovanni,[1] Sunder Sims-Lucas,[1] Caroline Miller,[2] Vincent H. Gattone,[2] V.P. Eswarakumar,[3] Carlton M. Bates.[1] *[1]Div of Nephro, Dept of Peds, University of Pittsburgh, PA; [2]Cell of Anat. and Cell Bio, Indiana University, IN; [3]Dept of Ortho, Yale, CT.*

Background: Renal development is governed by reciprocal signaling between the metanephric mesenchyme (MM) and the epithelial ureteric bud (UB). Abnormal signaling can result in structural urogenital abnormalities, and we have previously demonstrated that conditional deletion of FGF receptors (Fgfrs) 1 and 2 in the MM (*Fgfr1/2*$^{Mes-/-}$)led to severe dysgenesis and blocked UB elongation and branching. Our purpose was to determine whether Fgfr2 signaling mediated by the adaptor Frs2α, was critical for early MM and UB morphogenesis.

Methods: We generated compound mutant mice with *Pax3*cre mediated deletion of *Fgfr1* in renal mesenchyme and point mutations in the Frs2α binding site on Fgfr2 (*Fgfr1*$^{Mes-/-}$*Fgfr2*$^{LR/LR}$). We performed histological staining, 3D reconstructive imaging, real time PCR, immunohistochemistry, and in situ hybridizations against multiple targets in both embryonic and post-natal mice.

Results: Unlike *Fgfr1/2*$^{Mes-/-}$ mice, *Fgfr1*$^{Mes-/-}$*Fgfr2*$^{LR/LR}$ did not develop severe renal dysgenesis. However, E13.5 mutants exhibited non-cell autonomous UB defects, including hyperproliferative, dilated tips and reduced branching, without obvious histological abnormalities in the MM. The dilated mutant UB tips contained extremely long and/or bifurcated renal cilia. By P21, most of the parenchyma of *Fgfr1*$^{Mes-/-}$*Fgfr2*$^{LR/LR}$ kidneys was replaced by cysts. Embryonic analysis showed increased β-catenin expression in UB structures, which increased with age. Mutant UB structures also demonstrated elevated and ectopic expression of c-Ret and its targets Etv4/5 and increased phospho-ERK expression in UB tips and trunks. Mutants had decreased expression of Bmp4 in the MM, which may be driving the UB alterations including changes in Ret expression and signaling.

Conclusions: In conclusion, while Fgfr2 signaling through Frs2α is not required for early renal morphogenesis, it is part of a novel signaling axis which controls UB morphogenesis and cystic disease by regulating cilia morphogenesis and ureteric β-catenin and Ret expression.

Funding: NIDDK Support

TH-PO443

Dynamic Cell Movements in the Morphogenesis of Renal Tubules in Xenopus laevis Soeren S. Lienkamp,[1] John B. Wallingford,[2,3] Gerd Walz.[1,4] *[1]Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg, Baden Württemberg, Germany; [2]Molecular Cell and Developmental Biology & Institute for Cellular and Molecular Biology, University of Texas, Austin, TX; [3]Howard Hughes Medical Institute, University of Texas, Austin, TX; [4]Center for Biological Signaling Studies (BIOSS), University of Freiburg, Baden-Württemberg, Germany.*

Background: Epithelial tubules are universal building blocks of many organs including the vertebrate kidney. The morphogenetic programs that shape epithelial tubules and renal tubules in particular remain poorly defined. It has been suggested that active cell movement must occur. However, the active movement of renal tubular epithelial cells has not been observed in vivo.

The Xenopus pronephros is a fully functional excretory organ and consists of a single tubule, which is functionally remarkably similar to the mammalian metanephric tubule. Positioned right beneath the epidermis, it is perfectly well suited to study renal development in a living organism.

Methods: We used confocal time-lapse imaging of Xenopus embryos expressing fluorescent proteins within the embryonic kidney. Automated position and focus adjustment algorithms were used to correct for shifts in embryo position or growth. A high degree of spatial and temporal resolution was achieved to resolve movements of cells within a tissue, single cell behaviour, and subcellular protein dynamics in vivo.

Results: We find that tubulogenesis is a highly dynamic process that integrates proliferation, cell shape changes, and complex cellular rearrangements. Classic morphogenetic movements such as convergent-extension and rosette formation occur in the developing tubule. This process depends on myosin II and is disrupted by molecules that interfere with planar cell polarity signalling.

Conclusions: Formation of renal tubules requires active cell movement and dynamic rearrangement within the epithelium. Evolutionary conserved mechanisms of morphogenesis are driving forces. Disruption of morphogenetic cell movements leads to severe defects in tubule formation. This has potential implications for the understanding of hereditary renal malformations.

Funding: Government Support - Non-U.S.

TH-PO444

Modeling Polycystic Kidney Disease Using Human Induced Pluripotent Stem Cells Benjamin S. Freedman, Albert Q. Lam, Joseph V. Bonventre. *Medicine/Renal, Brigham and Women's Hospital, Boston, MA.*

Background: Induced pluripotent stem (iPS) cells are a powerful new way to model human disease in different cell types and during development.

Methods: We generated iPS cell lines from patients with autosomal dominant and autosomal recessive polycystic kidney disease (PKD-iPS cells) using retroviral transduction of four stem cell transcription factors. These are to our knowledge the first iPS cells from patients with kidney disease.

Results: PKD-iPS cells express pluripotency markers characteristic of embryonic stem cells and differentiate into all three germ layers including somatic cell lineages which are affected by PKD. Undifferentiated iPS cells possess primary cilia and express polycystin-1 and polycystin-2. Compared to control iPS lines from healthy patients, PKD-iPS lines exhibited normal cilia number and morphology but a dramatic reduction in polycystin-2 localization to the cilium. When iPS cells were embedded in three-dimensional extracellular matrix, they formed cysts of OCT4+, ZO-1+ epithelial cells surrounding empty central lumens lined with apical cilia. Differentiated iPS cells showed diverse patterns of polycystin-2 localization to the endoplasmic reticulum, plasma membrane, and primary cilium. In suspension culture, differentiated PKD-iPS cells formed spheroid embryoid bodies featuring sporadic, fluid-filled cysts which increased in the presence of 8-bromo-cAMP. When implanted underneath the kidney capsule of immunodeficient mice, PKD-iPS cells gave rise to large cystic teratomas.

Conclusions: These results establish new experimental models for human PKD and indicate that epithelial phenotypes of iPS cells can be explored to provide insight into underlying PKD pathophysiology.

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TH-PO445

Low Serum Cultured Adipose-Derived Mesenchymal Stem Cells, but Not Bone-Marrow Derived Mesenchymal Stem Cells, Ameliorate Rat Crescentic Glomerulonephritis by Functional Polarization of Macrophages into Immunoregulatory Phenotype

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Background: We have reported that adipose tissue-derived stem cells (ASC) promoted regeneration in a rat model of acute kidney injury. More recently, we have shown that ASC more strongly modulate T-cell immune reaction than bone marrow derived mesenchymal stem cells (BM-MSC). In the present study, we examined the renoprotective effects of ASCs focusing on their immunomodulatory properties for macrophages.

Methods: Crescentic glomerulonephritis was induced in WKY rats by intraperitoneal injection of anti-rat GBM IgG. Renal function and histology were assessed in animals treated with ASC or BM-MSC which were intravenously given every day from day 0 through day 5. For evaluation of ASC-driven functional polarization in macrophage, we cultured peritoneal macrophages with ASCs. Specific marker for macrophage phenotype (proinflammatory;M1 and immunoregulatory;M2) and IL-10 in culture supernatant were assessed by FACS and ELISA, respectively.

Results: Intravenous injection of ASC significantly prevented rats from renal dysfunction and proteinuria caused by anti-GBM IgG injection. The number of glomeruli with crescents was significantly decreased in ASC group compared to control group. Interestingly, glomerular infiltrates of M2 macrophages was increased only in ASC group despite comparable number of M1 macrophages to control group. Renal IL-10 concentration in diseased rats was higher in ASC group than in control group. In vitro co-culture system clearly demonstrated that ASC, but not BM-MSC, directly turned macrophage into M2 phenotype. Moreover, these effects of ASC were stronger in low serum culutured ASC than high serum cultured ASC.

Conclusions: ASC exerted profound immunoregulatory properties especially on macrophages and ameliorated glomerular injury in a rat model of anti-GBM glomerulonephritis. Our findings suggest that ASC transfer may provide a novel therapeutic strategy for patients with crescentic glomerulonephritis.

TH-PO446

Amniotic Fluid-Derived Stem Cells Ameliorate Renal Disease in the Remnant Kidney Model

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Background: Human amniotic fluid stem cells (hAFSC) are a class of fetal, pluripotent stem cells which exhibit high proliferative capacity, express mesenchymal and embryonic markers but do not undergo neoplastic transformation or induce teratoma transformation. The aim of the present study was to analyze the effects of intravenous injection of hAFSC in an experimental model of chronic renal disease, the 5/6 nephrectomy model (Nx).

Methods: hAFSC derived from second-trimester amniocentesis were isolated by plastic adhesion. After 4-7 passages, hAFSC characteristics were confirmed by flow cytometry and by their ability to differentiate into osteogenic, adipogenic and chondrogenic cells. Male Wistar rats (n=28) were divided into 4 groups: **S**, sham-operated, **S+hAFSCs**, sham rats receiving hAFSC; **Nx**, 5/6 nephrectomy; and **Nx+hAFSCs**, Nx rats receiving hAFSC. $5x10^5$ hAFSC were injected into the tail vein, and rats were followed for 30 days. The following parameters were analyzed: blood pressure, proteinuria, and kidney expression of α-smooth muscle actin (myofibroblasts) and ED1+cells (macrophages), analyzed by immunohistochemistry.

Results:

	BP (mmHg)	$\mathbf{U_{prot}}$ (mg/24h)	$\mathbf{S_{creat}}$ (mg/dL)	**Myofibroblast activation** (%)	**Macrophage infiltration** (cell/mm^2)
Sham (n=5)	123±3	10.9±1	0.5±0.1	0.33±0.08	17±3
Sham+hAFSC (n=5)	105±16	17.7±2	0.5±0.1	0.11±0.01	1±0.2
Nx (n=9)	183±4[a,b]	92.9±14[a,b]	1.1±0.1[a,b]	6.27±0.74[a,b]	50±8[a,b]
Nx+hAFSC (n=5)	132±9[a,b,c]	30.5±9[c]	0.7±0.1	0.44±0.18[c]	3±1[c]

Data are presented as mean±SEM: [a] $p<0.05$ *vs* Sham; [b] $p<0.01$ *vs* Sham+hAFSC; [c] $p<0.01$ *vs* Nx.

Conclusions: These preliminary results suggest that intravenous inoculation of hAFSC ameliorate renal disease in the remnant kidney model and that they may represent an alternative source for stem cell therapy.

Funding: Government Support - Non-U.S.

TH-PO447

Induction and Monitoring of Intermediate Mesoderm from Human iPSCs and ESCs

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Background: The differentiation method from pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), into kidney lineage remains to be developed. Kidney is derived from one of early embryonic germ layers, intermediate mesoderm (IM), and directing pluripotent stem cells into IM lineage is a crucial step for kidney regeneration.

Methods: We constructed a targeting vector and transduced it into human iPSCs (hiPSCs) to obtain reporter hiPSC lines for an IM marker gene OSR1 by homologous recombination. To differentiate IM cells from hiPSCs, we examined the combinational treatment of growth factors.

Results: We have efficiently generated hiPSC lines that contain an allele of OSR1 gene into which a green fluorescence protein (GFP) gene was knocked-in by homologous recombination. We have also established an induction protocol using combinational treatment of growth factors, in which differentiation of hiPSCs produced cultures consisting of more than 40 % OSR1+ cells. Furthermore, the cells expressed other IM marker genes, and could differentiate into multiple cell types included in IM derivative organs, such as kidney, gonad and adrenal cortex in vitro.

Conclusions: Our differentiation protocol can induce human pluripotent stem cells into IM cells with similar developmental potential to that in embryos, supplying systems for understanding the developmental mechanisms of IM lineage and cell sources for the regeneration of IM derivative organs.

TH-PO448

Selective Production of Hyaluronan by Epithelial Cells Is Necessary but Not Sufficient To Induce Tubulogenesis

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Background: Branching morphogenesis is a fundamental process in the development of kidney and mammary gland. Extracellular matrix plays an important role in tubulogenesis. We hypothesized that epithelial cells can modify their pericellular matrix to drive tubulogenesis in response to stimuli.

Methods: The role of hyaluronic acid (HA), a key component of the pericellular matrix, in epithelial tubulogenesis, was studied in three different in vitro models of epithelial tubulogenesis in 3D collagen gels: 1) renal MDCK cells in response to hepatocyte growth factor (HGF); 2) renal mCCD-N21 cells (spontaneous) and 3) mammary gland-derived J3B1A cells in response to transforming growth factor β1 (TGF-β1).

Results: Induction of tubulogenesis by either HGF or TGF-β1 strongly induced hyaluronan synthase 2 (HAS2) expression, a key enzyme involved in HA biosynthesis. Immunostaining revealed that HA is preferentially produced at the tips of growing tubes. Reduced HA production, either by pharmacological inhibition (4-MU) or by shRNA-mediated knockdown of HAS2, completely abrogated tube formation in all three cell lines. To assess whether HA production was sufficient by itself (i.e in absence of tubulogenic treatment) to drive the tubulogenic process, we analyzed the effect of inducible HAS2 overexpression in MDCK Tet-off cells. Overexpression of HAS2 in response to doxicyclin removal did not promote tubulogenesis but led to the formation of giant cysts. Similarly, after HGF treatment, HAS2-overexpressing cells did not display enhanced tubulogenesis, but formed enlarged disorganized structures. CD44, the major HA receptor, was expressed at the tips of the tubes formed by MDCK cells. Moreover, addition of CD44 blocking antibody to MDCK and mCCD-N21 cells grown in collagen gels blocked tubule elongation, suggesting that HA-induced tubulogenesis is mediated by CD44.

Conclusions: These results indicate that selective production of HA by epithelial cells is necessary but not sufficient to drive the tubulogenic process, and that non-selective overproduction of HA leads to disorganized cellular outgrowth in response to HGF.

Funding: Government Support - Non-U.S.

TH-PO449

Ectopic Ureteral Budding from the Wolffian Duct Results in Hypoplastic Kidneys but Not in Dysplastic Kidney Masaru Motojima,[1] Fumiyo Komaki,[1] Yoichi Miyazaki,[2] Fumio Niimura,[1] Taiji Matsusaka,[1] Iekuni Ichikawa.[1,3] *[1]Tokai University School of Medicine, Kanagawa, Japan; [2]Jikei University School of Medicine, Tokyo, Japan; [3]Vanderbilt University School of Medicine, Nashville, TN.*

Background: Mice carrying a null mutation of the *Foxc1* gene frequently develop a double collecting system. Since the kidney and urinary tract derived from normal budding can serve as a control within the same tissue specimen, these mice provide an ideal opportunity to ascertain the specific role of ectopic budding in the development of the kidney and ureter anomalies.

Methods: Tissue specimens were collected from *Foxc1*$^{ch/ch}$ mutants at various embryonic stages. The upper and lower pole kidneys were qualitatively and quantitatively examined histologically and by *in situ* hybridization (ISH) and immunohistochemistry.

Results: The upper pole kidneys of newborn *Foxc1*$^{ch/ch}$ mice were significantly more hypoplastic (kidney volume, 81±6% vs. lower pole, p<0.01), and contained significantly fewer glomeruli (68±6%, vs. lower pole, p<0.01). In those mice, the ureter, calyx and tubules in the medulla of the upper pole were abnormally dilated compared with the lower moeities. *In utero,* at E14.5, the stage just before formation of the first urine, the upper kidney was already smaller (43±6% vs. lower kidney p<0.01). At E12.5, the number of condensed mesenchymes assessed by *Pax2* ISH was significantly lower in the lower poles (59±8%, p<0.01). Neither morphological examination by Hematoxylin and Eosin staining nor immunostaining for nephrin, megalin, aquaporin-1 and Tamm-Horsfall protein revealed any dysplastic regions in either pole of newborn *Foxc1*$^{ch/ch}$ mouse kidneys. Of note, at birth, expression of Foxc1 was restricted to maturing podocytes.

Conclusions: Ectopic budding leads to kidney hypoplasia. However, ectopic budding alone does not result in kidney dysplasia unless gene(s) involved in the development of any intermediate structures of the nephron is affected, or ectopic budding occurs at a site substantially distant from normal budding so that adjacent mesenchymal cells are not fully committed to develop into renal parenchyma.

Funding: Government Support - Non-U.S.

TH-PO450

Can Stem Cells Prevent Progression of Kidney Disease? Aleksandra Rak-Raszewska,[1] Bettina Wilm,[1] Simon Kenny,[2] David Edgar,[1] Patricia Murray.[1] *[1]Cellular and Molecular biology, University of Liverpool, United Kingdom; [2]Department of Pediatric Surgery and Urology, Alder Hey Children's NHS Trust, Liverpool, United Kingdom.*

Background: Kidney disease can either be acute or chronic, the latter progressively worsening over time to become end stage renal disease (ESRD) - a stage when kidneys are non-functional. At present, the only treatment options for ESRD are transplantation or dialysis, which both have severe drawbacks. The incidence of ESRD is rising annually, therefore, in order to regenerate kidney tissue or prevent worsening of the kidney condition, a new therapy should be developed. One approach is to use embryonic stem cells (ESC). Therefore, we have first investigated the ability of ESC and their mesodermal derivatives to integrate into mouse embryonic kidney rudiments in an *ex vivo* assay. This study showed that differentiation of ESC into mesoderm promotes integration into ureteric bud, developing glomeruli and functioning proximal tubules. Building on this *ex vivo* study, we have set up an adriamycin nephropathy model to induce acute kidney injury, that with time can progress to chronic disease. This allows us to investigate the ability of ESC and their mesodermal derivatives to prevent the development of acute kidney disease in the short term or prevent progression to chronic disease in the longer term.

Methods: We have differentiated ESC *in vitro* into mesodermal cells. Following differentiation these cells were mixed with freshly dissected E13.5 mouse embryonic kidney rudiments and their nephrogenic potential was investigated in the *ex vivo* assay. For the adriamycin nephropathy *in vivo* model the ESC and their mesodermal derivatives were injected into injured mice. The analysis involved immunohistochemistry, biochemical and molecular analysis.

Results: The *ex vivo* data showed that ESC differentiated into mesoderm integrated into kidney structures more efficiently than undifferentiated ESC. They integrated into glomerular-like and tubular-like structures, and moreover they functioned appropriately to their location, e.g they differentiated into proximal tubular cells with functioning organic anion transporters. The *in vivo* experiments are still ongoing.

TH-PO451

Controlled Tubulogenesis of the Ureteric Bud from Dispersed Ureteric Epithelial Cells Using a Micropatterend Gel Peter V. Hauser,[1,2] Masaki Nishikawa,[1,2] Hiroshi Kimura,[3] Norimoto Yanagawa.[1,2] *[1]Renal Regeneration Laboratory, VAGLAHS at Sepulveda, North Hills, CA; [2]David Geffen School of Medicine, UCLA, Los Angeles, CA; [3]Institute of Industrial Science, University of Tokyo, Japan.*

Background: The initial step in renal development is the interaction of the ureteric bud (UB), an outgrowth of the Wolffian duct, with the metanephric mesenchyme. Our project aims to generate a tubular structure from dispersed renal progenitor cells *in vitro*, using a micropatterned agarose gel to control the growth and define the geometry of the structure.

Methods: A micropatterned agarose gel (3%) was casted from a silicone mask, in which the postive shape of the pattern was etched by photolithography. The pattern in the agarose gel, produced from the mask, contained rectangular cavities (3mm x150µm x150µm), into which dispersed mouse ureteric bud cells (CMUB-1, Probetex) suspended in collagen I (2.5%) were seeded (5.10^{5}cells/mold) by centrifugation (1200rpm,10min). The gel holding the cells was subsequently cultured in DMEM (10%FCS+P/S) at 37°C, 5% CO_2.

Results: After 24h *in vitro* culture, the embedded dispersed ureteric bud cells formed single layered tubular structures that contained a lumen. The tubular structures conformed to the shape and size of the cavities in the gel. Tubular formation was followed by detachment from the mold. The terminal ends of the tubular structure were multilayerd and closed. Laminin staining revealed a closed surface of the tubular structures. Aldosterone (100nM) positively effected tubular formation, while the addition of the factors HGF and EGF, or the matrixprotein fibronectin had no effect.

Conclusions: We conclude that micropatterned gels can be used to control the growth of geometrically defined substructures of the developing kidney from dispersed cells. These structures can be used as building elements for tissue engineering purposes in order to study renal development *in vitro*.

Ongoing studies are underway to examine the expression of ureteric bud markers (c-Ret, Wnt11, Wnt9b, Sox9) along the tubes and the effects of growth factors, such as GDNF on directional growth and branching of the generated tubules.

TH-PO452

Isolation of a Human Nephron Progenitor Population Sally Metsuyanim,[1,3,4] Orit Harari-Steinberg,[1,3] Dorit Omer,[1,3,5] Benjamin Dekel.[1,2,3,5] *[1]Pediatric Stem Cell Research Institute, Sheba Medical Center, Tel Hashomer, Israel; [2]Division of Pediatric Nephrology, Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Israel; [3]Sheba Center for Regenerative Medicine, Sheba Medical Center, Tel Hashomer, Israel; [4]Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel; [5]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.*

Background: A critical step in developing cell based therapies for renal disease is defining a suitable stem/progenitor cell population. Taking into account the bone-marrow derived and blood stem cells lack intrinsic capacities to generate nephron cell types identification of tissue-specific renal stem cells with wide nephrogenic potential has been a long sought after goal. In the mammalian kidney, fresh stem cells are induced into the nephrogenic pathway to form nephrons during development and no equivalent cell types can be traced in the adult kidney. Nevertheless, prospective isolation of such human nephron stem/progenitor cells has yet been accomplished.

Methods: Here we describe the generation of single cell suspensions from mid-gestation human fetal kidneys.

Results: Clonogenic analysis, expansion in serum-free defined conditions and cell sorting with the neural cadherin adhesion molecule 1 (NCAM1) identified a population that is highly clonogenic and specifically enriches for human transcripts of nephron stem/progenitor cells. After transplantation onto the chorio-allantoic membrane of the chick embryo these cells but not differentiated counterparts efficiently formed nephron's proximal, loop of henle and distal tubules. Finally, a single injection of nephron progenitor cells prevented death and renal failure in the SCID – glycerol induced acute kidney injury model.

Conclusions: These cells represent a previously unidentified intrinsic nephron precursor population and are a promising candidate for cell-based therapeutic strategies.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO453

Fibroblast Growth Factor Receptor Signaling Is Critical for Nephron Differentiation Sunder Sims-Lucas,[1] Caitlin M. Schaefer,[1] Seppo J. Vainio,[2] Carlton M. Bates.[1] *[1]Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland.*

Background: Reduced nephron number is one of the leading causes of renal failure in children and is linked with many adult onset diseases. Nephron development requires reciprocal signaling between the ureteric bud and the metanephric mesenchyme (MM). Previously in mice targeted deletion of fibroblast growth factor 8 (Fgf8) in the MM produced renal vesicles (RV) that failed to become mature nephrons. Our laboratory has shown that Pax3cre mediated inactivation of fibroblast growth factor receptor (Fgfr) 1 and 2 in the MM (both cortical stromal and nephrogenic lineages) leads to severe renal dysgenesis. Furthermore, Pax3cre-driven deletion of Fgfr1 and abrogation of the fibroblast growth factor receptor substrate 2α (Frs2α) binding site of Fgfr2 (*Fgfr1*$^{Mes-/-}$*Fgfr2*$^{LR/LR}$) led to cystic kidney disease. The purpose of this study was to determine the role of Fgfr signaling in the nephron lineage using a RV specific cre line.

Methods: Similar to the Pax3cre studies, we used Wnt4cre mice to delete Fgfr1 and 2 in the nephrogenic (RV) lineage (*Fgfr1/2*$^{RV-/-}$) and to delete Fgfr1 in the RV along with abrogation of the Frs2α binding site of Fgfr2 (*Fgfr1*$^{RV-/-}$*Fgfr2*$^{LR/LR}$). Kidneys were harvested from E13.5 to E18.5 and histology and marker analysis performed.

Results: *Fgfr1*$^{RV-/-}$*Fgfr2*$^{LR/LR}$ mice had phenotypically normal kidneys at all ages examined. However, E13.5 *Fgfr1/2*$^{RV-/-}$ mice had defective nephron formation with areas around many ureteric tips devoid of nephron precursors, leading to fewer total nephrons. 3 dimensional reconstructions at E13.5 showed a shift in the nephron distribution toward less mature structures than controls. By E18.5 *Fgfr1/2*$^{RV-/-}$ kidneys were much smaller than controls although they did possess some mature nephrons.

Conclusions: This study reveals that Fgfr signaling through Frs2α in RV is not required for nephron differentiation (and does not mediate the *Fgfr1*$^{Mes-/-}$*Fgfr2*$^{LR/LR}$ renal cystic phenotype). However, Fgfr signaling independent of Frs2α (or requiring multiple adapters) is critical in nephron differentiation and determination of final nephron number.

Funding: NIDDK Support

TH-PO454

Understanding the Mechanisms Downstream of Wnt7b Action in Renal Medulla Formation Latoya Ann Roker, Jing Yu. *Cell Biology, University of Virginia, Charlottesville, VA.*

Background: *Wnt7b* has been shown to mediate renal medulla formation, through canonical signaling to the medullary interstitium. *p57kip2*, a cyclin-dependent kinase inhibitor expressed in both the medullary interstitium and in podocytes, has also been shown to cause a renal medulla defect when ablated in mice, and is probably associated with Beckwith-Wiedemann syndrome including renal medulla anomaly when mutated. However, how *p57Kip2* regulates renal medulla formation, and whether and how it is regulated by *Wnt7b* signaling is unexplored.

Methods: We employed a combination of Luciferase assay in cell culture, Chromatin Immunoprecipitation (ChIP), immunohistochemistry, and mouse genetics to address these questions.

Results: We showed that the *p57kip2* expressing interstitial cells are canonical Wnt signaling responsive cells. A genomic region of *p57kip2* bearing putative Tcf/Lef binding sites responded to canonical Wnt signals in vitro. Furthermore, interstitial cell expression of *p57kip2* is necessary for normal renal medulla formation.

Conclusions: Our data suggest that *p57kip2* is a direct target of Wnt7b/canonical Wnt signaling and *p57Kip2* expressed in the renal interstitium plays a critical role in renal medulla formation.

TH-PO455

p53 in the Cap Mesenchyme Regulates Nephron Endowment Zubaida R. Saifudeen, Yuwen Li, Jiao Liu, Susana Dipp, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Background: The factors which determine nephron endowment or mass are not completely understood, and reduced nephron number is associated with susceptibility to hypertension and progressive renal disease. We recently reported that germ-line deletion of the transcription factor p53 in mice results in renal hypoplasia (50% reduction in nephron mass) (JASN 2009). This study examined the molecular mechanisms whereby p53 regulates nephron endowment.

Results: Confocal microscopy determined that p53 is expressed in the Six2/Pax2-positive cap mesenchyme (CM), suggesting that p53 has the potential to regulate self-renewal, survival, or migration behavior of renal progenitor cells. Genome-wide microarray comparing E15.5 p53$^{-/-}$ and p53$^{+/+}$ kidneys coupled with p53 ChIP-Seq and IPA network analysis revealed that p53 is a direct regulator of 107 CM genes. The top canonical pathways regulated by p53 include VEGF, PI3K/AKT, Myc-mediated apoptosis and DNA methylation and transcriptional repression. Novel p53 targets in the CM encompass developmental regulators - Osr1, Pbx1, Pax2, Cux1, Dach1, Cdh6 and VEGFA; chromatin remodelers - ARID1 and RBBP4; and regulators of cilia formation – IFT172 and KIF24. To determine the functional relevance of p53 in the progenitor cell population, we conditionally deleted *p53* from the CM by crossing Six2-CreGFP to p53-floxed mice (CM$^{\Delta p53}$). CM$^{\Delta p53}$ kidneys are small, exhibit thin, fused caps and reduced number of Six2/Pax2+ cells, and have significantly fewer number of Lhx1+ nascent nephrons. GFP^{+}/CM$^{\Delta p53}$ cells spread dorsally over the flattened ampullae failing to aggregate into discrete caps. Unlike the Six2 domain, the Meis1+ stroma is markedly expanded.

Conclusions: We conclude that p53 performs cell-autonomous functions in the CM progenitor domain via regulation of cell cycle, cell-cell signaling, chromatin remodeling, and cell death.

Funding: NIDDK Support

TH-PO456

***Robo1* and *Robo2* Play a Role in Murine Kidney Branching Morphogenesis and Podocyte Foot Process Architecture** Anna Pisarek-Horowitz, Qinggang Li, Xueping Fan, David J. Salant, Weining Lu. *Renal Section, Boston University Medical Center, Boston, MA.*

Background: Robo1 is a homolog of Robo2, both of which are transmembrane proteins involved in the control of axonal guidance and cell migration. *Robo2* is required for normal kidney induction and restricts the ureteric bud to a single site from the nephric duct. The most striking phenotype in homozygous *Robo2* knockout mice is multiple ureters with supernumerary ureteric buds and duplex kidney. Our recent studies show that *Robo2* is also required for normal podocyte structure and function. It is not known, however, if *Robo1* also plays a role in kidney development and acts synergistically with *Robo2*.

Methods: Immunohistochemistry; knockout mice analysis; real time RT-PCR; embryonic kidney organ culture; scanning electron microscopy.

Results: By immunohistochemistry with a specific Robo1 polyclonal antibody, we found that Robo1 was specifically expressed in mouse embryonic kidney, and is significantly downregulated after birth. Robo1 protein is also expressed in mouse developing glomeruli and its mRNA and protein expression are up-regulated in the glomeruli of *Robo2* knockout homozygous newborn kidney. To investigate the compound effect of *Robo1* and *Robo2* on kidney development, we analyzed *Robo1*$^{-/-}$*Robo2*$^{-/-}$ double knockout mice. Compared with *Robo2*$^{-/-}$ single homozygotes, all *Robo1*$^{-/-}$*Robo2*$^{-/-}$ double homozygous mice died within hours after birth. Interestingly, embryonic kidney organ culture and newborn histology analysis revealed that double homozygous mice manifested a severe branching morphogenesis phenotype and reduced glomerular number compared with *Robo2*$^{-/-}$ single homozygotes. The few glomeruli present at birth in a *Robo1*$^{-/-}$*Robo2*$^{-/-}$ double homozygote displayed abnormal podocyte foot process architecture on scanning electron microscopy.

Conclusions: These results indicate that *Robo1* functions synergistically with *Robo2* during kidney branching morphogenesis and podocyte foot process patterning.

Funding: NIDDK Support, Private Foundation Support

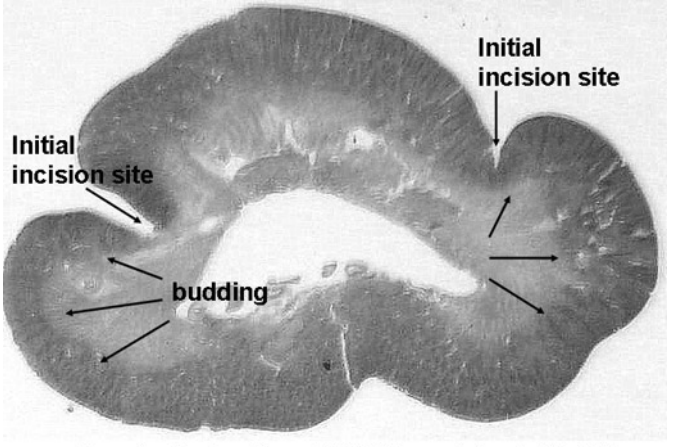
TH-PO457

Compensatory Renal Growth after Unilateral/Subtotal Nephrectomy in the Ovine Fetus Sebastien Sammut,[1] Luc Behr,[1] Mehrak Hekmati,[1] Kathleen Laborde,[3] Martine D. Lelievre-Pegorier.[2] *[1]IMM Research, Paris, France; [2]INSERM U872, Centre de Recherche des Cordeliers, Paris, France; [3]Physiology, Necker Hospital - Paris 5, Paris, France.*

Background: In adult, unilateral and subtotal nephrectomy result in compensatory renal growth but do not involve formation of new nephrons. During the period of active nephrogenesis *in utero*, it is not clear whether compensatory growth can occur and if so, whether more nephrons can be formed.

Methods: Ovine fetuses (n =18) underwent unilateral nephrectomy (1/2Nx, n =5), subtotal nephrectomy (5/6Nx, n =5) or sham nephrectomy (Sham, n=8) at 70 days of gestation. After 134 days, renal functional studies were performed, fetuses were euthanized and kidneys further analyzed.

Results: In 1/2Nx, kidney weight to body weight ratio was greater (5.13 ± 1.09 g/kg vs 2.48 ± 0.20 g/kg in Sham; P<0.05); the nephron number was similar (2658 ± 518 nephrons vs 2467 ± 367 respectively). In 5/6Nx, both catch up in kidney weight (4.62 ± 0.89 g/kg vs 2.48 ± 0.20 g/kg) and nephron number (2560 ± 694 nephrons vs 2467 ± 367) was observed in spite of initial severe 2/3 parenchymal reduction. At incision sites, parenchyma budding was observed, leading to a butterfly-like shape. In all groups, *in utero* glomerular filtration rate was similar. At the molecular level, caspase gene expression was significantly decreased (<0.01) in 5/6Nx. No differences in expression of Bcl2, Pax2, Wt1, VEGF or cRet were observed among the differents groups.



Longitudinal section of 5/6 kidney stained with H.E.

Conclusions: A significant compensatory growth occurs *in utero* after parenchymal reduction. Furthermore, nephron endowment develops after 5/6Nx during the period of active nephrogenesis in sheep. This is the first time such neo-nephrogenesis is described; the morphological pattern observed suggests a stimulation of ureteric bud branching, allowing a compensation of glomeruli number and function, despite 2/3 renal parenchyma reduction.

Funding: Government Support - Non-U.S.

TH-PO458

Mesenchymal Stem Cells Induce Renoprotection in the Podocyte Injury Model Rodrigo J. Ramalho, Amanda G. Pires, Rita C. Cavaglieri, Denise M. Malheiros, Irene L. Noronha. *Nephrology, Univ Sao Paulo, Brazil.*

Background: Several studies have shown that mesenchymal stem cells (mSC) protects against renal damage. However, the role of mSC in models of podocyte injury is still unclear. The aim of this study was to evaluate the potential effects of mSC in the puromycin model (PAN).

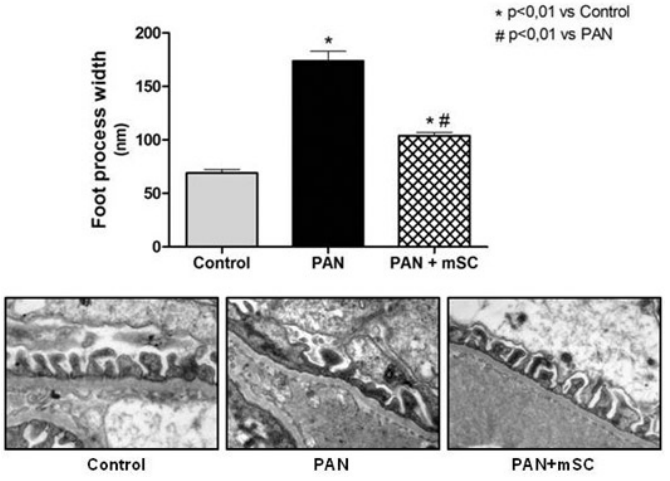
Methods: PAN model (150 mg/kg at day-0 and 5 mg/kg at day-15) was induced in Wistar rats (n=30), aggravated with unilateral nephrectomy. mSC from rat bone marrow were isolated and delivered at the kidney subcapsule. Rats were divided into three groups (Control, PAN, PAN+mSC) and followed for 60 days. The following parameters were analyzed: blood pressure (BP), proteinuria, histological changes, electron microscopy, immunohistochemistry, and real time PCR.

Results: mSC promoted a significant decrease of proteinuria (at 30th and 60th day) and of BP levels (at 60th day). In parallel, a marked proliferative activity was detected in PAN+mSC. Recovery of the podocyte marker WT1 was also observed in PAN rats treated with mSC.

	Proteinuria (mg/24h)		BP (mmHg)		PCNA (cels/mm²)	WT1 (%)
day	30	60	30	60	60	60
CONTROL	12.9±1.0	21.0±3.8	124±2	128±4	2.6±0.7	2.42±0.14
PAN	217.8±16.8#	79.4±6.4#	151±4#	161±5#	6.18±0.6#	1.45±0.12#
PAN + MSC	133.0±24.6#*	44.3±13.7*	154±3#	143±6*	15.0±3.0#*	2.06±0.16*

# p<0,05 vs Control; *p<0,05 vs PAN

There was no significant difference in glomerulosclerosis, interstitial fibrosis, macrophages, lymphocytes, and myofibroblasts. Electron microscopy showed a significant improvement of foot process width associated to mSC administration (figure). Relative mRNA levels of nephrin, podocin, synaptopodin, podocalyxin, WT1 and VEGF recovered partially with mSC.



Conclusions: In the PAN model, mSC induced renal protection characterized by decreased proteinuria and BP levels associated with high proliferative activity, podocyte recovery and improvement of foot process effacement.

Funding: Government Support - Non-U.S.

TH-PO459

Src Family Kinase Activity Promotes Ureteric Bud Branching While Limiting Ureteric Bud Segment Elongation during Mouse Fetal Kidney Development Alexander E. Davidovich,[1] Josephine Axis,[1] Deborah P. Hyink,[2] Kurt Amsler.[1] *[1]Department of Biomedical Sciences, New York College of Osteopathic Medicine of NYIT, Old Westbury, NY; [2]Division of Nephrology, Baylor University College of Medicine, Houston, TX.*

Background: The activity of Src Family Kinases (SFKs), a family of non-receptor tyrosine kinases, has been implicated in the pathogenesis of both Autosomal Dominant and Autosomal Recessive Polycystic Kidney Disease, disorders characterized by abnormal renal development. The involvement of this kinase family in normal fetal renal development, however, has not been determined.

Methods: Using a mouse fetal kidney organ culture system, we investigated the role of SFK activity in the regulation of fetal renal development. Fetal CD-1 mouse kidney pairs, isolated from gestation day 12 embryos, were separated and cultured on permeable membrane filters in DMEM/Ham's F12 medium containing 5% fetal bovine serum plus insulin/transferrin/selenium in either the absence or presence of 10 μM PP1, a well-characterized SFK inhibitor. Kidneys were imaged daily for three days, beginning from explantation, and parameters of ureteric bud (UB) branching morphogenesis were quantitated.

Results: Kidneys maintained in the presence of PP1 exhibited differences from their control counterparts in several aspects of UB branching. After two days in culture, PP1 produced: 1) a progressive inhibition of UB branch bifurcations (p=5.8 x10-17) reaching approximately 60% inhibition and 2) an inhibition of lateral branching (p=2.9 x10-5). In contrast, UB segment lengthening was not decreased by PP1 treatment. In fact, PP1 produced an increase in the UB primary branch segment length, with the greatest effect being observed on day 1 of treatment (p=0.002). Beginning after the first day of PP1 culturing, fetal kidneys exhibited morphologically abnormal UB tip regions suggesting a disruption of the normal ureteric bud-metanephric mesenchyme interaction.

Conclusions: Our data indicate that: 1) SFKs are activated under standard fetal mouse kidney organ culture conditions, 2) optimal SFK activity is necessary for UB branching, and 3) SFK functional levels may impede UB segment elongation.

TH-PO460

Purification of Differentiated Tubule Cells from Mouse Embryonic Stem Cells Using Flow Cytometry Ryuji Morizane, Toshiaki Monkawa, Hiroshi Itoh. *Internal Medicine, Keio University School of Medicine, Tokyo, Japan.*

Background: Although embryonic stem (ES) cell and induced pluripotent stem (iPS) cell have been utilized to generate various lineage cells *in vitro*, the induction of renal lineage cells from ES and iPS cells have remained to be elucidated. Recently, we found that Activin stimulate the expression of kidney specific protein (KSP) that is exclusively expressed in renal tubule epithelial cells and that it enhances the differentiation of ES and iPS cells to tubule cells.

Methods: In this study, we produced a monoclonal antibody to purify tubule cells differentiated from ES cells by flow cytometry, and we have established a method to generate tubule cells from ES cells *in vitro* by using Activin and flow cytometry with the monoclonal antibody. We designed the monoclonal antibody so that it binds to the extracellular domain of a protein called X which was exclusively expressed in renal epithelial cells, that is, ureteric buds, bowman's capsules, proximal / distal tubules and collecting ducts. We confirmed the specificity of anti-X antibody by flow cytometry, PCR and Western blot using ureteric bud cell line.

Results: We sorted X-positive cells from differentiated ES cells by flow cytometry using anti-X antibody after the induction with Activin. X-positive cells comprised about 1.5% of total differentiated ES cells, and were expressing mRNA of X much more than X-negative cells. X-positive cells also expressed aquaporin 2 (AQP2), aquaporin 3 (AQP3), KSP and E-cadherin, suggesting X-positive cells were similar to tubule cells in gene expression. To observe the formation of tubular structure *in vitro*, we transferred X-positive cells into non-adhesion dish after sorting by flow cytometry, and we found cell aggregates. After 24 hours of formation of cell aggregates, we embedded them in Matrigel ® and cultured with epithelial growth factor (EGF). The cells expanded outward, and formed a ring structure that resembled tubular structure.

Conclusions: In conclusion, we could induce tubule cells from mouse ES cells with Activin stimulation and cell selection using anti-X antibody. Currently, we proceed to characterizing X-positive cells induced by this method.

TH-PO461

Drugs Frequently Used in Premature Neonates Reduce Nephrogenesis in Embryonic Kidney Culture Ruud R.G. Bueters, Lisanne Kusters, Annelies Klaasen, Lambertus V. Heuvel, Michiel F. Schreuder. *Department of Pediatric Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Nephrogenesis ceases around the 36th week of gestation in man, with no additional nephron formation later in life. A lower number of nephrons leads to glomerular hyperfiltration, hypertension and renal impairment in the long run. Many preterm born children are treated with drugs that may reduce nephron formation. We investigate these drugs for effects on kidney growth and ureteric branching.

Methods: At embryonic day 13 embryo's were delivered from time pregnant mice through Caesarean section and kidneys were dissected. Subsequently embryonic kidneys were cultured on filters in control media. Three different concentrations (including clinical dose) of gentamicin and ceftazidime were added to the media. Starting kidney size was assessed by surface area measurements and kidneys were whole mount stained with anti-calbindin D-28K after 24 hours of culture. Visualization was performed by means of confocal microscopy and ureteric bud branching was evaluated by counting. Additionally, qPCR course experiments were performed with hydrolysis probes to investigate gene expression levels of Wt1, Sox9, Bmp7, Fgf8 and Gdnf. The genes Actb and Hmbs were used for normalization.

Results: Ureteric bud count indicates that 300 μM (high-dose) of gentamicin and 200 or 2000μM (mid- and high dose, respectively) of ceftazidime impair ureteric bud development. Additional qPCR analysis revealed a 2.5 fold down regulation in Fgf8 and a tendency to downregulation in Gdnf for high-dose ceftazidime. No clear changes in gene expression were noted for gentamicin due to lack of a dose response relationship. These results will be verified in additional experiments and at later time-points.

Conclusions: 300μM of gentamicine and 200 or 2000μM of ceftazidime impair ureteric bud formation already after 24 hours of treatment. Additionally, we suggest a role for Fgf8 and Gdnf in the mechanism behind ceftazidime induced bud impairment.

Funding: Private Foundation Support

TH-PO462

Notch Signal Is Essential to the Formation of Glomus and Proximal Tubule While It Inhibits the Extension and Differentiation of Distal Tubule in Xenopus Embryos Tomohisa Katada, Hiroyuki Sakurai. *Department of Pharmacology and Toxicology, Kyorin University, School of Medicine, Mitaka, Tokyo, Japan.*

Background: Notch signaling, a highly conserved cell-cell signaling system, has been shown to regulate cell differentiation and/or proliferation at various stages of animal development. This study aimed to elucidate the role of Notch signaling in the development of the pronephros in Xenopus embryos.

Methods: In order to enhance or inhibit Notch signaling, a conditional Notch-activating construct (NICD-hGR) or a conditional Notch-suppressing construct (XSu(H)1DBM-hGR) was injected into the blastomere of Xenopus embryos. These constructs turned on when dexamethasone (DEX) was added to the culture. wt1, XSMP-30 and gremlin were used as gene markers for glomus, proximal tubule and distal tubule, respectively.

Results: Constitutive activation of Notch signaling from late neurula stage on resulted in excessive formation of the glomus and malformation of the proximal tubule. Inhibition of Notch signaling starting at the same stage of development reduced expression of the gene markers for the glomus (wt1) and the proximal tubule (XSMP-30). These results demonstrated important roles of Notch signaling in proximal nephron development, consistent with previous reports by others. In addition, Notch activation at different time points inhibited distal tubule extension to different degrees. These shorter distal tubules, although they were positive for pan distal tubule marker, gremlin, lacked expression of

markers specific for a certain segment of the distal tubule such as claudin 19, claudin 14, or rhcg, suggesting that Notch overactivation inhibited not only extension but also differentiation of the distal tubule.

Conclusions: In summary, activation of Notch signaling defined proximal pronephros while proper inhibition was indispensible for distal tubule development. These results suggest that regulation of Notch activity plays an important role in development of Xenopus pronephros.

Funding: Government Support - Non-U.S.

TH-PO463

Role of Calcium Oscillations in Metanephric Mesenchyme Cells for Nephron Formation Georgiy R. Khodus,[1] Jacopo Maria Fontana,[1] Hjalmar Brismar,[2] Anita Aperia.[1] *[1]Karolinska Institutet, Sweden; [2]Royal Institute of Technology, Sweden.*

Background: Emerging evidence suggest that many of the processes governing the reciprocal interactions between the metanephric mesenchyme (MM) and ureteric bud (UB) are calcium dependent. Yet little is known about the origin of calcium signals in the metanephric kidney.

Methods: We have used laser scanning confocal microscopy to record calcium activity in explanted kidney rudiments derived from E14.0 rat embryo and cultured for 48 hours. Kidneys were loaded with the calcium sensitive dye "Oregon Green BAPTA-1", placed in a closed chamber at 35°C and slowly perfused with Krebs-Ringer solution. Calcium was recorded in MM cells bordering UB. The intensity of calcium traces was normalized and background was subtracted.

Results: During the recording time of 30 min, spontaneous calcium oscillations, here defined as at least four calcium peaks exceeding more than 10% of baseline, were observed in 34.0 ± 5.8 % of cells (mean ± std.dev.). The calcium oscillations were completely dependent on release of calcium from the intracellular stores and, to a lesser degree, on influx of calcium from the extracellular space.

To examine the functional role of the spontaneous calcium oscillations, the intracellular calcium stores of the kidney rudiments were depleted by partial inhibition of the SERCA pump with cyclopiazonic acid (CPA) during the 2nd day in culture. In kidney rudiments exposed to 5 μ M CPA, the relative number of cells with calcium oscillations was reduced from 35% to 7%. The UB tips were abnormally shaped and appeared swollen and there was a significant, 27 %, decrease in UB branching and formation of glomeruli in CPA treated kidney rudiments compared to non-treated kidney rudiments. GDNF secreted from the MM and acting on UB Ret-receptors, is a key feature of MM and UB interaction. GDNF secretion, which in the brain is known to be a calcium dependent process, was measured in condition media. Twenty four hours of CPA treatment caused a robust decrease of GDNF secretion.

Conclusions: We conclude that spontaneous intracellular calcium oscillations in MM cells, generated by calcium release from intracellular stores, are required for normal nephron development.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO464

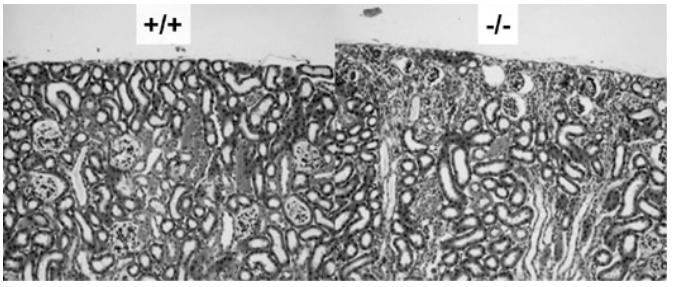
Early B Cell Factor 1 (Ebf1): A Novel Regulator of Renal Cortex Maturation and Glomerular Function Jackie A. Fretz,[1] Tracy Nelson,[1] Heino Velazquez,[2] Yougen Xi,[1] Lloyd G. Cantley,[2] Gilbert W. Moeckel,[2] Mark C. Horowitz.[1] *[1]Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, CT; [2]Internal Medicine- Nephrology, Yale University School of Medicine, New Haven, CT.*

Background: The maturation of the metanephros occurs via multiple coordinated signaling pathways, cytokines and transcription factors. We report here a previously unrecognized role of the helix-loop-helix protein Early B cell Factor 1 (Ebf1) as a novel transcription factor required for proper glomerular maturation.

Methods: Using the Ebf1-null mouse as a model of Ebf1-deficiency we characterized the biochemical, metabolic, and histological abnormalities in renal development that arise in the absence of this transcription factor.

Results: The expression of Ebf1 is both spatially and temporally regulated within the developing cortex and glomeruli. Initially Ebf1 expression is seen in the early condensed mesenchyme and appears to mediate glomerular maturation. However, Ebf1 expression is less restricted within the mature kidney and appears in both glomeruli and cortical tubular segments, but is excluded from the collecting duct and medulla. In the absence of Ebf1, developing kidneys show thinned cortices, reduced glomerular number, and features of the nephrogenic blastema that persists into adulthood. In the Ebf1-null mice, the glomeruli show abnormal vascularization, crescent-like structures and severely effaced podocytes by TEM. These mice exhibit albuminuria early and show elevated blood urea nitrogen (BUN) levels. Moreover, in glomeruli lacking Ebf1 the GFR is reduced by 66% and the expression of Wt1 as well as VEGF is decreased compared to wild type control animals.

Conclusions: Taken together these results present the first report of a novel and significant role of Ebf1 in glomerular development, maturation and in the maintenance of kidney integrity and function.



Funding: Other NIH Support - NIAMS, Veterans Administration Support

TH-PO465

Compensatory Growth of the Glomerulus in the Developing Mouse Kidney Ji Ma, Jianyong Zhong, Valentina Kon, Iekuni Ichikawa. *Pediatrics, Vanderbilt University, Nashville, TN.*

Background: Although glomerulogenesis ceases by postnatal day 3 (P3), the glomerular number remains preserved in rodents undergoing reduction in renal mass in the neonatal period. Our recent studies in developing mice reveal a dynamic change in glomerular number which peaks at P7, then decreases and stabilizes after P18. The present study examines the growth pattern of the developing glomeruli following injury of relatively mature glomeruli and the potential modulating effects of podocyte-derived vascular endothelial growth factor-A (VEGF) to promote compensatory recovery of glomerular number.

Methods: Glomerular injury was induced in relatively mature glomeruli within the deep cortex of mice carrying *Nphs1*-hCD25 transgene (NEP25) by a single i.p. injection of LMB2 at P4. Enhanced expression of podocyte VEGF was achieved by administration of doxycycline to *Nphs2*-rtTA/*tetO*-hVEGF (rtTA/VEGF) double transgenic mice starting from birth (P0).

Results: At P21, a time when normal glomerular maturation is nearly complete, there was no significant difference in body weight (BW) and kidney weight among groups. NEP25 transgenic pups showed glomerular destruction and sclerosis in the deep cortex. These glomerular damages were accompanied by significantly decreased glomerular volume especially in superficial glomeruli (Control: 9763±349 μm^3/g BW *vs*. NEP25: 7607±473 μm^3/g BW, $P<0.05$), which was restored by podocyte-specific induction of the VEGF transgene (NEP25/rtTA/VEGF: 10400±778 μm^3/g BW, $P<0.05$ *vs*. NEP25). The total number of glomeruli was not significantly different among groups. The ratio of superficial:deep glomerular number tended to increase in NEP25 *vs*. controls (6.24±0.66 *vs*. 4.29±0.55, $P=0.063$) and was accompanied by significantly decreased medullary thickness, and increased ratio of superficial:deep cortex thickness. Enhanced podocyte VEGF did not change the glomerular number and distribution.

Conclusions: Damage and loss of glomeruli during development can be compensated through activation of glomerulogenesis in the superficial cortex, which contains relatively immature nephrons. The recovery process involves VEGF-mediated angiogenesis that enhances glomerular growth following glomerulogenesis.

Funding: NIDDK Support, Private Foundation Support

TH-PO466

The Identification of MicroRNAs Regulating Gene Expression during the Birth Transition of the Developing Kidney by Next-Generation Sequencing James B. Tee. *University of Calgary.*

Background: The transition from fetal to newborn kidney is associated with several developmental changes that are involved in the maturation of both renal structure and function. Disruptions to the genetic regulation this process can be the contributing source for reduced renal function in children, including low nephron numbers and cystic kidney disease. MicroRNAs (miRNAs) act to reduce the expression of its target genes. Conventional screening of miRNA expression by pre-generated microarrays are typically limited by its older miRNA library source, lacking the evaluation of a growing number of novel miRNAs that would be detected by next-generation sequencing. The potential role of miRNAs in controlling the developmental processes that govern kidney development at birth have not been studied to date.

Methods: To evaluate this process, kidneys were extracted from rats at gestational days 21 and 22, birth, and 1 day of age. A cDNA library of small RNAs was generated from each timepoint and sequenced on the Illumina GA2. Raw data was aligned to the rat genome (rn4), and then manually parsed for the mature sequences of all 408 miRNAs known to date.

Results: Comparing the pre- and postnatal stages revealed 8 miRNAs that exhibited a >= 2-fold increase in expression. Predicted target mRNAs were generated for the top 5 miRNAs (miR-17-2, 30b, 99a, 16, 425) using a combination of miRanda-based algorithms. Correlation with existing gene expression data at these two stages revealed 32 matching target genes that were downregulated >= 2-fold, including those for uromodulin (p = 5.1E-5), annexin A1 (6.7E-6), and solute carrier family 12-1 (7.0E-3). Conversely, 20 miRNAs exhibited a >= 2-fold decrease in expression between the pre- to post-natal kidney. The predicted targets of the top 5 downregulated miRNAs (miR-542, 341, 378, 101a, 10b) correlated with the increased expression observed in 31 genes including polycystic kidney and hepatic disease 1 (p = 7.3E-3), NADPH oxidase 4 (2.5E-2) and solute carrier family 22a1 (6.9E-3).

Conclusions: The presented results provide a first look at miRNAs that may be involved in the regulation of gene expression governing the developmental processes of the kidney at birth transition.

Funding: Government Support - Non-U.S.

TH-PO467

Using the NCAM1 Marker for Prospective Isolation of Highly Clonogenic Human Adult Kidney Epithelial Cells with Unequivocal Tubular Regeneration Capacities Ella Buzhor,[1,3] Orit Harari-Steinberg,[1,3] Dorit Omer,[1,3] Benjamin Dekel.[1,2,3] *[1]Pediatric Stem Cell Research Institute, Sheba Medical Center, Tel Hashomer, Israel; [2]Division of Pediatric Nephrology, Safra Children's Hospital, Sheba Medical Center, Sackler Faculty of Medicine, Israel; [3]Sheba Center for Regenerative Medicine, Sheba Medical Center, Tel Hashomer, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.*

Background: There is an ongoing debate on existence of genuine epithelial progenitors in the adult kidney. Recent studies have re-assured that dedifferentiation of injured cells and not activation of an intra-tubular progenitors is taking place in regenerating kidneys. NCAM1 expressed only during nephrogenesis, no expression remains in adulthood. Re-expression of NCAM1 has been observed following ischemic injury in S3 proximal tubule cells.

Methods: In this report, surgical human kidney-tissue specimens were studied to prospectively isolate NCAM+ cells, define their phenotype and functional properties in vitro and in vivo.

Results: No in situ expression of NCAM1 was detected in the human nephron compartment. Nevertheless, human kidney epithelial cell (hKEpC) cultures, uniform for the CD24 and CD133 markers, up-regulated NCAM1 expression in 10-15% of the cells. Cell sorting of hKEpCs according to NCAM1, selected a cell subpopulation that overexpressed nephron progenitor (*Six2/Sall1/Pax2/Wt1*) and proximal tubule markers, as well as elevated *Vimentim* and reduced *E-cadherin*. *In vitro* functional assays revealed NCAM1+ cells to be highly clonogenic, slow-cycling and exclusively forming well-defined 'nephrospheres'. *In vitro* depletion of NCAM1+ cells from hKEpCs cultures resulted in loss of clonogenicity and decreased nephrosphere formation. *In vivo*, NCAM1+ cells grafted to the chorioallantoic membrane of the chick embryo generated renal tubules, predominantly of proximal and to a lesser extent of distal origin, while NCAM1- cells, mesenchymal stem cells or HEK293, failed to do so.

Conclusions: Thus, we have isolated a clonogenic, sphere-forming human adult kidney cell population that possess enhanced ability to generate renal tubules. These findings may ultimately pave the way for autologous cell therapy for renal diseases.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO468

CKD Impairs Functionality of Mesenchymal Stem Cells (MSC) in Rats Barbara Mara Klinkhammer, Monika Mallau, Anna Makowska, Rafael Kramann, Claudia R.C. van Roeyen, Eva Bücher, Simon Otten, Esther Stüttgen, Jurgen Floege, Uta Kunter. *Div. of Nephrology, RWTH University Hospital, Aachen, Germany.*

Background: MSC hold promise in many renal diseases, but little is known about the effects of CKD on long term MSC function. We isolated MSC from the bone marrow of CKD rats and tested their functionality in the acute anti-Thy1.1 nephritis model.

Methods: MSC from "healthy" male F344 rats, "CKD" rats 22 weeks after 5/6 Nx, and healthy MSC from transgenic "hPLAP" (*human placental alkaline phosphatase*) rats were analyzed *in vitro* for proliferation capacity, adipogenic differentiation, cellular senescence and growth factor production. To evaluate MSC function *in vivo*, anti-Thy1.1 nephritis was induced in F344 rats. On day 2 after disease induction, 250 000 MSC ("healthy", "CKD", "hPLAP") or DMEM were injected into the left renal artery. Untreated right kidneys served as controls. Rats were sacrificed at days 4 or 6.

Results: *In vitro*, "CKD" MSC exhibited spontaneous adipogenic differentiation, high levels of active SA-β-Gal, and reduced proliferation capacity (cell population doublings: 134±63 h vs. 52±23 h in "healthy" MSC, $p<0.006$). Culture supernatants of "CKD" MSC contained less TGFβ but similar amounts of $VEGF_{164}$ compared to "healthy" MSC. *In vivo*, treatment with "hPLAP" or "healthy" MSC decreased proteinuria on day 6 compared to "CKD" MSC or DMEM ("hPLAP" 9±6 vs. "CKD" 34±16 vs. DMEM 27±4 mg/24h, $p<0,001$ and n.s., respectively). BrdU positive cells and glomerular mitotic figures on day 4 increased in kidneys treated with "healthy" MSC whereas there was no difference between the "CKD" and DMEM group. On day 6 "healthy" or "hPLAP" MSC reduced mesangiolysis scores, while "CKD" MSC or DMEM did not (% damage reduction treated vs. untreated kidney: "healthy" 26±24; "hPLAP" 51±18; "CKD" -3±31; DMEM 2±21 ("hPLAP" vs. DMEM $p<0,001$; "CKD" vs. DMEM n.s.).

Conclusions: CKD induces a sustained loss of *in vivo* functionality in MSC, possibly by reducing cell proliferation, modulating the secretory phenotype and a shift towards adipogenic differentiation, i.e. changes that resemble cellular senescence. Autologous MSC from CKD patients might thus not be a suitable source for regenerative therapies.

Funding: Government Support - Non-U.S.

TH-PO469

The Embryonic Environment of the Metanephric Kidney Promotes p53 Activation Via Post-Translational Modifications and Mdm2 Cleavage Karam S. Aboudehen, Zubaida R. Saifudeen, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Background: In addition to its classical role as a guardian of the genome, p53 functions as a developmental regulator. In the embryonic mouse kidney, p53 prevents ectopic budding from the nephric duct by antagonizing GDNF-Ret signaling. Moreover, p53 regulates expression of a subset of renal function genes (RFG) via direct binding to the target promoters. Cellular p53 levels are normally kept low via proteosomal degradation mediated by interactions with the E3 ubiquitin ligase, Mdm2. In response to cellular stress (e.g., hypoxia, DNA damage) p53 is modified post-translationally, leading to Mdm2 dissociation. p53 also induces caspase-mediated cleavage of 90-kda Mdm2 to 60-kda Mdm2, which acts to stabilize p53. The mechanisms mediating p53 stabilization and activation in the embryonic kidney are unknown.

Results: Using specific antibodies to total and phosphorylated or acetylated p53, we demonstrate that, compared to postnatal and adult kidneys, embryonic and neonatal p53 is hyper-phosphorylated on serines 6, 9, 15, 20 and 392. Embryonic kidney p53 is also hyper-acetylated on lysines 373, 382, and 386, hence exhibits enhanced DNA binding affinity. Site-directed mutagenesis of critical serines and lysines of p53 demonstrated that these modifications exert differential effects on p53 stability and transcriptional activity. In vivo, $p53^{pSer392}$ is enriched in differentiating proximal tubules, whereas $p53^{ace\text{-}373,382}$ is expressed in the metanephric mesenchyme, nephron progenitors and collecting duct cells. Co-immunoprecipitation/Western blotting revealed that embryonic p53 exists mostly in a mono-ubiquitinated form, whereas, in postnatal and adult kidney, p53 is poly-ubiquitinated. This finding correlates well with the temporal switch from cleaved to intact forms of Mdm2 and dramatic reductions in p53 levels.

Conclusions: We conclude that the embryonic environment mimics the stress response pathway leading to enhanced p53 stability and DNA binding activity. Furthermore, the developmental switch in mdm2 protein species likely plays an important role in p53 degradation following the end of nephrogenesis.

Funding: NIDDK Support

TH-PO470

Gene-Environment Interactions Cooperate To Repress the Developmental Regulator, Pax2, in Renal Dysgenesis Lei Yan,[1] Nathaniel J.D. McLaughlin,[1] Xiao Yao,[1] Dimcho Bachvarov,[2] Zubaida R. Saifudeen,[1] Samir S. El-Dahr.[1] *[1]Pediatrics, Tulane University School of Medicine, New Orleans, LA; [2]Molecular Medicine, Laval University, Quebec, Canada.*

Background: Gene-environment interactions play an important role in the pathogenesis of human congenital disorders. Bradykinin B2 receptor-null (*Bdkrb2*$^{-/-}$) mice have normal kidney development; however, the *Bdkrb2*$^{-/-}$ progeny develop renal dysgenesis following gestational salt stress via the maternal diet. The transcription factor, Pax2, is significantly downregulated in *BdkrB2*$^{-/-}$ kidneys compared to wild-type pups. Humans with *Pax2* mutations have renal dysgenesis. We tested the hypothesis that embryonic salt stress cooperates with *BdkrB2* inactivation to repress *Pax2* gene transcription.

Results: *Bdkrb2*$^{-/-}$ mice were crossed to *BAC-GFP* transgenic mice carrying 30 kb of *Pax2* upstream elements. GFP protein and mRNA, surrogates of in vivo Pax2 transcriptional activity, from salt-stressed *Bdkrb2*$^{-/-}$;*Pax2-GFP* and wild-type embryonic kidneys were analyzed quantitatively by fluorescent microscopy and real-time PCR, respectively. GFP pixel area/intensity and mRNA levels were lower in E12.5 and E14.5 *Bdkrb2*$^{-/-}$ than *Bdkrb2*$^{+/+}$ kidneys ($p<0.05$). However, when E12.5 *Bdkrb2*$^{-/-}$ kidneys were grown ex vivo for 2 days to remove the embryonic stressor, GFP expression, the number of ureteric bud branching points and tips, and K-cadherin expressing renal vesicles normalized. Microarray analysis in E14.5 salt-stressed *BdkrB2*$^{-/-}$ and *BdkrB2*$^{+/+}$ kidneys revealed significant changes in 1% of probe sets. Upregulated genes (n=202) were linked to extracellular matrix synthesis and muscle development, whereas downregulated genes (n=164) belonged to transcription factors, cell cycle and fate and regulators of nucleic acid metabolism. Importantly, 28 upregulated and 13 downregulated genes have putative Pax2-responsive elements in their promoter regions. Moreover, 8 of these genes have mouse mutant phenotypes consistent with a major role in muscle, kidney, lymphatic and cardiovascular development.

Conclusions: We conclude that *Pax2* transcriptional activity is responsive to gene-environment interactions in vivo, representing a novel mechanism of renal dysgenesis.

Funding: NIDDK Support

TH-PO471

Metanephros Transplantation Contributes to Maintaining Blood Pressure in Diltiazem Treated Anephric Rats Shinya Yokote,[1,2] Takashi Yokoo,[1,2] Kei Matsumoto,[1,2] Yasunori Utsunomiya,[1] Tetsuya Kawamura,[1] Tatsuo Hosoya.[1] *[1]Division of Nephrology and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Nishi-Shimbashi, Minato-ku, Tokyo, Japan; [2]Project Laboratory for Kidney Regeneration, Institute of DNA Medicine, Jikei University School of Medicine, Nishi-Shimbashi, Minato-ku, Tokyo, Japan.*

Background: The kidney is an important organ in maintaining blood pressure. We have previously reported that transplanted metanephroi can reproduce some kidney function. The aim of the present study was to determine the metabolic function of transplanted metanephroi with particular reference to maintaining blood pressure during renal failure.

Methods: Ten-week-old male Wistar rats were transplanted with metanephroi in the paraaortic area (PA group; n=5) or in the omentum and epididymis (OE group; n=8), followed by bilateral nephrectomy. For comparison, we performed bilateral nephrectomies without transplantation on fourteen rats (non-transplanted group; n=9 and heminephrectomy control group; n=5). Nephrectomies were performed on the rats in the transplanted and non-transplanted groups two weeks after transplantation. Rats in the control group had sham operations performed. Hypotension was induced by intravenous infusion of diltiazem hydrochloride at a dose of 0.025mg/kg/min for 2 hours and at a dose of 0.05mg/kg/min for further 2 hours using a syringe pump. Mean arterial blood pressure (MAP) was monitored from the left femoral artery. Plasma renin activity (PRA) was analyzed every hour. Renin expression from the transplanted metanephroi was evaluated by RT-PCR and immunopathologically at the time of sacrifice.

Results: RT-PCR showed that metanephroi in the transplanted group expressed renin m-RNA. Metanephros transplantation significantly raised PRA and maintained MAP compared with the non-transplanted, anephric group. No significant differences between the transplanted and control groups were found with respect to PRA and MAP.

Conclusions: The present study has shown that transplantation of metanephroi produces PRA and contributes to maintaining MAP in a rat model of hypotension.

Funding: Government Support - Non-U.S.

TH-PO472

Branching Tubulogenesis of Renal MDCK Cells Requires HIF-1α and Its Target Gene FSP1 – Implications for a (Patho)Physiological Role in the Human Kidney Bjoern Buchholz, Sven Kroening, Gunnar Schley, Tina Gimm, Wanja M. Bernhardt, Kai-Uwe Eckardt. *Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: During nephrogenesis, outgrowth and branching of the ureteric bud is essential to generate the definitive renal collecting system and to determine the number of nephrons. During kidney development, the hypoxia-inducible transcription factor HIF-1α is expressed in medullary and cortical collecting ducts of various species. In addition, HIF-1α is stabilized in epithelial cells of ischemic and polycystic kidneys. Therefore, we wondered if HIF-1α might play a role in tubulogenesis and tubular repair and which target genes would be involved.

Methods: We studied in vitro branching tubulogenesis of Madin-Darby Canine Kidney (MDCK) cells, which originate from the collecting duct, according to their protein expression of HIF-1α. Therefore, we stabilized HIF-1α by hypoxia or the hypoxia-mimetic DMOG or inhibited HIF-1α with 17 AAG or chetomin. We also established two MDCK cell clones with a stable knockdown of HIF-1α and performed RT-PCR to test for relevant target genes. In addition, we used immunohistochemistry to examine developing kidneys of humans and rats as well as kidneys affected by hypoxia or ischemia.

Results: Both, hypoxia and DMOG enhanced in vitro tubulogenesis. In contrast, 17 AAG, chetomin and knockdown of HIF-1α significantly inhibited branching tubulogenesis. The metastasis-promoting protein FSP1 which has recently been shown to be a HIF-1α target gene was markedly decreased in the HIF-1α knockdown cells. Inhibition of FSP1 by Sulindac as well as knockdown of FSP1 by siRNA diminished in vitro tubulogenesis of MDCK cells. In addition, FSP1 was markedly expressed in tubules of developing human and rat kidneys, as well as in ischemic, hypoxic, and polycystic kidneys.

Conclusions: Branching tubulogenesis of MDCK renal collecting duct cells depends on HIF-1α. In vitro tubulogenesis of MDCK cells also depends on the HIF-1α target gene FSP1. FSP1 is highly expressed in tubular cells of developing kidneys and adult kidneys affected by hypoxia suggesting a physiological and pathophysiological role for FSP1/HIF-1α in vivo.

TH-PO473

An Alternative Splice Product of the HmPKD1 Gene Induces Mesenchyme to Epithelial Transition in COS-1 Cells Robert L. Bacallao, Clifford Babbey, Wei Min Xu. *Medicine, Indiana University and Richard Roudebush VAMC, Indianapolis, IN.*

Background: In a genome wide cDNA library construction using 5' cap probes, additional transcripts from the HmPKD1 locus were identified by Kimura et al, 2006 (Genome Research). We completed the sequence analysis and characterized the biological effects of this cDNA when expressed in COS-1 cells.

Methods: cDNA encoding a splice variant from the HmPKD1 locus was obtained from Dr. Sugano (University of Tokyo) and sequenced at the DNA sequencing facility at Indiana University. The clone was transiently transfected into COS-1 cells. Paired samples of transfected and untransfected were compared for E-cadherin and ZO-1 expression. In addition trans-epithelial resistance (TER) was measured using an Epithelial Volt Ohm meter (EVOM). Finally growth morphology in 3D collagen culture was assessed by multiphoton confocal microscopy.

Results: The cDNA sequence was mapped onto the HmPKD1 genomic sequence database at UC Santa Cruz. The start site of the cDNA maps to intron 40 of HmPKD1. The intron 40 sequence runs into exon 41 with a splice from the 3' end of exon 41 with the 5' end of exon 43. All the rest of the splices are the same as that observed in the full-length PKD1 gene.

Transiently transfected COS-1 cells develop a measurable TER over the course of 3 days post transfection. In contrast untransfected cells fail to develop a TER. Over the same time course, transfected cells homogeneously express ZO-1 and E-cadherin. When plated in 3D collagen culture, transfected COS-1 cells form cysts with a single monolayer of cells surrounding a lumen.

Conclusions: A alternative transcript from the HmPKD1 locus induces expression of epithelial markers in a normally mesenchymal cell line (COS-1). Transient transfected of this cDNA causes mesenchyme to epithelial transition and causes cyst formation in 3D collagen culture.

Funding: Clinical Revenue Support

TH-PO474

Low Serum Cultured Adipose Derived Stem Cells Ameliorate Zymosan Induced Severe Rat Peritonitis Model Hangsoo Kim, Masashi Mizuno, Kazuhiro Furuhashi, Takayuki Katsuno, Kaoru Yasuda, Takenori Ozaki, Waichi Sato, Naotake Tsuboi, Yasuhiko Ito, Enyu Imai, Shoichi Maruyama, Seiichi Matsuo. *Nagoya University Graduate School of Medicine, Japan.*

Background: We developed a novel culture system for low serum cultured adipose derived stem cells (LASCs) and have shown the therapeutic potential of LASCs in various animal models.

Methods: In this study, we focused on a fungal peritonitis in peritoneal dialysis(PD),which is a major problem since the fibrosis may progress rapidly and it may evolve into encapsulating peritoneal sclerosis (EPS) even when the peritoneal dialysis catheter is removed.

A rat model of fungal peritonitis was induced by administration zymosan daily for 5 days after scraping peritoneum mechanically. The rats were divided into two groups;LASC(L group) or vehicle(V group) administration intraperitoneally with PD solution(1.5% glucose,neutral liquid) every day.On day 5, thickness of peritoneum, infiltration of inflammatory cells, and deposition of complement etc. were compared between the groups.

Results: On day 5, microscopic findings in L group was less plaques and less edematous. Histologically,the thickness of peritoneum,infiltration of inflammatory cells and deposition of complement both C3 and membrane attack complex(MAC) in L group was significantly reduced than those in V group. In addition, the mesothelial layer on peritoneal surface in L group recovered earlier compared with that in V group.The proliferation of mesothelial cell was confirmed in vitro experiment.

Conclusions: Administration of LASCs into the peritoneal cavity suppressed the inflammation of peritonitis induced by zymosan, and the mesothelial cell layer in L group recovered earlier than that in V group. These data suggest that LASCs have the multiple effects to peritoneal damage.In the future, LASCs therapy may be useful for peritoneal injury during fungal infection of the peritoneum.

TH-PO475

Block of Par1 Signaling Inhibits Metanephric Kidney Growth and Differentiation In Vitro Kimberly J. Reidy,[1] Zhongfang Du,[1] David Cohen,[2] Anne Muesch,[2] Jonathan M. Barasch,[3] Katalin Susztak.[4] *[1]Pediatric Nephrology, Children's Hospital at Montefiore, Bronx, NY; [2]Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY; [3]Nephrology, Columbia University, New York, NY; [4]Nephrology, Albert Einstein College of Medicine, Bronx, NY.*

Background: We have identified expression of Partitioning defective Par1 polarity proteins in developing S-shape nephrons. Par1a/b are serine threonine kinases that are functionally redundant and genetic deletion of both results in embryonic lethality. Par1 and a complex of Par3/Par6/aPKC localize to distinct portions of cell membranes to establish polarity. Podocyte-specific deletion of aPKC induced foot process effacement, proteinuria and glomerulosclerosis. We hypothesized that Par proteins may contribute to nephron differentiation, and sought to define the temporal and spatial expression of Par1a/b during embryonic kidney development, and to examine the effect of Par1 signaling on nephrogenesis in vitro.

Methods: Par mRNA and protein expression of was examined using embryonic kidneys obtained from timed-pregnant Sprague Dawley rats. Localization of Par1a and 1b was examined by immunofluorescence (IF). To examine the effect of Par1 signaling, isolated metanephroi were infected with a GFP tagged dominant negative Adeno-GFP-DNPar1 construct or control Adeno-GFP constructs, induced with LIF, and cultured for 7-14 days.

Results: Par1 mRNA expression correlated with expression of factors involved with nephron segmentation including Notch2 and Lhx1. Par1a, 1b and Par3 proteins are expressed at low levels at E13, and expression increased at E15. Par1b phosphorylation was also regulated during nephrogenesis. In vitro blockade of Par1 signaling with the DNPar1 construct during nephrogenesis resulted in stunted growth. Early mesenchymal to epithelial transition was not inhibited, but expression of podocalyxin was impaired.

Conclusions: These data suggest that Par1a/b may contribute to nephron development, and studies are ongoing to examine their role in cell proliferation, apoptosis and differentiation during nephrogenesis.

Funding: NIDDK Support

TH-PO476

Both Excessive and Deficient Maternal Salt Intake Decrease Nephron Number and Cause Delayed Hypertension in the Offspring Grzegorz Piecha,[1] Marie-Luise Gross-Weissmann,[2] Eberhard Ritz,[3] Nadezda Koleganova.[2] *[1]Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland; [2]Institute of Pathology, University of Heidelberg, Germany; [3]Department of Internal Medicine, Division Nephrology, University of Heidelberg, Germany.*

Background: An adverse environment during fetal development has life-long consequences: hypertension, increased cardiovascular risk, renal malfunction. Using stereology we studiedwhether high salt intake in pregnancy modifies kidney structure as well as blood pressure in the offspring.

Methods: Sprague-Dawley rats were fed low (0.15% NaCl), medium (1.3%), or high (8.0%) salt diets during pregnancy and weaning. Offspring were weaned at 4 weeks of age and subsequently received a standard rodent diet. Blood pressure was measured by telemetry and albuminuria by a rat specific ELISA up to 52 weeks of age. The nephron number at 12 weeks of age was determined using design-based stereology.

Results: The nephron number was significantly lower in offspring of dams on low (males: 19100±3700 per kidney, females: 19000±2500) and high (m: 12100±600, f: 12800±2800) compared to medium salt intake (m: 32400±2500, f: 28500±6000). In male offspring of dams on both low and high salt intake baseline albumin excretion was higher than in offspring of dams on medium salt intake from 6 months of age. Albumin excretion increased after 1 and 2 weeks of high salt intake. The increase in albuminuria was significantly higher in both male and female offspring of dams on high and low salt diet respectively.

Conclusions: Systolic, diastolic, and mean arterial pressures were not significantly different between the offspring until 6 months of age. Starting at age 7 months systolic blood pressure was higher in offspring of dams on low (123±8 mmHg) and high (124±7) compared to medium (116±5) salt intake and remained higher until 12 months of age.

TH-PO477

The Expression of Prox1 Is Regulated by the Osmolality in Mouse Renal Medulla Yumi Kim, Wan-Young Kim, Sun-Ah Nam, Jin Kim. *Anatomy and MRC for Cell Death Disease Research Center, Catholic University of Korea, Seoul, Republic of Korea.*

Background: Prox1, prospero-related homeobox1 transcription factor, is known to be expressed in various internal organs and is related to those differentiations. We previously reported that Prox1 is expressed in the development of the ascending thin limb (ATL) of Henle's loop by transformation of the thick ascending limb (TAL) in the developing mouse kidney. The maximum urine osmolality develops progressively during postnatal life, while it is about half that of adults in neonates. Prox1 expression areas in the developing region of ATL change according to these osmolality increasing. The purpose of this study was to examine whether the expression and distribution of the Prox1 change with the osmolality in vitro and in vivo condition by immunohistochemistry and Western blot analysis.

Methods: We performed in vitro experiments with MDCK cells under various osmotic culture conditions. And adult male C57BL/6 mice were fed a low-potassium diet for 2 weeks for hypokalemic condition, and control animals received normal diet in vivo experiments.

Results: Prox1 was expressed strongly in nucleus and weakly in cytoplasm of MDCK cells under isotonic medium. In contrast, when MDCK cells were cultured in hypotonic or hypertonic medium, the nuclear immunoreactivity decreased while the cytoplasmic immunoreactivity weakly remained. Furthermore, after 2 wk on a low-potassium diet, urinary osmolality significantly decreased. In the normal adult kidney, the immunoreactivity for Prox1 in ATL was weak in initial part, but not in terminal part of renal papilla. However, Prox1 was expressed not only in initial part of renal papilla but also in terminal part of renal papilla in the potassium depleted mice. And the intensity of Prox1 immunoreactivity was increased in the potassium depleted mice compared to normal mice. Thus as seen both in vitro and in vivo, Prox1 was expressed in the osmotic condition within optimal range.

Conclusions: These results suggested that the expression of Prox1 is regulated by osmolality in the mouse renal medulla.

Funding: Government Support - Non-U.S.

TH-PO478

Abstract Withdrawn

TH-PO479

Diabetes Mellitus and Pup Sex Influence Congenital Kidney Abnormalities in the ACI Rat Pascale H. Lane. *Pediatrics, University of Nebraska Medical Center, Omaha, NE.*

Background: The ACI rat develops congenital abnormalities of the kidney and urinary tract (CAKUT) in 10-15% of offspring via an autosomal dominant mutation with variable penetrance. In humans, maternal diabetes mellitus (MDM) increases the risk of CAKUT by ~15%. We tested the hypothesis that MDM would increase the rate of CAKUT in ACI rats.

Methods: Female ACI rats received streptozocin (MDM) or saline (Control; C) on day 1. Tail-vein blood glucose >300 mg/dL 2-3 days later defined the presence of diabetes. They were then housed with males until signs of pregnancy developed. After spontaneous delivery, pups were weighed and sacrificed on day 3-5 of life. The anterior abdominal wall and peritoneal contents were removed, and the urinary tract photographed with a dissecting microscope. The kidneys were then removed and weighed.

Results: Maternal glucose at the time of pup harvest averaged 173±6 in C and 420±15 in MDM ($p<0.001$). 111 litters (61C and 50MDM) demonstrated no differences in litter size (C 6±0.2 vs MDM 6±0.3) or sex distribution (C 3±0.2 males vs MDM 3±0.2 males).

Pup Characteristics

	Male C	Female C	Male MDM	Female MDM	Significance
PW, g	8.6±0.1	8.3±0.1	6.3±0.1	5.9±0.1	M,S
KW:PW, mg/g	12.6±0.2	13.1±0.2	11.9±0.2	12.7±0.2	M,S
CAKUT, %	13	11	21	14	p=0.08

PW: pup weight; KW:PW: total kidney weight/pup weight; M: maternal metabolic state; S: pup sex

MDM reduced PW ($p<0.001$) and KW:PW ($p=0.007$). Females had lower PW ($p<0.00$) but higher KW:PW ($p<0.002$). No interactions of maternal metabolic state and pup sex were demonstrated. The rate of CAKUT did not differ significantly with maternal metabolic state (C 12% vs. MDM 17%; $p=0.09$), although a strong trend toward increased relative risk was present (RR 1.39; 95% CI 0.97 to 1.99). This trend was most pronounced in male pups (RR 1.55; 95% CI 0.97 to 2.50).

Conclusions: ACI rat offspring from MDM show a strong trend toward increased CAKUT, particularly in male pups. This model may help us understand the mechanism(s) through which MDM increases CAKUT and the particular sex-specific risk of this condition.

Funding: Private Foundation Support

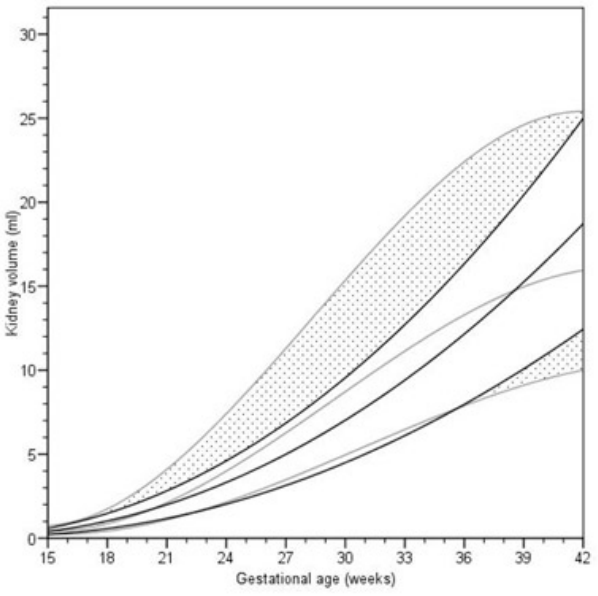
TH-PO480

Superior Size and Volume Charts for Fetal Kidney Development Derived from Longitudinal US Measuments Stefan Hendrik Van Vuuren,[1,2] Henny Damen Elias,[3] Robert H. Stigter,[4,3] Robert Van der Doef,[3] Tom P.V.M. De Jong,[2,6] Roel Goldschmeding,[1] Paul Westers,[5] Gerard H.A. Visser,[3] Lou Pistorius.[3] *[1]Pathology, UMCU, Utrecht, Netherlands; [2]Pediatric Renal Centre, UMCU, Utrecht, Netherlands; [3]Obstetrics, UMCU, Utrecht, Netherlands; [4]Obstetrics and Gynaecology, Deventer Hospital, Deventer, Netherlands; [5]Centre for Biostatistics, UU, Utrecht, Netherlands; [6]Pediatric Renal Centre, AMC, Amsterdam, Netherlands.*

Background: Kidney growth continues into puberty but available fetal growth charts suggest it halts soon after birth (Chitty et al, Prenat Diagn 2003;23:891-7). Therefore we established reference curves for sonographic size and volume of the fetal kidney and renal pelvis starting from the 15th week of gestation.

Methods: In a prospective longitudinal study the length and anteroposterior and transverse diameters of both kidneys and the anteroposterior and transverse diameters of the renal pelvises of 102 consecutive fetuses were measured by 4-weekly ultrasound examinations. We carried out multilevel statistical analysis for comparison of kidney dimensions with gestational age and femur length.

Results: Size charts for fetal kidney dimensions, kidney volume and renal pelvis were created and compared with previously published charts. These charts were significantly different from previous published charts by Chitty et al. (see figure).



Comparison of 3rd, 50th and 97th centiles for kidney volume estimation obtained in this study (black lines) and the 3rd, 50th and 97th centiles of Chitty[1] (gray lines).

The shadowed areas represent the potential loss of cases with abnormal growth by using the Charts presented by Chitty[1] in comparison with our data.

[1] Chitty LS, Altman DG: Charts of fetal size: kidney and renal pelvis measurements, Prenat Diagn 2003, 23:891-897

Furthermore, our charts showed a high correlation between kidney and femur length (r = 0.940), and between the kidney volume and the calculated volume based on femur length (r =0.941).

Conclusions: Longitudinal measurements have produced growth charts that differ from previous ones in that renal volume does not level off before birth, which is consistent with continued immediate postnatal growth. The added correlation charts with femur length will be useful to identify deviations in fetuses small or large for gestational age.

TH-PO481

The Role of Ubiquitin Specific Protease 40 in the Glomerular Endothelial Cells Hisashi Takagi,[1] Michael P. Madaio,[2] Kunimasa Yan.[1] *[1]Pediatrics, Kyorin University School of Medicine, Mitaka, Tokyo, Japan; [2]Nephrology and Kidney Transplantation Section, Medical College of Georgia, Augusta, GA.*

Background: The vascular endothelium is an essential structure of the glomerular tuft; however, the regulatory mechanism of vascularization in the developing glomerulus remains elusive. The ubiquitination pathway is a highly dynamic and coordinated process that regulates degradation of proteins within a cell, in which ubiquitin specific protease has the ability to remove ubiquitin from a ubiquitylated substrate. Novel glomerulus-enriched transcripts were recently reported, in which ubiquitin specific protease 40 (USP40) was found in mouse glomerulus.

Methods: Mouse USP40 cDNA was cloned from kidney cDNA library and used for generation of recombinant protein and establishment of stably expressing cell line. Polyclonal antibody was generated by using recombinant USP40. RT-PCR was done to determine expression of USP40 mRNA. Immunohistochemistry and immunoelectron microscopy were done to examine localization of USP40 in the mature and fetal kidney. siRNA experiment was done to examine the role of USP40 in podocalyxin biogenesis in the immortalized mouse endothelial cells.

Results: USP40 mRNA was abundantly expressed in the glomerulus compared with the rest of kidney. USP40 was specifically localized at microvascular endothelial cells intensely in the glomerulus, and moderately in the interstitium of mouse, rat, and human mature kidney. The subcellular localization of USP40 in the stable cell line and mouse glomerular endothelial cell line was observed in the cytoplasm, not in the specific organellas. Molecular migration of USP40 of cell lines, isolated glomeruli and the rest of kidney was revealed to be a 150-kDa. In the fetal kidney, USP40 was already recognized in the comma-shaped glomerulus. USP40 siRNA resulted in the drastic decrease of podocalyxin protein of glomerular endothelial cells.

Conclusions: The present data suggest that USP40 may play a crucial role in the glomerular development through interacting with ubiquitin, thereby regulating degradation of specific proteins of the microvascular endothelial cells including podocalyxin.

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TH-PO482

Gender Differences in Chronic Kidney Disease and Progression in Type 2 Diabetes Margaret K. Yu,[1] Courtney R. Lyles,[2] Bessie A. Young.[1,2,3] *[1]Division of Nephrology, University of Washington, Seattle, WA; [2]Department of Health Services, University of Washington, Seattle, WA; [3]Epidemiology Research and Information Center, VA Puget Sound, Seattle, WA.*

Background: The effect of gender on chronic kidney disease (CKD) and its progression in diabetes is not well defined. The objective of this study is to evaluate gender differences in CKD and CKD risk factors in type 2 diabetes.

Methods: The Pathways Study is a prospective cohort of ambulatory, diabetic patients from a large managed care system in Seattle, WA. Chi-square tests, two-sample t-tests, and logistic regression were used to determine gender differences in baseline CKD stage, risk factors, and progression. Risk of mortality was evaluated by Cox-proportional hazards modeling.

Results: Among 4800 enrollees, 4749 patients met entry criteria (48.6% women, 51.4% men). Women had a higher baseline prevalence of hypertension (45.0% vs 41.0%, p=0.008) and mean LDL (115.9 vs 107.8 mg/dL, p<0.001) than men. Fewer women than men had their LDL checked (60.1% vs 67.5%, p<0.001) or were prescribed a statin (26.5% vs 35.4%, p<0.001). Men had a higher prevalence of baseline microalbuminuria (16.5% vs 14.5%, p=0.032) and number of end organ complications (p<0.001). Women had a higher prevalence of moderate/severe CKD (GFR<60 ml/min, 35.9% vs 31.6%, p<0.001). In logistic regression models, women had a greater odds of progressing to a higher CKD stage (OR=1.34, 95% CI=1.13-1.60) but there was no difference in progression to ESRD at 5 years (p=0.451). Men had greater all-cause mortality at 5 years (HR=1.30, 95% CI=1.05-1.60) but after adjustment for diabetic complications the difference was no longer significant (HR=1.14, 95% CI=0.93-1.41).

Conclusions: Diabetic women had a higher prevalence of hypertension and hyperlipidemia. They were less likely than men to have their cholesterol checked or to have a statin prescribed. Women were more likely to progress to a higher CKD stage but there was no difference in progression to ESRD at 5 years. Men had a higher 5-year mortality which seems related to their higher number of end organ complications. Further studies on the effect of gender on CKD progression in diabetes are warranted.

Funding: NIDDK Support, Other NIH Support - NIMH, Veterans Administration Support

TH-PO483

Investigation of Trajectories of Renal Function Decline in Subjects with Type 1 Diabetes Mellitus and Proteinuria Jan Skupien, Adam Smiles, Marcus G. Pezzolesi, Monika A. Niewczas, Robert C. Stanton, James Warram, Andrzej S. Krolewski. *Joslin Diabetes Center and Harvard Medical School, Boston, MA.*

Background: The modern-day natural history of renal function decline in patients with type 1 diabetes mellitus (T1D) and nephropathy remains unknown.

Methods: We identified 244 patients under care of the Joslin Clinic who had T1D and proteinuria, with normal renal function. These patients were followed for 5 to 18 years (98% until 2008). The multiple measurements of serum creatinine obtained during follow-up and eGFR (estimated glomerular filtration rate) values calculated with CKD-EPI formula were used to determine long-term trajectories of renal function changes.

Results: In one-half of the cohort, renal function was stable or decreased slowly (non-decliners); in the other half, renal function decreased rapidly (-3.5 to -71 ml/min/1.73m2/year) with almost 100% risk of ESRD (decliners). The observed trajectories according to three periods of enrollment into the study are summarized in the Table.

Patterns of trajectories	Calendar year of enrollment			
	1991-1995	1996-2000	2001-2004	Total
	n=74	n=72	n=98	n=244
	Percent			
Non-decliners	35.1	48.6	56.1	47.6
Decliners				
linear slope	23.0	25.0	17.3	21.3
decelerating	13.5	5.5	6.2	8.2
accelerating	18.9	4.2	0.0	6.9
indeterminate	9.5	16.7	20.4	16.0

Most decliners had linear trajectory of eGFR loss. Transitions to slower rate of decline (deceleration) were rare. The risk of development of rapid decline in subjects with stable renal function was 28% in the sub-cohort with the longest follow-up.

Conclusions: Almost half of the patients with proteinuria had stable renal function and minimal risk of ESRD (end-stage renal disease) during their lifetime. The other half of the patients are at risk of ESRD, but time to onset varied between 2 and 20 years due to very variable rate of renal function decline, which was constant in the majority of the decliners. Infrequent decelerating trajectories suggest that the current therapies are ineffective in delaying renal function loss in decliners. It may be hypothesized that the risk of ESRD is primarily determined by currently non-modifiable, early, constitutional (possibly genetic) factors.

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TH-PO484

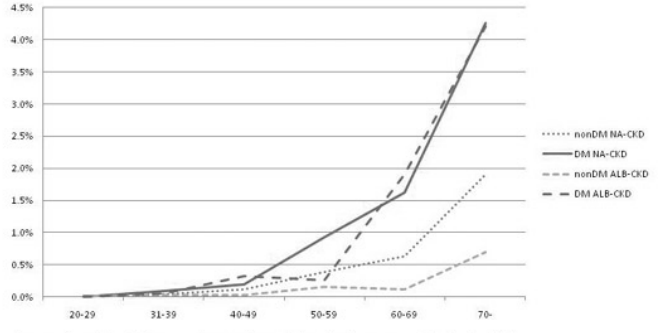
Normoalbuminuric Chronic Kidney Disease in the U.S. Population Amy K. Mottl,[1] Keunsang Kwon,[2] Susan L. Hogan,[1] Abhijit V. Kshirsagar.[1] *[1]University of North Carolina, Chapel Hill, NC; [2]Chonbuk National University, Jeonju, Korea.*

Background: At least one-third of diabetics have decreased estimated glomerular filtration rate (eGFR) < 60ml/min/1.73m^2 in the absence of albuminuria. Our goal was to determine the age-specific prevalence of nonalbuminuric CKD in a sample of the U.S. population and to describe any potential demographic and clinical differences from albuminuric CKD.

Methods: The National Health and Nutrition Examination Survey (NHANES) 2001-2008 cohort included 18,587 participants ≥ 20 years and with both serum creatinine and urine albumin:creatinine ratio (UACR) available for analysis. We determined stratum specific prevalence rates and 95% confidence intervals.

Results: Diabetes was present in 13% of the total population. Nonalbuminuric CKD was prevalent in 7% of diabetic and 3% of nondiabetic participants. Decreased eGFR with albuminuria was prevalent in 7% and 1% of diabetic and nondiabetic participants, respectively. There is a clear relationship between age and the prevalence of decreased eGFR (Figure 1). This relationship is equivalent in diabetics regardless of albuminuric status. In nondiabetics the effect of age is much stronger in the nonalbuminuric stratum. Moreover, in the nondiabetic stratum, nonHispanic Blacks have more albuminuric than nonalbuminuric CKD: 12% (8-17%) versus 3% (2-4%). Nondiabetic women and nonHispanic Whites have a preponderance of nonalbuminuric CKD: 68% (63-73%) versus 53% (45-61%) and 93% (91-95%) versus 78% (73-84%), respectively.

Conclusions: In conclusion, in the U.S. population the prevalence of nonalbuminuric CKD is greater in diabetic than nondiabetic people. Albuminuria is an effect modifier of the relationship between age and decreased eGFR in nondiabetic but not diabetic populations. Nondiabetic, nonalbuminuric CKD is more predominate in women and nonHispanic Whites whereas albuminuric CKD predominates in nonHispanic Blacks.



Age-specific relationship between the prevalence of albuminuric versus nonalbuminuric CKD in diabetic and nondiabetic participants of 2001-2008 NHANES.

TH-PO485

Renal Outcomes after Remission of Albuminuria in Diabetic Patients Nobue Tanaka, Tetsuya Babazono, Ko Hanai, Yasuko Uchigata. *Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan.*

Background: It is known that the reductions of albuminuria are observed in some of diabetic patients. However, the clinical course of albuminuria after the episode of remission has not been well described. We therefore performed this observational cohort study to evaluate the renal outcomes after remission of albuminuria in diabetic patients.

Methods: A total of 1237 albuminuric patients with diabetes were enrolled in this cohort study between 2003 and 2005, and were followed up until 2010. During the observational period, the remissions of albuminuria were found in 310 patients. There were 30 type 1 and 280 type 2 diabetic patients, and the mean (± SD) age at the remission of albuminuria was 58 ± 13 years, including 230 patients with remission from micro- to normoalbuminuria and 80 patients with remission from macro- to microalbuminuria. We assessed the cumulative incidence of re-progression of albuminuria from the onset of remission using the Kaplan-Meier method. The clinical factors associated with the progression of albuminuria were analyzed by the Cox proportional hazard model, adjusting for age, sex, systolic and diastolic blood pressure, estimated glomerular filtration rate (eGFR), urinary albumin-to-creatinine ratio (ACR), hemoglobin and glycated hemoglobin.

Results: The mean follow-up period after remission of albuminuria was 4.9 ± 1.5 years. Five-year cumulative incidences of the progression of albuminuria were 28.7% in normoalbuminuria and 35.0% in microalbuminuria. The clinical factors associated with the progression from normo- to microalbuminuria were higher urinary ACR (hazard ratio [HR] per log10 = 5.92, 95% confidence interval [CI] 1.16-30.3, p = 0.03), lower total cholesterol (HR = 0.99, 95%CI 0.98-1.00, p = 0.03) and higher triglyceride (HR per log10 = 4.11, 95%CI 1.54-10.99, p < 0.01). In microalbuminuric patients, lower eGFR at the remission of albuminuria was related to the progression to macroalbuminuria (HR; 0.97, 95%CI; 0.96-0.99, p < 0.01).

Conclusions: This longitudinal study suggests that the progression of albuminuria occur even after remission of albuminuria in a considerable number of diabetic patients.

TH-PO486

Control of Postprandial Hyperglycemia Is Critical for Renal Preservation in Diabetes Anil K. Mandal,[5812] Linda M. Hiebert.[0980] *[1]Dept of Medicine, Univ of Florida, Gainesville, FL; [2]Veterinary Biomedical Science, Univ of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Background: We have shown that 2 hour postprandial glucose (2hPPG) and dglucose (2hPPG-Fasting glucose) are significantly related to renal function change (Diab Res Clin Pract 2011; 91: 190 – 194). HbA$_1$c is most commonly used to determine glycemic control in diabetes mellitus (DM). We asked the question: Which glycemic parameters better predict renal function changes in long-term follow up of DM?

Methods: 25 adult patients with established DM were followed in office for an average 8.4 months. DM diagnosis was established by 2 hour postprandial glucose (2hPPG) above 200 mg/dL on two occasions. For brevity, Type I and Type II are not used. Mean age is (mean ± SD) 63.18 ± 11.55 years; males (n=11) and 67.36 ± 8.12 years females; (n=14). They are followed at 6 - 8 weeks with a laboratory of fasting and 2hPP basic metabolic panel consisting of fasting glucose (FG), 2hPPG, and BUN, Serum creatinine (Scr), HbA$_1$c, and average glucose (AVG), prior to office visit. dglucose was calculated. They are treated with a combination of insulin glargine (Lantus®) after breakfast and dinner; and regular insulin based on finger stick glucose 2 hours after each meal and at bed time. Spearman's rank correlation coefficients were obtained; measurements over a mean of 9.5 visits are averaged for each subject.

Results:

(n = 25; mean ± SEM)
FG (mg/dL) 157.5 ± 7.1 (115.3 – 216.3)
2hPPG (mg/dL) 239.9 ± 9.6 (137.6 – 380.4)
dglucose (mg/dL) 87.1 ± 8.9 (0.4 – 205)
HbA1c (%) 8.3 ± 0.3 (6-14)
AVG (mg/dL) 193.4 ± 9.1 (128 – 356)
F Scr (mg/dL) 1.4 ± 0.1 (0.7-2.7)
2hPP Scr (mg/dL) 1.5 ± 0.1 (0.8-3.1)

Correlations between (1) 2hPPG and dglucose and (2) F Scr and 2hPP Scr are statistically significant (-0.49 < r < -0.44; P < 0.03 in every case). Scr levels were at the normal range and decreased with glycemic control. No correlation was found between F Scr and HbA$_1$c (r = 0.099; P = 0.6382), or 2hPP Scr and HbA$_1$c (r = -0.012; P value = 0.9534).

Conclusions: 1) This study reconfirms that treatment of DM with insulin with dglucose of less than 100 mg/dL is a strong predictor of renal preservation in DM.

2) HbA$_1$c is not a dependable predictor of renal function change in DM.

TH-PO487

Prognostic Predictor for Renal Insufficiency and Its Association with Diabetes Mellitus in Patients with Acute Myocardial Infarction Chang Seong Kim, Joon Seok Choi, Jeong-Woo Park, Eun Hui Bae, Seong Kwon Ma, Myung Ho Jeong, Soo Wan Kim. *Department of Internal Medicine, Chonnam National University Medical School, Gwnagju, Korea.*

Background: The existence of diabetes mellitus (DM) and renal dysfunction may individually or simultaneously have a negative prognostic effect on patients with acute myocardial infarction (AMI). However, few studies are assessed the role of renal insufficiency and its association with DM in the context of AMI. The aim of this study was to investigate the interaction between renal dysfunction and DM in patients with AMI.

Methods: We analyzed a total of 9905 patients with AMI were enrolled in a nationwide prospective Korea Acute Myocardial Infarction Registry from November 2005 to August 2008. Patients were categorized into 4 groups using the presence of DM and renal insufficiency (GFR < 60 ml/min/1.73m^2). Group I : no DM and no renal insufficiency (n=5700); Group II : DM and no renal insufficiency (n=1730); Group III : no DM and renal insufficiency (n=1431); Group IV : DM and renal insufficiency (n=1044). The primary end points were major adverse cardiac events including a composite of all cause of death, myocardial infarction, target lesion revascularization and coronary artery bypass graft during a 12-month follow-up.

Results: During 12-month follow-up, the primary end points occurred in 1804 patients (18.2%). There were significant differences on composite MACE in 12-month follow-up among the 4 groups (Group I, 12.5%; Group II, 15.7%; Group III, 30.5%; Group IV, 36.5%; *p* value <0.001). There was stepwise increased in composite MACE with increasing stage of group. In a Cox proportional Hazards model, after adjusting for multiple covariates, going from group II to group IV, the mortality at 12-month was also increasing stepwise compared with Group I (HR 1.96, 95% CI 1.34-2.86, p=0.001; HR 2.42, 95% CI 1.62-3.62, p <0.001, respectively).

Conclusions: Renal insufficiency and its association with DM provide the increment of a composite MACE and the poor prognostic predictor in patients with AMI. Moreover, categorization using presence or absence of DM and renal insufficiency provides valuable information for early risk stratification in patients with AMI.

TH-PO488

Race and Obestity Strengthen the Association of Retinopathy and Low Glomerular Filtration Rate in Type 2 Diabetes Amy K. Mottl,[1] Keunsang Kwon,[2] Seema Garg,[1] Abhijit V. Kshirsagar.[1] *[1]University of North Carolina, Chapel Hill, NC; [2]Chonbuk National University, Jeonju, Korea.*

Background: In type 2 diabetes, decreased estimated glomerular filtration rate (eGFR) may occur in the absence of albuminuria or retinopathy. Determining which characteristics strengthen the relationship of low GFR and retinopathy may provide further insight to the risk and pathogenesis of microvascular complications. NonHispanic Blacks and obese individuals are known to have a greater risk for progression of retinopathy and nephropathy. We hypothesized these two factors may strengthen the association of diabetic eye and kidney disease.

Methods: The National Health and Nutrition Examination Survey (NHANES) 2005-2008 cohort included approximately 1300 participants with older onset diabetes who had retinopathy, serum creatinine and urinary albumin:creatinine ratio (UACR) data. We used multivariate regression analyses to assess the relationship between retinopathy and low eGFR and UACR. Covariates included age, gender, HbA1C, systolic and diastolic blood pressures. Analyses were stratified to evaluate ethnicity/race and body mass index (BMI) as effect modifiers of this relationship.

Results: Of 269 participants with decreased eGFR, 35% had no microalbuminuria and no retinopathy; 16.1% had retinopathy with no microalbuminuria; 27.1% had microalbuminuria and no retinopathy and 22% had both microalbuminuria and retinopathy. Multivariate logistic regression analyses stratified by ethnicity demonstrated retinopathy to be more significantly predictive of decreased eGFR in nonHispanic Blacks than nonHispanic Whites (OR=2.68 ; 95% CI 1.17, 6.14). Multivariate logistic regression analyses stratified by BMI demonstrated retinopathy to be more predictive of decreased eGFR in participants with a BMI ≥ 30 (OR = 2.62; 95% CI 1.26, 5.46). Analyses of albuminuria showed no association with retinopathy and no effect modification by ethnicity or BMI.

Conclusions: In older onset diabetes the absence of albuminuria and retinopathy is common among individuals with reduced eGFR. Retinopathy is predictive of low eGFR but not albuminuria. This relationship appears to be stronger in nonHispanic Blacks and obese individuals.

TH-PO489

Epicardial Fat Volume in Stage 3-5 CKD Patients and Its Associations with Obesity, CKD, and Coronary Artery Calcification Jasmine D. Kerr,[1] Rachel M. Holden,[1] Alexander R. Morton,[1] Robert Louis Nolan,[2] Jocelyn S. Garland.[1] *[1]Department of Medicine, Queen's University, Kingston, ON, Canada; [2]Department of Radiology, Queen's University, Kingston, ON, Canada.*

Background: Epicardial fat volume (EFV) is associated with coronary artery calcification (CAC) and may play a role in the pathogenesis of cardiovascular disease (CVD). It also correlates with the metabolic syndrome, a common co-morbid condition in chronic kidney disease (CKD). Our objectives were to quantify the EFV in stage 3-5 CKD patients and determine its relationship with CKD, metabolic syndrome, and CAC.

Methods: In this cross-sectional study, 95 pre-dialysis patients with stage 3-5 CKD underwent multi-slice cardiac CT for determination of their CAC scores and EFV. EFV was determined as the average volume of two slices at the level of the left main coronary artery.

Results: Mean age was 64 years, 57% were male, 80% had metabolic syndrome, 40% had diabetes, mean BMI was 32kg/m^2, and mean eGFR was 25mL/min/1.73m^2. Mean EFV was 5.01cm^3. Log UACR (r=0.33, P=0.001), abdominal waist circumference, (r=0.5, P=0.0001), HOMA-IR (r=0.4, P=0.001), log CAC score (r=0.29, P=0.006) and log FGF-23 (r=0.23, P=0.03) were significantly associated with EFV. eGFR and serum phosphorus level were not. By linear regression, log UACR (ß=0.60; 95% confidence interval, 0.10 to 1.10; P=0.02), abdominal obesity (ß=2.02; 95% confidence interval, 1.18 to 2.9; P<0.0001) triglycerides (ß=-1.34; 95% confidence interval, -2.64 to -0.03; P=0.045), and log CAC score (ß=0.42; 95% confidence interval, 0.04 to 0.80; P=0.03) were all independent risk factors for increased EFV.

Conclusions: Recognizing the limitation of comparing EFV across different study populations, the burden of EFV appears to be increased in this CKD cohort. EVF was associated with traditional and non-traditional CVD risk factors known to contribute to CAC. The association of FGF-23 and EFV is intriguing and warrants further study, but suggests a potential link between visceral fat, insulin resistance, and CAC in CKD. Studies are needed to clarify the role of epicardial fat in the etiology of CVD and to determine if EFV is a useful measure of cardio-metabolic risk in CKD patients.

TH-PO490

Prevalence of CKD in Type 2 Diabetes – First Results of a Nationwide Study in German Primary Care Physicians Ludwig F. Merker,[1] Baptist Gallwitz,[2] Katja Schoene,[3] Barbara Waldeck.[3] *[1]Diabetes and Kidney Center, Dormagen, NRW, Germany; [2]Med. Klinik 4, Universität Tübingen, Tübingen, Germany; [3]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany.*

Background: No systematic prospective nationwide database on the prevalence of chronic kidney disease (CKD) in type 2 diabetes is available.

Methods: We recruited 2500 consecutive type 2 diabetics from 250 primary care physicians randomized by epidemiologic aspects. The following data was collected by questionnaire: antidiabetic and antihypertensive medication, CKD status, hypertension and comorbidities. On site urinanalysis was carried out by dipstick, the same for microalbuminuria (MAU). Central lab included HbA1c, serum creatinine, ACR and lipid profile; eGFR (ml/min/1,73m²) was estimated using MDRD formula. Pts with signs of UTI were excluded from MAU screening. The study was performed according to GCP standards and approved by German ethics committees. Statistics are descriptive. To ensure data quality 10 % of the PCP's are monitored. Data collection will finish in July 2011. Here we present the first data.

Results: So far, data is available from 373 pts (213 male, 160 female) on average: age 69.9 yrs., BMI 30,4 kg/m2, HbA1c 6,8 %, diabetes duration 8,3 yrs., 79,9 % hypertension, duration 10,9 yrs., 96,3 % were treated, 37,3 % reached < 130/80 mm Hg. 84,5 % patients showed co morbidities, CKD 3 (eGFR 30-60) in 25,7 %; this was recognized in 43,8 % of the PCP. 55 % had MAU, 56,2 % had eGFR estimation and 9,2 % had clearance calculation (Cockr.-Gault) by the PCP. Elevated ACR was seen in 31,2 % (of which 2/3 were male). eGFR >60 without MAU was detected in 54,1 %, isolated MAU in 17,8 %, macroalbuminuria in 2,9 %, macroalbuminuria and eGFR <60 in 2,2 %, isolated eGFR <60 in 14,6 % and MAU as well as eGFR <60 was seen in 8,2 %.

Conclusions: CKD detection by MAU screening only is not sufficient. An eGFR calculation should always be performed when serum creatinine is measured. This should be mandatory in particular in elderly patients. Hypertension is nearly always detected, but treatment goals are not always met. The final study results are pending. These data were first presented at DDG on June 3rd, 2011 as a German language poster (#284).

Funding: Pharmaceutical Company Support

TH-PO491

Mineral Metabolism Disturbances and Atherosclerosis in Type 2 Diabetic CKD Patients Ana Paula Silva, Ana Pinho, Ana Cabrita, André Fragoso, Nelson Almeida Tavares, Nélio Santos, Pedro Neves. *Nefrology, Hospital Faro, Faro, Portugal.*

Background: Epidemiologic studies have demonstrated that the carotid intima-media thickness (CIMT) is a strong and independent predictor of cardiovascular events in both the general population and among those with renal disease.

Methods: In chronic kidney disease (CKD) patients, the disturbances of the mineral metabolism are also related with cardiovascular disease.

The purpose of this study was to evaluate the relationship between the carotid intima media thickness with the mineral metabolism (vitamin D, FGF23, PTH, phosphorus, calcium), inflammation (interleukin 6), oxidative stress (malonaldehyde) and insulin resistance (HOMA-IR) in a group of type 2 CKD diabetic patients.

In this cross-sectional study, we included 50 type 2 diabetic patients (f=18, m=32) with mild and moderate renal disease (mean estimated GFR = 50 ml/min). We measured the CIMT using the high-resolution B-mode ultrasonography.

The population was divided according to the CIMT in 2 groups: G1 (n=25) – CIMT ≥ 0.9 mm and G2 (n=25) - CIMT <0.9 mm. We performed descriptive statistics. Student *t* and Chi-Square tests were used for comparison between groups. We also used a single regression model to evaluate the relation between the CIMT with the FGF-23 and 25(OH) D3 levels.

Results: G1 showed higher malonaldehyde (3.7 *vs* 3.2 mg/dl, p = 0.013), PTH (173.4 *vs* 110.6 µg/ml, p = 0.014), FGF 23 (146.6 *vs* 73.8 ng/mL, p = 0.0001), IL6 (8.6 *vs* 5.8 pg/ml p = 0.008), TNFα (10.4 *vs* 7.5 pg/ml p = 0.001), and lower 25(OH)D3 (10,4 *vs* 24.9 ng/ml p = 0.004) levels. There were no differences regarding the other parameters, such as total cholesterol, phosphorus, HgA1c, HOMA-IR and eGFR. Furthermore, we found in the regression model, that FGF-23 (r=0.785 p=0.0001) and 25(OH)D3 (r= - 0.432 p= 0.002) were significantly related with CIMT.

Conclusions: We concluded that in diabetic patients with early stages of CKD there is a relationship between the mineral abnormalities and the CIMT, a known marker of subclinical atherosclerosis. Further prospective analysis are required to validate the clinical significance role of these new markers of cardiovascular in our renal patients.

Funding: NIDDK Support

TH-PO492

ATLANTIC DIP: Extra Counselling Required for Women with Diabetes and Microalbuminuria Embarking on Pregnancy Andrew Smyth,[1,2] Eoin O'Sullivan,[1] Louise A. Carmody,[1] Gloria E. Avalos,[3] Fidelma P. Dunne.[1,3] *[1]Endocrinology, Galway University Hospitals, Galway, Ireland; [2]Nephrology, Galway University Hospitals, Galway, Ireland; [3]School of Medicine, National University of Ireland, Galway, Ireland.*

Background: Pregnancies in patients with pre-existing diabetes are associated with higher rates of morbidity. This study aims to assess the effect of microalbuminuria on pregnancy outcomes in patients with established diabetes pre-pregnancy.

Methods: Pregnancies in patients with established diabetes at five centres between January 2005 and December 2009 were retrospectively reviewed. Outcome measures included birth outcome (live birth, stillbirth, miscarriage and delivery method), maternal complications (hypertension, pre-eclampsia, polyhydramnios, hemorrhage) and neonatal outcomes (gestational age, birth weight, birth height, shoulder dystocia, malformations, jaundice and hypoglycemia). Analysis was performed using PASW for Mac Version 18.0, p<0.05 was considered significant using Chi-square and t-test where appropriate.

Results: 132 pregnancies (81.8% (n=108) type 1 and 18.2% (n=24) type 2 diabetes) were included, with a mean age of 31.9+/-5.6 years and median duration of diabetes prior to pregnancy of 12.6 years (0.4-33.9years). Albumin:creatinine ratio (ACR) pre-pregnancy was available in 100% (n=132) with a mean of 6.55mg/mmol (0-214); 22% (n=29) of these had microalbuminuria. Maternal hypertension occurred in 18.5% (n=20), pre-eclampsia in 13% (n=14), polyhydramnios in 10.4% (n=11) and hemorrhage in 2.8% (n=3). Patients with microalbuminuria had higher rates of polyhdramnios (p=0.04) and hypertension (p=0.05), as well as higher systolic blood pressure early in pregnacy (128.6mmHg versus

119.4mmHg, p=0.01). Patients with microalbuminuria were more likely to have babies who were either large or small for gestational age (p=0.05). There was no association between microalbuminuria and birth or neonatal outcomes.

Conclusions: This study shows that the presence of pre-pregnancy microalbuminuria is associated with higher rates of maternal hypertension, polyhydramnios and larger or small babies. Future studies should explore ways of reducing morbidity within this cohort of pregnant women.

Funding: Government Support - Non-U.S.

TH-PO493

Recurrence of Diabetic Kidney Disease in Type 1 and Type 2 Diabetic Patients after Kidney Transplantation Izumi Nyumura,[1] Tetsuya Babazono,[1] Kazuho Honda,[2] Yasuko Uchigata.[1] *[1]Department of Diabetes Center, Tokyo Women's Medical University School of Medicine, Tokyo, Japan; [2]Department of Pathology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.*

Background: Renal lesions are more heterogeneous in type 2 than type 1 diabetic patients due to modification by aging and concomitant obesity, hypertension and lipid disorders. However, information is scarce regarding whether severity of recurrent diabetic kidney disease (DKD) in the renal allograft may differ in type 1 and type 2 diabetic patients. We therefore conducted this study to highlight the relationship between type of diabetes and clinical or histological parameters associated with the recurrence of DKD in renal allograft.

Methods: We studied 34 diabetic renal allograft recipients, 17 with type 1 and 17 with type 2 diabetes who underwent renal biopsy both in the intraoperative period and at 3-16 years after transplantation. Glomerular morphometrical analysis including mesangial area, capillary tuft number and capillary tuft area was performed by a computer-assisted image analyzer. The thickness of glomerular basement membrane (GBM) was evaluated by an electron microscopy. The rate of decline in estimated glomerular filtration rate (eGFR) and albuminuria were evaluated as clinical parameters.

Results: Recipient's age and body mass index (BMI) were significantly higher in type 2 than type 1 diabetes. There were no significant differences in terms of donor's age, posttransplant observational period, blood pressure, HbA1c and lipid parameters. Increase in mesangial glomerular areas and thickness of GBM were significantly greater in type 2 diabetes (p=0.032 and p=0.005), however, after adjustment for covariates including recipient's age, BMI, HbA1c, blood pressure and lipid parameters by covariance analysis, the statistical significance disappeared. Glomerular capillary number and area, the grade of tubulo-interstitial lesions, and vascular lesions, as well as the rate of decline in GFR and albuminuria were not different in the two groups.

Conclusions: Type of diabetes is less likely to be associated with the histological and clinical parameters of the recurrence of DKD in diabetic transplanted patients.

TH-PO494

Predictive Factors for Progression to End-Stage Renal Disease in Diabetic Patients with Chronic Kidney Disease Stage 4-5 Tetsuya Babazono, Izumi Nyumura, Ko Hanai, Nobue Tanaka, Yasuko Uchigata. *Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Although several factors have been shown to be associated with higher risk of the onset and progression of CKD in diabetic patients; predictive factors for ESRD in diabetic patients with later stages of CKD have never been determined. We therefore conducted this observational cohort study to identify clinical factors associated with higher risk of ESRD in diabetic patients with CKD stage 4-5.

Methods: We studied a total of 131 diabetic patients with CKD stage 4-5, 58 and 73 patients with stage 4 and 5, respectively. There were 39 women and 92 men and the mean age at the start of follow-up was 63 ± 12 years (range: 32-88 years). The primary endpoint was the composite of the start of RRT and death. Patients were observed at least 6 months. Effects of age, sex, urinary albumin-to-creatinine ratio (ACR) and eGFR at baseline, mean values of systolic and diastolic blood pressure, hemoglobin, glycated hemoglobin, serum albumin, uric acid, low- and high-density lipoproteins during the follow-up period on renal outcome were examined using the Cox proportional hazard model.

Results: The median follow-up period was 19 month (range: 6-137 months). Among 131 patients, 88 patients started dialysis and 10 patients died before initiation of dialysis. Five-year cumulative incidence of reaching the endpoint was 83.4% and the median follow-up period was 24.8 months. Among the covariates, higher urinary ACR and diastolic blood pressure, lower eGFR, serum albumin, and hemoglobin levels were significantly associated with higher risk of reaching the endpoints (p<0.05). Higher glycated hemoglobin and lower low-density lipoprotein levels had marginal effects (p<0.2).

Conclusions: Several clinical factors were identified as predictive factors of higher incidence of hard renal outcomes in diabetic patients with CKD stage 4-5. Whether modification of these factors may diminish the risk should be determined.

TH-PO495

Renal Outcome in Biopsy Proven Non Diabetic Renal Disease in a Type 2 Diabetic Multi-Ethnic Asian Population Soumita Bagchi,[1] Thomas Paulraj Thamboo,[2] Vathsala Anantharaman.[1] *[1]Nephrology Division, National University Health System, Singapore; [2]Pathology Department, National University Health System, Singapore.*

Background: Very few studies have investigated the factors affecting the outcome of non-diabetic renal disease (NDRD) in type 2 diabetics (DM). In this study we sought to determine the predictors for NDRD on renal biopsy in DM patients and define the correlates of a favorable renal outcome at 1 year.

Methods: 157 diabetics who had an adequate native kidney biopsy at the National University Hospital, Singapore from 2000 to 2010 were retrospectively reviewed.

Results: 87 (55.4%) patients had NDRD and 70 (44.6%) had diabetic nephropathy (DN). The predominant histopathologic diagnosis involved glomerular abnormalities (71.1%). On multivariate analysis, absence of retinopathy (P<0.001, OR=20.292) and sub-nephrotic proteinuria (P<0.001, OR=5.144) were significant predictors of NDRD.

After excluding 25 with ESRD at presentation and 13 lost to followup, 119 patients were evaluated for 1 year renal outcome. At follow-up, mean MDRD eGFR was higher for NDRD compared to those with DN (46.5±37.4ml/min vs 31.9±24.4ml/min, P<0.005) and proportion with eGFR<15ml/min was lower (8.5% vs 38.7 <0.001). DN on renal biopsy (P=0.001, OR=6.979), baseline serum albumin<35g/l (P=0.023, OR=4.976) and eGFR (P=0.006, OR=1.044) were independently associated with eGFR<15ml/min at 1 year.

34 (48.5%) of the 70 patients with NDRD studied for 1 year renal outcome, had stable or improving eGFR. In these patients, absence of hypertension (P=0.039, OR=5.089), absence of nephrotic range proteinuria (P=0.01, OR=7.183), eGFR<30ml/min at the time of biopsy (P=0.032, OR=4.472) and immunosuppressant use (P=0.003, OR=10.485) were independent predictors of stable or improving eGFR at 1 year.

Conclusions: Possibility of NDRD should be considered in DM patients with kidney disease since they have better renal outcome. Absence of retinopathy and sub-nephrotic proteinuria are independent predictors of NDRD. Absence of an active urinary sediment does not rule out NDRD. Finally, immunosuppressant use should be considered whenever applicable, since it is an independent modifiable predictor of favorable renal outcome in these patients.

TH-PO496

Correlation of Structural and Functional Abnormalities in Type 2 Diabetic Nephropathy with Overt Proteinuria Edmund J. Lewis,[1] Richard D. Rohde,[1] David J. Cimbaluk,[2] The Collaborative Study Group.[3] *[1]Nephrology, Rush University Medical Center, Chicago, IL; [2]Pathology, Rush University Medical Center, Chicago, IL; [3]Collaborative Study Group, Chgo, IL.*

Background: Our objective was to determine structure: function relationships in type 2 diabetic nephropathy (T2DN).

Methods: : Renal biopsies from 27 patients with T2DN were examined. These derived from the Irbesartan Diabetic Nephropathy pilot trial. Mean age 57±8.2 yrs; duration of diabetes: 15±10 yrs; mean BMI 33.5±7.6; mean serum creatinine (SD) (Scr) 1.6±0.5 mg/dl; mean creatinine clearance (Ccr) 63±28 ml/min; mean estimated GFR (eGFR) 52±20 ml/min/1.73m^2; mean proteinuria 4.7±4.2 G/day. Percent global sclerosis (GS) and semiquantitative tubular atrophy (TA) and chronic interstitial fibrosis (CIF) were measured. TA and CIF were scaled: 0 (0%); 1+ (<25%); 2+ (25-50%); 3+ (>50%).

Results: TA correlated with function; Ccr: 0/1+TA = 83.8±20.5 ml/min; 2+TA = 60.8±20.3 ml/min; 3+TA = 35.6±12.2 ml/min, P: 0/1+TA versus 2+TA = 0.04; P: 2+TA vs 3+TA = 0.04; P: 0/1+TA vs 3+TA = 0.00004.

Scr:0/1+TA = 1.3±0.8 mg/dl; 2+TA= 1.6 ±0.2 mg/dl, 3+TA = 2.0±0.2 mg/dl.

eGFR:0/1+TA = 64 ml/min/1.73m^2; 2+TA = 50.6 ml/min/1.73m^2; 3+TA = 35.6 m1/min/1.73m^2

% GS correlated with function:

Tertile 1 (n=9): Scr 1.1±0.2 mg/dl = 14.3% GS

P = 0.0007

Tertile 2 (n=9): Scr 1.6±0.1 mg/dl= 43.2% GS

P = 0.53.0

Tertile 3 (n=9): Scr 2.1±0.3 mg/dl= 50.8% GS

Tertile 1 vs 3: P = 0.0003

Correlation coefficients for % GS:

Scr: R^2 = 0.45; Ccr: R^2 = 0.50; eGFR: R^2 = 0.46

GS and TA were highly correlated: TA 0 = 0% GS; TA 1+ = 21% GS; TA 2+ = 38% GS, TA 3+ = 60% GS. Semiquantitative CIF did not correlate with creatinine parameters. Urine protein excretion did not correlate with TA, GS or CIF.

Conclusions: In this cross-sectional analysis of anatomic changes in the renal biopsy in T2 DN, TA and GS correlated with creatinine parameters of renal function. We found that a 0.5 mg/dl difference in Scr was associated with a mean threefold higher %GS. TA and GS correlated highly. T2 diabetes patients with advanced nephropathy have consistent structure: function relationships. These observations support the use of yearly creatinine parameters as clinical trial endpoints.

TH-PO497

Clinical Indications for Renal Biopsy in Diabetic Glomerulosclerosis with Superimposed Non-Diabetic Renal Disease Jon H. Steuernagle,[1] LaTonya J. Hickson,[2] Axel Pflueger,[2] Ziad El-Zoghby,[2] Sanjeev Sethi,[3] Mary E. Fidler,[3] Samih H. Nasr,[3] Lynn D. Cornell.[3] *[1]Internal Medicine, Mayo Clinic; [2]Nephrology and Hypertension, Mayo Clinic; [3]Anatomic Pathology, Mayo Clinic, Rochester, MN.*

Background: Patients with diabetes have been found to have non-diabetic renal disease (NDRD) on biopsy. The clinical reason for performing such biopsies is not well known from large sample studies.

Objective: To examine clinical indications for renal biopsies having diabetic glomerulosclerosis (DGS) and concomitant NDRD.

Methods: Our renal biopsy database was queried for DGS from 1994 to 2010 followed by review of each DGS report for NDRD. Renal pathologist comments on clinical indications for renal biopsy were recorded.

Results: Of 3575 biopsies with DGS, concomitant NDRD was present in 967 (27%). The most common NDRD was tubulointerstitial (69%), glomerular (29%) and atheroembolic vascular disease (2%). Tubulointerstitial NDRD included acute tubular necrosis (60%), interstitial nephritis (36%), and others (4%). Glomerular-NDRD included IgA (22%), pauci-immune crescentic (17%), membranous glomerulonephritis (16%), post-infectious (16%), membranoproliferative (8%), thrombotic microangiopathy (5%) and others (16%). Mean age was 59±14 years, 54% were male, and creatinine was 4.1±2.5 mg/dL at biopsy. Biopsy reasons included acute renal failure with or without proteinuria (74%), nephrotic range proteinuria (15%), proteinuria (2%), hematuria (1%), rapidly progressive renal failure (1%), and unknown (7%). Acute renal failure (79%) and nephrotic range proteinuria (11%) were primary biopsy reasons in tubulointerstitial NDRD similar to glomerular NDRDs such as IgA (58% and 25%, respectively).

Conclusions: NDRD is common among diabetic patients undergoing renal biopsy. NDRD is often treatable beyond conventional therapies utilized for DGS. Acute renal failure and nephrotic range proteinuria with or without other features help identify a potential need for biopsy in this group.

TH-PO498

Urinary Sulphate Excretion Is a Predictor for Progression of Diabetic Nephropathy Gudbjörg Andrésdóttir,[1] Stephan J.L. Bakker,[2] Henrik Post Hansen,[4] Hans-Henrik Parving,[3] Peter Rossing.[1] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Department of Internal Medicine, University Medical Center Groningen, Groningen, Netherlands; [3]Rigshospitalet, Copenhagen, Denmark; [4]Herlev Hospital, Denmark.*

Background: Hydrogen sulphide (H_2S) mediates smooth muscle relaxation and vasodilatation. Diabetes-related endothelial dysfunction may reduce it's biosynthesis. H_2S is converted to sulfite in the mitochondria, and then oxidized to thiosulphate and sulphate, these are excreted in the urine.

Methods: Urinary excretion of sulphate in type 1 diabetic patients with progressive diabetic nephropathy was measured to evaluate if sulphate excretion can predict progression of nephropathy. It was a post hoc study of a prospective, randomized, unmasked, controlled trial studying the effects of low/high protein diet, following 82 patients for 4 years. 72 patients (49 men) with 3 or more measurements of GFR (^{51}CR EDTA)were included in our study and rate of decline of GFR was determined. Sulphate excretion was measured by ion exchange chromatography in 24h urine at baseline.

Results: At baseline age [mean(SD)] was 40.3(7.9) years and GFR was 70(31) ml/min/1.73m2. Sulphate excretion [median(range)] was 11.1(1.9 - 32.2)mmol/day. During follow-up decline in GFR was 3.9(3.4) ml/min/year. U-sulphate was associated with age (p=0.001, r_s=-0.36) and SBP (p=0.049, r_s=-0.22). U-sulphate excretion showed a significant negative association with the rate of decline of GFR (p=0.016, r_s =-0.28). It was not associated with baseline values of; GFR, HbA1c, AER, cholesterol, assigned diet group, sex, smoking, use of antihypertensive medication or presence of cardiovascular disease. In multible linear regression models, when adjusted for the known progression promotors age, sex, blood pressure, Hba1c, smoking and albuminuria as well as diet group, u-sulphate excretion is highly significant (p=0.0002) and it's inclusion in the model increases adjusted r^2 from 6% in the model without sulphate, to 23% in the model with sulphate.

Conclusions: High urinary sulphate excretion at baseline is associated with slower rate of decline in GFR in diabetic nephropathy during four years of follow up, independent of known progression promoters.

TH-PO499

Close Association of Serum Fibroblast Growth Factor 23 with Diabetic Nephropathy in Type 2 Diabetic Subjects Miho Murata, Atsushi Aoki, San-E Ishikawa. *Department of Medicine, Jichi Medical University Saitama Medical Center, Saitama, Japan.*

Background: The present study was undertaken to determine alterations in serum fibroblast growth factor 23 (FGF-23) and 25(OH)Vitamin D_3 levels in subjects with diabetic nephropathy and atherosclerotic disorders.

Methods: 247 type 2 diabetic patients were enrolled in the present study. They were 149 males and 98 females, with the ages of 64.5±10.2 years. Serum levels of FGF-23 and 25(OH) Vitamin D_3 were measured by methods of ELISA. Serum creatinine, estimated glomerular filtration rate (eGFR) and urinary albumin and/or protein excretion were determined for classification of diabetic nephropathy. Flow-mediated dilatation (FMD), intima-media thickness (IMT) and calcification of carotid artery were examined for atherosclerosis.

Results: Serum FGF-23 levels were 35.7±1.1 pg/ml, ranging from 10.0 to 148.0 pg/ml. Serum 25(OH) Vitamin D_3 levels were 86.1±2.5 nmol/l, ranging from 28.3 to 239.3 nmol/l. We divided the diabetic subjects into two groups by the median level of FGF-23 (31.6 pg/ml). In the subjects of serum FGF-23 level more than 31.6 pg/ml (high FGF-23; n=124), BMI was significantly higher (25.4±3.9 vs. 23.6±3.5, p<0.001) and eGFR was significantly less (72.2±2.2 vs. 82.2±1.7 ml/min/1.73m^2, p<0.001) than those in low FGF-23 group. In single regression analysis, serum FGF-23 levels had a positive correlation with BMI (r=0.25, p<0.001), and a negative correlation with eGFR (r= -0.30, p<0.001). Serum FGF-23 levels were gradually increased according to the progression of diabetic nephropathy (p value in trend test: <0.001). Its elevation was significantly greater in the diabetic subjects with stage 4 than those with stages 1, 2 and 3A. There were no differences in FMD, IMT and vascular calcification between the two groups of high and low FGF-23. Also, we divided the subjects by median of 25(OH)Vitamin D_3 (79.2 nmol/l), and evaluated the differences between the two groups. However, there was no difference in any parameter at all.

Conclusions: These findings indicate that serum FGF-23 levels increase in association with progression of diabetic nephropathy, but not with atherosclerosis, in type 2 diabetic subjects.

TH-PO500

GDF-15 and hs-TnT Predict Progression of Nephropathy in Diabetes and Hypertension Merel E. Hellemons, Stephan J.L. Bakker, Dick de Zeeuw, L.E. Deelman, Hiddo Jan Lambers Heerspink. *Clin. Pharmacology, UMCG, Groningen, Netherlands.*

Background: Increased albuminuria is probably a sign of vascular damage. Biomarkers reflecting endothelial or vascular damage could be eligible to predict progression of albuminuria. We tested this in a prospective case-control study in type 2 diabetes (T2DM) and hypertension (HT).

Methods: We conducted a nested case-control study in the PREVEND-study. Progression of albuminuria (cases) was defined as transition from normo- to micro- or micro- to macro-albuminuria. Controls had non-progressive albuminuria during follow-up and were pair-matched for age, gender, and DM duration. We included 34 case/control pairs with T2DM and 75 pairs with hypertension (HT). The markers growth-differentiating factor-15 (GDF-15) and high-sensitivity Troponin T (hs-TNT) were measured at baseline and their contribution in predicting progression of albuminuria during follow-up (median 2.8 years) was assessed.

Results: GDF-15 was higher in cases than in controls in T2DM (1332±709 vs. 909±381 pg/mL, P<0.001) but not in HT. GDF-15 was independently associated with progression in albuminuria in T2DM (adjusted OR 5.4[1.2-25.0] per doubling of GDF-15) and in HT (2.94[1.20-7.20]) and contributed significantly to prediction of progression of albuminuria with a higher C-statistic and Integrated Discrimination Improvement (IDI) in T2DM and marginally in HT (table 1).

Hs-TnT was higher in T2DM and HT cases (6.8±5.8 vs. 4.0±2.9; P=0.002) and (8.2±5.4; vs. 6.7±4.9; P=0.05) respectively. Hs-TnT was independently associated with progression of albuminuria in HT (OR 2.70[1.13-6.33]) but not in T2DM (3.51[0.62-19.9]) and contributed significantly to prediction of progression of albuminuria in HT [table 1]. Hs-TnT marginally contributed to progression of albuminuria in T2DM.

Conclusions: We identified GDF-15 and hs-TnT as promising markers for progression of albuminuria in T2DM and HT, with GDF-15 superior in T2DM and hs-TnT superior in HT.

C-statistics and IDIs

T2DM	C-statistic	P	IDI	P
Baseline Model*	0.878			
Baseline Model + GDF-15	0.922	0.02	0.140	0.002
Baseline Model + hsTNT	0.894	0.16	0.061	0.04
HT				
Baseline Model	0.882			
Baseline Model + GDF-15	0.889	0.54	0.022	0.15
Baseline Model + hsTNT	0.905	0.08	0.046	0.01

*Albuminuria and eGFR

TH-PO501

Serum Cystatin C Concentration Reflects the Severity of Carotid Atherosclerosis in Type 2 Diabetes Patients without Chronic Kidney Disease Fang Liu, Ping Fu. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.*

Background: Atherosclerosis (AS) is the most common arterial complication of diabetes, which reflects the morbidity and mortality of cardiovascular events. This study is to investigate whether serum Cystatin C (Cys C) concentration correlates with the severity of carotid atherosclerosis (CAS) and whether it provides additional information on the risk for cardiovascular disease in patients with type 2 diabetes (T2DM).

Methods: The relationship between serum Cys C and the severity of CAS was investigated in 633 patients met the inclusion/exclusion criteria. Carotid intimal medial thickness (IMT) and the location and size of AS plaque were evaluated by Doppler ultrasound. Based on glomerular filtration rate (GFR) estimated by simplified MDRD

formula, the patients were divided into those with chronic kidney disease (DM-CKD) and without CKD (DM-NCKD). The relationship of serum Cys C with the severity of CAS was examined.

Results: Serum Cys C was closely correlated with GFR in all subjects and in DM-CKD patients, but not in DM-NCKD patients. In 396 DM-NCKD patients (62.5%) with the eGFR ≥60 ml•min–1•1.73 m–2, 210 patients (210/396, 53%) with a significant large number of carotid AS plaques had a higher serum concentration of Cys C than those (186/396, 47%) without AS plaques formation (1.05±0.27mg/L vs 0.89±0.22mg/L). Moreover, the serum concentration of Cys C was closely correlated with the severity of CAS (r=0.338, P<0.001), even after adjustment for confounding factors (r=0.14, P=0.005), such as age and duration of diabetes. Multiple linear regression analysis also showed closely correlation of Cys C with the severity of CAS.

Conclusions: Cys C is more than a sensitive marker of renal function, but a marker of CAS severity in T2DM patients without CKD. Besides DM-CKD, it might serve as an indicator of morbidity and mortality of cardiovascular events.

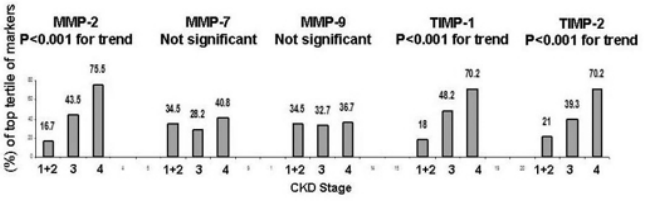
TH-PO502

Urinary Excretion of Markers of Metalloproteinases System and Their Associations with Reduced Renal Function in Subjects with Type 1 Diabetes and Proteinuria Jung Eun Lee, Monika A. Niewczas, William Walker, Adam Smiles, Jan Skupien, James Warram, Andrzej S. Krolewski. *Research Division, Joslin Diabetes Center, Boston, MA.*

Background: Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are thought to regulate extracellular matrix turnover contributing to renal fibrosis and to progression of chronic kidney disease (CKD). The aim of this study was to investigate profile of urinary excretion of MMPs/TIMPs system in subjects with type 1 diabetes (T1D), proteinuria and different CKD stages.

Methods: Glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease study equation. MMPs (MMP1, 2, 7, 9, 12) and TIMPs (TIMP 1-4) were measured on the Luminex platform and adjusted with urinary creatinine for analyses.

Results: This study included 302 patients with T1D and overt proteinuria, 85 (28%) were in CKD stage 3, 49 (16%) were in stage 4, and the others were in stage 1 or 2. The median albumin to creatinine ratio (ACR) was 781 (455-1644) mg/g Cr. Among detectable markers, urinary excretion levels of MMP-2, TIMP-1, and TIMP-2 were increased proportionally with more advanced CKD stages, whereas urinary excretion of MMP-7 and MMP-9 did not differ by CKD stages. The proportions of subjects with the highest tertile of biomarkers distribution stratified by CKD stages are presented in the figure.



Urinary MMPs and TIMPs were closely correlated among each other. In multiple linear regression analysis, urinary excretion of MMP-2 (β = -0.0088, P<0.0001), TIMP-1 (β = -0.0041, P<0.0001), and TIMP-2 (β = -0.0037, P<0.0001), but not of MMP7 and MMP9, were correlated with GFR inversely and independently from ACR.

Conclusions: Urinary excretion of distinct markers of MMPs/TIMPs was associated with more advanced CKD stages in T1D. Longitudinal studies are needed to evaluate the predictive values of the markers.

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TH-PO503

Urinary Transcriptome Analysis of Diabetic Kidney Disease Sang Youb Han,[1,2] Iram Aqeel,[3] Ae Seo Deok Park,[1] Michele H. Mokrzycki,[1] Vaughn W. Folkert,[1] Katalin Susztak.[1] *[1]Medicine, Albert Einstein College of Medicine, Bronx, NY; [2]Internal Medicine, Inje Univ. Ilsan-Paik Hosp., Goyang, Republic of Korea; [3]Medicine, NY Health and Hospitals Corperation, Bronx, NY.*

Background: Urinary transcript levels has been suggested as a noninvasive marker of renal damage in patients with various renal diseases. However, genome-wide transcriptome analysis of control and DKD urines have not been performed. Here we analyzed mRNA expression profiles of urinary sediments using Affymetrix microarray to determine the feasibility of this approach.

Methods: Random urine were collected from healthy controls and patients with DKD. Patients with DKD had a mean of 1.8 g/day of proteinuria, and significantly decreased GFR (mean 32.8 ml/min). Total RNA was extracted from 30 ml of urine using combination method of Trizol and Qiagen kit after centrifugation with 3000 RPM for 15 min. The quality and integrity of RNA were determined by using the Agilent Bioanalyzer. Total RNA were amplified using the Affymetrix 3' IVT kit and hybridized onto Affymetrix U133 expression arrays. Normalized gene expression data wer analyzed using the Genespring GX and Ingenuity Pathway Analysis Softwares. Transcripts with statistically significant differential expression were determined using the Benjamini-Hochberg corrected p<0.05 and fold changes > 2.0 parameters. In addition, we also examined individual gene expression levels using quantitative RT-PCR with primers specific for different renal cell types.

Results: Using statistical methods we identified 931 probesets with at least 2-fold change in their expression between control and DKD groups. The top 6 transcripts with the highest fold changes were ALB, ALDOB, BBOX1, DDAH1, GATM, and HPD. All these genes showed reduced expression in the DKD group. Network analysis indicated disturbances in protein trafficking, protein synthesis, cellular assembly and organization as the top differentially regulated pathways in DKD urine samples.

Conclusions: The urinary transcriptome analysis may be a useful non-invasive tool to evaluate diabetic nephropathy. Further prospective studies will be needed to elucidate the validity urine transcript levels in diabetes and chronic kidney disease.

Funding: NIDDK Support, Private Foundation Support

TH-PO504

Exercise-Induced Increases in Serum Retinol-Binding Protein 4 and Endothelial Progenitor Cells in Diabetic Subjects Atsushi Aoki, Miho Murata, San-E Ishikawa. *Medicine, Jichi Medical University Saitama Medical Center, Saitama, Japan.*

Background: Retinol-binding protein 4 (RBP4) is an adipokine as well as primary carrier for retinol in plasma, and synthesized in liver, adipose tissues and muscular tissues. Endothelial progenitor cells (EPCs) derived from bone marrow are involved in regulating vascular endothelial cells. We determined whether acute exercise load changes serum RBP4 and EPCs in type 2 diabetic subjects.

Methods: 62 type 2 diabetic subjects (65.1±8.1 years) were enrolled. Acute cardio pulmonary exercise (CPX) loaded until maximal oxygen intake was performed, and serum RBP4 and EPCs were determined before and after the CPX. Serum RBP4 was measured by ELISA, and CD34+/133+ cells as EPCs were determined by FACS.

Results: Serum RBP4 levels, but not the numbers of EPCs, were increased according to the progression of diabetic nephropathy (p value in trend=0.043) In the 30 subjects without nephropathy (stage 1), an exercise load promptly increased serum RBP4 from 48.2±23.5 to 54.3±23.1 μg/ml (p=0.0006). In the 32 subjects with nephropathy (stage 2, 3, and 4) the exercise load did not alter serum RBP4. By contrary, an exercise load significantly increased the numbers of EPCs in both the diabetic subjects with stage 1 (88.9±83.3 to 114.9±104.4 cells/100μl , p=0.0003),and stage 2, 3 and 4 (63.0±68.4 to 78.5±68.8 cells/100μl, p=0.005). The alteration in serum RBP4 (ΔRBP4) during the exercise had a positive correlation with eGFR (r=0.30 p=0.018). Also, the numbers of EPCs and the alteration of EPCs (ΔEPCs) during the exercise had positive correlations with serum RBP4 levels (the numbers of EPCs: r=0.402, p=0.006; ΔEPCs: r=0.310, p=0.04).

Conclusions: These findings indicate that acute exercise promptly increases serum RBP4 levels and the number of EPC in diabetic subjects without nephropathy, and that there may be some interaction of RBP4 with the induction of EPCs under acute exercise.

TH-PO505

Association of Serum Leptin Levels with Progression of Diabetic Kidney Disease in Patients with Type 2 Diabetes Ko Hanai, Tetsuya Babazono, Izumi Nyumura, Nobue Tanaka, Yasuko Uchigata. *Diabetes Center, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.*

Background: The association of leptin with diabetic kidney disease (DKD) remains controversial, and furthermore previous reports were limited by cross-sectional design. We therefore conducted this longitudinal study to clarify the association of serum leptin levels with progression of DKD in patients with type 2 diabetes (T2D).

Methods: This was a single-center observational cohort study on Japanese adult patients with T2D. Patients who had malignant diseases, glomerulonephritis diagnosed by biopsy, who had undergone renal replacement therapy or with lower limb amputation were excluded. Patients with an estimated GFR (eGFR) < 60 mL/ min/ 1.73 m^2 were excluded because serum leptin levels are increased due to decreased renal clearance. Then, of 668 patients, 380 and 356 were enrolled as the eGFR and ACR cohorts, respectively. Patients were classified into 3 groups by gender-specific tertile of leptin levels. Outcome measurements were the rate of change in eGFR and progression to a more advanced stage of albuminuria.

Results: During the mean follow-up period of 4.2 ± 1.2 years, the mean rate of change in eGFR was -1.66 ± 3.69 mL/ min/ 1.73 m^2/ year in the eGFR cohort. Patients with low or high leptin levels had a significantly steeper eGFR decline than those with mid-range leptin levels in both the univariate and the multivariate model (all p values < 0.05 vs. patients with mid-range leptin levels). In the ACR cohort, 34 patients showed progression of albuminuria during the mean follow-up period of 3.2 ± 1.6 years. Negative graded relationships between the hazard ratio for progression of albuminuria and 3 groups, based on leptin levels, were recognized in both the univariate and the multivariate model. Patients with the low leptin levels had a significantly elevated risk of progression of albuminuria than those with high leptin levels in both the univariate and the multivariate model (both p values < 0.05).

Conclusions: In this study of patients with T2D, both low and high serum leptin levels were risk factors for kidney function decline. Meanwhile, lower serum leptin levels were associated with progression of albuminuria.

TH-PO506

The Good and the Bad: The Relationships between 25 (OH) D and FGF-23 Levels with Cardiovascular Disease in Type 2 Diabetic CKD Patients Ana Paula Silva, Ana Cabrita, Ana Pinho, Nélio Santos, Pedro Neves. *Serviço de Nefrologia, Hospital de Faro, Faro, Portugal.*

Background: Cardiovascular disease is the main risk factor of morbidity and mortality in chronic kidney disease (CKD) patients and its relevance increases when diabetes is the etiology of the renal disease. Carotid wall thickness, left ventricular hypertrophy and vascular calcification are commonly used surrogates of cardiovascular disease in renal patients. Recently, the disturbances of the mineral metabolism were also related to cardiovascular disease, even in the early stages of CKD.

The aim of this study was to evaluate the relationships between vitamin D and FGF-23 levels with cardiovascular risk factors in a group of diabetic CKD patients.

Methods: In a cross-sectional study, we included 50 type 2 diabetic patients (f=18, m=32) with CKD stages 3 and 4. The mean age was 60.4 years and the mean estimated (MDRD) GFR was 50 ml/min. Exclusion criteria were uncontrolled hypertension, known prior cardiovascular disease, neoplasic or infectious disease, anti-inflammatory, phosphorus binders or vitamin D therapies.

We analyzed several cardiovascular parameters: the left ventricular mass index (LVMI), the carotid intima-media complex thickness (CIMT), the aortic augmentation index (AGI), the aortic pulse pressure (PP) and the difference between the 1st and 2nd peaks of the central pressure waveform in systole (Aix).

Results: Using the Pearson correlation, we found: a *positive* correlation between FGF-23 levels and LVMI (r=0,584 p=0,001), CIMT (r=0,785 p=0,001), AGI (r=0,485 p=0,001), PP (r=0,317 p=0,007) and Aix (r= 0,715 p= 0,001); an *inverse* correlation between 25 (OH) D levels and LVMI (r= - 0,633 p=0,001), CIMT (r= - 0,432 p= 0,002), AGI (r = -0,389 p= 0,004) and Aix (r= - 0,437 p= 0,001).

Conclusions: In our study, we found that the FGF-23 and the 25 (OH)D levels, known risk markers of cardiovascular disease in CKD patients, were related with some of cardiovascular parameters commonly used in the clinical practice.

Further studies are needed to better understand the physiopathology of these relationships and to ascertain if the correction of the FGF-23 and the 25 (OH)D levels improves the outcomes of our patients.

Funding: NIDDK Support

TH-PO507

Total Serum Free Light Chains Are an Important Prognostic Marker in Patients with Type II Diabetes

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Background: Polyclonal serum free light chains (FLCs) have recently been shown to be prognostic of overall survival and cardiac events in patients with chronic kidney disease (CKD). High FLC levels represent both degree of renal impairment and severity of inflammation. FLCs have also been reported to be elevated in diabetes, which is a major cause of CKD and cardiovascular disease. The purpose of this study was to determine if serum FLCs are associated with a shorter overall survival (OS) in type II diabetic patients.

Methods: A total of 527 patients (280 (53.1%) males; 247 (46.9%) females) were included. Baseline patient characteristics were: median (IQR) - systolic BP: 136 (26), diastolic BP: 82 (14), age: 54 (20), total cholesterol: 4.5 (1), HDL: 1.18 (0), LDL: 2.21 (1), ACR: 1.90 (6), BMI: 28 (7), eGFR: 75.6 (18.7), HBA1C: 6.6 (2), triglycerides: 2.3 (2) and total FLCs: 41.10 (21.6). The prognostic value of each variable was assessed using Kaplan Meier and Cox-regression analysis.

Results: A total of 166 (80 males and 86 females) had elevated FLC levels (normal range: 9-48 mg/L). By 4.5years 17/527 (3.2%) patients had died. Total FLC levels were significantly higher in patients who had died (52.8 mg/L) compared with those who were still alive (20.9 mg/L) (p=0.026). Patients with elevated FLC levels (>50mg/L) had a significantly shorter OS than patients with FLCs <50mg/L (p=0.014). The following variables were included as part of the univariate analysis: systolic BP, diastolic BP, age, gender, creatinine, smoker status, total cholesterol, HDL, LDL, ACR, BMI, eGFR, HBA1C, triglycerides and total FLCs. Only raised FLCs (HR=3.2, p=0.02), age>65 yrs (HR=3.6, p=0.03) and eGFR (HR=0.9, 95% CI=0.93-0.99, p=0.017) were identified to be prognostic of survival. Of these, only FLC>50mg/L were shown to be independently associated with OS in the multivariate analysis (HR=3.8; p=0.029).

Conclusions: To conclude, elevated serum FLCs (>50mg/L) were independently associated with a shorter overall survival in patients with type II diabetes.

Funding: Pharmaceutical Company Support

TH-PO508

Lower Serum Magnesium Level Predicts Adverse Renal Outcome and Mortality in Advanced CKD Patients with Type 2 Diabetes Mellitus

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Background: Hypomagnesemia is a common finding among patients with type 2 diabetes mellitus (DM). Magnesium deficiency in type 2 DM is suggested to cause poor glycemic control as well as various micro- and macrovascular complications. However, few studies have focused on the impact of hypomagnesemia on the progression of renal failure and mortality. The aim of this study was to determine whether hypomagnesemia is a risk factor for end-stage renal disease (ESRD) and death among type 2 DM patients with advanced renal failure.

Methods:

Study design: A retrospective cohort study

Settings and participants: CKD stage 3 to 5 patients with type2 DM who were not receiving renal replacement therapy and whose serum magnesium level was measured at least once between January and December 2008.

Outcome: Composite of ESRD and all-cause mortality

Predictor: The participants were stratified into the following 2 groups based on baseline serum magnesium concentration according to the previously published normal lower limit: Lower group (serum magnesium, <1.8 mg/dL) and Higher group (serum magnesium, ≥1.8 mg/dL).

Results: This study included 151 patients (men, 70 %; median age, 69 years; serum creatinine, 2.05 mg/dL; serum magnesium 1.9 mg/dL). At the median follow-up of 29 months, 37 patients had ESRD and 10 died. Unadjusted Kaplan-Meier analysis showed Lower group had significantly worse outcomes than the Higher group (p= 0.01); this persisted after adjustment for age, sex, estimated GFR, serum albumin, hemoglobin A1c, hypertension, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, diuretics use and history of cardiovascular disease (hazard ratio 2.40; 95% confidence interval 1.14, 4.73; p = 0.02). Although there was no adjustment for proteinuria due to 33% missing data, it must be noted that there was no significant difference in proteinuria between the Lower and Higher group among patients with available data.

Conclusions: Low serum magnesium level independently predicted adverse renal outcome and mortality in advanced CKD patients with type 2 DM.

TH-PO509

Significance of Urinary Full-Length and Ectodomain Forms of Megalin in Patients with Type 2 Diabetes Mellitus

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Background: Megalin mediates endocytic uptake of glomerular-filtered proteins in proximal tubule cells (PTCs). Also, it undergoes regulated intramembrane proteolysis (RIP) by which its extra- and intracellular domains are cleaved for putative signal transduction. To develop efficient novel biomarkers associated with the pathogenesis of PTC injury, particularly in diabetic nephropathy (DN), we investigated urinary megalin excretion in patients with type 2 diabetes mellitus (T2DM).

Methods: Sandwich ELISAs were carried out with monoclonal antibodies against the amino (A-megalin assay) and carboxyl (C-megalin assay) termini of megalin to analyze its urinary forms.

Results: We found that the A-megalin assay detected mainly a megalin ectodomain form, whereas the C-megalin assay identified a full-length form. The A-megalin assay targets were primarily in the soluble fraction, whereas the C-megalin assay targets were in both soluble and insoluble fractions. In 52 T2DM patients, urinary C-megalin levels tended to be elevated in line with increased albuminuria and were associated positively with plasma inorganic phosphate (Pi) and negatively with hemoglobin (Hb) levels, independently of age and sex. Other urinary biomarkers such as albumin, NAG, α_1-microglobulin, β_2-microglobulin, L-FABP, NGAL and type IV collagen did not show such a combined association with Pi and Hb levels. In contrast, urinary A-megalin levels were increased in T2DM patients with normo- and microalbuminuria but not in those with macroalbuminuria. In addition, urinary A-megalin levels were negatively associated with fractional excretion of Na.

Conclusions: Urinary full-length megalin excretion as measured by the C-megalin assay appears to be linked to chronic progression of DN, Pi dysregulation, and anemia, whereas urinary excretion of megalin ectodomain as measured by the A-megalin assay may be associated with RIP-related distinctive mechanisms involved in renal Na handling in T2DM patients.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

TH-PO510

YKL-40 Levels Are Associated with Coronary Calcium Score in Type 2 Diabetic Patients with Albuminuria but without Known Cardiovascular Disease

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Background: Type 2 diabetic patients (T2D) with albuminuria have a poor prognosis, primarily due to increased morbidity and mortality from cardiovascular disease (CVD). Early detection of asymptomatic CVD is essential to improve treatment and prognosis. In this cross-sectional study we investigated the inflammatory biomarker YKL-40, which seems to reflect early atherosclerosis, in relation to asymptomatic significant coronary artery disease in T2D patients with elevated urinary albumin excretion rate and the association with NT-proBNP and coronary calcium score (CCS).

Methods: A cohort of 200 T2D patients with elevated urinary albumin excretion rate (micro- or macroalbuminuria), but normal plasma creatinine and without a history of CVD were invetigated. Patients with plasma NT-proBNP levels above the median and/or coronary calcium scores (CCS) > 400 (n=133), were examined by myocardial scintigraphy and coronary angiography. YKL-40 levels were measured in all patients with a commercial ELISA assay (Quidel, USA).

Results: Median (IQR) level of YKL-40 was 89 (55-142) ng/mL. YKL-40 was higher in patients with significant CAD (110 ng/mL vs. 82 ng/mL, p= 0.005).YKL-40 was associated with plasma NT-proBNP (R=0.2, p=0.0001) and CCS (R=0.22, p=0.002). Only weak associations (R<0.3) were found between YKL-40 and traditional risk factors as age, known diabetes duration, vibration threshold, BMI and plasma creatinine. In a logistic regression model, YKL-40 in the third tertile was associated with significant CAD, however when adjusting for sex and age these associations were no longer significant

Conclusions: In patients with T2D and albuminuria but without known CVD, YKL-40 levels are associated with CCS, a non invasive marker of coronary calcification, but not independently associated with CAD. This could prove helpful in future risk assessment.

Funding: Private Foundation Support

TH-PO511

Phosphorous as an Early Marker of Morbidity and Mortality in Type 2 CKD Diabetic Patients Ana Paula Silva, Ana Cabrita, Ana Pinho, André Fragoso, Marilia Faisca, Nelson Almeida Tavares, Pedro Neves. *Nephrology, Hospital Faro, Faro, Portugal.*

Background: Disturbances of the mineral metabolism, namely the phosphorous (P) level, have been related to increased morbidity and mortality in the general as well in the chronic kidney population.

However, studies conducted to assess the impact of P in stages 3 and 4 of renal disease are scarce. The purpose of this study was to evaluate the impact of P levels in a homogeneous population of type 2 chronic kidney disease (CKD) patients.

Methods: ± 25.1ml/min. Exclusion criteria were: previous cardiovascular disease, uncontrolled hypertension, neoplasic or infectious diseases. The mean follow-up was 47.6 months. We divided the population in 3 groups, according to the P tertiles. GI (n=39) - P <3.60 mg/dl; G2 (n=39) - P > 3.70 and < 4.60 mg/dl; G3 (n=41) P ≥ 4.60 mg /dl

Several laboratory parameters were analyzed: albumin, hemoglobin, estimated glomerular filtration rate (MDRD), markers of inflammation (interleukin 6, TNFa), insulin resistance (HOMA-IR), the left ventricular mass index (LVMI) and the hospitalization days caused by cardiovascular events.

In the analysis we used descriptive statistics, ANOVA for comparison among groups and the Kaplan-Meier method to compare the survival of the 3 groups.

Results: Patients in the higher P tertile (G3), showed lower estimated GFR (p = 0.0001), and hemoglobin (p = 0.030) levels; they also showed higher HOMA-IR (p = 0.0001), LVMI (p=0.0001), IL6 (p = 0.0001), TNF (p = 0.0001) and more hospitalization days (p = 0.0001) when compared with other groups. Using the Kaplan Meier analysis we found that the survival of the G1, G2 and G3 at 24 months was respectively : 93%, 86% and 71% (log-rank = 6.88 p=0.032).

Conclusions: In conclusion, we found in a group of type 2 diabetic CKD patients (stages 3 and 4) that higher P levels, even under the limits of therapeutic intervention, were associated with increased morbidity and mortality. Further studies are needed to verify if an earlier intervention regarding the P metabolism will be associated with improved outcomes in our patients.

Funding: NIDDK Support

TH-PO512

FGF23 and Asymptomatic Coronary Artery Disease in Type 2 Diabetic Patients with Elevated Urinary Albumin Excretion Rate Christel Joergensen,[1] Henrik Reinhard,[1] Orlando M. Gutierrez,[2] Myles S. Wolf,[3] Hans-Henrik Parving,[4] Peter Karl Jacobsen,[1,4] Peter Rossing.[1] *[1]Steno Diabetes Center, Denmark; [2]University of Alabama at Birmingham; [3]University of Miami, Miller School of Medicine; [4]Copenhagen University Hospital, Rigshospitalet, Denmark.*

Background: Coronary artery disease (CAD) is the major cause of morbidity and mortality in type 2 diabetic patients. Elevated FGF23 levels have been associated with surrogate markers (endothelial dysfunction and arterial stiffness) of cardiovascular disease in non diabetic subjects with early chronic kidney disease. We investigated the association between FGF23 and coronary calcium score (CCS) as well as asymptomatic CAD in type 2 diabetic patients with urinary albumin excretion rate > 30mg/24h.

Methods: A cross-sectional study including 200 type 2 diabetic patients without clinical signs of CAD. Patients with p-NT-proBNP>45.2ng/L and/or CCS>400 were stratified as high risk patients of CAD (n=133). High risk patients were examined by myocardial perfusion imaging (MPI; n=109), and/or CT-angiography (CTA; n=20), and/or coronary angiography (CAG; n=86). The patients were divided according to tertiles of FGF23.

Results: Median (range) FGF23 level was 71.3 (18.3- 992.2) RU/ml. All patients had normal creatinine levels. FGF23 was not associated with Agatston CCS intervals 1-5 (p=0.82). In 70(35%) patients, significant CAD was demonstrated by MPI and/or CAG. Compared to patients without significant CAD, FGF23 was not significantly higher in patients with significant CAD, 70.0 (18.3-992.2) RU/ml and 77.0 (36.0-783.0) RU/ml respectively; p=0.14. In a logistic regression model adjusted for conventional cardiovascular risk factors, increasing FGF23 was associated with CAD, OR 1.35 (0.6-3.1); p=0.47 for second vs. first tertile and OR 2.1 (0.9-4.7); p=0.07 for third vs. first tertile; although not statistically significant.

Conclusions: In high risk type 2 diabetic patients with elevated urinary albumin excretion rate, elevated levels of FGF23 are not significantly associated with asymptomatic coronary artery disease.

TH-PO513

Kidney Function and Albuminuria in Patients with Type 2 Diabetes Treated with Exenatide BID vs. Insulin Glargine Pamela W. Anderson,[1] Manjiri Pawaskar,[1] Katherine R. Tuttle,[2] Qian Li,[3] Byron Hoogwerf,[1] Matthew Reynolds.[3] *[1]Eli Lilly and Company, Indianapolis, IN; [2]Sacred Heart Medical Center, Spokane, WA; [3]United BioSource Corporation, Lexington, MA.*

Background: Exenatide twice daily (EXE BID) is a GLP-1 receptor agonist excreted via the kidney indicated to improve glycemic control in adults with type 2 diabetes (T2D). There have been postmarketing reports of decreased kidney function during treatment with EXE BID. This study examined changes in kidney function and incidence of albuminuria over 1-year exposure of EXE BID vs. insulin glargine in patients with T2D treated in usual clinical practice.

Methods: A retrospective analysis was performed using the General Electric (GE) electronic medical record database, comprised of patients who initiated EXE BID (n=4,494) or IG (n=5,424) between 11/1/2006 and 04/30/2009. The 2 groups were 1:1 propensity score matched on baseline demographic, clinical, and resource use variables yielding 2,683 matched pairs. Measures of kidney function or damage included serum creatinine (SCR), urinary albumin/creatinine ratio (UACR), and estimated GFR (eGFR). eGFR was calculated from SCR using the modified MDRD equation.

Results: Both EXE BID and IG groups had less than 1 ml/min/1.73m^2 of change in eGFR from baseline to 1 year, which was not statistically different across groups (IG: 76.6 to 76.7 ml/min/1.73m^2 and EXE BID: 75.7 to 75.8 ml/min/1.73m^2). Also, the number of patients in each stage of chronic kidney disease was not significantly different at baseline and endpoint across the 2 groups. Baseline UACR was significantly higher in the IG group (120 vs. 42 mg/g for EXE BID group, p=0.03) despite propensity score matching. There was no longer a significant difference in UACR after 1 year between the groups (101 vs. 52 mg/g, p=0.51), and there was not a significant change from baseline in either group (11 vs. 8 mg/g, p=0.19 for EXE BID and IG, respectively).

Conclusions: Patients in both IG and EXE BID groups had stable eGFR after 1 year of treatment. Clinical data from a large, diverse patient population in usual care practice did not detect adverse changes in kidney function or damage during treatment with EXE BID.

Funding: Pharmaceutical Company Support

TH-PO514

Renoprotective Efficacy of an L/N-Type Calcium Channel Blocker in Early Diabetic Nephropathy Complicated by Hypertension Shinya Fukumoto, Eiji Ishimura, Yohei Mima, Takaaki Maeno, Eiji Kimoto, Ken Wakikawa, Katsuhito Mori, Tetsuo Shoji, Shigeichi Shoji, Masanori Emoto, Masaaki Inaba. *Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan.*

Background: The renoprotective efficacy of cilnidipine, an L/N-type calcium channel blocker (CCB), in hypertensive patients with chronic kidney disease has been reported, but the efficacy in early diabetic nephropathy has not been studied. We examined differences in efficacy between L/N and L type CCBs for early nephropathy.

Methods: The present study was a multicenter crossover study pre-registered as a CLEARED study. It involved 90 outpatients (57 males and 33 females; age, 65±9 ys) with early nephropathy complicated by hypertension. Their urinary albumin excretion (UAE) was 50±42 mg/gCr, and eGFR was 73±19 ml/min. Forty-nine patients were being treated with inhibitors of the rennin-angiotensin system (RAS). For the group (group A for 69 patients) with switching to cilnidipine after administration of an L-type CCB for more than 6 months and the group with switching from cilnidipine to an L-type CCB (group B for 21 patients), we investigated changes in UAE 6 months before switching, at the time of switching, and 6 months after switching.

Results: Groups A and B each exhibited significant change in neither blood pressure (BP) nor HbA1c level after switching. Group A had a significant increase in UAE during an L-type CCB (52±8 → 92±19 mg/gCr, p<0.01), but a significant decrease in UAE 6 months after switching to cilnidipine (→ 50±10 mg/gCr, p<0.01). Group B exhibited no significant change in UAE during cilnidipine, and no significant change in UAE for 6 months after switching to an L-type CCB. Factors related to increase in UAE in both groups were examined by logistic regression analysis. Cilnidipine was found to be a significant factor reducing the risk (OR: 0.246, 95% CI, 0.074-0.823, p<0.05) independent of age, sex, BP, RAS inhibitors, and HbA1c.

Conclusions: The L/N-type CCB cilnidipine exhibited excellent renoprotective efficacy in patients with early diabetic nephropathy compared with L-type CCBs, and switching from an L-type CCB to cilnidipine had beneficial effects.

TH-PO515

Ameliorating Day- and Night-Time Blood Pressure Control by Combined ECE/NEP Inhibition with Daglutril on Top of Losartan: A Randomized, Cross-Over, Placebo-Controlled Clinical Trial in Patients with Type 2 Diabetes and Albuminuria Irene M. Van Der Meer,[1,3] Aneliya Parvanova Ilieva,[3] Giuseppe Remuzzi,[1,2] Ariela Benigni,[2] Piero Ruggenenti.[1,2] *[1]Unit of Nephrology, Azienda Ospedaliera Ospedali Riuniti di Bergamo, Bergamo, Italy; [2]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [3]Department of Internal Medicine, HAGA Ziekenhuis, Den Haag, Netherlands.*

Background: Albuminuria reduction and optimal blood pressure (BP) control are of utmost importance for patients with type 2 diabetes (T2DM) and nephropathy. Endothelin-1 receptor antagonism reduces BP and albuminuria, but fluid retention frequently occurs. No study has investigated the antihypertensive and antiproteinuric effects of the combined endothelin-converting enzyme/neutral endopeptidase (ECE/NEP) inhibitor daglutril.

Methods: In a randomized, double-blind, placebo-controlled cross-over trial, 42 micro- or macroalbuminuric patients with T2DM and stable BP control were randomized to treatment with daglutril (300 mg/day) followed by placebo or the reverse. Each treatment lasted 8 weeks and was given on top of losartan (100 mg/day). Change in 24-hour albuminuria was the primary outcome. Secondary outcomes were changes in office and ambulatory BP, renal hemodynamics and glomerular size-selectivity.

Results: Mean±SD 8-week changes for daglutril versus placebo in daytime systolic and diastolic BP were -2.3±8.0 versus 3.2±9.4 mmHg (P=0.03) and 0.3±4.7 versus 1.8±5.5 mmHg (P=0.19), respectively. Corresponding changes in night-time systolic and diastolic

BP were -5.7±10.3 versus 4.0±7.8 mmHg (P<0.001) and -3.3±5.4 versus 2.0±5.9 mmHg (P<0.001), respectively. No significant changes were observed in 24-hour albuminuria. Peripheral edema was observed in four patients, but no heart failure occurred.

Conclusions: Daglutril has no effect on albuminuria, but reduces day- and night-time systolic BP and night-time diastolic BP on top of losartan in T2DM patients. Its safety profile compares favourably to endothelin-1 receptor antagonists. Ameliorating BP control by combined ECE/NEP inhibition provides a new approach to hypertension that may be of value in reducing the incidence of cardiovascular disease in this high-risk population.

Funding: Pharmaceutical Company Support

TH-PO516

Effects of Lipid-Lowering Treatment on Platelet Reactivity and Platelet-Leukocyte Aggregation in Diabetic Patients with Chronic Kidney Disease – A Randomized Trial

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Background: Diabetes mellitus (DM) is associated with hyperreactive platelets and increased platelet-leukocyte aggregation (PLA), but the impact of concomitant chronic kidney disease (CKD) has been much less studied. Platelet- and leukocyte activation may contribute to the high incidence of atherosclerotic cardiovascular disease in patients with DM and chronic kidney disease. Lipid-lowering treatment (LLT) with statins may have favorable effects on platelet activation and inflammation, and ezetimibe co-treatment provides additional cholesterol-lowering.

Methods: After a placebo run-in period, the effects of simvastatin alone (S) or simvastatin+ ezetimibe (S+E) were compared in a randomized, double-blind, cross-over study on platelet reactivity, PLA formation, and inflammatory parameters.18 DM patients with estimated glomerular filtration rate (eGFR) 15-59 ml/min x 1.73 m^2 (CKD stages 3-4) (DM-CKD) and 21 DM patients with eGFR >75 ml/min (DM only) were included.

Results: PLAs were elevated at baseline in DM-CKD compared to DM only patients (p<0.001). S+E reduced PLAs among total leukocytes and neutrophils in DM-CKD patients (p=0.01 for both). Platelet reactivity did not differ between patient groups or with LLT. Plasma sCD40L (p<0.001), elastase (p=0.01) and vWf (p<0.01) were elevated in DM-CKD compared to DM only patients. S+E tended to reduce sCD40L in DM-CKD patients, but LLT did not influence vWF or elastase.

Conclusions: In conclusion, DM patients with CKD stages 3-4 had increased platelet-leukocyte aggregation and inflammatory activity compared to DM patients with normal GFR. Simvastatin+ezetimibe decreased PLAs in DM patients with concomitant CKD, but did not influence inflammatory parameters in either patient group.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-PO517

Effect of Thiazides on GFR Decline in Diabetes Mellitus Type 2

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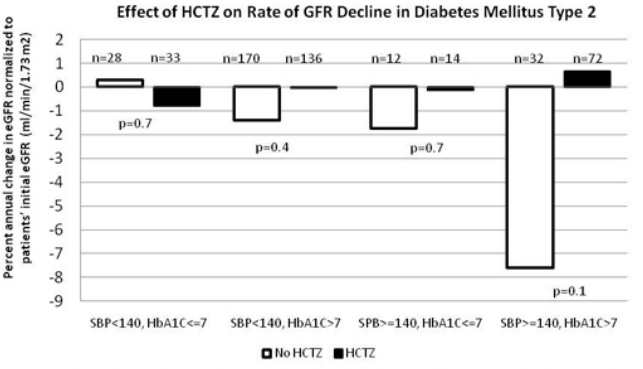
Background: Poor glycemic control and the use of diuretics in DM2 patients can both induce relative volume depletion. The combined effects on volume depletion could potentially induce intermittent acute tubular necrotic episodes and result in acceleration of kidney function decline over time. The current study examines the effect of hydrochlorothiazide (HCTZ), a mild diuretic, on the annual percentage of change in the estimated (MDRD) glomerular filtration rates (eGFR) in DM2 patients stratified by glycemic control and blood pressure.

Methods: This is a retrospective study involving record retrieval of patients with DM2 and hypertension evaluated for any reason at UCLA-OVMC during 2009-2010. Data retrieved include age, gender, use of thiazides and inhibitors of the renin angiotensin system, eGFR, hemoglobin A1C (HbA1C), blood pressure, and albuminuria to creatinine ratio (ACR). Patients were stratified based on systolic blood pressure <= or > 140 mmHg and HbA1C <= or > 7%. Differences in the annual percentage change in eGFR during the duration of follow-up between any 2 groups comparing those receiving thiazides vs. not were based on student's t-test. A p-value <0.05 was considered statistically significant.

Results: 497 patients' records were included. There were 71.8% female, with mean +/- standard deviation age of 57 +/- 9 years, duration of follow-up of 601 +/- 157 days, systolic blood pressure of 134 +/- 14, diastolic blood pressure of 70 +/- 9 mmHg, HbA1C of 8.2 +/- 1.4%, and ACR of 141 +/- 521 mcg/mg. 97.2% of patients were receiving either an angiotensin converting enzyme inhibitor or angiotensin receptor blocker. The percentages of change in the eGFR for the 4 groups are shown.



There is a trend for slower decline in GFR among patients receiving hydrochlorothiazide compared to those who did not across all groups stratified for blood pressure and hemoglobin A1C.

Conclusions: The use of thiazides in DM2 patients with poor glycemic control does not accelerate but appears to be protective against eGFR decline.

TH-PO518

Abstract Withdrawn

TH-PO519

Effect of Atrasentan in Subjects Taking Maximum Doses of Renin Angiotensin-Aldosterone System Inhibitors (max-RAAS) for Diabetic Nephropathy (DN)

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Background: RAAS inhibitors reduce albuminuria in subjects with DN, though >50% taking max-RAAS have residual albuminuria. Low dose atrasentan, a highly selective endothelin A receptor antagonist, reduces residual albuminuria. These analyses evaluated the efficacy and safety of atrasentan + max-RAAS in subjects with DN.

Methods: This was a randomized, double blind trial of subjects with type 2 diabetes and albuminuria on stable doses of RAAS inhibitors, an eGFR >20mL/min/1.73m^2, a UACR of 100-3000mg/g and a NT-pro-BNP level <500pg/ml who were allocated to placebo (PBO), or atrasentan 0.25 mg, 0.75 mg, or 1.25 mg QD for 8 weeks. This abstract is focused on 33 subjects taking max-RAAS out of 89 participating in the trial. Treatment-by-subgroup interaction was tested using ITT population.

Results: The treatment-by-subgroup interaction for change from baseline (BL) to final log UACR was not significant (P=0.816), suggesting subjects taking max-RAAS or taking lower than max-RAAS responded similarly to atrasentan. BL demographics were similar between the groups. Results are shown in the table. The small sample sizes prevented robust statistical comparisons.

		Atrasentan		
Change from BL to Week 8 mean (SE)	PBO (N=9)	0.25 mg (N=5)	0.75 mg (N=8)	1.75 mg (N=11)
UACR (% change)	5%	-27%	-32%[1]	-35%[2]
Systolic Blood Pressure, SBP mm Hg)	0.8 (4.6)	-1.4 (6.4)	-12.2 (5.0)	-6.5 (4.2)
eGFR (mL/min/BSA)	3.2 (2.6)	4.1 (3.9) N=4	-5.3 (2.8)	-0.2 (2.4)
Subjects with a 40% Reduction UACR	11%	40%	63%[3]	36%[4]
Edema (% of subjects)	11%	40%	13%	58%

P-values vs. PBO
1. 0.134
2. 0.087
3. 0.050
4. 0.319

Conclusions: Subjects in the 0.75 mg and 1.75 mg groups had numerically greater % changes in UACR compared with PBO, and subjects in the 0.75 mg group also had a numerically greater reduction in systolic blood pressure (SBP) and eGFR, indicating that atrasentan could be useful for subjects with residual albuminuria while taking max-RAAS. Edema, mostly mild, was higher in subjects taking atrasentan, but was not dose-dependent in those on max-RAAS. With the caveat of small sample size, larger studies are needed to confirm these findings.

Funding: Pharmaceutical Company Support

TH-PO520

Aliskiren Added to Losartan Has Beneficial Effects across Different Levels of Albuminuria in Hypertensive Patients with Type 2 Diabetes and Nephropathy: The AVOID Study Frederik I. Persson,[1] Julia Lewis,[2] Edmund J. Lewis,[3] Peter Rossing,[1] Hans-Henrik Parving.[4,5] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]School of Medicine, Vanderbilt University, Nashville, TN; [3]Rush University Medical Center, Chicago, IL; [4]Dept. of Medical Endocrinology, University Hospital of Copenhagen, Copenhagen, Denmark; [5]Faculty of Health Sciences, Aarhus University, Aarhus, Denmark.*

Background: Aliskiren 300 mg once daily added to standard treatment with losartan 100 mg once daily and optimal antihypertensive therapy has antiproteinuric effect in hypertensive patients with type 2 diabetes and nephropathy. However, the beneficial impact may depend on the level of baseline albuminuria. Consequently we performed a post-hoc analysis of the AVOID study to determine which group of patients benefits the most from the addition of direct renin inhibition.

Methods: In AVOID, 599 patients (eGFR>30 ml/min/1.73m2) with type 2 diabetes, hypertension and nephropathy received 6 months' aliskiren (150 mg titrated to 300 mg after 3 months) or placebo added to losartan 100 mg and optimal antihypertensive therapy. The primary endpoint, changes in early morning urinary albumin creatinine ratio (UACR), was assessed by tertiles of baseline UACR levels.

Results: Patients were divided into tertiles of baseline UACR (<340 mg/g, ≥340 mg/g – 788 mg/g and >788 mg/g respectively). The antiproteinuric effect of aliskiren treatment was consistent across subgroups of baseline UACR with a between-treatment ratio of 0.78 (95% CI 0.62, 0.98) p=0.032 in the lowest tertile; 0.79 (0.63, 0.99) p=0.039 in the middle tertile and 0.84 (0.67, 1.05) p=0.13 in the highest tertile, independent of baseline BP.

Conclusions: This *post hoc* analysis of the AVOID study suggests that renin inhibition with aliskiren 300 mg once daily added to losartan 100 mg once daily plus optimal antihypertensive therapy provides reductions in UACR of equal magnitude across different levels of baseline albuminuria.

Funding: Pharmaceutical Company Support

TH-PO521

Cholecalciferol Reduces Urinary Angiotensinogen in Early Diabetic Kidney Disease Christopher K. Johnson,[1] Alexandra V. Flynn,[1] Hiroyuki Kobori,[2] Bryan R. Kestenbaum,[1] Ian H. de Boer.[1] *[1]University of Washington, Seattle, WA; [2]Tulane University, New Orleans, LA.*

Background: The renin-angiotensin system (RAS) is an important therapeutic target in diabetic kidney disease. Tissue and urinary levels of angiotensinogen (AGT) reflect intrarenal RAS activation and oxidative stress. In animal models, vitamin D receptor agonists downregulate the RAS and reduce tissue and urinary AGT. We investigated whether treatment with cholecalciferol (vitamin D3) decreases urinary AGT in early human diabetic kidney disease.

Methods: In a randomized, double-blind, clinical trial, 22 participants with type 2 diabetes mellitus, albuminuria (urinary albumin-creatinine ratio (ACR) 30-1000mg/g), and estimated glomerular filtration rate >= 60 mL/min/1.73m^2 were assigned to treatment with cholecalciferol 2000 IU daily or matching placebo for one year. All participants were treated with RAS inhibitors. Spot urinary AGT and interleukin-6 (IL-6) concentrations were measured by ELISA at baseline, three months, and one year and indexed to urinary creatinine. We analyzed effects of treatment using linear mixed models.

Results: On treatment, participants in the cholecalciferol group had a 64% decline in urinary AGT (95% CI -83%, -25%; p = 0.006) while patients in the placebo group had a 15% decline (95% CI -51%, +47%); between group difference 58% (95% CI -83%, +6%; p = 0.07). Participants in the cholecalciferol group had a 16% decline in urinary IL-6 (95% CI -39%, 16%), while patients in the placebo group had a 1% decline (95% CI -28%, +36%). Participants in the cholecalciferol group had a 22% decline in urinary ACR (95% CI -50%, 23%), while patients in the placebo group had a 5% decline (95% CI -40%, +50%).

Conclusions: Among individuals with type 2 diabetes and albuminuria on treatment with a RAS blocker, daily cholecalciferol supplementation reduced urinary AGT excretion. This may reflect further downregulation of the intrarenal RAS, possibly due to decreased oxidative stress.

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TH-PO522

Analysis of Peripheral Edema in Subjects Taking Atrasentan for Diabetic Nephropathy (DN) Blai Coll,[1] Shihua Wen,[1] Yili Pritchett,[1] Mark E. Molitch,[2] John J. Brennan,[1] Donald E. Kohan,[3] Dennis L. Andress.[1] *[1]Abbott; [2]Northwestern U Feinberg Sch of Med; [3]U of Utah.*

Background: Low dose atrasentan, a highly selective endothelin A receptor antagonist, reduces residual albuminuria in subjects with DN. Peripheral edema is the most frequently reported adverse event (AE) associated with endothelin A receptor blockade. The goal of these analyses was to characterize edema events in subjects taking atrasentan.

Methods: This was a randomized, double blind trial of subjects with type 2 diabetes on stable doses of RAAS inhibitors having an eGFR >20mL/min/1.73m^2 and albuminuria of 100-3000mg/g creatinine (UACR), who were allocated to placebo (PBO), 0.25mg, 0.75mg, or 1.75mg of atrasentan daily for 8 weeks. The protocol was later amended to have serum NT-pro-BNP >500pg/mL as an exclusion.

Results: 89 subjects were randomized: 23 to PBO and 22 to each atrasentan dose group. Baseline (BL) demographics were similar among groups. UACR lowering was different from PBO in the 0.75mg and 1.75mg groups. 21 subjects reported treatment-emergent edema. Of those 21 subjects, 13 were taking diuretics at BL. The incidence of mild/moderate edema was: PBO:2/23, 0.25mg:4/22, 0.75mg:5/22, and 1.75mg:10/22; none reported severe edema. 62% of the treatment-emergent edema were reported in the first 4 weeks. Of the 21 subjects with edema, 6 resolved by treatment end, 9 resolved during the 30 day follow-up after treatment, 5 had continued edema at the end of the study, and 2 subjects were discontinued: 1 in the 1.75mg group from angioedema and 1 in the 0.75mg group from congestive heart failure (BL NT-pro-BNP, 4400 pg/ml). 7 subjects started diuretics during the study. Variables associated with a higher risk of edema by logistic regression were treatment group (1.75mg, OR =17.9, *P*=0.005) and BL UACR (≤300 or >300mg/g, OR=6.1, *P*=0.012).

Conclusions: Edema occurrence with atrasentan therapy was dose-dependent, mostly mild/moderate, and appeared during the first 4 weeks. Less than 1/3 of subjects initiated diuretics during treatment and 71% resolved edema before the end of study. These data suggest that with appropriate patient selection, low dose atrasentan may be safe for long-term clinical trials.

Funding: Pharmaceutical Company Support

TH-PO523

Determinants of Hyperkalemia in Diabetic (DM) Patients Treated with an ACE Inhibitor (ACEI) and a Mineralocorticoid Receptor Antagonist (MRA): Clinical Implications for Obviating Hyperkalemia Murray Epstein,[1] Bruce Beckerman,[2] John Vincent,[2] Harry Shi.[2] *[1]University of Miami School of Medicine, FL; [2]Pfizer, Inc., New York, NY.*

Background: Although combined RAAS blockade with an MRA and ACEI generally produces a greater reduction in albuminuria/proteinuria than ACEI alone, it tends to cause hyperkalemia. This post hoc analysis of the 067 study (CJASN 1:940-951, 2006) assessed whether estimated baseline GFR (eGFR) predicts hyperkalemia. Sequential measurements of serum potassium (sK) during the study defined its temporal profile.

Methods: 268 patients with type 2 DM, albuminuria, hypertension, eGFR >60 mL/min/1.73m^2 and sK ≤5.0 mmol/L were randomized to receive enalapril 20mg plus either the MRA eplerenone (EPL) 50mg, EPL 100mg, or placebo for 12 weeks. sK was measured at baseline and every 2 weeks during the trial to determine the incidences of sustained hyperkalemia (sK >5.5 mmol/L on two consecutive occasions 1 to 3 d apart) and severe hyperkalemia (sK >6.0 mmol/L on any occasion).

Results: Pertinent data were available for 259 subjects. The Table displays the incidence of sustained and severe hyperkalemia by treatment and GFR quartile (eGFR, MDRD equation). EPL reduced albuminuria in doses as low as 50 mg/day (41% reduction in UACr). Only 6 patients in the EPL groups had severe or sustained hyperkalemia, and hyperkalemia did not correlate with eGFR. Evaluation of time to onset of hyperkalemia failed to confirm a particular time period when enhanced monitoring of sK would be beneficial.

Conclusions: This analysis demonstrates that a dosing regimen of the MRA EPL 50mg with an ACEI or an ARB in type 2 DM patients confers the desired anti-albuminuric benefit with minimal risk of hyperkalemia.

Incidence of hyperkalemia by treatment and eGFR quartile

Baseline eGFR (mL/min/1.73m²)	Hyperkalemia	Placebo/ENAL	EPL50 / ENAL	EPL100 /ENAL
		N=88	N=89	N=82
		n (%)	n (%)	n (%)
<72 (N = 64)	Sustained	0 (0.00)	1 (1.12)	0 (0.00)
	Severe	1 (1.14)	0 (0.00)	0 (0.00)
72 – <88 (N=66)	Sustained	0 (0.00)	0 (0.00)	0 (0.00)
	Severe	0 (0.00)	1 (1.12)	0 (0.00)
88 – <104 (N=67)	Sustained	0 (0.00)	0 (0.00)	0 (0.00)
	Severe	0 (0.00)	0 (0.00)	2 (2.44)
≥104 (N=62)	Sustained	0 (0.00)	0 (0.00)	0 (0.00)
	Severe	0 (0.00)	0 (0.00)	1 (1.22)

Funding: Pharmaceutical Company Support

TH-PO524

Efficacy and Safety of Dapagliflozin in Patients with Type 2 Diabetes and Moderate Renal Impairment Donald E. Kohan,[1] Paola Fioretto,[2] James List,[3] Weihua Tang.[3] *[1]University of Utah Health Sciences Center, UT; [2]University of Padova Medical School, Italy; [3]Bristol-Myers Squibb, NJ.*

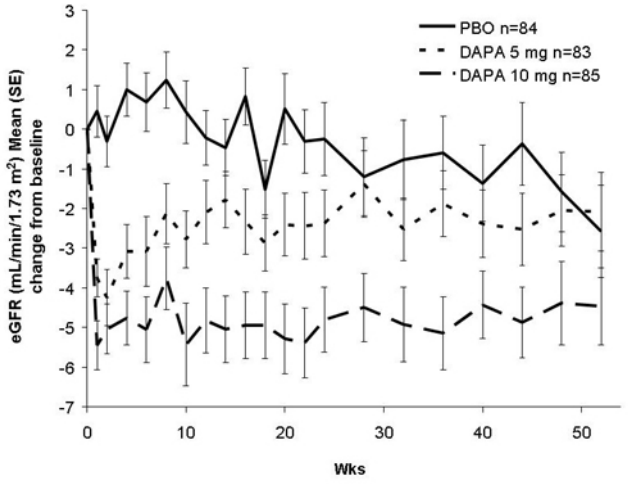
Background: Dapagliflozin (DAPA), a selective SGLT2 inhibitor, promotes urinary glucose excretion (UGE).

Methods: The effects of DAPA on HbA1c, renal function and safety were assessed in patients (pts) with type 2 diabetes (T2D) and moderate renal impairment (eGFR 30–59 mL/min/1.73m^2). Pts (n=252), aged ≥ 18 yrs, HbA1c 7.0–11.0%, were randomized to receive DAPA (5 or 10 mg/d) or placebo (PBO) for 52 wks.

Results: Mean decreases in HbA1c were similar for PBO (-0.32%) and DAPA (-0.41%, -0.44%) at the 24-wk endpoint. At 52 wks, mean UGE with DAPA 5 and 10 mg (27 and 22 g/g creatinine (Cr)) was about half that seen in pts with normal renal function. DAPA 5 and 10 mg decreased PBO-subtracted weight (-2.44 and -2.98 kg) and blood pressure (systolic: -6.22 and -9.14 mm Hg; diastolic: -1.09 and -2.83 mm Hg). DAPA dose-dependently reduced eGFR at wk 1 but then stabilized eGFR vs a continued gradual decline with PBO. Values of urinary albumin:Cr>1800 mg/g were reported in more pts receiving PBO (10.8%) than DAPA (5 mg 7.2%; 10 mg 7.1%). More pts receiving PBO (8%) discontinued due to hyperkalemia vs DAPA 5 (1%) and 10 mg (5%). Renal adverse events, hypotension, fractures, and genital infections were more common on DAPA than PBO. Urinary tract infections were similar among groups. Mean serum phosphorus and

magnesium were slightly increased with DAPA, but remained in the normal range. Serum uric acid decreased and parathyroid hormone increased with DAPA vs PBO.

Conclusions: In summary, DAPA did not improve HbA1c in pts with T2D and moderate renal impairment. Further research is needed to determine if DAPA exerts a renoprotective effect; however potential benefits must be balanced against possible risks in this population.



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TH-PO525

Influence of Renal Function on Dapagliflozin Pharmacodynamics in Patients with Type 2 Diabetes Mellitus Sreeneeranj Kasichayanula, Xiaoni Liu, Melanie Pe Benito, Frank Lacreta, David W. Boulton. *Bristol-Myers Squibb, NJ.*

Background: Dapagliflozin (DAPA), a selective inhibitor of the renal sodium-glucose cotransporter-2 (SGLT2), is currently in development for the treatment of type 2 diabetes mellitus (T2DM). DAPA reduces hyperglycemia independently of insulin by promoting urinary glucose excretion that is proportional to the filtered load of glucose, the product of plasma glucose and the glomerular filtration rate (GFR).

Methods: This open-label, parallel study was conducted to evaluate the pharmacokinetics/pharmacodynamics (PD), safety and tolerability of DAPA in patients with T2DM and normal renal function or, mild, moderate or severe renal impairment (CLcr of 51–80, 30– 50, and <30 ml/min, respectively, excluding patients on dialysis) and healthy subjects after single (50 mg/d) and subsequent multiple (20 mg/d for 7 days - T2DM patients only) doses of DAPA. Background antihyperglycemic medications, excluding exenatide, were allowed for subjects on stable therapy.

Results: Systemic DAPA exposure (AUC) was higher and urinary glucose clearance and changes in fasting serum glucose were lower with decreasing creatinine clearance. Following a single, 50 mg dose of DAPA, systemic DAPA exposure was 28%, 52% and 75% higher and fasting renal glucose clearance (mL/min) values were 53%, 77%, and 85% lower in T2DM patients with mild, moderate, and severe renal impairment, respectively, in comparison to subjects with normal renal function. The corresponding steady-state glucose clearance values, with DAPA 20 mg/d for 7 days, were 42%, 83%, and 84% lower. Mean 24-h urinary glucose excretion (mg) was similarly reduced with increasing degrees of renal impairment. There were no marked differences in serum electrolytes, including sodium, between groups at baseline or after treatment. DAPA was well-tolerated in all subjects, with no discontinuations due to drug-related adverse events.

Conclusions: The PD effects of DAPA are reduced as renal function decreases, despite higher systemic DAPA exposure, due to a decreased GFR and subsequent decrease in filtered glucose load. These results suggest that DAPA may have reduced efficacy in patients with moderate to severe renal dysfunction.

TH-PO526

Influence of Renal Function on Dapagliflozin Pharmacokinetics in Healthy Subjects and in Patients with Type 2 Diabetes Mellitus Sreeneeranj Kasichayanula, Xiaoni Liu, Melanie Pe Benito, Ming Yao, Frank Lacreta, William Humphreys, David W. Boulton. *Bristol-Myers Squibb, NJ.*

Background: Dapagliflozin (DAPA), a selective inhibitor of the renal sodium-glucose cotransporter-2 (SGLT2), is under development for the treatment of type 2 diabetes mellitus (T2DM). The majority of DAPA is glucuronidated by the enzyme UGT1A9 to the inactive metabolite, DAPA 3-O-glucuronide (D3OG). D3OG is mainly cleared via renal excretion. As the kidney is the primary target of DAPA action, this open-label, parallel study was conducted to assess the pharmacokinetics (C_{max} and AUC of DAPA and D3OG) of single and multiple doses of DAPA in patients with T2DM and normal renal function or, mild, moderate or severe renal impairment and healthy subjects.

Methods: Criteria for mild, moderate and severe renal impairment were CLcr of 51–80, 30– 50, and <30 mL/min, respectively, excluding patients receiving dialysis. Subjects received single (50 mg/d) and subsequent multiple (20 mg/d for 7 days in T2DM patients only) oral doses of DAPA (doses up to 10 mg were studied in phase 3 trials).

Results: Single dose pharmacokinetics of DAPA were similar in healthy subjects and patients with T2DM and normal renal function while exposures to DAPA and D3OG were incrementally higher with decreasing renal function. Compared to patients with T2DM with normal renal function, the steady-state geometric mean C_{max} values for DAPA [D3OG] were 4% [20%], 6% [37%], and 9% [52%] higher in subjects with mild, moderate, and severe renal impairment, respectively, and the corresponding geometric mean values for DAPA AUC0-τ were 32% [54%], 60% [110%], and 87% [169%] higher, respectively. Isolated human kidney microsomes showed a high activity of UGT1A9 and a higher rate of D3OG formation relative to human liver and intestinal microsomes (3- and 109-fold higher, respectively).

Conclusions: These findings indicate that a substantial proportion of DAPA metabolism occurs via UGT1A9 in the kidney. The less than 2-fold increase in D3OG exposure suggests that a dose adjustment of DAPA is likely not needed in patients with renal impairment.

TH-PO527

Nox5-Dependent Reactive Oxygen Species Production in Human Podocytes Exposed to Diabetic Stimuli Chet E. Holterman,[1] Moin Saleem,[3] Chelsea Towaij,[1] Rhian Touyz,[1] Mark E. Cooper,[2] Chris R. Kennedy.[1] *[1]Kidney Research Center, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; [3]University of Bristol, Bristol, United Kingdom.*

Background: Reactive oxygen species (ROS) production, via the NADPH oxidase family of enzymes (Noxs), plays a critical role during filtration barrier injury in diabetic nephropathy (DN). While most studies have focused on the role of Nox4 we here examine the role of Nox5 in DN and ROS induced podocyte damage in response to classic diabetic stimuli.

Methods: *In vivo,* Nox5 protein expression was compared by immunofluorescence in human kidney biopsies obtained from non-diabetic and diabetic/albuminuric individuals. *In vitro*, a conditionally immortalized human podocyte line (hPOD) was exposed to various diabetic stimuli including TGF-b (1-10 ng/ml), advanced glycation end-products (AGE), high glucose (25 mM) and equibiaxial mechanical stretch (10% elongation, 0.5 Hz). Nox5 expression was determined by quantitative RT-PCR (qPCR), and western blotting.

Results: Biopsies from diabetic individuals with confirmed albuminuria revealed higher immunodetectable Nox5 expression in glomerular structures vs non-diabetic controls. RT-PCR verified that Nox5β is the predominant isoform present in hPODs while qPCR demonstrated that Nox5 mRNA expression is significantly upregulated in hPODs in response to high-glucose/stretch (>2fold), TGF-b (>2 fold), and AGE (>4fold). As expected Nox5 protein levels are also higher in hPODs in response to diabetic stimuli.

Conclusions: Upregulation of Nox5 in diabetic kidney occurs in response to classic diabetic stimuli. This may in part be responsible for ROS–induced podocyte damage and filtration barrier dysfunction observed in diabetic kidney disease. Mouse models with podocyte targeted Nox5 expression may elucidate the role of Nox5 in the development and progression of DN.

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TH-PO528

Preservation of Endothelial GTP Cyclohydrolase I Activity by Metformin Prevent the Development of Diabetic Nephropathy Kengo Kidokoro, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Increased oxidative stress is involved in the pathogenesis of diabetic vascular complications. Uncoupling of endothelial nitric oxide (NO) synthase (eNOS) via oxidation of tetrahydrobiopterin (BH4), a cofactor required for NO production, plays a major role in of oxidative stress generation. GTP cyclohydrolase I (GTPCH I) is the rate-limiting enzyme in BH4 biosynthesis, and hyperglycemia accelerates GTPCH I degradation via inhibition of AMP-activated protein kinase (AMPK) activity. We hypothesized that preservation of GTPCH I activity and improvement of NO availability either pharmacologically or genetically could prevent development of diabetic renal complications.

Methods: We used male C57/BL6 mice (WT) and spontaneously diabetic Ins2Akita mice (AKITA) and treated them with metformin (Met; 300 mg/kg/day), an oral hypoglycemic drug and a potent AMPK activator as well, for 4 weeks. Next, human glomerular endothelial cells (hGECs) were exposed to normal glucose (5 mM), high glucose (30 mM D-glucose), or hyperosmotic control (5 mM D-glucose + 25 mM mannitol) in the presence or absence of Met. We also examined the effect of overexpression of GTPCH I on diabetic renal complications by crossing endothelium-specific GTPCH I transgenic mice with AKITA (GCHtg/AKITA).

Results: Urinary albumin excretion (UAE) was significantly suppressed in AKITA/Met compared with AKITA without any alteration in blood glucose level. Renal BH4 level and GTPCH I expression were increased in AKITA/Met compared with AKITA. Levels of phospho-AMPK, GTPCH I, and eNOS dimer were reduced in hGECs exposed to high glucose, but Met attenuated the alterations. UAE was significantly suppressed in GCHtg/AKITA. Exacerbated superoxide production and diminished bioavailable NO were noed in the glomeruli of AKITA. Biochemical analysis showed eNOS was uncoupled in AKITA. In GCHtg/AKITA, serum levels of BH4 were preserved and glomerular eNOS was re-coupled.

Conclusions: Preservation of endothelial GTPCH I expression by metformin and resultant improvement of BH4 biosynthesis prevented development of renal complications in diabetic milieu.

Funding: Government Support - Non-U.S.

TH-PO529

Fusion of Bone Marrow-Derived Cells with Renal Tubules Contributes to Renal Dysfunction in Diabetic Nephropathy Tomohisa Yamashita,[1] Mineko Fujimiya,[2] Satoshi Yamamoto,[1] Masayuki Koyama,[1] Yusuke Okazaki,[1] Shutaro Ishimura,[1] Marenao Tanaka,[1] Masato Furuhashi,[1] Hideaki Yoshida,[1] Tetsuji Miura.[1] *[1]2nd Department of Internal Medicine, Sapporo Medical University, Sapporo, Hokkaido, Japan; [2]2nd Department of Anatomy, Sapporo Medical University, Sapporo, Hokkaido, Japan.*

Background: We previously reported that in diabetes proinsulin-producing bone marrow-derived cells (BMDCs) appear in various organs and these cells undergo fusion with hepatocytes and neurons in the dorsal root ganglia. Fusion cells are polyploidy and produce tumor necrosis factor (TNF)-alpha, suggesting that diabetes reprograms gene expression in BMDCs by turning on ''inappropriate'' genes, ultimately causing diabetic complications. In this study, we assessed whether the same mechanism is involved in the DN.

Methods: We performed bone marrow transplant (BMT) from green fluorescent protein transgenic mice to C57BL/6J mice and produced diabetes by streptozotocin (STZ) or feeding a high-fat diet. Immunofluorescence staining and fluorescence in situ hybridization for Y-chromosome were performed and observed by confocal laser scanning microscopy.

Results: In the diabetic mice, massive infiltration of BMDCs occurred in the tubular interstitium, and tubular epithelial cell loss and basement membrane abnormality were prominent. The damaged tubular epithelial cells and BMDCs were positively stained with proinsulin and TNF-alpha. Cell fusion between BMDCs and renal tubules is confirmed by the presence of Y chromosome in female mice that received BMT from male mice. 15.4% of tubular epithelial cells contain Y chromosome in STZ-diabetic mice, 8.6% in HFD-diabetic mice, but only 1.5% in nondiabetic mice. In addition, some nuclei contain two Y chromosomes or a large single Y chromosome in STZ-diabetic mice.

Conclusions: This study demonstrated that diabetes causes nuclear fusion between BMDCs and renal tubular epithelial cells at more than 10-fold higher frequency than nondiabetic mice. Chromosomal abnormality induced by the cell fusion may play a crucial role in DN.

TH-PO530

Proximal Tubule Endocytosis by Kidney Injury Molecule-1 Mediates Early Diabetic Nephropathy Takaharu Ichimura,[1] Shan Mou,[2] Huiping Zhao,[1] Sheng Xiao,[2] Heung-Myong Woo,[1] Suetonia Palmer,[1] Craig R. Brooks,[1] Chang Wang,[1] Joel M. Henderson,[3] Vijay K. Kuchroo,[2] Joseph V. Bonventre.[1] *[1]Renal/Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; [2]Center for Neurologic Disease, Brigham & Womens' Hospital, Boston, MA; [3]Pathology, Boston University Medical Center, Boston, MA.*

Background: Diabetes mellitus is the leading cause of end-stage renal failure. Although diabetic nephropathy (DN) is characterized by glomerulopathy and albuminuria, disease progression best correlates with tubular degeneration and interstitial fibrosis. Kidney Injury Molecule-1 (KIM-1), a scavenger receptor highly expressed by injured proximal tubule cells, is present in the urine of patients with DN and high levels are associated with progression of albuminuria.

Methods: The ability of KIM-1 to mediate uptake of advanced glycosylation end products (AGEs) was determined in kidney epithelial cells in culture. To study the effects of mouse Kim-1-mediated endocytosis on DN disease progression, diabetes was induced in normal mice and mice which contain a targeted mutation of the extracellular mucin domain which prevents endocytosis. DN was induced by combining streptozotocin injection, unilateral nephrectomy and high fat diet.

Results: High glucose levels enhanced Kim-1 expression in cultured kidney epithelial cells via a process that was reactive oxygen species dependent. Kim-1 augmented tubule cell uptake of AGEs and oxidized lipids, two components of the proximal tubular fluid in patients with diabetes mellitus. Mutant mice rendered diabetic developed less albuminuria, brush border loss and fewer interstitial myofibroblasts than normal mice. DN-associated glomerular enlargement, GBM thickening and sclerosis were also diminished by Kim-1 mutation. Kim-1 dependent ingestion of oxidized lipids and AGEs and enhanced caspase-3 activity was inhibited in primary tubule cells from Kim-1 mutant animals.

Conclusions: Kim-1 expression facilitates tubular injury in early DN by endocytic processes which trigger a proinflammatory response leading to interstitial fibrosis. These observations suggest Kim-1 as a novel drug target for prevention and treatment of DN.

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TH-PO531

Diabetic Nephropathy (DN) in Insulin-Deficient Mouse Models: Longitudinal Functional & Ultrasonic Dumentation of Progressive Decline in Glomerular Filtration Rate (GFR) & the Role of Reduced Oxidative/ Nitrosative Stress in Metformin Renoprotection Jian Xu,[1] Meghan Pantalia,[1] Alexander Lau,[1] Bonnie Eby,[1] Chris Skaggs,[1] Shujie Yu,[1] Hongtao Liu,[1] Jian-Xing Ma,[2] Kai Lau.[1] *[1]Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Progress in DN research has been hindered by few models with renal failure (RF). In those showing RF, there are concerns of collateral damages or inaccurate GFR estimated by plasma inulin kinetics after bolus injection.

Methods: Here we measured GFR by renal clearance (C) of inulin (in) during steady-state infusion to document RF in male Ove 26 & Akita mice & study the mechanim of renoprotection by metformin. We profiled serial Ccre by HPLC & defined its utility & pitfalls.

Results: At 7 mon, Cin (in µl/min) fell in Ove 26 mice (225 vs. 401/mouse in control, 919 vs 1,319/100g body [B] weight [W], or 332 vs. 813 /g kidney [K]W). Ultrasound showed larger K volume (0.52 vs. 0.23 cc), which correlated (r=0.80) with heavier KW (845 vs. 510 mg). Chronic metformin prevented RF in Ove mice (Cin=404) & blocked the increases in nitrotyrosine-containing proteins in diabetic kidneys. Echogenicity was 24% higher in Akita at 10.5 mon, showing chronic scarring. Ccre (in µl/min), normal at 4 mon (436 vs. 468), showed hyperfiltration at 6 mon (1,477 vs. 581), normalized at 9 mon (672 vs. 717) but finally fell by 13 mon (240 vs. 700). RF was confirmed by reduced Cin (128 vs. 375/ mouse, 591 vs. 1,213/100g BW, or 256 vs. 941/g KW). Ccre correlated with Cin (r=0.8), gave similar estimates of GFR fall, but over-estimated GRF by 80 %.

Conclusions: 1. With chronicity, untreated Ove 26 & Akita diabetics develop renal failure like man, validating their utility in studying DN. 2. Metformin is renoprotective by reducing oxidative/nitrosative stress. 3. Analyzed by HPLC, Ccre separates diabetic from normal but over-estimates GFR by 80%. It defines the evolution from hyper- to hypo-filtration non-invasively & corroborates falls in GFR found by renal Cin. 4. Ultrasound identifies hypertrophy non-invasively & detects chronic scarring with disease progression.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support, Clinical Revenue Support

TH-PO532

Uremia-Induced Increases in Rodent Hepatic 11bHSD1 and Associated Excessive Gluconeogenesis Contributes to Insulin Resistance in Non-Diabetic Chronic Kidney Disease Ananda Chapagain, Julius Edward Kieswich, Steven Michael Harwood, Martin J. Raftery, Magdi Yaqoob. *Translational Medicine and Therapeutics, WHRI, London, United Kingdom.*

Background: Insulin resistance (IR) is common in chronic kidney disease (CKD). However the mechanism underlying this has yet to be elucidated. Abnormally elevated hepatic gluconeogenesis can cause hyperglycemia and hyperinsulinemia, secondary to upregulation of key gluconeogenic genes in particular phosphoenolpyruvate carboxykinase (PCK1), leading to elevated hepatic glucose production.

Methods: 11b-hydroxysteroid dehydrogenase type I (11bHSDI) catalyses intracellular conversion of the inactive glucocorticoid cortisone to active cortisol and promotes hepatic gluconeogenesis. We investigated the role of elevated hepatic 11bHSD1 in uremia-induced IR.

Rodent models of non-diabetic uremia were created using subtotal nephrectomy and adenine-induced uremia. Sham or uremic animals were gavaged with vehicle or carbenoloxone (CX)50mg/kg for two weeks. In separate experiments,insulin and glucose tolerance tests were perfomed in each of the groups and serum and organs harvested and snap-frozen for analysis.

Results: Uremic rats displayed hyperinsulinemia and abnormal glucose and insulin tolerance. This was associated with upregulation of 11bHSD1 and PCK1. Additionally, levels of the gluconeogenic coactivator PPARg co-activator 1alpha (PGC1a) were increased. Uremia was also associated with increased concentrations of plasma triglycerides, non-esterified fatty acids and cholesterol, alongwith elevated expression of key lipogenic genes; fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), steroid-regulatory element binding protein-1c (SREBP1c), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR).Treatment with CX for two weeks corrected hyperinsulinemia, improved glucose tolerance and insulin sensitivity and reduced expression of PCK1 and PGC1a along with correction of dyslipidemia and expression of FAS, ACC, SREBP1 and HMGCR significantly.

Conclusions: Our results indicate that elevated expression of 11bHSD1 contributes to IR and dyslipidemia in uremia, and confirms 11bHSD1 inhibitors as a novel therapeutic target for management of IR in patients with CKD.

Funding: Government Support - Non-U.S.

TH-PO533

Inhibition of the p66 Longevity Gene Prevents Diabetic Nephropathy in Mutant Akita Diabetic Mice Himanshu Vashistha,[1,4] Pravin C. Singhal,[2] Ashwani Malhotra,[2] Surya V. Seshan,[3] Krzysztof Reiss,[4] Leonard G. Meggs.[1,4] *[1]Department of Nephrology, Ochsner Clinic Foundation, New Orleans, LA; [2]Division of Nephrology, North Shore LIJ Health System, Great Neck, NY; [3]Department of Surgical Pathology, Weil Cornell Medical School, New York, NY; [4]Department of Medicine, LSUHSC, New Orleans, LA.*

Background: The absence of an experimental model that faithfully mimics diabetic nephropathy (DN) in humans, has been a major limitation in dissecting the pathobiology of DN and in identifying novel molecular targets for therapeutic intervention. The recent development of Akita (Ins2+/C96Y) diabetic mice, lacking the bradykinin 2 receptor (B2R-/-) is a significant advance, in that hyperglycemia (HG) is more durable and sustained , than with inbred strains requiring streptozotocin injection, with a disease phenotype that more closely approximates DN in humans.

Methods: The p66-/- mouse is the unique genetic model of increased resistance to oxidative stress, aging and apoptosis. To test if this loss of function mutation, will delay or prevent DN, p66 was deleted for the genome of mutant Akita diabetic mice, by homologous recombination with the genome of p66-/- mouse. RNAi was used to deliver p66shRNA to human podocytes, which were maintained under HG conditions.

Results: Triple mutant Akita (p66-/-/Ins2+/C96Y/B2R-/-) mice at 11-12 mo of age, show marked attenuation of mesangial sclerosis and tubular injury, with a striking reduction in urinary albumin excretion. Podocytes/glomerulus did not differ from non-diabetic wild type (wt) control mice, with minimum evidence of foot process effacement. In vitro studies, with human podocytes expressing p66shRNA show inhibition of HG-induced ROS production, local angiotensin II generation and apoptosis.

Conclusions: We speculate p66 is a target for therapeutic intervention in glomerular diseases, where ROS have been implicated in metabolic, inflammatory and toxic injury.

Funding: NIDDK Support

TH-PO534

Macrophage Liver X Receptor α Ameliorates Renal Lesions in Hyperlipidemic Diabetic Mice Eva Kiss, Mahnaz Bonrouhi, Katja Wagenblass, Hermann-Josef Groene. *German Cancer Research Center (DKFZ) Heidelberg.*

Background: Abnormal lipid metabolism and renal accumulation of lipids are pathophisiological cofactors of diabetic nephropathy. Liver X receptors (LXR) α and β are nuclear receptors activated by oxidized cholesterols. LXR/RXR heterodimers regulate genes linked to lipid and carbohydrate homeostasis and inhibit inflammatory gene expression in macrophages.

Methods: In this study the effects of systemic and macrophage specific LXR activation were analyzed on renal damage in hyperlipidemic diabetic mice. Diabetes was induced by streptozotozin in LDLR-deficient (LDLR-/-) mice on standard chow or western diet with or without the nonselective synthetic LXR-agonist, GW3965 for 20 weeks. The role of macrophage-LXRα mediated effects were analyzed in mice with macrophage overexpression of LXRa.

Results: Hyperglycemia and hyperlipidemia acted synergistically in inducing renal injury (mesangial matrix increase, podocyte damage, foam cell formation) and altering renal function. LXR activation by GW3965 inhibited significantly impairment of renal function by a 60% higher creatinine clearance, reduced glomerular and interstitial mononuclear cell infiltrate (Mac-2+, F4/80, CD3) and the number of interstitial myofibroblasts (αSMA+) by 40-50%. Renal expression of genes involved in cholesterol efflux (ABCA1, ABCG1) was upregulated concomitant with the downregulation of proinflammatory/ profibrotic cytokines (TNFα, TGFβ) and oxidative enzymes (Nox-2) in GW3965 treated hyperlipidemic diabetic mice. Overexpression of LXRα in macrophages was sufficient to significantly ameliorate hyperglycemia or/ and hyperlipidemia induced renal damage and albuminuria. *In vitro* in macrophages LXR activation by GW3965 or 25-OH-Cholesterol as well as LXRa overexpression significantly reduced the proinflammatory/ profibrotic activity (TNFα, MCP-1, collagen I) induced by glycated LDL.

Conclusions: LXR activation attenuated diabetic and hyperlipidemic renal lesions attesting to the potent regulatory role of these nuclear receptors in metabolic diseases.

Funding: Government Support - Non-U.S.

TH-PO535

Renal Effects of Inhibiton of Toll like Receptor Signaling in Type-2 Diabetic Mice Jin Joo Cha,[1] Young Sun Kang,[1] Young Youl Hyun,[1] Mihwa Lee,[1] Jung Eun Kim,[1] Deok Hwa Nam,[1] Hye Kyung Song,[1] Ji Eun Lee,[2] Hyunwook Kim,[2] Sang Youb Han,[3] Kum Hyun Han,[3] Dae R. Cha.[1] *[1]Internal Medicine, Korea University Medical College, Ansan, Kyeonggido, Republic of Korea; [2]Internal medicine, Wonkwang University Medical College, Gunpo, Kyeonggido, Republic of Korea; [3]Internal Medicine, Inje University Medical College, Koyang, Kyeonggido, Republic of Korea.*

Background: Chronic inflammation caused by high concentration of glucose and free fatty acids (FFAs) is one of the major pathogenesis of type2 DM. Recent evidences suggest that the activation of toll-like receptor(TLR) signaling, which is involved in various innate immune responses, induces peripheral insulin resistance and mediates central insulin and leptin resistance. Present study was performed to investigate the renal effects of TLR signaling blockade in the diabetic mouse.

Methods: Eight-week old db/db mice were treated for 12 weeks with intraperitoneal GIT27, which targets the function of macrophages through inhibition of TLR4 and TLR2/6-mediated signaling pathways, at a dose of 20mg/kg/day. Another group of db/db mice were treated with control vehicle.

Results: GIT27 treated db/db mice showed decrease in HbA1c at 3months, improved glucose tolerance, lower lipid profile without impact on body weight and food consumption. GIT27 treatment also markedly decreased urinary albumin excretion(30.24±17.38 vs 6.88±10.50ug/d, p=0.02), decreased pro-inflammatory cytokine synthesis(IL-2, TNF-α, p<0.05), improved tissue lipid metabolism(cholesterol, triglyceride, lipid peroxidase level in the kidney, liver, fat tissue) and improved glomerulosclerosis compared to control db/db group. In cultured podocytes and adipocytes, high glucose with FFAs stimulation increased TLR4 expression and pro-inflammatory cytokines synthesis, which effects were abolished by GIT27 treatment.

Conclusions: In summary, GIT27 treatment improved insulin resistance, dyslipidemia and proteinuria in type 2 diabetic mice. These results suggest TLR pathway inhibition might have a direct protective role in diabetic kidney disease.

TH-PO536

Resveratrol Prevents Diabetic Nephropathy Via Activating the AMPK-SIRT1-PGC-1 Pathway in db/db Mice Sungjin Chung, Min-Young Kim, Ji Hee Lim, Hoon Suk Park, Byung Ha Chung, Hyun Wha Chung, Seok Joon Shin, Hyung Wook Kim, Yong-Soo Kim, Yoon-Sik Chang, Cheol Whee Park. *Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: AMPK and SIRT1 play key roles in the regulation of lipid and glucose homeostasis and for controlling oxidative stress. Many of resveratrol's effects are consistent with the activation of SIRT1-AMPK-PGC-1α and the modulation of their targets, including PPARs and FoxOs. In this study, we sought to uncover the mechanism by which resveratrol affects diabetic nephroapthy.

Methods: The *db/db* and *db/m* mice were treated with or without resveratrol starting at 8 weeks of age for 12 weeks.

Results: The *db/db* mice treated with resveratrol had decreased albuminuria, and resveratrol ameliorated glomerular matrix expansion compared to the control *db/db* mice even under the same degree of hyperglycemia. Resveratrol decreased inflammatory cell infiltration and the intrarenal free fatty acid and triglyerides contents associated with increased phospho-Thr^{172} AMPK-SIRT1- PGC-1α signaling and activation of its key downstream effecters the PPARα- ERR-1α expressions. Furthermore, resveratrol decreased the activity of PI3K-Akt phosphorylation and FoxO3a phosphorylation, which resulted in a decrease of proapototic Bax and increases in the anti-apoptotic Bcl-2 and anti-oxidant SOD1 and SOD2 expressions in the *db/db* mice. Consequently, resveratrol reversed the diabetes-induced lipid accumulation in the kidney and the increased renal apoptotic cells and oxidative stress that is reflected by the serum 8-hydroxydeoxyguanosine (8-OH-dG) and the urinary 8-OH-dG and isoprostane levels.

Conclusions: Our results suggest that resveratrol prevents diabetic nephropathy in *db/db* mice by activation of AMPK-SIRT1-PGC-1α signaling, which results in prevention of lipotoxicity, apoptosis and oxidative stress in the kidney.

TH-PO537

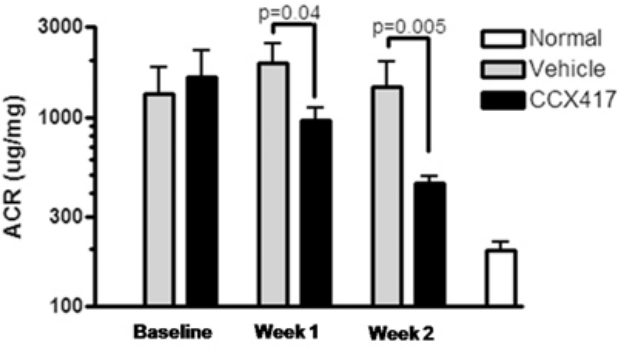
CCR2 Inhibition in Diabetic Mice Results in a Rapid and Robust Improvement of Renal Function Tim Sullivan, Zhenhua Miao, Bin N. Zhao, Robert D. Berahovich, Lisa C. Seitz, Jay P. Powers, Linda Ertl, Shichang Miao, Ruiping Zhao, Ton Hy Dang, Shirley Liu, Pirow Bekker, Thomas J. Schall, Juan C. Jaen. *ChemoCentryx, Inc., Mountain View, CA.*

Background: Diabetic Nephropathy represents one of the major consequences of uncontrolled diabetes. The chemokine receptor CCR2 has been implicated in the recruitment of blood monocytes into kidney in response to hypertension and hyperglycemia. In addition, parenchymal renal cells are thought to upregulate CCR2 under those pathological conditions. We set out to assess the therapeutic benefit of CCR2 antagonism in a mouse model of diabetic nephropathy.

Methods: CCX417 (a small molecule CCR2 antagonist, analog of the clinical compound CCX140-B) was dosed daily to male db/db mice (age 12-19 weeks). Weekly assessments included body weight, fasting plasma glucose, serum clinical chemistry, and 24 hr urinary volume and output of albumin, creatinine and glucose.

Results: Treatment with CCX417 significantly reduced urinary albumin excretion (UAER) and albumin:creatinine ratio (ACR). Statistically significant improvements in UAER and urinary ACR were noted as early as 48 hours after initiation of CCX417 treatment. Serum markers of renal function were also improved after 14 days of CCX417 treatment: serum creatinine and blood urea nitrogen. The benefits seen on renal function preceded significantly reduced fasting plasma glucose levels. Significant reductions in urinary output were also seen with CCR2 antagonism.

Conclusions: Robust and rapid improvements of albuminuria, serum markers of renal function, and hyperglycemia were seen following pharmacological intervention with a small-molecule CCR2 antagonist in a rodent model of diabetic nephropathy. These results support the clinical evaluation of CCR2 antagonists, such as CCX140-B, for the treatment of diabetic nephropathy.



Funding: Pharmaceutical Company Support

TH-PO538

A Novel Role for Liver X Receptors in Diabetic Nephropathy Xiaoxin Wang,[1] Monika Patel,[2] Hannah Danielle Santamaria,[1] Weidong Wang,[1] Nathaniel L. Solis,[1] Liru Qiu,[1] Carolyn L. Cummins,[2] Moshe Levi.[1] *[1]university of colorado denver; [2]University of Toronto.*

Background: The liver X receptor (LXR) belongs to the nuclear receptor superfamily of ligand-activated transcription factors. Oxysterols and oxidized derivatives of cholesterol are endogenous ligands for LXR. Activation of LXR induces the transcription of genes involved in reverse cholesterol transport and prevents inflammation. We hypothesized that these properties of LXR agonists would make them useful in the modulation of diabetic nephropathy. However, certain LXR agonists also induce de novo fatty acid synthesis which may limit their efficacy. Recently, a novel LXR agonist N,N-dimethyl-3beta-hydroxy-cholenamide (DMHCA) has been developed that does not have undesirable lipogenic effects and therefore facilitates the exploration of LXR function in the diabetic kidney.

Methods: In this study we induced type 1 diabetes in western diet fed DBA/2J mice using multiple low dose streptozotocin injections and treated mice for 10-wk after diabetes was confirmed with the LXR agonist DMHCA at a dose of 80mg/kg body weight/day.

Results: We found that LXR agonist treatment improves proteinuria (DBA/Con: 123±26mg/mg; DBA/STZ: 286±41mg/mg; DBA/STZ/DMHCA: 137±22mg/mg; $p<0.01$), podocyte loss, mesangial expansion, and tubulointerstitial fibrosis. In addition LXR treatment prevented renal lipid accumulation, macrophage infiltration, inflammation, and oxidative stress. In contrast induction of diabetes in the LXR a/b double knockout mice resulted in exaggerated diabetic nephropathy characterized by increased urinary albumin and nephrin excretion, and increased inflammation and lipid accumulation.

Conclusions: These results therefore indicate a novel and an important role for LXR in modulation of diabetic nephropathy.

TH-PO539

Effect of Physical Exercise on Urinary Albumin and N-acetyl-β-glucosaminidase, and Podocyte Numbers in Diabetic KK-A^y Mice Yuji Ishikawa, Tomohito Gohda, Mitsuo Tanimoto, Keisuke Omote, Saori Yamaguchi, Masako Furukawa, Maki Murakoshi, Shinji Hagiwara, Yasuhiko Tomino. *Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan.*

Background: Exercise is generally recommended for the management of type 2 diabetes, but its effects on diabetic nephropathy are still unknown. We hypothesized that appropriate physical exercise improves early diabetic nephropathy via attenuation of chronic inflammation and oxidative stress.

Methods: Type 2 diabetic KK-A^y mice, a spontaneous diabetic nephropathy model, underwent two different kinds of exercise for one hour five days a week (moderate intensity) and 30 minutes three days a week (low intensity) for 8 weeks. Sedentary mice or those undergoing exercise regimens causing no significant body weight loss were used. We examined the urinary excretion of albumin and N-acetyl-β-glucosaminidase (NAG), number of podocytes in glomeruli, renal expressions of hypoxia inducible factor (HIF)-1α and monocyte chemotactic protein (MCP)-1, and biomarkers of oxidative stress such as urinary 8-hydroxydeoxyguanosine (8-OHdG) and serum superoxide dismutase (SOD).

Results: Exercise reduced urinary albumin excretion and NAG, and also maintained the number of podocytes in the exercised KK-A^y mice independently of improvements of body weight and hyperglycemic status. However, the moderate-intensity exercise increased expression of HIF-1α in the kidneys. Sedentary KK-A^y mice showed increased expression of MCP-1 in the tubules, urinary 8-OHdG levels, and decreased serum SOD levels compared with exercised KK-A^y mice.

Conclusions: On the whole, low-intensity exercise attenuates progression of early diabetic nephropathy without affecting renal ischemia. Reductions in rates of urinary albumin change and urinary NAG, and maintained podocyte numbers, with parallel improvements in oxidative damage and chronic inflammation are related to the beneficial effects of exercise in diabetic kidney disease.

TH-PO540

Fractalkine/CX3CR1 Mediates Extracellular Matrix Accumulation in Diabetic Kidney Kyung Hee Song, Jehyun Park, Hunjoo Ha. *Department of Bioinspired Science, College of Pharmacy, Ewha Womans University, Seoul, Republic of Korea.*

Background: Fractalkine (FKN, CX3CL1) functions not only as a chemokine but also as an adhesion molecule and plays an important role in the recruitment of macrophages into the kidney through binding to its receptor CX3CR1. Previous studies have demonstrated that FKN/CX3CR1 play a role in ischemic and protein-overload renal injury. However, their role in diabetic renal injury and the mechanism involved in have not been clearly understood. This study examined whether FKN/CX3CR1 mediates diabetic stimuli-induced extracellular matrix (ECM) accumulation in the kidney.

Methods: Streptozotocin (STZ; 50 mg/kg/day) was intraperitoneally administered for 5 days in male CX3CR1 wild type (WT) and null C57BL/6J mice. Mouse mesangial cells (MMCs) transfected with siRNA for CX3CR1 were used to further elucidate the direct effect of FKN/CX3CR1 on ECM upregulation.

Results: At 12 weeks after the induction of diabetes, equivalent hyperglycemia was observed in diabetic WT and CX3CR1 null mice. However, parameters of diabetic renal injury including increased plasma creatinine, kidney to body weight ratio, glomerular volume, fractional mesangial area, urinary protein excretion, and accumulation of renal fibronectin, collagen, and macrophage were much less severe in diabetic CX3CR1 null mice than diabetic WT mice. In MMCs, 30 mM high D-glucose, 100 μM oleic acid, 10 ng/ml of transforming growth factor-β1 upregulated FKN and CX3CR1 mRNA and protein expression along with ECM expression, which was significantly attenuated by CX3CR1 siRNA. More importantly, FKN itself increased ECM synthesis in MMCs, and CX3CR1 siRNA abrogated FKN-induced ECM synthesis.

Conclusions: These results demonstrated that FKN/CX3CR1 may play an important role in diabetic renal injury through ECM upregulation, apart from macrophage infiltration, and suggest that FKN/CX3CR1 system may become an effective therapeutic target for the prevention of diabetic renal injury.

TH-PO541

Resveratrol Attenuates Diabetic Nephropathy by Modulating Angiogenic Factors Donghai Wen, Xinzhong Huang, Chuan-Ming Hao. *Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China.*

Background: Angiogenesis plays an important role in the pathogenesis and progression of diabetic nephropathy(DN). In the present study, we investigated the therapeutic potential of resveratrol, a natural polyphenol with anti-angiogenic activity, in DN.

Results: In a type 1 diabetic rat model induced by streptozocin, urine albumin excretion(13.81±1.25 *vs* 1.53±0.38mg/24h), kidney weight(2.26±0.11 *vs* 1.94±0.04g) and creatinine clearance rate(0.32±0.05 *vs* 0.09±0.02ml/min/100g body weight) were increased, and were suppressed by eight weeks treatment of resveratrol(6.55±0.57mg/24h;1.91±0.07g;0.18±0.03ml/min/100g body weight;respectively). Morphological studies revealed increased glomerular diameter, mesangium expansion, and glomerular basement membrane thickness in diabetic rats(211.67±14.05 *vs* 158.33±6.66nm), which were also reduced by resveratrol treatment(179.67±7.64nm). In addition, treatment of the diabetic rats with resveratrol significantly alleviated renal fibrosis detected by increased expression of fibronectin, PAI-1, CTGF, type IV collagen and TGF-β1. In the diabetic kidney, increased expression of VEGF, Flk-1 and angiopoietin 2, and reduced expression of Tie-2 were observed. Theses changes in angiogenic growth hormones and their associated receptors were attenuated by resveratrol treatment. No change in angiopoietin 1 expression was detected among each group of rats. In vitro, resveratrol significantly down-regulated high glucose-induced VEGF and Flk-1 expression in cultured mouse glomerular podocytes and endothelial cells, respectively. However, these effects of resveratrol were blacked by knocking-down silent information regulator 1(Sirt1) using RNA interference, consistent with a role of Sirt1 in mediating the effect of resveratrol. On the other hand, up-regulation of Sirt1 in cultured endothelial cells by transfecting the cells with Sirt1 plasmid reduced Flk-1 expression. Increased permeability and cellular junction disruption of cultured endothelial cells caused by VEGF were also inhibited by pretreatment with resveratrol.

Conclusions: Resveratrol may attenuate DN via modulating the angiogenesis system.

Funding: Government Support - Non-U.S.

TH-PO542

mTOR Regulates Nox4-Mediated Podocyte Loss in Diabetic Renal Injury Assaad Antoine Eid, Bridget M. Ford, Jeffrey L. Barnes, Yves C. Gorin, Goutam Ghosh-Choudhury, Hanna E. Abboud. *Medicine/Nephrology, University of Texas Health Science Center at San Antonio, TX.*

Background: Glomerular podocyte apoptosis represents a critical mechanism for excessive loss of urinary albumin that eventuates in kidney fibrosis. Pharmacological doses of the mTOR inhibitor rapamycin reduce albuminura in diabetes by unknown mechanism.

Methods: We explored the hypothesis that mTOR mediates podocyte injury in diabetes.

Results: High glucose (HG) induces apoptosis of cultured podocytes and increases the levels of Nox4 and NADPH oxidase activity. HG also inhibits the phosphorylation of AMPK on the activating site Thr^{172}, increases the phosphorylation of tuberin on its inactivating sites Thr^{1462}. HG also activates mTOR and enhances the phosphorylation of its substrate S6 kinase. Inhibition of mTOR by low doses of rapamycin prevents HG–induced expression of Nox4, NADPH oxidase activity and podocyte apoptosis. Inhibition of mTOR had no effect on AMPK or tuberin phosphorylation indicating that mTOR is downstream of these two signaling molecules. In isolated glomeruli of OVE26 type 1 diabetic mice, there is similar decrease in the phosphorylation/activation of AMPK, enhanced phosphorylation of tuberin on the inactivating site Thr^{1462} and activation of mTOR and S6 kinase together with increase in Nox4 and NADPH oxidase activity. Inhibition of mTOR by low and clinically relevant doses of rapamycin reduces podocyte apoptosis, glomerular basement membrane thickening and attenuates albuminuria.

Conclusions: Our data provide evidence for understanding a novel function of mTOR in Nox4-derived ROS generation and podocyte apoptosis that contribute to urinary albumin excretion in type 1 diabetes. Thus mTOR inhibition may represent a therapeutic modality of diabetic kidney disease.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

TH-PO543

Increased Plasma Glucose and Reduced Albuminuria in AMP-Activated Protein Kinaseβ1 Null Mice with Streptozotocin-Induced Type 1 Diabetes
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Background: Aim: To determine the effect of type 1 diabetes on glucose metabolism and kidney function in mice lacking the metabolic regulating kinase AMP-activated protein kinase (AMPK).

Methods: Background: AMPK is a master metabolic regulator considered to have an important role in development of insulin resistance. In order to determine whether AMPK might play a role in glucose metabolism in the absence of insulin, we induced diabetes in mice lacking the β1 subunit of AMPK (AMPKB1 -/- mice).

Type 1 diabetes was induced by 5 i.p. injections of streptozotocin (STZ). Mice were sacrificed at 4 weeks post-injection.

Results: There was a significant increase in plasma glucose in AMPKβ1-/- mice at days 28 compared with WT controls (e.g. day 28, 31.1+8.0 vs 20+8.8 mmol/L, $p<0.05$). Insulin concentrations were similar in both groups. Plasma adiponectin was reduced in AMPKβ1-/- mice compared with WT ($p<0.05$), but was similar in the diabetic mice groups. Despite the difference in plasma glucose, diabetic AMPKβ1-/- mice had less albumin excretion than WT at 28 days (ACR 64.7+11.8 vs 34.2+9.5 mg/mmol, $p<0.001$). Creatinine clearance was unchanged. Kidneys from diabetic AMPKβ1-/- mice also had less α-smooth muscle actin accumulation than diabetic wild type controls by Western blot ($p<0.01$) but no difference in TGF-β1 mRNA expression. Administration of the AMPK activator metformin 600 mg/kg to diabetic C57Bl/6 mice for the last 7 days of the STZ model was associated with significantly increased albumin excretion (ACR 63.4±20.6 vs 94.8±26.4 mg/mmol, $p<0.05$) but no change in plasma glucose.

Conclusions: These studies confirm that AMPK contributes to glucose homeostasis independent of any effect on insulin signaling. Within the kidney, reduced AMPK signaling was associated with less albuminuria and markers of fibrosis. The data suggest that anti-diabetic drugs which activate AMPK, such as metformin and the glitazones, may have deleterious effects on kidney survival in diabetes.

Funding: Government Support - Non-U.S.

TH-PO544

Translational Regulation of microRNA-192/194 by Transforming Growth Factor Beta 1 in Chronic Kidney Disease
Robert H. Jenkins, John Martin, Aled O. Phillips, Timothy Bowen, Donald Fraser. *Institute of Nephrology, Cardiff University, Cardiff, Wales, United Kingdom.*

Background: Tubulointerstitial fibrosis is a key determinant of CKD and epithelial-to-mesenchymal transition (EMT) of proximal tubular epithelial cells (PTC) by transforming growth factor beta 1 (TGF) is integral to this process. We have linked changes in expression of microRNAs, notably miR-192, to EMT in diabetic nephropathy. miR-192 is also important in renal electrolyte transport and cell cycle control. The role of miR-192 in kidney disease is complex, with increased and decreased miR-192 expression linked to adverse outcome in various patient groups and animal models. A limitation to our current knowledge is the regulation of miR-192. Previously, we demonstrated the transcriptional co-regulation of miR-194-2/192 and repression by TGF. The purpose of the current study was to identify factors regulating transcription.

Methods: An in vitro study in PTC using RT-qPCR, luciferase reporter constructs, EMSA, and siRNA knockdown.

Results: Analysis of the miR-194-2/192 promoter identified binding sites for hepatocyte nuclear factor 1 (HNF1) and tumor suppressor p53. Analysis of RNA from 20 human tissues detected miR-192/194 expression in a subset of epithelial tissues, including intestine, kidney and liver. HNF1A/B expression was also restricted to the same subset of tissues, while p53 was ubiquitously expressed. RT-qPCR demonstrated decreased HNF1A/B mRNA expression in response to TGF, but there was no change in p53. Transcription factor binding to both HNF1 and p53 sites in the miR-194-2/192 promoter was detected by EMSA. Specificity was confirmed using probes incorporating point mutations to the key residues within each binding site. Supershift analysis confirmed binding of HNF1A/B. Binding to the HNF1 site decreased in response to TGF, while binding to the p53 site remained constant. Reporter constructs with mutations to the HNF1 or p53 binding sites abolished promoter activity. siRNA knock down of HNF1 resulted in decreased miR-192/194 expression.

Conclusions: These data identify a requirement for p53 and HNF1 to enable miR-192/194 expression in PTC. TGF repression is mediated via a decrease in HNF1A/B binding to the promoter.

Funding: Private Foundation Support

TH-PO545

Physiological Levels of Endostatin Suppress Glomerular Vascular Leak and Inflammation in Diabetic Nephropathy
Yuki Udagawa,[1] Ryota Kimura,[1] Nobunori Satoh,[1] Yoshihiko Ueda,[2] Osamu Yokosuka,[3] Makoto Ogawa,[4] Yuki Hamano.[4] *[1]Clinical Education and Research, Graduate School of Pharmaceutical Sciences, Chiba University; [2]Pathology, Dokkyo Medical University Koshigaya Hospital; [3]Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University; [4]Nephrology, Chiba University Hospital, Japan.*

Background: Disruption of angiogenesis balance to favor enhanced angiogenesis represents a key step in a number of pathological disorders. The potential role of physiological levels of endogenous inhibitors of angiogenesis is poorly understood in diabetic nephropathy. Collagen XVIII (Col18) is a component of highly specialized extracellular matrix associated with basement membranes of epithelia and endothelia. Proteolytic cleavage within its C-terminal domain releases a fragment, endostatin, which has anti-angiogenesis effects. Streptozotocin (STZ) has been commonly used to induce hyperglycemic diabetes and its toxicity leads to vascular injury and inflammation. The aim of this study is to investigate the effect of endogenous endostatin on early vascular leak and inflammation in the kidney of STZ-induced hyperglycemic mice.

Methods: Diabetic nephropathy was induced in Col18/endostatin-null (KO) and wild-type (WT) mice by injection of STZ. Animals injected with citrate buffer only served as controls. At 14 weeks, blood and urine samples were collected and mice were sacrificed to obtain tissues for histological study.

Results: In contrast to animals only injected with citrate buffer, mice that received STZ developed hyperglycemia. No differences in the degree of hyperglycemia and blood pressure were seen between diabetic KO and WT mice. Albuminuria and mean glomerular volume were significantly increased in diabetic KO mice compared to diabetic WT mice. Lack of endostatin led to increased macrophage accumulation, expression of MCP-1 and TNF-α, but not of VEGF, and number of $CD31^+$ blood vessels in the kidney of diabetic mice. $CD31^-CD34^+$ cells were also observed in the glomerulus from diabetic KO mice.

Conclusions: Physiological levels of endostatin, a fragment of collagen XVIII, can serve as a suppressor of renal angiogenesis and inflammation in early stages of diabetic nephropathy.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

TH-PO546

Angiotensin II Type II Receptor (AT_2R) Deficiency Accelerates the Development of Nephropathy in Type I Diabetes Via Oxidative Stress and ACE2
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Background: Since the functional role(s) of angiotensin II (Ang II) type II receptor (AT_2R) in type I diabetes is unknown, we hypothesized that AT_2R is involved in decreasing the effects of type I diabetes on the kidneys.

Methods: We induced diabetes with low-dose streptozotocin (STZ) in both AT_2R knock-out (AT_2R KO) and wild type (WT) male mice aged 12 weeks and followed them for 4 weeks. Three subgroups [non-diabetic, diabetic and insulin-treated diabetic (Rx insulin implant)] were studied. Systolic blood pressure (SBP), physiological parameters, glomerular filtration rate (GFR), renal morphology, gene expression and apoptosis were assessed.

Results: After 4 weeks of diabetes, compared to WT controls, AT_2RKO mice clearly developed features of early diabetic nephropathy (DN), such as renal hypertrophy, tubular apoptosis, and progressive extracellular matrix (ECM) protein accumulation as well as increased GFR. AT_2RKO mice presented hypertension unaffected by diabetes. Renal oxidative stress (measured as heme oxygenase 1 (HO-1) gene expression and reactive oxygen species (ROS) generation) and intrarenal renin angiotensin system components, such as angiotensinogen (Agt), AT_1R and angiotensin-converting enzyme (ACE) gene expression, were augmented whereas angiotensin-converting enzyme-2 (ACE2) gene expression was decreased in renal proximal tubules (RPTs) of AT2RKO mice. The renal changes noted above were significantly enhanced in diabetic AT_2RKO mice, but attenuated in insulin-treated diabetic WT and AT_2RKO mice. The renal changes noted above were significantly enhanced in diabetic AT_2RKO mice, but partially attenuated in the insulin-treated diabetic WT and AT_2RKO mice.

Conclusions: AT_2R deficiency accelerates the development of DN, which appears to be is mediated, at least in part, via heightened oxidative stress and ACE/ACE2 ratio in RPTs.

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The Protective Role of miR-29 in Diabetic Kidney Disease: Mechanism and Therapeutic Potential Haiyong Chen,[1] Xiang Zhong,[2] Xiao Ru Huang,[3] Xiaoming Meng,[1] Hui Y. Lan.[1,3] *[1]Department of Medicine & Therapeutics, Chinese University of Hong Kong, Hong Kong, China; [2]Department of Chemical Pathology, Chinese University of Hong Kong, Hong Kong, China; [3]Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China.*

Background: TGF-β/Smad signaling plays a vital role in renal fibrosis including diabetic nephropathy (DN). However, general blockade of TGF-β signaling may promote renal inflammation. Thus, identification of TGF-β/Smad3-dependent miRNAs related to fibrosis may be the key step towards to develop specific therapy for DN. We have previously shown that miR-29 is TGF-β/Smad3-dependent miRNA in renal fibrosis, thus, we hypothesized that miR-29 may have therapeutic potential for diabetic kidney.

Methods: miR-29 expression and diabetic kidney injury was examined in db/db mice. The therapeutic effect of miR-29 on diabetic kidney disease was determined by delivering a Dox-inducible miR-29 to 10-week db/db mice using an ultrasound-microbubble-mediated-technique. The regulating mechanisms of miR-29 under diabetic conditions was investigated in tubular cells lacking Smad2 or Smad3 and in a stable mesangial cell line with Dox-inducible miR-29 overexpression and knockdown.

Results: In db/db mice, miR-29 was reduced by 60% over the 20 week time, which was associated with a marked increase in TGF-β/Smad3 signaling, renal fibrosis (collagen I, IV), and microabluminuria (all $p<0.01$). Ultrasound-mediated gene therapy restored normal levels of miR-29 in db/db mice, resulting in inhibition of diabetic kidney injury as described above (all $p<0.01$). In vitro, addition of AGEs induced a loss of miR-29 (60%↓) and increased collagen I & IV(all $p<0.01$), which was further significantly enhanced in miR-29 knockdown mesangial cells, but blocked in cells overexpressing miR-29. Moreover, we found that AGEs-downregulated miR-29 expression was Smad3-depedent, but not Smad2.

Conclusions: miR-29 is lost in the diabetic kidney of db/db mice and is mediated by TGF-β/Smad3. Overexpression of miR-29 is able to attenuate diabetic kidney disease in db/db mice and in vitro under diabetic conditions. Thus, miR-29 may represent a novel therapy for diabetic kidney complication.

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Spironolactone Diminishes Urinary Albumin Excretion in Type 1 Diabetic Patients with Microalbuminuria: A Randomized Placebo-Controlled Crossover Study Stine Nielsen,[1] Frederik I. Persson,[1] Erik Frandsen,[4] Takeshi Sugaya,[2] Dietmar Walter Zdunek,[5] Katrine Jordan Pedersen,[1] Hans-Henrik Parving,[3] Peter Rossing.[1] *[1]Steno Diabetes Center; [2]Riken Kobe Institute, Japan; [3]University Hospital of Copenhagen, ; [4]Glostrup Hospital; [5]Roche Diagnostics.*

Background: It has been shown, that adding the aldosterone receptor blocker spironolactone to standard renoprotective treatment, including renin-angiotensin-aldosterone system (RAAS) blockade in diabetic patients, may provide additional renoprotection. These studies have mainly been done in overt diabetic nephropahy. We examined the early renoprotective effect of spironolactone on markers of glomerular and tubular damage in type 1 diabetic patients with microalbuminuria.

Methods: We performed a double-blind, randomized, placebo controlled, crossover study with microalbuminuria using spironolactone 25 mg or placebo once daily for 60 days, added to optimal antihypertensive treatment including RAAS blockade.

After each treatment period, endpoints were evaluated: urinary(u)-albumin excretion/24hour(h), 24h blood pressure, glomerular filtration rate (GFR) and markers of tubular damage: urinary liver-type fatty-acid binding protein (LFABP), neutrophil gelatinase associated lipocalin (NGAL) and kidney injury molecule 1 (KIM1).

Results: All patients completed the study. During spironolactone treatment, u-albumin excretion was reduced with 60% (21-80) from 90 mg/24h to 35mg/24h when compared with placebo ($p=0.01$). Blood pressure (24h) did not change during spironolactone treatment ($p>0.2$ for all comparisons), and GFR decreased from 78(8) to 72(6) mL/min/1.73m^2 ($p=0.003$). The tubular markers u-LFABP, u-NGAL and u-KIM1 did not change during treatment ($p>0.3$ for all comparisons). Treatment was well-tolerated, two patients had severe hyperkaliemia (plasma potassium=5.7 mmol/L), which were treated with diuretics and dietary intervention.

Conclusions: Spironolactone treatment on top of standard renoprotective treatment lowers urinary albumin excretion in microalbuminuric patients with type 1 diabetes, and thus may offer additional renoprotection independent of blood pressure.

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Prostaglandin E2 EP1 Receptor and Its Role in the Development of Diabetic Albuminuria Jean-Francois Thibodeau,[1,2] Anthony Carter,[2] Chris R. Kennedy.[2] *[1]Cellular & Molecular Medicine, University of Ottawa, Canada; [2]Ottawa Hospital Research Institute, Ottawa.*

Background: Inhibition of cyclooxygenase (COX)-derived prostaglandin (PG) synthesis reduces albuminuria in diabetic nephropathy (DN). However the specific PG receptor(s) targeted by such inhibition have not been well defined. We hypothesized that one such receptor-the PGE2 EP1 receptor, contributes to the pathogenesis of albuminuria in DN.

Methods: To test this hypothesis, gene-targeted EP1-null male mice (EP1$^{-/-}$), on an FVB/N background were subjected to a low-dose streptozotocin (stz) model of type 1 diabetes (5 day i.p., 50 mg/kg bw; Na-citrate as vehicle) and studied for 16 weeks. Blood pressures were obtained by tail cuff plethysmography while glomerular filtration rate was measured by FITC-inulin clearance. Albuminuria was determined by ELISA of 24 h urine collections. Kidney fibronectin, megalin and cubilin expression were assessed by immunoblot and immunohistochemistry.

Results: The onset of diabetes had no impact on systolic blood pressure, yet both WT-stz and EP1$^{-/-}$-stz groups became equivalently hyperglycemic (>35 mM) and were hyperfiltering to similar degrees at 16 weeks (WT-stz, 26.1 ± 3.5 vs. WT, 10.3 ± 2.1 µl.min^{-1}.g.bw^{-1}, $p<0.01$; EP1$^{-/-}$-stz, 28.6 ± 3.9 vs. EP1$^{-/-}$, 11.1 ± 2.5 µl.min^{-1}.g.bw^{-1}, $p<0.01$). Renal fibronectin levels increased in WT-stz mice but not in EP1$^{-/-}$-stz mice (WT, 0.809 ± 0.08 vs WTstz, 2.06 ± 0.21; EP1$^{-/-}$ 1.33±0.23 vs. EP1$^{-/-}$stz 1.33 ±0.075 a.u., $p<0.01$). Importantly, albuminuria was significantly reduced in EP1$^{-/-}$-stz mice at 16 weeks (WT-stz, 1546 ± 282 vs EP1$^{-/-}$stz, 526 ± 110 µg/24hours, $p<0.001$, n=7-11). Lastly, immunodetectable megalin but not cubilin expression was decreased significantly in WT-stz mice – while levels were preserved in EP1$^{-/-}$-stz mice (WT, 1250 ± 193 vs WTstz, 769 ± 81; EP1$^{-/-}$, 1242± 209 vs. EP1$^{-/-}$stz, 1416± 252 a.u., $p<0.05$, n=4-6).

Conclusions: These findings suggest that the PGE2 EP1 receptor contributes to early DN-induced albuminuria. Despite similar indices of gross glomerular damage, the mechanisms underlying this effect may be due to subtle injury to the filtration barrier along with altered post-glomerular megalin-mediated albumin processing by the proximal tubule.

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TH-PO550

Conditional Ablation of Macrophages Ameliorates Diabetic Nephropathy Alaa S. Awad. *Medicine, Penn State College of Medicine, Hershey, PA.*

Background: Monocyte/macrophage recruitment correlates strongly with the progression of renal impairment in diabetic nephropathy (DN). However, the direct role of macrophages and/or macrophage/podocyte interaction in DN is not known. We hypothesize that macrophages contribute to direct podocyte injury and/or abnormal podocyte niche leading to DN.

Methods: Experiments were conducted in CD11b-DTR mice treated with diphtheria toxin (DT) or vehicle (mutant DT) following streptozotocin (STZ) induced diabetes.

Results: We first established a dose, route and time response curves of DT in CD11b-DTR mice and we were able to chronically deplete kidney macrophages but not B cells, T cells or neutrophils.

We further demonstrated that macrophage depletion using DT in diabetic CD11b-DTR mice significantly attenuated diabetic albuminuria to normal range (5-fold reduction; $p<0.05$) compared to diabetic CD11b-DTR mice treated with vehicle despite comparable blood glucose levels after 6 wks of diabetes.

Vehicle-treated diabetic CD11b-DTR mice showed significant increases in kidney macrophages ($p<0.05$) compared with control mice using Mac-2 staining and flow cytometry and was associated with an early phase of increased M1/M2 macrophages followed by a late phase of increased M2/M1 macrophages. In contrast, kidney macrophages in DT-treated diabetic CD11b-DTR mice were similar to control mice.

In vitro, we demonstrate that podocytes grown on high glucose media are associated with significant increase ($p<0.005$) in macrophage migration from those grown in normal glucose media using transwell migration assay and this effect was completely blocked with the addition of anti-MCP-1 antibody. In addition, classically activated M1 macrophages ($CD11b^+LY6C^+LY6G^-TNF\text{-}^{high}IL\text{-}10^{low}CD206^{low}$); but not alternatively activated M2 macrophages ($CD11b^+LY6C^+LY6G^-TNF\text{-}\alpha^{low}IL\text{-}10^{high}CD206^{high}$) induced podocyte permeability *in vitro*.

Conclusions: These findings provide evidence that macrophages directly induce diabetic renal injury; mainly by altering podocyte permeability through the pro-inflammatory M1 but not the anti-inflammatory M2 subsets.

Attenuating the deleterious effect of macrophages on podocytes could provide a new therapeutic approach to the treatment of DN.

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TH-PO551

Fenofibrate Ameliorates Diabetic Nephropathy Via Inhibiting the Canonic Wnt Pathway Rui Cheng, Xuemin He, Ti Zhou, Ying Chen, Jian-Xing Ma. *Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Recent clinical and animal studies have reported that Fenofibrate, a ligand of peroxisome proliferator-activated receptor-α (PPARα), has a protective effect against diabetic nephropathy. However, the underlying mechanism is not clear. Recently, we have demonstrated that aberrant activation of the canonical Wnt pathway plays a pathogenic role in diabetic retinopathy and nephropathy. The objective of the present study is to investigate whether Fenofibrate ameliorates diabetic nephropathy by inhibiting the canonical Wnt pathway.

Methods: Diabetes was induced in rats by one single intraperitoneal injection of Streptozotocin. Diabetic rats were fed with standard chow diet or chow diet containing Fenofibrate. Renal function was evaluated by measuring the urine albumin level and levels of collagenIV, ICAM-1, TNF-α and CTGF. Primary human renal proximal tubular epithelial cells (HRPTCs) were co-treated with high glucose and different concentrations of Fenofibrate. The levels of LRP6, a key co-receptor of Wnt ligands, β-catenin, an essential effector of the Wnt pathway, and CTGF, a target gene of the Wnt pathway, were determined by Western blot analysis.

Results: Fenofibrate treatment reduced albuminuria, decreased collagen IV accumulation and attenuated ICAM-1 and TNF-α expression in the kidneys of diabetic rats, demonstrating protective effects against diabetic nephropathy. Fenofibrate decreased renal levels of LRP6 and β-catenin in the diabetic rats. In HRPTCs, levels of phosphorylated LRP6, β-catenin and CTGF were significantly up-regulated by high glucose, and Fenofibrate attenuated the increases in a concentration- and time- dependent manner.

Conclusions: These results suggest that PPARα has a regulatory role in Wnt signaling, and the protective effect of Fenofibrate against diabetic nephropathy is, at least in part, through inhibiting the canonical Wnt pathway.

TH-PO552

Early Outgrowth Bone Marrow Cells Attenuate Renal Injury and Dysfunction Via a Novel Antioxidant Mechanism in Type 2 Diabetes Yanling Zhang, Darren A. Yuen, Andrew Advani, Kim Connelly, Richard E. Gilbert. *St. Michael's Hospital, Toronto, Canada.*

Background: Despite RAS blockade and antihypertensive therapy, diabetic nephropathy remains the commonest cause of ESRD. While bone marrow derived cells potentially provide a new therapeutic strategy, most experimental studies have been undertaken using healthy donor cells. In the clinical setting, however, the dysfunction of autologous cells from patients with diabetic nephropathy may limit their effectiveness. Accordingly, we assessed the therapeutic efficacy of donor cells derived from both healthy and diabetic animals, delivered to a mouse model of diabetic nephropathy.

Methods: db/db mice were randomized to receive a single intravenous injection of PBS or 0.5 x 10^6 early outgrowth cells (EOCs) cultured from the bone marrow of db/m mice (non-diabetic) or db/db (diabetic) mice. Outcome parameters including mesangial expansion, glomerular hypertrophy, tubular apoptosis and oxidative stress were assessed 4 weeks after cell infusion.

Results: Untreated db/db mice developed mesangial matrix expansion and tubular epithelial cell apoptosis in association with increased reactive oxygen species (ROS) and thioredoxin interacting protein (TxnIP), a negative regulator of the key thiol-reducing enzyme, thioredoxin. EOCs derived from both diabetic and non-diabetic mice were equally effective. Without affecting blood glucose and blood pressure, EOCs not only attenuated both mesangial and peritubular matrix expansion, as well as tubular apoptosis, but also diminished ROS and TxnIP over expression in the kidney of db/db mice (all $p<0.05$). Consistent with a so-called endothelial progenitor type profile, analysis of cell surface markers revealed that EOCs were CD34 and VEGFR2 positive.

Conclusions: This study shows firstly that EOCs from both healthy and diabetic donors preserve kidney structure and function in experimental diabetic nephropathy in association with modulation of oxidative stress. The equally beneficial effects of cells from healthy and diabetic donors highlight the potential of autologous cell therapy in the clinical setting of diabetic nephropathy.

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TH-PO553

The Role of Growth Differentiation Factor 15 in Streptozotocin Induced Diabetes in Mice Magdalena Mazagova, Maaike Goris, Robert H. Henning, L.E. Deelman. *Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands.*

Background: Growth differentiation factor 15 (GDF15), a distant TGF-beta family member, is an early response gene up-regulated after induction of diabetes. Futhermore, GDF15 has recently emerged as an promising biomarker in patients with cardiovascular dysfunction and in patients suffering from type I and II diabetes. In this study, we investigated whether genetic deletion of GDF15 modulates initiation of renal damage in early experimental diabetes.

Methods: The study was performed in wt male mice and male GDF15 knockout mice. Mice were made diabetic by repeated injections with a low dose of streptozotocin (STZ 50mg/kg i.p. for 5 days). A group of healthy mice served as control. Mice were sacrificed, one and two weeks after the last STZ injection. For biochemical analyses, urine and blood samples were collected. Kidney sections were frozen for isolation of mRNA and protein. Remaining kidney sections were fixed in paraformaldehyde and examined by immunohistochemistry.

Results: Injection with 50 mg/kg STZ resulted in similar elevated blood glucose levels in GDF15 ko and wt mice (ko day 7: 21.4+/-2.7mM, ko day 14: 20.5+/-3mM, wt day 7: 19.4+/-2.4mM, wt day 14: 19.9+/-1.3mM,). GDF15 ko mice showed more renal pre-fibrosis (SMA) than wt mice after two weeks of diabetes (GDF15 ko day 14: 292.6+/-22.5%, wt day 14: 184+/-17.3%). GDF15 ko mice demonstrated higher renal expression levels as of day 7 for Collagen1 (5.8+/-1.2, $P<0.05$), KIM-1 (23.1+/-4.3, $P<0.05$), ICAM (3.14+/-0.4, $P<0.05$), compared to wt mice renal expression of Collagen1 (2.1+/-0.4), KIM-1 (6.1+/-1.8), ICAM (1.7+/-0.2). Furthermore, GDF15 ko showed increased urine volume, increased food intake and elevated urinary glucose loss compared to wt.

Conclusions: Our study demonstrates that genetic deletion of GDF15 augments renal damage, indicating that GDF-15 is a protective factor in the early initiation of diabetic kidney disease.

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Application of Self-Fabricated mRNA Array To Detect Urinary Gene Expression Profiling in Patients with Diabetic Nephropathy Min Zheng, Linli Lv, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

Background: The initiation and progression of diabetic nephropathy (DN) is complex which involves multiple mechanisms. Quantification of mRNA expression in urinary sediment has emerged as a novel strategy for studying renal diseases. It might be a promising approach for searching new biomarkers with DN by screening related gene expression in urine sediment. Considering the numerous molecules involved in DN development, a high-throughput platform with parallel detection of multiple genes is needed. In this study, we applied a high-throughput mRNA array to analyze urinary mRNAs in patients with DN.

Methods: A total of 9 subjects including 6 DN patients and 3 normal controls were studied. These patients with DN were assigned into two groups according to their urinary albumin excretion (UAE): group A (UAE>300 mg/g, n=3) and group B (30≤UAE≤300 mg/g, n=3)Potential molecules which may be involved in the pathogenesis and progression of DN were selected as the target mRNAs. mRNA array containing 88 genes were fabricated. The target mRNAs included tubular injury markers (n=9), epithelial-to-mesenchymal transition markers (n=12), podocyte markers (n=10), cytokines (n=28), extra-cellular matrix (n=6), signal pathway (n=19) and RAS system (n=4). Urinary cell pellet was collected from each study participant. Relative abundance of these target mRNAs from urinary pellet was quantified.

Results: A total of 30 mRNAs were significantly increased in DN patients compared with normal controls ($p<0.05$). Among these genes, α-actin4, CDH2, ACE, FAT1, synaptopodin, COL4α, twist, NOTCH3 mRNA expression were 15-fold higher than those in normal controls. In contrast, urinary TIMP-1 mRNA was significantly decreased in DN patients ($p<0.05$). It was shown that CTGF, MCP-1, PAI-1, ACE, CDH1, CDH2 mRNA varied significantly among the 3 study groups, and their mRNA levels increased with DN progression ($p<0.05$).

Conclusions: Our results suggested that mRNA array could serve as a high-throughput and sensitive tool for detecting mRNA expression in urinary sediment, which might be useful in searching new biomarkers for diabetic nephropathy.

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The Effect of Oral Anti-CD3 Antibody on Lipid Metabolism and Diabetic Nephropathy in db/db Mice Hyunwook Kim,[1] Jin Joo Cha,[2] Mihwa Lee,[2] Hye Kyung Song,[2] Deok Hwa Nam,[2] Jung Eun Kim,[2] Young Youl Hyun,[2] Ji Eun Lee,[1] Sang Youb Han,[3] Kum Hyun Han,[3] Young Sun Kang,[2] Dae R. Cha.[2] *[1]Division of Nephrology, Department of Internal Medicine, Wonkwang University College of Medicine Sanbon Hospital, Gunpo-si, Korea; [2]Division of Nephrology, Department of Internal Medicine, Korea University College of Medicine Ansan Hospital, Ansan-si, Korea; [3]Division of Nephrology, Department of Internal Medicine, Inje University College of Medicine Ilsan Baik Hospital, Goyang-si, Korea.*

Background: Recent advances revealed an emerging prominent role of different T lymphocytes from adaptive immunity in obesity-related adipose tissue inflammation. Oral anti-CD3 antibody, as an inducer of regulatory T cell, was recently reported to ameliorate inflammatory milieu in diabetes. Therefore, we asked whether oral anti-CD3 antibody has beneficial effects on progression of kidney disease in diabetic db/db mice.

Methods: Eight-week old db/db mice were divided into control and treatment group administered with 5-consecutive day treatment of oral anti-CD3 antibody. After 12-week study period, mice were killed for analyses.

Results: 2 days after the completion of 5-consecutive day treatment, we found a significant increase both in CD4+CD25-LAP+ and CD4+CD25+FoxP3+ regulatory T cells in spleen of treated animals compared with control. Over 12-week study period, while there were no significant differences in insulin resistance status or blood glucose levels, the treated animals showed significantly improved serum cholesterol and triglyceride levels as well as gained less cholesterol and triglyceride accumulation in both kidney and liver. In addition, lipid hydroperoxide levels in the kidney, adipose tissue, and liver were significantly suppressed and urinary levels of inflammatory cytokines such as IL-2, IL-1β, and TNF-α and 8-isoprostane were also reduced in treated animals. Furthermore, we found a significant decrease in urinary protein excretion and the preservation of renal histologic changes in the treated animals.

Conclusions: Thus, our results suggest that oral anti-CD3 antibody treatment has a protective role in progression of diabetic kidney disease possibly via regulatory T cell modulation.

TH-PO556

Early Kidney Response to Diabetes Mellitus in Mice Lacking Sglt2 Volker Vallon,[1] Maria Gerasimova,[1] Ken Platt,[2] Jean M. Whaley,[3] Scott C. Thomson,[1] Timo M. Rieg.[1] *[1]Depts of Medicine & Pharmacology, UC San Diego & VASDHCS, San Diego, CA; [2]Lexicon Pharmaceuticals, Inc, The Woodlands, TX; [3]Bristol-Myers Squibb R&D, Pennington, NJ.*

Background: The Na-glucose transporter SGLT2 mediates glucose reabsorption in the early proximal tubule and most glucose reabsorption by the kidney, overall. Inhibitors of SGLT2 are developed as new antidiabetic drugs which reduce renal glucose reabsorption

thereby lowering blood glucose levels. We studied gene-targeted mice lacking *Sglt2* (-/-) to elucidate its role in early diabetes where SGLT2 may also contribute to kidney growth and to glomerular hyperfiltration by lowering NaCl delivery to the macula densa.

Methods: Diabetes was induced by low-dose streptozotocin (STZ: 50 mg/kg ip on 5 days). GFR was determined by FITC-inulin kinetics in awake mice.

Results: At 3 and 5 weeks post STZ, hyperglycemia was less in *Sglt2*-/- than wild type (WT) mice (280±13 vs. 434±20 and 290±8 vs. 445±15 mg/dl; n=21-31, P<0.001), while food and fluid intake were increased and body weight modestly reduced to similar levels in both. For a given level of hyperglycemia, urinary glucose to creatinine ratios were greater in *Sglt2*-/- compared with WT mice consistent with lower renal glucose reabsorption in the absence of SGLT2. Induction of STZ-diabetes resulted in similar plasma concentrations of Na^+, K^+, and aldosterone, and a modestly greater hematocrit in *Sglt2*-/- vs. WT (45.5±0.6 vs. 42.3±0.5 %; n=9-10; P<0.05). The STZ-induced increase in GFR observed in WT (291±11 (in non-diabetics) to 383±12 µl/min; n=11/16; P<0.001) was absent in *Sglt2*-/- (292±6 to 308±10 µl/min, n=8/14; NS). However, the STZ-induced increase in kidney weight was similar in *Sglt2*-/- (345±5 (in non-diabetics) to 385±8 mg, n=15/24, P<0.01) and WT (344±6 to 397±15 mg, n=12/19; P<0.01).

Conclusions: We conclude that lack of SGLT2 lowers blood glucose levels in early STZ-diabetes mellitus and prevents glomerular hyperfiltration but not the increase in kidney weight.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO557

The ZDSD Rat, a Translational Model of Obesity, Metabolic Syndrome and Diabetes That Expresses the Characteristics of Human Diabetic Nephropathy Richard G. Peterson,[1] Karen M. Zimmerman,[2] Vincent H. Gattone.[2] *[1]PreClinOmics, Indianapolis, IN; [2]Anatomy, Indiana University School of Medicine, Indianapolis, IN.*

Background: Most previously available rodent models of type II diabetes have leptin/leptin receptor mutations. The ZDSD rat was selectively inbred over a period of 10 years to be a model of type 2 diabetes without these defects. This model is also an excellent model of pre-diabetes/metabolic syndrome.

Methods: The ZDSD rat was developed by crossing lean ZDF rats to polygenic obese CD rats. Selective inbreeding has resulted in a consistent, translational model of metabolic syndrome and type 2 diabetes.

Results: During the pre-diabetic state, ZDSD male rats are obese with: hypertension, glucose intolerance, insulin resistance, hyperlipdemia, hyperglycemia, elevated glycated Hb and increased cardiovascular and inflammatory biomarkers. In the overtly diabetic state, the model exhibits complications very much like the human condition such as delayed wound healing, osteoporosis and nephropathy. Findings include increased kidney biomarkers, urinary: albumin, beta-2 microglobulin, cystatin-C, Kim-1, clusterin, osteopontin, GSTY b-1 and RPA-1; and serum: NGAL, beta-2 microglobulin, GST alpha, and von Willebrand factor. Morphologically, light and electron (transmission and scanning) microscopy revealed mesangial expansion with sclerosis (Kimmelstiel-Wilson-like nodules), thickened glomerular basement membranes, podocyte effacement and glomerulo-sclerosis. These markers and conditions become discernable within reasonable times after diabetes develops. By 8-12 weeks of diabetes there are significant elevations in most of the kidney biomarkers and morphological changes such as mesangial expansion, nodular sclerosis, thickened GBM and podocyte effacement.

Conclusions: As opposed to the ZDF or ZSF1 rat models which have leptin defects, the ZDSD model has a more reproducible nephropathy, exhibiting biomarkers and histopathology more similar to those seen in human diabetic nephropathy. These data indicate that the ZDSD rat model, in the overtly diabetic state, demonstrate that it is potentially the best translational rodent model for diabetic nephropathy available at this time.

Funding: NIDDK Support

TH-PO558

ROCK1 Promotes Diabetic Nephropathy by Triggering Mitochondrial Fission through Serine Phosphorylation of Drp1 Jianyin Long, Wenjian Wang, Farhad R. Danesh. *Department of Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Mitochondrial dysfunction has been proposed as the underlying molecular mechanism for microvascular complications of diabetes. However, the signaling pathways by which hyperglycemia leads to mitochondrial dysfunction are poorly understood. In this study, we used a genetic approach to determine the role of ROCK1 on mitochondrial dynamics and progression of diabetic nephropathy (DN).

Methods: We generated db/db:ROCK1$^{-/-}$ mice as a model of type 2 diabetes, and a conditionally inducible knock-in mouse expressing a constitutively active (cA) ROCK1 mutant by targeting cA-ROCK1 into ROSA 26 locus. We then crossed Rosa$^{cA\text{-}ROCK1}$ transgenic mice with a tamoxifen- inducible podocyte-specific Cre transgenic line.

Results: We found that targeted deletion of ROCK1 in diabetic db/db mice abrogated albuminuria, while podocin Cre cA-ROCK1 mice developed significant albuminuria. Using electron microscopy, we observed a marked increase in mitochondrial fission in the podocytes of diabetic db/db mice, which was prevented in diabetic db/db:ROCK1$^{-/-}$ mice. Importantly, podocin Cre cA-ROCK1 mice developed significant increase in mitochondrial fission in the glomeruli. We also found that mitochondrial recruitment of dynamin-related protein 1 (Drp1), a major component of the mitochondrial fission machinery, was significantly decreased in the glomeruli of db/db:ROCK1$^{-/-}$ mice, while its mitochondrial recruitment was enhanced in glomeruli from podocin Cre cA-ROCK1 mice. Western blot analysis using phospho Drp1 antibodies indicated that ROCK1 knockdown by dominant negative ROCK1 or siRNA, abrogated high glucose induced phosphorylation of Drp1-Ser^{600}, but not the phosphorylation of Drp1-Ser^{579}. *In vitro* kinase assay indicated that purified ROCK1 kinase phosphorylates bacterially expressed Drp1 at Ser^{600} in the consensus ROCK1 phosphorylation motif. Finally, we found phosphorylation of Drp1 at Ser^{600} promoted its mitochondrial translocation, resulting in enhanced mitochondrial fission and increased mitochondrial apoptosis.

Conclusions: Collectively, we identify a previously unrecognized role of ROCK1 and mitochondrial fission in the pathogenesis of DN.

Funding: NIDDK Support

TH-PO559

The Role of Epidermal Growth Factor Receptor in Peroxisome Proliferator Activated Receptor Gamma Mediated Sodium and Water Transport in the Proximal Tubule Sonia Saad,[1] Dania Yaghobian,[1] Rachel Yong,[1] Muh Geot Wong,[1] Xinming Chen,[1] Darren J. Kelly,[2] Carol A. Pollock.[1] *[1]Medicine, Kolling Institute, Sydney, NSW, Australia; [2]Medicine, St Vincent Hospital, Melbourne, Vic, Australia.*

Background: Cellular sodium and water transport are dysregulated in diabetes mellitus. We have demonstrated that EGFr regulates high glucose-induced Na reabsorption via the sodium-hydrogen exchanger-3 (NHE3) in human proximal tubule cells (PTCs). PPARγ agonists, such as Pioglitazone, are used in patients with diabetes, but their use is limited by fluid retention. We have shown that PPARγ agonist induces NHE3 and the water channel AQP1 in PTCs. The role of EGFr in PPARγ agonists mediated increase of sodium and water retention in the proximal tubule are not known.

Methods: PTCs were exposed to 5mM, 25mM glucose (HG) or 3µM Pioglitazone. P-EGFr, NHE3, PPARγ and AQP1 protein expression were measured by Western blot. The role of EGFr was elucidated using the EGFR tyrosine kinase inhibitor PKI166. The specific role of PPARγ in HG mediated increase of P-EGFr, NHE3 and AQP1 was determined by effectively silencing PPARγ using siRNA technique. The expression of PPARγ and P-EGFr was determined in diabetic Ren2 rats with and without pioglitazone. The interaction between PPARγ and EGFr was determined by CHIP assay and the effect of TZDs on EGFr activation by luciferase assay.

Results: Exposure of PTCs to both HG and pioglitazone increased protein expression of P-EGFr, NHE3, AQP1 and PPARγ (P<0.05). EGFr and PPARγ are increased in diabetic rats and further increased in the presence of pioglitazone. Pioglitazone increased expression of NHE3 and AQP1 was abolished with PKI166 (P<0.05). HG induced increase of P-EGFr, NHE3 and AQP1 was significantly decreased with PPARγ siRNA (P<0.05). Pioglitazone induced PPARγ binding to EGFr promoter and subsequent downstream activation.

Conclusions: Our data suggest a role of EGFr in mediating PPARγ induced Na reabsorption via NHE3 and water reabsorption via AQP1 channels in the proximal tubule. This suggest that EGFr inhibition may have the potential to direct future therapeutic targets in DN, as well as limiting salt and water retention which currently restricts the use of PPARγ agonists.

Funding: Government Support - Non-U.S.

TH-PO560

The Effect of Oral Anti-CD3 Antibody Treatment on High Fat Induced Obese Mice Hyunwook Kim,[1] Jin Joo Cha,[2] Young Youl Hyun,[2] Young Sun Kang,[2] Mihwa Lee,[2] Jung Eun Kim,[2] Deok Hwa Nam,[2] Hye Kyung Song,[2] Ji Eun Lee,[1] Sang Youb Han,[3] Kum Hyun Han,[3] Dae R. Cha.[2] *[1]Internal Medicine, Wonkwang University Medical College, Gunpo, Kyeonggido, Republic of Korea; [2]Internal Medicine, Korea University Medical College, Ansan, Kyeonggido, Republic of Korea; [3]Internal Medicine, Inje University Medical College, Koyang, Kyeonggido, Republic of Korea.*

Background: Obesity is considered as a state of chronic inflammation, and it has been shown that pro-inflammatory cytokines expressed in obesity are linked to the development of insulin resistance. Regulatory T-cells(T-regs) have shown to be down-regulated in obese experimental model and recent studies demonstrated the effect of induction of T-regs by oral anti-CD3 antibody in ameliorating insulin resistance and metabolic derangement in ob/ob mice. We investigated the effect of oral anti-CD3 antibody treatment in high fat induced obese mice on diabetic nephropathy, insulin resistance and lipid metabolism.

Methods: C57BL/6(B6) mice, aged 8 weeks, were fed with either normal fat diet(n=3) and high fat diet. After 4 weeks, high fat fed mice were divided into 2 groups. One group(n=8) were fed once a day for 5 consecutive days with anti-CD3 antibody (5ug/feeding).

Results: Although there were no significant differences in glucose tolerance test, insulin and HOMA-IR level, anti-CD3 antibody treatment group showed significantly lower kidney weight, decreased urinary albumin excretion, improved plasma lipid (cholesterol, triglyceride) profile and liver tissue lipid metabolism. Urinary 8-isoprostane level and pro-inflammatory cytokines, glomerulosclerosis index were also decreased in the treatment group. To confirm the effect of anti-CD3 antibody on increased T-regs, $CD4^+LAP^+$ lymphocytes were measured by FACS analysis. There was a trend in increase of spleen $CD4^+LAP^+$ cells after 1 month. However, the level was undetectalbe after 3 months.

Conclusions: Our study suggest that oral anti-CD3 antibody treatment before the disease onset in high-fat induced obesity might have a beneficial effect on lipid metabolism and obesity induced kidney disease. The low percentage of $CD4^+LAP^+$ lymphocytes might be related to the low and chronic inflammatory state of obesity.

TH-PO561

Peroxisomal Dysfunction Mediates Renal Injury in Diabetic Catalase Null Mince Inah Hwang, Jiyoun Lee, Jehyun Park, Hunjoo Ha. *Department of Bioinspired Science, Division of Life & Pharmaceutical Sciences, the Center for Cell Signaling & Drug Discovery Research, and College of Pharmacy, Ewha Womans University, Seoul, Korea.*

Background: Overproduction of reactive oxygen species (ROS) under hyperglycemia plays an important role in diabetic vascular complications, including diabetic nephropathy (DN). Plasma free fatty acid (FFA) as well as glucose is increased under the diabetic milieu and peroxisome and mitochondria participates in cellular FFA oxidation; therefore, we investigated whether deficiency of catalase, a major antioxidant enzyme in peroxisome, determine accelerated diabetic renal injury through peroxisomal dysfunction and abnormal renal FFA metabolism associated with increased ROS.

Methods: Experimental diabetes was induced by multiple injections of low dose streptozotocin into catalase knock-out (CKO) and wild-type (WT) C57BL/6 mice. Mesangial cells transfected with siRNA for catalase were used to further elucidate the role of endogenous catalase in peroxisomal and mitochondrial function regulating FFA oxidation.

Results: At four weeks after the induction of diabetes, equivalent high plasma glucose and FFA levels were observed in diabetic WT and diabetic CKO mice. However, parameters of DN including urinary albumin excretion, glomerular hypertrophy, and accumulation of extracellular matrix (ECM) proteins along with markers of oxidative stress were much more severe in diabetic CKO mice than diabetic WT mice. Catalase deficiency in CKO mice and mesangial cells induced defects in peroxisomal and mitochondrial biogenesis and FFA oxidation leading to renal lipid accumulation. Catalase deficiency in mesangial cells also increased mitochondrial ROS.

Conclusions: The present data provide unprecedented evidence that FFA-induced peroxisomal dysfunction exacerbates diabetic renal injury, and that endogenous catalase is an important antioxidant that protects the kidney through peroxisomal and mitochondrial fitness under diabetic stress.

TH-PO562

Deficiency of Matrix Metalloproteinase-2 Accelerated Renal Injury in Streptozotocin-Induced Diabetic Mice Nana Obara,[1] Kei Fukami,[1] Sho-Ichi Yamagishi,[2] Yoshimi Takamiya,[1] Yusuke Kaida,[1] Seiji Ueda,[1] Seiya Okuda.[1] *[1]Division of Nephrology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan; [2]Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume, Fukuoka, Japan.*

Background: Matrix metalloproteinase-2 (MMP-2) is one of the potent metalloproteinases . Accumulation of ECM in the late stage diabetic kidney are, at least in part, by down-regulation of MMP-2. However, whether MMP-2 could affect in early stage of diabetic nephropathy has not been elucidated. Therefore, we investigated the effects of MMP-2 deficiency on renal injury in early stage of diabetic mice.

Methods: Diabetes was induced by streptozotocin (STZ) (50mg/kg) in male MMP-2 knockout mice (MMP-2 KO) and C57BL/6J mice (Ctrl). After 16 weeks, cortical MMP-2 expression and activity were measured by zymography and real-time PCR. Albuminuria and N-acetyl-β-D-glucosaminidase (NAG) were measured by enzyme-linked immunosorbent assay (ELISA). Alfa-smooth muscle actin (α-SMA) was evaluated by western blots. Glomerular fibrosis and tubulointerstitial injury were evaluated by PAS and Masson-trichrome stain, respectively.

Results: Plasma levels of glucose and HbA1c were increased by about 2-3-folds in 16-week diabetic (DM) mice compared with non-DM Ctrl mice (plasma glucose; 490.3+/-7.5 mg/dl, HbA1c; 9.91+/-0.22 % in 16-week diabetic mice). MMP-2 expression and activity in the kidney cortex of diabetic mice were significantly increased. Serum levels of BUN and creatinine (Cr) and albuminuria were significantly increased in MMP-2 KO DM mice compared with Ctrl DM mice (BUN; 23.2+/-1.7 *vs* 39.8+/-6.0 mg/dl, p<0.01, Cr; 0.07+/-0.01 *vs* 0.17+/-0.04 mg/dl, p<0.01, Albuminuria; 55.9+/-11.9 *vs* 89.3+/-13.8 μg/day, p<0.05). Further, glomernlar and tubulointerstitial injury, and cortical α-SMA expression were enhanced in DM mice, which were further increased in MMP-2 KO DM mice.

Conclusions: Renal MMP-2 is activated in early stage of diabetic nephropathy, and deletion of MMP-2 is further exacerbated. Diabetes-induced overexpression and activation of MMP-2 may be, at least in part, by compensatory action.

TH-PO563

VEGF165b Modifies Glomerular Permeability and Slows Progression of Albuminuria in Animal Models of Diabetic Nephropathy Sebastian Oltean,[1] Joane Ferguson,[1] Amit Kaura,[1] Chris R. Neal,[1] Steve Harper,[1] David O. Bates,[1] Andy Salmon.[1,2] *[1]Microvascular Research Laboratories, University of Bristol, United Kingdom; [2]Academic Renal Unit, University of Bristol, United Kingdom.*

Background: VEGF165b, an alternatively-spliced isoform of VEGF, decreases glomerular permeability to water (LpA/Vi) when directly administered to isolated intact glomeruli ex vivo, or transgenically over-expressed in podocytes in vivo [1].We investigated whether VEGF165b decreases both glomerular permeability to water and albuminuria in animal models of diabetic nephropathy.

Methods: All animals were made diabetic using standard streptozotocin protocols. Glomerular permeability to water (LpA/Vi) was determined as previously described (Salmon et al, J Physiol 2006). uACR ratio was determined from spot urine collection. (*p<0.05 vs control animals; #p<0.05 vs diabetic WT animals; ‡p<0.05 vs sham-treated diabetic animals; all ANOVA)

Results: Sprague-Dawley rats developed proteinuria (2-fold)* six days after diabetes induction with streptozotocin (STZ) (single 45mg/kg injection i.p). Elevated glomerular permeability to water in diabetic glomeruli (1.7-fold vs sham)* was blocked by 1hr incubation in 1nM VEGF165b (0.9-fold vs sham)#.

C57Bl/6 mice expressing human VEGF165b in podocytes (nephrin promoter: nephVEGF165b), or wild-type litter-mates (WT), were injected with 100mg/kg STZ i.p. daily for 3 days to induce diabetes. As compared with diabetic-WT mice, diabetic nephVEGF165b mice had less albuminuria (68% reduction)#, lower LpA/Vi (45% reduction)#, less mesangial matrix expansion (20% reduction)#, and less GBM thickening (15% reduction)#.

DBA2J mice were injected 5 consecutive days with STZ (50mg/kg i.p.). After 2 consecutive weeks of hyperglycemia (>20mmol/L), baseline urinary albumin-creatinine ratio (uACR) was measured, and mice received saline or 1μg VEGF165b injections i.p. twice weekly for 6 weeks. uACR increased progressively to 4.6-fold above baseline at 6 weeks in the sham-treated group, but only to 1.2-fold‡ in the VEGF165b treated group.

Conclusions: Our study suggests the potential of using VEGF165b as a therapeutic agent to slow diabetic nephropathy progression.

[1] Qiu et al , JASN 2010

TH-PO564

Activation of Renal Genes Encoding Complement Components in Diabetic Nephropathy and Ischemia: Hypothesis Generation by RNAseq Discovery Katherine J. Kelly,[1] Jesus H. Dominguez.[1,2] *[1]Medicine/Nephrology, Indiana University School of Medicine, Indianapolis, IN; [2]Medicine/Nephrology, Roudebush VA Medical Center, Indianapolis, IN.*

Background: We have demonstrated accelerated progression of nephropathy in obese, diabetic ZS (F1 hybrids of Zucker and spontaneously hypertensive heart failure) rats following a single episode of renal ischemia.

Methods: We now examined the nature of renal inflammation in diabetic nephropathy using whole transcriptosome RNA sequencing to evaluate transcript expression in three groups: lean, obese-diabetic/sham surgery, obese-diabetic/postischemia (2 weeks).

Results: The nephropathy in this model is characterized by increased serum creatinine (1.8 ± 0.1 in obese-diabetic/postischemia vs 0.4 ± 0.4 mg/dl in lean rats), proteinuria, marked renal inflammation with fibrosis. An average of 43M (50 nt) reads were detected from each sample and the total number of unique and validated gene transcripts was 16536. Components of the classical (C1q, C2, C3, C4) complement pathway were significantly increased, 1.5 to 2.5 fold, in the obese-diabetic/sham group when compared to the lean group. The alternate complement component 6 was increased 4 fold in the obese-diabetic/sham group. Expression was further increased in the obese-diabetic/postischemia group: 2-5 fold for components of the classical pathway and 8.8 fold for C6. In addition, regulators of the complement system were significantly downregulated in the diabetic kidneys. These include CD55, "decay accelerating factor," and CD59 which inhibits the membrane attack complex. Complement receptors were increased (for example 42 fold increase in CR2 in obese-diabetic/sham group compared to lean) consistent with the inflammatory phenotype observed. Although complement components have long been described in diabetic nephropathy biopsies, this common finding has been viewed as non-specific.

Conclusions: These results support the hypothesis that in diabetes/obesity the kidney is an active participant in complement mediated renal injury by upregulating key complement genes. These critical injury pathways, acting in situ, may amplify inflammation and contribute to chronic renal injury.

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TH-PO565

Systems Genomics Studies Implicate Xanthine Oxidase in Diabetes-Induced Podocyte Depletion Haiying Qi, Ilse S. Daehn, Erwin P. Bottinger. *Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Diabetic nephropathy (DN) risk in diabetics is controlled by genetic susceptibility. Podocyte depletion is a hallmark of experimental murine and human DN. Inbred C57BL/6(B6) are resistant and DBA/2J(D2) are susceptible to diabetes-induced glomerular lesions [Qi, et al. Diabetes,2005],including podocyte depletion [Qi H, et al. ASN 2009].

Methods: BXD recombinant inbred strains and parental B6 and D2 strains were made diabetic using streptozotocin (STZ) low-dose protocol and analyzed for QTL mapping using GeneNetworks tools. In a 2nd experiment, diabetes was induced in 8 wk-old D2 mice in the absence or presence of xanthine oxidase inhibitor(XOI) allopurinol,administered with mouse chow.

Results: The QTL mapping study identified two loci on Chr 13 and Chr 17, respectively, that were significantly associated with diabetes-induced podocyte depletion(DIPL). Xanthine dehydrogenase(Xdh) gene was localized under the peak of Chr 17 QTL, and Xdh gene expression was strongly increased by diabetes in glomeruli of D2, but not B6. Xdh and xanthine oxidase(XO) are interconvertible forms of the same enzyme encoded by the Xdh gene. Circulating XO activities were not different between diabetic B6, and non-diabetic B6 and D2 mice, but significantly(65%) elevated in diabetic D2 mice. Thus, Xdh is a candidate gene for DN susceptibility in D2 mice. Next, we treated non-diabetic and diabetic D2(STZ)with XOI. XOI had no effect on time of onset and levels of hyperglycemia

at 6 or 12 wks of diabetes. XOI mediated significant protection against diabetes-induced albuminuria and DIPL in D2 mice.

podocyte number per glomerular section and ACR ratio

N=5/group	Podocytes/glom. Section	Podocytes/glom. Section	ACR (mg/mg)	ACR (mg/mg)
	6wk	12wk	6wk	12wk
D2 ctr	11.40±0.81	11.49±1.12	28.25±8.51	34.48±7.95
D2 ctr + XOI	11.16±0.67	12.58±0.13	36.69±13.60	27.09±10.72
D2 STZ	9.17±0.77*	8.67±0.48*	358.79±158.06	383.22±193.09
D2 STZ + XOI	11.16±0.53#	10.63±0.26#	190.96±73.97#	143.15±143.27#

*compared with D2 ctr, #compared with D2 STZ , p<0.05

Conclusions: We propose that Xdh/XO may mediate DN susceptibility. Future studies will test XDH locus variants in human DN and evaluate the therapeutic/preventive potential for XOI in human DN.

Funding: NIDDK Support

TH-PO566

Metallothionein Overexpression May Underlie Tubular Injury in Type 2 Diabetic Nephropathy

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Background: Oxidative stress is thought to play an important role in the onset and progression of diabetic nephropathy (DN). On the other hand, chronic hypoxia and TGF-β1 contribute to the development of renal fibrosis in DN.

Methods: For investigation of whether other key factors contribute to the pathogenic mechanism of DN, we analyzed microarray data using Affymetrix GeneChip (Rat Gene 1.0 ST Array) with microdissected juxtamedullary proximal tubules in a rat model of type 2 diabetes, Zucker Diabetic Fatty (ZDF) rats.

Results: A total of 27,342 transcripts were analyzed, among them, 47 were upregulated (>2.0-fold) in the ZDF rat compared with the lean control rat. One of them which were upregulated in diabetic renal tubules was metallothionein(MT)-1. MT is a cysteine-rich protein with low molecular weight, and act as an antioxidant against the toxicity of metals, ischemia, and ROS. By addressing the pathological role of MT, we first studied the regulation of MT expression in human renal proximal tubular epithelial cells (HRPTECs). Hypoxia (1% O_2) slightly increased MT mRNA levels, and insulin (100 nM) markedly enhanced MT mRNA levels up to 2~3 fold. Whereas, TGF-β1 (2.5 ng/ml) decreased MT mRNA levels down to ~50 % of the control in normoxia. Immunofluorescence of the kidney sections of 17 weeks old ZDF rats, showed significantly enhanced MT expression specifically in the tubules, not in the glomeruli, which also demonstrated strong intensity of HIF-1α, hypoxic probe pimonidazole, and oxidative stress marker 8-hydroxy-2'-deoxyguanosine, compared with lean control rats. Intriguingly, MT was faintly expressed in severe fibrotic kidneys in 39 weeks old ZDF rats, as well as control rats.

Conclusions: In conclusion, our data, for the first time, suggest that DN may be associated with the significant serial alterations of renal tubular MT expression in response to hypoxia, insulin or profibrotic TGF-β1, and that enhanced expression of MT may be an early pathogenic event reflecting abnormalities of antioxidant systems in DN.

Funding: Government Support - Non-U.S.

TH-PO567

SGLT1 Can Reabsorb 70% of the Filtered Glucose Load in Mice Lacking SGLT2

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Background: SGLT2 (S2) reportedly reabsorbs 90% of the filtered glucose (G) load. Inhibiting S2 to increase urinary G excretion (UGE) is a promising approach for improving glycemic control in diabetic patients, but clinical trials suggest that SGLT inhibitors highly selective for S2 over SGLT1 (S1) block G reabsorption by only 16-50%. We studied the role of S1 in renal G reabsorption in the absence of S2.

Methods: We generated S1 knockout (KO), S2 KO, S1 & S2 double KO (DKO), and wildtype littermate (WT) mice. Mice fed a high fat diet with fructose as the only carbohydrate (G-free diet) had 24 hr UGE measured; S2 KO and DKO mice were studied with or without a sc dose of 300 mg phlorizin (Ph). Other S2 KO and WT mice were either fed G-free diet and studied with an oral G tolerance test (oGTT), or were fed G-containing diet, exposed to vehicle (Veh) or streptozotocin (STZ, to induce diabetes), and then followed for blood G and HbA1c levels.

Results: All mice appeared healthy on G-free diet. Table 1 shows male UGE data; females showed the same pattern. All data are presented as mean ± SD.

Table 1. 24 hr UGE (mg)

WT (6)	S1 KO (10)	S2 KO (6)	S2 KO+Ph (6)	DKO (6)	DKO+Ph (6)
1 ± 1*	11 ± 4*	184 ± 70*	505 ± 271	681 ± 131	720 ± 106

(N); * different from each other group, p<0.001

G AUC (mg.hr/dl) values during oGTT at 10-12 wks of age were lower for male S2 KO (336 ± 89, n=22) relative to WT (442 ± 112, n=21) mice (p<0.01); females showed the same pattern. 40 wks after STZ or Veh treatment, 50 wk old male WT-STZ mice had HbA1c (%) values (7.7 ± 3.1, n=5) that were higher than values for S2 KO-STZ (5.1 ± 0.5, n=8), WT-Veh (4.9 ± 0.4, n=7) or S2-Veh (4.8 ± 0.6, n=7) mice (p<0.05 for each comparison); blood G values showed the same pattern.

Conclusions: 1) S2 is the major renal G transporter, but S1 can reabsorb 70% of filtered G in the absence of S2. 2) Mice lacking S2 can display improved glucose tolerance despite UGE that is only 30% of maximum. 3) Mice lacking S2 show improved glycemic control after exposure to diabetogenic doses of STZ. 4) S2 KO mice on G-free diet closely model humans receiving highly selective S2 inhibitors in terms of effects on UGE and glucose tolerance.

Funding: Pharmaceutical Company Support

TH-PO568

Intradialytic Parenteral Nutrition (IDPN) Increases Serum Prealbumin (PA) Levels in Malnourished Hemodialysis (HD) Patients – A Prospective, Multicenter, Open, Phase-IV-Study

Tobias A. Marsen, Roman Fiedler, Helmut Mann. *DPN Study Group, Germany.*

Background: Malnutrition (MA) is increasingly becoming a clinical problem in maintenance HD patients. Since PA is a nutritional parameter whose increase was shown to be positively correlated with patient survival and decreased morbidity, it was the goal of this study to assess PA and other nutritional parameters during 16 weeks (w) of IDPN.

Methods: 140 patients on HD with MA (albumin <35 g/l, PA <250 mg/l, phase angle α<4.5°assessed by bioimpedance, subjective global assessment score (SGA)≤grade B) were enrolled and 77 patients were evaluated per protocol. Patients were randomized to receive IDPN 3x/w in the study group (SG, n=34) and compared to the control group (CG, n=43). IDPN was compounded according to guideline recommendations. Oral supplements were allowed in both groups. Endpoints were defined as differences between baseline (BL) and 4 w of treatment (4WT) or BL and end of treatment (ET). Primary endpoint: PA. Secondary endpoints: Early (≥15% at 4WT) rise of PA, phase angle, SGA, albumin, transferrin, quality of life (SF12). Power calculation was established for PA (32 patients per study group) and statistical evaluation was performed according to an adaptive design [Bauer, Köhne: Biometrics 1994]. Statistical calculation: t-test, significance: p<0,05.

Results: There are no significant differences in BL data of both groups. SG shows a significant advantage (p<0,05) in change of PA (mean increase SG: +29,8 mg/l) compared to CG (mean decrease CG: -1,9 mg/l). A relevant early PA increase ≥15% after 4WT is more frequently achieved in SG (44%, n=15). Phase angle, SGA, albumin, transferrin, SF12 show no significance.

Conclusions: IDPN in malnourished HD patients significantly increases PA. It also results in early PA rise. Since increase in PA within 3 months of nutritional support predicts improved survival (Cano: JASN 2007) it is accepted as a positive marker for patient prognosis. Our data demonstrate a positive influence on PA after 16 w IDPN, thus favouring IDPN as a beneficial option of therapy. Due to small patient numbers there is a lack of statistical power to evaluate responsiveness of secondary endpoints in this study.

Funding: Pharmaceutical Company Support

TH-PO569

A Reduced Appendicular Skeletal Muscle Mass Is Associated with Total Mortality in Male Hemodialysis Patients

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Background: Decreased skeletal muscle mass and increased abdominal adiposity are often observed in dialysis patients. However, it remains unclear whether either sarcopenia or abdominal adiposity is associated with clinical outcomes.

Methods: We measured appendicular skeletal muscle mass (ASM), a sum of the muscle mass in the four limbs, and trunk fat mass (TFM) from a dual energy X-ray absorptiometry, and determined a skeletal mass index (SMI) as ASM/height^2 and a trunk fat mass index (TFMI) as TFM/height^2 (kg/m^2) in hemodialysis (HD) patients aged from 41 to 75 years old (male/female=165/83, time on HD: 10±7 years). We also followed all patients for the 5 years, and examined the impact of changes of body composition on total mortality.

Results: SMI was positively correlated with serum creatinine (r=0.37, p<0.01) and albumin (r=0.21, p=0.01), while negatively with age (r=-0.25, p<0.01) in male patients. In contrast, no association was found between SMI and clinical parameters in female patients. TFMI was significantly correlated with total cholesterol (p<0.05) and triglyceride (p<0.01) in both sexes. In male patients, there was also a significant association of TFMI with hemoglobin (r=0.23, p<0.01) and carotid artery intima-medial thickness (CA-IMT) (r=0.21, p<0.05). During the follow-up, 27 male and 20 female patients had expired. Cox-hazards analysis after the adjustment for co-morbid risk factors revealed that male patients with the lowest tertile of SMI (≤6.5kg/m^2) (n=48) had a significantly higher risk for total mortality (RR: 8.31 folds [95%CI, 1.45-47.51], p<0.02) when compared with those with the highest tertile of SMI (>8.3 kg/m^2) (n=49). In contrast, SMI did not associate with 5-year mortality in female patients. FMI also did not relate to total death in both sexes.

Conclusions: These findings suggest that SMI less than 6.5kg/m^2 was a significant determinant of total mortality in male HD patients.

TH-PO570

Prognostic Value of Geriatric Nutritional Risk Index for Cardiovascular and All-Cause Mortality in End-Stage Renal Disease Patients on Chronic Hemodialysis

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Background: Malnutrition is a prevalent complication in end-stage renal disease (ESRD) patients on hemodialysis (HD), and is associated with cardiovascular (CV) morbidity or poor survival. Geriatric nutritional risk index (GNRI) has been recently developed as a more accurate tool for malnutrition status. We investigated the prognostic value of the GNRI at start of HD therapy for mortality in ESRD patients.

Methods: A total of 1,568 ESRD patients who started HD therapy were examined. The GNRI was calculated as follows; GNRI = (14.89 × albumin) + [41.7 × (body weight / body weight at BMI of 22)] Thereafter, the patients were divided into quartiles according to GNRI levels; quartile 1 (Q1): < 84.9, Q2: 85.0-91.1, Q3: 91.2-97.2 and Q4: > 97.3, and were followed up for up to 10 years.

Results: During follow-up period (mean 63±42 months), 363 patients died including 180 CV deaths. Kaplan-Meier survival rates for 10 years were 57.9, 73.3, 80.8 and 89.2% for CV mortality, and were 32.1, 51.9, 61.3 and 73.8% for all-cause mortality in Q1, Q2, Q3 and Q4, respectively (both p<0.0001). Even after adjustment for other risk factors, GNRI was an independent predictor for both mortality.

GNRI and mortality

	Cardiovascular mortality		All-cause mortality	
	HR (95%CI)	P for trend	HR (95%CI)	P for trend
GNRI > 97.3	Reference	<0.0001	Reference	<0.0001
91.2 - 97.2	1.56 (0.87-2.79)		1.59 (1.09-2.30)	
85.0 - 91.1	1.90 (1.07-3.38)		1.74 (1.20-2.52)	
< 84.9	3.37 (1.96-5.80)		2.92 (2.05-4.15)	

Upon receiver operating characteristic (ROC) analysis, area under curve (AUC) for CV mortality was larger in GNRI (0.71) compared to albumin (0.64) and BMI (0.63) alone. AUC for all-cause mortality was also 0.70, 0.63 and 0.63 in GNRI, albumin and BMI, respectively.

Conclusions: GNRI at starting HD therapy could strongly predict CV and all-cause mortality in ESRD patients. This simple marker might be clinically useful for the assessment of malnutrition status in HD patients.

TH-PO571

Body Composition and Mortality in Prevalent Hemodialysis (HD) Patients: HEMO Study

Rebecca Filipowicz,[2] Tom H. Greene,[1,2] Guo Wei,[1] Srinivasan Beddhu.[1,2] *[1]VA; [2]Univ Utah.*

Background: Both higher body size (as indicated by body mass index- BMI) and higher muscle mass (as indicated by serum creatinine- SCr) are associated with better survival in HD patients (pts) but the relative importance of muscle vs. body size is not established.

Methods: The HEMO Study was a 2 X 2 factorial design study conducted by NIDDK to evaluate the effects of flux and dialysis dose on outcomes in anuric HD pts. Details of HEMO Study have been published elsewhere. In the current study, the associations of SCr, BMI and the ratio of Scr to BMI with time to death were examined in Cox proportional hazards models.

Results: The mean age, SCr, and BMI were 58 ± 14 yrs, 10.2 ± 2.9 mg/dL, 25.5 ± 5.3 kg/m², respectively. 56% were men, 63% black, and 45% had DM. There were 860 (47%) deaths over an average 2.85 years of follow-up.

Associations* of SCr, BMI and SCr/BMI with mortality (n=1,775).

Model 1	HR (95% CI)
For each change in SD of SCr	0.68 (0.63, 0.74)
For each change in SD of BMI	0.86 (0.80, 0.93)
Model 2	
For each change in SD of SCr/BMI	0.82 (0.75, 0.89)

*All models are adjusted for age, gender, race, and HEMO trial interventions

The results indicate that patient differences in Scr level are substantially more strongly predictive of mortality than patient differences in BMI, relative to the SD of each factor. Furthermore, each SD increase in the ratio SCr/BMI was associated with 18% lower risk of death. In subgroup analyses, each SD increase in the SCr/ BMI ratio was associated with lower risk of death in those with BMI< 25 (HR 0.69, 95% CI 0.2-0.78), BMI 25-29.9 (HR 0.72, 95% CI 0.57 -0.90) and BMI 30 (HR 0.53, 95% CI 0.37-0.76).

Conclusions: Both SCr and BMI are associated with lower risk of death in HD pts. However, in the HEMO Study, the magnitude of the association of SCr with mortality was stronger than the magnitude of the association of BMI with mortality, and higher SCr/BMI ratio was also associated with lower risk of death. Interventions targeting muscle mass at any level of BMI may improve survival in HD pts.

TH-PO572

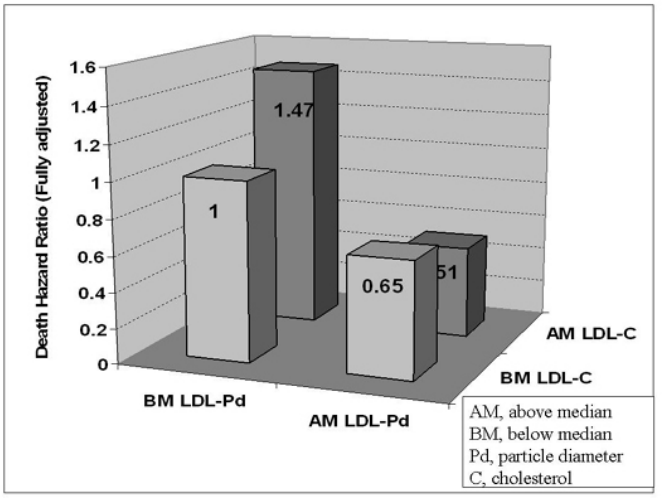
Novel Lipoprotein Subfraction and Size Measurements in Prediction of Mortality in Maintenance Hemodialysis Patients

Nazanin Noori,[1] Michael Caulfield,[2] Susanne B. Nicholas,[3] Miklos Z. Molnar,[1] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center; [2]Quest Diagnostics Nichols Institute; [3]David Geffen School of Medicine at UCLA; [4]Salem VA Medical Center.*

Background: Studies on CKD patients (pts) are often limited to conventionally measured total cholesterol and LDL-C. What remains to be determined is what measures of lipoprotein help better identify high risk dialysis pts & whether LDL-"altering" strategies are more effective in this population than LDL-lowering medications.

Methods: Mortality-predictability of LDL particle diameter and lipoprotein subfraction concentrations, measured by novel ion mobility, was examined in a cohort of 235 MHD pts who were followed for up to 6 years using Cox models with incremental levels of multivariate adjustment: (A) Case-mix variables. (B) Lipids included LDL and HDL-C and TG concentrations. (C) Malnutrition-inflammation complex syndrome (MICS) variables included serum or blood levels of phosphorus, albumin, creatinine, calcium, ferritin, hemoglobin, nPNA; & BMI. (D) Adjustment for CRP, IL-6, & TNFα.

Results: Over 6 yrs, 71 pts (31%) died. Conventional lipid profile was not associated with mortality. The death hazard ratio (HR, 95% confidence interval) of the highest versus lowest quartiles of very small and large LDL-particle concentrations were 2.14(1.00-4.62) and 0.47(0.20-0.99), respectively. As figure shows, across increasing quartiles of the LDL particle diameter, death HR were 1.0, 0.93(0.46-1.87), 0.43(0.21-0.89), and 0.45(0.31-1.00), respectively.



Conclusions: Whereas conventional lipid profile cannot predict mortality in MHD, novel lipoprotein measures such as larger novel LDL particle diameter or higher large LDL particle concentrations appear predictive of greater survival, while higher very small LDL particle concentration is associated with higher death risk.

Funding: NIDDK Support

TH-PO573

The Predictive Value of the Changes of Nutritional and Anthropometric Parameters for Survival and Hospitalization Outcome in the Hemodialysis (HEMO)Study

Chi-Ting Su,[1,2] Francis Pike,[3] Mark L. Unruh.[4] *[1]Nephrology, National Taiwan University Hospital, Yun-Lin, Taiwan; [2]GSPH, Genetics, U. Pittsburgh; [3]Biostatistics; [4]Renal-Electrolyte Division, University of Pittsburgh Medical Center.*

Background: While previous works have focused on relationships between baseline measures and all-cause mortality in end-stage renal disease, markers of nutrition vary over time and may affect mortality due to either infections or cardiovascular disease. We conducted the study to examine whether changes in nutritional status,anthropometric parameters and Karnofsky performance index would predict cause-specific mortality in hemodialysis patients.

Methods: Anthropometric assessments were measured in HEMO study and functional status was measured by Karnofsky index.Index of coexistent diseases score,baseline demographics,blood studies were covariates.The definition of cause-specific mortality in the HEMO study has been described elsewhere.Cox-regression model was used to examine patient survival along with the decline of anthropometric parameters,and performance status to mortality.

Results: There were 1404 patients,in HEMO study,with at least one follow up anthropometric measurements.The difference of mid-arm muscular circumference(MAMC) between baseline and the data in the first visit was significant(p=0.07).Lower serum albumin and MAMC were associated with increased mortality(p<.0001)and cardiac mortality. Patients with higher Karnofsky index had less all-cause mortality and death from congestive heart failure(p=0.01).The decline of MAMC was associated with all-cause mortality,death from infection(p=0.01)and time to 1st cardiac hospitalization or cardiac death(p=0.03). Adjusted survival analysis revealed declining MAMC is an independent predictor for

all-cause mortality(HR:1.4,p<.0001),death from infection(HR:1.46,p=0.05),and time to 1st cardiac hospitalization(HR:1.25,p=0.0002).

Conclusions: In patients undergoing maintenance hemodialysis,nutritional status and functional performance status are associated to survival.The decline of MAMC is a predictor for patient mortality.Serum albumin,MAMC and Karnofsky index are key modifiable predictors to consider as targets for improving patient outcomes.

TH-PO574

Global Variation of Nutritional Status in Children Undergoing Peritoneal Dialysis Joshua Zaritsky, Dagmara Borzych-Duzalka, Anja K. Büscher, Annabelle N. Chua, Vera Hermina Koch, Maria Lipka, Viola M. Pinto, Lesley Rees, Sara Testa, Patricia G. Vallés, Franz S. Schaefer, Bradley A. Warady. *International Pediatric Peritoneal Dialysis Network.*

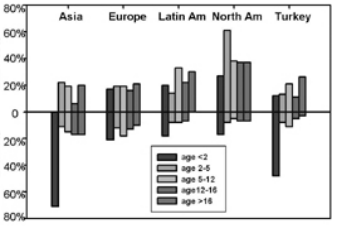
Background: Despite the availability of clinical guidelines, it remains unknown if nutritional practices and outcomes vary globally for children receiving peritoneal dialysis (PD).

Methods: We examined the nutritional status of 1466 children from 78 centers in 30 countries participating in the International Pediatric Peritoneal Dialysis Network (IPPN). Body Mass Index (BMI) and height measurements were normalized to WHO (2006) and CDC (2000) standards for children < and > age 5, respectively (table).

Results: Enteral feeding practices for patients < age 5 varied widely with the highest rate seen in North America. Analysis of BMI distribution by age showed high rates of BMI <5% for ages <2 in Asia and Turkey and BMI >85% (overweight) for ages 2-5 in North America (figure). Height SDS were poor in Asia, Latin American and Turkey. Multivariate analysis identified age (OR 0.94 (0.91-0.97)), residual urine output (OR 0.96 (0.93-0.99)) and residence in India (OR 3.83 (1.41-10.5)) as factors associated with a BMI <5%, while enteral feeding (OR 1.76 (1.1-2.8)) and residence in the USA were associated with a BMI >85%.

Region	BMI SDS (Mean±SE)	BMI <5% (%)	Height SDS (Mean±SE)	Enteral Feeding Age <5 (%)
Asia	-0.2±0.1	18	-2±0.1	4
Europe	-0.2±0.06	15	-1.6±0.07*	46
Latin Am	0.2±0.06*	8	-2.3±0.07	35
North Am	0.5±0.09*	8	-1.6±0.1*	72
Turkey	-0.2±0.08	12	-2.6±0.09	6

*p<0.001 versus other regions



Conclusions: These findings demonstrate considerable global variation in nutritional status and suggest provision of calories alone does not correct growth failure in children receiving chronic PD. Future studies are needed to further examine nutritional practice patterns including underutilization as well as excessive use of enteral feeding in some age groups/regions, and to determine what, if any association exists between a BMI <5% or >85% and unfavorable outcomes in pediatric PD patients.

Funding: Pharmaceutical Company Support

TH-PO575

Validation of Measurement of Hydration of Fat Free Mass in Volume Expanded Dialysis Patients Alessio Molfino,[1,2,3] Burl R. Don,[1] George A. Kaysen.[1] *[1]Nephrology, UC Davis, Davis, CA; [2]Clinical Nutrition, University of Rome; [3]Clinical Medicine, Sapienza University of Rome, Italy.*

Background: Fat mass (FM) is measured with dual-energy X-ray absorptiometry (DXA), but is expensive and not portable. Multifrequency bioimpedance spectroscopy (BIS) measures total body water (TBW) and intracellular and extracellular water (ICW and ECW). Adiposity is calculated by subtracting Fat Free Mass (FFM) from weight assuming fractional hydration of FFM of 0.73. Hemodialysis patients (HD), however, have non physiologic expansion of ECW.

Methods: We estimated the hydration of FFM in healthy adult subjects (C) and HD with BIS using a formula that allows ECW and ICW to vary (TBW/FFM = (1 +ECW/ICW)/(1.569+1.16*(ECW/ICW)) (Wang Z. et. al. AJP Endo Metab 276:995,1999). We then derived a value for FM accounting for the effects of varying expansion of ECW to be applied to HD and C. FM was measured by DXA in 11 C (6 male and 5 female) median age 32.5 y (23-53 y), weight 76.5 kg (47.9-102.8 kg), body mass index (BMI; kg/m2) 26.5 (17.5-29.8), and in 8 male HD with median (range) age 44.5 y (28-55 y), weight 102.5 kg (73-119.3 kg), BMI of 31.15 (24.7-36.9). We measured TBW, ECW and ICW with BIS and calculated FM using either Weight - TBW/.73 or with the formula to account for variations in ECW/ICW to estimate FM.

Results: ECW/ICW was significantly greater in HD than in C (0.860 vs 0.780; p=0.01) and estimated hydration of FFM by use of the formula significantly greater in HD than in C (0.724 0.004 vs 0.7195 0.003; P=0.048). FM (Kg) estimated by DXA, BIS and the formula were not different in C (20.0 2.7, 20.3 2.9, 19.4 3.0, p=n.s. and correlated r2 p< 0.001) or in HD (27.8 3.2, 28.4 2.9, 27.6 2.9, p=n.s and correlated r2 p< 0.001.). Neither Bland-Altman plot regressed (r2=0.00). Assumption of hydration of FFM of 0.73 provided a measure of FM 0.6 kg > DXA in HD and 0.3 kg > in C. The model provided a measure of FM 0.2 kg < DXA in HD and 0.6 kg < in C.

Conclusions: Expansion of ECW in HD is statistically significant, however the effect on hydration of FFM is insufficient to cause any deviation from values derived using a hydration value of 0.73, validating the use of BIS to estimate adiposity in HD patients.

Funding: Private Foundation Support

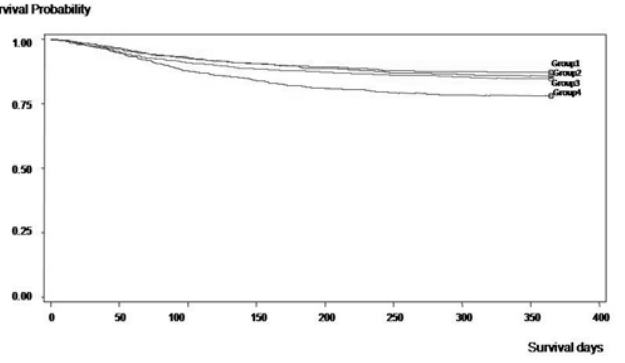
TH-PO576

Nutrition Status Prior to Starting Hemodialysis Is Strongly Associated with One-Year Mortality in Hemodialysis Patients Eiichiro Kanda,[1] Jenna O. Krisher,[2] William M. McClellan.[3] *[1]Tokyo Kyosai Hospital, Japan; [2]ESRD Network 6; [3]Emory University.*

Background: Malnutrition is a common complication in hemodialysis patients. We examined nutritional status prior to starting hemodialysis to determine whether it was independently correlated with one-year mortality among incident hemodialysis patients.

Methods: We enrolled 6304 incident hemodialysis patients in North Carolina, South Carolina and Georgia(ESRD Network 6 area) and followed up these individuals for up to one year. Prehemodialysis nutritional status was assessed using data from the Centers for Medicare & Medicaid Services (CMS) 2728 Form. Nutritional status was evaluated on the basis of body mass index (BMI), serum albumin level, and geriatric nutritional risk index (GNRI) (calculated from height, weight and serum albumin levels). Patients were categorized into quartile groups by GNIR. Mortality was evaluated using a fully adjusted Cox proportional hazard model.

Results: The mean age (SD) was 61.3 (15.0) years old; female 47.9%; diabetes 46.2%; mean BMI 29.1 (9.0) kg/m^2; mean serum albumin 3.02 (0.72) mg/dl; 5.9% under the care of a dietitian prior to dialysis; GNRI median 62.7 (IQR 50.8-90.7). Nutrition status prior to hemodialysis was correlated with mortality: low BMI (less than 23) hazard ratio (HR) 1.32 (95% confidence interval 1.15-1.52); hypoalbuminemia (less than 3.8 g/dl), 2.09 (1.59-2.75). The group with the lowest GNRI, Group 4 (<50.8), had an HR of 1.41 (1.16-1.71) relative to Group 1 (GNRI>90.7)



Patient with higher geriatric nutritional risk index (GNRI) showed lower risk of mortality.
Log-Rank test P<0.0001, Wilcoxon signed rank test P<0.0001
Group 1; 90.7 <= GNRI
Group 2; 62.7 <= GNRI < 90.7
Group 3; 50.8 < GNRI < 62.7
Group 4; GNRI < 50.8

Patients who were under the care of dietitians had higher serum albumin levels (t-test, P<0.0001) and lower mortality, HR 0.74 (0.55-0.99) than patients who were not.

Conclusions: In dialysis patients, nutritional status prior to starting hemodialysis is associated with early mortality risk. Moreover results suggest that the control of nutritional status may decrease the risk.

TH-PO577

Quality of Sleep and Daytime Sleepiness in Chronic Hemodialysis – A Study of 400 Patients Sonia Maria Holanda Almeida Araujo,[1] Andre Pantaroto,[2] Nicole Araujo,[2] Constance Almeida de Alencar Araújo,[1] Gilson Almeida,[3] Elizabeth De Francesco Daher,[1] Pedro Bruin,[1] Veralice Meireles Sales Bruin.[1] *[1]Medicina, Universidade Federal do Ceara, Fortaleza, Ceara, Brazil; [2]Medicina, Faculdade de Medicina de Jundiai, Jundiai, Sao Paulo, Brazil; [3]Medicina, Faculdade Christus, Fortaleza, Ceara, Brazil.*

Background: Impaired sleep has potential health consequences in chronic hemodialysis patients. To date, this issue has not been examined in studies involving a large number of subjects. We aimed to identify factors associated with poor sleep quality and excessive daytime sleepiness (EDS) in dialysis patients.

Methods: This is a cross-sectional observational study, involving 400 patients (59% male) from three hemodialysis centers (SD-HEMOFOR). Quality of sleep was evaluated by the Pittsburgh Sleep Quality Index (PSQI); EDS by the Epworth Sleepiness Scale (ESS); risk of obstructive sleep apnea (OSA) by the Berlin questionnaire and comorbidity severity by the Charlson Comorbidity Index (CCI).

Results: Poor sleep quality (PSQI>5) was found in 227 individuals (57%) and was associated with older age (p=0.001), diabetes (p=0.03), heart failure (p<0.005); hypoalbuminemia (p=0.01), low transferrin saturation (TSAT) (p=0.009), higher CCI score (p=0.01) and depression (p<0.005). Independent factors were older age, heart failure, low TSAT and depressive symptoms. Daytime somnolence was present in 108 patients (27%) and was independently associated with stroke (OR=2.84 CI= 1.03-7.76), lower hemoglobin concentration (OR=2.45 CI= 0.95-3.03) and high risk of OSA (OR=1.65 CI= 1.03-2.63). High risk of OSA (N=120; 30%), was associated with hypertension (p<0.001), overweight/obesity (p=0.001), older age (p=0.003) and symptoms of depression (p=0.01).

Conclusions: Poor sleep quality and EDS are prevalent on chronic hemodialyis. Heart failure, low TSAT and depressive symptoms are independently associated with poor sleep quality. Stroke, anemia and high risk of OSA are independently associated with EDS. These results provide new insight into possible treatment strategies.

Funding: Government Support - Non-U.S.

TH-PO578

High Dietary Fiber Intake Associates with Lower Indoxyl Sulfate Concentrations in Chronic Kidney Disease Liesbeth Viaene, Bjorn K.I. Meijers, Bert Bammens, Pieter Evenepoel. *Nephrology, University Hospital, Leuven, Belgium.*

Background: Mounting in vitro and clinical evidence indicate that indoxyl sulfate (IndS), a protein-bound uremic toxin originating from bacterial protein fermentation in the colon, is involved in the pathogenesis of accelerated cardiovascular (CV) disease in chronic kidney disease (CKD) patients. High dietary fiber intake suppresses protein fermentation and is associated with decreased CV morbidity and mortality in both CKD and non-CKD populations.

Methods: We performed a cross-sectional observational study to investigate associatons between dietary fiber intake and serum IndS levels. Fasting blood samples were collected and analyzed for routine biochemistry and IndS (HPLC) in 195 stable CKD patients (105 male; age 65±16 year). Dietary intake was estimated through a dietary history and nutrient content was calculated using Becel® Nutritional Software.

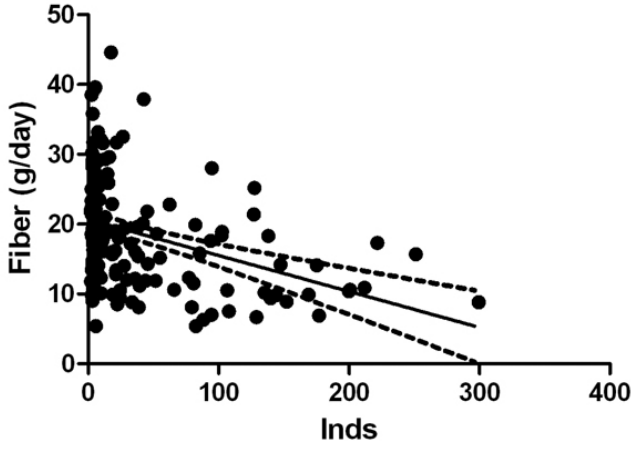
Results: Demographics and biochemistry are summarized in table 1.

Demographics and dietary intake

	CKD1-2	CKD3	CKD4-5	CKD5D	P
Male (%)	28	55	64	62	0.01
Age (y)	44	68	69	70	<0.0001
BMI (kg/m²)	25	28	27	26	0.2
Inds (µM)	4	7	18	88	<0.0001
Total kcal/day	2162	1808	1647	1717	0.0007
Protein (g/d)	84	78	69	71	0.006
Carbohydrate (g/d)	269	209	207	215	0.0002
Fiber (g/d)	24	22	20	13	<0.0001

Median values are shown

Total energy intake, as well as intake of dietary protein, carbohydrate, fat and fiber intake were lower in more advanced CKD stages. Of all macronutrients, only dietary fiber intake was associated with Inds levels (β=-0.06; p<0.0001), independent of eGFR, age, sex and BMI.



Conclusions: Dietary fiber intake is inversely associated with indoxyl sulfate concentrations in CKD patients, independent of eGFR. Suppressing indoxyl sulfate serum levels might be one of the mechanisms through which dietary fiber could decrease CV morbidity and mortality.

TH-PO579

Olfactory Dysfunction of ESRD Patients Was Recovered by Initiation of Dialysis Young-Il Jo,[1] Ju-Young Moon,[2] Sang-Woong Han,[3] Dong Ho Yang,[4] Sang-Ho Lee,[2] Hyeong Cheon Park,[5] Sug Kyun Shin.[6] *[1]Nephrology, Konkuk University Hospital, Seoul, Republic of Korea; [2]Nephrology, Kyung Hee University Hospital at Gangdong, Seoul, Republic of Korea; [3]Nephrology, Hanyang University Guri Hospital, Guri, Republic of Korea; [4]Nephrology, Bundang CHA Hospital, Sungnam, Republic of Korea; [5]Nephrology, Yonsei University Gangnam Severance Hospital, Seoul, Republic of Korea; [6]Nephrology, NHIC Ilsan Hospital, Goyang, Republic of Korea.*

Background: Olfactory function was impaired in chronic kidney disease patients. Some studies showed that olfactory function of ESRD patients receiving dialysis did not differ from those of healthy controls. However, there was no prospective study following initiation of dialysis on olfactory function in ESRD patients. This prospective study was designed to clarify the effect of dialysis on olfactory function of ESRD patients who initiate hemodialysis (HD) or peritoneal dialysis (PD).

Methods: Fifty-two ESRD patients who initiated maintenance HD (n=16) or PD (n=36) were recruited from 6 hospitals. Olfactory function was tested by using the Cross-Cultural Smell Identification Test (CC-SIT) before the initiation of the first dialysis and then at 3 and 6 months after initiation of dialysis.

Results: There were no differences in mean smell score before initiation of dialysis and baseline characteristics such as age, diabetes, HbA1C or hs-CRP. In ESRD patients not receiving dialysis, the smell score was negatively correlated with ages (*r=-0.179, p=0.02*) and did not differ between DM and non-DM patients and between male and female. The smell scores increased significantly after initiation of HD and PD. In ESRD patients on HD, the smell scores increased from 7.6±2.5 at baseline to 8.4±3.0 at 3 months (*p=0.060* vs. baseline) and to 9.3±2.5 at 6 months (*p=0.014* vs. baseline). PD patients also showed a significant increase of the smell scores after initiation of PD: 8.4±2.2 at baseline, 9.1±1.8 at 3 month (*p=0.034*), 9.2±1.8 at 6 month (*p=0.039*). Improvement of smell scores following HD or PD had no relation to gender, diabetes or age.

Conclusions: These results suggest that smell disturbance in patients with ESRD could be recovered by initiation of HD or PD.

TH-PO580

Increased Levels of Serum Parathyroid Hormone and Fibroblast Growth Factor-23 Are the Main Factors Associated with the Progression of Vascular Calcification in Long-Hemodialysis Patients Guillaume Jean, Charles Chazot. *Hémodialyse, NEPHROCARE, Tassin, France.*

Background: Vascular calcifications (VCs) are frequently observed in hemodialysis (HD) patients and have been associated with poor outcomes. The aim of the present study was to assess the frequency and the factors associated with the progression of VCs using a semi-quantitative X-ray score.

Methods: We included all prevalent HD patients between January 2006 and January 2007, with initial radiological scores ranging from 0 to 3 according to the severity and extent of the VCs (abdominal aorta, iliac, femoral, popliteal, and radial arteries). Patients were classified as non-progressors or progressors according to the change in their VC score after 3 years. We analyzed the values of the means of all biological data and treatment doses.

Results: Among the 85 patients still alive after 3 years, 47 were classified as non-progressors and 38 as progressors (44.7%). No regression in VC score was observed. Among all the studied parameters, only serum PTH and fibroblast growth factor (FGF)-23 levels were increased in the progressor group. On evaluating the association between patients with serum PTH and FGF-23 < or ≥ the median values (190 pg/ml and 3000 RU/ml, respectively), we found that only exhibiting high levels of both parameters is associated with the risk for VC progression (odds ratio, 5.8; 1.7–19.8, $P = 0.004$). Hyperphosphatemia (<10%), and especially, hypercalcemia (1%), and hyperparathyroidism (HPT >; 585 pg/ml = 0%) were infrequently observed. Calcitriol analogs (38%), cinacalcet (15%), dialysate calcium (mean 1.48 mmol/l), and calcium- (10%) and non-calcium based phosphate binders (38%) were prescribed on an individual basis.

Conclusions: After 3 years, VC progression was observed in 44.7% of prevalent HD patients using long-dialysis sessions and an individual therapeutic strategy. The main factor associated with VC progression was the association of higher serum PTH and FGF-23 levels. It remains to be seen whether patients should be treated to lower their PTH value, even in the target range, using calcitriol analogs, calcimimetics, parathyroidectomy, or by modifying the Klotho-FGF-23 axis.

TH-PO581

Greater Reductions in Mortality over Time for Obese and Extremely Obese Patients Compared to Normal or Underweight Patients Who Begin Dialysis Therapy: A Cohort Study from 1995-2005 Austin G. Stack,[1,2] Hoang Thanh Nguyen,[1] Bhamidipati V.R. Murthy,[2] Arif I.F. Mutwali.[1] *[1]Regional Kidney Centre, Letterkenny Genral Hoapital, Letterkenny, Donegal, Ireland; [2]Division of Renal Disease and Hypertension, University of Texas Medical School, Houston, TX.*

Background: Prior studies have identified an inverse correlation between body mass index (BMI) and survival with obese patients experiencing the greatest benefit. The relationship between changing trends in body size and associated mortality risks among new dialysis patients has not been previously explored.

Methods: We compared trends in mortality among underweight, normal, overweight, obese, and extremely obese patients who began dialysis therapy between 1995-2005 using data from the US Renal Data System. National incidence data were available on new patients (N=851,480) between May 1995 and December 2005 and all patients were followed until Oct 2006. Mortality risks were compared across 4 calendar periods (1995-1996, 1997-1999, 2000-2002 and 2003-2005) with the generation of adjusted hazard ratios[1] (HR) 95% Confidence intervals) using multivariable Cox regression analysis. All analyses were conducted using SAS V 9.01.

Results: From 1995-2005 the prevalence of overweight, obesity and extreme obesity increased while the prevalence of normal and underweight decreased. The multivariable relative mortality risks for each weight category by calendar period are illustrated:

Prevalence (%)	1995-96	1997-99	2000-02	2003-05
Underweight (< 18.5)**	10.8	9.0	5.1	4.4
Normal weight (18.5-24.9)**	43.8	41.3	38.5	35.8
Overweight (25-29.9)**	26.7	28.3	30.3	30.8
Obese (30-39.9)**	16.6	18.8	22.6	25.0
Extreme Obese (> 40)**	2.1	2.7	3.5	4.1
Relative Risk Death[1,2]	Referent			
Underweight (< 18.5)	1.00	0.98	1.03	1.01
Normal weight (18.5-24.9)	1.00	0.98*	0.91**	0.88**
Overweight (25-29.9)	1.00	0.97**	0.90**	0.86**
Obese (30-39.9)	1.00	0.95**	0.88**	0.83**
Extreme Obese (> 40)	1.00	0.91*	0.83**	0.78**

[1]Each weight category modeled separately
[2]Adjusted for 17 demographic, clinical and laboratory variables, *P<0.001 **P<0.0001

Conclusions: The increasing prevalence of overweight, obesity and extreme obesity was associated with a trend of falling mortality risks from 1995-2005. The larger the BMI category, the proportionally greater the reduction in relative mortality risks over time with the survival of underweight patients unchanged. Increasing BMI among successive incident dialysis cohorts is associated with temporal improvements in overall survival.

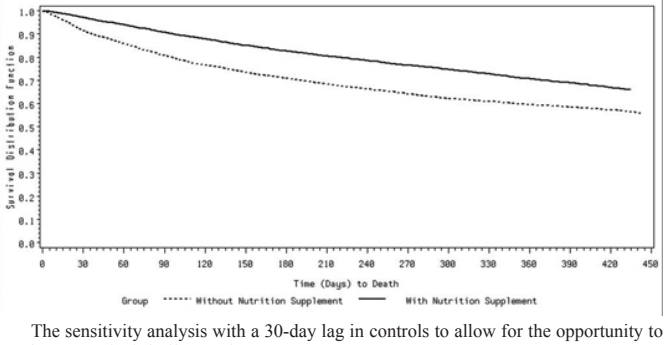
TH-PO582

Effect of Oral Nutritional Supplements on Mortality in Hemodialysis Patients Eduardo K. Lacson, Weiling Wang, Barbara L. Zebrowski, J. Michael Lazarus, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

Background: We evaluated whether supervised oral nutritional supplement (ONSP) at every treatment impacts survival in hemodialysis (HD) patients.

Methods: All chronic in-center HD patients in Fresenius Medical Care North America facilities, with albumin ≤3.5 g/dL in Q4-2009 without oral supplements in the prior 90-days were eligible for ONSP until albumin was ≥4.0 g/dL. Patients prescribed ONSP in Q4-2009 were study patients (supplement start date defined as Day 1 of follow-up), the rest were controls (date of 1st albumin ≤3.5 g/dL defined as Day 1 of follow-up). Case-mix data as of Day 1 and lab values for hemoglobin, phosphorus and eKt/V from the prior 90-days were recorded. Mortality was tracked for 1 year up to12/31/2010. Cox models were used to compare one-to-one propensity score and geographic region-matched groups. The propensity score used age, gender, race, diabetes, vintage, BMI, cause of ESRD, hospitalization in the prior 30 days, albumin, hemoglobin, phosphorus, eKt/V, access type and an indicator for incident patients (i.e. vintage ≤90 days).

Results: Propensity score plus region matched patients (N=4,354 pairs) had similar mean age of 64.9 years, 49% male, 58% white/38% black, mean vintage of 3.5 years, and mean BMI 30.2. They had 41% fistulas, 21% grafts, 38% catheters, and baseline albumin of 3.27 g/dL. Fewer study patients had diabetes (61.3% vs. 63.4%, p=0.04). The median time to receive supplement was 18 days (mean 23 days). The HR=0.67 (p<0.0001) for ONSP vs. controls. KM survival curves are shown.



The sensitivity analysis with a 30-day lag in controls to allow for the opportunity to receive ONSP had similar results.

Conclusions: Geographic region and propensity score-matched patients with albumin ≤3.5 g/dL who were prescribed and received supervised ONSP exhibited significantly better one-year survival.

TH-PO583

Leptin, NPY and Cortisol Concentration Changes in 4-Hour Hunger Test in Patients with Renal Failure Stanislaw Niemczyk,[1] Katarzyna Romejko-Ciepielewska,[1] Ewa Paklerska,[1] Longin Niemczyk,[2] Katarzyna Szamotulska,[3] Joanna Matuszkiewicz-Rowinska.[2] *[1]Military Institute of Medicine; [2]Medical University of Warsaw; [3]National Research Institute of Mother and Child.*

Background: Malnutrition is a common finding in end-stage renal disease. Weight loss is present in approximately 40% of dialysis patients. Hormone disturbances in end-stage renal disease may be responsible for malnutrition. High leptin concentration in patients with renal failure inhibits food intake and may lead to the loss of weight. Cortisol increases leptin concentration. NPY is an orexigenic peptide. Leptin inhibits NPY synthesis in hypothalamus.

Methods: The study involved 87 pts (37 HD pts and 50 controls). Blood samples for leptin, NPY and cortisol measurement were taken twice (at 8 a.m., after a 12-hour fast, and at noon, after 4 hours of prolonged fasting).

Results: During a 4-hour fast, we observed a decrease in leptin levels in all HD patients, with statistically significant differences in HD patients with normal body weight (p=0,006). We also observed high leptin concentration in fasting state in HD malnourished (0,14-225) and obese (0-275) patients. NPY levels were slightly reduced in undernourished HD patients. We observed a fall of cortisol concentration in 4-hour hunger test in all HD patients, with statistically significant differences in HD obese (p=0,034) and normal weight patients (p=0,013). Cortisol concentration in fasting state were higher in all HD patients in comparison with the control group, we observed the statistically significant differences between malnourished (p=0,010) and obese (p=0,018) pts.

Conclusions: High fasting leptin concentration and no significant reduction in these concentration during prolonged fasting in undernourished HD patients indicate an association between leptin and the development of malnutrition. This association may result from reduced NPY levels. HG fasting leptin concentration in HD obese patients is a result of leptin resistance in this group of patients. High cortisol concentration in HD malnourished patients may be responsible for inhibition of food intake and the loss of weight.

TH-PO584

Site and Size of Vascular Calcifications Are Different in Dialysis Patients with Various Underlying Diseases Hiromichi Suzuki, Tsutomu Inoue, Hirokazu Okada, Tsuneo Takenaka. *Department of Nephrology, Saitama Medical University, Iruma gun, Saitama, Japan.*

Background: It is well known that vascular calcification (VC) contributes to increased cardiovascular disease in dialysis patients and is used as a marker of the severity of vascular disease. However, VC occurs in vessels of various diameters and no definitive studies have determined the significance of VC in different vessels. The aim of this work was to learn if there was any association between the site of VC in the arteries and the underlying disease in dialysis patients.

Methods: This was an observational and cross-sectional study that included 78 dialysis patients. Using computed tomography (CT) scans, the total volume of VC in the thoracic and abdominal aorta and in the arteries of the lower limbs with a density more than 130 Hounsfield Units were semi-quantitatively determined as the sum of all voxels. Clinical characteristics and laboratory variables were determined by cardiac echography, dual-energy x-ray absorptiometry and pulse wave velocity.

Results: The patients (66% men, 40% diabetic) had median age anddialysis period of 62.3 yr and 76.7 months, respectively. VC in the thoracic aorta was present in 92%, in the abdominal aorta in 90% and in the lower limbs in 90% of the patients. All three lesions correlated significantly with each other. Stepwise regression was applied in which the independent variables were identified from the univariate analyses. Significant associations were seen for the following: the prevalence of calcification in the thoracic aorta with age, presence of diabetes, and calcium supplement; the abdominal aorta with period of dialysis, elevations of both systolic and diastolic blood pressure and levels of serum albumin; arteries of the lower limbs with presence of diabetes mellitus, use of sevelamer and cinacalcet and serum levels of intact parathyroid hormone and albumin.

Conclusions: The presence and extension of VC in thoracic and abdominal aortas and in arteries of lower limbs might be regulated in a complex manner and the use of these variables as a marker of the severity of vascular disease should proceed with caution.

TH-PO585

Impact of High Flux Membrane Dialysis on Clearance of Cardiac Troponin T in Asymptomatic Hemodialysis Patients Azharuddin Mohammed, Simon Fletcher, Daniel Zehnder. *Department of Nephrology, University Hospitals Coventry, Coventry, United Kingdom.*

Background: Cardiac troponin T (or I) level at baseline and 6-12 hours later is used to diagnose acute myocardial injury in HD patients. It is not uncommon for the 1st or the 2nd troponin sample collection timing to coincide when the patient is on dialysis. This questions whether collecting a timed troponin sample during dialysis offers reasonable diagnostic accuracy, and if dialysis impacts their clearance significantly.Aim of this study is to assess the impact of dialysis on clearance of troponin T.

Methods: 111 asymptomatic maintenance HD patients using Nipro high flux dialysis membrane and dialysing in-centre had undergone midweek cardiac troponin T testing predialysis. Of these 111 patients, 94 had both pre and post dialysis troponin T checked using Roche E170 immunoassay. Using local troponin T cut off range we divided patients into 3 groups. Troponin T level of <0.01 ug/L are classed as normal(A), 0.01-0.10 ug/L are classed as high(B) and a troponin T of >0.1 ug/L are classed as very high(C).A change in troponin T concentration and a class change post dialysis is then analysed.

Results: Prevalence of troponin T in the 3 groups were 22.5 %(25) in group C, 74.7% (83) in group B and 2.7 %(3) in group A (n=111). We then analysed those who had both pre and post dialysis troponin T checked (n=94). Mean troponin T in group B was 0.045 ug/L (0.1-0.10) and 0.26ug/L (0.11-1.69) in group C. Percentage of patients in the three groups pre and post dialysis were 3.2% Vs 4.25%, 76.5% Vs 80.8% and 20.2% Vs 14.9% respectively for group A, B and C. Mean troponin T change after dialysis in group B was 0.007 ug/L and 0.03 ug/L in group C; p=0.296. A sub analysis of group C (n=19) showed none of the patients changed to group A after dialysis, but 5 patients changed to group B; p=0.318. In group B (n=72), 1 patient changed class to group A.

Conclusions: Dialysis using high flux membrane has shown a minimal clearance of troponin T which is not statistically significant. Scheduled timely serial measurement of troponin T can be undertaken with a reasonable diagnostic accuracy to evaluate for acute myocardial injury in HD patients even if they are on dialysis.

TH-PO586

Prognostic Importance of Serum Magnesium in Peritoneal Dialysis (PD) Patients (pts) Morrell M. Avram, Hitesh Kapupara, William Hartman, Jyotiprakas Chattopadhyay. *Avram Division of Nephrology, SUNY Downstate University Hospital at LICH, Brooklyn, NY.*

Background: Lower serum magnesium is a significant predictor of mortality in hemodialysis pts. We have reported that lower serum magnesium is associated with poorer nutritional status, impaired cellular health and increased inflammation in PD pts. This study was designed to investigate the prognostic value of serum magnesium for mortality in PD pts.

Methods: Sixty two PD pts were enrolled in this study between November 2000 to May 2008. On enrollment, demographic, clinical and biochemical data were recorded. Pts were followed to May, 2011.

Results: Mean age was 54±16 (SD) years, 55% were female, 65% were African American and 24% were diabetic. Mean enrollment serum magnesium was 1.597±0.28 (SD) mEq/L (range:0.8-2.1 mEq/L). Maximum follow-up period was 10.46 years. During the follow-up period, 26 pts (42%) expired. Pts who survived had higher enrollment serum magnesium compared to those who did not survive (1.76 vs. 1.50 mEq/L, p=0.028). Pts were stratified into 2 groups by enrollment serum magnesium. Upon 10.46 years of observation, pts with enrollment magnesium >1.6 mEq/L had significantly better cumulative survival (Kaplan Meier) than that of pts with magnesium<1.6 mEq/L (p=0.02). In Cox's multivariate proportional hazard analysis, after adjusting for age, race, gender, diabetes, months on dialysis at enrollment, serum albumin, creatinine and C-reactive protein, serum magnesium was a significant predictor of mortality (Relative risk: 0.947, p=0.014). Months on dialysis at enrollment (Relative risk:1.022, p=0.002), albumin (Relative risk=0.131, p=0.04) and C-reactive protein (Relative risk=1.055, p=0.04) were also significant predictors of mortality in this model.

Conclusions: In conclusion, enrollment lower serum magnesium level is a strong independent predictor of long-term survival in PD pts. Factors affecting serum magnesium concentration in PD pts should be investigated in more detail. Current dialysate magnesium concentration is 0.5 mEq/L. Higher concentration should be considered in order to prevent magnesium depletion.

Funding: Private Foundation Support

TH-PO587

Bone Mineral Density in Patients Receiving Hemodialysis Therapy for More Than 30 Years Shigeru Otsubo,[1] Takashi Akiba,[2] Kosaku Nitta.[3] *[1]Department of Blood Purification, Sangenjaya Hospital, Tokyo, Japan; [2]Department of Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [3]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Chronic kidney disease-mineral and bone disorder is a regular complication of hemodialysis patients. On the other hand, the number of patients receiving hemodialysis therapy for more than 30 years has been increasing. No information seems to be presently available concerning bone mineral density (BMD) in such patients. We investigated tartrate-resistant acid phosphatase (TRACP) 5b and the BMD, of extremely long-term hemodialysis patients.

Methods: Ninety-three outpatients receiving maintenance hemodialysis at our Hospital were enrolled. TRACP-5b was measured using a novel fragments-absorbed immunocapture enzymatic assay and two monoclonal antibodies. BMD was assessed using dual-energy X-ray absorptiometry scans. The absolute BMD values for the 1/3 distal radius on the side not containing the vascular access were reported. We classified the patients according to the duration of hemodialysis therapy (less than 10 years (n=57), 10-20 years (n=10), 20-30 years (n=8), or more than 30 years (n=18)) and compared them.

Results: The TRACP 5b level was higher in more than 30 years group (1192.4 ± 484.1 mU/dL), compared with each of the other groups (479.6 ± 253.3 mU/dL in less than 10 years group (p<0.0001), 456.7 ± 296.4 mU/dL in 10-20 years group (p<0.0001), 509.6 ± 256.3 mU/dL in 20-30 years group (p<0.0001)).The BMD and Z score was 0.59 ± 0.17 g/cm^2 and -0.26 ± 1.42 in less than 10 years group, 0.59 ± 0.16 g/cm^2 and -1.50 ± 2.33 in 10-20 years group, 0.45 ± 0.12 g/cm^2 and -2.23 ± 1.16 in 20-30 years group and 0.43 ± 0.13 g/cm^2 and -3.21 ± 1.86 in more than 30 years group. The BMD was lower in more than 30 years group than in less than 10 years group (p=0.019) and 10-20 years group (p=0.013). The Z sore was reduced gradually according to the duration of hemodialysis and was significantly lower in more than 30 years group than in less than 10 years group (p<0.0001) and 10-20 years group (p=0.009).

Conclusions: In patients receiving more than 30 years hemodialysis therapy, the BMD was reduced.

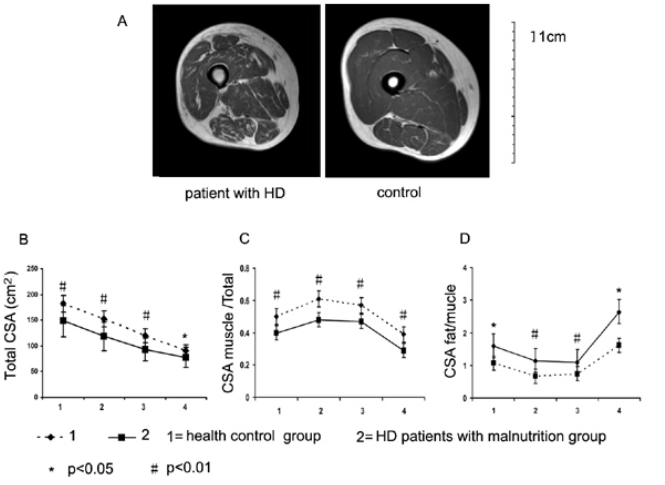
TH-PO588

Endogenous Glucocorticoid and Insulin Resistance Induced Muscle Wasting in Hemodialysis Patients Huiling Wang. *Division of Nephrology, Jimin hospital, Shanghai, China.*

Background: Patients undergoing advanced chronic kidney disease often present muscle wasting, to causes of poor physical function and living quality. The endogenous glucocorticoids (eGC) may trigger skeletal muscle atrophy by interaction with insulin-rsistance. However, the relationship between eGC lever or insulin-resistance and muscle wasting still need systematically addressed in clinic

Methods: We selected 70 patients who undertaken maintenance hemodialysis (MHD) without diabetes, and presenting malnutrition status evaluated by subjective global assessment (SGA); 30 healthy adults as control. Magnetic resonance imaging (MRI) of lower leg was determined the cross-sectional area (CSA) of muscle and fat.The serum corticosterone, fasting insulin and insulin resistance(HOMA-IR) was measured.

Results: The results showed there were no significant differences in gender and blood glucose. The bodyweight and body mass index, serum albumin were decreased in MHD. The serum corticosterone and the fasting insulin increased 4-5 times, the HOMA-IR was increased significantly compared with control. The MRI results showed the length of thighbone was no difference between MHD patients and the control group. The total cross-sectional area (CSA) of leg was significantly reduced in MHD. The morphologic observation showed muscle atrophy in MHD, the muscle CSA/total CSA ratio was lower, but the fat CSA/muscle CSA ratio was higher in MHD. The Spearman correlation analysis showed the eGC, fasting insulin levels and HOMA-IR was positively correlated to the MQSGA and MRI fat CSA; fat /muscle ratio; but negatively correlated with MRI muscle CSA and muscle /total ratio



Conclusions: In summary, dialysis patients with protein energy wasting characterized as decreased BMI and loss muscle mass, but fat content was increased. A high level of endogenous corticosteroids and insulin resistance might be related to muscle wasting

Funding: Government Support - Non-U.S.

TH-PO589

Effect of Fasting and Refeeding on Global mRNA Expression on Skeletal Muscle of ESKD Patients Manish Suneja, John B. Stokes, Victoria S. Lim. *Internal Medicine, University of Iowa Hospital and Clinics, Iowa City, IA.*

Background: The effects of prolonged fasting (physiological low insulin state) and refeeding (high insulin state) on global mRNA expression in skeletal muscle of End Stage Kidney Disease patients are not known.

Methods: We studied five ESKD (3 male and 2 female) and seven healthy adult human subjects (3 male and 4 female).The subjects fasted for 40 hours, and then were refed with a mixed meal. Muscle biopsies were obtained from vastus lateralis muscle at the end of the 40 hour fast, followed by a second biopsy 6 hours later. Exon expression arrays were used to analyze RNA isolated from the muscle biopsy samples. We studied the differential gene expression in muscle during fasting and refeeding conditions and compared it with control subjects.

Results: Out of > 17,000 mRNAs measured, 344 and 670 genes increased with fasting whereas 349 and 514 genes decreased with fasting in normal controls and ESKD patients respectively ($P \leq 0.05$). There were only 80 genes in common which were regulated by fasting in both the groups ($P \leq 0.05$). Out of these 80 genes, 30 were upregulated; 37 were downregulated; and 13 genes were discordantly regulated . One of the prominent effect of fasting in both the groups was to increase levels mRNAs encoding known mediators of insulin/IGF1 signaling including IGF1 receptor (*IGF1R*), insulin receptor substrate-2 (*IRS2*) and GRB2-associated binding protein 1*(GAB1)*. Interestingly, fasting also induced mRNAs encoding known inhibitors of insulin/IGF1 signaling like thioredoxin-interacting protein (*TXNIP*). There was also a differential gene expression noted in the two groups with regards to insulin/IGF1signaling. Promoters of insulin signaling {insulin receptor (*INSR);* insulin receptor substrate-4(*IRS4)*} and inhibitor of insulin receptor/signaling {PTP1B (*PTPN1*) } were upregulated in the control group but not in the ESKD group.

Conclusions: Fasting and refeeding induces some common set of genes in both groups. Differential gene expression was noted in the two groups with regards to insulin/IGF1signaling. These differential changes in gene expression in the muscle may be responsible for high level of insulin resistance seen in ESKD patients.

Funding: Private Foundation Support

TH-PO590

Body Composition Monitoring in Chronic Hemodialysis and Kidney Transplant Patients Sylvie Sulkova, Roman Safranek, Michaela Kubisova, Lydia Habanova, Petr Moucka, Katerina Petranova, Miroslav Merta. *Department of Nephrology, Gerontology and Metabolic Care, Medical Faculty and Teaching Hospital, Hradec Kralove, Czech Republic.*

Background: Chronic hemodialysis (HD) patients are at increased risk of malnutrition. Simple assessment, e.g. BMI calculation are not suitable for HD patients. The aim of our work was to assess one-year monitoring of body composition of HD patients and compare it with kidney transplant patients.

Methods: Body composition was assessed in 63 HD patients (22 females, 64 (54; 71) years) in two-month interval for one year. Bioimpedance spectroscopy (Body Composition Monitor) was used to estimate LTI (lean tissue index, kg/m^2), FTI (fat tissue index, kg/m^2). BMI (body mass index, kg/m^2) was calculated. Body composition of HD patients was compared with 100 kidney transplant patients (40 females, 57 (51; 70) years, 53 (20; 99) months after kidney transplant, serum creatinine 123 (94; 156)µmol/l). Data are given as median (lower; upper quartile).

Results: BMI, LTI, and FTI at the beginning of the study were 28.8 (26.2; 33.8)kg/m^2, 13 (11.4-14.8)kg/m^2, and 15.5 (10.8; 18.3)kg/m^2, in HD patients and 28.3 (25; 31.2) kg/m^2, 14.9 (12; 17.2)kg/m^2, 12.8 (8.7; 16.8)kg/m^2 in kidney transplant patients. Compared to reference range for normal population, we observed low LTI in 44% and 33% of HD and kidney transplant patients and high FTI in 65% and 60% of HD and kidney transplant patients, respectively. We observed no change in BMI during the course of the study in the group of HD patients, but changes in lean and fat tissue index in individual patients.

Conclusions: Bioimpedance spectroscopy shows low lean and high fat tissue index in HD patients. Similar results we obtained in a group of kidney transplant patients, which indicates profound defect in tissue composition regulation both in HD as well as in transplant status. We observed no influence of kidney graft function and time from kidney transplant on body composition. Body composition monitor is a useful tool for monitoring of fat and lean tissue mass in both HD and transplant patients and reveals changes of body composition in individual patients that may help in management of the patients.

Funding: Government Support - Non-U.S.

TH-PO591

Body Composition and Physical Performance in Maintenance Hemodialysis (MHD) Patients Jun Chul Kim,[1,2] Bryan B. Shapiro,[1,2] Kamyar Kalantar-Zadeh,[1,2,3,4] Usama Feroze,[1,2] Janos Porszasz,[1] Rachelle Bross,[1,2] Joel D. Kopple.[1,2,3,4] *[1]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; [2]Harold Simmons Center for Chronic Disease Research and Epidemiology, Torrance, CA; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]UCLA School of Public Health, Los Angeles, CA.*

Background: MHD patients often display protein-energy wasting, sarcopenia and diminished physical performance. This study was undertaken to assess the relationship between body composition and physical performance in MHD patients.

Methods: Body composition, assessed by dual-energy x-ray absorptiometry and body mass index (BMI), were compared to three measures of physical performance: 6-minute walking distance, sit-to-stand testing and stair climbing. At present, 28 patients undergoing MHD for ≥6 months have been examined in this ongoing study.

Results: Patients were 53±13SD years, 36% female; 36% diabetic; dialysis vintage was 53±48 months. Unadjusted analyses indicated that 6-minute walking distance correlated with lean body mass (LBM) (r= 0.358; p= 0.031), LBM index (LBMI) (kg of LBM/m2 height; r= 0.322; p= 0.047) and % body fat (r= 0.435; p= 0.010); stair climb correlated with LBM (r= 0.341; p= 0.038), LBMI (r= 0.366; p= 0.028) and possibly with LBM of both legs combined (r= 0.318; p = 0.050) (Figures A and B). Sit-to-stand did not correlate with any body composition measure. There were no associations between BMI (range, 19.8-44.2 kg/m2) and physical performance.

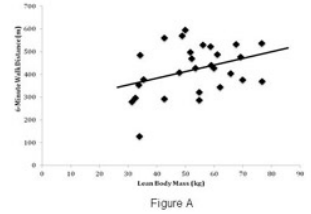


Figure A

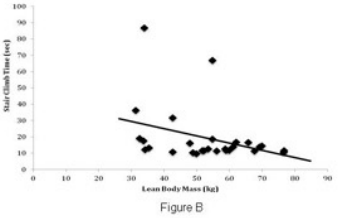


Figure B

Conclusions: These preliminary findings indicate that in MHD patients body composition, and especially LBM, was associated with certain measures of physical performance, and particularly with 6-minute walking distance and stair climbing.

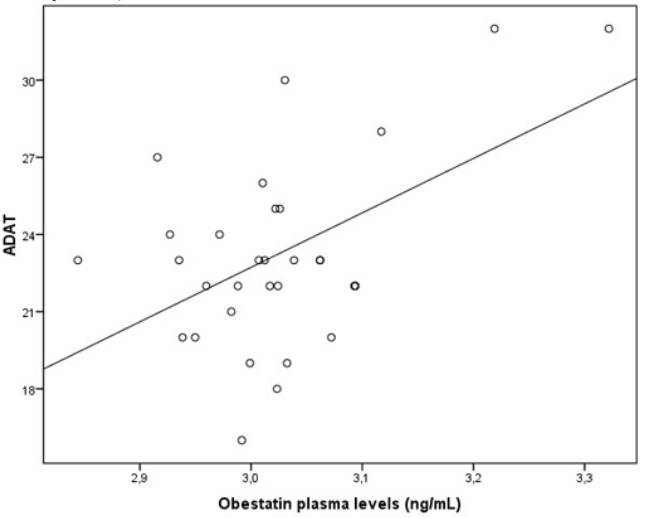
TH-PO592

Plasma Obestatin Levels Are Negatively Correlated with Appetite in Hemodialysis Patients Cristiane Moraes,[1] Julie Lobo,[2] Milena Barcza Stockler-Pinto,[2] Amanda Barros,[1] Denis Fouque,[3] Denise Mafra.[1] *[1]Department of Clinical Nutrition, Nutrition Faculty, Federal University Fluminense, Niterói, Rio de Janeiro, Brazil; [2]Institute of Biophysic Carlos Chagas Filho, Health Sciense Centre, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]Department of Nephrology, Hôpital E.Herriot, Univ Lyon, Lyon, France.*

Background: Obestatin hormone, an appetite suppressant which seems to be up regulated in hemodialysis (HD) patients, appear of interest in chronic kidney disease since the majority of these patients suffer of protein- energy wasting (PEW). The purpose of this study was to analyze plasma obestatin levels in HD patients and its correlation with appetite and biochemical parameters.

Methods: Thirty- four HD patients (64.7% men, 46.6 ± 15.3 yr, BMI, 23.2 ± 4.0 kg/m2, 30.5 ± 6.7% body fat, 57.0 ± 36.0 months on dialysis) were studied. Obestatin levels were measured using the enzyme immunometric assay methods. Anthropometric parameters were recorded and, the appetite was assessed with Appetite and Diet Assessment Tool (ADAT) where the higher score means lower appetite.

Results: Obestatin levels (3.0 ± 0.1pg/mL) and ADAT values did not present differences between gender or BMI groups (cutoff point of BMI 25.0 kg/m2). Obestatin levels were positively correlated with ADAT values (r= 0.5; p= 0.004; Figure 1) and negatively correlated creatinine (r=-0.4; p=0.019). ADAT and creatinine were negatively correlated (r= -0.4; p= 0.019).



Conclusions: Obestatin levels are increased in HD patients with poor appetite and these patients present loss of muscle mass according to creatinine. Therefore high plasma obestatin could be related to PEW.

Funding: Government Support - Non-U.S.

TH-PO593

Association of Adipokines with Cardiovascular Mortality in Patients on Hemodialysis Shoichi Maruyama,[1] Kaoru Yasuda,[1] Hirotake Kasuga,[2] Yoshinari Yasuda,[1] Tomoki Kosugi,[1] Waichi Sato,[1] Naotake Tsuboi,[1] Yasuhiko Ito,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]Department of Nephrology, Nagoya University Graduate School of Medicine, Naogya, Aichi, Japan; [2]Department of Nephrology, Naogya Kyoritsu Hospital, Nagoya, Aichi, Japan.*

Background: The role of adipokines for cardiovascular disease remains controversial in chronic hemodialysis (HD) patients. We prospectively investigated the association of adipokine with cardiovascular mortality in patients on HD.

Methods: Plasma adiponectin, leptin, resistin and tumor necrotic factor-α (TNF-α) were measured in pre-dialysis blood sample in 203 patients on HD (age: 65±11 years, diabetes: 49%, duration of HD: 8.1±7.1 years). Geriatric nutrition risk index (GNRI) as a parameter of nutrition status was also calculated from individual serum albumin levels and body mass index. Patients were followed up for 5 years.

Results: During follow-up period (mean 54±14 months), 27 patients (13.3%) died due to cardiovascular disease. On Cox analysis, only adiponectin (HR: 1.07, 95%CI: 1.02-1.12, P = 0.01) and leptin (HR 0.94, 95%CI 0.88-0.99, P = 0.021) were significantly associated with cardiovascular mortality, respectively. Based on cut-off levels using ROC analysis (11.7µg/ml, AUC=0.72 for adiponectin, and 3.5ng/ml, AUC=0.74 for leptin, respectively), 5-year freedom from cardiovascular mortality was lower in the high adiponectin group than in the low adiponectin group (77.3% vs. 94.1%, P = 0.0006), and was lower in the low leptin group than in the high leptin group (72.3% vs. 93.7%, P = 0.0001), respectively. After adjustment for other risk factors, high adiponectin and low leptin were independent risk factors of cardiovascular mortality (HR: 2.54, 95%CI: 1.01-6.59, P = 0.045, and HR:2.82, 95%CI:1.12-7.14, P = 0.029, respectively). Adiponectin levels were negatively correlated with GNRI levels (r = 0.36, P < 0.0001), and leptin levels were positively correlated with GNRI (r = 0.49, P < 0.0001).

Conclusions: In contrast to general population, high adiponectin and low leptin were associated with an increased risk of cardiovascular mortality in patients on HD. These paradoxical associations were hypothesized to be affected from prevalent malnutrition status in HD patients.

TH-PO594

Phase Angle, a Bioimpedence Analysis (BIA)-Derived Parameter of Body Composition and Cellular Health, Predicts Long-Term Survival in Hemodialysis (HD) Patients (Pts) Neal Mittman, Brinda Desiraju, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, SUNY Downstate University Hospital at LICH, Brooklyn, NY.*

Background: BIA has been validated as a useful tool to measure body composition in HD pts. Phase angle (PA), a BIA-derived parameter, has been associated with cellular health in dialysis pts. The objective of this study was to explore the relationship between PA, clinical, and biochemical characteristics in HD pts.

Methods: Fifty-eight HD pts in our urban center were enrolled from 2000, clinical data was recorded, and pts were followed over time.

Results: Mean age was 61 yrs. Fifty-seven percent were women, 37% were diabetic, and 79% were of African descent (79%). Mean dialysis vintage was 81 mo. Mean PA was 5.17 degrees. PA decreases with age, and men had significantly higher PA (6.17 v. 4.42 degrees, p=0.001). Pts with PA≥6 degrees had better cumulative survival compared to those with lower PA (p<0.005). In Cox's multivariate analysis, after adjusting for age, gender, diabetic status and dialysis vintage, PA was an independent predictor of mortality (RR=0.617, p=0.012). Therefore, for each degree higher, the relative mortality decreases by 38%. The correlates of PA are shown below:

Correlations of PA with age and biochemical markers

Variables	Correlation Coefficient	p
Age (years)	-0.35	0.007
Albumin (g/dl)	0.36	0.019
Creatinine (mg/dl)	0.51	0.001
Hematocrit (%)	0.32	0.037
Months on dialysis	-0.24	0.078
Survival period (years)	0.39	0.004

Conclusions: As previously noted, PA is positively correlated with survival, and negatively correlated with age. Higher PA also correlated with arkers of somatic (creatinine) and visceral (albumin) protein stores, serum potassium and hematocrit. In summary, PA is an independent predictor of mortality risk, likely related to its reflection of cellular health and nutritional status in dialysis pts, and therefore may be a useful prognostic tool in this population. The use of serial BIA measurements to assess risk and impact survival, ideally in association with aggressive management of protein calorie malnutrition, should be evaluated in prospective trials.

Funding: Private Foundation Support

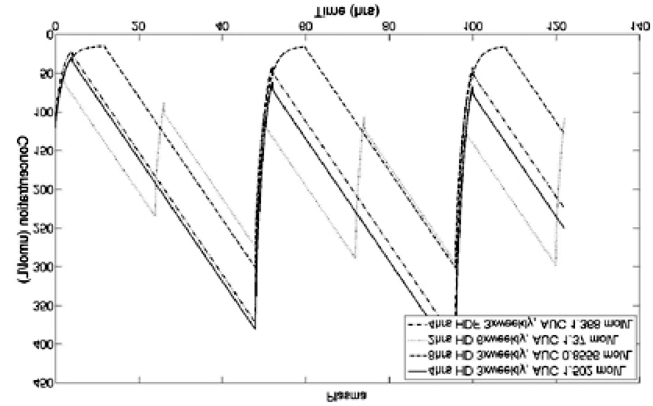
TH-PO595

Optimizing Oxalate Removal in Primary Hyperoxaluria by Hemodialysis Colin A. Hutchison,[1] Richard T. Keir,[2] Anne Bevins,[3] Neil D. Evans,[2] Paul Cockwell.[1] [1]*Renal Unit, University Hospital Birmigham, United Kingdom;* [2]*University of Warwick, United Kingdom;* [3]*The Binding Site Group Ltd, Birmingham, United Kingdom.*

Background: Primary hyperoxalemia is frequently associated with end stage renal disease which in turn is complicated by early cardiovascular disease secondary to oxalosis. It is therefore necessary to optimize the removal of oxalate by hemodialysis to minimize this complication. The purpose of this study was to evaluate the removal of oxalate by different dialysis schedules in a patient recently returned to dialysis following a failed kidney transplant.

Methods: During a three month period the clearance of oxalate was studied with the following dialysis modalities and schedules: high flux hemodialysis (HD), 2 hrs 6x week, and 4 hrs or 8 hrs 3x week; hemodiafiltration (HDF), 4 hrs 3x week; and high cut-off (HCO) hemodialysis, 4 hrs 3x week. A two compartment mathematical model was used to simulate oxalate clearance.

Results: The absolute clearance rate of oxalate with HDF was significantly higher compared with HD alone. However the simulations of the different modalities determined that the greatest reduction in area under the curve (oxalate exposure per week) was achieved with 8 hrs of HD three times per week.



This represented the importance of clearing the extra-vascular compartment by time alone. This was a 43% reduction from the exposure when HD was just 4 hrs three times per week. In comparison there was only a 10% reduction in the area under curve for HDF (4hrs, 3x week). HCO membranes provide clearance rates which were equivalent but not superior to standard HD.

Conclusions: To provide optimal removal of oxalate in a chronic dialysis patient the duration of dialysis was the most significant factor.

TH-PO596

Metabolic Profiling of Frailty in Hemodialysis Patients Quinlyn A. Soltow,[1] Rebecca H. Zhang,[2] Dean P. Jones,[1] Nancy G. Kutner.[2] [1]*Department of Medicine, Emory University;* [2]*Department of Rehabilitation Medicine, Emory University, Atlanta, GA.*

Background: The phenotype of frailty provides criteria that define individuals who lack functional reserve and are at risk for functional decline. An ongoing special USRDS study (ACTIVE/ADIPOSE) is focusing on identifying components and progression of frailty dimensions in prevalent hemodialysis (HD) patients. As an adjunct to this study, our group is using metabolomics to investigate specific metabolites and metabolic patterns unique to frailty.

Methods: A subset (n = 29) of prevalent patients undergoing HD in Atlanta GA dialysis clinics and participating in ACTIVE/ADIPOSE were age- and sex-matched with an equal number of healthy controls. All were assessed for frailty characteristics and supplied plasma samples, which were compared using a dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) method developed at Emory to generate global metabolic profiles.

Results: Mean (sd) age of HD patients = 54.8 (13.1); mean (sd) age of healthy controls = 53.7 (14.7); 62% of each cohort were male. Frailty status was defined by the Fried criteria of recent weight loss, exhaustion, low physical activity, slow walk time, and low grip strength. The DC-FTMS-based metabolomics method detected over 10,000 metabolic features in plasma samples from 58 subjects. Using the following data reduction techniques, Principal Component Analysis separated metabolic patterns unique to HD and frailty status, while False Discovery Rate identified 120 metabolic features that differed significantly by frailty status in HD patients. Among these significant features, many nutritionally relevant metabolites matched to metabolomics databases, including various amino acid dipeptides and tripeptides, lipids, and others.

Conclusions: Metabolic patterns detected by DC-FTMS are altered in HD patients and change according to frailty status, and metabolic features relating to nutritional status and protein metabolism can be identified that change with frailty progression. Metabolic profiling is a useful tool for detection of potential biomarkers or patterns of metabolites to predict frailty in hemodialysis patients and to evaluate interventions that delay functional decline.

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TH-PO597

Changes in Pre-Dialysis Weight Relate to Key Laboratory Parameters in Hemodialysis Patients Richard Amerling,[1] Penny Faith Palmiero,[2] James F. Winchester,[1] Len A. Usvyat,[2] Nathan W. Levin,[2] Peter Kotanko.[2] [1]*Division of Nephrology and Hypertension, Beth Israel Medical Center, New York, NY;* [2]*Renal Research Institute, New York, NY.*

Background: Low hemoglobin (Hb) and serum albumin (SA) levels are predictors of poor outcome in hemodialysis (HD) patients (pts). Fluid overload expands intravascular volume and lowers Hgb and SA. Given that these parameters are measured pre-HD, we hypothesized that fluid overload lowers SA and Hgb and confounds their use as prognostic indicators.

Methods: We performed a retrospective record review in 5510 in-center HD pts treated in RRI clinics in February and March 2011. For each pt we identified the first date in February and March 2011 when Hb, SA, and pre-HD weights were recorded. Simple linear regression analysis was used to assess the relationship of Δpre-weight (ΔPW) [March pre-HD weight – February pre-HD weight] versus ΔHb [March Hb – February Hb] and ΔSA [March SA – February SA]. Pts were divided into quintiles of ΔPW.

Results: We found a weak yet significant inverse correlation between ΔPW and ΔHgb (r =-0.15; P<0.001) and ΔPW and ΔSA (r = -0.12; P<0.001). A 1 kg decrease in pre-HD weight was associated with a Hb rise of 0.08 g/dL and an albumin rise of 0.012 g/dL. Dividing patients into quintiles of ΔPW suggests a dose response relationship between ΔPW and ΔSA and Hb (ANOVA with post-hoc Tukey test demonstrated significant differences between Δalbumin and ΔHgb between all quintiles of ΔPW).

Month to month weight change vs. ΔSA and ΔHb

Weight change quintile (range;kg)	Mean ΔSA (g/dL)	Mean ΔHb (g/dL)
1 (<-1.3)	0.043	0.35
2 (-1.3 to -0.4)	0.033	0.144
3 (-0.3 to 0.4)	0.01	-0.02
4 (0.4 to 1.3)	-0.02	-0.048
5 (>1.3)	-0.042	-0.262

ΔPW remained a significant predictor of ΔSA and ΔHgb using simple linear regression models adjusted for Feb pre-HD weight, gender, race, vintage, and age.

Conclusions: We demonstrate that short-term changes in pre-HD weight, which are likely to reflect differences in extracellular volume, are significantly and inversely correlated with changes in Hb and SA. Volume status should be considered when interpreting Hb and SA. Volume overload may partially explain association of low Hb and SA with poor outcomes in HD patients.

Funding: Private Foundation Support

TH-PO598

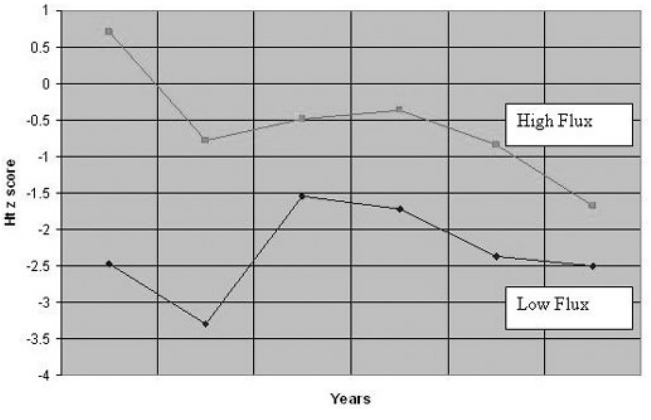
Impact of Membrane Flux on Growth and Mineral Metabolism in Children with End Stage Renal Disease on Hemodialysis Nataliya Chorny,[1] Gail Prado,[1] Sreevidya Kusuma,[1] Amrit Bhangoo,[2] Anil K. Mongia.[1] *[1]Pediatric Nephrology, SUNY Downstate, Brooklyn, NY; [2]Pediatric Endoctrinology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Growth retardation in children with ESRD is attributed to various factors including efficacy of hemodialysis, proinflammatory state and bone disease. High flux dialyzers are efficient in removal of both small non-protein bound and middle molecules like β2-microglobulin and may reduce inflammation.

Methods: We hypothesized that children on high flux membranes will show better growth and improved mineral metabolism.

Retrospective review of 21 children on long term hemodialysis from 2006-2011. Patients divided into two group based on high flux (Optiflux and Polyflux) or low flux dialyzers (DZ). One patient in high flux group received rGH, but was discontinued. We measured height, weight, height and weight Z scores, change in height and weight Z scores and laboratory variables including serum albumin, CO2, Hemoglobin, serum ferritin, calcium, phosphorus, PTH, urea reduction rate (URR), kt/V. Statistical analysis was done using t-test

Results: Ages at onset ranged from 2 years to 21 years, 10 were males. 14 were AA, 4 Hispanic. Height and weight Z scores though not statistically significant were better in patient on High flux dialysis compared to patient on Low flux dialyzer (Fig 1)



Mean Predialysis Values

Parametes	Low Flux DZ	High Flux DZ	P-value
Hemoglobin	11±1.34	11 ±5.76	0.875
URR	75±38.8	75±38.2	0.244
Kt/V	1.94 ±1.075	1.72±0.8	0.405
Albumin	4.±0.26	4±1.13	0.453
Ferritin	382.±240	65.2±43.5	0.055
CO2	20 ±7.7	22±7.3	0.07
Phosphorus	4.9±0.69	4.6±1.6	0.576
Calcium	8.9 ±0.55	8.9 ±4.45	0.912
PTH	295±187.5	449±346.	0.204

Conclusions: In children on chronic dialysis, growth may be improved by using High Flux dialysis. This may be related to better uremic toxin removal, acidosis improvement and reduced dialysis induced inflammation.

TH-PO599

The Role of Nutritional Scores for Serial Monitoring of Nutritional Status in Hemodialysis Patients Ilia Beberashvili,[1] Ada Azar,[2] Inna Sinuani,[1] Hadas Kadoshi,[2] Gregory Shapiro,[1] Leonid Feldman,[1] Zhan Averbukh,[1] Joshua Weissgarten.[1] *[1]Nephrology, Assaf Harofeh Medical Center, Zerifin, Israel; [2]Nutrition, Assaf Harofeh Medical Center, Zerifin, Israel.*

Background: Simple prediction scores could help identify hemodialysis patients at high risk for protein-energy wasting (PEW). We compared the longitudinal performance of the malnutrition-inflammation score (MIS) and geriatric nutritional risk index (GNRI) - the two nutritional scores for patients on maintenance hemodialysis.

Methods: Nutritional scores, dietary energy and protein intake, nutritional biomarkers, anthropometry and bioimpedance analysis were performed at baseline and at 6, 12 and 18 months following enrollment in 75 prevalent hemodialysis patients (43% women, mean age 64.8±11.9 years). Additionally the patients underwent simultaneous assessment of MIS and GNRI at baseline, calculated by two independent examiners. The study period encompassed 46.8 ±16.4 months.

Results: GNRI had higher inter-observer agreement (weighted k-score 0.96) than MIS (weighted k-score 0.62). Longitudinally, one-unit increase in MIS over time, controlling for demographic and clinical parameters, was associated with a 0.4089 kcal/kg/day reduction in daily energy intake (P<0.001) and with a 0.0135 g/kg/day reduction in nPNA (P=0.015). GNRI did not correlate with the change over time of dietary intake. Longitudinal changes of both scores were associated with appropriate changes over time in levels of biomarkers of nutrition, inflammation (IL-6) and body composition parameters. Both scores expressed significant associations with prospective hospitalization, whereas only MIS was associated with mortality in our cohort: the Cox proportional hazard-calculated relative risk for death for each one-unit increase in the MIS was 1.15 (95% CI, 1.03 to 1.3; P = 0.015).

Conclusions: Both, MIS and GNRI are valid tools for the longitudinal assessment of nutritional status of hemodialysis patients, whereas the MIS has lower inter-observer reproducibility, but is more comprehensive than GNRI.

TH-PO600

Serum Creatinine to Body Weight Ratio – A Simple Measure of Body Composition? Rebecca Filipowicz,[1] Talat Alp Ikizler,[3] Glen Morrell,[1] Guo Wei,[1] Tom H. Greene,[1,2] Srinivasan Beddhu.[1,2] *[1]VA; [2]Univ Utah; [3]Vanderbilt.*

Background: Body composition is not routinely measured in clinical practice in HD pts. Therefore, we examined whether SCr (a marker of muscle mass in HD pts) to weight (Wt) ratio is associated with measures of body composition, inflammation and functional ability.

Methods: This study is a secondary analysis of an ongoing longitudinal study of nutritional status in HD pts. 116 pts who underwent at least one DEXA scan for measurement of body composition were included. Ht, wt and 6-min walk distance were measured on a non-HD day. Intra-abdominal fat at the L4-L5 level and muscle area at mid thigh level were meausred with MRI. hsCRP was measured using Roche MODULAR P analyzer. hsCRP > 10 mg/L was defined as ↑ CRP. Generalized estimating equations (GEE) were used to fit a pooled cross-sectional regression model relating outcomes to concurrently measured SCr/Wt across 4 study visits.

Results: Baseline characteristics by SCr/wt tertiles

	SCr/wt)0.14	SCr/wt 0.10-0.14	SCr./wt ⟨0.10
SCr, mg/dL	12.0±3.0	9.6 ±1.7	6.9 ±1.9
Wt, kg	65±16	83±15	89 ±18
Age,yr	43± 16	51±14	58±15
Men, %*	58	55	59
DM, %	11	55	62
CHF,%	11	15	33
CVD,%	11	23	26
BMI, kg/m²	23.2± 4.5	29.0±4.7	30.8±5.7
Intra-abdominal fat, cm²	79±43	140±68	167±65
Muscle area, cm²*	109±29	108±28	104±26
DEXA fat mass/wt ,%	25±9	34±9	39±18
6-min walk distance,m	372±109	313±110	244±78

*p=ns As shown in the table, ↓ SCr/wt ratio was associated with ↑ visceral fat and ↑ DEXA fat mass to body wt ratio. In a multivariable GEE regression, adjusted for age, gender, race and DM, compared to the highest tertile of Scr/Wt, the lowest tertile was associated with ↓6 min walk distance (β -61, 95% CI -94 to -29 m). Similarly, compared to the highest tertile, the lowest tertile was associated with ↑odds of CRP (OR 2.91, 95% CI 1.15 to 7.41). Results were similar when these models were further adjusted for wt.

Conclusions: SCr/Wt is associated with direct measures of body composition and correlates with inflammation and physical function. The specific functional form of SCr/Wt which scales optimally with body composition needs to be determined.

Funding: NIDDK Support, Other NIH Support - National Center for Research Resources

TH-PO601

Severity of Glucose Intolerance Is a Morbidity and Long-Term Mortality Risk Factor in Non-Diabetic (NDM) Hemodialysis (HD) Patients (pts) Neal Mittman, Brinda Desiraju, Thomas S. Guagliardo, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, S.U.N.Y. Downstate University Hospital at L.I.C.H., Brooklyn, NY.*

Background: Patients with chronic kidney disease have impaired glucose tolerance, a known cardiovascular risk factor, irrespective of etiology. Serum fructosamines (SF), including glycated albumin, have been evaluated as superior markers of glycemic control to HbA1c (A1c) in this population. We have reported that SF, but not A1c, is associated with infection and hospitalization in DM HD pts (KI, 2010). We are unaware of any published work associating degree of glucose intolerance in NDM HD pts with outcomes.

Methods: We enrolled sixty NDM (A1c≤5.6%, no history DM), measured SF (corrected for albumin, AlbSF), and followed them for up to 6 years.

Results: Mean age was 54 yr, and they were evenly divided by gender. The majority (78%) were of African descent. Mean A1c was 5.1% (range, 4.4-5.6). 75% had SF above normal range (normal ≤265µmol/L; mean, 285; range 187-378). There was no association of A1c with morbidity or mortality during 2 and 5 years of observation. AlbSF, on the other hand, was correlated with frequency ($p<0.001$) and duration of hospitalization ($p<.03$) over both periods of observation. In addition, univariate Cox regression analysis revealed that AlbSF was associated with increased mortality risk. In Cox's multivariate analysis, after adjusting for age, gender, and dialysis vintage, AlbSF remained a significant independent mortality predictor (See Table).

Variables	Two years observation	Five years observation
	Relative Risk (p)	Relative Risk (p)
Age (years)	1.025 (p=0.36)	0.99 (p=0.98)
Gender (female vs. male)	0.39 (p=0.23)	0.47 (p=0.17)
Months on dialysis	0.99 (p=0.84)	1.004 (p=0.18)
AlbSF (µmol/g)	1.01 (p=0.003)	1.007 (p=0.004)

Therefore for each µmol/g increase in AlbSF, there was a 1% and 0.7% greater relative mortality risk over 2 and 5 years, respectively.

Conclusions: In conclusion, severity of glucose intolerance in NDM HD pts, as measured by SF, is highly associated with interim and long-term outcomes. These results need to be confirmed in large prospective trials, including therapeutic interventions aiming to normalize or reduce SF levels.

Funding: Private Foundation Support

TH-PO602

Early Time-Dependent Changes in Serum Albumin Predict 2-Year Survival in Maintenance Hemodialysis Jorge P. Strogoff-de-Matos,[1] Giselly R. Pereira,[1] Jorge Reis-Almeida,[1] Adrian Marcos Guinsburg,[2] Cristina Marelli,[2] Ana Beatriz Lesqueves,[3] Marcos Sandro Fernandes de Vasconcelos,[3] Eufronio D'almeida,[3] Frederico Ruzany,[3] Jocemir R. Lugon.[1] *[1]Division of Nephrology, Universidade Federal Fluminense, Niteroi, Brazil; [2]Fresenius Medical Care, Buenos Aires, Argentina; [3]Fresenius Medical Care, Rio de Janeiro, Brazil.*

Background: Our aim was to assess the predictive value of early time-dependent changes in serum albumin (sAlb) on late outcome in hemodialysis (HD) patients.

Methods: In this observational study, 1,775 incident patients on HD from 25 dialysis facilities had the sAlb measured at admission and 3 months later. The time-dependent change in sAlb was calculated as the ratio of 3-month sAlb: initial sAlb, and expressed as percentage. Patients were split into two groups according to initial sAlb (below or above the median) and followed-up for 2 years. The risk of death associated with the early change in sAlb was calculated by a Cox regression model with adjustment by age, gender and diabetes.

Results: Patients were 59% males, 20.7% diabetics, 50±16 years old, and have a median sAlb of 3.8 g/dL. The 2-year survival was significantly higher in the group with initial sAlb above the median (94.3% vs. 85.5%, P< 0.0001). The adjusted hazard ratio (HR) of death was 0.88 (95% confidence interval [CI] 0.78 to 0.99; P=0.04) for each 10% incremental in sAlb among patients in the group with initial sAlb below the median. Conversely, the adjusted HR of death was 1.35 (95% CI 1.21 to 1.50; P=0.0001) for each 10% reduction in the group with initial sAlb above the median.

Conclusions: Early time-dependent sAlb changes have a significant impact on 2-year survival rate. Patients with initial low sAlb can have good outcomes if an early elevation in sAlb is seen, whereas those with initial satisfactory levels can switch to a bad prognosis in case of early reduction in sAlb.

TH-PO603

Risk Factors for Depressive Symptoms in a Large Population on Chronic Hemodialysis Sonia Maria Holanda Almeida Araujo,[1] Nicole Araujo,[2] Andre Pantaroto,[2] Constance Almeida de Alencar Araújo,[1] Gilson Almeida,[3] Pedro Bruin,[1] Veralice Meireles Sales Bruin,[1] Elizabeth De Francesco Daher.[1] *[1]Medicina, Universidade Federal do Ceara, Fortaleza, Ceara, Brazil; [2]Medicina, Faculdade de Medicina de Jundiai, Jundiai, Sao Paulo, Brazil; [3]Medicina, Faculdade de Medicina Christus, Fortaleza, Ceara, Brazil.*

Background: Despite significant effect in quality of life, depressive symptoms have not been sufficiently evaluated as an important parameter in hemodialysis patients. We aimed to identify depressive symptoms, and study risk factors in a large group of individuals with end-stage renal disease (ESRD) on chronic hemodialysis.

Methods: Cases were analyzed according to the presence/absence of depressive symptoms. All individuals were investigated by interview and all variables were measured concurrently. Depressive symptoms were evaluated by the Beck Depression Inventory (BDI-II≥16) and poor sleep quality by the Pittsburgh Sleep Quality Index (PSQI>5).

Results: In 400 patients (59% male), depressive symptoms were present in 77 (19.3%). Depressive symptoms were more common in women and were independently associated with poor quality sleep (P=<0.005), unemployment (P=0.005), diabetes (P=0.005), hypoalbuminemia (P=0.01), heart failure (P=0.01) and pruritus (P=0.03).

Conclusions: Women with ESRD on chronic hemodailysis are at increased risk of depression. Furthermore, unemployment and the presence of diabetes, hypoalbuminemia, heart failure and pruritus should raise suspicion for this diagnosis. Depressive symptoms are also independently associated with poor quality sleep and studies about the effects of sleep hygiene therapy on depressive symptoms are warranted.

Funding: Government Support - Non-U.S.

TH-PO604

Correlation of Nutritional Status with Depression and Cognitive Function in Hemodialysis Patients Young Rim Song, Jwa-Kyung Kim, Jung-Woo Noh, Ja-Ryong Koo. *Division of Nephrology and Kidney Research Institute, Hallym University Sacred Heart Hospital, Anyang, Kyunggi-do, Korea.*

Background: Malnutrition, depression and cognitive impairment are in themselves challenging problems in hemodialysis patients, but diagnosis and treatment are often suboptimal. Nutritional status and its association with cognitive function and depression remains uncertain

Methods: Seventy seven patients with maintenance hemodialysis were enrolled as part of this cross sectional study. Nutritional assessment was undertaken with a bio-impediance spectroscopy device, the Body Composition Monitoring (BCM), handgrip strength (HGS), a seven-point subjective global assessment (SGA) and serum biomarkers (albumin, prealbumin, cholesterol, CRP). HGS was measured before and after HD sessions on the non-fistula side with dynamometer (Jamar) and the highest value was used for analysis. Muscle function (MF) loss was defined as less than the 10th percentile of population-based reference in Korea. Neuropsychological test was performed by Mini-Mental State Examination in the Korean version of CERAD assessment packer (MMSE-KC) and Beck depression Inventory (BDI) during the 1st hour of dialysis.

Results: The mean age was 59.2 years and the median dialysis duration was 39.5 months. 56.6 % were men, 58 % had diabetes. The Mean MMSE and BDI score were 27.1 ± 3.1 and 20.5 ± 10.8, respectively. 68.9 % had MF loss and 51.3 % had moderate to severe depression. In the multivariate analysis, BDI score correlated with handgrip strength (r=-0.342, p=0.003), SGA score (r=-0.226, p=0.050) and Lean tissue index (r=-0.247,0.035). MMSE score correlated with age (r=-0.337, p=0.003), handgrip strength (r=0.259, p=0.026), lean tissue index (r=0.273, p=0..019), fat tissue index (r=,-0.345, p=0.003), albumin (r=0.283, p=0.013). After adjustment for other clinical variables, Patients with MF loss were more likely to be depressed (adjusted OR=3.78,95% CI 1.2-12.7).

Conclusions: Handgrip strength was associated with depression, independently of age, sex, albumin, proalbumin and CRP levels. MMSE score was not associated with nutritional status.

TH-PO605

Effects of Antioxidants on Postprandial Metabolic Response to a Fat- and Carbohydrate-Rich Meal in Patients with Chronic Kidney Disease Tetsu Miyamoto, Abdul Rashid Tony Qureshi, Björn Anderstam, Tae Yamamoto, Peter Stenvinkel, Bengt Lindholm, Jonas Axelsson. *Renal Medicine and Baxter Novum, Karolinska Institute, Stockholm, Sweden.*

Background: Metabolic and inflammatory pathways are closely interrelated with an impaired sequestration of nutrients. No study has investigated impact of anti-oxidants on the postprandial metabolic and inflammatory response in end-stage renal disease patients (pts).

Methods: A randomized, double-blind study comparing fasting and postprandial circulating biomarkers of glucose and lipid homeostasis and inflammation was conducted in 5 non-diabetic hemodialysis (HD) pts and 9 matched controls assessed at 30, 60, 120, 180 and 240 min after a standardized meal consisting of 75 g of milk fat, 80 g of carbohydrates and 6 g of proteins/m^2. Subjectsts were tested 4 times: at baseline, and after 7 days treatments of anthocyanins (MP865, 240 mg/day), N-acetylcysteine (NAC, 600 mg/day) and placebo (lactose, 200 mg/day) respectively.

Results: NAC resulted in increased plasma NAC levels (17.2±4.1 mmol/L) and decreased circulating homocysteine levels (14.1±3.0 mmol/L after placebo vs 10.3±3.1mmol/L after NAC, p=0.03). Following the meal, glucose increased in a similar manner in the two groups, while insulin and C-peptide increased more in HD-pts. After NAC treatment, a smaller area under the curve of change during the 4 hour postprandial period (ΔAUC_{4hr}) of insulin was observed compared to baseline (p=0.03) and placebo treatment (p=0.08) in controls. However, these effects of NAC on insulin were not observed in HD-pts. HDL- and LDL- cholesterol marginally decreased in both groups with no effects of the treatments. Unexpectedly, IL6 slightly decreased over time in pts (ΔAUC_{4hr} = -3.3±1.2 hr pg/ml) in contrast to controls (ΔAUC_{4hr} = 0.8±1.2 hr pg/ml, $p<0.001$). No effects of the treatments on ΔAUC_{4hr} of IL-6 or TNF-a were detected in pts and control subjects.

Conclusions: The postprandial state in non-diabetic HD-pts is characterized by impaired insulin sensitivity and slight decrease in circulating proinflammatory cytokines. Treatment with anti-oxidants had no impact on selected postprandial metabolic and inflammatory markers in HD-pts.

Funding: Private Foundation Support

TH-PO606

Which Frequency for Cobalamin Supplementation in Pre-Dilutional Hemodiafiltration? Carlos Frangié, Thierry Baranger, Frank Bergé, Valerie Drouillat, Emmanuelle Rosier, Piotr Seniuta. *Néphrologie et Dialyse, Polyclinique Bordeaux Nord, Bordeaux, France.*

Background: The technical data of high permeability dialyzers in pre-dilutional hemodiafiltration (HDF)show a high clearance of the cobalamin. The cobalamin is a vitamin not produced by the human body and its serum rate depends on dietary intake. Its was reported that an overdose of cobalamin may increase risk of neoplasia. In chronic HDF, intravenously or orally cobalamin supplementation is common.

Methods: A retrospective study followed by a prospective study, were performed on patients of our HDF centers 1 (C1) and 2 (C2). Retrospective study over a period of one year (June 2009-June 2010), analyzed cobalamin serum level among 50 patients in C1 (28 women, 22 men) and 36 patients in C2 (15 women, 21 men). Patients of C1 had received a weekly injection of cobalamin intravenously; the patients of C2 did not receive supplementation since June 2009. The prospective analysis was conducted over a period of 6 months, using monthly intravenous cobalamin injection for patients of C1, C2 patients constituting the control group without supplementation.

Results: Retrospective analysis shows that C1 patients, all had an overdose of cobalamin. Patients in the C2, initially overdosed in cobalamin, had presented a significant decrease in serum after 6 months ($p = 0{,}0002$) while remaining in the normal range, and this rates remained stable after one year ($p = 0.3$). The prospective analysis showed decreased but remaining high normal serum cobalamin in patients of C1, and stability in normal in patients of C2.

Conclusions: In vitro studies of high permeability dialyzers with pores up to 30 kDa, showed a very high clearance of cobalamin (1,335 KDa). Mean daily intakes of cobalamin (in order of 4 mg) cannot compensate per-dialytic clearance. In this study, serum level of cobalamin may remains normal without supplementation, and cobalamin binding to high molecular weight protein could be an explanation.

In pre-dilutional HDF, quarterly or biannual cobalamin supplementation seems to be sufficient. A prospective study is underway in our centers, using quarterly cobalamin , intravenous (C1, n = 130) or orally (C2, n = 72) supplementation.

TH-PO607

A Pilot Study of Nuclear Magnetic Resonance (NMR) Lipid Profiling in End Stage Renal Disease Hania Kassem, Manish P. Ponda. *Nephrology, New York University School of Medicine, New York, NY.*

Background: Lipoproteins can predict coronary heart disease risk. Hemodialysis (HD) patients suffer from accelerated cardiovascular disease. They also tend to have atherogenic changes in the lipid profile, including hypertriglyceridemia and low HDL. However, conventional therapy has been disappointingly ineffective in lowering cardiovascular risk for this population. Nuclear Magnetic Resonance (NMR) lipid analysis can determine lipoprotein particle size and number, and can complement the standard lipid profile. NMR lipid analysis has been previously studied in the dialysis population, but not in comparison to matched controls.

Methods: We sought to assess, using NMR analysis, lipoprotein particle properties in patients on hemodialysis as compared to matched controls without kidney disease.

After obtaining informed consent, fasting plasma samples were collected from 21 patients undergoing in-center intermittent hemodialysis and from 13 controls (without kidney disease) matched for gender, age and presence of diabetes. Both routine lipid analysis and NMR spectroscopy were performed by Liposcience (Raleigh, NC). Means of different lipoprotein particle numbers and size were compared between both groups.

Results: Both groups were well matched for age, gender and presence of diabetes. There was a statistically non-significant higher frequency in statin use in HD subjects. Subjects on HD had significantly lower total cholesterol, HDL and LDL cholesterol levels. Using NMR analysis, HD subjects had lower numbers of LDL and HDL particles. The percentage of small LDL particles in relation to total LDL particles was not different between groups The mean size of VLDL, LDL and HDL particles was not different between groups.

Conclusions: The dyslipidemia of hemodialysis patients is largely captured by the standard lipid profile. These patients have similar particle profiles as compared to matched controls without kidney disease. Larger studies are required to detect more subtle changes that may impact cardiovascular risk.

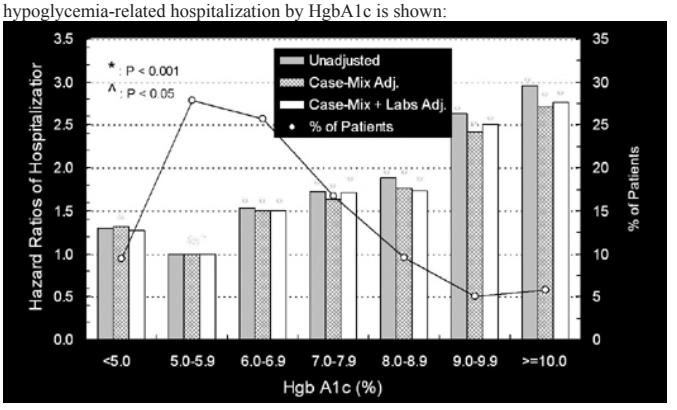
TH-PO608

Hypoglycemia-Related Hospitalization in Diabetic Hemodialysis Patients Eduardo K. Lacson,[1] Weiling Wang,[1] J. Michael Lazarus,[1] Mark E. Williams.[2] *[1]Fresenius Medical Care, North America, Waltham, MA; [2]Joslin Diabetes Center, Boston, MA.*

Background: We evaluated patient characteristics and factors that may associate with hospitalization for hypoglycemia in diabetic maintenance hemodialysis (HD) patients.

Methods: All 24,751 diabetic chronic HD patients in Fresenius Medical Care North America facilities as of 01/01/03, with a hemoglobin-A1c (HgbA1c) in Q4 '02 were included. Baseline characteristics (age, gender, race, BSA, DM Type, vintage, vascular access), insulin use, and labs (eKt/V, albumin, hemoglobin, calcium, phosphorus, creatinine and white cell count) were recorded. Over the next three years, hospital ICD-9 diagnoses for hypoglycemia were found in 4.1% of patients (N=1,017; i.e. "cases"), while the rest (N=23,734) became controls. Stepwise logistic regression (entry: univariate $p \leq 0.1$) and Cox models were used to relate baseline HgbA1c and other variables to hypoglycemia-related hospitalization.

Results: Cases tended to be younger (61.6 vs.63.8 years), but with slightly less BSA (1.81 vs. 1.86 m^2), over-representation of Type 1 DM (10.8% vs. 5.3%) and insulin use (54.3% vs. 46.7%), and higher mean HgbA1c (7.35% vs. 6.75%), all $p<0.0001$. All other case-mix factors and labs were similar, except for calcium (9.21 vs. 9.29, $p<0.001$). The stepwise model indicated that HgbA1c, BSA, DM Type-1, insulin use, male gender and calcium were associated with hypoglycemia-related hospitalization. The hazard ratios for hypoglycemia-related hospitalization by HgbA1c is shown:



Conclusions: Insulin use as well as higher HgbA1c associated with greater risk for hypoglycemia-related hospitalization. The latter finding requires further investigation. We speculate that attempts at glycemic control in high HgbA1c patients may create situations where changes in medications, diet, and clinical conditions lead to hypoglycemia – at times requiring hospitalization.

TH-PO609

New Method To Predict Lean Body Mass Using Estimated Creatinine Excretion in Dialysate and Equilibrated Urea Kt/V in Japanese Maintenance Hemodialysis Patients Tomohito Matsunaga. *Kidney Center, Eijinkai Hospital, Osaki, Miyagi, Japan.*

Background: The assessment of Lean Body Mass (LBM) is quite important to monitor the nutrition status of maintenance hemodialysis patients. Some equations were presented previously. The purpose of this study is to predict LBM using estimated creatinine excretion (CrE) and equilibrated urea Kt/V (eKt/V) in Japanese maintenance hemodialysis patients.

Methods: The 208 patients who have received MHD were analyzed (107 males, 97 females; mean age 61.4±10.5 years; duration of HD treatment 13.7±6.8 years). All patients received four-hour HD session three times per week with ultra-pure dialysate.

The first pre-, post-dialysis levels of BUN and Creatinine (Cr) were measured in the week. LBM and Fat mass were measured using BIA (Inbody 3.0™) after the first post-dialysis. Estimated CrE was calculated the K/DOQI guidelines formula. To avoid the post-dialysis rebound, post-dialysis level of Cr was corrected using single pool Kt/V (spKt/V) and eKt/V. Actual CrE in dialysate were measured by collecting total amount of dialysate during one dialysis session.

Results: The mean levels of LBM, Fat mass, body mass index (BMI) and post-BW were 42.36±7.99 Kg, 13.87±5.36 kg, 22.6±3.13 kg/mand 56.2±9.4 kg and spKt/V, eKt/V, estimate CrE and actual CrE were 1.43±0.25, 1.25±0.21, 1755.4±416.4 mg/session and 2089.7±510.3 mg/session respectively. Estimated CrE was positively correlated with actual CrE ($r=0.8955$ $p<0.001$). Multiple linear regression analysis showed that LBM was significantly correlated with estimated CrE, Body Height (BH), eKt/V and sex ($r=0.9656$, $p<0.001$). The obtained equation of regression was as follows: estimated LBM = 0.005 x estimated CrE + 0.333 x BH (cm) – 11.63 x eKt/V – 1.741 x Sex (male=0, female=1) – 4.486 and estimated Fat mass = post- BW – estimated LBM. Fat mass was correlated with estimated Fat mass ($r=0.9438$, $p<0.001$).

Conclusions: In conclusion, LBM can be predicted using estimated CrE obtained from the first pre-, post-, dialysis levels of Cr and eKt/V in Japanese MHD patients.

TH-PO610

Clinical Correlation of Subclinical Fluid Overload in Peritoneal Dialysis Patients Bonnie Kwan, Cheuk-Chun Szeto, Chi-Bon Leung, Philip K.T. Li. *Dept of Medicine & Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong.*

Background: Chronic intravascular hypervolaemia may be an important contributing factor of cardiovascular disease in peritoneal dialysis (PD) patients. We explored the relation between body composition parameters, dialysis adequacy, nutritional status, and degree of arterial stiffness in stable chronic PD patients.

Methods: Prevalent chronic PD patients in our dialysis unit underwent clinical assessment. Those with overt fluid overload were excluded. Past medical history of cardiovascular events was noted. Body composition and arterial pulse wave velocity was measured, dialysis adequacy and nutritional status was assessed.

Results: Study included 122 PD patients, 55.7% male, 51.6% diabetic. Within the parameters of body composition, over-hydration (OH) was found to have a significant correlation with total body water ($r = 0.474$, $p < 0.001$) and extracellular water ($r = 0.755$, $p < 0.01$). There was no correlation with intracellular water, lean tissue mass (LTM), fat mass or percentage of fat mass though there was a significantly negative correction with

percentage of LTM (r = -0.241, p = 0.07). OH is common, with 72.1% having OH of >1 litre, while 20.5% had OH >5 litres. OH is more common in male patients, and in diabetic patients.

OH has a significant positive relationship with blood pressure, body mass index, body weight and pulse wave velocity of the carotid-femoral territory. Degree of comorbidity assessed using Charlson's score, and serum albumin, has a significant positive correlation with parameters of OH and a significant inverse correlation with LTM. OH has a modest positive correlation with peritoneal transport characteristics and dialysis adequacy, though not with residual renal function.

After a mean follow-up of 12 months, 7 patients died, 2 were converted to hemodialysis, and 2 had transplants.

Conclusions: Fluid overload is common in PD patients. Markers of fluid overload had good correlation with the degree of comorbidity, systolic BP, serum albumin and pulse wave velocity. Longer follow-up would be useful in assessing the relationship of OH with clinical outcomes.

TH-PO611

Serum Nutritional Markers, Body Composition and Mortality in Peritoneal Dialysis (PD) Patients (pts) Morrell M. Avram, Zoe Hartman, Jyotiprakas Chattopadhyay. *Avram Division of Nephrology, SUNY Downstate University Hospital at LICH, Brooklyn, NY.*

Background: Malnutrition and abnormal levels of some body composition parameters are important predictors of mortality. In this study we have investigated the relationships between visceral and somatic protein stores such as albumin, creatinine and body composition and outcomes in PD pts.

Methods: We enrolled 62 PD pts in this study between November 2000 to May 2008. On enrollment, demographic, clinical and biochemical data were recorded. Pts were followed to May 2011. Body composition was determined by bioelectrical impedance analysis (BIA).

Results: Mean age was 54 years. At enrollment, the mean (±SD) serum albumin and creatinine were 3.71±0.59 g/dL and 11.38±4.2 mg/dL respectively. Mean phase angle (PA) and extracellular mass/body cell mass ratio (ECM/BCM) were 6.06±1.6 degrees and 1.21±0.2 respectively. Correlations of albumin and creatinine with body composition parameters are shown in the Table 1.

Table 1. Correlations of albumin and creatinine with BIA parameters

Variables		Albumin (g/dL)	Creatinine (mg/dL)
BMI(Lbs/in^2)	r	0.28	0.48
	p	0.027	<0.0001
BCM (Lbs)	r	0.31	0.51
	p	0.016	<0.0001
ECM/BCM	r	-0.42	-0.24
	p	0.001	0.07
ECW/ICW	r	-0.44	-0.26
	p	<0.0001	0.049
Reactance (Ohms)	r	0.42	-0.03
	p	0.001	0.82
Phase angle (degrees)	r	0.49	0.28
	p	<0.0001	0.031

BMI: Body mass index; BCM: Body cell mass; ECM/BCM: Extracellular mass/body cell mass ratio; ECW/ICW: Extracellular water/intracellular water ratio;

Albumin and creatinine directly correlated with body mass index (BMI), body cell mass (BCM) and PA. Albumin and creatinine inversely correlated with ECM/BCM ratio and Extracellular water/intracellular water ratio (ECW/ICW). During the study period 26 pts expired. Pts who survived had significantly higher albumin (p=0.005), creatinine (p=0.018), PA (p=0.017) and lower ECM/BCM ratio (p=0.04) compared to those who expired. By univariate Cox's regression analysis, albumin (Relative risk=0.17, p<0.0001), PA (Relative risk=0.58, p<0.0001) and ECM/BCM ratio (Relative risk=1.033, p=0.001) were strongly associated with mortality.

Conclusions: In PD pts there is a strong correlation between serum nutritional markers and BIA derived body composition parameters and their ability to predict survival. It may be useful to incorporate BIA parameters in the management of malnutrition and overall health in these pts.

Funding: Private Foundation Support

TH-PO612

The Effects of Different Hemodialysis Modalities on Protein Nutrition: Low-Flux HD, High-Flux HD and Online HDF Tai Yeon Koo, Joon-Sung Park, Chang Hwa Lee, Chong Myung Kang, Gheun-Ho Kim. *Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Republic of Korea.*

Background: Malnutrition is associated with mortality in hemodialysis(HD) patients, and dialysis flux may affect nutrition. Previous studies have reported conflicting results regarding impact of flux intervention on nutrition.This study was performed to compare protein nutrition between patients undergoing three different HD modalities(low-flux HD(LFHD),high-flux HD(HFHD) and online hemodiafiltration(OL-HDF)).

Methods: 13 patients who had maintained LFHD for more than 2 years, were switched to HFHD, and then switched to OL-HDF 2 years later.We longitudinally followed nutritional parameters(dry weight,body mass index(BMI),normalized protein catabolic rate(nPCR),serum albumin,total protein and cholesterol),HD adequacy(Kt/V_{urea},urea reduction ratio(URR) and serum $ß_2$-microglobulin) and inflammatory parameter(hs-CRP).

Results: Dry weight, BMI and serum cholesterol were not changed over three different modalities.nPCR was increased when LFHD was switched into HFHD, and maintained by OL-HDF.When LFHD was switched into HFHD, serum total protein and albumin were initially decreased, but recovered later.With initiation of OL-HDF, serum total protein and albumin were decreased.Compared with LFHD, OL-HDF increased Kt/V_{urea} ,URR and middle molecular clearance.

Comparison of nutritional parameters between LFHD,HFHD and OL-HDF

	Total protein(g/dl)	Albumin(g/dl)	Normalized protein catabolic rate(g/kg/d)	Kt/Vurea	Urea reduction ratio(%)
LFHD baseline	7.1±0.3	4.8±0.3	1.1±0.2	1.6±0.2	70±4.8
HFHD12 mo	7.1±0.4	4.6±0.4	1.3±0.3*	1.6±0.3	72±6.2
HFHD24 mo	7.4±0.3*	4.8±0.3	1.3±0.3*	1.6±0.3	73±6.0
OL-HDF12 mo	7.1±0.3#	4.3±0.3*#	1.3±0.3*	1.8±0.3*	75±4.5*
OL-HDF24 mo	7.1±0.6#	4.2±0.5*#	1.3±0.3*	1.7±0.4*	74±5.5*

Values are described as mean±SD.* p<0.05 vs.LFHD.#p<0.05 vs.HFHD(24 mo).LFHD,low-flux HD;HFHD,high-flux HD;OL-HDF,online HDF

hs-CRP was not affected by three different modalities.

Conclusions: Compared with LFHD, HFHD and OL-HDF may be more efficient in removal of uremic toxins, resulting in improvement of appetite and protein intake. However HFHD and OL-HDF may have negative protein balance probably because of more protein loss during dialysis.

TH-PO613

Association between Metabolic Alkalosis and Malnutrition-Inflammation Complex Syndrome in Maintenance Hemodialysis Patients Kawin Tangdhanakanond, Daranee Chewaproug, Eric J. Bloom, Rasib Raja. *Department of Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

Background: Metabolic alkalosis is a common finding in maintenance hemodialysis patients partly due to the use of high-bicarbonate dialysate bath and/or malnutrition. Malnutrition-Inflammation Complex Syndrome (MICS) is generally associated with poor dialysis outcomes including increased mortality. However, there is limited and conflicting evidence regarding the association between metabolic alkalosis and MICS in hemodialysis patients.

Methods: We conducted a retrospective analysis on 94 hemodialysis patients at an outpatient hemodialysis unit. All patients received maintenance hemodialysis 3 times per week with 35 mmol/L bicarbonate bath. Demographic and laboratory data including various markers of inflammation and nutritional status were analyzed and compared between 2 stratified groups based on predialysis serum bicarbonate level: the higher bicarbonate group (bicarbonate ≥26 mmol/L, n=51) and the lower bicarbonate group (bicarbonate <26 mmol/L, n=43)

Results: Lower mean predialysis BUN (mg/dL) (53.16 ± 13.38, 60.84 ± 16.20, p=.014), and lower normalized protein nitrogen appearance or nPNA (0.89 ± 0.22, 1.00 ± 0.27, p=.031) were shown in the higher bicarbonate group. However, other markers of nutritional status and inflammation including serum albumin, prealbumin, creatinine, ferritin, as well as lipid profiles were not statistically different between the 2 groups. Urea reduction ratio and Kt/V were also similar between the 2 groups. Even though not significant, mean C-reactive protein (mg/L) was lower in the higher bicarbonate group (21.34 ± 38.05, 30.55 ± 77.36, p=.52).

Conclusions: Our study suggested that metabolic alkalosis in hemodialysis patients might be associated with poor dietary nutrition rather than chronic inflammation. A large prospective study with measurement of various nutritional and inflammatory markers is warranted for further investigation.

TH-PO614

Changes in Plasma Ghrelin Concentrations and Feelings of Hunger or Satiety in Hemodialysis and Continuous Peritoneal Dialysis Patients with Chronic Renal Failure in Post- and Pre-Prandial Periods Yoshitaka Miyaoka, Toshiyuki Nakao. *Nephrology, Tokyo Medical University, Shinjyuku-ku, Tokyo, Japan.*

Background: Ghrelin (G) is known as an appetite-stimulating hormone. However, little is known about the changes in plasma G level and their association with feelings of hunger or satiety in hemodialysis (HD) or continuous peritoneal dialysis (CAPD) patients.

Methods: We measured the plasma concentration of G and assessed the hunger feelings in 51 chronic dialysis patients (36 HD, 15 CAPD), and in 10 healthy controls for comparison. Feeling of hunger or satiety was assessed using a hunger-satiety score (H-S score) which were rated as grade 5. Blood samples and H-S scores were obtained both postprandially (1 hour after breakfast) and preprandially (5 hours after breakfast, just before lunch) on the same day. In the HD patients, 4-hour HD treatment was performed between the postprandial and preprandial periods. In CAPD patients, a 2.5% glucose PD solution was dwelled between the postprandial and preprandial periods.

Results: The mean plasma G levels (fmol/ml) in the postprandial period was no significant differences among the patient groups. In the preprandial period, mean plasma G levels were significantly lower (p<0.05) in the HD patients than in the other patient groups: 12.6±12.2 (HD patients), 24.5±24.5 (CAPD patients), 26.9±13.3 (HD patients on non-HD day), 38.3±22.8 (controls). Compared with the plasma G level in the postprandial period,

that in the preprandial period in the HD patients was significantly lower (p=0.007), while those in the other groups were significantly higher (p<0.05). The mean H-S score (points) in the preprandial period were significantly higher than those in the postprandial period in each patient group, with no significant differences among the patient groups.

Conclusions: Plasma G levels are reduced by HD even in fasting patients. Feelings of hunger or satiety synchronize with plasma G levels excerpt patients receiving HD treatment.

TH-PO615

Nutritional Intervention Strategy in Malnourish Chronic Hemodialysis Patients: Results of a 12-Months Prospective Study Ignace Mpio, Christine Cleaud, Walid Arkouche, Jean-Christophe Szelag, Nouredine Boumendjel, Carlos Cardozo, Alejandra Lenz, Denis Fouque, Maurice Laville. *AURAL, Lyon, France.*

Background: Undernutrition (UN) in chronic hemodialysis (CHD) is a major clinical problem because it affects negatively the survival of patients (pts). Several therapeutic and dietary approaches are described. This work aimed to evaluate the effects of nutritional strategy in our hemofialysis-pts

Methods: The study was conducted during 12 months in 132 CHD (49 women , 83 men, mean age was 66 ± 16 years (median 71 years) dialysis vintage 72 ± 74 months (48 months)). The nutritional care (NC) was based on the action of a multidisciplinary team (nephrologists, dietetican, nurses) in order to improve a better compliance. The protocol consist of an optimization of dietary protein intake and energy with, if required, oral supplementation at home and especially during dialysis session (20 g of protein, 400 Kcal) .

We analyzed serum albumin (ALB), CRP, nPCR, and Kt / V at initiation (D0), at 6 month and at endpoint (M12). Undernutrition was defined as severe with ALB<35g/L, mild with ALB between 35 and 38g/L.

Results: At the end of the study the nutritional status of pts is reversed. The frequency of patients with optimal nutritional status was 68% at month 12 while 59% of subjects were malnourished at D0. A M12 the ALB was > 38 g / L under the effect of 3 potentials factors: the improvement of the inflammatory condition, Kt/V, and a protein intake > 1 g / kg / d. The inflammatory status was determining because ALB was higher in pts with CRP <10mg / L vs CRP ≥ 10 mg / L (40.2 vs. 37.7 g / L, p = 0.002 *), there was an inverse correlation between CRP and ALB (R^2 = 0,080, p = 0.002 **). (*Mann-Whitney test, ** ANOVA test) Five pts (4%) died in 1 year.

Conclusions: Our study showed an improvement of the nutritional status of patients. The success of the NC in HDC malnourished pts can be obtained with a multidisciplinary involvement, under an effective dialysis dose and an absence of inflammatory state.

TH-PO616

Prospective Comparison on the Progression of Abdominal Aortic Calcification According to Dialysis Modality in End-Stage Renal Disease Patients Moon-Jae Kim. *Division of Nephrology & Hypertension, Inha University School of Medicine, Incheon City, Korea.*

Background: In end-stage renal disease patients on dialysis, the prevalence, degree and progression of arterial calcifications are greater than in general population. However, there have been few reports whether the progression of abdominal aortic calcifications (AAC) is different or not according to dialysis modality. The purpose of this study was to compare the incidence of AAC progression between hemodialysis (HD) and peritoneal dialysis (PD) patients.

Methods: HD and PD patients who had been on dialysis for ≥ 3 years were included in this observational, prospective 1-year study. AAC was assessed with plain lateral lumbar spine x-ray using anterior-posterior abdominal aorta calcification score at the baseline and 12 month. AAC progression was defined as the increase in the length of linear calcification or new appearance of calcification.

Results: Thirty one patients were progressed (Group 1) and 68 were unchanged (Group 2) in AAC. In group 1, 11 were HD and 20 were PD patients. In group 1, the AAC scores at baseline were 7.9±5.8 in HD and 8.5±6.0 in PD and, after 1 year, 10.2±5.7 in HD and 10.7±6.5 in PD, respectively. In group 2, AAC scores at baseline and after 1 year were unchanged in HD (6.3±7.0) and PD (6.6±7.2). There were no differences in the mean laboratory values of previous 2 years, the percentages of diabetes (51.6 vs. 38.2%), the presence of residual renal function (32.3 vs. 42.6%) and the presence of coronary artery disease (12.9 vs. 5.9%) between group 1 and 2. However, there was significant difference in the percentages of baseline AAC score of more than 25 percentile (AAC score = 1) (90.3 vs. 55.9%, p<0.001), body mass index (25.1±4.0 vs. 23.2±3.2 kg/m^2, p=0.012), duration of dialysis (6.6±2.8 vs. 5.7±3.0 years, p=0.047), corrected calcium product phosphate (53.5±12.2 vs. 48.5±10.9 mg^2/dL^2, p=0.046), total cholesterol (148±28 vs. 179±37 mg/dL, p<0.001), triglyceride (133±80 vs. 172±101 mg/dL, p=0.038), and LDL-cholesterol (78±24 vs. 100±30 mg/dL, p<0.001) between group 1 and 2.

Conclusions: Dialysis modality seems not to affect differently on the progression of abdominal aortic calcification.

TH-PO617

Enteral Nutrition Is Superior Than Intradialytical Parenteral Nutrition in Hemodialysis Children Yolanda Fuentes, Georgina Toussaint, Saul Valverde, Ana M. Hernández, Maria I. Garcia-Roca, Mara Medeiros. *Hospital Infantil de Mexico Federico Gomez, Mexico, DF, Mexico.*

Background: Protein-energy wasting syndrome is present in 30 to 60% of the patients with chronic kidney disease. The etiology is complex and multifactorial. The intradialytical parenteral nutrition (IDPN) has been used in adults with an improvement of nutritional status, reduction in mortality rates and regression of the net negative whole body muscle protein balance. There is scarce experience regarding IDPN in children; the intervention is expensive with no studies comparing oral supplementation with IDPN.

The aim of this study was to examine the effectiveness of IDPN compared to oral supplementation to improve the nutritional state in children on hemodialysis.

Methods: Prospective, crossover, randomized trial in chidren (6 -1 7 years) on hemodialysis program, with an ABN (anthropometric and bioelectric impedance analysis) score < 10.33. During dialysis procedure they received 3 months the intervention A (oral supplementation) or B (IDPN), designed to provide a third of the required daily caloric intake. Protein RDA for age plus 0.5 mg/kg/day. There was no washout period and patients were switched to the other arm to complete the crossover design. Nutritional status was evaluated monthly using the Anthropometry-Bioimpedance-Nutritional score (ABN Score).

Results: Twenty one patients completed 6 months of treatment. Eleven started with IDPN and 10 with enteral supplementation. The ABN score improved after 6 months of nutritional intervention from 8 ± 1.6 to 9.3 ± 1.7 (p=0.001). After 3 months of enteral supplementation the ABN improved from 8.2 ± 1.8 to 9.04 ± 1.5 p=0.04, and after 3 months of IDPN the ABN score improved from 8.6 ± 1.5 to 9.2 ± 1.8 p=0.17

Conclusions: Nutritional intervention (oral and IDPN) is safe and well tolerated in children with ESRD in hemodialysis. The improvement is significant after enteral intervention but not after IDPN.

Funding: Pharmaceutical Company Support

TH-PO618

Changes in Concentration of Thyroid Hormones, RT3, Conversion Ratios and Ratios of Thyroid Hormones to Carrier Proteins in a Single Hemodialysis and Hemodiafiltration Longin Niemczyk,[1] Stanislaw Niemczyk,[2] Katarzyna Szamotulska,[3] Wieslaw Klatko,[4] Zbigniew Bartoszewicz,[1] Ivanna Dubchak,[2] Malgorzata Gomolka,[2] Urszula Syta,[2] Janusz Wyzgal,[1] Joanna Matuszkiewicz-Rowinska.[1] *[1]Medical University of Warsaw; [2]Military Institute of Medicine; [3]National Research Institute of Mother and Child; [4]Hospital, Ciechanow, Poland.*

Background: Previous studies have not explained influence of a single HD and HDF on thyroid function.

AIMS: To assess changes in concentration of TSH, thyroid hormones (TH), conversion ratios (CR) and ratios of thyroid hormones to carrier proteins (TH/P) immediately after and 6h after HD and HDF.

Methods: Patients aged 56±15 years, undergoing HD (n=23) or HDF treatment (n=15) were investigated. Concentrations of TSH, TT4, FT4, TT3, fT3, rT3 and TBG were measured before, immediately after and 6 hours after single dialysis. General linear model for repeated measurements was applied in statistical analysis. Non-normally distributed variables were log-transformed.

Results: Concentration of TSH, TH, rT3, CR and TH/P before dialysis (means ± SD) as well as type of observed trend is shown in the table (∪,∩ - quadratic changes, ↑ - increase, ↓ - decrease). There were no significant differences in the shape of the changes between HD and HDF group, except deeper transient reduction in TSH concentration in HDF.

	HD	HDF		
	before dialysis		Trend before-afer-6h after	p-value trend
TSH [µIU/ml]	1,71±1,07	1,87±1,53	∪ - with interaction	<0,001
TT4 [nmol/l]	80,70±22,89	83,50±12,93	↑	<0,001
TT3 [nmol/l]	1,29±0,31	1,28±0,25	↑	<0,001
fT4 [pmol/l]	13,50±2,35	14,61±1,60	↑	<0,001
fT3 [pmol/l]	4,04±0,78	3,80±0,62	↑	<0,001
rT3 [pmol/l]	306,23±219,17	333,28±150,63	↑	<0,001
TT3/TT4	0,017±0,004	0,016± 0,004	∪	<0,001
fT3/fT4	0,304±0,061	0,264±0,060	∪	<0,001
rT3/TT4	3,673±1,620	4,019±1,904	↑	<0,001
rT3/TT3	243,69±160,136	285,836±216,074	↑	<0,001
fT4/TT4	0,173±0,028	0,177±0,023	↓	<0,001
fT3/TT3	3,234±0,723	3,021±0,368	↓	<0,001
TT4/TBG	3,571±0,569	3,483±0,576	∩	<0,001
TT3/TBG	0,058±0,011	0,054±0,016	∩	0,084

Conclusions: Single dialysis causes important temporal changes in concentration of TSH, TH, rT3, TH/P, and CR. The character of these changes is similar in HD and HDF, apart fromTSH concentration.

TH-PO619

Uremic Retention Solutes in Hemodialysis Patients Yoshiharu Itoh,[1] Kaori Kikuchi,[1] Atsuko Ezawa,[1] Toshimitsu Niwa.[2] *[1]Kureha Corp.; [2]Nagoya Univ.*

Background: We found 12 uremic retention solutes such as indoxyl sulfate, p-cresyl sulfate were significantly increased in serum level of chronic kidney disease rats compared to normal rats. These solutes were significantly decreased in serum level after administration of oral charcoal adsorbent, AST-120.

In this research, we checked the presence of these solutes in hemodialysis patients and healthy subjects and measured their protein binding ratios. We also evaluated effects of solutes on ROS production in HUVEC.

Methods: We assayed serum levels of these 12 uremic retention solutes and phenylacetyl glycine and CMPF in 45 hemodialysis (HD) patients before and after HD and in 8 healthy subjects by LC/ESI-MS/MS.

Free fraction of their serum levels in HD patients was measured by ultra-centrifugation method and their protein binding ratios were calculated from free and total fraction of their serum levels.

We evaluated the effect of their solutes on ROS production in HUVEC by using fluorescence probe, CM-H_2DCFDA.

Results: Twelve solutes except indoxylacetyl glycine and phenylacetyl glycine were detected in serum of HD patients and healthy subjects. Serum levels of 11 solutes except 4-ethylphenyl sulfate (4EtPhS) were significantly higher in HD patients than in healthy subjects.

Indoxyl sulfate (IS), p-cresyl sulfate (PCS), phenyl sulfate (PhS), indoleacetic acid, CMPF and 4EtPhS showed protein binding ratios more than 90%. Ratios in serum level of HD patients to healthy subjects were much higher in free fraction than in total fraction in most solutes such as IS, PCS, PhS.

Conclusions: We confirmed serum levels of 11 uremic retention solutes were significantly higher than in HD patients, as well as in CKD rats.

A significant relationship was observed between protein binding ratios and reduction rates by HD. IS, PCS, CMPF, 4EtPhS showed reduction rate less than 30%, with more than 90% protein binding ratios.

Funding: Pharmaceutical Company Support

TH-PO620

The Effects of On-Line Hemodiafiltration on Inflammatory Markers and other Clinical Findings: Crossover Study Won Min Hwang, Sung Ro Yun. *Department of Nephrology, Konyang University Hospital, Daejon, Republic of Korea.*

Background: On-line hemodiafiltration(HDF) is a method that increase the removal of middle molecules by diffusive and convective solute transport component. The aims of this study are to evaluate the effects of on-line HDF on inflammatory markers and other clinical findings.

Methods: Forty patients on thrice – weekly high flux hemodialysis (HF-HD) were switched to on-line HDF for 1year. We measured intradialytic blood pressure, solute clearance rate(Kt/V, urea reduction ratio (URR, %)), anemia(transferrin saturation(%), ferritin, hemoglobin, hematocrit), nutrition(albumin), bone markers(calcium, phosphate, intact-PHT, alkaline phosphatase), electrolyte (Na, K, Cl), total CO2 and β2-microglobulin at HF-HD, six weeks and one year after switching of on-line HDF. Also, inflammatory markers (hs-CRP, TNF-α), quality of life(SF-36) and itching score(psoriasis area and severity index, PASI) were compared at that times.

Results: The URR (76.68 ± 5.01 vs. 78.31 ± 5.26, %), Kt/V (1.76 ± 0.26 vs. 1.86 ± 0.29) were significantly higher in patients treated with six weeks after on-line HDF compared with those treated with HF-HD. Hb, tCO2, alkaline phosphatase, high density lipoprotein(HDL) level was significantly increased. Itching score (7.5 ± 2.8 vs. 6.1 ± 1.4, p = 0.039) was improved. However, no change of inflammatory markers (hs- CRP, TNF-α) and clinical markers were measured on one year after except the level of β2- macroglobulin(31.19 ± 9.84 vs. 27.88 ± 9.18, p = 0.009)

Conclusions: This study shows that solute clearance rate, itching score, anemia, bone metabolism, and acidosis were improved by the switching from HF-HDF to on-line HDF for 6 weeks (early response). But, these effects of clinical findings and inflammatory markers were not shown between 6 weeks and one year after on-line HDF (late response).

TH-PO621

Epicardial Adipose Tissue as a Marker of Inflammation in Dialysis Luis Gerardo D'Marco,[1] Cristina Karohl,[2] Paolo Raggi.[3] *[1]Ruiz y Paez University Hospital, Bolivar, Venezuela; [2]Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; [3]Division of Cardiology and Department of Medicine, Emory University, Atlanta, GA.*

Background: Epicardial adipose tissue (EAT) is fat confined within the pericardial sac and it is believed to have a local inflammatory and pro-atherogenic effect. EAT can be measured using Multi Slide Computer Tomography (MSCT). The association of EAT as direct measures of region specific adipose tissue in chronic kidney disease (CKD) has not been shown to date.

Methods: Thirty seven CKD-5D patients (mean dialysis duration 26±30 months, 70% males, 57% African-American, 48% diabetic) underwent MSCT to measure EAT and coronary artery calcium (CAC). CAC distribution in quartiles of EAT was assessed. Finally, we measured patients' body mass index and categorized them as normal (BMI 18.5 - 25 kg/m^2) and abnormal BMI (BMI > 25 kg/m^2).

Results: The mean BMI was 28.6 ± 5.7 kg/m^2, and the mean EAT volume was 66 ± 29 ml and 102 ± 69 ml for normal and abnormal BMI groups. There was a direct correlation between dialysis vintage and EAT while BMI showed an inverse relationship with dialysis vintage (*P=0.05*). In this small sample size, the association of CAC and EAT was only marginally significant (*P=0.7*). EAT was significantly correlated with age (*P=0.02*), history of cardiovascular disease (*P=0.03*) and diabetes (*P=0.001*).

Conclusions: The direct association of EAT with dialysis vintage in the face of an inverse association of BMI with dialysis duration, and the marginally significant association of EAT and CAC appear to support the notion that chronic hemodialysis is a state of chronic inflammation, malnutrition and a proatherogenic condition.

TH-PO622

Effect of Insulin Infusion on Liver Protein Synthesis during Hemodialysis Mark Reinhard,[1] Jan Frystyk,[2] Bente Jespersen,[1] Per R. Ivarsen.[2] *[1]Department of Renal Medicine, Aarhus University Hospital, Aarhus N, Denmark; [2]Aarhus University Hospital, Denmark.*

Background: Hemodialysis (HD) is a catabolic procedure that may contribute to the high frequency of protein-energy wasting among patients on maintenance HD. The aim was to investigate the effect of insulin infusion on liver protein synthesis during HD.

Methods: In a randomized cross-over study 10 non-diabetic, HD patients (M/F:7/3, median age 59 years, range 33-79) received 1) no treatment (NT), 2) glucose infusion (G) (10% glucose, 2.5 mL/kg/h), 3) glucose-insulin infusion (GI) (10% glucose added 30 units of NovoRapid® per liter, 2.5 mL/kg/h) during a standardized 4 h HD. During infusions blood glucose (BG) was maintained at 8.0-10.0 mmol/L by additional glucose infusion. Glucose and glucose-insulin infusions were commenced 2 h prior to HD and continued throughout the dialysis. Fasting blood samples were collected before infusions were started (baseline) and followed by a meal. After HD start, blood samples were collected every hour for hormones and every second hour for inflammatory markers until 2 hours post-HD.

Results: Data presented as mean±SD. BG concentrations were comparable at baseline in the three treatment groups (p=0.37). BG levels from baseline to end of dialysis were significantly different only in the NT group compared with the G group (p=0.008). During all three treatments there was an overall increase in serum albumin (37.3±2.0 to 40.5±2.9 g/L, p<0.001 for each treatment) and fibrinogen (11.3±1.7 to 12.8±2.0 µmol/L, p<0.0001 for each treatment), but no significant differences between treatments over time for either albumin (p= 0.24) or fibrinogen (p=0.14). Preliminary data on 4 patients showed a postprandial increase in bioactive insulin-like growth factor I (IGF-I) 3 h after baseline (from 0.83±0.34 to 1.32±0.51 µg/L) with no difference between treatments (p=0.71) and a corresponding decrease in IGF-binding protein 1 (IGFBP-1) 5 h after baseline (from 348±131 to 157±78µg/L) with no difference between treatments (p=0.58). However after 5 h, IGFBP-1 increased during NT, but remained suppressed following the infusions (p=0.02).

Conclusions: Neither glucose nor glucose-insulin infusion appear to add to the anabolic effects of a meal on liver protein synthesis.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-PO623

Effects of Six Versus Three Times Per Week Hemodialysis on Depressive Affect and Mental Health: Frequent Hemodialysis Network (FHN) Trials Mark L. Unruh, Brett Larive, Glenn M. Chertow, Paul W. Eggers, Amit X. Garg, Jennifer J. Gassman, Maria E. Tarallo, Fredric O. Finkelstein, Paul L. Kimmel, The FHN Trial Group, Rosio Ramos. *NIDDK, Bethesda, MD.*

Background: Patients undergoing maintenance hemodialysis have a significant burden of poor mental health. More frequent hemodialysis has been associated with gains in general mental health and improved depressive affect in observational studies. We studied the effects of frequent in-center and nocturnal dialysis on depression and mental health in randomized trials.

Methods: A total of 332 patients were randomized to frequent (six times per week) as compared with conventional (three times per week) hemodialysis in the Frequent Hemodialysis Network Daily (n=245) and Nocturnal (n=87) Trials. Adjusted change in scores over 12 months on the Beck Depression Inventory (BDI), RAND Short-form health survey 36-item (SF-36) self-reported mental health composite score (MHC) and emotional subscale.

Results: In the Daily Trial, subjects randomized to frequent as compared with conventional in-center hemodialysis demonstrated no significant change in mean BDI (adjusted mean change of -2.0 ± 0.7 vs. -0.4 ± 0.7; p=0.10), but experienced clinically significant improvements in MHC (3.7 ± 0.9 vs. 0.2 ± 1.0; P<0.01) and emotional subscale (5.1 ± 1.6 vs. -0.6 ± 1.7; p=0.01). The effects of frequent in-center hemodialysis did not appreciably differ according to age, sex, race, or education status. In the Nocturnal Trial, there were no significant changes among subjects randomized to nocturnal as compared with conventional hemodialysis in BDI (adjusted mean change of -2.00 ± 1.2 vs. -0.3 ± 1.2; p=0.30), MHC (3.1 ± 1.6 vs. -0.8 ± 1.7; p=0.08) or emotional subscale (3.5 ± 2.7 vs. -2.2 ± 2.7; p=0.13).

Conclusions: Daily in-center hemodialysis, as compared with conventional in-center hemodialysis, improved general mental health but had no significant effect on depressive affect Increased frequency nocturnal dialysis had no significant effects on depressive affect or mental health, however, the magnitude of the treatment effects were consistent with those observed in frequent in-center dialysis.

Funding: NIDDK Support

TH-PO624

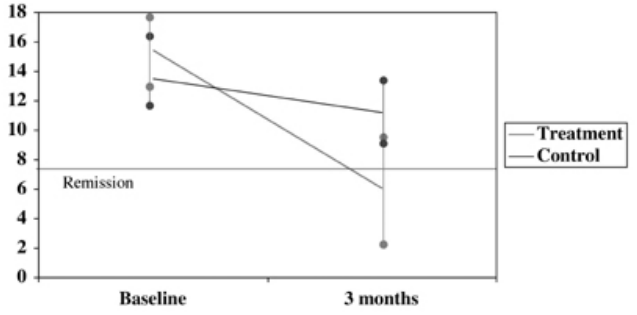
Chairside Cognitive Behavioral Treatment of Depression in Hemodialyzed Patients Is Effective: A Randomized Controlled Trial Daniel Cukor,[1] Nisha Ver Halen,[1] Paul L. Kimmel.[2] *[1]Psychiatry and Behavioral Science, SUNY Downstate Medical Center, Brooklyn, NY; [2]NIDDK, NIH, Bethesda, MD.*

Background: Depression is common, and associated with morbidity and mortality in ESRD patients, but there have been few treatment studies. Non-drug psychotherapeutic treatments, which are effective in the general population, have been under-studied in HD samples.

Methods: We designed an individual standardized intervention for depression in ESRD HD patients. The ten week, once weekly psychosocial intervention (CBT) was administered chairside during dialysis. It taught both cognitive and behavioral skills focused on reducing depressive affect. Subjects randomly assigned to the control group waited for 3 months before getting the intervention. Assessments were at baseline, 3 and 6 months.

Results: The sample included 36 participants (18 cases, 18 controls), 97% African / Caribbean American. 60% were born outside the US. At baseline the population was moderately depressed according to both self-reported (Beck Depression Inventory [BDI] mean = 23.2 ± 8.8) and clinician-administered scales (Hamilton Depression Scale [HAM] mean = 13.5 ± 5.5) Subjects assigned to the treatment condition showed mood improvement (BDI 11.2 ± 7.4, HAM 6.5 ± 6.1) at 3 months when compared to the wait-list controls (BDI 15.6 ± 9.0, HAM 11.2 ± 5.2). There was a significant interaction effect between group and time, indicating the intervention group improved significantly more than controls by both self report (p=.038) and clinician administered (p=.008) depression measures. The intervention group mean HAM scores (<7) reflect remission.

Hamilton-D ratings (M ± SD)pre and post treatment for the treatment (n=18) and control (n=18) groups



Conclusions: A non-drug depression intervention in ethnic minority HD patients is feasible and effective. CBT may be a cost-effective, clinically applicable approach to reducing HD patient depression which has been associated with increased morbidity and mortality.

Funding: NIDDK Support

TH-PO625

Neurocognitive Function and the KDQOL Cognitive Function (CF) Subscale Eric P. Sorensen, Mark J. Sarnak, Hocine Tighiouart, Lena M. Giang, Bethany A. Kirkpatrick, Tammy Scott, Daniel E. Weiner. *Tufts Medical Center.*

Background: Cognitive impairment is common and underdiagnosed in ESRD. Prior work suggests that poor performance on the KDQOL cognitive function (CF) subscale can help identify cognitive impairment in ESRD. We tested this hypothesis using a detailed neurocognitive screen assessing multiple cognitive domains.

Methods: The 3-question KDQOL-CF asks patients to quantify on a 6-point scale if they react slowly to things said and done, have difficulty concentrating or thinking, or become confused. KDQOL-CF results were converted to a 100-point scale; a higher score is consistent with better performance. Cognitive tests were administered at the start of a dialysis session. In primary analyses, cognitive outcomes were quantified using principal components analysis as memory and executive function domains. Regression models adjusted for age, sex, race, education and ESRD cause. Consistent with prior work, performance of a KDQOL-CF score ≤60 also was also assessed.

Results: Of 168 patients, 144 had complete data. Age was 62±17 years; 51% were men, 30% black, 90% high school graduates, and 33% had diabetes causing ESRD. KDQOL-CF score was 76±19; 40 (24%) had scores ≤60. In univariate and adjusted analyses, there was no significant association between KDQOL-CF score and memory (p=0.16 and p=0.33) or between KDQOL-CF score and executive function (p=0.13 and p=0.46). CF scores were most highly associated with depression, assessed by CESD>15 (p=0.002 for trend). The KDQOL-CF, using a cutpoint of 60, performed poorly in predicting cognitive function, including lower executive and memory factor scores and impaired performance on individual cognitive tests (figure).

Conclusions: The KDQOL-CF score is a poor determinant of neurocognitive performance in hemodialysis patients.

Test / Factor	Metric	N	Sensitivity	Specificity	PPV	NPV
Executive Factor	>0.5 SD Below this Population Mean	31%	0.33	0.80	0.43	0.72
Executive Factor	>1 SD Below this Population Mean	15%	0.27	0.77	0.18	0.85
Memory Factor	>0.5 SD Below this Population Mean	36%	0.29	0.78	0.43	0.66
Memory Factor	>1 SD Below this Population Mean	14%	0.30	0.77	0.18	0.87
Recall*	>1 SD Below General Population	54%	0.27	0.81	0.62	0.49
Delayed Recall*	>1 SD Below General Population	8%	0.15	0.76	0.05	0.91
Recognition*	>1 SD Below General Population	26%	0.23	0.77	0.26	0.74
Block Design*	>1 SD Below General Population	37%	0.27	0.76	0.40	0.64
Digit Symbol*	>1 SD Below General Population	59%	0.34	0.85	0.77	0.47
Trails A#	>1 SD Below General Population	66%	0.31	0.84	0.78	0.39
Trails B#	>1 SD Below General Population	63%	0.29	0.80	0.70	0.40
Digit Span *	>1 SD Below General Population	24%	0.38	0.82	0.39	0.80
COWAT^	Lowest Quartile of General Population	54%	0.25	0.78	0.58	0.46
Mental Alternations	<15 Alternations	70%	0.21	0.72	0.64	0.28
CESD	Score >15	30%	0.37	0.82	0.46	0.76

*Standardized for age; ^Standardized for age and education; #Standardized for age, sex and education

Funding: NIDDK Support

TH-PO626

Cognitive Function in Hemodialysis (HD) Patients Mark J. Sarnak, Hocine Tighiouart, Tammy Scott, Lena M. Giang, Eric P. Sorensen, Bethany A. Kirkpatrick, Daniel E. Weiner. *Tufts Medical Center, Boston, MA.*

Background: There are few large, generalizable studies of detailed cognitive testing in HD patients, and comparisons with the general population. We hypothesized that multiple domains of cognitive function, particularly those relating to vascular disease (sub-cortical function), may be compromised.

Methods: Detailed neurocognitive testing was performed in 324 HD patients from 6 Boston-area HD units. One sample t-tests were used to evaluate differences in cognitive function between HD patients and normative data.

Results: Mean age was 63 years, 78% were white, 54% men, and 17, 48, 35, and 37%, had a history of stroke, diabetes, heart failure, and coronary disease, respectively. Fifty percent were high school graduates. Despite preserved MMSE, HD patients had lower scores on tests of learning and on tests of subcortical function, with 37.5% scoring more than 1.5 SD below the population norm on 2 or more tests of subcortical function.

Cognitive tests in study sample vs reference data

Cognitive Test	Description of Test	HD Patients		Normative Data	Comparisons	
		Mean±SD	Rescaled	Reference ±SD	One-sample t-test P-value	> 1.5 SD below reference
MMSE	Screen	26.7±2.9	NA	Normal ≥ 24	NA	13.7%
NAART	Intelligence	102.3±12.2	NA	100±15	P<0.001	1.6%
Delay Recall	Primary Cortical (Memory)	4.4±2.7	10.5±2.6[a]	10±3	P<0.001	1.3%
Recall Total	Primary Cortical (Learning)	23.8±7.2	7.5±3.2[a]	10±3	P<0.001	29.2%
Recognition	Primary Cortical (Recognition)	20.7±3.0	9.2±3.1[a]	10±3	P<0.001	11.9%
Block Design	Primary Subcortical	26.1±10.6	8.7±2.8[a]	10±3	P<0.001	12.4%
Digit Symbol	Primary Subcortical	40.1±17.0	6.8±2.6[a]	10±3	P<0.001	34.4%
Trials A	Primary Subcortical	61.3±39.6	38.2±9.6[b,c]	50±10	P<0.001	38.2%
Trials B	Primary Subcortical	171.0±88.1	37.1±11.3[b,c]	50±10	P<0.001	39.5%

[a]Normalized for subject age. [b]Normalized for age, gender and education level. [c]T scores for test performance.

Conclusions: There is a high prevalence of cognitive impairment in HD patients, particularly as it relates to subcortical function. We hypothesize that the latter may be due to a higher prevalence of clinical and subclinical cerebrovascular disease in HD patients.

Funding: NIDDK Support

TH-PO627

Comparison of Cognitive Function across ESRD Treatment Modalities: A Systematic Review Deidra C. Crews,[1] Priscilla Auguste,[1] Tanjala S. Purnell,[1] Raquel Greer,[1] Temitope Olufade,[1] Julio Lamprea,[1] Johanna Sheu,[2] Patti Ephraim,[1] Hamid Rabb,[1] Neil R. Powe,[3] L. Ebony Boulware.[1] *[1]Johns Hopkins University; [2]Harvard University; [3]San Francisco General Hospital.*

Background: Patients with ESRD are encouraged to make informed decisions about their choice of renal replacement treatment (RRT). Limitations in cognitive function are common among ESRD patients. However, the quality and quantity of evidence regarding differences in cognitive function among patients treated with different RRTs is unknown.

Methods: We performed a systematic review of published studies describing differences in patients' cognitive function across different RRTs (hemodialysis-HD, peritoneal dialysis-PD, kidney transplant-TX). We identified relevant articles (English, published after 1987) from PubMED and hand-searched bibliographies. We abstracted data on 5 domains of cognitive function. Two independent reviewers assessed studies' quality (e.g. selection bias and validity of outcome assessment). We calculated standardized effect sizes (Cohen's d) of associations between each RRT and outcome.

Results: Of 110 potentially relevant studies, 15 reported RRT comparisons, with 26 comparisons of relevant outcomes (Table). Most studies were from outside the U.S. (80%), and all were observational (11 cross-sectional, 2 cohort and 2 pre-post designs). Studies ranged from very low to moderate quality (only 4 adjusted for important confounders

such as educational level) and suggested better or no different cognitive outcomes among patients on PD and TX compared to patients on HD.

Comparison of Published Studies on Cognitive Function Outcomes by RRT Number of studies favoring each treatment

Study Design (N studies)	HD vs. PD			HD vs. TX			PD vs. TX			*Dialysis vs. TX		
	HD	Neither	PD	HD	Neither	TX	PD	Neither	TX	Dialysis	Neither	TX
Memory												
Cross-sectional (2)		1										1
Pre-Post Transplant (1)												1
Concentration												
Cross-sectional (2)			2									
Pre-Post Transplant (2)					1						1	
Learning												
Cross-sectional (1)			1									
Pre-Post Transplant (1)											1	
Overall Cognition												
Pre-Post Transplant (1)					1							
Self-Reported Cognition												
Cross-sectional (8)		4	2					2			1	
Cohort (2)		2										
Total Study Comparisons	0	7	6	0	2	0	0	2	0	0	3	2

Abbreviations: HD=hemodialysis; PD=peritoneal dialysis; TX=transplant
*Dialysis includes HD and PD patients combined

Conclusions: Current evidence suggests PD and TX patients have better or no different cognitive function than HD patients. However, the quality of evidence is poor. Prospective studies, preferably assessing patients prior to the start of RRT, are needed to better inform patients' choice of RRT.

Funding: NIDDK Support, Private Foundation Support

TH-PO628

High Prevalence of Cognitive Impairment in All Age Groups of Hemodialysis Patients Hristos Karakizlis, Maren Bodden, Christoph Klein, Richard Dodel, Joachim Hoyer. *Dept. of Nephrology and Neurology, Philipps-University, Marburg, Hessen, Germany.*

Background: Cognitive impairment correlate with chronic disease and can cause substantially reduced compliance for therapy. Recently a high prevalence of moderate and even severe cognitive impairment was found in senior patients (pts) from a U.S. hemodialysis patient cohort. We tested cognitive function in a German dialysis cohort and delineated the impairment of specific cognitive function domains by use of an elaborated neuropsychological test battery.

Methods: We performed a population based study in the regional government district Mittelhessen, Germany. All outpatient hemodialysis pts aged ≥18 years were screened for their cognitive function with Mini Mental State Examination (MMSE). Furthermore in pts capable for more intensive testing (MMSE >24) we performed a neuropsychological test battery (CERAD) examining the five cognitive domains verbal and visual memory, constructional praxis, two executive functions.

Results: From a total of 330 patients inclusion criteria were met by 298 patients of whom 200 (67.1%; mean age 68.6 yrs) agreed to take part in the study. 94 pts (47%) scored within normal range (MMSE 30-28), whereas 69 pts (35%) showed mild cognitive impairment (MMSE 27-25) and 36 pts (18%) suffered from moderate to severe cognitive impairment (MMSE ≤24). Cognitive impairment (MMSE <28) was high in senior (≥65 yrs) pts (66%), but was also detected in 30% of younger aged patients (<65 yrs). CERAD testing confirmed the high rate of cognitive impairment in the younger patient group, especially verbal fluency and executive functions were affected.

Conclusions: The results demonstrate an extraordinary high prevalence of cognitive impairment in hemodialysis patients affecting more than half of the patients. In particular almost every fifth patients has moderate or severe cognitive impairment which usually results in a significant reduction of the patients' ability to comply with treatment. The results clearly demonstrate that cognitive impairment is not confined to senior patients but affect the patients younger than 65 yrs. The knowledge of individual cognitive impairment can help to individually adjust therapeutic guidance of hemodialysis patients.

TH-PO629

Development of a Predictive Mortality Risk Score from the ARO European Hemodialysis (HD) Cohort Jurgen Floege,[1] Marc Froissart,[2] Iain A. Gillespie,[3] Daniele Marcelli,[4] Sharon Richards,[3] Kai-Uwe Eckardt.[5] *[1]Nephrology, RWTH University of Aachen, Aachen, Germany; [2]International Development Nephrology, Amgen Europe GmbH, Zug, Switzerland; [3]CfOR, Amgen Ltd, Uxbridge, United Kingdom; [4]Nephrocare, FME, Bad Homburg, Germany; [5]Nephrology, Universitätsklinikum Erlangen, Erlangen, Germany.*

Background: HD patients exhibit a high mortality risk, yet there is no widely accepted predictive mortality risk score for these patients. A large European HD cohort was used to assess predictors of mortality and derive a predictive score.

Methods: We derived and internally validated a mortality risk score in HD patients, using both modifiable and non-modifiable risk factors, in 8132 HD patients (38% incident and 62% prevalent) randomly assigned to either the development or validation dataset. The methodological approach was derived from the predictive model used in the Framingham Heart Study. The risk score was determined using a baseline Cox regression model and derived separately for 1- and 2-year mortality. Continuous variables were introduced as categorical in the model.

Results: Increasing age, history of cancer, diabetes and peripheral vascular disease, as non-modifiable factors, and use of anticoagulant therapy were associated with an increased risk of mortality. Lower BMI was associated with a higher mortality risk, the inverse of the relationship observed in community-based datasets. Mortality risk was higher when Hb was <10 g/dL and lower when Hb was >12 g/dL. PTH values <150 pg/mL indicated higher mortality risk. Use of vitamin D was associated with a slightly lower risk. The magnitude of the difference between the development and the validation datasets was assessed by the c-statistic which reached 0.69 (0.67, 0.70). The resulting clinical additive score [range -9 to +20] conveyed a 2-year mortality risk of 2% to >90%. This allowed building up a simple heat map for risk assessment in an individual patient.

Conclusions: A predictive clinical score for 1- and 2-year mortality risk developed in a large European HD database is now available as a simple clinical tool. An external validation and refinement of the score is currently being conducted in a 12000 incident HD patient database.

Funding: Pharmaceutical Company Support

TH-PO630

Mortality and Race in Pediatric End-Stage Renal Disease: Who Is Dying before Transplant? Sandra Amaral,[1] Rachel E. Patzer,[2] Nancy G. Kutner,[3] William M. McClellan.[2] *[1]Pediatrics, Emory University, Atlanta, GA; [2]Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA; [3]Rehabilitation Medicine, Emory University, Atlanta, GA.*

Background: Children with End-Stage Renal Disease (ESRD) have increased risk of morbidity and mortality. Kidney transplantation may mitigate these risks and prolong patient survival and quality of life. Yet, some children with ESRD never proceed to transplantation due to premature death. Little is reported about racial differences in mortality risk after incident ESRD.

Methods: We performed a retrospective cohort study of all incident ESRD patients < 21 years of age from January 2000 through Sept. 2008, followed through Sept. 2009. We examined rate of death among dialysis patients by race/ethnicity using multivariable-adjusted Cox models. We censored patients at death or end of follow-up and excluded patients who received a transplant. We considered neighborhood poverty and patient's health insurance at incident ESRD as measures of socioeconomic status.

Results: Among 8, 146 pediatric ESRD patients, 896 (9.7%) deaths occurred. 735 (82%) died before waitlisting and 161 (18%) died on the waiting list. Median time to death after incident ESRD was 463 days; mean age at death was 14.9 yrs. Compared to the incident ESRD population, a greater proportion of patients who died were black (vs. white or Hispanic). In adjusted analyses, the effect of race on death was significantly modified by health insurance, with Hispanics experiencing lower rate of mortality across all levels of insurance status. This was most striking in Hispanics with no health insurance who had 64% lower rate of death (HR=0.36; 95% CI: 0.18-0.71) compared to whites with no insurance, whereas black patients with no health insurance had 59% higher (HR=1.59; 95% CI: 0.95-2.67) rate of death compared to whites.

Conclusions: We found that blacks with no health insurance had a greater rate of death after incident ESRD compared with whites, while Hispanics had significantly lower rate of death after incident ESRD vs. the other racial groups regardless of insurance status. Further studies are needed to elucidate why these racial differences in mortality exist.

TH-PO631

In-Center Daily Hemodialysis and Patient Survival: A Multinational Cohort Study Rita Suri,[1] Robert M. Lindsay,[1] Brian Bieber,[2] Ronald L. Pisoni,[2] Amit X. Garg,[1] Peter Austin,[3] Louise M. Moist,[1] Bruce M. Robinson,[2] Yun Li,[4] Cécile Couchoud,[5] Eduardo K. Lacson,[6] Deborah Lynn Zimmerman,[7] Gihad E. Nesrallah.[1] *[1]U Western Ontario; [2]Arbor Research Collaborative for Health; [3]Institute for Clinical and Evaluative Sciences; [4]U Michigan; [5]Agence de la Biomédecine; [6]Fresenius Medical Care North America; [7]U Ottawa.*

Background: Increasing hemodialysis frequency from 3 to 6 times per week improves left ventricular mass and health-related quality of life. However, the effects of increased frequency on survival remain uncertain.

Methods: We identified 556 patients from France, the United States, and Canada in the International Quotidian Dialysis Registry, who received daily hemodialysis ≥5 times per week from January 2001 to August 2010. Using propensity-score based matching techniques, we matched 318 of these patients to 575 contemporaneous patients receiving conventional, 3 times weekly, hemodialysis in the Dialysis Outcomes and Practice Patterns Study. All patients had session times of less than 5 hours, and received hemodialysis in the clinic or hospital setting. We compared mortality rates between groups using Cox proportional hazards regression.

Results: Mean dialysis frequency in the daily group was 5.8+0.5 sessions/week. Mean weekly treatment time was 15.7+4.3 hours for daily patients and 11.9+1.0 hours for conventional patients. During 1382 patient-years of follow-up, 170/893 patients died. Patients receiving daily hemodialysis had a significantly higher mortality rate than patients receiving conventional hemodialysis (15.6 vs. 10.9 deaths per 100 patient-years: hazard ratio 1.6; 95% CI 1.1-2.3; p=0.023). Similar results were observed in pre-specified subgroup and sensitivity analyses.

Conclusions: In contrast to previous studies, in-center daily hemodialysis was not associated with any appreciable mortality benefit. As we cannot exclude the possibility of residual confounding, these findings require confirmation in an adequately powered randomized trial. Until then, decisions to undertake daily hemodialysis should not be based on assertions of improved survival.

TH-PO632

Relationship of Missed and Shortened Hemodialysis [HD] Treatments to Hospitalization and Mortality Chamberlain I. Obialo,[1] W. Hunt,[2] Khalid Bashir,[1] Philip Zager.[2] *[1]Renal Section, Dept of Medicine, Morehouse School of Medicine, Atlanta, GA; [2]Dialysis Clinic, Inc., Albuquerque, NM.*

Background: Missed and shortened HD treatments are more common in US than in Europe and Japan. The factors that predict these events and their relationships to clinical outcomes have not been previously studied in a nationally representative sample of US HD patients. The present study was conducted to explore: the frequency of missed and shortened treatments by race, treatment schedule, geographic region: and the effect of these events on mortality and hospitalization.

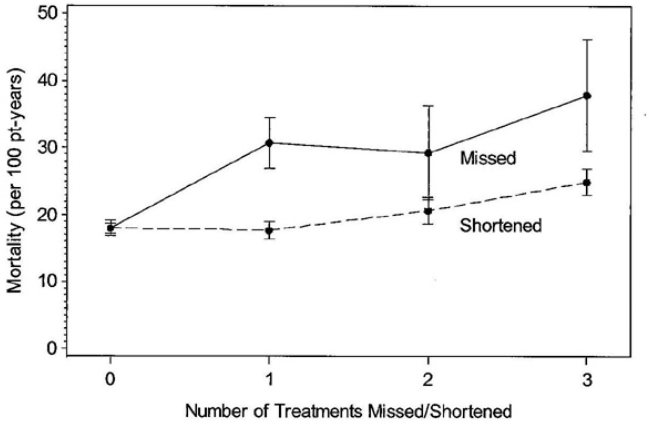
Methods: We studied a prevalent cohort of 15,340 HD patients treated in facilities operated by Dialysis Clinic Inc (DCI) between January 2007 and June 2008. The cohort consisted of 48% non-Hispanic whites [NHW], 41% African Americans [AA], 6% Hispanics, 2% Native Americans [NA], 2% Asians and 1% unknown.

Results: The percent of missed and shortened treatments differed significantly by race.

Race/Ethnicity	AA	NA	Hispanic	NHW	Asian
Missed HD(%)	3.1*	2.9*	2.8*	1.8	0.9*
Shortened HD(%)	18.4*	15.0*	17.0*	12.6	8.9*

* p<0.001 vs. NHW

Missed and shortened treatments were more frequent among patients treated on the Tuesday, Thursday, Saturday vs. the Monday, Wednesday, Friday schedule (2.9% vs. 2.1% missed and 16.4% vs. 14.9% shortened, both p<0.0001). The frequency of missed and shortened treatments differed slightly by geographic region and was lowest in the northeast. Missed and shortened treatments were associated with increased mortality.



Similar associations were also observed for hospitalization (not shown).

Conclusions: The frequency of missed and shortened HD differed by race,dialysis schedule and geographic region and was associated with increased hospitalization and mortality. Increased adherence with prescribed dialysis may decrease morbidity and mortality.

Funding: Clinical Revenue Support

TH-PO633

Declining Mortality in US Dialysis Patients: A General Population Effect-Or More? The United States Renal Data System Robert N. Foley,[1,2] David T. Gilbertson,[1] Areef Ishani,[1,3] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN; [3]Minneapolis VAMC, Minneapolis, MN.*

Background: Substantial reductions in mortality have occurred in US dialysis patients, in spite of increasing burdens of comorbid illness and successive failures of large, multicenter trials to define salutary interventions. Hence, we set out to address the hypothesis that mortality reductions in dialysis populations reflect general-population phenomena.

Methods: Defined as annual mortality rates in prevalent dialysis patients as numerator and mortality rates in the U.S. population as denominator, we compared age-specific mortality ratios for 1998 and 2008.

Results: Dialysis mortality rates fell in all age-groups except 15 to 24 years. In contrast, age-specific dialysis-general population mortality ratios fell in only 3 of the 8 subgroups examined: age 25 to 34 years (ratio 55.6 in 1998/ratio 46.6 in 2008), 35 to 44 years (44.7/37.2) and 45 to 54 years (28.6/23.8). When prior duration of dialysis therapy was taken into consideration declines in mortality ratio were only apparent in dialysis patients with duration ≤ 1 year, among the following subgroups: age 25 to 34 (68.5/50.5), 35 to 44 (48.2/38.9) and 45 to 54 (29.5/23.3).

Conclusions: Mortality reductions in dialysis patients > 55 years reflect general-population trends. Mortality reductions in younger patients, in contrast, may exceed general population expectations.

Funding: NIDDK Support

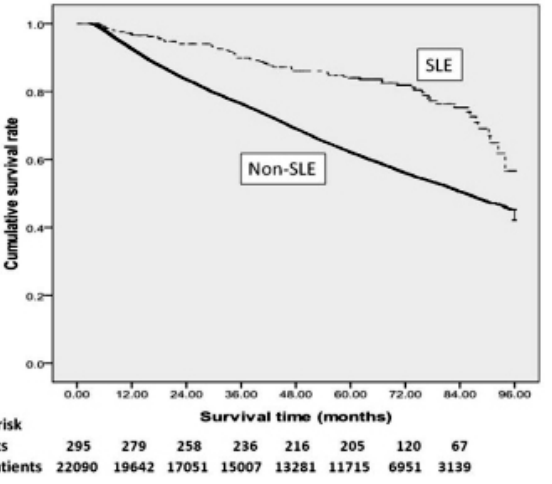
TH-PO634

Long-Term Outcomes and Predictors for Mortality among SLE Dialysis Patients: National Cohort Study in Taiwan Chih-Chiang Chien. *Departments of Nephrology, Chi-Mei Medical Center, Tainan, Taiwan.*

Background: To compare the prognosis of patients with systemic lupus erythematosus (SLE) receiving dialysis and non-SLE patients receiving dialysis and determine the factors that affect survival after dialysis.

Methods: We used the National Health Insurance Research Database and collected data on patients who started maintenance dialysis from 2001 to 2003. The patients were followed from initiation of dialysis until death, discontinuing dialysis, or the end of 2008. We performed a Kaplan-Meier analysis of the cohort and used multivariate Cox regression analysis to identify significant predictors of survival.

Results: Of the 22,394 dialysis patients studied, 303 (1.35%) had SLE. Patients with SLE were predominantly women and younger than the other patients (39.4 ± 15.3 years vs. 59.4 ± 14.2 years, p<0.001). The Kaplan-Meier survival curves for dialysis patients with and without SLE are illustrated.



The 1- and 5-year cumulative survival rates were 97.6% and 84.0% in patients with SLE, and 92.7% and 62.2% in patients without SLE. After adjusting for age, gender, dialysis modality, and comorbidities, we did not find a significant survival difference between the two patient groups after 8 years of follow-up. Multivariate analysis showed that older age (≥65), male gender, and the presence of diabetes were independent predictors of mortality (p<0.05) among SLE patients.

Conclusions: The long-term survival outcome was similar between the SLE and non-SLE patients undergoing dialysis. The factors affecting patient mortality were not all identical in these two groups.

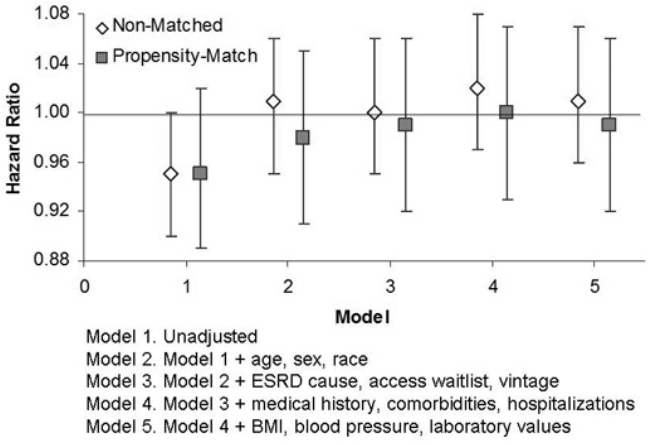
TH-PO635

Single Use Versus Reprocessed Dialyzers and Mortality Daniel E. Weiner, Hocine Tighiouart, Klemens B. Meyer. *Tufts Medical Center, Boston, MA.*

Background: There is limited overall evidence either supporting or discouraging dialyzer reprocessing.

Methods: To explore the long-term medical utility of dialyzer reuse, we compiled patient level data for all prevalent hemodialysis patients with Medicare coverage treated at a DCI facility on January 1, 2005, merging DCI data with USRDS data to better define comorbidity and outcomes based on medical claims. Reuse status was determined by patient dialyzer prescription. The primary outcome was time to all-cause mortality through July 2009, ascertained with both DCI and USRDS data; sensitivity analyses examined transplant and PD-censored mortality. Primary analyses used Cox regression with extensive comorbidity adjustment while secondary analyses matched patients based on their propensity to reuse. A sandwich estimator accounted for center effect.

Results: There were 9,051 Medicare patients included; 5735 were reuse patients in reuse facilities and, of 3316 non-reuse patients, 2641 were in non-reuse facilities. Reuse patients were more likely to be African American, Hepatitis C negative, and had more grafts and fewer catheters. Dialysis vintage was 3 months longer, blood pressure slightly higher and heparin use substantially lower in non-reuse patients. Propensity score matching successfully paired 2533 patients in each groups. Results of primary and propensity matched analyses were similar and revealed no significant difference in outcomes by reuse status (Figure), with a hazard ratio for reuse of 1.01 (0.96, 1.07) and 0.99 (0.92, 1.06) in multivariable adjusted non-matched and propensity-matched analyses, respectively. Analyses censoring at modality change were similar to those in the figure.



Conclusions: Dialyzer reuse is not associated with mortality when compared to single use. The decision to reuse therefore should be based on other factors, including resource utilization and cost and environmental concerns.

Funding: Private Foundation Support

TH-PO636

Early Start of Renal Replacement Therapy in Critically Ill Patients Improves Renal Outcome but Not Survival Anne Lerner-Gräber, Timo Speer, Danilo Fliser, Matthias Klingele. *Department of Internal Medicine 4, Saarland University Medical Centre, Homburg / Saar, Germany.*

Background: To assess the relationship between serum creatinine and urea levels at the beginning of renal replacement therapy (RRT) on mortality and need for RRT after discharge in critically ill patients.

Methods: Observational study of all patients requiring RRT in 2 internal medicine and 4 surgical intensive care units (ICUs) of a tertiary hospital between 03/2007 and 08/2009. Patients being on chronic RRT before admission to the hospital were excluded. Serum creatinine and urea levels at the start of RRT, mortality and recovery of kidney function during hospitalization and after discharge (mean follow-up period 511 days) were assessed.

Results: In total, 583 patients and 5694 RRTs were included for analysis. The in-hospital mortality was 68.4 % (n = 399), whereas the mortality rate in survivors after discharge who were not lost to follow-up (n = 140, 24.0 %) was 25.7 %. There was no statistically significant difference of serum creatinine and urea levels at initiation of RRT between those patients who died or who survived. After discharge, in 20 survivors no recovery of renal function after acute kidney injury could be observed and they required RRT due to persistent renal insufficiency. In these patients serum creatinine (261 ± 151 vs. 475 ± 282 µmol/L, p=0.000) as well as serum urea levels (20.8 ± 11 vs. 30.8 ± 16.5 mmol/l, p=0.007) were significantly higher at initiation of RRT compared to those in whom renal function recovered after discharge. Of note, the rise of creatinine or urea levels between admission and start of dialysis treatment are strong predictors of recovery of renal function after discharge (β=0.458, p=0.000; β=0.419, p=0.000).

Conclusions: In critically ill patients with kidney injury requiring RRT on the ICU, lower serum creatinine and urea levels at the start of RRT signalize better renal outcome after hospital discharge, while mortality is not improved.

TH-PO637

Predictors of Hospitalizations in Maintenance Hemodialysis (HD) Patients Jochen G. Raimann,[1,2] Len A. Usvyat,[1] Peter Kotanko,[1,2] Nathan W. Levin.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center.*

Background: Despite technological advances in hemodialysis (HD) technology, mortality and hospitalization (hosp) rates remain high in these patients (pts) (USRDS 2010). This is of substantial clinical and economic impact. This analysis attempts to identify significant predictors of hosp in HD pts.

Methods: Pts receiving HD in RRI clinics for more than 365 days from Jan 1, 2008 to Dec 31, 2010 were included. Clinical parameters, hosp days and admissions during the observation period were averaged per patient for the entire observation period. Two multivariate regression models were developed aiming to predict hosp days (Model 1) and number of admissions (Model 2), respectively, during the observation period. Both models were adjusted for age, gender, race, HD vintage, diabetes (DM), HD access, pre HD systolic and diastolic blood pressure (SBP, DBP), pre HD temperature (temp), interdialytic weight gain (IDWG), post HD weight (wt), difference of post HD wt and prescribed target wt (Δ TW), enPCR, Kt/V, albumin, total calcium, ferritin, hemoglobin (Hgb), potassium, phosphorus, transferrin saturation (TSAT) and treatment time.

Results: 5758 incident and prevalent HD patients (age 60.5±15.4 years, 55% male, 51% blacks, BMI 28.7±12.8 kg/m^2), with an average of 1.74 hosp admissions lasting 11.8 days per year, were studied. Model 1 (adjusted R^2 0.25, P<0.05) significantly predicted hosp days and Model 2 (adjusted R^2 0.25, P<0.05) hosp admissions. **Table 1** shows significant predictors and unstandardized coefficients.

Conclusions: This analysis showed a significant relationship between hosp and potentially modifiable indicators of fluid overload (IDWG, Δ TW), anemia management (Hgb, TSAT) and vascular access (catheter). Knowledge of predictive parameters may aid the the identification of pts at risk and thus help to prevent hosp admissions.

Table 1: Predictors of Hospitalization.

	Model 1			Model 2		
	B	S.E.	P-value	B	S.E	P-value
Intercept	-59.7	50.2	0.23	**15.93**	**6.50**	**0.01**
Age [yrs]	0.01	0.02	0.70	0	0	0.53
Male [0/1]	-0.23	0.43	0.59	0.02	0.06	0.74
Black race [0/1]	0.09	0.44	0.84	0.04	0.06	0.50
Other Race [0/1]	-1.08	0.80	0.18	**-0.23**	**0.104**	**0.03**
HD Vintage [yrs]	0.08	0.05	0.13	0	0.01	0.94
Diabetes [0/1]	**2.54**	**0.42**	**<0.001**	**0.35**	**0.06**	**<0.001**
Catheter [0/1]	**4.95**	**0.54**	**<0.001**	**0.70**	**0.07**	**<0.001**
Pre HD SBP [mmHg]	**-0.07**	**0.02**	**<0.001**	**-0.01**	**0**	**<0.001**
Pre HD DBP [mmHg]	**0.08**	**0.03**	**0.01**	**0.02**	**0**	**<0.001**
Pre HD Temp [°F]	**1.97**	**0.51**	**<0.001**	0.02	0.07	0.82
IDWG [kg]	**1.77**	**0.30**	**<0.001**	**0.23**	**0.04**	**<0.001**
Post wt [kg]	**-0.12**	**0.01**	**<0.001**	**-0.14**	**0**	**<0.001**
Δ TW [kg]	**0.42**	**0.05**	**<0.001**	**0.05**	**0.01**	**<0.001**
enPCR [g/kg/d]	-0.16	0.92	0.86	**-0.35**	**0.12**	**<0.01**
eKT/V	-0.28	0.85	0.75	**0.26**	**0.11**	**0.02**
Albumin [g/dL]	**-13.61**	**0.66**	**<0.001**	**-1.61**	**0.09**	**<0.001**
Calcium [mEq/L]	0.63	0.36	0.09	**-0.10**	**0.05**	**0.04**
Ferritin [ng/mL]	**0**	**0**	**0.01**	0	0	0.06
Hgb [mg/dL]	**-4.46**	**0.29**	**<0.001**	**-0.66**	**0.04**	**<0.001**
Potassium [mEq/L]	0.47	0.44	0.28	**0.12**	**0.06**	**0.04**
Phosphorus [mg/dL]	-0.03	0.21	0.89	0.05	0.03	0.05
TSAT [%]	**-0.25**	**0.03**	**<0.001**	**-0.03**	**0**	**<0.001**
Tx time [mins]	0	0	0.79	0	0	0.07

TH-PO638

Does Combination Therapy with Peritoneal Dialysis and Hemodialysis from the Initiation of Renal Replacement Therapy Improve the Patients' Prognosis? Atsushi Ueda,[1] Aki Hirayama,[2] Kei Nagai,[1] Kunihiro Yamagata.[3] *[1]Kidney & Dialysis Center, Namegata District General Hospital, Namegata, Ibaraki, Japan; [2]Integrated Medicine, Tsukuba University of Technology, Tsukuba, Ibaraki, Japan; [3]Internal Medicine, University of Tsukuba, Ibaraki, Japan.*

Background: Peritoneal dialysis and hemodialysis (PD+HD) combined therapy is an alternative method to supplement the weak points of PD. Usually this combined therapy is started on the halfway of renal replacement therapy (RRT) and its beneficial effects were already reported. However, the advantage when the combination is carried from the beginning of RRT is little known. The aim of this study is to evaluate the advantages of PD+HD therapy from the initiation of RRT by a retrospective cohort study.

Methods: Ninety-three end-stage renal patients initiated to RRT in our hospital were employed. The patients received an adequate explanation about the three treatment method and selected by own idea. Thus they were divided into three groups: hemodialysis (HD, n=65),peritoneal dialysis (PD, n=18) and HD+PD (n=10). Residual renal function(RRF), peritoneal permeability using peritoneal equilibration test data, serum beta-2-microglobulin (β_2MG) and survivorship curve for three years of every six months were measured.

Results: The RRF rapidly decreased in the HD group compared to PD or PD+HD, and there was no difference between PD and PD+HD group. The peritoneal permeability was not different between PD and the PD+HD group. When the dialysis therapy was introduced, β_2MG did not admit a significant difference in HD and the PD+HD group. However, the value of the β_2MG after 1 year and two years of the HD group increased significantly (HD after 1 year 31.7±9.8 vs. PD+HD 19.4±5.4µg/L and after 2 years 31.4±8.5 vs. 20.6±3.0µg/L, p<0.05). Kaplan-Meier analysis revealed that PD+HD was the most excellent of the prognosis compared other therapies .

Conclusions: PD+HD combination therapy from the initiation of RRT showed advantages in the remaining kidney function, serum β_2MG values and survival rate compared to monotherapy with conventional HD or PD alone. Therefore it was concluded that PD+HD therapy has the possibility of becoming the most excellent selection at the start of RRT.

TH-PO639

Health Literacy and Outcomes in Hemodialysis Patients Jamie Green,[1] Maria K. Mor,[2] Anne-Marie Shields,[2] Mary Ann Sevick,[1,2] Robert M. Arnold,[1] Paul M. Palevsky,[1,2] Michael J. Fine,[1,2] Steven D. Weisbord.[1,2] *[1]Medicine, University of Pittsburgh, PA; [2]VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: Although limited health literacy is common among chronic hemodialysis patients, its associations with patient outcomes are not well characterized. We sought to evaluate the associations of limited health literacy with dialysis treatment adherence, hospitalizations, and mortality.

Methods: Using the Rapid Estimate of Adult Literacy in Medicine (REALM), we measured the baseline health literacy of 260 chronic hemodialysis patients enrolled in a randomized clinical trial of symptom management strategies. We used Poisson regression to evaluate the independent associations of limited health literacy (REALM scores <61) with dialysis adherence (abbreviated and missed treatments) and hospitalizations, and Cox regression to characterize the independent association of limited health literacy with mortality over a follow-up period of up to 24 months. The analyses adjusted for potential confounders and used multiple imputation to account for missing data.

Results: Overall, 41 of 260 patients (16%) demonstrated limited health literacy. Limited health literacy was independently associated with a greater number of abbreviated dialysis treatments (9.1% of sessions v. 8.9% of sessions; incident rate ratio = 1.2; 95% CI 1.1-1.3) and missed treatments (3.6% of treatments v. 1.7% of treatments; incident rate ratio = 1.4; 95% CI 1.1-1.7). There was a non-statistically significant trend towards a higher risk of hospitalization among patients with limited health literacy (2.7 hospitalizations per year v. 2.2 hospitalizations per year; incident rate ratio = 1.2; 95% CI 1.0-1.4). Limited health literacy was not associated with a higher risk of mortality (hazard ratio = 1.3; 95% CI 0.6-2.9).

Conclusions: Patients receiving chronic hemodialysis with limited health literacy are more likely to shorten and miss dialysis treatments and may be at greater risk for hospitalization. Interventions to address limited health literacy may increase adherence and reduce health resource utilization in this chronically ill patient population.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO640

Relationship between Outpatient "AKI", Proteinuria, Diagnosis Diabetes, Race and Rate of Decline in Renal Function, Nine Years Prior to Dialysis Initiation Steven J. Rosansky,[1,2] James W. Hardin,[2] Frankie Richards,[1] Kathlyn Sue Haddock,[1] Ann M. O'Hare,[4] William F. Clark.[3] *[1]Research, Dorn Research Institute, Columbia, SC; [2]Biostatistics, University of SC School of Public Health, Columbia, SC; [3]Nephrology, University of Western Ontario, London, ON, Canada; [4]Nephrology, VA Seattle, Seattle, WA.*

Background: AKI has been examined as a discharge coded, inpatient phenomenon. Predialysis creatinine based e GFR is commonly used in the decision to initiate dialysis,which may not account for AKI episodes and temporarily higher serum creatinine . Proteinuria is a primary determinant of renal function trajectory.

Methods: The current study examines the change in MDRD e GFR (ml/min/1.73m^2/yr) using initial and final serum creatinine, to determine the change of renal function by: AKI; diagnosis diabetes; urine proteinuria and race. Growth curve analysis examined the effect of covariates on renal function decline. We studied 212 chronic dialysis patients with at least: one urinalysis, ≥3 creatinine values over ≥3 years. The method of creatinine determination was the same throughout the study 1989-2008. Diabetics were identified by any HgbA1 C of ≥ 6%. Quarterly "AKI"episodes were defined by sequential serum creatinine values of a 50 percent increase from the prior serum creatinine in a 90-day window.

Results: In the study population:one AKI occurred in 67%, 2 or more in 23%; 50% were diabetic; 9.2 years follow-up:mean of 41 creatinine and 20 urinalysis measures per patient. Patients were divided by : <2plus protein; ≥2plus protein(using all UA data); 3 AKI groups: none, 1, 2 or more episodes; diabetic/non diabetic,white/non white.

Pre-dialysis Change in Renal Function

Co-Variates	N	ml/min/1.73m^2/yr
Urinalysis Results		
< 2+ proteinuria	29	6.0
≥ 2+ proteinuria	183	6.26
AKI Episodes		
None	68	6.27
One	92	6.58
Two or more	52	5.59
Diagnosis Diabetes		
Yes	103	6.0
No	109	6.46
Race		
White	139	6.58
Black	74	5.47

Conclusions: In conclusion, there was no significant difference in predialysis rate of e GFR decline by AKI frequency,proteinuria,race ,diagnosis diabetes.Nevertheless,AKI episodes must be considered in the evaluation of predialysis e GFR data and the decision to initiate dialysis

Funding: Veterans Administration Support

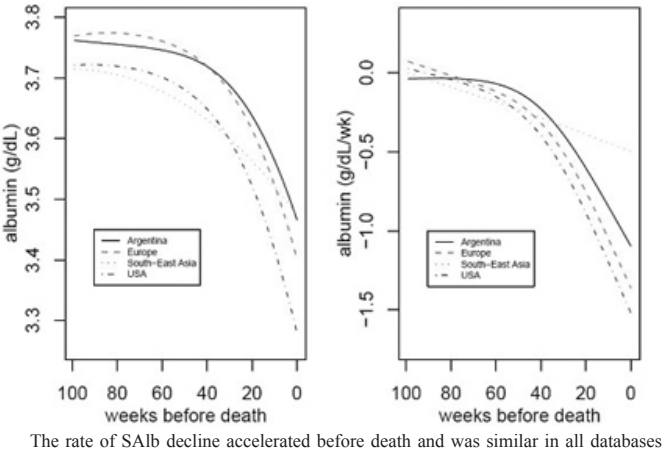
TH-PO641

Serum Albumin Dynamics before Death in Chronic Hemodialysis (HD) Patients – Results of an International Study Michael Etter,[1] Yuedong Wang,[2] Claudia Barth,[3] Mathias Schaller,[7] Adrian Marcos Guinsburg,[4] Daniele Marcelli,[5] Cristina Marelli,[4] Adam Tashman,[2] Len A. Usvyat,[6] Nathan W. Levin,[6] Peter Kotanko.[6] *[1]FMC Asia Pacific, Hong Kong, Hong Kong; [2]University of California - Santa Barbara, Santa Barbara, CA; [3]Kuratorium für Dialyse und Nierentransplantation, Cologne, Germany; [4]FMC Latin America, Buenos Aires, Argentina; [5]FMC Europe, Bad Homburg, Germany; [6]RRI, NY, NY; [7]University of Cologne Medical Center, Germany.*

Background: HD patients (pts) experience mortality rate between 14-20% per year. In a US HD cohort a decline of serum albumin (SAlb) before death was described (Kotanko 2009). Here, we investigate if the SAlb decline holds for a globally diverse HD population.

Methods: HD databases from FMC clinics in Europe, Asia, Latin America, RRI clinics in US, and KfH in Germany were queried. SAlb was standardized to the BCG. Pre-death SAlb dynamics were analyzed by estimating the mean SAlb level before death and its 1st derivative using quintic splines.

Results: 27807 HD pts from 23 countries were studied (Europe 17 [N=12333; age 71.7, 59% males]; South-East Asia 4 [N=1484; age 68; 53% males]; Argentina [N=10517; age 63.1; 58% males]; USA [N=3473; age 69.9; 56% males]). SAlb levels prior to death [mean (SD); g/dL] were 3.53 (0.63); 3.45 (0.59); 3.63 (0.54); 3.3 (0.62); same order as above. In all regions, SAlb levels dropped between 0.24 (South-East Asia) and 0.44 (USA) mg/dL in the 2 years preceding death in female pts (left).



The rate of SAlb decline accelerated before death and was similar in all databases (right).

The results were identical in male pts and in pts from KfH (not shown).

Conclusions: This international study corroborates previous findings in US HD pts. Insights into the pre-death biology are key to the development of alert systems to facilitate timely diagnostic and therapeutic interventions.

TH-PO642

Frequency of Pulmonary Embolism and Associated Mortality in Patients with Chronic Kidney Disease and End Stage Renal Disease Ankit Sakhuja, Gagan Kumar, Puneet Sood, Rahul S. Nanchal, Aaron T. Dall. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Background: Chronic Kidney Disease (CKD) and End Stage Renal Disease (ESRD) are growing problems in the United States. Studies are not clear with respect to frequency of Pulmonary Embolism (PE) in these patients.

Methods: We analyzed the data from the Nationwide Inpatient Sample database from the year 2007. Adult patients with ESRD, CKD and PE were identified using appropriate ICD-9-CM codes as previously used. The primary outcomes measured were frequency of PE in patients with CKD and ESRD. Secondary outcome was all cause in-hospital mortality.

Results: Of 32,759,253 estimated adult discharges, 30,119,186 had normal kidney function, 1,799,785 had CKD and 840,282 had a discharge diagnosis of ESRD. The unadjusted frequency of PE was 0.97% in patients with CKD and 0.59% in patients with ESRD in comparison to 0.83% in patients with normal renal function ($p < 0.001$). On multivariate regression analysis, patients with CKD are more likely to have a PE (OR 1.27; 95% CI: 1.14 - 1.42) and those with ESRD are less likely to have a PE (OR 0.69; 95% CI: 0.61 - 0.78) when compared to patients with normal renal function. The all cause in-hospital mortality in patients with PE increased with decline in renal function. 7.2% of patients with normal renal function who had a PE died, this number rose to 12.6% for those with CKD and 17.2% for those with ESRD ($p<0.0001$).

Conclusions: Patients with CKD have a higher frequency of PE whereas patients with ESRD have a lower frequency of PE in comparison to those with normal renal function. The all cause in-hospital mortality in patients with PE is higher in those with CKD in comparison to those with normal renal function and is highest in those with ESRD.

TH-PO643

The Effect of Censoring for Transplantation When Analyzing Survival on Dialysis: Applying Competing Risk Analysis to ERA-EDTA Registry Data
Marlies Noordzij, Karlijn J. Van Stralen, Kitty J. Jager. *ERA-EDTA Registry, Department of Medical Informatics, Academic Medical Center, Amsterdam, Netherlands.*

Background: When studying patient survival on dialysis treatment, kidney transplantation (Tx) is often considered as a censored observation, while it is in fact an event that competes with the occurrence of death. Kaplan-Meier (KM), a conventional method for unadjusted survival analysis, can handle only one outcome and may therefore be inappropriate in the presence of competing risks. We therefore compared the results of KM with those of the Cumulative Incidence Competing Risk (CICR) method; an alternative method specifically designed for analyzing competing risks.

Methods: Based on data of incident dialysis patients from countries participating in the ERA-EDTA Registry we studied 1, 2, and 5 years patient survival of patients starting dialysis between 1999 and 2003. The probabilities of separate events, i.e. death, Tx and event-free survival (EFS) from day 91 after the start of dialysis onwards were calculated using both the standard KM and the CICR methods.

Results: Data analysis was based on 69,081 patients from Europe. When studying 1-year patient survival on dialysis, both methods yielded similar results, i.e. probabilities of death, Tx and EFS of 17%, 7%, and 76%, respectively, with an expected total of 100%. For 2-years survival the KM method yielded probabilities of 31% for death, 16% for Tx and 58% EFS (total 105%). These probabilities were 29%, 13% and 58%, respectively, for the CICR method (total 100%). Finally, after 5-years of follow-up, the difference between the methods was even more pronounced. The probabilities of death (61%) and Tx (34%) were overestimated by KM (EFS 25%) to a total of 120%, while the CICR method yielded lower probabilities of 51%, 24% and 25%, respectively, adding up to 100%.

Conclusions: The KM method overestimates the risk of both mortality and Tx with percentages as high as 10% at 5 years after the start of dialysis Therefore, this method is inappropriate to analyze patient survival in the presence of competing risks and using the CICR method is recommended.

TH-PO644

Time to Death from Cessation of Dialysis in an Elderly Veteran Population
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Background: An extensive review shows a paucity of literature on time to death after cessation of dialysis in end stage renal disease (ESRD) patients. A common question nephrologists often face from patients is the length of time before death after dialysis is withdrawn. In a report by Murray et al in 2006 using the United States Renal Data System (USRDS) data from 2001 to 2002, the time to death after stopping dialysis was not looked at.

Methods: We conducted a single center retrospective chart review of 174 adult veteran dialysis patients between January 2002 and May 2011 to determine length of time until death after withdrawal from dialysis. We also looked at DNR status, place of death, and vascular access at death.

Results: The 174 patients had a mean age of 69.0±10.9 years and the following characteristics: 97.1% male, 68.4% diabetic, 93.7% hypertensive, and 59.8% had coronary artery disease. Diabetes and hypertension were the most common cause of renal failure and accounted for 65% of the patients. Mean duration on dialysis was 3.25 years, and vascular access at death was catheter (43.7%) or fistula (48.8%). 74.7% of patients had a DNR, of which only 1.7% did not have it honored. 37.9% of patients withdrew from dialysis. Mean time to death after stopping dialysis was 10.7 days with a standard deviation of 15.5 days. Place of death was 48.3% hospital, 33% hospice, 6.9% nursing home, 5.2% home, 4.6% dialysis unit.

Conclusions: Our results show that withdrawal from dialysis (37.9%) is much more common than that reported by the USRDS. This could be due to the characteristics of our cohort, an elderly veteran population. Mean time to death after cessation of dialysis (10.7 days) is also slightly higher than that reported in the literature. Further studies regarding withdrawal of dialysis as well as patient and family satisfaction with the dying process are needed.

TH-PO645

Cause of Death in a Veteran Dialysis Population: Death Certificate vs. Chart Review
Rizwan Badar,[1,2] Xiao Wan,[1,2] Seyed-Ali Sadjadi,[1,2] Navin Jaipaul,[1,2] Ruth Peeters.[1] *[1]Nephrology, VA Loma Linda Healthcare System, Loma Linda, CA; [2]Nephrology, Loma Linda University Medical Center, Loma Linda, CA.*

Background: According to USRDS, "other" is the most common cause of death (COD) in dialysis patients with ESRD, followed by cardiac death. This information is gathered from the ESRD Death Notification Form 2746, which is completed by nephrologists, other physicians, or non-physicians. Often times the death certificate (DC), which is frequently inaccurate, is used to obtain this information. Determining the COD is difficult due to co-morbidities, low autopsy rate, and lack of physician training.

Methods: We conducted a single center retrospective chart review (CR) of 174 adult veteran dialysis patients between January 2002 and May 2011 to determine the correlation between CR and DC COD. A panel of trained physicians determined the CR COD and extracted the DC COD, and the two were categorized and compared by calculating the Pearson correlation coefficient (r). Statistical analysis was performed using SPSS, version 17.0.

Results: The 174 patients had a mean age of 69.0±10.9 years and the following characteristics: 97.1% male, 68.4% diabetic, 93.7% hypertensive, and 59.8% had coronary artery disease (CAD). Mean duration on dialysis was 3.25 years, and vascular access at death was 43.7% catheter vs. 48.8% arteriovenous. The most common categorized COD by CR and DC are shown in Table 1. The correlation between CR and DC COD was statistically significant ($p<0.001$) but weak ($r= 0.42$).

Table 1. Cause of Death* by Death Certificate vs. Chart Review

	DC COD	CR COD
Cardiac, n(%)	45(37.5)	24(20.0)
Infectious, n(%)	37(30.8)	41(34.2)
Other, n(%)	22(18.3)	35(29.2)
Malignancy, n(%)	11(9.2)	11 (9.2)
Vascular, n(%)	5(4.2)	9(7.5)

*54 patients with missing DC were excluded

Conclusions: Our findings demonstrate poor correlation between COD by CR and DC. In addition, infection and not "other" or CAD, was the leading cause of death in this elderly veteran dialysis population. We speculate that death certificates are often inaccurate due to lack of physician training and bias in coding, and that ESRD death notification form shortcomings limit the ability to properly report the accurate cause of death.

TH-PO646

Health Related Quality of Life and All-Cause Mortality in Patients with Diabetes on Dialysis
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Background: The purpose of the study was to compare HRQOL in dialysis patients with and without diabetes (DM), and assess the mortality risk. Secondly, to assess HRQOL in diabetes patients on dialysis (DDM) with non-dialysis diabetes patients (DM) and patients with diabetic foot ulcers (DFU).

Methods: A 36-item Short-Form Health Survey 36 (SF-36) was used in 301 prevalent dialysis patients of whom 78 had DM, and in 221 nondialysis patients with DM and 127 with DFU, and compared with a general population sample (n=5903). Physical and mental component summary scores (MCS/PCS) were calculated in dialysis patients. Kaplan Meier plot and Cox proportional hazard model were used for mortality analysis in dialysis patients, and data censored for renal transplantation.

Results: DDM had lower HRQOL than other dialysis patients (MSC 46 (IQR 38 -54) vs 50 (40-57), $p=0.04$ and PCS 33 (28-43) vs 37(31-45) $p=0.05$). During follow-up (median 3.6 years), cumulative survival was reduced for DDM (log rank test, $p =0.04$), and DM remained a significant predictor for mortality (hazard ratio 1.59 (95% CI 1.02-2.46) after multiple adjustments. There were significant differences in the eight SF-36 subscales between the DDM, DFU, DM and the general population sample ($p<0.001$ for all, ANOVA), and the differences were most pronounced for physical function, role limitation physical, general health (GH) and vitality (VT). DDM differed significantly from DFU in GH 37 (33-42) vs 46 (41-40) ($p<0.001$) and VT 39 (34-44) vs 47 (43-51) ($p=0.001$)

Conclusions: The very low perceived PCS and MSC in dialysis patients with DM compared to nonddiabetes patients in dialysis and even to non-dialysis patients with DM, and the reduced survival of diabetes patients in dialysis, should lead to increased focus to prevent progression to end stage renal failure, and increased use of preemptive renal transplantation. Physical exercise training should be utilized to overcome or assist in physical function impairment in this patient group.

TH-PO647

Mortality Difference between Older and Younger ESRD Patients Is Smaller 2 Years after Dialysis Initiation Compared to the First 2 Years of RRT
Joachim H. Beige. *Nephrology and KfH Renal Unit, Hospital St. Georg, Leipzig, Saxony, Germany.*

Background: Patients with end.stage renal disease (ESRD) requriring renal replacement therapy (RRT) are subjected to an excess mortality risk compared with general population. In the older ESRD patients, such risk is furtherone exaggerated. That issue impacts on decision-making on the initiation of RRT in the elderly. Data are needed, to estimate mortality in older RRT patients.

Methods: KAPLAN-MEIER survival analyis of all patients of a chronic dialysis unit (hemo- and peritoneal dialysis) affiliated with a nephrological department of an academic teaching hospital from 2000 to 2010 (n=1679). Calculation of time from initiation to death and from initiation plus 2 years to death. Censoring of all non-death dialysis stop events (change to other center, change to transplantation, change to renal function, lost of follow-up).

Results: There was a strong significant overall mortality difference in patients 65 y or younger compared to patients over 65 y (Fig., LogRank $p<0.0001$). However, mortality difference in third, fourth, fifth and sixth year after RRT initiation in younger patients (0.10; 0.11; 0.11; 0.18) compared to older patients (0.24; 0.27; 0.19; 0.35) was not as strong as in the the first 2 years ($p=0.04$).

Conclusions: The excess mortality of ESRD patients >; 65 y in need of RRT compared to younger ESRD patients peaks during the first two years after RRT initiation. After 2 years,

patients in their high seventh or eighth life decade exhibit a yearly mortality of about 20%. From such epidemiological reasoning, stable long-term RRT in the elderly is a satisfying, life-prolonging and adequate treatment modality.

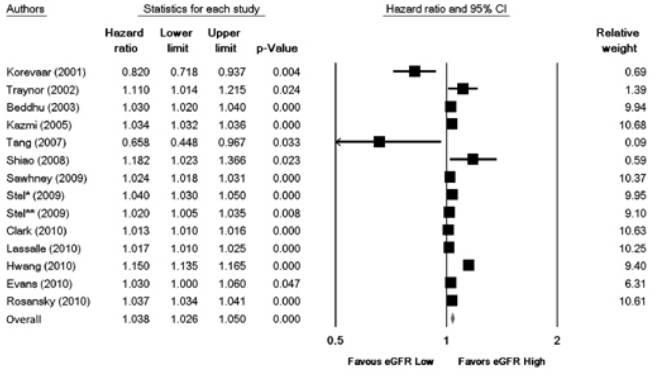
TH-PO648

Estimated Glomerular Filtration Rate (eGFR) at Initiation of Dialysis and Mortality in Patients with Advanced Chronic Kidney Disease (CKD): A Meta-Analysis Paweena Susantitaphong, Sarah Altamimi, Motaz Ashkar, Bertrand L. Jaber. *Medicine, St. Elizabeth's Medical Center, Boston, MA.*

Background: The number of patients with advanced CKD initiating dialysis is on the rise worldwide. Recent data suggest that initiation of dialysis at higher eGFR may be associated with worse clinical outcomes. We conducted a meta-analysis to examine the association of eGFR at initiation of dialysis with mortality in patients with advanced CKD.

Methods: We searched for randomized controlled trials, prospective and retrospective cohort studies in MEDLINE through March 2011. We selected studies that focussed on early versus late initiation of dialysis (defined by eGFR or creatinine clearance) and mortality among patients with advanced CKD. Using random-effects model meta-analysis, we computed the adjusted hazard ratio of eGFR at initiation of dialysis and all-cause mortality.

Results: Sixteen observational studies and one randomized controlled trial were identified (n = 1,365,933). By meta-analysis, which was restricted to the 14 cohort studies with retrievable data (n = 468,188), higher eGFR (per 1 mL/min/1.73 m^2 increase) at initiation of dialysis was associated with a higher pooled adjusted hazard ratio (HR) for all-cause mortality (1.038; 95% CI 1.026, 1.050; P<0.001, Figure). Among the 7 studies that adjusted analytically for nutritional variables, higher eGFR remained associated with all-cause mortality (adjusted HR 1.028; 95% CI 1.013, 1.044; P<0.001) compared to those that did not (adjusted HR 1.053; 95% CI 1.034, 1.072; P<0.001).



*Cohort initiating dialysis in 1999

**Cohort initiating dialysis in 2003

Conclusions: This meta-analysis demonstrates that higher eGFR at initiation of dialysis is associated with a higher mortality risk among patients with advanced CKD, which is independent of nutritional status. This concerning observation requires further study.

TH-PO649

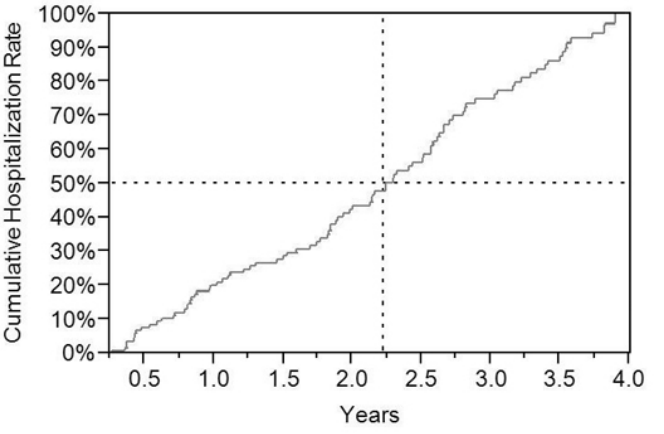
Hospitalizations in Older Incident Hemodialysis Patients Kimberly L. Schoonover,[1] LaTonya J. Hickson,[2] Suzanne M. Norby,[2] Marie C. Hogan,[2] Amy W. Williams.[2] *[1]Internal Medicine, Mayo Clinic; [2]Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Hospitalizations account for high morbidity in older chronic hemodialysis (HD) patients. Better understanding of the cause and predictors of hospitalization may provide a means for intervention.

Objective: To determine rate, cause and predictors of hospitalizations in older incident chronic HD patients.

Methods: Review of hospitalizations in older (age ≥65 years) HD patients initiating therapy from 2007-2009 and remaining on therapy for ≥3 months.

Results: Of 125 patients, mean age was 76±7 years, 72% were male, 52 % were diabetic, and 59% had coronary artery disease. At first HD, 41% had arteriovenous fistula/graft (AVF/G) present. 51% started HD in the hospital. Mean dialysis duration was 1.8±1.0 years. At least one hospitalization occurred in 90 (72%) patients had at least one hospitalization. Mean first hospital stay was 5±5 days. 67 (54%) had two, 46 (37%) had three, and 36 (29%) had four or more hospitalizations. The estimated cumulative hospitalization rate was 50% at 2.2 years (figure). Primary admission diagnoses for the first hospitalization included cardiovascular (32%), infection (17%), gastrointestinal (13%), access (6%), vascular (6%), neurology (4.5%), trauma (4.5%), endocrine (3%), pulmonology (3%), malignancy (2%), and other (9%). The most common cardiovascular diagnoses were acute coronary syndrome, atrial fibrillation, and heart failure exacerbation. Predictors of ≥1 hospitalization included: first dialysis location in hospital (HR 1.8, CI 1.1-3.0, p=0.02) and absence of (AVF/G) at first HD start (HR 1.9, CI 1.2-3.2, p=0.01).



Conclusions: Hospitalizations are common in older incident hemodialysis patients and are often recurrent. Location of dialysis initiation and patient preparedness for therapy may provide insight into degrees of risk for recurrent hospitalizations.

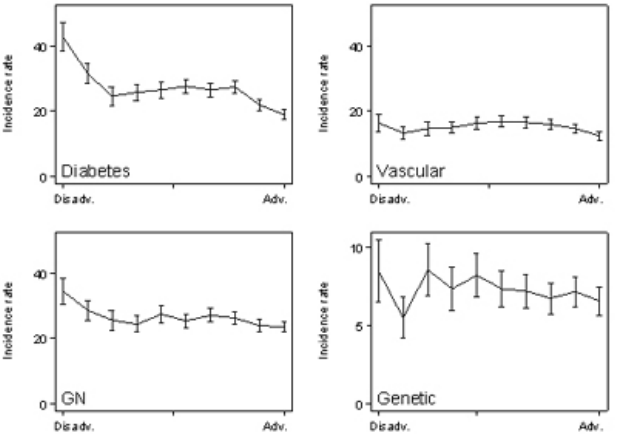
TH-PO650

Socioeconomic Gradients of ESKD Incidence Vary with Age and Primary Renal Disease Stephen P. McDonald, Philip A. Clayton, Blair S. Grace. *ANZDATA Registry, Adelaide, SA, Australia.*

Background: An inverse relationship between socioeconomic status (SES) and the incidence of renal replacement therapy (RRT) has been shown in several settings. In Australia, public hospitals provide access to RRT under a government-funded scheme without a cost barrier. However, previous studies have suggested an inverse relationship exists, not explained by higher RRT among Indigenous people in Australia. We examined relationships between SES and RRT incidence across various groups, including age and primary renal disease.

Methods: Residential postcodes of non-Indigenous people who started RRT in Australia from 1999 to 2009 (N=19,411) were mapped to SES using standard concordance files from the Australian Bureau of Statistics. RRT incidence rates were compared across SES deciles using Poisson regression to adjust for age, gender and year.

Results: The overall incidence rate of RRT decreased with increasing SES advantage (RR per decile 0.96 [95% CI 0.96 – 0.97]). A more marked gradient was noted for ESKD attributed to diabetic nephropathy (in contrast there was lesser or no gradient observed for reno-vascular or genetic diseases).



Trends across the SES spectrum were similar for males and females. However, the SES gradient was more marked among people aged 40-69 years (RR 0.94 [0.94 – 0.95] per decile) than older people (RR per decile 1.00 [1.00–1.01]). The gradient was also more marked among those who lived in major cities (RR per decile 0.90 [0.88–0.92]) compared with more remote locations (RR per decile 0.98 [0.92–1.02]).

Conclusions: A clear, inverse relationship exists in Australia between rates of RRT and SES. The differential is most marked among those with diabetic nephropathy: either the incidence of diabetes is higher among people with lower SES, or diabetic nephropathy progresses more rapidly. The SES effect is most marked among people of working age, and attenuated among older people.

Funding: Government Support - Non-U.S.

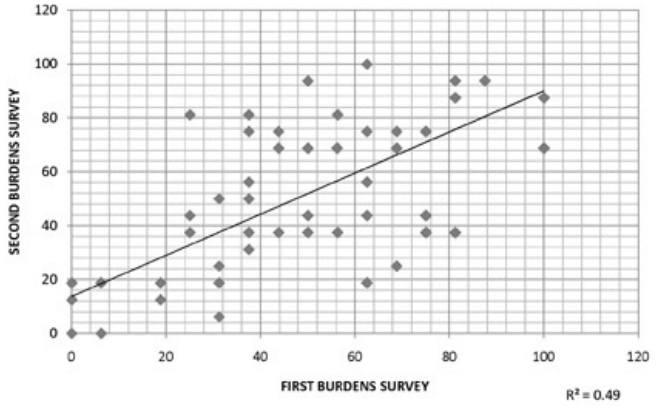
TH-PO651

Lessons Learnt from Consecutive Health-Related Quality of Life (KDQOL-SF) Surveys in a Mayo Clinic Stable Hemodialysis Patient Population Macaulay A. Onuigbo.[1,2] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI.*

Background: The Kidney Disease Quality of Life Short Form (KDQOL-SF), a well validated epidemiological research tool, is a short-form health survey that measures ESRD patients' self-assessment of functioning and well-being as determined by 3 component scores of physical component summary (PCS), mental component summary (MCS) and kidney disease component summary. More recently, there is increasing application of KDQOL-SF scores as a measure of the ESRD patients' health-related quality of life (HRQOL).

Methods: A prospective ongoing administration of KDQOL-SF assessments by certified and trained Dialysis Social Workers as part of ongoing established KDQOL care of HD patients was repeated in 56 patients between October 2008 and September 2010, at least six months between the two consecutive assessments.

Results: A total of 112 administrations of the KDQOL-SF assessments to the 56 HD patients were analyzed; they were administered a mean of 13 (6-22) months apart. PCS mean score increased from 32 to 34 and mean of burden score increased from 47 to 49. Overall, all the indices showed a trend towards improved higher scores at the second testing. When fortuitously, the second testing was administered within days of an acute illness or hospitalization, patient scores were reduced, albeit transiently.



Conclusions: In general, HRQOL scores in stable HD patients tend to get better with more time on dialysis. More of such studies are warranted to enhance the use of this research tool in the improved care of ESRD patients on maintenance HD. Such surveys must be avoided within 30 days of acute illnesses and hospitalizations.

TH-PO652

Usefulness of Assessing Dialysis-Related Symptom by Serum Level of β2-Microglobulin as a New Surrogate Marker Evaluating the Quality of Life among Dialysis Patients Atsuhiro Kanno, Ikuto Masakane, Satoko Ito, Minoru Ito, Kiyotaka Yabuki. *Yabuki Hospital.*

Background: The level of β2-microglobulin [β2-M] is strongly associated with the presence of carpal tunnel syndrome and dialysis-related amyloidosis in chronic dialysis patients. However, it is not fully clarified whether serum level of β2-M is related to the presence of other common problems among dialysis patients, though history of dialsis is not necessarily a long-term.

Methods: A total of 342 patients performed with chronic maintenance hemodialysis [HD] or hemodiafiltration [HDF] in our three facilities were participated and they were evaluated by the self-rating scored questionnaire based on fifth graded face scales from 0 (none) to 4 (very strong) according to the severity of symptoms. They include 20 physical and psychological common dialysis-related symptoms. Odds ratio for each of moderate to severe (score = 2, 3, 4) or severe (score = 3, 4) levels of these complaints were calculated using a multiple logistic regression model adjusted for confounding factors including age, gender, years of dialysis, history of diabetes mellitus, Kt/V, normalized protein catabolic rate, body mass index, predialytic values of hemoglobin, albumin, β2-M, sodium, potassium, phosphate.

Results: Among participants, 146 (42.7%) patients were treated with HDF and the overall mean values were 66.7 years for age and 19.3% of participants had a history of dialysis for more than 10 years (median 5.4 years). There was a significant relationship between β2-M and moderate to severe lethargy (odds ratio [OR] 1.02, P =0.024), loss of interest (OR 1.05, P =0.0028), reduction of blood pressure during dialysis (OR 1.06, P =0.0056) and a sense of thirst (OR 1.06, P =0.0028).

Conclusions: In our study, it was shown that predialytic serum level of β2-M was significantly associated with various dialysis-related symptoms. There is a possibility that β2-M would be a useful clue for the presence of common complaints, though history of dialysis was not necessarily a long-term. It is considered to be verified that an effort to diminish the level of β2-M, as a surrogate marker evaluating the quality of life, would contribute to relieve these symptoms.

TH-PO653

Frailty Is Associated with Earlier Dialysis Initiation in End-Stage Renal Disease Yeran Bao,[1] Lorien S. Dalrymple,[2] Glenn M. Chertow,[3] George A. Kaysen,[2] Kirsten L. Johansen.[1] *[1]Medicine, UCSF, San Francisco, CA; [2]Medicine, UC Davis, Davis, CA; [3]Medicine, Stanford University, Palo Alto, CA.*

Background: Over the last decade, patients have been started on dialysis at progressively higher levels of residual kidney function (early start). Some have suggested higher mortality with early start, while others argue that this could reflect a tendency for sicker patients to start early. We hypothesized that frailty, a predictor of adverse outcomes in end-stage renal disease (ESRD), is associated with dialysis initiation at a higher level of kidney function.

Methods: We undertook a cross-sectional study using data from the Comprehensive Dialysis Study (CDS), a United States Renal Data System (USRDS) special study. We examined the baseline prevalence of frailty, its associated clinical characteristics, and its relationship with early start of dialysis. Frailty was defined using a modification of Fried's criteria. Early start was defined as starting dialysis at an estimated glomerular filtration rate (eGFR)>10 ml/min/1.73 m^2. We used multivariate logistic regression to model the relationship between clinical characteristics and frailty and the association between frailty and early start.

Results: Among a total of 1,576 CDS participants included in the study, the prevalence of frailty was 73.3%. Female gender, being on Medicaid, higher disease burden, and higher eGFR were associated with higher odds of frailty. Each 1 ml/min/1.73m^2 increase in eGFR at dialysis initiation was associated with an 8% higher odds of being frail (OR 1.08, 95% CI 1.05-1.11, p<0.001). When the association between frailty and early start was examined, frail patients were nearly twice as likely to start dialysis at an eGFR>10 ml/min/1.73 m^2 (OR 1.91, 95% CI 1.50-2.44, p<0.001) after adjusting for age, gender, race, Medicaid status, tobacco use, serum albumin, serum hemoglobin, early nephrology referral, erythropoietin use and disease burden.

Conclusions: In light of the recent trend of early start and the lack of evidence to support it, our study highlights that frailty may be an important clinical measure as we assess the timing and effectiveness of renal replacement therapy.

Funding: NIDDK Support, Veterans Administration Support

TH-PO654

HIV Infection in ESRD (1995-2007): As Incidence Falls, Prevalence Rises Paul L. Kimmel, Dmitry Vishniakov, Paul W. Eggers. *NIDDK, NIH, Bethesda, MD.*

Background: Access to highly active antiretroviral therapy (HAART) since 1996 has changed HIV infection from a fatal to a chronic illness. Previous ESRD hemodialysis patient studies showed incidence rates plateaued in the 1990's. We report updated trends in incidence, hospitalizations and prevalence of ESRD HD HIV infection.

Methods: A retrospective cohort study of HIV infected ESRD patients was performed, using the CMS Medical Evidence Form where HIV was listed as a primary cause of ESRD and/or Medicare billing data where ESRD HD patients had at least 1 hospitalization or 2 outpatient encounters with an HIV/AIDS diagnosis.

Results: The number of incident cases of ESRD HD HIV infection was relatively stable (mean 816±45 per year), but the incidence rate decreased from 3.0 (1995) to 2.7 per million US population (2007), and from 1.14 to 0.67% of incident ESRD patients (1995 to 2007). Mean incident age increased from 39.0±0.3 to 44.7±0.4 y, black patients decreased from 90.4 to 86.2%, and women increased from 25.3 to 36.0% of the incident population. Median survival tripled from 0.9 (1995) to 2.72 y (2005). In contrast, prevalent HIV ESRD increased linearly from 1,971 to 6,741 cases. Prevalence rate increased from 7 to 12 HIV infected per 1000 ESRD patients from 1995 to 2007 respectively (0.3 to 0.6% of the ESRD HD population). The ESRD program hospitalization rate was 0.218 in 2007, while in HIV infected patients it was 22.4% higher (0.267). There was an approximately 2.5 fold increase in the number of hospitalizations for infection in HIV patients compared to uninfected patients, a disparity not seen in other diagnostic groups.

Conclusions: While the incidence of HIV infection has remained numerically stable, and has decreased in comparison to the increase in the total ESRD incident population, this does not explain the program prevalence of HIV infection. Survival has increased dramatically, leading to doubling in the albeit low prevalence of HIV infection in the ESRD population over the past decade. While access to HAART in the US has resulted in a decrease in incidence, the number and age of HIV infected patients has grown as patient survival improves. HIV infection in HD patients will remain a concern for the foreseeable future.

Funding: NIDDK Support

TH-PO655

Risk of Infection-Related Hospitalizations by Dialysis Modality: A Propensity-Score Matched Cohort Jean-Philippe Lafrance,[1,3] Elham Rahme,[2] Sameena Z. Iqbal,[2] Michel Vallee.[1,3] *[1]Université de Montréal, Canada; [2]McGill University, Canada; [3]Centre de Recherche Hôpital Maisonneuve-Rosemont, Canada.*

Background: Infection is the second leading cause of admission after cardiovascular disease among patients receiving maintenance dialysis. How peritoneal dialysis (PD) compare to hemodialysis (HD) in terms of overall infection-related hospitalization risk is not known.

Methods: We conducted a retrospective cohort study using a database linking administrative databases and a dialysis registry in Quebec, Canada, between January

2001 and December 2007. We included all incident adult patients with at least 90 days of dialysis. Patients with a pre-emptive kidney transplant were excluded. A propensity score for dialysis modality was estimated by multivariable logistic regression for each patient using demographics, body mass index, smoking, co-morbidities, and initial laboratory data. PD patients were 1:1 matched to HD patients by this propensity score. Risk of infection-related admissions was estimated using a counting-process survival model by Prentice, Williams and Peterson, which accounts for recurrent outcomes. Missing data was imputed using a multiple imputation technique.

Results: At 90 days after dialysis initiation, we identified 925 patients on PD and 4933 patients on HD. 1830 patients were matched (915 PD and 915 HD). All variables were well balanced in each group including: Mean age (PD: 58.7 vs HD: 58.4 years), male sex (PD: 58.8% vs HD: 59.5%), and diabetes (PD: 40.6% vs HD: 40.0%). During the study period, 338 patients were hospitalized once for an infection, 119 twice, and 101 at least three times. PD was associated with an increased risk of infection-related hospitalizations compared to HD (unadjusted hazard ratio: 1.65, 95% confidence interval: 1.43, 1.89).

Conclusions: PD patients are at higher risk of infection-related hospitalizations than HD patients. Better knowledge of epidemiology of infection-related hospitalizations may help to prevent this high-burden morbidity among chronic dialysis patients.

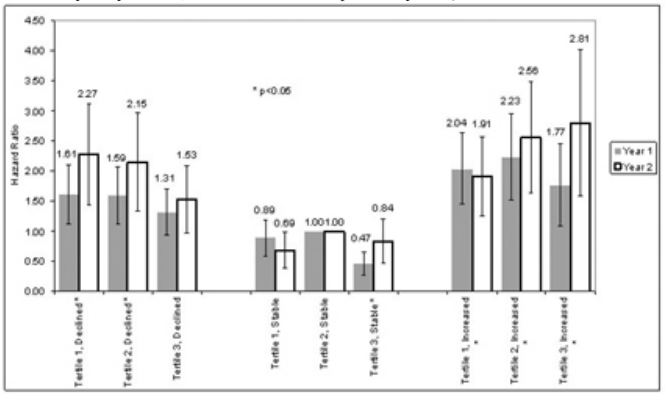
TH-PO656

Body Temperature in Hemodialysis (HD) Patients: A Novel Indicator of Patient Outcomes Len A. Usvyat,[1] Frank Van der Sande,[2] Jeroen Kooman,[2] Jochen G. Raimann,[1] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute, NY, NY; [2]University of Maastricht Hospital, Netherlands.*

Background: Pre-HD body temperature (BT) is important routine indicator, however, only BT notably below or above normal range is usually considered relevant. Since little is known about the significance of BT changes over time in HD patients (pts), this study aimed to explore this area.

Methods: Data of all RRI HD pts beginning HD b-n Jan 1, 2000 and Feb 28, 2010 were analyzed. Pts' baseline pre-HD BT (BTBL) was computed as an average of the first 30 days on HD; the slope of BT change (BTSLOPE) was computed by linear regression using all BT in the 1st yr. Pts were stratified according to (a) BTBL tertile: <36.47, 36.47 to 36.71, >36.71 °C, and (b) BTSLOPE: increase: >0.01 °C/month, decrease: <-0.01 °C/month, and stable: BT between -0.01 and +0.01 °C/month. Cox proportional hazards models adjusted for demographics, labs, and multiple comorbids were constructed to assess the relationship b-n BT and BTSLOPE and survival in the 1st and 2nd year on HD.

Results: 7003 pts were studied (avg age 62.1 years; 57% males, 53% white). Cox analysis demonstrated that pts with stable BT have better outcomes, while patients with increasing BT have poorest outcomes in the first year of dialysis. Over all three BTBL tertiles, pts with stable BTSLOPE had the best outcomes (hazard ratios of 0.89, 1.00, and 0.47, respectively). The relationship between BTSLOPE and BTBL groups did not change materially in pts who survived to the second year on HD, using BTBL calculated in the first 30 days of year two, and BTSLOPE computed in year 2).



Conclusions: Increases and to a lesser extent decreases in BT from all BTBL levels are associated with a unfavorable effect on survival. Dynamics of BT in incident HD pts should receive attention as a novel potential indicator of outcomes in HD pts.

TH-PO657

Diabetic End Stage Renal Disease in the Indigenous Population of Guam Saied Safabakhsh, Andrew Charfauros, Joseph Chargualaf, Brittany L. Balajadia, Ali H. Ahangari. *Micronesian Institute for Disease Prevention & Research.*

Background: Diabetic Nephropathy is the most common cause of patients acquiring End Stage Renal Disease (ESRD) in the United States. The purpose of our study was to establish the frequency, familiar patterns, and common complications of diabetic ESRD patients in the indigenous (Chamorro and other Pacific Islanders) population of Guam.

Methods: This retrospective study was conducted at four Hemodialysis (HD) centers on Guam. Relevant information from their medical records were recorded and patients participated in a 5 minute questionnaire. Patients with incomplete data were removed from the study.

Results: The total number of subjects in all four HD centers was 410 and a total of 334 subjects were accounted for during finalization of data. 263 (79%) of all subjects were diabetic and 246 (74%) of all subjects were Pacific Islanders. Of the 246 Pacific Islanders, 208 (84%) were diabetic, 180 (87%) of the diabetic Pacific Islander subjects had a family history of diabetes, while 92 (44%) had a family history of kidney disease in their first degree relatives. 48 (75%) of the Pacific Islanders had peripheral vascular disease (PVD), 158 (68%) had retinopathy and 148 (66%) had coronary artery disease (CAD).

Conclusions: Diabetes is the number one cause of ESRD and has a strong familiar pattern among the indigenous population of Guam. Complication of diabetes including CAD, PVD and retinopathy are highly prevalent among Pacific Islanders in Guam. This may be due to late diagnosis, poor diabetic management and possibly genetic predispositions involved in this population.

TH-PO658

Hemoglobin A1C Levels among Diabetic ESRD Patients with Possible Relationship to a-Erythropoietin Therapy and Inflammation Saied Safabakhsh, Jamie Pangelinan, Josephine Meno, Brittany L. Balajadia, Ali H. Ahangari. *Micronesian Institute for Disease Prevention & Research.*

Background: The objective of this study was to determine if Erythropoietin (a-Erythropoietin) therapy and inflammation affected A1C levels among Diabetic End Stage Renal Disease (ESRD) patients.

Methods: All subjects with ESRD at the three free standing dialysis units on Guam, a U.S. territory in Micronesia, were identified. Data from their medical records were collected and patient questionnaires from all subjects were completed.

Results: Out of 410 total subjects on dialysis, 341 participated in the study. 267 (78%) is caused by diabetes, 43 (13%) is caused by hypertension, 16 (5%) by Glumerolonephritis, 12 (3%) were f other causes. The average A1C level among diabetic subjects was 7.0 (ranging from 2 to 12.7). The average random blood sugar on three different occasions was 189 g/dL (ranging from 60 to 637). Out of 341 subjects, 247 (72%) were on Erythropoietin Therapy (500-20,000 units/treatment). Out of 247, 159 were diabetic subjects. Out of 159 diabetic subjects, 101 (67%) had high Ferratin levels (greater than 500 units). The average Ferratin level was 831 units (ranging from 16 to 1,971). Computed analysis graphs showed that A1C levels did not correlate with average random blood glucose levels with respect to Erythropoietin dosage and inflammatory assays separately.

Conclusions: Our results indicate that A1C levels may be affected by Erythropoietin Therapy and acute or chronic inflammation (established by high Ferratin levels). A1C readings alone may not be a good method for the management of diabetic dialysis patients, nor is it a good indicator of true glucose control among patients who have inflammation or are on Erythropoietin Therapy. We recommend combination of A1C and random blood sugars for better management of diabetes for patients who are on dialysis. Clinicians who care for diabetic dialysis patients should take into consideration the evaluation of patients for Erythropoietin dosage and inflammatory assays for better and more accurate management of their diabetes.

Funding: Private Foundation Support

TH-PO659

Low Serum Bicarbonate Is Associated with an Increased Risk of Death in the US Population Kalani L. Raphael, Guo Wei, Tom H. Greene, Alfred K. Cheung, Srinivasan Beddhu. *Internal Medicine, University of Utah, Salt Lake City, UT.*

Background: Low serum bicarbonate levels are associated with a higher risk of CKD progression and death in people with CKD, however, the association between low serum bicarbonate levels and death in the general population is unknown.

Methods: Adult NHANES III participants were grouped into one of four serum bicarbonate categories according to the baseline value: ≤20, 21-25, 26-30, and >30mM. The hazard of death for each group, compared to the 26-30mM group, was determined after adjusting for age, gender, race, CKD, albuminuria, CHF, lung disease, and diuretic use.

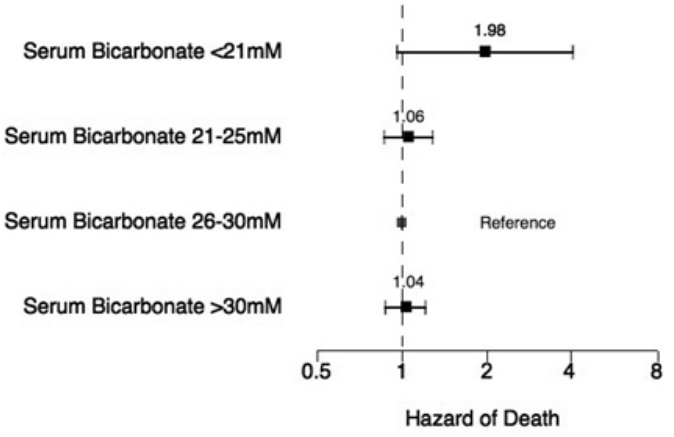
Results: Those with serum bicarbonate ≤20mM were more likely to be female, African-American, and have CHF, CKD, and higher albuminuria.

Characteristics of NHANES III participants by serum bicarbonate

Serum HCO3- (mM)	≤20 (0.7%)	21-25 (23.6%)	26-30 (52.2%)	>30 (23.5%)	p
Age(yrs)	44.7 (20.1)	42.0 (16.7)	45.2 (16.8)	46.6 (17.7)	<0.001
Men(%)	31.3 (21.0, 43.9)	36.9 (34.5, 39.3)	49.6 (47.9, 51.3)	55.4 (53.2, 57.6)	<0.001
African-American Race(%)	15.5 (9.2, 24.9)	12.3 (10.3, 14.6)	10.6 (9.3, 12.0)	9.4 (7.7, 11.5)	0.396
CKD(%)	17.0 (8.6, 30.8)	5.7 (4.6, 7.1)	5.5 (4.8, 6.3)	6.2 (5.4, 7.2)	0.004
Diuretic use(%)	8.5 (3.6, 19.0)	4.3 (3.3, 5.5)	7.2 (6.4, 8.1)	10.5 (9.0, 12.2)	<0.001
Albumin:Cr (mg/gm)	111.4 (690.7)	30.2 (217.3)	23.9 (171.1)	22.9 (172.0)	0.106

Percentages shown as percent (95% C.I.); Continuous measures shown as mean (standard deviation).

Compared to the reference group, those with serum bicarbonate ≤20mM had a two-fold increased hazard of death (HR 1.98, 95%CI 0.97-4.06), whereas no difference was detected in the 21-25mM and >30mM groups.



Conclusions: Serum bicarbonate levels ≤20mM appear to be associated with an increased risk of death in the general population even after adjusting for demographic factors, CKD and other possible confounders.

Funding: NIDDK Support, Other NIH Support - National Institutes of Health and the National Cancer Institute grant 1KM1CA156723

TH-PO660

Time-Dependent Effects of Dietary Sodium Bicarbonate on Renal Disease Progression in 5/6 Nephrectomized Rats Sejoong Kim,[1] Nam Ju Heo,[2] Yun Kyu Oh,[2] Ki Young Na,[2] Kwon Wook Joo,[2] Jin Suk Han.[2] *[1]Internal Medicine, Gachon University of Medicine and Science, Korea; [2]Internal Medicine, Seoul National University College of Medicine, Korea.*

Background: Sodium bicarbonate therapy ameliorated metabolic acidosis (MA) and the decrease in glomerular filtration rate (GFR) only after the control of blood pressure (BP) in 5/6 nephrectomized rats with casein diet. Recent clinical trials, however, showed beneficial effect of sodium bicarbonate in patients with early and late stage chronic kidney disease. We evaluated time-dependent beneficial effect of sodium bicarbonate to prevent the decline in GFR and correct MA in rats with a remnant kidney.

Methods: Sprague-Dawley rats ate dietary sodium bicarbonate (NaHCO3) or sodium chloride (NaCl) with 20% casein without any antihypertensive medication for 12 weeks after 5/6 nephrectomy.

Results: After treating casein diet, alkali-treated group had higher levels of serum bicarbonate than control group (20.3 ± 0.55 vs. 15.6 ± 0.61 mmol/L at week 4, 25.0 ± 1.73 vs. 13.1 ± 1.29 mmol/L at week 10, and 31.3 ± 3.38 vs. 17.0 ± 0.93 mmol/L at week 12). After week 4, systolic blood pressure in the two groups was over 160 mm Hg, and there was no difference between the groups. At week 4, glomerular filtration rate (GFR) in NaHCO3 group was higher than in NaCl group (0.36 ± 0.09 vs. 0.15 ± 0.01 mL/min/100g BW, p=0.011). After week 10, there were no differences in GFR between the two groups (0.14 ± 0.03 vs. 0.15 ± 0.04 mL/min/100g BW at week 10, and 0.07 ± 0.03 vs. 0.07 ± 0.01 mL/min/100g BW at week 12). At week 4 and week 10, glomerulosclerosis (GS) and tubulointerstitial damage indices (TI) in NaHCO3 group were less severe than in controls. In contrast, at week 12, GS in the alkali-treated group was more profound than in control group, and there was no difference in TI damage between the two groups.

Conclusions: Dietary sodium bicarbonate had short-term beneficial effects in ameliorating metabolic acidosis and preventing the decrease in GFR without correction of blood pressure. However, these effects did not sustain after 10 weeks.

TH-PO661

Dietary Determinants of Urinary Citrate Excretion Ernest I. Mandel,[1,2] Eric N. Taylor,[2,3] Gary C. Curhan.[1,2] *[1]Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [3]Division of Nephrology and Transplantation, Maine Medical Center, Portland, ME.*

Background: General strategies for prevention of kidney stones include restricting animal protein intake and increasing fruit and vegetable intake. While these strategies may increase citrate excretion by altering dietary acid-base balance, the independent dietary predictors of urinary citrate excretion have not yet been explored.

Methods: We conducted a cross-sectional study within a sub-cohort of 2612 individuals from the Health Professionals Follow-up Study (older men), Nurses' Health Study I (older women), and Nurses' Health Study II (younger women) who provided two 24-hour urine collections for previous studies of nephrolithiasis. Dietary data were ascertained from the 131-item Willett food frequency questionnaire. Lifestyle and clinical data were culled from responses to biennial questionnaires. Multivariable linear regression was used to quantify the predictors of urinary citrate excretion.

Results: After adjusting for dietary, lifestyle, and clinical factors, those in the highest quintile of non-dairy animal protein intake had urinary citrate excretion 148 mg/d (95% confidence interval, 70.9, 224.5), 76 mg/d (12.3, 139.0), and 57 mg/d (8.3, 122.3) lower than those in the lowest quintile for older men, older women, and younger women respectively. Conversely, those in the highest quintile of dietary potassium intake had citrate excretion 155 mg/d (79.3, 231.4), 121 mg/d (51.7, 189.3), and 56 mg/d (-16.4, 127.7) higher than those in the lowest quintile. Among older men, those with daily vitamin C intake of 1000 mg/d or more had citrate excretion 146 mg/d (47.7, 244.4) lower than those consuming less than 90 mg/d. Both dairy protein intake and urinary sodium excretion, as a marker of sodium intake, were associated with higher citrate excretion.

Conclusions: In addition to non-dairy animal protein and potassium intake, dietary intakes of vitamin C, dairy protein, and sodium are significant independent predictors of urinary citrate excretion in certain populations.

Funding: NIDDK Support

TH-PO662

Alkali Therapy from Prenatal Period Is Superior to Postnatal Alkalization in NBC1 W516X Knock-In Mice Featuring Severe Metabolic Acidosis and Early Lethality Yu-Wei Fang,[1,2] Sung-Sen Yang,[3] Min-Hua Tseng,[1] Shih-Hua P. Lin.[1,3] *[1]Graduate Institute of Medical Science, National Defense Medical Center, Taipei, Taiwan; [2]Division of Nephrology, Department of Medicine, Shin-Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan; [3]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan.*

Background: We have created the Na^+/HCO_3^- cotransporter 1(NBC1)W516X knock-in mice as model of isolated proximal renal tubular acidosis(KI 2011). These mice had early lethality associated with severe metabolic acidosis and may be used for evaluating the deleterious effects of metabolic acidosis on cell and organ dysfunction.

Methods: $NBC1^{W516X/W516X}$ mice were divided into 3 groups: (1) non-alkali treatment controls, (2) prenatal alkalization since post-coitus by feeding with 280 mM sodium bicarbonate ($NaHCO_3$) in drinking water and 7.5% $NaHCO_3$ 100 µl/7g intraperitoneally since day 6 after birth(PreTx) and (3) The same alkalization since day 6 after birth(PostTx). Blood acid-base status, renal function, organ abnormalities, and survival rate were assessed.

Results: $NBC1^{W516X/W516X}$ mice with severe metabolic acidosis and associated anemia, volume depletion, prerenal azotemia, several organ abnormalities (kidney atrophy, splenomegaly, reduced bone density) exhibited early lethality before weaning (21 days). Both Pre-Tx and Post-Tx alkali administration in NBC1W516X/W516X mice markedly prolonged the survival well beyond weaning, dramatically decreased protein catabolism, and attenuated several organ abnormalities. Pre-Tx group achieved a significantly higher bicarbonate (15.5±1.4 mmol/l in Pre-Tx, n=7 v.s 7.3±1.7 mmol/l in Post-Tx, n=12, $p<0.05$) and pH (7.29±0.03 in Pre-Tx, n=7 v.s 7.14±0.05 in Post-Tx, n=12, $p<0.05$) accompanied by a significantly less splenomegaly and higher body weight than Post-Tx group. On day 60 after birth, the survival rate was significantly higher in Pre-Tx than Post-Tx group (57 % vs 33%, $P<0.05$).

Conclusions: Alkali Therapy from prenatal period is superior to postnatal alkalization in treatment of severe metabolic acidosis in $NBC1^{W516X/W516X}$ mice. Prenatal alkali therapy can also apply to some inherited diseases featuring metabolic acidosis before birth.

TH-PO663

Deletion of Glutamine/Neutral Amino Acid Transporter ASCT2 (ATB0) Does Not Alter Acid-Base Regulation under Baseline Condition or in Response to Acid Loading Faraaz Siddiqui, Manoocher Soleimani, Hassane Amlal. *Internal Medicine, University of Cincinnati, OH.*

Background: Glutamine metabolism through ammoniagenesis plays an important role in the regulation of acid-base homeostasis. Several glutamine/neutral amino acid transporters are expressed in the proximal tubule cells but their role in glutamine transport is not well understood. An amino acid transport system ASCT2 (ATB0) is reported to transport glutamine and is expressed in the brush border membranes (BBM) of kidney proximal tubule and large intestine, however, its role in glutamine metbolism and acid excretion is unknown.

Methods: To understand the role of ASCT2 in glutamine metabolism and acid excretion, mice with genetic deletion of ASCT2 (KO) were generate. KO and wild-type(WT) mice were placed in metabolic cages and subjected to 280 mM NH4Cl loding for 6 days to indcue metabolic acidosis.

Results: he ASCT2 mutant mice (KO) showed complete absence of ASCT2 expression in both kidney and colon. They are fertile, have normal growth and did not exhibit any acid-base abnormalities at steady-state. urinary NH4+ excretion increased from 67 to 753 µmole/day in KO and from 62 to 751 µmole/day in WT mice after 6 days of acid loading, indicating that the capacity of KO mice to excrete daily acid load is not hampered by the deletion of ASCT2. In the cortex of acid-loaded animals, the expression of apical glutamine transporter BoAT1 was not altered in KO vs. WT mice, whereas the expression of BBM gamma glutamyl transferase (GGT), which converts glutamine to glutamate, was significantly increased (+38%, $P<0.02$) in KO vs. WT mice. Interestingly, the expression of basolateral glutamine transporter SN1 was reduced by 46% ($P<0.002$) in KO vs. WT animals.

Conclusions: These studies indicate that the deletion of ASCT2 results in compensatory activation of other mechanisms including GGT, which lead to increased ammoniagenesis and enhanced daily net acid excretion in response to acid loading.

Funding: Clinical Revenue Support

TH-PO664

Angiotensin II-Stimulated Ammonia Production in Cultured Mouse Proximal Tubule Cells Is Modulated by Type 2 Angiotensin II Receptors: Effect of pH Glenn T. Nagami, Alexandria K. Plumer, Evelyn M. Warech. *Nephrology Section, Dept of Medicine, VA Greater Los Angeles Healthcare System & UCLA, Los Angeles, CA.*

Background: We previously found that type 2 angiotensin II (Ang II) receptors (AT2Rs) reduced Ang II-stimulated total ammonia production (TAP) in proximal tubule segments from non-acid loaded control mice but not in segments from 18-h acid-loaded mice and that the greater modulating effect was associated with higher brush border membrane AT2R levels. The purpose of this study was to examine the effects of pre-exposure of cultured S1 proximal tubule cells (derived from an SV40 large T antigen mouse) to normal (7.4) or low (7.0) pH on: a) Ang II-stimulated TAP in the presence or absence of the AT2R antagonist PD123319 (PD) and b) AT2R cell surface expression levels.

Methods: S1 proximal tubule cells were grown to confluence in DMEM: Hams F12 media with 7% fetal bovine serum maintained in 35% O_2 and 5% CO_2. The growth medium was changed to serum-free medium (overnight) and then changed to pH 7.4 or 7.0 medium for specified periods. TAP was measured in the medium using an enzymatic ammonia assay. Cell surface proteins were biotinylated and isolated with streptavidin beads. AT2Rs were detected by immunoblot analysis.

Results: After exposure to pH 7.4, cells acutely treated with Ang II (10^{-9}M) + PD (10^{-6}M) displayed higher TAP compared to cells treated with Ang II alone (7.9±0.2 vs 7.0±0.2 nmol/min/mg prot, N=6, $p<0.05$). After exposure to pH 7.0 for 2h, cells treated with Ang II+PD displayed TAP that was similar to cells exposed to Ang II alone (8.9±0.3 vs 8.7±0.2, N=6, n.s). AT2R cell surface protein expression was 2.2±0.3-fold higher in cells pre-incubated for 2 h at pH 7.4 vs cells incubated at pH 7.0 ($p<0.05$, N=5). The reduction in expression levels with low pH was blocked by colchicine.

Conclusions: Although we previously reported that low pH enhanced the stimulatory effect of Ang II on TAP in proximal tubule cells by increasing AT1R expression, the present results suggest that Ang II-stimulated TAP can be inhibited by AT2R activation and that the colchicine-sensitive reduction in AT2R expression with low pH may also contribute to the enhanced stimulatory effect of Ang II on TAP.

Funding: Veterans Administration Support

TH-PO665

Rescuing Kidney Anion Exchanger I Trafficking Mutants Emmanuelle Cordat, Carmen Chu, R. Todd Alexander. *Physiology, University of Alberta, Edmonton, AB, Canada.*

Background: The anion exchanger I (AEI) is a membrane glycoprotein that exchanges chloride for bicarbonate. It is found in erythrocytes (eAEI), and in the basolateral membrane of alpha intercalated cells (kAEI). Mutations in the gene encoding the anion exchanger 1 can cause hereditary spherocytosis and/or distal renal tubular acidosis (dRTA). Some dRTA mutants, although functional in red blood cells or when expressed in Xenopus oocytes, are retained intracellularly in tubular epithelial cells. We investigated whether these mutants can be rescued to the cell surface, where they may be functional.

Methods: We tested chemical treatments and lower temperature incubations on two recessive dRTA mutations, G701D and C479W, and a dominant mutation, R589H, of kAEI. We examined the folding, stability, and degradation of wildtype (WT) and rescued trafficking kAE1 mutants.

Results: Our experiments showed that glycerol and DMSO can rescue the trafficking of kAEI mutants G701D and R589H, but not C479W. Incubation at 30C restored trafficking of G701D but not of R589H. Functional studies revealed increased ion exchange activity in some of the rescued G701D and R589H mutants compared to non-treated conditions, although activity remained compromised compared to WT levels. Interestingly, rescued mutant kAE1 proteins are degraded more rapidly than WT kAE1, which could be explained by a limited improvement in the folding of treated mutant kAE1. All three mutant kAE1 proteins are slightly stabilized by proteasome inhibitors but inhibition of the lysosome appears to completely stabilize the G701D mutant, suggesting that G701D is degraded via the proteasomal and lysosomal pathways. Since G701D kAE1 can reach the Golgi even in untreated cells, we hypothesize that in the presence of a lysosomal inhibitor, G701D traffics to the plasma membrane but is endocytosed and degraded more rapidly than WT kAE1.

Conclusions: Our experiments suggest that some trafficking dRTA mutants can be rescued, thus opening the door to alternative treatments for dRTA patients.

This work was funded by SickKids Foundation, WCHRI, AI-HS, and CIHR

Funding: Government Support - Non-U.S.

TH-PO666

The Cytosolic Mutant L522P of NBCe1 Has a Dominant Negative Effec Osamu Yamazaki, Hideomi Yamada, Masashi Suzuki, Shoko Horita, Motonobu Nakamura, George Seki, Toshiro Fujita. *Internal Medicine, Tokyo University, Tokyo, Japan.*

Background: Homozygous mutations in the electrogenic Na-HCO3 cotransporter NBCe1 cause proximal renal tubular acidosis (pRTA) and ocular abnormalities. The defective membrane expression of NBCe1 causes familial migraine (PNAS 107,15963,2010). While the homozygous Δ65bp mutation causes pRTA, the full-brown ocular abnormalities, and hemiplegic migraine, the heterozygous Δ65bp mutation, which forms hetero-oligomer complexes with WT, causes glaucoma and migraine through a dominant negative effect. In this study, we examined whether the L522P mutant of NBCe1, associating with pRTA and hemiplegic migraine, has a similar dominant negative effect.

Methods: Two-electrodes voltage-clamp method in *Xenopus* oocytes and cell pH measurement in HEK293 cells were used to determine the NBCe1 activity.

Results: L522P alone had no electrogenic activity, but decreased the NBCe1 current by 67% when co-expressed with WT in *Xenopus* oocytes. In HEK293 cells, L522P was predominantly expressed in cytoplasmic regions. When co-expressed with WT, L522P and WT showed cytoplasmic overlapping with the diminished membrane expression of WT. Functional analysis confirmed that L522P alone showed no transport activity, but significantly reduced the net NBCe1 activity by 49% when co-expressed with WT. Co-immunoprecipitation analysis using myc- and GFP-tagged constructs confirmed the association between WT and L522P. To examine the role of L522 in more details, we also examined the properties of artificial mutants L522I and L522R in HEK293 cells. L522I was properly expressed in the plasma membrane, showing the transport activity comparable to WT. By contrast, L522R was predominantly expressed in cytoplasmic regions, showing no transport activity. While the co-expression of L522I with WT increased the net NBCe1 activity compared with WT alone, the co-expression of L522R with WT significantly reduced the net NBCe1 activity by42%.

Conclusions: These results indicate that the introduction of either a large structural change (L522P) or a positive charge (L522R) in the critical amino acid L522 impaired the proper trafficking of NBCe1, resulting in the acquirement of dominant negative effect.

Funding: Government Support - Non-U.S.

TH-PO667

The N-Terminus of NBCe1-A-TM1 Tightly Interacts with the Cytoplasmic Domain Quansheng Zhu, Rustam Azimov, Liyo Kao, Debra Newman, Weixin Liu, Ira Kurtz. *Department of Medicine, UCLA.*

Background: NBCe1-A is expressed on the basolateral membrane of the renal proximal tubule where it plays a critical role in bicarbonate reabsorption. The transporter consists of 1035 amino acids and spans the lipid bilayer 14 times. We have recently determined that the transmembrane segment 1 (TM1) is involved in forming part of the NBCe1-A substrate translocation pathway; however, the actual size of TM1 in the lipid bilayer has remained elusive. The importance of TM1 in NBCe1-A is also exemplified by a TM1-S427L mutation that causes proximal RTA. NBCe1-A-TM1 was previously assumed to have a similar length to that of AE1-TM1 based on the sequence homology, where the N-terminus is connected to the cytoplasmic domain with a highly flexible loop. In this study, we analyzed the folding of the N-terminal cytoplasmic region (Cys389 - Gln424) of NBCe1-A-TM1 using the substituted cysteine accessibility method combined with extensive chemical stripping and functional transport assays. Our findings have uncovered that NBCe1-A-TM1 is structurally unique: 1) TM1 contains 33 residues that starts at amino acid Ala410; 2) the region of Arg394 - Leu406 is inaccessible to aqueous media even after 3 M sodium carbonate stripping; 3) the region prior to Arg394 is increasingly accessible to the aqueous media; 4) substitution of any of the three charged residues, Asp405, Lys409 or Asp416 impairs NBCe1-A membrane processing; 5) surprisingly, cysteine substitution of residues in the Cys389 - Gln424 region affects transporter function only minimally. On the basis of the results, we conclude that the NBCe1-A-TM1 is significantly longer than AE1-TM1 and interacts with the cytoplasmic domain tightly.

Funding: NIDDK Support

TH-PO668

TM 5 and 6 Play an Essential Role in Forming the NBCe1-A Dimer Interface Quansheng Zhu, Liyo Kao, Debra Newman, Weixin Liu, Ira Kurtz. *Department of Medicine, UCLA.*

Background: The basolateral electrogenic sodium bicarbonate cotransporter NBCe1-A mediates the absorption of the filtered bicarbonate load in the proximal tubule. We previously determined that NBCe1-A consists of 14 transmembrane (TM) helices and exists as a dimer in the lipid bilayer. The molecular mechanism underlying the formation of the NBCe1-A dimeric interface is currently unknown. To address this question we performed substituted cysteine scanning mutagenesis coupled with disulfide cross-linking studies on NBCe1-A dimers expressed in HEK-293 cells. The results show that: 1) individual cysteine substituted amino acids at the beginning (Tyr567-Ser582) and the end (Gln643-Asp647) of extracellular loops 3 are self cross-linked to form NBCe1-A dimers in the plasma membrane; 2) the cross-linked dimers are sensitive to DTT treatment indicating disulfide bond formation; 3) NBCe1-A dimers are resistant to NEM pre-treatment suggesting the cross-linking is formed during protein synthesis. Biotin maleimide labeling analysis revealed that the extracellular aqueous/lipid interface of TM 5 is at amino acid Tyr566 and TM 6 is at Ile648, indicating that extracellular loop 3 consists of 82 amino acids. Our experiments have demonstrated for the first time that the NBCe1-A dimeric interface is formed by the self-association of TM 5 of each monomer and the self-association of TM 6 of each monomer.

Funding: NIDDK Support

TH-PO669

Interplay between Glycosylation and Disulfide Bonding Defines NBCe1-A Extracellular Topography Quansheng Zhu, Liyo Kao, Rustam Azimov, Debra Newman, Weixin Liu, Ira Kurtz. *Department of Medicine, UCLA.*

Background: A common feature among sodium-driven bicarbonate transporters within the SLC4 family is the presence of four cysteines (Cys) in the glycosylated extracellular loop 3 (EC-loop 3). We recently showed that EC-loop 3 is the largest highly exposed surface loop in NBCe1-A and interestingly, resides at the dimer interface. In the present study, we determined the significance of the unique pattern of disulfide bond formation

among the four Cys in the NBCe1-A EC-loop 3. NBCe1-A constructs carrying one to four Cys residues in various combinations in EC-loop 3 were expressed in HEK-293 cells and subjected to an SDS-PAGE gel shift assay, chemical stripping, DTT treatment and chemical probing analysis. The function of each construct was analyzed in the HEK-293 cells. Our results show that: 1) the four cys in EC-loop 3 are in a folded conformation and intra-disulfided; 2) cys 1, 2 and cys 3, 4 form two intra-monomeric disulfide bonds; 3) the first disulfide bond determines the number of glycosylation sites in the loop, and glycosylation guides the disulfide bond formation between cys 3 and 4; 4) in the absence of the four cys residues, NBCe1-A traffics normally to the plasma membrane, however, each monomer becomes triply glycosylated and most of the introduced cys in loop 3 become self cross-linked. The findings indicate that EC-loop 3 in NBCe1-A is highly structured. We predict that the two loops from each of the NBCe1-A monomers interact to form a domain-like structure on the surface of the transporter resembling receptors such as G-protein coupled receptors.

Funding: NIDDK Support

TH-PO670

Towards the High-Resolution Structure of hhNBCn1 Natalia Abuladze, Nathaniel Magilnick, Kirill Tsirulnikov, Alexander Pushkin. *Medicine, UCLA, Los Angeles, CA.*

Background: Integral membrane proteins account for ~30% of all proteins, and human membrane proteins are the targets for ~50% of therapeutic drugs. Despite these facts, high resolution three-dimensional (3D) structures of less than 300 unique membrane proteins have been solved at atomic resolution mostly by X-ray crystallography, of which eukaryotic membrane proteins account for less than 10%. Recent advances of cryo-electron microscopy (cryoEM) and single-particle reconstruction have solved the structures of macromolecular complexes at ~4Å providing a new tool that may be used for structural studies of eukaryotic membrane proteins.

Methods: We are using cryoEM for structural study of the human heart electroneutral sodium bicarbonate cotransporter NBC3 (hhNBCn1) that plays an important role in the intracellular pH and sodium regulation. hhNBCn1 fused at the N-terminus with the 6His tag was expressed in Sf9 cells, solubilized in 50 mM Tris-HCl, pH 8.0, containing dodecyl maltoside (DDM), Roche complete protease inhibitor cocktail and pepstatin, and purified in the presence of 0.03% DDM on DEAE-cellulose DE-52 (Whatman) and Ni-IMAC FF (GE HealthCare).

Results: Purified hhNBCn1 was homogeneous by SDS-PAGE and immunoblotting but a mixture of different oligomeric forms was present. hhNBCn1 dimers were further purified by size-exclusion chromatography on Sephacryl S-300 (GE HealthCare). As a preliminary step of structural characterization, hhNBCn1 dimers were negatively stained with 0.5% uranyl acetate and imaged on a FEI Tecnai F20 electron microscope operated at 200 kV and a magnification 50,000X. Analysis of images suggested that the hhNBCn1 dimer consists of a larger and a smaller domain. 3D reconstruction from ~5,000 randomly selected dimer particle images agreed with the proposed 2-doman model but suggested that particles of more than one conformation are present. To separate hhNBCn1 dimers with different conformations, we performed hhNBCn1 crystallization assuming that a certain crystal contains structurally uniform particles. Several similar shape hhNBCn1 microcrystals (<2 μ) were obtained in the presence of MPD. They will be solubilized and used for cryoEM and 3D reconstruction. Supported by AHA.

Funding: Private Foundation Support

TH-PO671

Structural Study of AE1 Chloride-Bicarbonate Exchanger Kirill Tsirulnikov, Nathaniel Magilnick, Natalia Abuladze, Alexander Pushkin, Ira Kurtz. *Department of Medicine, UCLA.*

Background: In erythrocytes and the collecting duct, AE1 (SLC4A1) mediates 2 major functions: 1) Cl^-/HCO_3^- exchange, and 2) support of the structural integrity of erythrocytes via interaction with cytoskeletal proteins. AE1 (~100 kDa) consists of a ~55 kDa membrane domain responsible for anion-exchange, and a cytoplasmic domain, which functions as an anchoring site for membrane-associated proteins. We have recently generated a 3D model of full-length AE1 using electron tomography of negatively stained AE1 dimers purified from bovine erythrocytes. In the present study the model was refined using single particle 3D reconstruction. Approximately 1,000 images of AE1 dimers negatively stained with uranyl formate were recorded on a FEI Tecnai F20 electron microscope operated at 200 kV and 50,000 magnification with 4kX4k CCD camera. 330,000 particles were automatically picked using Batchboxer (EMAN). 33,000 particles were randomly selected from this data set and used for 3D reconstruction and structure refinement using EMAN. To avoid a model-bias problem in the structure refinement, we used both a ball and a rod as starting models. The independent 3D reconstructions gradually converged into a single model, suggesting that a correct model was generated. The model has a shape like a bottle gourd of 16 nm consisting of larger and smaller domains connected with two narrow pillar-like regions. The reported X-ray structure of the recombinant dimeric cytoplasmic domain of AE1 fits well the smaller domain in our model. Different groups of AE1 dimers were identified that varied only in the size and shape of the connector region between the domains. This flexibility of the connector region accounted for the different orientations of the cytoplasmic domain relative to the membrane domain. We propose that the connector is an important region in AE1 required for the flexibility of the erythrocyte membrane. It is possible that the residues in AE1 deleted in patients with South East Asian Ovalocytosis belong to the connector region, providing a potential explanation for the increased erythrocyte rigidity in this disorder.

Funding: Private Foundation Support

TH-PO672

CO_2/NH_3 Selectivities and Inhibitor Sensitivities of Mammalian Aquaporins Raif Musa-Aziz,[1,2] R. Ryan Geyer,[1] Xue Qin,[1] Walter F. Boron.[1] *[1]Physiol and Biophys, Case Western Reserve University, Cleveland; [2]Physiol & Biophys, University of Sao Paulo, Brazil.*

Background: The 13 mammalian aquaporins (AQP0-12) have diverse localization patterns and can be divided into 2 major families: orthodox AQPs and aquaglyceroporins. Previously we expressed mammalian AQP1, AQP4-M23 and AQP5 in *Xenopus* oocytes and used video microscopy to compute osmotic water permeability (P_f), and used microelectrodes to record the transient rise in surface pH (ΔpH_S) caused by CO_2 or NH_3 influx. Subtracting the respective values for day-matched, H_2O-injected control oocytes yielded the channel-specific values P_f* and ΔpH_S* (semiquantitative index of gas permeability). We had found that AQP1, 4-M23, and 5 each has a characteristic CO_2/NH_3 and CO_2/H_2O permeability ratio. Also, we had shown that pCMBS (blocks 4 H_2O pores) reduces AQP1's $(\Delta pH_S^*)_{NH3}$ by ~100% and reduces $(\Delta pH_S^*)_{CO2}$ by ~40%. DIDS (does not block H_2O pores) has no effect on $(\Delta pH_S^*)_{NH3}$, but reduces $(\Delta pH_S^*)_{CO2}$ by ~60%.

Methods: Here, we extend our study to determine if other AQPs (0-9) exhibit CO_2/NH_3 selectivity, and use inhibitors to explore the CO_2 and NH_3 pathways through AQP6 and AQP9.

Results: $(\Delta pH_S^*)_{CO2}$ differed significantly from 0 for AQP0, AQP1, AQP4-M23, AQP5, AQP6, and AQP9. $(\Delta pH_S^*)_{NH3}$ differed significantly from 0 for AQP1, AQP3, AQP6, AQP7, AQP8, and AQP9. P_f* differed significantly from 0 for all AQPs tested except AQP6. The ratio $(\Delta pH_S^*)_{CO2}/P_f$* fell in the sequence AQP6 (∞)>AQP5>AQP4-M23>AQP1≅AQP9>AQP0. The ratio $(\Delta pH_S^*)_{CO2}/(-\Delta pH_S^*)_{NH3}$ fell in the sequence AQP4-M23 (∞)=AQP5>AQP6>AQP1>AQP9>AQP4-M1≅AQP2=0. Additionally, for AQP6, pCMBS (1 mM) increased P_f* by 70%, but has no effect on $(DpH_S^*)_{NH3}$ or $(\Delta pH_S^*)_{CO2}$. DIDS (100 mM) has no effect on P_f* or $(\Delta pH_S^*)_{NH3}$, but reduces $(\Delta pH_S^*)_{CO2}$ by 50%. For AQP9, pCMBS reduces P_f* by 60%, $(-\Delta pH_S^*)_{NH3}$ by 100%, and $(\Delta pH_S^*)_{CO2}$ by 50%. DIDS has no effect on P_f* or $(\Delta pH_S^*)_{NH3}$, but reduces $(\Delta pH_S^*)_{CO2}$ by 50%.

Conclusions: In summary, we show that mammalian AQPs exhibit a diverse range of selectivities for CO_2 *vs* NH_3 *vs* H_2O. Also, with AQP9—as with AQP1—H_2O, NH_3, and 50% of CO_2 move through monomeric (pCMBS-sensitive) H_2O pores, whereas 50% of CO_2 takes another (DIDS-sensitive) pathway that is still unidentified.

Funding: Other U.S. Government Support, Government Support - Non-U.S.

TH-PO673

Anion Exchanger 4 Expression in β-Intercalated Cells Is Characterized by a Unique Subapical:Lateral Pattern and Regulated by Acid:Base Status Jeffrey M. Purkerson, George J. Schwartz. *Pediatrics, University of Rochester Medical Center, NY.*

Background: Adaptation of the rabbit CCD to acidosis is characterized by a reduction in HCO_3 secretion by β-intercalated cells (β-ICs), due in part to down-regulation of apical $Cl:HCO_3$ exchanger (pendrin) activity. Here, we examined expression of AE4 in the CCD to better understand the function of another anion exchanger that may contribute to HCO_3 transport in the collecting duct.

Methods: Immunofluorescence staining of kidney sections showed co-labeling of PNA (peanut agglutinin), and AE4+ cells and failed to demonstrate co-expression of AE4 and AE1 indicating that AE4 is expressed in β-ICs. We performed 3D-reconstructions of images obtained via confocal microscopy of microdissected CCDs and RT-qPCR of kidney cortex.

Results: 3D reconstructions showed that AE4 expression in β-ICs occurs in a hollow barrel-like pattern located underneath the apical PNA cap.

AE4 was localized below the tight junction as defined by ZO-1 staining.We examined regulation of AE4 by acid/base status in normal (urine pH: 8.2±0.22, HCO_3: 26± 3.4 mM), 3 day acid (urine pH: 4.7±0.38, HCO_3: 16±3), and rabbits immediately transitioned from 3 day acid to alkali loading for 16-18 h (recovery: urine pH: 8.0±0.28, HCO_3: 28±4.8). Morphometric analysis of AE4 staining revealed that acidosis reversibly reduced depth and area of the AE4 "barrel" structure in β-ICs by 30±0.1 and 44±0.1%, respectively. Recovery from acidosis restored AE4 "barrel" to near normal size. Similar to pendrin, AE4 mRNA abundance was reduced by acidosis to 36±3% of normal, and was increased to 118±17% of normal upon recovery.

Conclusions: AE4 is expressed in the CCD and may act in conjunction with pendrin to regulate HCO_3 secretion in β-ICs during alkali-loading perhaps via intercellular $Cl:HCO_3$ exchange or by facilitating HCO_3 trafficking from the blood to the urine.

Funding: NIDDK Support

TH-PO674

Deletion of the Cl^-/HCO_3^- Exchanger Pendrin Downregulates Calcium-Absorbing Proteins in the Kidney and Causes Calcium Wasting Sharon L. Barone,[1,2] Jie Xu,[1,2] Hassane Amlal,[1] Manoocher Soleimani.[1,2] *[1]Medicine, University of Cincinnati, OH; [2]Research Services, Veterans Administration, Cincinnati, OH.*

Background: The epithelial calcium channel ECaC (TRPV5) and the Cl^-/HCO_3^- exchanger pendrin (SLC26A4) are expressed on the apical membrane of tubular cells in the distal nephron and play essential roles in calcium reabsorption and bicarbonate secretion, respectively. ECaC works in tandem with the basolateral Na/Ca exchange to reabsorb the filtered calcium in the distal nephron. ECaC expression and/or activity is downregulated *in vitro* by extracellular acidic pH. We hypothesized that the acidic urine in pendrin KO mice might affect the expression and or activity of ECaC/calbindin/Na/Ca exchange pathway and alter urine calcium excretion.

Methods: Northern hybridization, immunoblot analysis and immunofluorescent labeling were performed and urine calcium excretion rates were measured.

Results: Immunolocalization studies demonstrate that pendrin and the Na/Ca exchanger are expressed by distinct cell types within the distal convoluted and connecting tubules. In the collecting duct we observe the expression of pendrin but not the Na/Ca exchange. Our results confirm that deletion of pendrin causes acidic urine (urine pH 4.9 in ko Vs. 5.9 in wt mice, $p<0.03$). We further observe that pendrin deletion downregulates the calcium-absorbing molecules ECaC and Na/Ca exchange in the kidney. These changes were associated with a ~100% increase in 24 hr urine calcium excretion in pendrin null mice (~8.1 µmoles/24 hrs in wt Vs. 17.6 in pendrin KO mice, $p<0.01$). Subjecting the wild type and mutant mice to oral bicarbonate loading for 12 days increased the urine pH to ~8 in both genotypes, normalized the expression of ECaC and Na/Ca exchange, and reduced the urine calcium excretion in pendrin null mice to levels in wt mice.

Conclusions: We propose that acidic urine caused by pendrin impairment can play an important role in the pathogenesis of calcium, uric acid and possibly cystine stones. Future studies should investigate whether single nucleotide polymorphisms in pendrin gene are associated with the currently unexplained acidic urine in humans with calcium or uric acid stone.

Funding: NIDDK Support, Veterans Administration Support

TH-PO675

Acid Decreased the Expression of Ca-Sensing Receptor in Type-B of Mouse Kidney Collecting Duct Intercalated Cells Yukiko Yasuoka,[1] Yuichi Sato,[2] Yuichiro Izumi,[3] Hiroshi Nonoguchi,[4] Katsumasa Kawahara.[1] *[1]Dept of Physiology, Kitasato U. Sch. of Med, Sagamihara, Kanagawa, Japan; [2]Dept of Mol. Diagnostics, Kitasato U. Sch. of Allied Health Sci, Sagamihara, Kanagawa, Japan; [3]Epithelial Systems Biology Labolatory, NHLBI, National Institutes of Health, Bethesda, MD; [4]Division of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

Background: The renal collecting duct serves the fine-tuning of renal acid-base homeostasis. Morphological changes, such as hypertrophy and cell height, of intercalated cells (IC) may contribute to the cell function in acid and alkali secretion during metabolic acidosis (Ac) and alkalosis (Al), respectively.

Methods: By using a quantitative *in situ* hybridization technique and immunohistochemistry, in mice (C57B/6J) kidney, we examined changes in the Ca-sensing receptor (CaSR) mRNA and protein expression at each segment of the distal tubules and the cortical collecting duct (CCD) after (1) 6-d $CaCl_2$-loading, (2) Ac or Al induced by NH_4Cl- or $NaHCO_3$-intake (6 d), and (3) furosemide-injection (24-hr).

Results: Moreover, by defining cell types of IC using a double-staining technique with anti-AE1 and AE4 antibodies in the CCD, we determined that CaSR mRNA was expressed not in type-A but in type-B cells of the IC throughout the CCD. The CaSR mRNA expression was moderately high in thick ascending limb of Henle's loop (TAL), macula densa (MD), distal convoluted tubule (DCT), and CCD (TAL, MD > DCT > CCD) in control. Interestingly, although the CaSR mRNA and protein expression was unchanged in all segments examined during Al and furosemide-infusion, it was significantly decreased only in IC-B during Ac and $CaCl_2$-loading. Cell height of IC-A increased during Ac and Ca-loading, whereas cell-height of IC-B increased during Al. No morphological changes occurred after furosemide-injection. All these results indicate that the CaSR expression in IC-B cells was decreased during either acid-loading or secondary acidosis by hypercalcemia and was independent of both hypercalciuria and hypercalcemia.

Conclusions: Unique expression of CaSR in the IC-B suggests that CaSR may be responsible for alkali secretion at least during alkalosis.

TH-PO676

Expression of the Ammonia Transporter Family Members, Rh B Glycoprotein and Rh C Glycoprotein, in Foxi1 Null Mice Ki-Hwan Han,[1] Hyun-Wook Lee,[2] Alexandra F. Kovar,[2] Sven Enerback,[3] Jill W. Verlander,[2] David Weiner.[2,4] *[1]Department of Anatomy, Ewha Womans University, Seoul, Korea; [2]Renal Division, University of Florida, Gainesville, FL; [3]Department of Biomedicine, University of Gothenburg, Sweden; [4]Nephrology Section, NF/SGVHS, Gainesville, FL.*

Background: Mice lacking the transcription factor Foxi1 have an intercalated cell-differentiation defect and develop renal tubular acidosis. Rh B glycoprotein (Rhbg) and Rh C glycoprotein (Rhcg) are ammonia transporters critical for renal acid excretion. This study's purpose was to examine Rhbg and Rhcg expression in Foxi1 null mice.

Methods: Kidneys from Foxi1 null and wild-type mice processed for immunohistochemistry. Cell-specific expression was examined using double-immunolabel immunohistochemistry.

Results: In control mice, basolateral Rhbg was heterogeneous, strong in intercalated cells and weak in principal cells. In Foxi1 null mice, in cortical collecting duct (CCD) and outer medullary collecting duct (OMCD) Rhbg immunoreactivity was homogeneous; cells with strong Rhbg label were not present. Rhcg in control mice was apical and basolateral, and greater in intercalated than in principal cells. In Foxi1 null mice, Rhcg was homogeneous and no intensely labeled cells were present in CCD and OMCD. However, Rhbg and Rhcg expression in CCD and OMCD of Foxi1 null mice was similar to or greater than expression in principal cells in wt mice. In connecting segment cells (CNT), both Rhbg and Rhcg expression was intense and unchanged by Foxi deletion. Finally, both AE1-positive and pendrin-positive intercalated cells in Foxi1 null mouse CNT, CCD and OMCD. CA II expression was present in most CCD and OMCD cells in Foxi1 null mouse, consistent with the known CA II expression in mouse principal cells.

Conclusions: Rhbg and Rhcg expression is regulated through two separate pathways; one downstream of Foxi1-dependent stimulation of A-type intercalated cell and non-A, non-B cell development and one independent of Foxi1 and primarily present in CNT cells and principal cells.

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

TH-PO677

Expression and Localization of the Renal Ammonia Transporter, Rhcg, in Male Reproductive Tract Hyun-Wook Lee,[1] Jill W. Verlander,[1] Jesse Bishop,[1] Mary Handlogten,[1] Sijo Parekattil,[1] Ki-Hwan Han,[3] David Weiner.[1,2] *[1]Univ. of Florida, Gainesville, FL; [2]Ewha Womans Univ., Seoul, Korea; [3]NF/SGVHS, Gainesville, FL.*

Background: The Rhesus glycoprotein, Rhcg, is an ammonia transporter that is essential for normal renal ammonia excretion. Global Rhcg deletion decreases renal ammonia excretion and impairs male fertility.

Methods: We performed the first detailed study of Rhcg expression in male reproductive organs using immunoblots, qRT-PCR, and immunolocalization and began to examine functional requirement for Rhcg in male reproduction using cell-specific Rhcg deletion.

Results: Immunoblots and qRT-PCR demonstrated Rhcg protein and mRNA in testis and epididymis, with highest expression in the epididymal head. Rhcg immunolabel was present in testis at the luminal surface in a subset of seminiferous tubules. Immunogold electron microscopy demonstrated Rhcg expression in Sertoli cells, spermatids and sperm. Rhcg immunolabel was present on cilia of ciliated cells in efferent ducts, and was apical in a subset of cells in epididymis. To determine cell-specific expression, we double immunolabeled for H-ATPase. In initial segment and head of epididymis, Rhcg was expressed on principal cell stereocilia. In upper body of epididymis, only principal cell microvilli had Rhcg immunolabel. In middle body, lower body and tail of epididymis, clear cells expressed apical Rhcg. In vas deferens, Rhcg was apical in a subpopulation of epithelial cells. We then identified that Ksp-cadherin-Cre-positive, floxed Rhcg mice had Rhcg deletion from epididymis, but not testis or sperm, as well as the previously reported renal collecting duct Rhcg deletion. Collecting duct and epididymis Rhcg deletion in sires did not alter litter size compared to Cre-, floxed Rhcg control sires.

Conclusions: 1) Rhcg is expressed in sperm and in a heterogeneous pattern in specific cell types in testis and epididymis, suggesting ammonia transport by Rhcg affects one or more components of male fertility; 2) unlike global Rhcg deletion, collecting duct and epididymal Rhcg deletion does not alter male fertility, 3) the complex distribution of Rhcg in male reproductive organs suggests it contributes to multiple components of male fertility.

Funding: NIDDK Support, Veterans Administration Support

TH-PO678

Renal Rhcg Expression Is Increased during Lithium-Induced Nephrogenic Diabetes Insipidus in Rats Robert J. Walker,[1] Jennifer J. Bedford,[1] Gerard Davis,[1] Frederiek E. Vos,[1] David Weiner,[23] John P. Leader.[1] *[1]University of Otago, New Zealand; [2]University of Florida, Gainsville, FL; [3]Renal Section, NF/SGVHS, Gainesville, FL.*

Background: Lithium is used to treat bipolar disorders but is associated with nephrogenic diabetes insipidus and less commonly an impaired ability to handle an acid load. The role of ammonia via the putative NH3 transporter Rh C Glycoprotein (Rhcg) is unknown.

Methods: 11 participants (8 females) on lithium therapy were matched with 6 healthy volunteers (3 females). They received an acute dose of ammonium chloride (100 mg/kg body weight). Urine and blood samples were taken at the time and 2, 4, and 6 hours later. Rats maintained for 6 months on a lithium diet were also given an acute dose of ammonium chloride (100 mg/kg body weight). Urinary acidification was measured and the kidneys subsequently removed for immunohistochemistry and western blots for Rhcg expression.

Results: At baseline neither the humans nor rats had evidence of a metabolic acidosis. Humans on lithium showed a decreased ability to respond to an acute metabolic acid load and developed a more marked hyperchloremic metabolic acidosis and a less acidic urine. Similarly rats on chronic lithium treatment failed to respond as well to an acute acid load as did control rats. In controls urinary pH fell to 6.5 ± 0.1 and remained low for 6 hours whereas in the lithium rats urinary pH only fell to 6.7± 0.1 and returned to pH 7.0 after 2 hours The ammonia transporter, Rhcg in the outer medulla of rat kidneys showed an increase both with western blots (100 ± 37, control: 154 ± 17, lithium; $p<0.001$) and by immunohistochemistry.

Conclusions: Humans on long-term lithium therapy, demonstrated a reduced ability to excrete an exogenous acid load. This was also evident in our chronic lithium-exposed rat model. Immunohistochemical examination of rat kidneys following long term exposure to lithium revealed a substantially altered and increased expression of Rhcg. This was confirmed with western blots. We presume that this increased expression is a response to prevent the development of lithium-induced metabolic acidosis.

Funding: Government Support - Non-U.S.

TH-PO679

Specific Firing of Renal Afferent Innervation: Which Role Play Acid-Sensing Ion Channels? Wolfgang Freisinger, Tilmann Ditting, Sonja Heinlein, Johannes Schatz, Roland Veelken. *Medical Clinic 4, Nephrology and Hypertension, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany.*

Background: Dorsal root ganglion neurons with projections to the kidney exhibit a significantly higher portion of tonically firing neurons (repetitive firing) upon electrical stimulation than neurons with non-renal projections. As renal nerve endings are exposed to pH-changes in e.g. inflammation, we tested the hypothesis that tonically firing renal neurons exhibit currents upon acidic stimulation significantly different from neurons with other firing patterns predominantly linked to non-renal sites.

Methods: Dorsal root ganglion (DRG) neurons from Sprague Dawley Rats (Th11-L2) were characterized according to firing response (tonic – sustained firing vs. phasic – single firing). Furthermore, inward currents due to acid stimulation (pH 5,3) -consisting either of a large transient component and a smaller, sustained component or only sustained component- were measured in voltage clamp recordings and analyzed in terms of the putatively involved channels (ASIC or TRPV1) by adding the respective blockers (Amiloride, Capsazepine).

Results: 40 DRG neurons were examined. Tonic firing pattern was found more frequently in renal DRG neurons than in non-renal neurons (47% vs.10%. $p<0.05$), as shown previously. There was no significant difference in cell size (Ø 40,8 vs. 40,3µm). Tonic cells had higher capacity (120 vs. 70 pF) and lower membrane potential (-49 vs. -53,6 pF). Upon acidic stimulation, only cells with tonic firing exhibited an additional afferent transient current (12750±8750pA vs. 210±390pA), which could be blocked by amiloride (12750±8750pA vs. 710pA±1017pA, $p<0.05$).

Conclusions: For the first time we could show that tonically firing neurons, which are found predominantly in renal afferent innervations, exhibit transient inward currents blocked by the channel blocker amiloride, pointing to the presence of ASIC channels. The expression of these channels in an organ subjected to acid changes appears fascinating. The correlation with a tonic firing pattern as yet unclear, but these channels could influence membrane potential thus leading to higher excitability of these neurons in vivo.

TH-PO680

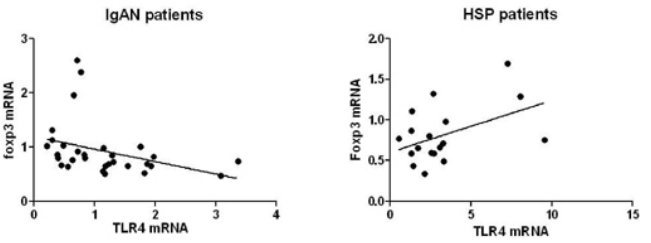
Different Modulation of Regulatory T Cells by Toll like Receptor Activation in Primary IgA Nephropathy and in Henoch-Schoenlein Purpura Nephritis: A Factor Regulating the Different Clinical Outcome? Elisa Loiacono,[1] Roberta Camilla,[1] Valentina Daprà,[1] Laura Morando,[1] Rachele Gallo,[1] Licia Peruzzi,[1] Margherita Conrieri,[2] Manuela Bianciotto,[2] Francesca Maria Bosetti,[2] Rosanna Coppo.[1] *[1]Nephrology, Dialysis, Transplant, R. Margherita Hosp, Turin, Italy; [2]Emergency Pediatrics, R. Margherita Hosp, Turin, Italy.*

Background: Primary IgA Nephropathy (IgAN) and IgAN related to Henoch Schoenlein purpura (HSP) have common histological features but different clinical outcomes. Children mostly show remission in IgAN related to HSP vs progression in primary IgAN. In both cases a dysregulation of innate immunity results in failure of mucosal antigen elimination and/or altered IgA synthesis. Among the innate immunity actors, toll-like receptors (TLR) modulate regulatory T (Treg) cells,that express the transcription factor Forkhead box P3(Foxp3)involved in suppressing pro-inflammatory responses. We had reported increased TLR expression in mononuclear cells(PBMC)of patients with primary IgAN.

Methods: We investigated 21 children with HSP(7.9±2 y.o; 10 with and 11 without urinary abnormalities) and 13 children with primary IgAN(10.7±3 y.o.).

TRL2, TLR4 and TLR9 mRNAs, Foxp3 mRNA (Treg marker) and TGF mRNA (Th17 pro-inflammarory cells marker)were quantified in PBMC of these children and in healthy controls by real time PCR(Taqman), normalizing with Abl mRNA.

Results: We observed a significant inverse correlation between TLR4 and Foxp3 mRNAs in PBMC from children with primary IgAN (r: -0.35, $p < 0.05$) while in cases of HSP TLR4 and Foxp3 mRNAs were directly correlated (r: 0.46, $p< 0.05$).



Conclusions: These data suggest that in primary IgAN TLR4 hyperexpression and dowregulation of anti-inflammatory Tregs may turn into a pro-inflammatory and chronic outcome, while an enhanced Treg activity in front of a similar innate immunity engagement may favor the recovery in children with HSP.

TH-PO681

Activation of Innate Immunity in Henoch-Schoenlein Purpura with and without Nephritis: Increased Toll-Like Receptors Expression in Circulating Lymphomononuclear Cells Roberta Camilla,[1] Elisa Loiacono,[1] Valentina Daprà,[1] Laura Morando,[1] Rachele Gallo,[1] Margherita Conrieri,[2] Manuela Bianciotto,[2] Francesca Maria Bosetti,[2] Licia Peruzzi,[1] Alessandro Amore,[1] Rosanna Coppo.[1] *[1]Nephrology Dialysis Transplant, R. Margherita H., Torino, Italy; [2]Pediatric Emergency, R. Margherita H., Torino, Italy.*

Background: Innate immunity is involved in pathogenesis of primary IgA nephropathy (pIgAN).We reported an overexpression of TLR4 in circulating mononucleates(PBMC) from patients with pIgAN. Henoch-Schoenlein purpura (HSP)is a systemic vasculitis with renal features of pIgAN.

We aimed at investigating whether in PBMC from patients with HSP TLRs are upregulated as in pIgAN and the differences in cases with and without renal involvement.

Methods: TLR2, TLR4, and TLR9 expression was detected in BPMC of 28 HSP children (4-13 y.o.,mean age 7.6 ±2y) at clinical onset: 13 had urinary abnormalities(hematuria, proteinuria > 0.250 mg/day in 9/13). Children control groups were 15 pIgAN (5-16 y.o.,mean age 10.9±3y), 27 idiopathic nephrotic and 40 healthy subjects (HC). TRLs were detected by flow cytometry, expressed as MIF and mRNAs were quantified by real time PCR(Taqman).

Results: Patients with HSP showed, in comparison to HC increased expression of TLR2 (4.2±1.5 vs 3.0±0.6 MIF, p=0.02), TLR4 (2.2±0.7 vs 1.7±0.4 MIF, p=0.02), and TLR9 mRNA (2.9±2.0 vs 0.7±0.4, p=0.005). No difference was found in HSP with or without renal involvement. HSP showed, in comparison with pIgAN, higher levels of TLR9 and TLR4 mRNAs (TLR9: 2.9±2.0 vs 0.9±0.6, p=0.01; TLR4: 2.9±2.3 vs 1.6±1.2, p=0.004).

No modification in expression of TLR2,4,9 and corresponding mRNAs was detected in disease control groups in respect to HC.

Conclusions: In conclusion, this study shows for the first time an activation of TLRs during HSP suggesting an activation of innate immunity similar to what found in pIgAN.

Funding: Government Support - Non-U.S.

TH-PO682

The Expression of TLR9 in the Tonsils and Peripheral Blood of IgA Nephropathy Patients and It's Variation after Tonsillectomy Gang Wu,[1] You-Ming Peng,[1] Lin Sun,[1,2] Hong Liu,[1] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]Departments of Pathology and Medicine, Northwestern University, Chicago, IL.*

Background: TLR9 is a member of the toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. The expression of TLR9 in the tonsils and peripheral blood in IgAN patients and the changes after tonsillectomy is unclear.

Methods: 55 patients with IgAN (n=28) or chronic tonsillitis (Non-IgAN) (n=27), and 6 normal persons were enrolled into the study. Both the tonsils from chronic tonsillitis patients (n=27) and the peripheral blood from normal persons (n=6) were as control. Pathological changes of tonsillar tissues were observed by immunohistochemistry. The expression of TLR9 in B cells in tonsil was tested by immunohistochemistry and FCM(Flow cytometry), and the expression of TLR9 in peripheral blood from IgAN patients before and after tonsillectomy was tested by FCM as well.

Results: Immunohistochemistry showed that most of TLR9 positive cells were localized at the epithelium of tonsil, and a minor part at germinal centres, follicular and interfollicular area of tonsil. Compared to non-IgAN group,TLR9 positive cells of IgAN were significantly increased ($P<0.05$) showed by FCM. The expression of TLR9 on B cells tested by FCM, both in tonsil and peripheral blood from IgAN , were higher than control groups ($P<0.05$). After tonsillectomy, the expression of TLR9 on B cells of IgAN patients, was significantly decreased showed by FCM, even remain with proteinuria and hematuria; bue it had no obvious effect on the expression of TLR9 on B cells of normal persons.

Conclusions: Our data indicate that IgAN patients have an high expression of TLR9 in tonsils and peripheral blood, which could induce mucosal(innate) immune response.

Funding: Government Support - Non-U.S.

TH-PO683

A Multi-Center Validation Study of the Oxford Classification of IgA Nephropathy in China: ***Working Group on Validation Study of the Oxford Classification of IgA Nephropathy in China*** Cai-Hong Zeng,[1] Wei-Bo Le,[1] Shao-Shan Liang,[1] Hong-Bing Shen,[2] Agnes B. Fogo,[3] Zhi-Hong Liu.[1] *[1]Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China; [2]Department of Epidemiology and Biostatistics & Ministry of Education Key Lab for Modern Toxicology, School of Public Health, Nanjing Medical University, Nanjing, China; [3]Renal/EM Division, Department of Pathology, Vanderbilt University Medical Center, Nashville.*

Background: The Oxford classification of IgA nephropathy (IgAN) identified four pathologic lesions (MEST) for prediction of renal prognosis of IgAN independent of the clinical features. However, the limited number of patients and their heterogeneous origin necessitate validation studies in larger cohorts.

Methods: In this study, we validated the Oxford results using a cohort of 1026 adults with IgAN recruited from 18 centers in China.

Results: 31% of the patients in this cohort received immunosuppression and 27% prednisone, both similar to the Oxford cohort. The mean follow-up was 53 months. Compared with the Oxford cohort, the patients in the current study had slightly milder clinical course, as suggested by lower proteinuria (median, 1.3 vs 1.7 g/24h) at biopsy, and slower decline rate of eGFR (-1.5±10.5 vs -3.5±8.5ml/min per 1.73m^2 per year) during follow-up. In addition, there were lower proportions of patients with endocapillary proliferation(E, 11%) and mesangial hypercellularity (M1, 43%) in our cohort compared with the Oxford cohort, but higher proportions of segmental sclerosis or adhesion (S, 83%) and necrosis lesions (15% vs 2.3% in Oxford). The frequencies of crescents and severity of tubular atrophy/interstitial fibrosis were similar in both cohorts.

Conclusions: Our studies also confirmed a similar predictive value of the MEST lesions for renal outcome in Chinese adults with IgAN. On the other hand, we also reassessed the prognostic value of crescents and necrosis in IgAN, but no conclusive results were obtained.

TH-PO684

The Role of Focal Segmental Glomerulosclerosis in the Progression of IgA Nephropathy Liliany P. Repizo, Elerson Costalonga, Lilian P.F. Carmo, Igor Marques, Leonardo Abreu Testagrossa, Denise Maria Avancini Costa Malheiros, Lectícia Jorge, Rui Toledo Barros, Viktoria Woronik. *Departament of Nephrology, University of Sao Paulo, SP, Brazil.*

Background: Lesions morphologically identical with focal segmental glomerulosclerosis(FSGS) may appear in IgA nephropathy(IgAN). The aim of this study was to evaluate the role of FSGS in IgAN.

Methods: We analyzed 165 patients with biopsy-proven IgAN at our center from 1999 to 2009, 57 patients meet inclusion criteria of age > 18 years, biopsies containing at least 8 glomeruli and follow up longer than 2 years. Biopsies were reviewed and classified according to Columbia classification of FSGS in the context of IgAN. The primary endpoint was reduction of at least 50% of the initial eGFR (estimated by MDRD-simpilfied formula) and/or ESRD.

Results: It was possible to classify 30 of 57(52.7%) cases among the subtypes of FSGS. The FSGS lesions group cases were subdivided as follows: 25 NOS category, 4 tip lesions and 1 collapsing form. Of the remainder, no FSGS lesions group, 8 cases(14%) had glomerular lesions not definably FSGS and 19 cases (33,3%) didn't show lesions on light microscopy aside from mild mesangial prominence in some. The clinical and histological features are summarized in Table 1.

Clinical and histologic Features

N	No FSGS Lesions(27)	FSGS Lesions(30)
AGE(y)	33(25 – 42)	33(25 - 50.2)
Female	15(55,5%)	17(56.6%)
eGFRi(ml/min)*	74(50 - 91)*	34.5(25.5 - 64.7)*
Proteinuriai(g/day)	2.1(0.8-4.4)	2(1 - 4)
Hypertension*	11(40.7%)*	21(70%)*
Follow up	4.5(3 - 6.1)	4.8 (3.2 - 7.4)
eGFRend(ml/min)*	74(53 - 100)*	31.5(16.7 - 63.2)*
Interstitial Fibrosis>25%*	6(22.2%)*	18(60%)*
Crescents	7(25.9%)	13(43.3%)
Endpoint	3(11.1%)	10(33.3%)

Results are shown as median ± IQR * p < 0.05;

Finally, in univariate analysis FSGS lesions group showed a tendency to have a worse renal outcome at the end of follow-up (p0.06; OR 4.0 95% CI 0.9 – 16).We couldn't analyze the impact of FSGS subtypes on outcome because the low number of patients.

Conclusions: Our results underline the usefulness of monitoring all IgAN patients for FSGS, given their potential negative impact on renal outcome.

Funding: Government Support - Non-U.S.

TH-PO685

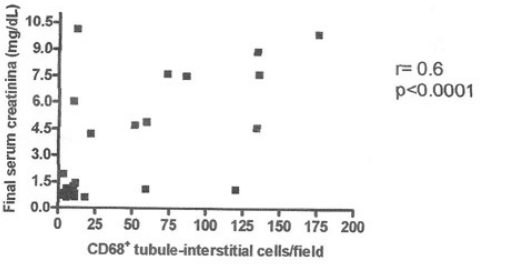
CD68 Positive Cells in Renal Biopsy Predict Long Term Prognosis in Proliferative Lupus Nephritis Cristiane Bitencourt Dias,[1] Patrícia Malafronte,[1] Jin Lee,[2] Aline Lázara Resende,[1] Cilene Carlos Pinheiro,[1] Denise Maria Avancini Costa Malheiros,[2] Rui Toledo Barros,[1] Viktoria Woronik.[1] *[1]Nefrologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; [2]Patologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.*

Background: No long-term assessment about macrophages in renal histology in lupus nephritis(LN)is known. The aim was to describe relations of renal outcomes with tecidual macrophages (CD68+) expressed in biopsy obtained on the diagnosis.

Methods: Thirty four female patients with proliferative LN were prospectively followed-up. Immunohistochemical was performed with monoclonal antibody anti-CD68 in renal biopsy. Patients were stratified in two groups according to renal outcome: GFR ≤ 60 mL/min/1.73m2 (n=14) and GFR > 60mL/min/1.73m2 (n=20). Patients received prednisone and cyclophosphamide on induction.

Results: The groups with better and worse renal outcome were not diferent between age (25.0±8.3 vs 26.0±11.5 years), activity index(5.0±1.6 vs 4.3±2.5), initial proteinuria (3.8±2.2 vs 5.4±2.7 g/day), systemic sledai (12.0(12.0–16.0)vs 12.0(8.0–12.0), and follow-up (3.6±0.6 vs 3.5 (2.7–4.0)years). The group with better renal outcome showed less chronicity index (1.0(0.0 - 4.0)vs 4.0(2.0–5.0)p=0.04), initial serum creatinine (1.4(1.0–2.1) vs 2.6±1 mg/dL p =0.03) and CD68+ tubule-interstitial (6.1(4.5–10.5)vs 65.8 ± 58.0 cells/field p=0.0006). Tubule-interstitial expression of CD68+ cells showed positive correlation with final serum creatinine (r=0.6, p<0.0001) and patients with CD68+ expression over 50 cells/field showed worse renal outcome p=0.003, with relative risk of 3.2.

Figure 1. Correlation between CD68$^+$ tubule-interstitial and final serum creatinine



Conclusions: CD68+ cells tubule interstitial expression on renal biopsies predict long term GFR. Values more than 50 CD68+ cells/field showed relative risk of 3.2 to develop worse renal outcome.

TH-PO686

Lupus Nephritis Versus "Lupus-Like" Glomerulonephritis: Are the Same Disease? Daniela Loss Mattedi, Roberto Savio Silva Santos, Janaina De Almeida Mota Ramalho, Lectícia Jorge, Rui Toledo Barros, Viktoria Woronik. *Departament of Nephrology, University of São Paulo, São Paulo, SP, Brazil.*

Background: Patients who present with typical histological lesions but do not fulfill the American Rheumatism Association criteria for the diagnosis of systemic lupus erythematosus (SLE) may represent a diagnostic problem. The aim of this study was to compare the clinicopathologic features of patients with SLE and lupus-like GN.

Methods: We performed a retrospective study of 10 patients with lupus-like GN, defined by: (1) immunofluorescence microscopy(IF) staining for granular glomerular IgG, IgM, C3 and C1q ≥ 1+ (0 to 4+ scale); (2) negative antinuclear antibodies (ANA titer ≤ 1:80) and negative anti-dsDNA antibodies; and (3)without extra-renal lupus activity. Cases were randomly matched with 20 patients with classical LN IV, according to baseline clearance (MDRD simplified formula) and time of follow-up. Treatment was decided by the clinical staff based on literature protocols.

Results: The clinical and histological features are summarized in Table 1.

Clinical and Histologic Features

	Lupus-like GN	Class IV LN
n	10	20
Age(yr)	42(35-47)*	27(20-33)*
Female	6(60%)	18(90%)
Serum Creatinine	1.4(0.9-2.3)	1.4(0.7-2.1)
eGFRi	47.5(28-90)	49.5(28-96)
Serum Albumine	2.4(2.0-2.8)	2.6(1.9-3.3)
Proteinuria	5.3(2.9-8.2)	3.9(1.2-7.5)
Hematuria	9(90%)	13(65%)
Hb	10.1(8.2-12)	11.2(10.3-13.1)
C3	72.5(50-99)	72(47-88)
ANA	0*	16(80%)*
anti-dsDNA	0*	12(60%)*
No. Glomerulos	23(12-32)	13(7-21)
Interstitial Fibrosis>10%	9(90%)	19(95%)
Crescents	1(10%)*	19(95%)*
Follow up	24(11.5-72)	24.7(14-76)
eGFRend	75(52-93)	72.6(30-98)
Albumin end	4.2(2.4-4.3)	4.2(4.0-4.6)
C3 end	117(73-145)	91(26-116)
Proteinuriaend(g\day)	0.85(0.3-5.1)	0.9(0.4-4.1)
eGFR\year	0.75(-9.6 - 8.6)	1.9(-23.8 - 45)

Results are shown as median IQR * p < 0.05

The immunosuppressive therapy used did not differ between groups and included prednisone, cyclophosphamide and mycophenolate. At the end of follow-up, one patient progressed to end stage renal disease in "Lupus-like" GN group and 2 patients in class IV LN group.

Conclusions: In our study, "Lupus-like" GN was very similar to LN regarding the clinical features, therapeutic response and outcome.

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TH-PO687

Worse Renal Outcome with MPO-ANCA-Associated Nephritis Compared to PR3-ANCA-Associated Nephritis

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Background: End-stage renal disease is a most debilitating outcome in ANCA associated vasculitis (AAV). Renal involvement and high age at diagnosis have been shown to predict ESRD, however, more prognostic factors are needed.

Methods: In order to analyse the impact of serological specificity on renal outcome clinical and laboratory data at presentation were retrieved from all patients diagnosed during a 13 year-period (1997-2009) within a defined population in southern Sweden. All patients were followed until the end of 2010 and renal survival among proteinase 3 (PR3)-ANCA+ and myeloperoxidase (MPO)-ANCA+ patients were studied by Kaplan-Meier analysis.

Results: A total of 121 patients with AAV, renal involvement and positve ANCA tests (PR3=62; MPO=59) were included in the study. The clinical and laboratory characteristics are listed in the table below. Patients with PR3-ANCA had significantly higher inflammatory activity and larger number of organ involved at diagnosis. Among MPO- ANCA+ patients, 19 (32%) developed end-stage renal disease compared to 10 (16%) of the PR3-ANCA+ patients (p=0.032). These results were consistent after correction for sex, age and creatinine at diagnosis. There was no significant difference in the mortality rate between the two groups.

Demographics, clinical and laboratory features in 121 patients with AAV and renal involvement

	PR3-ANCA	MPO-ANCA	p-value
Number	62	59	
Sex (M/F)	40/32	30/29	0.090
Age, median(range)	69 (19-88)	71 (32-92)	0.304
CRP, median(range)	124 (13-294)	50 (1-295)	<0.001
Hemoglobin,g/l,median(range)	104 (75-155)	103 (72-142)	0.097
>3 organ systems	47 (76 %)	35 (59 %)	0.04
Creatinine, median (range)	237 (53-1214)	259 (44-1369)	0.107
Death during follow-up	22 (35%)	25 (42%)	0.461
ESRD during follow-up	10 (16%)	19 (32%)	0.032

Conclusions: In this population-based cohort of AAV, MPO-ANCA + had a doubled risk of developing ESRD compared with PR3-ANCA + patients, despite similar renal function at diagnosis. These findings should be taken into consideration when prescribing treatment and stratifying patients in therapeutic trails.

TH-PO688

A Multi-Center Study on Renal Transplantation in ANCA-Associated Vasculitis

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Background: Anti-Neutrophil Cytoplasmic Antibody (ANCA)-associated glomerulonephritis (AAGN) can recur in a renal allograft. The aim of this study is to evaluate the outcome of renal transplantation in Dutch patients with AAGN with special focus on disease recurrence and graft survival.

Methods: Patients were retrospectively recruited through the Dutch national pathology database PALGA. The patients had to have 1 of the following diagnoses: Wegener's granulomatosis, microscopic polyangiitis or renal limited vasculitis, AND there had to be at least 1 transplant biopsy. Biopsies and clinical data were available for an interim analysis on 55 patients (median follow-up: 60 months). Biopsies were scored according to Banff '09. Renal recurrence was scored according to the new histopathologic classification of AAGN.

Results: Five years after transplantation, 15 grafts were lost: 4 due to recurrence (1 mixed, 1 focal, 2 crescentic class). In total 8 patients had a recurrence: 5 intra- and extrarenal, 2 intrarenal (2 focal, 2 crescentic and 3 mixed class) and 1 extrarenal. Five years after transplantation, 29 patients had a functioning graft: 20 serum creatinine <200µmol/L, 7 serum creatinine ≥200µmol/L, 2 unknown. There were 23 histologically proven acute rejections in 20 patients.

Conclusions: This is one of the largest studies to date investigating long-term outcome of renal allografts in AAGN patients. The recurrence rate in this cohort was 2.0% per patient year of follow-up. Five years graft-survival is 70.4%. Disease recurrence in the kidney has led to graft loss in most cases (4 out of 7).

TH-PO689

Histopathologic Classification of Glomerular Lesions in ANCA-Associated Glomerulonephritis Does Not Reliably Predict Clinical Outcome

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Background: A recently published classification schema identified 4 classes of GN associated with antibodies to neutrophil cytoplasmic antigens (ANCA) based on the appearance of glomeruli; focal (F), crescentic (C), mixed (M) and sclerotic (S) and found class to be an independent predictor of clinical outcomes in a European multicenter cohort of 100 subjects(1). We performed a ten year retrospective analysis of all 21 adults with ANCA-associated GN at a Northern New England center.

Methods: Biopsies were classified according to strict criteria of Berden(1). Statistical analysis was by ANOVA.

Results: Biopsies were readily categorized: 8 as class F; 4 C; 6 M and 3 S. Mean # glomeruli 11.1. Follow up 42.4 (2-122) mo. Mean age 68.1 yr with trend to older age in class S. 3862% were male. Treatment did not differ between groups. Mean entry eGFR 24.4 ml/min. There was a trend to lower entry eGFR in the S class, but no significant difference between groups or in entry eGFR between the non-S classes. Mean changes in eGFR at one yr and follow up were +8.24 and +18.75 ml/min respectively. There was a trend to greater improvement in class C and decline in function in class S, in contrast to the data of Berden(1), which showed greatest improvement in F. 8 reached ESRD: 2 C; 3 F; 2 M and 1 S. 4 died: 1 C; 3 F.

Conclusions: We describe a ten year of ANCA-associated GN in 21 subjects in a population similar to the index study. We did not detect correlation of clinical outcomes with pathologic class. We did not confirm that subjects with F histology had higher eGFR at entry or greater improvement. We conclude that the schema proposed by Berden(1) is not consistently reliable for clinical prognostication in individual patients. Our conclusion is limited by the small number of subjects (n=21) relative to the index study (100), and by wider variation in length of follow up. Our study points to a need for continuing collaborative data collection in this rare but important disease.

[1] Berden et al JASN 2010 1628-36

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TH-PO690

Clinical Implications of the New Pathological Classification of Pauci-Immune Small Vessel Vasculitis of the Kidney

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Background: We aimed to study the clinical correlation of the recent international working of renal pathologists (IWGRP) classification of small vessel renal vasculitis.

Methods: Clinical details and renal biopsy blocks of consecutive patients from 2001 to 2010 diagnosed to have pauci-immune renal vasculitis at our institution were retrieved and histological features re-evaluated by two pathologists to assess the clinicopathologic correlates of eGFR at presentation, follow up and the IWGRP classification.

Results: Of the 12,200 renal biopsies, 107(0.9%) were pauci-immune renal vasculitis (median age 48 (12-82)yrs; M:F = 1.5:1). The mean serum creatinine and proteinuria at presentation were 5.8±4.6mg/dl &1.6±2.1g/d. cANCA was positive in 28% and pANCA in 34.6%. Predictors of eGFR at presentation<30ml/min/1.73sq.m., were glomerular neutrophilic infiltrates(0.005), eosinophilic infiltrates(0.035), karyorrhectic debris(0.020), segmental sclerosis(0.007) and interstitial fibrosis (p<0.001). Based on IWGRP classification of the 9.3% focal , 29.9%crescentic, 55.1% mixed and 5.6% sclerotic lesions, 9.1, 39.0,44.2 and 7.8% respectively had an eGFR≤30ml/min/1.73sq.m. at presentation (p= < 0.001). Among the 28 cases followed up , these groups showed a mean eGFR at 1 year after diagnosis of 49.8±28.2, 46.3±23.4, 71.6±27.9 and 5.3 respectively, with the highest increment in eGFR in the crescentic group. eGFR at 3,6 and 12 months were independently predicted by the IWGRP class (temporally increasing β) and eGFR at presentation (temporally decreasing β).

Conclusions: The IWGRP class and eGFR at presentation are independent predictors of outcome in renal small vessel vasculitis. As interstitial fibrosis is predictive of eGFR at presentation, it could be incorporated into the IWGRP classification.

TH-PO691

Histologic Classification of Pauci-Immune Glomerulonephritis: Outcomes Predictors

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Background: Although renal biopsy is the gold standard for the diagnosis of pauci-imune glomerulonephritis, its pathologic classification remains a controversial issue. A recently developed classification proposes four general categories of lesions: focal, crescentic, mixed, sclerotic. The aim of our study is to access its prognostic value.

Methods: We analyzed 58 patients with biopsy-proven pauci-imune glomerulonephritis at our center from 2000 to 2010, 51 patients were included in analysis(5 excluded for missing

data and 1 for insufficient number of glomeruli on biopsy) Biopsies were reviewed and classified according to the new classification. X^2, one-way ANOVA, and multiple logistic regression analyses were performed as appropriate. The glomerular filtration rate (GFR) was estimated using modified MDRD simplified equation.

Results: At baseline, the mean age was 45 ± 20 yrs, and 37.2% of the patients were males. ANCA test was positive in 19 (37,3%), negative in 22 (43,1%) and missing in 10 (19,6%). Mean eGFR at entry was 19 ± 24 ml/min/1,73m². Regarding the histological features, cases were subdivided as shown in table.

Renal outcome according to class

	eGFR Entry	n	eGFR 12 Months	n
Focal	110 ± 41	2	129 ± 67	2
Crescentic	8,4 ± 4,1	9	25,8 ± 6,8	4
Mixed	23 ± 19	17	56,7 ± 31,8	9
Sclerotic	12,7 ± 11,4	23	28,7 ± 12,6	4
Total		51		19

Results are shown as mean± SD

As depicted in Table, the renal biopsy categories were correlated to the degree of renal function at presentation(p = 0,02), and showed a tendency at 1 year follow-up(p=0,08). We couldn't analyze the impact of focal subtype on outcome because the low number of patients. Finally, logistic regression analysis showed that sclerotic class was significantly associated with a worse renal outcome after one year of follow-up, even after adjustment initial eGFR (OR 4.7, 95%CI 1.17-19.2 p=0.02).

Conclusions: In our study, the histologic classification was correlated with renal function at baseline and was associated to ESRD at 1 year regardless of eGFR.

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TH-PO692

Clinical Characteristics of Japanese Patients Having Anti-Neutrophil Cytoplasmic Antigen-Associated Glomerulonephritis with Immune Deposits Yasufumi Takahashi, Makoto Harada, Yuji Kamijo, Makoto Higuchi. *Nephrology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan.*

Background: Anti-neutrophil cytoplasmic antigen-associated glomerulonephritis (ANCA-GN) is pathologically characterized as a pauci-immune type of necrotizing crescentic glomerulonephritis. However, several earlier studies investigating European/American patients reported that ANCA-GN was often accompanied by the glomerular immune deposits. The deposition rate and the clinical significance of the immune deposits were controversial, and there are no data concerning these issues in Japanese patients with ANCA-GN.

Methods: We retrospectively investigated 27 Japanese patients with rapidly progressive kidney impairment who were positive for serum ANCA (MPO-ANCA 24 cases, PR-3 ANCA 3 cases) and exhibited biopsy-proven necrotizing crescentic glomerulonephritis. The immune deposits were confirmed by two methods, immunofluorescence microscopy and electron microscopy. The pathological and clinical data were compared between immune-deposits-positive and negative groups.

Results: Among these 27 cases, 8 cases exhibited the glomerular immune deposits (30%). The clinical parameters at the time of admission, including serum creatinine (2.3 vs 2.5 mg/dl) and ANCA titer (298 vs 556 EU), did not differ between the immune-deposits-positive and negative groups, while massive proteinuria was significantly detected in the immune-deposits-positive groups (2.8 vs 1.1 g/day, P=0.029). However, there was no significant difference in kidney survival rate (75 vs 90%), survival rate (100 vs 84%), or the incidence of extrarenal organ injury (63 vs 53%), between the groups throughout the observation period (26 months).

Conclusions: Glomerular immune deposits were unexpectedly detected at a high rate in Japanese ANCA-GN patients. The clinical course of the ANCA-GN for 2 years after diagnosis was not altered, but the level of proteinuria was markedly enhanced by the glomerular immune deposits, suggesting that they had a potential effect as an aggravation factor of kidney dysfunction. An additional study investigating the long-term effects of the glomerular immune deposits in ANCA-GN is needed in the future.

TH-PO693

Identification of Early Pathologic Features of Focal Segmental Glomerulosclerosis by Repeat Renal Biopsy Cai-Hong Zeng, Hong-Guang He, Feng Xu, Qing-Yan Zhang, Xi Tang, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China.*

Background: To explore the early pathologic features of focal segmental glomerulosclerosis (FSGS).

Methods: We analysed 10 patients (8 males and 2 females) whose first renal biopsies showed minimal change lesions while second biopsies indicated FSGS (3 cases classified as NOS, 3 cellular, 2 tip, 1 perihilar, and 1 collapsing)

Results: The mean age at the time of the first biopsy was 22.9±6.7 years. The mean interval time of the two biopsy collections was 22.3±16.6 months. The proportion of segmental glomeruloscleorosis was 11.4±7.2 (3.2~22.2) %. At the first biopsy, there were no global glomerulosclerosis and mesangial proliferation. The splitting of Bowman's basement membrane (BBM) at tubular pole were seen in 7 cases, inflammatory infiltration surrounding glomeruli in 6 cases, and tubular cell refluxing into Bowman's capsule in 6 cases. These numbers increased to 10, 7 and 10, respectively, at the repeat biopsy. The proportions of glomeruli with above lesions in the cases were 69.1±33.6%, 19.7±5.7%, 17.9±6.1% at first biopy, and were 87.4±10.1%, 19.8±10.3%, 23.4±10.9% at second biopsy. Acute tubular-interstitial lesions (ATIL) were observed in both of the biopsies of 9 cases, of which, 4 cases had aggravated ATIL, 4 ameliorated, and 1 unchanged. Chronic tubular-interstitial lesions were present in one case at first biopsy, but in 9 at the repeat biopsy. 7 cases had more severe interstitial inflammatory infiltration at repeat biopsy compared with that at first biopsy. Arteriole hyalinosis was present in 2 cases at the first biopsy and then in 6 cases at the second biopsy. Foot process effacement ≥ 50% of glomeruli, denudation of food process from GBM, and cytoplasm vacuolation were observed in 6, 4, and 5 cases at first biopsy, respectively, and in 9, 6, and 9 cases at second biopsy.

Conclusions: In conclusion, splitting of BBM at tubular pole, inflammatory infiltration surrounding glomeruli, and tubular cell refluxing into Bowman's capsule, ATIL, denudation of foot process from GBM, and cytoplasm vacuolation are the early lesions of FSGS.

TH-PO694

Low Birth Weight-Related Nephropathy Has Similar Pathological Changes Both in Glomeruli and in Tubular Cells with Those of Mitochondrial Cytopathy Toshiyuki Imasawa,[#1] Moritoshi Kadomura,[#1] Takehiko Kawaguchi,[#1] Yutaka Yamaguchi,[#2] Hiroshi Kitamura.[#1] *[1]Division of Nephrology, Chiba-East National Hospital, Chiba-City, Chiba, Japan; [2]Yamaguchi's Pathology Laboratory, Matsudo-city, Chiba, Japan.*

Background: Glomerular involvement in patients with mitochondrial cytopathy is a focal segmental glomerulosclerosis (FSGS). In addition, it was recently reported that granular swollen epithelial cells (GSECs), which include increasing damaged mitochondria, are specific pathological changes in distal tubuli or collecting ducts in patients with mitochondrial cytopathy. On the other hand, adults who were born with low birth weight (LBW) have high risk of kidney damages and sometimes showed FSGS lesions in their glomeruli. Here, we assessed their tubular changes and mitochondrial gene mutations.

Methods: Five adult patients with FSGS lesions who were born under 2500g, two mitochondrial cytopathy patients, and five obesity-related FSGS patients who were born over 2500g, were analyzed in this study.

Results: In all five patients with LBW, glomeruli showed hypertrophy and FSGS perihilar variant lesions. Interestingly, in four of five patients, GSECs, which were previously reported only in patients with mitochondrial cytopathy, were also apparently observed in collecting ducts. A part of these tubular cells were dropping out of tubular arrangement with chromatin condensation. Mitochondrial gene mutations in blood, urine sediments, and kidney specimens were checked by PCR-Luminex assay. Any mitochondrial gene mutations were not detected in these patients with LBW. Two mitochondrial cytopathy patients also had FSGS perihilar variant lesions in glomeruli and had GESCs in their tubuli. PCR-Luminex assay in all blood, urine sediments, and kidney specimens revealed mitochondrial gene mutations (3243 A to G). Although five obesity-related nephropathy patients had FSGS perihilar variant lesions, their tubular cells did never show GSECs. Now, we try to stain kidney specimens for COX IV.

Conclusions: These results suggest that mitochondria should be associated with the etiopathogenesis of LBW-related nephropathy probably by functional defects.

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TH-PO695

Bilirubin Attenuate Arteriolopathy and Tubular Proteinuria by Inhibition of NADPH Oxidase in Cyclosporine Induced Nephropathy Sewon Oh,[1] Ki Young Na,[1,2] Suhnggwon Kim,[2] Ho Jun Chin.[1,2] *[1]Department of Internal Medicine, Seoul National University Bundang Hospital, Kyeong-Kido, Republic of Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background: Epidemiological studies have shown that patients with hyperbilirubinemia have a lower incidence of cardiovascular disease and strokes. In addition, bilirubin has been reported as an endogenous antioxidant by inhibition of reactive oxygen stress. We determined whether injection of bilirubin could attenuate vascular or tubular injury though inhibition of nicotinamide adenine inucleotide phosphate (NADPH) oxidase subunits in cyclosporine (CsA) induced nephropathy.

Methods: Male Sprague Dawley rats were administered vehicle, CsA, CsA + bilirubin (BIL), and BIL. Bilirubin was injected intraperitoneally three times for one week (60mg/kg). All groups kept on a 0.05% low salt diet were treated with vehicle (VH) or CsA for 4 week (15mg/kg/day, subcutaneous). Physiologic and histologic changes were studied in addition to the concentration of urine Kidney injury molecule-1 (Kim-1), urine Neutrophil gelatinase associated lipocalin (NGAL) by ELISA, and the protein expression of NADPH oxidase 4 (NOX4) and p22phox NADPH oxidase subunit by western blot. Urine hydrogen peroxide was measured using 2',7'-dichloro-dihydrofluorescein diacetate.

Results: There was no difference on renal function between CsA group and CsA+bilirubin group (1.69 ± 0.93 vs. 1.71 ± 0.35, *P*=0.967). Arteriolopathy was improved in CsA + BIL group compared than CsA group (20.5± 3.4 % vs. 37.3± 9.0 %, *P*=0.004). Also, CsA + BIL group showed lower concentration of urine Kim-1 than CsA group (0.85±0.34 vs. 2.28±1.52 ng/mL, *P*=0.025). Urine NGAL did not show significant difference (0.7± 0.3 vs. 1.5± 1.1 μg/mL, *P*=0.117). CsA + BIL group was found lower expression of NOX4 and p22phox NADPH oxidase subunit than CsA group (*P*=0.011 and *P*=0.028, respectively). Urine hydrogen peroxide was lowered in CsA + BIL group (444.6±74.9 vs. 242.2±140.5, *P*=0.022).

Conclusions: Bilirubin reduced reactive oxygen species by inhibiting NADPH oxidase and attenuate arteriolopathy and tubular proteinuria in CsA induced nephropathy.

TH-PO696

Cyclosporine A Causes Maturation Failure in Embryonic-Type Glomeruli Persisting after Birth Shinsuke Fujita, Keisuke Sugimoto, Kouhei Miyazaki, Mitsuru Okada, Tsukasa Takemura. *Pediatrics, Kinki University School of Medicine, Osakasayama, Osaka, Japan.*

Background: Cyclosporine A (CyA) is administered to patients to enable withdrawal of corticosteroids or to maintain remission in patients with intractable nephrotic syndrome (NS). While, chronic renal injury caused by CyA is a major concern. Early starting CyA treatment in life might be a risk factor for CyA nephropathy, however the cause to develop nephropathy remains unknown.

Methods: We analyzed renal histologic and immunohistologic findings in 22 children with NS who did (n=5) or did not (n=17) develop CyA nephropathy despite appropriately low serum CyA concentrations being maintained over 2 years. We performed staining for type IV collagen α1, laminin β1 and laminin β2 to discriminate embryonic-type from mature glomeruli. Staining patterns were used to semiquantitatively assess glomerular immaturity (glomerular immaturity index; GII).

Results: In follow-up biopsy specimens, residual embryonic-type, collapsed embryonic-type and sclerotic glomeruli that had failed to differentiate were observed. Patients with early-onset CyA nephropathy had a high GII. In patients with a high GII, arteriopathy developed early in CyA treatment. Arteriopathy was observed mostly near embryonic-type glomeruli. Taken together, these glomeruli (surviving embryonic-type, collapsing embryonic-type, and sclerotic glomeruli) essentially equaled the total number of embryonic-type glomeruli in specimens obtained before CyA treatment.

Conclusions: A need for caution in CyA therapy for patients with NS, even for a relatively short course of administration, because some patients may have embryonic-type glomeruli or immature arterioles that predispose them to CyA nephropathy.

TH-PO697

The Mechanism of the Development of Segmental Glomerular Sclerosis in Idiopathic Membranous Nephropathy Megumi Fukui,[1] Akiko Mii,[1] Akira Shimizu.[2] *[1]Internal Medicine (Division of Neurology, Nephrology, and Reumatology), Nippon Medical School, Tokyo, Japan; [2]Pathology, Nippon Medical School, Tokyo, Japan.*

Background: Membranous nephropathy (MN) with focal segmental sclerosis is associated with a poorer prognosis than MN without these lesions. The mechanism of the development of segmental sclerosis in MN is still uncertain.

Methods: We selected renal biopsy cases of idiopathic MN cases with segmental sclerosis (n=26/ total 250), and assessed the development of segmental sclerosis, focusing on the glomerular endothelial cell injuries, organization and thickening of GBM, and VEGF expression in podocytes.

Results: The average age of these cases is 62.4±9.77 years. About 77 % (n = 20) of these cases developed nephrotic syndrome. eGFR (mean 57.36±18.1 mg/dl) was significantly lower compared to MN without segmental sclerosis. In histopathology, the most common pattern of segmental sclerosis in MN was characterized by glomerular capillary collapse with extracellular matrix accumulation. All MN cases with segmental sclerosis showed endothelial cell injuries that characterized by obliteration and loss of glomerular capillaries with disappearance of CD34+ endothelial cells. Interestingly, in all cases with segmental sclerosis, narrowing of glomerular capillaries with endothelial cell injuries was evident even in non-sclerotic areas. In deed, by computer assessed morphometric analysis, MN cases with segmental sclerosis had significantly to smaller glomerular capillaries and larger matrix area in glomeruli than MN cases without sclerotic legion. No difference of VEGF expression on podocytes was detected between MN cases with and without segmental sclerosis (1.20±0.72 /0-4 semiquantitative score in MN with sclerosis vs. 1.35±0.86 /0-4 scores in MN without sclerosis, p=0.64). However, significant difference of the thickness of GBM was evident between MN cases with and without segmental sclerosis (1.48±0.70 μm in MN with sclerosis vs. 1.02±0.48 μm in MN without sclerosis, P<0.05).

Conclusions: The glomerular capillary and endothelial cell injuries may be induced by GBM organization and thickening with subepithelial deposits that contributed to the development of segmental sclerosis in MN.

Funding: Private Foundation Support

TH-PO698

Differences in Expanded CD8$^+$ T Cell Subsets Indicate Distinct Immune Responses Cause idiopathic and Secondary Membranous Nephropathy Aki Kuroki, Kei Matsumoto, Tadao Akizawa. *Division of Nephrology, Department of Medicine, Showa University, Tokyo, Japan.*

Background: Although idiopathic membranous nephropathy (IMN) and secondary forms of membranous nephropathy (sMN) share similar clinical and histological features, discovery of phospholipase A2 receptor 1, a target antigen in IMN indicates that they are caused by different immunologic processes. In this study, to define whether there are distinct immune responses in IMN and in sMN, we analyzed peripheral lymphocytes subsets.

Methods: We collected peripheral blood samples from patients with IMN, sMN and normal individuals (normal control, NC). sMN group included patients with lupus nephritis, and patients without clinical features of secondary disease, but whose renal tissues were positive for C1q, and/or negative for IgG4 staining, the findings, which were suggestive of sMN. We analyzed peripheral lymphocytes subsets using flow cytometry and analyzed the relationship between clinical parameters. Kruskal-Wallis' test and Spearman's rank correlation were used for statistical analysis.

Results: The most prominent difference between IMN and sMN was subsets of CD8$^+$ T cells. In the IMN group, CD8$^+$ T cells decreased, which was resulted from decrease in CD8$^+$ naïve T cells. However, CD8$^+$effector-memory T cells increased, and level of CD8$^+$effector-memory T cells correlated with the levels of proteinuria. In contrast, in the sMN group, CD 28 null CD8$^+$ T cells increased, although level of those cells was not correlated with any of clinical parameters. Another difference was level of CD25high CD4$^+$ regulatory T cells, which decreased only in the IMN group.

Conclusions: These results indicate that although histological findings are similar, immunologic processes underlying the deposition of immune complex are distinct in idiopathic and in secondary membranous nephropathy, and specific therapeutic approach is required.

Funding: Pharmaceutical Company Support

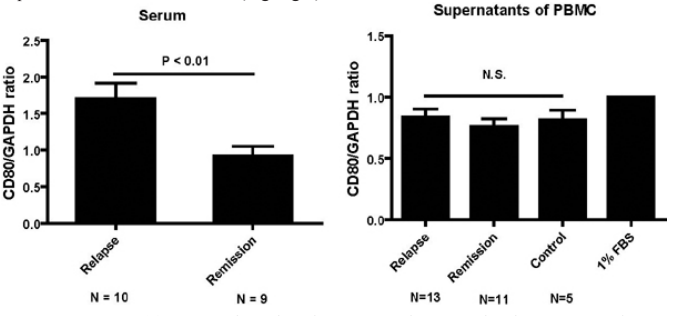
TH-PO699

Serum Factor from Minimal Change Nephrotic Syndrome Patients Increases Podocyte CD80 Expression Takuji Ishimoto,[1] Richard J. Johnson,[1] Eduardo H. Garin.[2] *[1]Division of Renal Diseases & Hypertension, University of Colorado Denver, Aurora, CO; [2]Division of Pediatric Nephrology, University of Florida, Gainesville, FL.*

Background: Proteinuria in Minimal Change (MC) patients is thought to be due to a circulating factor released by patients lymphocytes. In MC patients in relapse, glomeruli express CD80 and CD80 urinary excretion is increased. Because CD80 is known to mediate proteinuria, we have evaluated the effect of serum and supernatants of Peripheral Blood Mononuclear Cells (PBMC) cultures from MC patients on CD80 podocyte expression.

Methods: Thirteen MC patients were studied in relapse and 11 in remission. Five normal subjects served as controls. Immortalized human podocytes were incubated with 15% of MC patients serum or with 15% of 4 to 10x concentrated supernatants of PBMC cultures from normal controls and from MC patients. PBMC (1 x 10^6/ml) were incubated with 15% FBS for 72 h prior to obtain the supernatants. RNA was extracted at 6 h, and QPCR for CD80 was done.

Results: Serum albumin was 2.18 +/- 0.53 (g/dl) and urinary protein/creatinine ratio 11.2 +/- 5.12 (mg/mg) in relapse, and 4.1 +/- 0.73 (g/dl) and 0.19 +/- 0.12 (mg/mg) in remission respectively. MC patients in relapse serum significantly increased podocyte CD80 expression when compared to that seen in remission ($p< 0.01$) (Fig. left). No difference in CD80 expression among the groups were seen when podocytes were incubated with supernatants of PBMC cultures (Fig. right)



Conclusions: (1) MC patients in relapse serum increases in vitro CD80 podocyte expression. This finding can explain, at least in part, the mechanism of proteinuria in these patients. (2) The lymphocyte is not involved in the CD80 mediated proteinuria in MCD patients.

Funding: NIDDK Support

TH-PO700

Routine Microscopic Examination of Urine Sediment Can Identify Urinary Podocytes Masanori Hara. *Pediatrics, Yoshida Hospital, Tsubame, Niigata, Japan.*

Background: Podocytopenia is involved in the progression of glomerular sclerosis. One of the causes for podocytopenia is a detachment of podocytes from the glomerular basement membrane into urine. To detect urinary podocytes has a diagnostic value to predict ongoing severe podocyte injury. In this study we evaluated the usefulness of routine microscopic examination of urine as a simple method compared to the previous immunofluorescent (IF) examination.

Methods: Urine samples from patients with various glomerular diseases such as IgA nephropathy, Schoenlein Henoch purpura nephritis, lupus nephritis (N=25) were used. Urine was centrifuged at 1800 rpm for 5 minutes and the sediment was mixed with Sternheimer staining solution and examined carefully for characteristics of the cells appeared in urine sediment using conventional light microscope. For IF study urine sediment was suspended in PBS and then the cells in the sediment were trapped using BD SurePath™ Pap Test on the slide glass. The slide glass was processed to conventional IF procedure using anti-podocalyxin antibody. The cytological characteristics for podocytes was examined on the podocytes isolated from urine sediment using magnetic beads coupled with anti-podocalyxin antibody and then stained by Sternheimer solution.

Results: Cytological characteristics of urinary podocytes was as follow; 1) 15-30 um in diameter, 2) round or oval shape, 3) the margin of cells is mostly smooth, sometimes having processes, 4) the nucleus is located mostly in the center or sometimes deviated slightly, 5) often binucleated, 6) high N/C ratio, 7) large and clear nuclear staining, and 8)

violet (C30M70Y0K0 by CMYK color model) cytoplasmic color. The cells matched for all of the above criteria were regarded as podocytes and the number was counted. The time consumed for whole procedure by routine microscopic examination is within 30 minutes, although that by IF examination is about 3 hours. The number of podocytes by routine microscopic examination was well correlated with that by IF examination (Spearman, N=32, p<0.0001).

Conclusions: Routine microscopic examination of urine sediment by Sternheimer staining is very simple but reliable on identifying urinary podocytes.

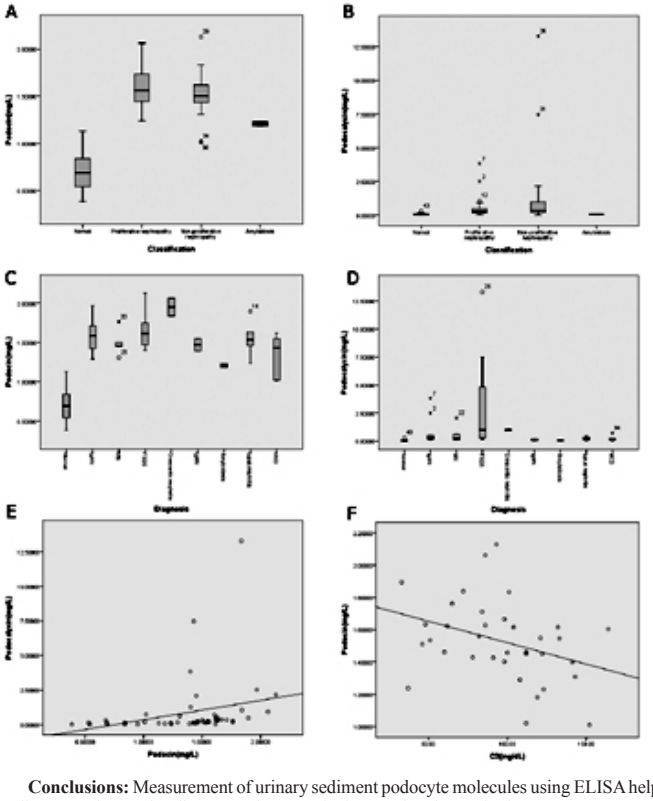
TH-PO701

ELISA Analysis of Urinary Sediment Podocin, Podocalyxin in the Patients with Different Glomerular Diseases Bin Zhu, Yi Lin, Xiaoling Zhu, Sen Zhong, Caifeng Zhu, Ying Lu. *Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Zhejiang University of Chinese Medicine, Hangzhou, Zhejiang, China.*

Background: To investigate the urinary sediment podocin and podocalyxin in the patients with biopsy proven glomerulonephritis using ELISA method.

Methods: Patients with biopsy proven glomerulonephritis were enrolled for the analysis of urinary sediment podocin and podocalyxin measured using ELISA.

Results: We collected totally 40 patients (15 Males and 25 females). There were 19 proliferative glomerulonephritis cases (IgAN: 10 cases; Crescentic glomerulonephritis: 2 cases; IgMN:2 cases; Lupus nephritis IV(A/G): 5 cases);19 non-proliferative glomerulonephritis cases (MCD: 5 cases; FSGS: 8 cases; MN: 6 cases) and 2 patients with renal amyloidosis. 10 healthy persons were enrolled as the control. Results: Urinary podocin and podocalyxin in the healthy person was the lowest. There was no significant difference in the urinary podocin and podocalyxin between the proliferative glomerulonephritis group and the non-proliferative glomerulonephritis group(P>0.05). The urinary podocin and podocalyxin was increased significantly in the patients with crescentic glomerulonephritis as compared with the patients with other renal disease (podocin: P<0.05; podocalyxin: P>0.05). Then the urinary podocin was gradually decreased in the order as FSGS, IgAN, MN, Lupus nephrits, MCD, IgMN. The urinary podocalyxin was gradually decreased in the order as FSGS, IgAN, Lupus nephrits, MN, IgMN, MCD. The urinary podocin and podocalyxin in the patients with renal amyloidosis was the lowest among the patients. Urinary podocalyxin correlated positively to urinary podocin . Urinary podocin correlated negatively to serum C3.



Conclusions: Measurement of urinary sediment podocyte molecules using ELISA help to diagnose glomerular disease. Urinary podocin correlated negatively to serum C3.

Funding: Government Support - Non-U.S.

TH-PO702

Clinical Implication of Detection of Urinary Podocyte-Associated mRNA Profile in Various Stages of Diabetic Nephropathy Min Zheng, Linli Lv, Jie Ni, Kun Ling Ma, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: Podocyte injury and subsequent excretion in urine play a crucial role in the pathogenesis and progression of diabetic nephropathy (DN). Quantification of messenger RNA (mRNA) expression in urinary sediment by real-time PCR is emerging as a noninvasive method of screening DN-associated biomarkers. We hypothesized that the urinary mRNA profile of podocyte-associated molecules may provide important clinical insight into the different stages of diabetic nephropathy.

Methods: DN patients (N=51) and healthy controls (N=13) were enrolled in this study. DN patients were divided into a normoalbuminuria group (UAE<30 mg/g, n=17), a microalbuminuria group (UAE 30~300 mg/g, n=15), and a macroalbuminuria group (UAE>300 mg/g, n=19), according to their urinary albumin excretion (UAE). Urinary cell pellet was collected from each study participant. Total RNA was extracted and cDNA was synthetized. Relative mRNA abundance of synaptopodin, podocalyxin, CD2-AP, α-actin4, and podocin were quantified by real-time PCR technology, and correlations between target mRNAs and clinical parameters were examined.

Results: The urinary mRNA levels of all genes studied were significantly higher in the DN group compared with controls (p<0.05), and mRNA levels increased with DN progression. Urinary mRNA levels of all target genes positively correlated with both UAE and BUN. The expression of podocalyxin, CD2-AP, α-actin4, and podocin mRNA correlated with serum creatinine (r=0.457, p=0.001; r=0.329, p=0.01; r=0.286, p=0.021; r=0.357, p=0.006, respectively). Furthermore, podocalyxin mRNA was found to negatively correlate with eGFR (r=-0.349, p=0.01). The ROC curve analysis shown that all target genes were effectively able to discriminate between DN patients and normal controls, with an AUC above 0.5.

Conclusions: The urinary mRNA profiles of synaptopodin, podocalyxin, CD2-AP, α-actin4, and podocin were found to increase with the progression of DN, which suggested that quantification of podocyte-associated molecules will be useful biomarkers of DN.

Funding: Government Support - Non-U.S.

TH-PO703

Lectin Microarrays of Intact Urinary Exosomes Indicate Distinct Glycosylation Profiles Jared Q. Gerlach,[1] Anja Krüger,[1] Shirley Hanley,[1] Susan Gallogly,[1] Marie C. Hogan,[2] Christopher James Ward,[2] Lokesh Joshi,[1] Matthew D. Griffin.[1] *[1]Regenerative Medicine Institute (REMEDI) and Glycoscience Group, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland; [2]Dept. of Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Urine exosome-like vesicles (ELVs) contain many nephron-derived glycoproteins but characterization of their surface glycosylation is limited and may be compounded by Tamm-Horsfall protein (THP) contamination. Intact ELVs and purified THP were profiled by lectin microarray and flow cytometry (FCM) to compare and contrast their glycosylation profiles.

Methods: 43 lectins were spot-printed on Nexterion® hydrogel slides. ELVs, isolated from healthy adult urine by ultracentrifugation (UC) or ultrafiltration (UF) were labeled with lipophilic dyes (CM-DiI, PKH26), incubated with lectin arrays and laser-scanned at 5μm resolution with fluorescence intensities generated for each lectin. Specificity was determined based on ≥50% inhibition with haptenic sugars. For FCM, ELVs were adsorbed to 4μm latex beads then incubated with biotinylated lectins and fluorochrome-labeled streptavidin prior to analysis on a FACSCanto® cytometer. Identical protocols were applied to AF647®-labeled THP.

Results: *N*-acetyl-glucosamine-binding lectins (LEL, WGA) had the highest fluorescence intensities with ELVs on microarrays and FCM suggesting high *N*-linked surface glycosylation. The α-fucose-specific lectin UEA-I exhibited negative or minimal binding to ELVs in either format. The galactose/*N*-acetyl-galactosamine-binding lectin SNA-II and the sialic acid-binding lectin SNA-I also bound ELVs on arrays, suggesting *O*-linked modifications and α2-6-linked terminal sialic acid, respectively. UC- and UF-prepared ELVs had closely comparable array profiles. THP and ELV profiles were similar but with distinct differences in relative intensities, e.g. β-galactose-binding lectin ABL (THP higher) and SNA-II (ELVs higher).

Conclusions: Microarrays and FCM allow rapid, ELV-specific glycosylation profiling, providing a basis to screen for protein glycosylation abnormalities in kidney disease and to facilitate urine ELV isolation.

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

TH-PO704

Refinement and Technical Validation of Assay for Urinary microRNAs Taro Horino, Takayuki Tsuji, Robert A. Star, Peter S.T. Yuen. *NIDDK, NIH, Bethesda, MD.*

Background: We previously showed that miRNAs in the urine exosomal fraction are biomarkers for acute and chronic kidney disease, but our assay required large 4 ml samples. Non-exosomal urinary miRNAs from glomerular filtration, tubular secretion, and/or cell death, are also potential biomarkers. Therefore, we developed a more sensitive purification/assay system, then measured and validated the distribution of miR27b in different fractions of normal urine.

Methods: We centrifuged healthy human or rat urine at 17,000 x g for 15 min, retained the 17k pellet, then ultracentrifuged the 17k supernatant at 200,000 x g for 1 h to obtain the exosomal fraction (200k pellet). We purified miRNA by Qiagen miRNeasy kit or TRIzol/miRNeasy. We measured miR27b or miR192 concentration by Taqman (RT-PCR) using synthetic mature miRNA (Dharmacon) standards. We validated assays by the Standard Addition Method (SAM; add 4 known increasing amounts of synthetic miRNA to an unknown sample, then extrapolate back to 0 addition).

Results: We readily detected miR27b in 100 µl of normal rat urine (30 amol/ml, validated by SAM). 20% of miR27b from normal rat urine was in the 17k pellet and 10% in the 200k pellet by direct measurement or SAM. The 200k supernatant contained 70% (direct measurement) or 60% (SAM) of urine miR27b. In contrast, human urine 200k sup (but not the exosomal fraction) contained an endogenous inhibitor which caused the assay to underestimate [miR27b]. Recovery of pure miR27b purified by miRNeasy or TRIzol/miRNeasy was 70% and 80%, respectively; this recovery was inhibited 29% by 5 µl of human urine (n=4 $p<0.05$). EDTA treatment, gel filtration, or dialysis could not remove the endogenous inhibitor.

Conclusions: We developed and validated a sensitive assay for urinary miRNAs that needs only 100 µl of urine. A lower volume requirement will allow miRNA measurements on archived samples. Urinary miR27b was mainly in the 200k supernatant in normal rats. The distribution in normal human urine could not be accurately determined due to endogenous inhibitory activity. Unless the inhibitor(s) can be removed, the Standard Addition Method may be needed to accurately assay miRNAs in unfractionated human urine or non-exosomal human urine fractions.

Funding: NIDDK Support

TH-PO705

Ultrastructural Pathology in IgG4-Related Kidney Disease Shinichi Nishi,[1] Hideki Fujii,[1] Yoko Takeda,[1] Keiji Kono,[1] Kentaro Nakai,[1] Shunsuke Goto,[1] Takako Saeki.[2] *[1]Divsioni of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan; [2]Department of Internal Medicine, Nagaoka Red Cross Hospital, Nagaoka, Niigata, Japan.*

Background: Autoimmune or allergic disorders were suspected to be pathogenic mechanisms of IgG4-related kidney disease (IgG4KD) characterized by tubulointerstitial nephritis (TIN). In order to explore pathogenic mechanisms we evaluated the distribution of electron-dense deposits (EDDs) in the interstitium and glomeruli in seven cases with IgG4KD.

Methods: Subjective seven cases were diagnosed according to the criteria of IgG4KD working group in the Japanese Society of Nephrology. Routine light, immunofluorescene and electron microscopy, as well as immunohistochemical and indirect immunofluorescence studies for IgG subclasses were performed. A clinicopathological study was conducted in all the cases.

Results: The distribution of the EDDs was variable, with incidence of deposition being highest in the mesangium (71.4%) and tubular basement membrane (85.7%). EDDs were also observed in the interstitium between collagen fibres, with the deposition rate being 71.4%. Some cases showed no immunological abnormalities, hypocomplementaemia or positive anti-nuclear antibody, but showed EDDs in their kidney. The histochemical and immunohistochemical studies for IgG4 did not indicate that the EDDs were immune complexes containing IgG4.

table1. Distribution of EDDs and serological abnormality

	Subepithelial space	Mesangium	Bwoman's capsule	TBM	Interstitium	Low complement	ANF positive
case 1	0	0	0	0	+	+	+
case 2	2+	0	0	+	+	+	0
case 3	0	+	+	+	+	+	+
case 4	0	+	+	+	+	+	+
case 5	+	+	0	+	0	0	ND
case 6	+	+	0	+	0	+	+
case 7	2+	+	+	+	+	0	0
rate of EDDs	57.1%	71.4%	42.9%	85.7%	71.4%		

ANF: anti-nuclear factor, ND: not detected

Conclusions: Although EDDs were found frequently in the interstitium and glomeruli of cases with IgG4KD, they appear to develop independently of autoimmune or allergic disorders. Further investigation is essential to determine the mechanism responsible for the development of IgG4KD from both immunological and pathological points of view.

TH-PO706

A Novel Case of Nephrotic Syndrome Caused by Immune-Mediated Severe LCAT Deficiency Satoshi Takahashi,[1] Keiju Hiromura,[1] Mayuko Tsukida,[1] Yuko Ohishi,[1] Hiroko Hamatani,[1] Noriyuki Sakurai,[1] Toru Sakairi,[1] Hidekazu Ikeuchi,[1] Akito Maeshima,[1] Takashi Kuroiwa,[1] Michio Nagata,[2] Yoshihisa Nojima.[1] *[1]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; [2]Department of Pathology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Background: Lecithin-cholesterol acyltransferase (LCAT) is a key enzyme in maintaining cholesterol homeostasis. In familial LCAT deficiency (FLD), abnormal lipid deposition causes renal injury and nephrotic syndrome, frequently leading to end-stage renal disease. We found a case of nephrotic syndrome due to acquired LCAT deficiency and examined the mechanism of inhibition of LCAT activity.

Methods: [Case report] A 63-year-old Japanese woman was admitted to our hospital because of edema. Laboratory data revealed U-Pro 4.1 g/day, Alb 2.5 g/dL, S-Cr 0.58 mg/dL, HDL-Chol 2 mg/dL, LDL 51 mg/dL and LCAT activity <50 U. A renal biopsy specimen showed that marked accumulation of foam cells and lipid deposits in most glomeruli, together with depositions of immune complexes along the capillary wall. By the steroid treatment, LCAT activity and urinary protein level returned to normal within 2 month. The re-biopsy specimen at 5 month after the initiation of treatment showed disappearance of foam cells and marked reduction of lipid deposits and immune complexes.

Results: The mixing test with the patient's serum and healthy control serum revealed the existence of an inhibitory factor to LCAT activity in the patient serum. The purified IgG fraction from the patient's serum also suppressed LCAT activity of normal serum. Co-immunoprecipitation study showed that protein G beads preincubated with the patient's serum could capture LCAT of healthy control serum, demonstrating that the patient's serum contained an antibody against LCAT.

Conclusions: This is the first report to describe the patient with an inhibitory anti-LCAT antibody and glomerular lesions comparable to those of FLD. This case would be important in considering the pathogenesis of glomerular lipid depositions and the possibility of treatment of renal injury in FLD with enzyme replacement therapy.

Funding: Other U.S. Government Support

TH-PO707

Renal Monoclonal Immunoglobulin Deposition Disease: A Report of 64 Cases from a Single institution Samih H. Nasr,[1] Anthony M. Valeri,[2] Lynn D. Cornell,[1] Mary E. Fidler,[1] Sanjeev Sethi,[1] Nelson Leung.[3] *[1]Pathology, Mayo Clinic, Rochester, MN; [2]Nephrology, Columbia University, New York, NY; [3]Nephrology, Mayo Clinic, Rochester, MN.*

Background: Monoclonal immunoglobulin deposition disease (MIDD) is a rare dysproteinemia-related renal disease. In order to better define the disease's clinical-pathologic spectrum and prognosis, we report the largest single-center series.

Methods: The characteristics of 64 MIDD patients who were seen at Mayo Clinic, Rochester, between 1992-2011, are provided.

Results: The 64 cases of MIDD included 51 cases of light chain deposition disease (LCDD)(kappa in 84% and lambda in 16%), 7 cases of heavy chain deposition disease (HCDD)(gamma in 6 and alpha in 1), and 6 cases of light and heavy chain deposition disease (LHCDD)(IgG kappa in 4, IgA kappa in 1, IgA lambda in 1). The mean age at diagnosis was 56 yrs and 36% of patients were ≤50 yrs. The M:F ratio was 1.9:1. Clinical evidence of dysproteinemia was present in 97% of patients including overt MM in 59%. M-spike was detected on SPEP in 73% and on UPEP in 81%. Serum free light chain (FLC) ratio was abnormal in all 51 patients (100%) tested. Presentation included renal insufficiency (97%), proteinuria (97%), hematuria (62%), and hypertension (83%). Nephrotic-range proteinuria and nephrotic syndrome (NS) were more common in HCDD than LCDD. By definition, all cases showed diffuse linear monoclonal protein deposition along GBMs and TBMs on IF with corresponding punctate electron dense deposits on EM. Nodular mesangial sclerosis was seen in 61% of cases. During an average of 34 mos of follow-up, 57% had stable/improved renal function, 4% had worsening renal function and 39% progressed to ESRD. The mean renal survival was 64 mos while the mean patient survival was 90 mos. Independent predictors of renal and patient survivals were creatinine at biopsy and the presence of lytic bone lesions, respectively.

Conclusions: Serum FLC ratio is abnormal in all MIDD patients. Nodular sclerosing glomerulopathy is present in most but not all cases. NS is more common in HCDD than LCDD. The prognosis for MIDD is improving. The degree of renal impairment at diagnosis is a predictor of renal survival.

TH-PO708

Myeloproliferative Neoplasm-Related Glomerulopathy Samar M. Said,[1] Nelson Leung,[2] Sandra Herrmann,[2] Sanjeev Sethi,[1] Lynn D. Cornell,[1] Mary E. Fidler,[1] Joseph P. Grande,[1] Vivette D. D'Agati,[3] Samih H. Nasr.[1] *[1]Pathology, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic, Rochester, MN; [3]Pathology, Columbia University, New York, NY.*

Background: Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders affecting the erythroid, granulocytic and megakaryocytic lineages. The characteristics of MPN-related glomerulopathy are undefined.

Methods: We evaluated the features of 11 patients with MPN-related glomerulopathy who were identified by searching our nephropathology laboratory archives from 2000-2010.

Results: There were 8 men and 3 women with a mean age of 73 yr (range 60-87 yr) at biopsy. The type of MPN was primary myelofibrosis in 8 patients, chronic myelogenous leukemia in 1, polycythemia vera in 1 and essential thrombocythemia in 1. Indications for biopsy were nephrotic-range proteinuria (mean 6.8 g/day) and chronic renal insufficiency (mean creatinine 2.5 mg/dl). Nephrotic syndrome, hypoalbuminemia, edema, and hematuria were present in 4, 9, 6, and 3 patients, respectively. The mean time from diagnosis of MPN to biopsy was 7.2 yrs (range 1-17 yrs). Histologically, mesangial sclerosis and hypercellularity were seen in all 11 cases, segmental sclerosis in 8, features of chronic thrombotic microangiopathy (TMA) in 9, and intracapillary infiltrating hematopoietic cells in 4. On follow-up, 4 progressed to ESRD and the remaining 7 had persistent renal dysfunction, despite the individualized institution of RAS blockade, immunosuppression and treatment directed to the hematologic disorder.

Conclusions: We describe a novel and under-recognized form of glomerulopathy associated with MPN that enlarges the spectrum of glomerular diseases associated with hematologic neoplasms. MPN-related glomerulopathy appears to be a late complication of MPN, particularly primary myelofibrosis. It is characterized clinically by heavy proteinuria and renal insufficiency and histologically by variable degrees of mesangial sclerosis and hypercellularity, segmental sclerosis, TMA, and intracapillary hematopoietic cells.

Prognosis is guarded. Greater awareness of this entity and larger studies are needed to define the optimal therapy.

TH-PO709

Association of Pathological Features of Renal Biopsies with Clinical Manifestations in Patients with Immunoglobulin Light Chain Amyloidosis – An Analysis of 170 Cases in a Chinese Centre Ying Yao, Su-Xia Wang, Youkang Zhang. *Department of Nephrology, Peking University First Hospital, Beijing, China.*

Background: This study aimed to investigate the association of pathological features of renal biopsies with clinical manifestations in patients with AL.

Methods: All patients with biopsy-proven renal amyloidosis were collected from our institute during 1988 and 2010(n=194). Immunohistochemistry with a panel of antibodies against Ig κ and λ light chains, AA protein, transthyretin, lysozyme, apolipoprotein A-I, and fibrinogen were performed on paraffin sections of renal biopsy. The severity of amyloid deposition in glomeruli, vessels and tubulointerstitium)were scored according to the histopathologic grading system proposed by Sait S(*Arch Pathol Lab Med.* 2010;134:532–544).

Results: AL was diagnosed in 170 cases (accounted for 87.6% of renal amyloidosis), with a ratio of λ light chain in 87%. The others included 1 case of secondary amyloidosis and fibrinogen Aα-chain amyloidosis respectively, 22 cases unclassified. In AL patients, the mean age was 56.8 years (range 26-83 years) with 108(63.5%) males. Multiple myeloma was diagnosed in 12 cases; M protein was detected in 74.3% (55/74) patients by serum or urine IFE. The level of urine protein and incidence of renal insufficiency was significantly higher in patients with diffuse marked glomerular deposition (score 5) than in patients with lesser score(p=0.004, 0.000). Patients with marked vascular amyloid deposition (score 4) or overt interstitial amyloid deposition(score≥2) had a significant higher incidence of liver involvement than that with lesser scores(p=0.048, 0.014). Patients with coexistence of amyloid and immune complexes had a higher level of proteinuria (8.46 vs 5.68 g/24h, P=0.001). Patients with AL-κ showed a higher frequency of liver involvement and marked vascular amyloid deposition than AL-λ(p=0.001, 0.006).

Conclusions: AL is the predominant pattern of renal amyloidosis in China. Diffuse marked glomerular amyloid deposition and coexistence with immune complexes were related with higher degree of proteinuria. Patients with AL-κ were more likely to have liver involvement and pervasive vascular amyloid deposition.

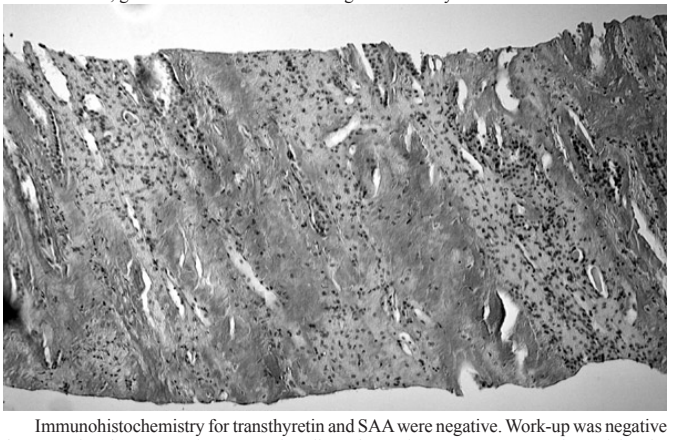
TH-PO710

Medullary Amyloidosis Associated with Apolipoprotein A-IV Sanjeev Sethi,[1] Jason David Theis,[1] Michelle Shiller,[1] Cynthia C. Nast,[2] Julie A. Vrana,[1] Ahmet Dogan.[1] *[1]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [2]Pathology, Cedars Sinai Hospital, Los Angeles.*

Background: Hereditary and familial forms of amyloidosis include transthyretin, fibrinogen A-a, lysozyme, gelsolin, apolipoprotein A-I and A-II associated amyloidosis. We describe a novel amyloidosis limited to the renal medulla.

Methods: A 52-year old man presented with 10-year history of increased urinary frequency, no significant proteinuria andgradual loss of renal function. There was no family history of renal disease. Serum creatinine had gradually risen to1.97 mg/dL with an eGFR of 33 ml/min.

Results: Renal biopsy showed large amounts of Congo red-positive amyloid restricted to the medulla; glomeruli and vessels were negative for amyloid.



Immunohistochemistry for transthyretin and SAA were negative. Work-up was negative for monoclonal gammopathy. Laser microdissection and mass spectrometry showed that the amyloid was composed of large amounts of apolipoprotein A-IV. MS/MS showed 100, 96 and 73 spectra in 3 microdissected samples that matched to apolipoprotein A-IV.

LC-MS/MS proteomic data by spectra from three samples prepared from 3 separate LMD of Congo red positive medullary tissue

Apolipoprotein A-IV	100	96	73
Apolipoprotein E	61	47	45
Serum Amyloid Protein	16	16	20
Ig gamma 1	11	9	9
Apolipoprotein A- I	4	7	4
Ig gamma 2	9	7	7

Genetic analyses detected 3 sequence variants which appeared to be common polymorphisms in the apolipoprotein A-IV gene. The following three sequence variants of Apolipoprotein A-IV gene were detected: c.548G>A, p.T29T (Thr29Thr); c. 1678G>A, p.S147N (Ser147Asn); and c.2378G>T, p.Q380H (Gln380His).

Conclusions: This is the first report of renal amyloidosis associated with apolipoprotein A-IV deposition, and renal involvement appears to be restricted to the medulla. The diagnosis may be missed if the renal biopsy consists of only renal cortex.

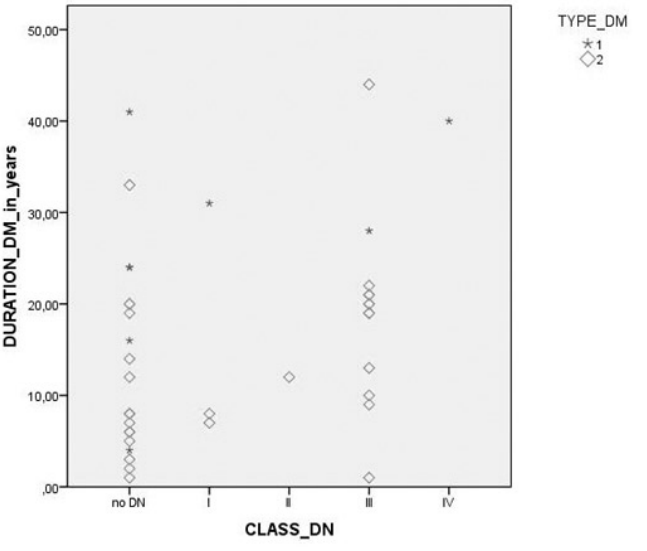
TH-PO711

Duration of Diabetes Mellitus Type 1 and Type 2 Correlates with Succeeding Stages I through IV of Diabetic Nephropathy: An Autopsy Study Elisabeth J. Valk, Hans J. Baelde, Jan A. Bruijn, Ingeborg M. Bajema. *Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Background: The new pathologic classification of diabetic nephropathy (DN) classifies DN into 4 groups, but it is unknown whether these represent progression from group I through IV in the course of the disease. We examined the relationship between duration of diabetes mellitus (DM) type 1 and type 2 and occurrence of DN and its allocation to the classes of the classification of DN (JASN 2010, 21:556-563).

Methods: We retrieved clinical data and paraffin embedded renal slides at autopsy from the archives of the LUMC of 31 patients with DM type 1 and type 2, from 1984 to 2004. Presence of DM was the main inclusion criterion. Patients with more than 15 years DM duration of whom autopsy materials showed no signs of DN were excluded. We also excluded patients with a combined pancreas and renal transplantation. There was 1 patient of whom a previous renal biopsy was available. In case of histologically proven DN, the lesions were scored as consistent with class I through IV.

Results: There were 4 patients with DM type 1 and 27 with DM type 2. 18/31 patients had histologically proven DN (3 patients type 1 DM and 15 patients type 2 DM). Figure 1 shows the distribution among classes according to duration of diabetes. There was a positive significant correlation ($r = 0.688$; $p < 0.001$) between duration of DM and the different groups of DN. Type 2 DM patients contributed mostly to this correlation (for this group, $r = 0.669$; $p < 0.001$). The renal biopsy that was available was performed 5 weeks before death and no transition of classes was shown.



Conclusions: Our preliminary results show that in the combined patient groups of type 1 and type 2 DM, there is a significant association between the duration of diabetes and DN class, which suggests that the histopathological classes indeed represent continuous stages of DN.

TH-PO712

The Pathologic Classification of Diabetic Nephropathy Is Correlated with the Clinical Characteristics of Diabetes Mellitus Fumihiko Yasuda,[1] Emiko Fujita,[1] Akiko Mii,[1] Megumi Fukui,[1] Yukinari Masuda,[2] Akira Shimizu.[2] *[1]Internal Medicine (Division of Neurology, Nephrology, and Rheumatology), Nippon Medical School, Tokyo, Japan; [2]Pathology, Nippon Medical School, Tokyo, Japan.*

Background: Nephrotic syndrome (NS) is a common complication of diabetic nephropathy (DN) . Recently, the renal pathology society (RPS) proposed the pathologic classification of DN (J Am Soc Nephrol 21: 556-563, 2010). In the present study, we examined the pathologic characteristics of DN cases with NS, using the RPS pathologic classification.

Methods: We selected renal biopsy cases of DM (n=62) , and assessed the clinical characteristics, focusing on the proteinuria, clinical stages of DM, and stages of chronic kidney disease (CKD). The renal biopsy was classified using the RPS pathologic classification of DN, and pathologic characteristics were correlated with clinical stages of DM, CKD stages, and proteinuria.

Results: We divided DM cases into 2 groups; DM with NS (n=31) and DM without NS (n=31). In clinical and pathologic characteristics of DM with NS group had tendency of high serum Cr (2.2±1.7 vs 1.5±1.1 mg/dl), low eGFR (36.3±21.9 vs 54.3±27.8 ml/min/1.73m2), high CKD stage, and high grade of pathologic classification than those of DN without NS. In the cases with pathologic class II (mesangial expansion), proteinuria was low levels, but the NS developed, accompanying with the long term DM history or non-DM glomerular disease, including membranous nephropathy. In the cases with pathologic class III (nodular glomerulosclerosis), the NS occurred together with the development of frequent nodular lesions. Importantly, the degree of pathologic classes was significantly correlated with the degree of clinical stages of DM (r=0.308, p<0.05), CKD stages (r=0.306, p<0.05), and levels of proteinuria (r=0.275, p<0.05).

Conclusions: In DM cases with NS, DN was characterized by the development of nodular glomerular sclerosis (Class III) with high clinical stages of DM and CKD stages. The pathologic class II cases with NS had a complication of non-DM glomerular diseases or a long term DM history. Importantly, the RPS pathologic classification of DN was correlated with the clinical characteristics of DM.

Funding: Private Foundation Support

TH-PO713

Implication of Urinary KIM-1 mRNA Detection in Patients with Diabetic Nephropathy Linli Lv, Min Zheng, Min Wu, Kun Ling Ma, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicime, Nanjing, Jiangsu Province.*

Background: Tubular injury has been found as an important early feature of diabetic nephropathy (DN) in addition to glomerulopathy, while it is still difficult to be early evaluated. The aim of this study was to investigate the role of KIM-1 mRNA expression in the urinary sediment for evaluating the progression of DN.

Methods: A total of 46 patients with DN and 14 healthy controls were studied. To evaluate progression of DN, patients were divided into groups based on the degree of albuminuria: Normoalbuminuria (UAE<30 mg/g, n=20), microalbuminuria (UAE 30~300 mg/g, n=11), and macroalbuminuria group (UAE>300 mg/g, n=15). Clinical data including albuminuria, blood urea nitrogen (BUN), and serum creatinine were recorded at baseline for each of the groups. Urinary KIM-1 mRNA expression was measured, and correlations with renal functional parameters were investigated.

Results: Urinary KIM-1 mRNA was significantly increased in patients with DN compared with healthy controls (p=0.006). Overall comparison showed that KIM-1 mRNA increased with increasing levels of albuminuria. It was the lowest in the control group, increased in the normoalbuminuria, microalbuminuria group, and was the highest in the macroalbuminuria group. KIM-1 mRNA strongly correlated with serum creatinine (r=0.459, P <0.001), BUN(r=0.478, p<0.001), and eGFR (r=-0.433, p=0.001). Moreover, KIM-1 mRNA was significantly higher in microalbuminuria group compared with normal control. Urinary KIM-1 mRNA could separate patients with DN from normal controls with AUC of 0.7430.743(p=0.006, 95% confidence interval (CI), 0.620-0.866). At the threshold that gave the maximal sensitivity and specificity for KIM-1 mRNA was 52.2 percent and the 100 percent (P<0.001) respectively.

Conclusions: The mRNA expression of KIM-1 in urinary sediment may reflect tubular damage in early diabetic nephropathy. KIM-1 mRNA could reflect the disease severity and be a novel biomarker for early detection of DN.

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TH-PO714

Dysregulated Balance of Th17 and Th1 Cells in Type 2 DM with Diabetic Nephropathy Young-Il Jo,[1] SolAh Park,[2] Eun Hye Seo,[1] Bokung Kim,[3] Seunghyun Lee.[2] *[1]Nephrology, Konkuk University Hospital, Seoul, Republic of Korea; [2]Department of Microbiology, Konkuk University School of Medicine, Seoul, Republic of Korea; [3]Department of Physiology and Biotechnology, Konkuk University School of Medicine, Seoul, Republic of Korea.*

Background: The etiological cause of diabetic nephropathy has yet not to be clarified. Recent evidences indicate that immune related factors may contribute to pathogenesis of type 2 DM (T2DM) with nephropathy and Th17 cells play an important role in the immunopathogenesis of autoimmune tissue inflammation. This research was designed to assess the frequency of Th17 and Th1 cells and the balance between Th17 and Th1 cells in T2DM patients with diabetic chronic kidney disease (CKD).

Methods: Thirty-eight T2DM patients with diabetic CKD, 9 T2DM patients without nephropathy, 11 non-diabetic CKD and 17 healthy controls were recruited. Peripheral blood mononuclear cells were collected and stimulated with phorbol myristate acetate and inomycin. The frequency of Th17 cells producing IL-17 and Th1 cells producing IFN-γ was measured by using flow cytometry. Expression of Th17-associated chemokine receptors CCR4 and CCR6 on $CD4^+$ T cells were assessed.

Results: T2DM patients with diabetic CKD had an increased frequency of Th17 cells compared with other groups, whereas for the frequency of Th1 cells, there was no difference between all groups, indicating an altered balance of Th17 and Th1 cell responses in T2DM with diabetic CKD. In patients with diabetic nephropathy, the frequency of Th17 cell increased only those with eGFR 60-120 ml/min/1.73m^2 but not healthy controls and T2DM patients without nephropathy. In addition, the frequency of Th17 cells in diabetic nephropathy showed a decreasing tendency with decreased eGFR. However, compared to T2DM patients with diabetic CKD, subjects with non-diabetic CKD did not reveal difference according to eGFR. In T2DM with diabetic CKD, the ratio of Th1-to-Th17 cells showed an opposite tendency with the frequency of Th17 cells: the ratio of Th1-to-Th17 cells an increasing tendency with decreased eGFR.

Conclusions: These results suggest that the dysregulated balance of Th17 and Th1 cells may play an role in the pathogenesis of T2DM with nephropathy.

TH-PO715

H,K-ATPases Participate in Mineralocorticoid-Induced Hypertension I. Jeanette Lynch,[1,2] Megan Greenlee,[1,2] Michelle L. Gumz,[1,2] Charles S. Wingo.[1,2] *[1]Division of Nephrology, University of Florida, Gainesville, FL; [2]Nephrology Section, Dept of Veteran Affairs, Gainesville, FL.*

Background: We have previously shown that in response to eight days of desoxycorticosterone pivalate (DOCP) treatment wild type (WT) mice develop a hypochloremic metabolic alkalosis and this response is abolished in $HK\alpha_1$ and $HK\alpha_2$ double null ($HK\alpha_{1,2}^{-/-}$) mice (Greenlee *et al.* JASN 22:49-58, 2011). Additionally, metabolic balance studies showed that this DOCP treatment caused significant Na retention in WT mice, but not in $HK\alpha_{1,2}^{-/-}$ mice. Thus, we hypothesized that renal H,K-ATPases are required for maximal DOCP mediated Na reabsorption and an increase in systemic arterial blood pressure (BP). Furthermore, we asked whether $HK\alpha_{1,2}^{-/-}$ mice had altered protein abundance for the α subunit for the epithelial Na channel (ENaC).

Methods: Age matched WT and $HK\alpha_{1,2}^{-/-}$ male mice were studied in three separate experiments: 1) ENaC subunit mRNA and protein expression levels were evaluated in kidneys from WT and $HK\alpha_{1,2}^{-/-}$ mice; 2) food and water intake, body weight, and urinary aldosterone levels were measured in *ad libitum* or pair fed mice of both genotypes; 3) BP, heart rate (HR) and locomotor activity were measured in WT and $HK\alpha_{1,2}^{-/-}$ mice in control and DOCP-stimulated conditions using radiotelemetry.

Results: We observed that: 1) renal medullary αENaC protein expression was reduced by 45% in $HK\alpha_{1,2}^{-/-}$ mice versus WT; 2) $HK\alpha_{1,2}^{-/-}$ mice consumed more food per body weight and had greater urinary output than WT; 3) pair feeding caused substantial weight loss and increased urinary aldosterone in $HK\alpha_{1,2}^{-/-}$ but not WT mice; 4) whereas two month survival was 100% in DOCP-treated WT mice (N=9/9), it was 43% in DOCP-treated $HK\alpha_{1,2}^{-/-}$ mice (N=3/7); 5) $HK\alpha_{1,2}^{-/-}$ mice had significantly greater (~10%) HR and reduced locomotor activity; 6) DOCP-treatment significantly increased BP in WT mice during the active phase but there was no significant increase in BP in the $HK\alpha_{1,2}^{-/-}$ mice during this time period.

Conclusions: These data strongly support a role for H,K-ATPases in sodium conservation and suggest that the H,K-ATPases are involved in the action of mineralocorticoids to increase systemic arterial blood pressure.

Funding: NIDDK Support

TH-PO716

Dysfunction of Gap Junctions Stimulates COX-2 Expression in Juxtaglomerular Renin-Secreting Cells Jian Yao, Ying Zhu, Masanori Kitamura. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Background: Gap junctions (GJs), formed by the specific proteins called connexin (Cx), play an important role in the control of juxtaglomerular (JG) functions. Mice lacking Cx40 or Cx45 have been shown to have markedly elevated levels of COX-2 in JG cells and develop renin-dependent hypertension. At present, the mechanisms involved are unclear. It is thought to be related to the disruption of GJ-mediated renin-suppressive signals from the neighboring cells. Besides communication-dependent actions, GJs also regulate cell phenotypes through communication-independent way. We, therefore, asked whether COX-2 expression in renin-secreting cells could be directly affected by dysfunction of GJs.

Methods: Renin-secreting As4.1 cells were cultured and assay for GJ protein expression and funciton by immunofluorescent staining, Western blot analysis, and diffusion of Lucifer yellow (LY) after single cell injection. The expression of COX-2 and other related signaling molecules were detected by Western blot analysis.

Results: 1) Renin-secreting As4.1 cells were characterized to express Cx43 and Cx45 and have functional gap junctional intercellular communication (GJIC). 2) Incubation of As4.1 cells with GJ inhibitor α-glycyrrhetinic acid (α-GA) resulted in a time- and concentration-dependent inhibition of GJIC and suppression of phosphorylated levels of Cx43, which was associated with markedly elevated levels of COX-2. 3) This effect of α-GA on COX-2 was mimicked by two active structural analogues carbenoxolone and β-glycyrrhetinic acid that can disrupt GJIC, but not inactive analogue glycyrrhizic acid that cannot disrupt GJIC. In addition, it was also achieved by structurally different GJ inhibitor lindane. 3) α-GA caused a time- and concentration-dependent activation of AKT and MAPK. Suppression of these kinases with specific inhibitors largely abolished its COX-2-elevating effect.

Conclusions: Taken together, our study indicates that dysfunction of GJs in JG renin-secreting cells elevates COX-2 expression. GJs could directly influence renin synthesis and secretion through modulation of COX-2 in a communication-independent way.

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TH-PO717

Interleukin-1β Regulates Renin Expression in the Renal Cortex Independently of Cyclo-Oxygenase-2 Erika I. Boesen, David M. Pollock. *Georgia Health Sciences University, Augusta, GA.*

Background: The proinflammatory cytokine interleukin-1β (IL-1β) is produced by a number of cell types within the kidney during health and disease, however its impact on renal function is incompletely understood. Cyclo-oxygenase-2 (COX-2), which is upregulated by IL-1β, is an important regulator of renin production. We therefore hypothesized that IL-1β regulates renin expression in the renal cortex.

Methods: Ren1 and COX-2 gene expression in renal cortical tissue was assessed by quantitative RT-PCR following 14-day treatment of male Sprague-Dawley rats with recombinant human IL-1 receptor antagonist (IL-1Ra; 2 mg/kg/d s.c.) or of male C57BL/6 mice with IL-1β (10 ng/h s.c.) or their respective vehicles. The role of COX-2 in IL-1β-induced Ren1 expression was more directly tested by co-treatment of mice with the selective COX-2 inhibitor celecoxib.

Results: After treatment of rats with IL-1Ra for 14 days s.c. (n = 8), renal cortical Ren1 mRNA expression was significantly reduced by 39% compared to vehicle-treated rats (n = 7; 2-ΔΔCT of 0.63 ± 0.1 vs 1.0 ± 0.1 respectively, $P < 0.01$) and COX-2 mRNA expression was reduced by 52% (2-ΔΔCT of 0.5 ± 0.1 vs 1.2 ± 0.3, $P < 0.05$). There were no significant differences in renal cortical IL-1β protein levels (6.2 ± 2.1 vs 7.0 ± 1.6 pg/mg total protein; ELISA), mean arterial pressure (111 ± 2 vs 114 ± 2 mmHg; telemetry) or sodium intake (2.8 ± 0.2 vs 2.8 ± 1.0 mmol/d) between vehicle and IL-1Ra-treated rats. In mice, infusion of IL-1β (10 ng/h; n = 7) s.c. for 14 days produced significant ($P < 0.05$) increases in both renal cortical Ren1 (2-ΔΔCT of 2.0 ± 0.5) and COX-2 mRNA (3.7 ± 0.5) compared to vehicle-treated mice (n=6; 2-ΔΔCT of 1.0 ± 0.1 and 1.1 ± 0.2 respectively). In separate groups of IL-1β-infused mice, inhibition of COX-2 with celecoxib (30 mg/kg/d p.o.) did not reduce renal cortical Ren1 mRNA levels (2-ΔΔCT of 1.0 ± 0.1 vs 1.0 ± 0.2 in IL-1β-infused mice not receiving celecoxib; n = 3-4).

Conclusions: Our data indicate that IL-1β stimulates renin expression independently of its effects on COX-2 expression.

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TH-PO718

Effects of Chronic Direct Renin Inhibition on Urine Levels of Renin, Soluble Prorenin Receptor, and Ang II in Cyp1a1-Ren2 Transgenic Rats with Ang II-Dependent Malignant Hypertension Minolfa C. Prieto, Catherine Howard, Gregory Shamitko, Dale M. Seth, Kenneth D. Mitchell. *Physiology and Hypertension and Renal Center of Excellence, School of Medicine, Tulane University Health Sciences Center, New Orleans, LA.*

Background: Cyp1a1Ren2 transgenic rats [TGR(Cyp1a1Ren2)] with malignant hypertension exhibit augmented expression of renin, prorenin receptor (PRR), and the soluble form of this receptor (sPRR) in the renal medulla, suggesting that exacerbation of these RAS components may be a leading mechanism for intrarenal Ang II generation in this model. However, the effects of direct renin inhibition on these RAS components and urinary levels of Ang II have not been established.

Methods: In this study we determined the effects of chronic direct renin inhibition with Aliskiren (60 mg/kg/d for 10 d) on renin, PRR, sPRR, and Ang II content in the urine of Cyp1a1Ren2 rats with inducible Ang II-dependent malignant hypertension.

Results: Compared to control non-induced rats (n=6), Cyp1a1Ren2 rats (n=6) rats exhibited elevated arterial pressures (198±6 vs. 137±7 mm Hg; $p<0.001$), increased protein levels of renin (2.3±0.1 vs. 1.0±0.0 IDU) and even greater of prorenin (4.2±0.3 vs. 1.0±0.1 IDU) in the urine as measured by Western blot. Aliskiren prevented the development of hypertension (141±10 mm Hg; $p<0.01$) and decreased urinary renin/proremin protein levels.. Aliskiren did not alter the PRR and sPRR protein levels in the urine of Cyp1a1Ren2 rats. Urinary excretion of renin increased in the hypertensive rats ($1.3x10^{-6} \pm 2.3x10^{-7}$ vs. $2.0x10^{-7} \pm 2.6x10^{-8}$ Enzyme Units/day; $p<0.001$) and did not change with Aliskiren ($2.8x10^{-6} \pm 2.5x10^{-7}$). However, the urinary excretion of Ang II in Cyp1a1Ren2 rats was increased (6186±1962 vs. 463±89 fmol/d; $p<0.001$) and partially prevented by Aliskiren (3415±424 fmol/day; $p<0.05$).

Conclusions: In Cyp1a1Ren2 rats with malignant hypertension, augmentation of renin/prorenin and sPRR in the urine contributes to increased uAng II content. The Aliskiren-mediated decreased in urinary renin/prorenin protein levels and urinary excretion of Ang II may reflect decreased intrarenal Ang II generation and, thus, the contribution to the antihypertensive effects of Aliskiren in Ang II-dependent malignant hypertension.

Funding: Other NIH Support - Institute of Developmental Award (IdeA) Program of NCRR, Pharmaceutical Company Support, Private Foundation Support

TH-PO719

Targeted Disruption of Aquaporin-1 Leads to Impaired Recruitment of Juxtaglomerular Cells in Response to Chronic Stimulation of the Renin-Angiotensin System Kirsten Madsen,[1,2] Anne Robdrup Tinning,[1] Armin Kurtz,[3] Boye Jensen.[1] *[1]Department of Cardiovascular and Renal Research, University of Southern Denmark, Denmark; [2]Department of Clinical Pathology, Odense University Hospital, Denmark; [3]Department of Physiology, University of Regensburg, Germany.*

Background: Aquaporin-1 (AQP-1) mRNA is expressed in juxtaglomerular granular (JG) cells that synthesize and release renin. It was hypothesized that AQP-1 is necessary for recruitment of JG cells and contribute in the pathway for renin release.

Methods: AQP-1 knock-out (KO) and wild-type (WT) mice were given a diet with 0.004% NaCl (LS) for 7 days and enalapril (ACEI, 0.1 mg/ml) in the drinking water for 3 days. Plasma renin concentration (PRC) and renal renin mRNA expression was determined. Renal vasculature was dissected from acid-macerated kidneys and afferent arterioles were counted as either 1) JG-cell negative; 2) with JG cells in a juxtaglomerular position or 3) with upstream JG-cell recruitment. Isolated perfused kidneys were used to determine renin release in response to standard stimuli. Perfusion-fixed kidneys from ACEI-treated mice were immunostained for renin and alpha-actin and serial sections were used to 3D-reconstruct the renal vasculature.

Results: At baseline, there were no differences in PRC, renal renin mRNA, renin release from isolated perfused kidneys or tail-cuff blood pressure in AQP-1 KO mice compared to WT mice. PRC was stimulated significantly in response to LS-ACEI treatment in both genotypes but reached a significantly higher level in AQP-1 WT. Renin mRNA level was elevated significantly and to a similar extent in both genotypes by LS-ACEI. In AQP-1 KO mice, the number of afferent arterioles with recruitment was significantly lower after LS-ACEI treatment compared to WT, while the number of arterioles with juxtaglomerular JG-cells was increased in AQP-1 KO mice. 3-D reconstruction of the vascular tree confirmed a predominantly juxtaglomerular position of renin-positive cells after ACEI in AQP-1 KO mice.

Conclusions: AQP-1 is not necessary for baseline regulation of renin secretion or expression but contributes to the recruitment of JG-cells and PRC during chronic stimulation of the renin-angiotensin system.

Funding: Private Foundation Support

TH-PO720

Regulation of Renal Renin Release by Proteinase-Activated Receptors Klaus Höcherl, Frank Schweda. *University of Regensburg, Institute of Physiology, Regensburg, Germany.*

Background: Proteinase-activated receptors 1-4 (PAR1-4) are highly expressed in the kidney and are involved in the regulation of renal hemodynamics and tubular function. Since evidence had suggested a role of PARs in the control of the renin system, we investigated the effects of the respective PAR subtypes on renin release.

Methods: In the present study, we evaluated the impact of PAR activation on renin secretion rates (RSR) and kidney perfusate flow using the proteinase thrombin and PAR1-, PAR2-, and PAR4-activating peptides (TFLLR-NH_2, SLIGRL-NH_2 and AYPGKF-NH_2, respectively) in the isolated perfused mouse kidney model.

Results: The proteinase thrombin, that besides its role in the coagulation cascade activates PARs, dose-dependently reduced perfusate flow and inhibited renin secretion rates (RSR) that had been prestimulated by the ß-adrenoreceptor agonist isoproterenol. Direct activation of PAR1 by TFLLR mimicked the effects of thrombin on RSR and vascular tone. The PAR1-activating peptide TFLLR dose-dependently and reversibly decreased isoproterenol-induced RSR and perfusate flow. Moreover, stimulation of RSR by bumetanide, prostaglandin E_2 or by a decrease in renal perfusion pressure was also inhibited by TFLLR. Similarly, TFLLR decreased perfusate flow under these conditions. However, the increase in RSR and perfusate flow induced by a calcium-free perfusate was not influenced by TFLLR. The PAR2-activating peptide SLIGRL concentration-dependently increased basal RSR and perfusate flow. The stimulations of RSR and perfusate flow induced by isoproterenol were further increased by SLIGRL. The stimulatory effects of SLIGRL on RSR were clearly attenuated by L-NAME. In contrast, the PAR4-activating peptide AYPGKF did not modulate RSR or perfusate flow. In addition, PAR1 and PAR2 immunoreactivity were detected in the juxtaglomerular region and were co-localized with renin immunoreactivity.

Conclusions: Our data provide evidence that PAR1 activation inhibits renin secretion and induces renal vasoconstriction likely via a calcium-dependent mechanism, whereas PAR2 activation stimulates renin release and induces vasodilation mainly via the release of nitric oxide.

TH-PO721

Paricalcitol Lowers Plasma Renin Activity and Improve Blood Pressure Control in the 2-Kidney, 1-Clip Hypertensive Rat Model Olivier Phan, Marc P. Maillard, Michel Burnier. *Division of Nephrology, University of Lausanne CHUV, Switzerland.*

Background: Vitamin D has been shown to regulate renin expression in juxtaglomerular cells. The aim of the study was to compare the effects of two vitamine D analogs, paricalcitol and calcitriol on plasma renin activity (PRA), blood pressure (BP) and heart weight (HW) in a high-renin model of hypertension i.e the 2-kidney one-clip rat model.

Methods: Male wistar rats were used at 150 gr. Hypertension was induced by clipping the left renal artery. After 10 days, rats were randomly assigned (based on the normal distribution of baseline body weights) into 3 groups with a standard diet: calcitriol (80 ng/kg), paricalcitol (240 ng/kg), and control (vehicle) with an intraperitoneal injection every 3 days for a total of 4 injections (N=11/group). A sham group was also created as sham control.

Results: 24 h before sacrifice, a catheter was inserted into the right femoral artery to measure BP. The rats were placed in large Plexiglas tubes without noise or visual stimulation for two hours. The catheter was attached to a combination pressure transducer, and arterial blood pressure (BP) was collected using computerized data acquisition software.

	Heart Rate (/mn)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	Heart weight (gr)	PRA (ng/ml/h)
Sham control	361±13	142±8*	96±8*	113±9*	1.03±0.1*	1.53±0.38*
Clip control	366±7	193±9	141±7	166±8	1.36±0.06	4.81±1.27
Clip calcitriol	365±8	182±10	129±7	154±8	1.07±0.07*	3.28±0.48
Clip paricalcitol	354±9	163±7*	119±6*	139±7*	1.02±0.06*	1.62±0.3*

* p<0.01 vs clip control

Conclusions: In this model, only paricalcitol is associated with a significant decrease in BP and plasma renin activity. However, both paricalcitol and calcitriol reduce cardiac hypertrophy suggesting a BP-independent effect on cardiac mass.

Funding: Government Support - Non-U.S.

TH-PO722

Effect of Murine Recombinant ACE2 on Blood Pressure and Serum ACE2 Activity Minghao Ye, Jan A. Wysocki, Daniel Batlle. *Division of Nephrology & Hypertension, Dept. of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Background: There is increasing interest in the potential therapeutic action of Angiotensin-converting enzyme 2 (ACE2), but studies in mouse models have been limited by the lack of tools to amplify ACE2 activity. We developed murine recombinant ACE2 (mrACE2) to circumvent the immunogenicity of human rACE2 when given to mice.

Methods: mrACE2 was purified using Ni-Sepharose followed by size-exclusion chromatography. After 19 hr of incubation in vitro with purified mrACE2 (4ng), Ang II (10-9M) disappeared completely, thereby confirming that mrACE2 effectively cleaves this peptide. Then, Ang II (0.2mg/kg) was given to mice pre-treated with vehicle or mrACE2 (1.0mg/kg IP).

Results: Serum ACE2 activity was markedly increased in mrACE2-pretreated mice (244.2±6.7 vs. vehicle 1.19±1.2, RFU/ul/hr). In vehicle mice, Ang II bolus resulted in a rapid increase in SBP (30 sec) (from 102±3.1 to 173±4.4 mmHg, n=8), which was markedly blunted by mrACE2 (from 109±5.2 to 139±8.3 mmHg, n=7). The SBP recovery was much faster compared with mice pretreated with vehicle (at 5 min, 106±8.2 mmHg vs. 168±3.9 mmHg, respectively, p<0.0001). Whether baseline ACE2 activity plays a role in blood pressure regulation was examined by inhibiting ACE2 pharmacologically. We reasoned that a pharmacologic approach to ACE2 inhibition would be more useful to examine the effect of ACE2 on blood pressure under conditions where Ang II was not infused. Accordingly we administered MLN-4760, a specific ACE2 inhibitor. Acute administration of MLN-4760 (1.0 mg/kg IP) was associated with a rapid but transient increase in SBP as compared to control mice that received vehicle (ΔSBP11.4±2.7 vs.0.1±3.1 mmHg, p< 0.01). This effect of MLN-4760 was associated with a complete inhibition of serum ACE2 activity.

Conclusions: mrACE2 markedly reduces hypertension by increasing serum ACE2 activity. Pharmacological ACE2 inhibition results in an increase in blood pressure showing that baseline ACE2 activity is needed to provide sustained Ang II degradation in the face of continuous Ang II formation. mrACE2 will provide a tool to examine the therapeutic potential of ACE2 amplification in vivo.

Funding: NIDDK Support

TH-PO723

Disrupted Angiotensin-Converting Enzyme2 (ACE2) Enhances Angiotensin II Induced Renal Fibrosis Via Smurf2-Mediated Degradation of Renal Smad7 Zhen Liu,[1] Xiao Ru Huang,[2] Haiyong Chen,[1] Hui Y. Lan.[1,2] *[1]Department of Medicine & Therapeutics, Chinese University of Hong Kong, Hong Kong, China; [2]Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China; [3]Department of Chemical Pathology, Chinese University of Hong Kong, Hong Kong, China.*

Background: ACE2 negatively regulates angiotensin II(Ang II) signaling by cleaving AngII to Ang 1-7, thereby counterbalancing the detrimental effect of Ang II. It is known that Ang II mediates renal fibrosis via TGF-β/Smad-dependent and independent mechanisms. We now hypothesized that ACE2 may interact with Smads to block Ang II-induced renal fibrosis.

Methods: The hypertensive kidney disease was induced in ACE2 knockout (KO) mice via subcutaneous Ang II infusion. Overexpression of renal Smad7 to rescue the loss of ACE2 in Ang II-induced renal fibrosis was examined. Mechanisms of disrupted ACE2 promoted Ang II-mediated renal fibrosis was also studied in vivo and in ACE2 KO mesangial cells.

Results: After Ang II infusion, mice with ACE2 KO developed more severe renal dysfunction (protienuria and serum creatinine, p<0.05), renal fibrosis (30-40%↑ in a-SMA, COL1, COL3), and inflammation (40-45%↑ in T cells, macrophages, IL-1b, and TNFa), which was associated with higher levels of intrarenal Ang II (40%↑), reduced Ang 1-7 (30%↓), and increased Ang II (AT1R and ERK1/2), TGF-β/Smad3, and NF-kB signaling. Enhanced Smad3 and NF.kB activation in ACE2 KO mice was largely due to degradation of renal Smad7(50%↓) mediated by the E3 ligase Smurf2 (40%↑), which was confirmed by the ability of blocking AT1 receptor or knocking down Smurf2 to prevent Ang II-induced degradation of Smad7 in ACE2 KO cells. In contrast, ultrasound-mediated Smad7 gene therapy was able to rescue Ang II-induced progressive renal injury including TGF-b/Smad3-mediated renal fibrosis and NF.kB-driven inflammation in ACE2 KO mice.

Conclusions: Disrupted ACE2 enhances Ang II-induced renal injury which is associated with a loss of renal Smad7. Enhanced AT1-Smurf2-dependent Smad7 ubiquitin degradation may be an essential mechanism by which loss of ACE2 promotes progressive renal injury in Ang II-induced hypertensive nephropathy.

Funding: Government Support - Non-U.S.

TH-PO724

Effects of Renin-Angiotensin System Component Ang-(1-7) on Podocyte Injury In Vitro Induced by Patients' Serum of Preeclampsia Yong Gu,[1,2] Guixiang Chen,[1] Jianying Niu.[1] *[1]Division of Nephrology, Fifth People's Hospital of Shanghai, Fudan University, Shanghai, China; [2]Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China.*

Background: Renin angiotensin system (RAS) is very complicated and multistrata endocrinium, including various components. Recently a new member of RAS components Ang-(1-7) and its specific receptor Mas were found that they have extensive effects, which could counteract the effects of AngII. The podocyte injury may play a role in the pathogenesis of proteinuria in preeclampsia. Our previous study showed that the decreased serum and urinary Ang-(1-7) may be a cause of podocyte injury in preeclampsia. In order to further study the effect of Ang-(1-7) on podocyte in preeclampsia, we use patients' serum of preeclampsia to preincubate podocyte and then add Ang-(1-7) to observe the change of podocyte specific proteins.

Methods: The podocyte was cultured in vitro and the patients' serum of preeclampsia was collected sterile for preincubating podocyte. They were divided into 4 groups: normal control (NC); normal pregnant serum (NP) group; preeclampsia model (PE) group and Ang-(1-7) + preeclampsia [Ang-(1-7) + PE] group. The morphologic change of podocyte was observed by microscope; the changes of nephrin, CD2AP, F-actin, ZO-1 and Mas receptor were examined by immunofluorescence. Western blot was used to examine the expression of Mas receptor.

Results: 1. The expression of nephrin, F-actin and ZO-1 on podocytes was significantly decreased in PE group than NC and NP group; but their expression in Ang-(1-7) + PE group was significantly increased than in PE group. The expression of CD2AP has no significantly difference among four groups.

2. There exist Mas receptors on podocytes in all groups which was examined by immunofluorescence and Western blot. The expression of Mas receptor on podocytes in PE group was significantly decreased than NC and NP group; but it was increased significantly in Ang-(1-7) + PE group than in PE group.

Conclusions: Ang-(1-7) could protect podocyte from injury in vitro induced by patients' serum of preeclampsia. The effect may be associated with integration of Ang-(1-7) and its specific Mas receptor.

TH-PO725

Identification of Stox1 Transcription Factor as a Specific Repressor of Placental Renin and Its Deficiency Leads to Vascular Defects and Gestational Hypertension Keizo Kanasaki,[#1, #2] Soo Bong Lee,[#1] Daisuke Koya,[#2] Raghu Kalluri.[#1] *[1]Division of Matrix Biology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Division of Diabetes and Endocrinology, Kanazawa Medical University, Uchinada, Kahoku, Ishikawa, Japan.*

Background: Storkhead-box protein 1 (Stox1) was identified as a candidate gene associated families with higher incidence of hypertensive crisis during pregnancies. Stox1 is a putative transcriptional factor with unknown function.

Methods: We generated Stox1 deficient mice (Stox1-/-) and analyzed pregnancy-associated hypertensive phenotype.

Results: Here, we demonstrate that Stox1-/- mice specifically exhibit pregnancy-associated hypertension, vascular defects and basement membrane abnormalities. Pregnant Stox1-/- mice display higher urine albumin excretion when compared to wild-type control pregnant mice. Non-pregnant females and males with deletion of Stox1 are normal despite ubiquitous expression of Stox1 in the control mice. Renin expression is significantly elevated in the placentas of Stox1-/- mice, but not in the kidney. RNA silencing and promoter analysis in the human cytotrophoblasts demonstrate that renin is negatively regulated by transcriptional action of Stox1, via its 3'UTR. Losartan, an angiotensin receptor I antagonist, completely ameliorates all pregnancy associated abnormalities in Stox1-/- mice.

Conclusions: These results indicate that Stox1 is a key regulator of blood pressure during pregnancy, and mice deficient for Stox1 serve as a genetic model for human gestational hypertension, a disease that affects about 8% of all pregnancies, causing significant morbidity.

Funding: NIDDK Support

TH-PO726

Effect of Cyclosporine A Administration in Pregnant Rats on Blood Pressure and Glomeruli Number in Their Offspring Marcin Adamczak,[1] Natalia Slabiak-Blaz,[1] Nadezda Koleganova,[2] Eberhard Ritz,[3] Andrzej Wiecek.[1] *[1]Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland; [2]Department of Pathology, University of Heidelberg, Germany; [3]Department of Internal Medicine, University of Heidelberg, Germany.*

Background: Successful kidney transplantation allows women previously suffered from chronic kidney disease stage 5 to get pregnant. However, the immunosuppressive therapy may influence development in the offspring i.e. may cause the hypertension and chronic kidney disease (CKD) in adulthood. The aim of the study was to assess the effect of exposure to cyclosporine A (CsA) during gestation on blood pressure and glomeruli number in the offspring.

Methods: Eight pregnant Sprague-Dawley rats were assigned into two groups (n=4 in each group). In the first group CsA in a dose 3mg/kg/day and in the second group the corresponding volume of solvent were given, respectively. The substances were

administered from the 10th day after the fertilization till the 7th day after the delivery, subcutaneously, once a day. At 7 and 11 weeks of age in the offspring blood pressure was measured. 12 weeks after delivery the experiment was terminated and the number of glomeruli was calculated using unbiased stereological method.

Results: Systolic (SBP) and diastolic blood pressure (DBP) in the offspring of the females treated with CsA during gestation (n=34) was higher compared to the offspring from mothers treated only with solvent (n=31) (7th week - SBP: 125±5 vs. 117±6mmHg, p<0.001; DBP: 82±6 vs. 77±6mmHg p<0.001; 11th week - SBP: 132±9 vs. 126±7mmHg, p<0.05; DBP: 89±8 vs. 83±7mmHg, p<0.001). The number of glomeruli was lower in the kidneys of offspring from mothers treated with CsA compared to the offspring of mothers treated only with the solvent (19553±769 vs 23974±842, p<0.001).

Conclusions: 1. Treatment with CsA during pregnancy may lead to arterial hypertension in the offspring. 2. Exposure on CsA during fetal life influence also on kidney development which results in 18% fewer glomeruli and this may be an important factor participating in the pathogenesis of CKD later in their life.

Funding: Government Support - Non-U.S.

TH-PO727

Offspring of Mother Rats Exposed to Cigarette Smoke Condensate Are Characterized by Elevated Blood Pressure and Reduced Urinary Sodium Excretion

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Background: It has been suggested that disturbances of fetal development caused by exposure to cigarette smoke (as a result of maternal smoking) increase the risk of hypertension and chronic kidney disease in the adult life. The aim of the present, experimental study, was to assess the impact of exposure of pregnant rats to cigarette smoke condensate on blood pressure and natriuresis in their offspring.

Methods: Sprague-Dawley rats on day 10 of pregnancy were randomly allocated (5 animals in each group) to twice daily application on oral mucosa cigarette smoke condensate (CSC) containing nicotine or solvent until delivery. Albuminuria, creatinine clearance and urinary sodium, potassium and calcium excretion were measured in 12 weeks old offspring.

Results: At 12 weeks of age significantly elevated systolic blood pressure was found in offspring exposed to CSC during the fetal period (n=54) compared to controls (n=51) (122±7 vs. 116±8 mmHg; respectively, p<0.001). Offspring of mother rats exposed to cigarette smoke condensate did not differ from the control offspring with respect to body weight, albuminuria, creatinine clearance and urinary potassium and calcium excretion, respectively. In contrast, offspring of mother rats exposed to cigarette smoke condensate are characterized by both significantly lower urinary sodium excretion (531±242 vs. 846±384 µmol/24 hours; p=0.004) and urinary sodium/creatinine ratio (8.1±4.1 vs. 9.6±4.2 mmol/mmol; p=0.04).

Conclusions: 1. Exposure of pregnant rats to cigarette smoke condensate causes a blood pressure increase and a reduction of urinary sodium excretion in their offspring. 2. Such effect of cigarette smoke condensate on kidney function may have a consequence on development of arterial hypertension and also chronic kidney disease in the adult life.

Funding: Government Support - Non-U.S.

TH-PO728

Aggravated Phenotypes of Preeclampsia in Mice Bearing 4 Copys of Ace Gene

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Background: Preeclampsia is pregnancy-associated hypertension with proteinuria. Excess sFlt-1, an endogenous VEGF inhibitor of placental origin has been implicated to cause hypertension, proteinuria and glomerular endotheliosis, all features of preeclampsia. sFlt-1 antagonizes VEGF and induces endothelial dysfunction . Human *ACE* polymorphisms leading to elevated activity of ACE are associated with preeclampsia.

Methods: We tested whether an increase in ACE aggravation of preeclampsia using mice having 4 copys of *Ace* gene.

Results: Adenoviral mediated sFlt-1 overexpression to the non-pregnant female mice increased BP of both mutant mice and WT mice. All of mutant sFlt-1 mice developed ascites approximately10 days after injection, and body weight (BW) also increased (average gain was 1.7 ± 0.6 g). However, none of WT sFlt-1 mice developed ascites and BW did not increase (average gain was –0.45 ± 0.06 g). Mutant sFlt-1 mice showed higher daily urinary albumin excretion (288 ± 42 mg/day vs. 150 ± 60 in WT sFlt-1) and lower GFR (170 ± 55 ml/min vs. 493 ± 109 in WT sFlt-1). Mutant sFlt-1 mice had less glomerular open capillary volume (20.5 ± 3.8 % vs. 34.7 ± 10.5 % in WT sFlt-1 mice), suggesting mutant sFlt-1 mice have more severe endotheliosis than WT sFlt-1 mice.

Conclusions: We conclude that mice having 4 copies of *Ace* gene develop more sever preeclampsia than WT mice. The mechanism underlying this phenomenon is under studying.

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TH-PO729

Hydrogen Sulfide: A Protective Gas for Preeclampsia?

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Background: Hydrogen sulfide ($H_{[sup]2[/sup]}S$) is emerging as a regulator of various physiologic functions like blood pressure regulation and neurotransmission. It is protective during renal hypoxia and has strong antioxidant properties. Endogenous $H_{[sup]2[/sup]}S$ is produced from cysteine by the enzymes cystathionine-ß-synthase (CBS) and cystathionine-γ-lyase (CSE). CSE ko mice have increased blood pressure and reduced endothelium-mediated vasorelaxation. CBS ko mice are growth restricted and die within 4 weeks after birth. Preeclampsia (PE) is accompanied by maternal hypertension, placental hypoxia and growth restriction. Therefore, we studied the placental expression profiles of CBS and CSE during PE and healthy pregnancies.

Methods: Placental biopsies were taken from patients with severe early-onset PE (n=10) and healthy pregnant women (n=9) who underwent indicated caesarian. CBS and CSE mRNA was quantified using RT-PCR. Immunohistochemistry (IHC) was performed on frozen tissue with MoABs against CBS and CSE. Colocalizaton studies for CSE/CBS and CD31 (endothelium) were performed using immunofluorescence staining.

Results: Mean time of delivery was 31 (range 27-35) wks in PE and 40 (range 38-42) wks in controls. RT-PCR showed a 3-fold decrease (p<0.05) of CBS mRNA expression in the PE placentae compared to controls. CSE mRNA was unaltered compared to controls. In both groups, IHC showed CBS and CSE-specific endothelial staining of the fetal vessels in both stem- and chorionic villi. CBS was also expressed by placental macrophages, the Hofbauer cells.

Conclusions: As the CBS expression is down regulated in severe early-onset PE, endogenous H2S production is likely to be decreased. We propose that H_2S is an angiogenic mediator during pregnancy and that deficiency of $H_{[sup]2[/sup]}S$ might partly underlie the pathogenesis of PE. This is supported by the endothelial localization of CBS/CSE since PE is accompanied by endothelial dysfunction. Therefore, H_2S may have protective and therapeutic potential in PE.

TH-PO730

Angiotensin II Type 1 Receptors Regulate Renal Medullary Endothelin B Receptor Function in Rats Fed a Low Salt Diet

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Background: We previously reported that angiotensin (Ang) II hypertensive rats have impaired endothelin B(ETB)receptor-mediated natriuresis. However, the physiological role if Ang II affects ETB receptor function is not known. Thus, we hypothesized that stimulation of endogenous Ang II with a low salt diet attenuates ETB-dependent natriuresis.

Methods: Experiments were designed to determine the diuretic and natriuretic responses to intramedullary infusion of an ETB receptor agonist sarafotoxin 6c (S6c) in rats fed a normal (0.4% NaCl) or low (0.02% NaCl) salt diet for 2 weeks.

Results: In rats on a normal salt, S6c increased urine flow (3.6±0.5 vs. 10.6±1.8 µl/min; p<0.05) and sodium excretion (0.35±0.06 vs. 1.22±0.23 µmol/min; p<0.05). In contrast, rats fed a low salt diet did not display an increase in water (4.3±0.8 vs. 6.2±1.4 µl/min) or sodium (0.26±0.07 vs. 0.34±0.13 µmol/min) excretion during S6c infusion. Moreover, western blotting showed that rats fed a low salt diet expressed less ETB receptors in renal inner medulla compared to a normal salt diet (0.73±0.05 vs. 1.00±0.07 A.U., normalized to actin; p<0.05). We next hypothesized that the reduced ETB receptor function in rats fed a low salt diet is mediated by Ang II type 1 (AT1) receptors. Rats fed a normal or low salt diet were treated with the AT1 antagonist, candesartan (5 mg/kg/d), for 2 weeks. Candesartan restored ETB-induced water (7.5±1.4 vs. 17.7±1.9 µl/min, p<0.05) and sodium (0.46± 0.09 vs. 1.02±0.21 µmol/min, p<0.05) excretion in rats fed a low salt diet. S6c also increased urine flow (4.4±0.6 vs. 10.5±2.6 µl/min, p<0.05) and sodium excretion (0.61±0.09 vs. 1.36±0.42 µmol/min, p<0.05) in candesartan-treated rats fed a normal salt diet.

Conclusions: These findings support the hypothesis that AT1 receptors regulate renal medullary ETB receptor function in a low salt diet model as a means of salt and water retention.

TH-PO731

Localization and Regulation of the Angiotensin II Type 1 Receptor-Associated Protein Arap1 in the Kidney

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Background: Arap1 is an interacting protein of angiotensin II type 1 (AT1) receptors and it facilitates increased AT1 receptor surface expression in vitro. Here we assessed the tissue localization and regulation of Arap1 in vivo.

Results: Arap1 was found in various organs of the mouse with an order of expression of heart kidney>aorta>adrenal gland≈liver≈testis≈spleen>brain, as determined by RT-PCR. In the kidney, Arap1 was found along a cortical-medullary gradient, with cortical expression levels exceeding those of the inner medulla by 130%. Arap1 protein was localized to the vasculature of the renal cortex including the juxtaglomerular cells; at lower levels, Arap1 was also present in vessels of the medulla. Arap1 furthermore was expressed in glomerular podocytes. Similar like in mice, Arap1 mRNA was detected in all kidney zones of the human kidney. Arap1 expression was regulated by various stimuli. Thus, a

high salt diet (4% NaCl [w/w], 7d, n=5) upregulated renal Arap1 expression in mice by 47% compared to controls (.6% NaCl [w/w], n=5, p=.01). Similarly, AT1 antagonism (losartan, 30 mg/kg/d, 7d), enhanced Arap1 mRNA expression by 52% (n=5 each, p<.01), whereas Ang II infusion (osmotic mini pumps, 7d, 2µg/kg/min Ang II) reduced Arap1 mRNA levels compared to vehicle by 34% (p<.01, n=7 each). Conditions of high Ang II levels, like unilateral kidney artery stenosis (48 hrs, n=5) or water restriction (48 hrs, n=6), all suppressed Arap1 levels compared to controls (-64% and -62% in the clipped and contralateral kidney, respectively, and -28% after water restriction; p<.01 vs. control each). Changes in Arap1 mRNA expression were paralleled by changes in Arap1 protein levels. Similar like in vivo, Arap1 mRNA and protein were suppressed by Ang II in a time- and dose-dependent manner in cultured mesangial cells (down to 32% of control), and this could be blocked by losartan.

Conclusions: In summary, Arap1 is highly expressed in the renal vasculature and its expression appears to be suppressed by AT1 receptor activation. Thus, Arap1 may serve as a local modulator of vascular AT1 receptor function in vivo.

TH-PO732

Candesartan Prevents High-Fat Diet-Induced Hypertension and Renal Injury in Spontaneously Hypertensive Rat Via Angiontensin II Receptor-PI3K/Akt/FoxO Signaling Pathway Sungjin Chung, Ji Hee Lim, Min-Young Kim, Seok Joon Shin, Hyung Wook Kim, Yong-Soo Kim, Yoon-Sik Chang, Cheol Whee Park. *Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: We have recently demonstrated that intrarenal renin-angiotensin system (RAS) would be involved in high-fat diet-induced renal lipotoxicity. Because the intracellular pathways involved in renal lipotoxicity remain to be determined, we examined the participation of FoxO transcription factors in renoprotective effects of RAS inhibition in spontaneously hypertensive rat (SHR) fed a high-fat diet.

Methods: SHR and WKY were treated with either a normal diet (SHR-C or WKY-C) or a high-fat diet (SHR-HF or WKY-HF) with or without candesartan or hydralazine for 12 weeks.

Results: Intrarenal lipid accumulation were significantly increased in SHR-HF and WKY-HF compared with SHR-C and WKY-C. In SHR-HF, systolic BP was more elevated than those in SHR-C but the increased BP were normalized by treatment with candesartan or hydralazine. Renal morphology showed that mesangial expansion and inflammation were significantly enhanced in SHR-HF. A high-fat feeding resulted in activation of the intrarenal RAS, which findings were shown by immunohistochemical stainings of renin and angiotensin II and Western blot analysis for renin and angiotensin II type 1 (AT1) receptor. These changes were associated with increases in the activity of intrarenal PI3K-Akt phosphorylation and FoxO3a phosphorylation, consequently leading to increases in urinary 8-OH-deoxyguanosine levels and intrarenal active caspase-3 expression, indicating enhanced oxidative stress and apoptosis in the kidney. Treatment with candesartan, but not hydralazine, normalized all of these abnormalities via repression of angiotensin II AT1 receptor and upregulation of FoxO3a.

Conclusions: A high-fat diet resulted in the downregulation of FoxO transcrition factor, and candesartan ameliorated high-fat diet-induced hypertension and renal damage through AT1 receptor-PI3K-Akt-FoxO3a pathway, suggesting that AT1 receptor inhibition and subsequent FoxO activation could provide beneficial effects against renal lipotoxicity.

TH-PO733

ACE Inhibition Attenuates Renal Injury in Mice Lacking Endothelial Nitric Oxide Synthase Wataru Kitagawa,[1,3] Katsuyuki Tanabe,[1] Yoshifuru Tamura,[1] Jelena Klawitter,[2] Christopher J. Rivard,[1] Richard J. Johnson,[1] Takahiko Nakagawa.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Department of Anesthesiology, University of Colorado Denver, Aurora, CO; [3]Division of Nephrology and Rheumatology, Aichi Medical University, Nagakute, Aichi, Japan.*

Background: The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in the development and progression of renal injury. Non-specific nitric oxide synthase inhibition (by L-NAME) is a known cause for RAAS activation while the specific role of endothelial nitric oxide synthase (eNOS) remains unclear. Our group previously documented that eNOS deficiency accelerated renal injury in the aging mice as well as in the mouse remnant kidney model. However, the role of RAAS has not been studied in these models. Here, we examined if renal injury due to eNOS deficiency depends on RAAS activation.

Methods: 5-month-old C57BL/6 wild type (WT) and Nos3tm1UNC (eNOS-KO) mice were divided into vehicle-treated (each n=10) or enalapril-treated (each n=10) groups. Enalapril was administered in the drinking water at a dose of 20mg/kg/day for 9 months.

Results: Blood pressure was significantly elevated in eNOS-KO mice at 14 months compared with WT mice (118.1±8.4 vs. 101.6±6.1 mmHg, p<0.005). Enalapril treatment significantly lowered the blood pressure in both groups (85.6±5.7 in eNOS-KO vs. 80.7±5.3 mmHg in WT). Urine albumin excretion was increased in eNOS-KO mice (but not in WT mice), whereas it was significantly reduced with enalapril treatment (167.4±64.1 vs. 27.0±9.6 µg/mg creatinine). Histologically, the development of glomerular sclerosis was found only in eNOS-KO mice, and it was significantly prevented with enalapril treatment (5.70±2.26 vs. 2.36±2.21 in % glomerulus, p<0.005). Activation of RAAS in eNOS-KO mice was proven by higher serum aldosterone concentrations compared with WT mice whereas enalapril significantly reduced serum aldosterone levels in eNOS-KO mice (346.3±150.6 vs. 166.8±40.8 pg/ml, p<0.05).

Conclusions: The eNOS deficiency causes RAAS activation in the mouse. Long-term ACE inhibititor treatment effectively prevents renal injury and hypertension in mice lacking eNOS.

TH-PO734

Distinct In Vitro and In Vivo Phenotypes of KCNJ5 Mutations in Hereditary Hypertension Ute I. Scholl,[1] Carol J. Nelson-Williams,[1] Peng Yue,[2] Robert J. Wyatt,[3] Michael J. Dillon,[4] Robert Couch,[5] Lisa Arel Hammer,[6] Anita Farhi,[1] WenHui Wang,[2] Richard P. Lifton.[1] *[1]Yale School of Medicine; [2]New York Medical College; [3]University of Tennessee Memphis; [4]University College London; [5]University of Alberta; [6]University of Michigan.*

Background: We have recently identified mutations in an adrenal potassium channel, KCNJ5, as a cause of primary aldosteronism in humans (Choi et al., Science 2011). Recurrent somatic mutations were found in aldosterone-producing adenomas, and an inherited mutation in a family with massive adrenal hyperplasia. All mutations were located in and near the channel selectivity filter, producing increased Na+ conductance and depolarization, the signal for aldosterone production and cellular proliferation in adrenal glomerulosa.

Methods: We report 9 patients from 4 kindreds with primary aldosteronism, and demonstrate mutations in KCNJ5 in all. Using heterologous expression in 293-T cells, flow cytometry and electrophysiology, we study the impact of these mutations on channel physiology and cell survival.

Results: All families had mutations at G151, different from the T158A mutation in the one family reported to date. Two families had G151R mutations, identical to one of the common mutations in APAs, and the other was G151E, which is never seen in APAs. Patients with G151R mutations developed adrenocortical hyperplasia. Their hypertension was poorly controlled by antihypertensives including spironolactone, leading to bilateral adrenalectomy in childhood. In contrast, G151E subjects treated with spironolactone had excellent control of blood pressure. Imaging, and, where available, histopathology, revealed no evidence of adrenal hyperplasia. Compared with the effect of the G151R mutation, G151E led to a much larger Na+ conductance, and consequently dramatic lethality in 293-T cells.

Conclusions: The absence of hyperplasia in subjects with G151E mutations is likely attributable to increased cell death in vivo, raising the question whether G151E or additional inherited or somatic mutations in KCNJ5 may be present in patients with primary aldosteronism without overt adenoma or hyperplasia.

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TH-PO735

ROMK Knockout in Rats Is Associated with Decreased Blood Pressure Xiaoyan Zhou, Zuo Zhang, Myung Shin, Sarah Horwitz, John Levorse, Wanda Sharif-Rodriguez, Denis Streltsov, Maya Dajee, Melba Hernandez, Yi Pan, Olga Price, Li Wang, Gail M. Forrest, Daphne Szeto, Yonghua Zhu, Yan Cui, Leslie Balogh, Yan G. Ni, Kathleen A. Sullivan. *Merck Research Laboratories, Merck & CO., Rahway, NJ.*

Background: ROMK channels mediate potassium recycling and facilitate sodium reabsorption through the $Na^+/K^+/2Cl^-$ cotransporter in the loop of Henle and potassium secretion at the cortical collecting duct. Human genetics studies indicate that ROMK homozygous loss-of-function mutations cause type II Barter's syndrome, featuring polyuria, renal salt wasting, and hypotension; Humans heterozygous for ROMK mutations identified in the Framingham Heart Study have reduced blood pressure. ROMK null mice recapitulate many of the features of type II Barter's syndrome. The aim of our study is to investigate the effects of knocking out ROMK on blood pressure in rats.

Methods: Zinc Finger Nuclease (ZFN) technology was used to generate ROMK knockout rats in the Dahl salt sensitive rat background. Rats were fed either a low salt (0.25% NaCl) or a high salt diet (4% NaCl), their blood pressure and renal function were monitored for 8 weeks.

Results: None of ROMK -/- survived beyond 4 weeks. The ROMK +/- rats, in which kidney ROMK mRNA level was decreased by 50% as compared to the wild type littermates, showed decreased systolic blood pressure: ~5 mmHg or ~15 mmHg lower than the wild type littermates while fed the low salt or high salt diet respectively. No significant differences in body weight, food intake, water intake, urine volume, urinary electrolytes and PGE2 excretions, GFR, and plasma electrolyte concentrations, were observed between the ROMK +/- rats and the wild type littermates.

Conclusions: The finding from this study, together with the human and mice genetics evidence, strongly supports the role of ROMK in blood pressure regulation.

Funding: Pharmaceutical Company Support

TH-PO736

Mesenchymal Stem Cells (MSC) Improved Renal Function and Reduced Renovascular Hypertension Elizabeth B. Oliveira-Sales,[1] Edgar Maquigussa,[1] Patricia Semedo,[1] Luciana Guilhermino Pereira,[1] Niels O.S. Camara,[1] Cassia Bergamaschi,[2] Ruy Campos,[2] Mirian A. Boim.[1] *[1]Medicine, Renal Division, UNIFESP, Sao Paulo, Brazil; [2]Physiology, UNIFESP, Sao Paulo, Brazil.*

Background: Renovascular hypertension induced by 2 Kidney-1 Clip (2K-1C), is a renin-angiotensin-system (RAS)-dependent model, leading to vascular rarefaction and renal failure. This work investigated the effects of mesenchymal stem cells (MSC) treatment on the blood pressure, renal function and RAS components in ischemic kidney 2K-1C rats.

Methods: Three weeks after left renal artery occlusion, fluorescently tagged MSC (2x105cells/animal) were weekly injected into the tail vein. Animals were sacrificed 6 weeks after clipping (two cell administration). Animals were divided in the following groups: control (n=5), control MSC-treated (n=2), 2K-1C (n=8) and 2K-1C MSC-treated (n=7).

The systolic blood pressure (SBP) was monitored using tail cuff recording method. Renal function was estimated by plasma and urine creatinine, proteinuria and urinary sodium and potassium. Protein levels of angiotensinogen (AGTN, renin, ACE, Ang II receptors types 1 (AT1) and 2 (AT2) were determined in the renal medulla of clipped kidney by western blot. Tracking assay by flow cytometry showed that labeled MSC were present in the cortex and medulla in the clipped kidney.

Results: MSC had no detectable effects in control animals and significantly reduce the SBP by 22% (from 224 ± 8 to 173 ± 6 mmHg). Proteinuria was increased in 2K-1C compared to control animals (15±3 vs 80±19 mg/24h, p<0.05) and it was significantly reduced after MSC treatment (48±8 mg/24h). Other renal function parameters were unchanged in all groups. AGTN, renin, ACE and AT1 protein expressions were elevated in clipped kidneys (49, 25, 30 and 43%, respectively) and the MSC treatment normalized all these expressions. In contrast, AT2 levels were significantly decreased in clipped kidneys (60%) and it was almost normalized after MSC treatment.

Conclusions: In conclusion MSC therapy suppressed the intrarenal RAS and improved renal function in the 2K-1C model. Whether the improvement of renal function was responsible for the MSC-reducing hypertension effects needs to be investigated.

TH-PO737

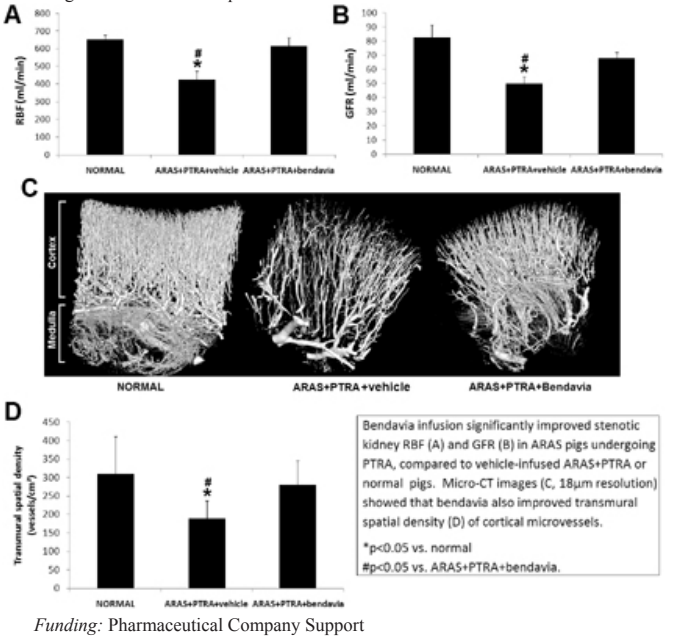
MTP-131 Reduces Renal Injury after Percutaneous Transluminal Renal Angioplasty (PTRA) in Swine Atherosclerotic Renal Artery Stenosis (ARAS) Alfonso Eirin, John R. Woollard, Xiang-Yang Zhu, James Krier, Xin Zhang, Stephen C. Textor, Lilach O. Lerman. *Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: MTP-131 (bendavia) is a novel compound that inhibits opening of the mitochondrial permeability transition pore, apoptosis, and cardiac reperfusion injury in animals and humans, but its potential for improving PTRA outcomes in ARAS is unknown.

Methods: Pigs were treated after 6 weeks of ARAS or control with PTRA+stenting (or sham), with adjunct continuous infusion of bendavia (0.05 mg/kg IV, 30 min before to 3 hrs after PTRA) or vehicle (n=7 each). Single-kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were studied 4 weeks later using multi-detector CT, and renal microvascular architecture *ex-vivo* by micro-CT.

Results: Four weeks after successful PTRA blood pressure decreased to normal levels in all PTRA-treated pigs (p>0.05 vs. Normal). Stenotic kidney GFR and RBF remained decreased in ARAS+PTRA+vehicle, but were restored in ARAS+PTRA+bendavia (p<0.05 vs. ARAS+PTRA; p>0.05 vs. normal). Furthermore, spatial density of cortical microvessels was normalized in these pigs (Figure).

Conclusions: Intra-venous infusion of bendavia at the time of PTRA restored renal hemodynamics and function and attenuated microvascular rarefaction in swine ARAS. Bendavia shows a unique therapeutic potential for improving kidney function and outcomes following PTRA in chronic experimental ARAS.



Funding: Pharmaceutical Company Support

TH-PO738

Aggravated Inflammation in Malignant Versus Non-Malignant Course of Experimental Renovascular Hypertension Karl F. Hilgers,[1] Lisa Jagusch,[1] Nada Cordasic,[1] Kerstin U. Amann,[2] Roland Veelken,[1] Andrea Hartner.[3] *[1]Nephrology & Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Nephropathology, University of Erlangen-Nuremberg, Erlangen, Germany; [3]Pediatric and Adolescent Medicine, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: The reason(s) why malignant nephrosclerosis develops in some cases of hypertension but not in others are largely unknown. We hypothesized that malignant nephrosclerosis in experimental renovascular hypertension exhibits a more pronounced macrophage infiltration.

Methods: Two-kidney, one-clip renovascular hypertension was induced in rats; controls (CON) were sham operated. To distinguish malignant hypertension (MH) from non-malignant hypertension (NMH) without using arbitrary definitions, we performed split-half analyses for two factors: weight loss, and the number of characteristic vascular lesions (onion skin lesions and fibrinoid necroses) per kidney section of the nonclipped kidney. Animals in the upper half for both criteria were defined as MH whereas those in the lower half for both criteria were NMH. Macrophage infiltration in the nonclipped kidney was counted (ED-1 stain), and the gene expression of macrophage chemoattractant proteins was measured by real-time RT-PCR.

Results: After 5 weeks, mean arterial pressure was elevated to the same degree in MH (207±10 mmHg, N=13) and NMH (201±4 mmHg, N=15; p=0.4 versus MH) compared to CON (113±3 mmHg, N=25, P<0.001). MH exhibited higher serum aldosterone, left ventricular hypertrophy, and interstitial fibrosis of the nonclipped kidney, compared to NMH and CON (all p<0.05). Macrophage infiltration was present in NMH (8.9±0.8 vs. 5.7±0.5 cells in CON, p=0.009) but more pronounced in MH (14.7±1.4, p<0.001 vs. NMH and CON). Osteopontin expression was increased 29fold in NMH (p=0.03 vs. CON) and 65fold in MH (p<0.01 vs. NMH and CON). MCP-1 expression was not altered in NMH (1.4fold, p=0.447) but increased 2.8fold in MH (p<0.01 vs. NMH and CON).

Conclusions: Macrophage infiltration is much more pronounced in malignant renovascular hypertension than in the non-malignant course of the disease. MCP-1 is induced only in malignant nephrosclerosis and may contribute to its pathogenesis.

Funding: Government Support - Non-U.S.

TH-PO739

Sestrin2 Regulates Activation of Peroxiredoxin and Mediates Dopamine D2 Receptor/Paraoxonase 2-Induced Decrease in Renal ROS Production Yu Yang, Santiago Cuevas Gonzalez, Yanrong Zhang, Laureano D. Asico, Ines Armando, Pedro A. Jose. *Research for Molecular Physiology, CNMC, George Washington University, Washington, DC.*

Background: We have shown that the D2 dopamine receptor (D2R) decreases renal reactive oxygen species (ROS) production and regulates blood pressure (BP), in part, via positive regulation of paraoxonase 2 (PON2), an enzyme that protects against cellular oxidative stress. Sestrin2 is a conserved antioxidant protein that regulates intracellular ROS level by regenerating over-oxidized peroxiredoxin (Prx-$SO_{2/3}$H). We hypothesized that renal sestrin2 may be involved in counter-regulating renal ROS production via the D2R, contributing to maintain normal BP.

Results: *In vitro* treatment of human renal proximal tubular cells with the D2R agonist, quinpirole (24h, 1μM), decreased ROS production (DCFDA method) by 42% (P<0.05; n=3). This effect was associated with increased protein expression of PON2 (1.3-fold, P<0.05, n=3) and sestrin2 (1.4-fold, P<0.01, n=4), and decreased protein expression of Prx-$SO_{2/3}$H (-55%, P<0.01, n=3). In contrast, silencing D2R (siRNA, 20 nM, 48h) down-regulated PON2 (-25%, P<0.05, n=3) sestrin2 expression (-48%, P<0.05, n=3), and increased Prx-$SO_{2/3}$H protein expression (1.75-fold, P<0.05, n=3) and ROS production (1.4-fold, P<0.01, n=4). Silencing PON2 (siRNA, 10nM, 48h) decreased sestrin2 (-32%, P<0.05, n=3) and increased Prx-$SO_{2/3}$H protein expressions (1.7-fold, P<0.05, n=3) and ROS production (1.4-fold, P<0.01, n=4). Silencing sestrin2 (siRNA, 20 nM, 48h) increased Prx-$SO_{2/3}$H protein expression (2.2-fold, P<0.01, n=3) and ROS production (1.3-fold, P<0.01, n=4). *In vivo* selective renal silencing of sestrin2 by subcapsular infusion of sestrin2 siRNA (3 μg/day, 7 days) in mice increased systolic (117±4 vs. 92±2 [vehicle-treatment] mmHg, P<0.01, n=3) and diastolic BP (86±2 vs. 67±3 [vehicle treatment] mmHg, P<0.01, n=3)

Conclusions: These results suggest that renal sestrin2 is positively regulated by D2R and PON2 and contributes to maintain normal BP. The negative regulation of peroxiredoxin activation by sestrin2 mediates, in part, the inhibitory effect of renal D2R on ROS production.

Funding: NIDDK Support, Other NIH Support - HL68686, HL23081, HL074940, HL092196

TH-PO740

Aliskiren Ameliorates Insulin Resistance and Aortic Endothelial Dysfunction in Fructose-Fed Rats Te-Chao Fang.[1,2,3] *[1]Internal Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [2]Institute of Medical Sciences, Tzu-Chi University, Hualien, Taiwan.*

Background: Renin-angiotensin system plays a major role in the pathogenesis of cardiovascular diseases. The objective of this study was to examine the effects of aliskiren, a direct renin inhibitor, on the aortic endothelial function of fructose-fed hypertensive rats.

Methods: Male Wistar Kyoto rats weighing 200 to 230 g were divided into 4 groups (n=6 for each group). Group Con: rats were fed standard chow diet for 8 weeks; Group Fru: rats were fed high fructose diet (60% fructose) for 8 weeks; Group FruA: rats were fed high fructose and were coinfused with aliskiren (100 mg/kg.day), and Group FruB: rats were treated as Group Fru, but aliskiren was administered 4 weeks later. Isolated vascular ring experiments, systolic blood pressure (SBP), homeostasis model assessment-insulin resistance (HOMA-IR), oral glucose tolerance test (OGTT) and related blood profiles were measured.

Results: By the end of week 4 and 8 of sustaining a high fructose diet, SBP had increased significantly from 110 ± 5 to 143 ± 4 and 140 ± 5 mmHg ($p< 0.05$), respectively. When fructose-induced hypertension had been established (from 112 ± 4 to 140 ± 3 mmHg, $p< 0.05$), subsequent aliskiren treatment for 4 weeks reversed the elevated SBP. Concurrent aliskiren treatment restored the development of hypertension. Additionally, a high fructose diet also had significantly higher HOMA-IR values at week 4 and 8 (21.25 ± 2.08 and 21.28 ± 3.1 from 6.15 ±1.59, respectively; $p< 0.05$), and concurrent or subsequent administration of aliskiren significantly reduced the HOMA-IR values. OGTT showed that fructose feeding resulted in insulin resistance, and co-administration or superimposition of aliskiren significantly ameliorated insulin resistance of fructose-fed rats. The percent of endothelium-dependent aortic relaxation in the Group FruA and Group FruB were significantly higher than that in the Group Fru (63.46 ± 5.50 and 60.10 ± 8.01 than 39.97 ± 7.44 %, respectively; $p<0.05$).

Conclusions: This study demonstrates that aliskiren does not only prevent but also ameliorates hypertension and aortic endothelial dysfunction in fructose-fed rats.

Funding: Private Foundation Support

TH-PO741

Vitamin B 6 Prevents the Hypertension Induced by Chronic Fructose Administration & Averts the Cardiovascular Complications in Akita Diabetic Mice: Role of Reduced Oxidative Stress Meghan Pantalia, Uzma Hajiyani, Shuangxi Wang, Bonnie Eby, Alexander Lau, Becky Pennington, Pedro Lozano, Kai Lau. *Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Hyperglycemia is known to stimulate ROS & glycation end-products (AGE). We recently showed benefits of blocking receptors (R) of AGE in preventing diabetic nephropathy & of up-regulating AMPK with metformin in preventing diabetic cardiomyopathy & hypertension. Since vit B6 is known to inhibit AGE, we studied its effects in 2 models.

Methods: Male Akita & controls were randomzied to vit B6 or nothing starting at 7 weeks. We monitored BP by tail-cuffs & heart function by echo. Normal male mice were also randomized to either fructose, fructose + vit B6, or dextrose, adjusted for 1.4 g/d. Endothelial function was studied by aortic relaxation.

Results: Akita mice had significant systolic (S) hypertension (in torr) at 5 mon (119 vs. 111) & 8 mon (127 vs. 109). Diastolic (D) BP (85 vs. 74) & mean arterial BP (MABP) (99 vs. 86) were elevated at 8 mon. In Akita mice, vit B6 normalized SBP at both ages & DBP & MABP at 8 mon. At 5 mon, Akita mice had reduced LV end-diastolic (ED) volume (V), stroke volume (SV) & cardiac output (CO) (7 vs. 13 ml/min). Vit B6 preserved LVEDV, SV & CO (12 ml/min). At 10 mon, LVEDV, SV & CO remained low in Akita, but fully corrected by vit B6. Peripheral vascular resistance was elevated in Akita (15 vs. 8 torr. min/ml) but normalized by vit B 6. Fructose raised fasting insulin (0.58 vs. 0.28 ng/ml) & total cholesterol (131 vs. 95 mg%), but blocked by vit B6. Blood glucose & Hgb A1c were unaltered by fructose/vit B6. Dextrose did not alter BP. Fructose raised SBP (123 vs. 114), DBP (95 vs. 84) & MABP (104 vs. 94), but these elevations were prevented by vit B6. Relaxation of aorta from Akita was reduced but normalized by vit B6. In cultured cells, vit B6 activated AMPK & eNOS.

Conclusions: 1. Vit B6 confers anti-hypertensive benefits to insulin- deficient & resistant states. 2. In fructose-induced metabolic syndrome, it lowers plasma cholesterol & insulin. 3. In Akita diabetics, it prevents cardiomyopathy & endothelial dysfunctions, pututatively, by raising AMPK and eNOS.

Funding: Veterans Administration Support, Private Foundation Support, Clinical Revenue Support

TH-PO742

Effect of L/N-Type Calcium Channel Blocker Cilnidipine on Adriamycin-Induced Cardiomyopathy and Nephropathy in Spontaneously Hypertensive Rats Shizuka Aritomi, Kazumi Niinuma, Mai Kawakami, Tomoyuki Konda. *Research Center, Ajinomoto Pharmaceuticals Co., Ltd., Kawasaki, Kanagawa, Japan.*

Background: Chronic treatment with adriamycin (ADR) is known to induce cardiomyopathy and nephropathy via activation of sympathetic nervous system (SNS) and renin-angiotensin system (RAS). It has been reported that cilnidipine, L/N-type calcium channel blocker (CCB) suppresses SNS and RAS. In this study, we investigated the effect of cilnidipine on the ADR-induced cardiomyopathy and nephropathy in SHR comparing with that of L-type CCB.

Methods: ADR (1.5 mg/kg) was administered intravenously to SHR rats (12 weeks of age) once a week for 3 weeks. Control rats were administered saline (unaffected group, n=10). One week after the last administration of ADR, rats were divided into 3 groups and administered either the vehicle (untreated group, n=16), cilnidipine (20 mg/kg; cilnidipine group, n=16), or amlodipine (3 mg/kg; amlodipine group, n=16) for 4 weeks.

Results: There was no difference in antihypertensive effect between cilnidipine group and amlodipine group throughout the experimental period. ADR caused an increase in the urinary albumin excretion (UAE), urinary protein excretion (UPE), and N-acetyl-ß-D-glucosaminidase excretion (UNAG), indicating the progression of renal dysfunction in this animal model. Although both cilnidipine and amlodipine suppressed these levels, cilnidipine suppressed the increase of UAE and UPE more effectively than amlodipine. Cardiac contractile function was assessed by echocardiography, and ADR exacerbated its dysfunction. Chronic treatment with cilnidipine also resulted in an improvement of cardiac function, but amlodipine did not. In addition, cilnidipine group showed a lower level of RAS than amlodipine group.

Conclusions: The results of the present study indicate that the L/N-type CCB cilnidipine suppresses the progression of renal and cardiac dysfunction induced by ADR. Since such effect was not observed by L-type CCB amlodipine treatment, blockade of N-type calcium channel might play an important role for protection of both renal and cardiac function. And the mechanism of cilnidipine's action can be partly explained by its suppressive action in RAS.

TH-PO743

A Synthetic Serine Protease Inhibitor Camostat Mesilate Inhibited the Proteolytic Activation of gENaC in the Kidney of the Aldosterone-Infused Rats Kohei Uchimura,[1] Yutaka Kakizoe,[1] Tomoaki Onoue,[1] Manabu Hayata,[1] Jun Morinaga,[1] Teruhiko Mizumoto,[1] Masataka Adachi,[1] Taku Miyoshi,[1] Naoki Shiraishi,[1] Sakai Yoshiki,[2] Kimio Tomita,[1] Kenichiro Kitamura.[1] *[1]Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [2]Research Headquaters, Ono Pharmaceutical Co., Ltd, Osaka, Japan.*

Background: ENaC plays an important role in the regulation of blood pressure by modulating Na reabsorption in the kidney. ENaC consists of a, b, and g subunits, and the activation of ENaC is mainly regulated by aldosterone in living body. It was reported that aldosterone induced a molecular weight shift of gENaC from 85 to 70 kDa, and recently this shift has been considered as the result of proteolytic cleavages by serine proteases and necessary for the activation of ENaC from the in vitro experiment. But detail mechanisms about the cleavage of gENaC in vivo are still unclear. In order to study the role of serine proteases in this cleavage in vivo, we administrated a synthetic serine protease inhibitor camostat mesilate to aldosterone-infused rats.

Methods: The SD rats (n = 6) were kept for 10 days under the following conditions: (1) Control, (2) aldosterone infusion, (3) aldosterone infusion+camostat treated with free access to water and chow. After 10 days, rats were sacrificed under anesthetic conditions with pentobarbital sodium.

Results: Camostat decreased 70kDa form of gENaC and produced the new about 75kDa form with increase of urinary Na/K ratio, suggesting that camostat inhibited one site of the dual cleavages of gENaC and suppressed the activation of ENaC. Prostasin is one candidate serine protease involved in the cleavage of gENaC in these model rats, because prostasin was shown to cleave this subunit in vitro and its excretion into urine was increased by aldosterone. Camostat inhibited protease activity, activating processing and urinary secretion of prostasin.

Conclusions: These results suggest that prostasin is one important serine protease in the pathogenesis of aldosterone-induced salt sensitive hypertension. A synthetic serine protease inhibitor, camostat mesilate, would be a new strategy in the treatment of salt-sensitive hypertension in human.

TH-PO744

The Sodium Chloride Cotransporter (NCC) and Prostasin, a Regulator of the Epithelial Sodium Channel (ENaC), Are Urinary Biomarkers for Hyperaldosteronism Nils van der Lubbe, Pieter Martijn Jansen, Anton H. Van den Meiracker, Alexander H. Danser, Robert Zietse, Ewout J. Hoorn. *Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.*

Background: Urinary exosomes are vesicles derived from renal tubule epithelial cells that have been shown to contain many disease-associated proteins. Here, we hypothesize that the aldosterone-sensitive sodium transporters could serve as biomarkers for aldosteronism.

Methods: Urinary exosomes were studied in a rat model of hyperaldosteronism and in patients with essential hypertension or primary aldosteronism (n = 5/group). Rats were adrenalectomized and then received vehicle or aldosterone. Primary aldosteronism was defined as an elevated aldosterone to renin ratio and unsuppressible aldosterone in response to volume expansion. Blood pressure and the number and type of antihypertensive drugs were similar in the two groups of hypertensive patients. Exosomes were isolated from 24h urine using ultracentrifugation. Phosphorylated NCC and prostasin, a protease regulating ENaC, were studied by immunoblotting. Prostasin rather than ENaC was studied, because the latter has not been identified in urinary exosomes.

Results: The animal model was confirmed by finding higher plasma aldosterone and lower urinary sodium in rats treated with aldosterone. In the urinary exosomes of these rats, the abundances of pNCC and prostasin were increased 3.9 and 1.8-fold compared to controls ($p < 0.05$ for both). Patients with primary aldosteronism also had higher pNCC and prostasin abundances compared to patients with essential hypertension (1.4 and 1.9-fold), although this did not reach statistical significance ($p = 0.1$ and $p = 0.2$, respectively).

Conclusions: In animals and patients with hyperaldosteronism, the abundance of pNCC and prostasin in urinary exosomes is increased. These results suggest that these aldosterone-sensitive proteins may be used as urinary biomarkers for primary aldosteronism. To validate these results, we are currently expanding the number of patients tested.

TH-PO745

Fat Distribution and Regulation of the Renin Angiotensin System in Healthy Humans Ann A. Zalucky, David Donald McTavish Nicholl, Michelle C. Mann, Brenda Hemmelgarn, Darlene Y. Sola, Sofia B. Ahmed. *University of Calgary.*

Background: Obesity, a major risk factor for chronic kidney (CKD) and cardiovascular disease (CVD), is associated with up-regulation of the renin angiotensin system (RAS), activity which is deleterious to kidney and CV function. The ideal measure of adiposity associated with increased risk in humans is unclear. We sought to determine the relationship between measures of fat distribution and angiotensin (AngII) dependent-control of blood pressure (BP) in healthy humans.

Methods: Thirty-eight healthy non-obese subjects (15 men, 23 women) were studied in high salt balance. Body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), body adiposity index, body surface area, fat mass%, and total body water% were measured. BP and circulating components of the RAS were measured at baseline and in response to graded AngII infusion (3ng/kg/min x 30 min followed by 6 ng/kg/min x 30 min). The primary outcome was the association between adiposity measures (BMI, HC and WHR) and the BP response to AngII challenge at 60 minutes.

Results: BMI was associated with baseline BP (SBP: r=0.544, p<0.001; DBP: r=0.324, p=0.047) and HC was associated with baseline SBP in women (r=0.491, p=0.02). However, BMI was not associated with the BP response to AngII (SBP: p=0.8, DBP: p=0.7), overall or when stratified by gender. Conversely, HC was non-significantly associated with both SBP (r=0.3, p=0.076) and DBP (r=0.2, p=0.3) responses to AngII challenge, a relationship that achieved significance in men (SBP: r=0.543, p=0.045; DBP: r=0.58, p=0.03) but not in women (SBP: p=0.3; DBP: p=0.7). While BMI was not associated with any circulating RAS components at baseline or in response to AngII challenge, WHR was a predictor of baseline PRA (r=0.563, p=<0.001), AngII (r=0.468, p=0.006) and aldosterone (r=0.355, p=0.03) as well as the PRA response to AngII infusion (r=0.514, p=0.001). Other anthropometric parameters were not associated with any of the responses to AngII challenge.

Conclusions: Fat distribution, as measured by HC and WHR, is associated with vascular RAS activity in healthy humans and may have implications for assessment and control of obesity-mediated hypertension.

TH-PO746

Underlying Mechanisms of Protection in AngiotensinII (AII)-Induced Salt-Sensitive Hypertension (A-SSHT) by Tubular Overexpression of Liver-Type Fatty Acid-Binding Protein (L-FABP) Ken Osaki,[1] Yusuke Suzuki,[1] Takeshi Sugaya,[2] Akira Nishiyama,[3] Satoshi Horikoshi,[1] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]CMIC Co Ltd., Tokyo, Japan; [3]Department of Pharmacology, Kagawa University Faculty of Medicine, Kagawa, Japan.*

Background: At the last ASN meeting, we reported that overexpression of L-FABP in proximal tubules protects against A-SSHT, which was induced by high salt diet after systemic AII infusion for 4 weeks. The objective of the present study is to examine the underlying mechanisms in L-FABP-mediated protection using transgenic mice which have tubular overexpression of L-FABP in the proximal tubules (L-FABPTg), and the murine proximal tubular cell line transgene with (mProxTg) or without (mProx) L-FABP.

Methods: 1) mProx and mProxTg were subjected to AII-stimulation (10 nmol/L) with or without pretreatment of tempol. mRNA expressions of angiotensinogen (Ag), oxidative stress and inflammatory markers were analyzed.

2) We also examined the expressions of these markers in L-FABPTg and their wild type littermates (WT) with A-SSHT.

Results: 1) AII-stimulation induced mRNA expressions in mProx of HO-1 and MCP-1 peaked at 12 and 36 hr, and Ag peaked at 36 hr. These expressions were significantly attenuated in mProxTg (P<0.05). Tempol pretreatement in mProx also significantly attenuated these expressions at 36 hr (P<0.05).

2) L-FABPTg with A-SSHT showed significant attenuation of renal Ag, 4-HNE, HO-1 and MCP-1 expressions with significant improvement of hypertension and interstitial T-cell infiltration.

Conclusions: It was found that tubular overexpression of L-FABP may protect against A-SSHT through attenuation of AII-induced oxidative stress and exacerbation of intrarenal RAS activation and inflammatory responses.

Funding: Government Support - Non-U.S.

TH-PO747

A Novel Mechanism Explaining Salt Sensitivity: Post-Transcriptional Regulation of 11β-Hydroxysteroid Dehydrogenase Type 2 by miRNA Mina Rezaei,[165875] Thomas Andrieu,[165880] Bernhard Dick,[150254] Brigitte Frey,[150256] Felix J. Frey.[8985] *[1]University of Berne; [2]University of Berne; [3]University of Berne; [4]University of Berne; [5]Nephrology and Hypertension, University of Berne, Switzerland.*

Background: Decreased activity of 11β-hydroxysteroid dehydrogenase (11β-HSD2), protecting the mineralocorticoid receptor from cortisol, induces salt sensitivity in humans and rodents. The mechanisms for tissue specific and inter-personal diverse expression of 11β-HSD2 are unknown. We hypothesized for the first time that miRNAs determine 11β-HSD2 activity.

Methods: The 11β-HSD2 activity in salt sensitive Sprague Dawley (SD) and salt insensitive Wistar (W) rats was investigated, the differences between the target sequence of miRNAs in the HSD11B2-3'UTR were analyzed and the role of dicer to regulate 11β-HSD2, was studied. The urinary ratio of corticosterone/dehydrocorticosterone (B/A) and their metabolites were assessed by gas chromatography/mass spectrometry.

Results: B/A ratio was increased in SD when compared to W rats (0.8±0.2 vs. 0.5±0.1, p<0.05), while (tetrahydrocorticosterone+5α-tetrahydrocorticosterone)/tetradehydrocorticosterone was decreased (0.5±0.1 vs. 1.3±0.3, p<0.001), indicating a diminished 11β-HSD2 activity in SD rats.

To demonstrate whether miRNAs are involved in the diminished 11β-HSD2 activity observed in vivo, the 3'UTR of HSD11B2 of SD and W rats were sequenced and cloned downstream of a reporter gene. Different cell lines (SW620, HT29, HCT116) were transfected with these constructs. The SD 3'UTR construct showed a lower reporter activity than W 3'UTR construct, an effect continuously reversed by cloning various mutated sequences progressively restoring the sequence of the 3'UTR of HSD11B2 found in W rats. These data indicate that the 3'UTR regulates 11β-HSD2 activity. To determine whether miRNAs modulate the expression of 11β-HSD2 we measured the expression of 11β-HDSD2 in HCT116 wild type and HCT116 dicer KO cells. Dicer KO HCT116 cells had an increased expression of 11β-HSD2.

Conclusions: These data indicate for the first time that the 3'UTR and miRNAs determine 11β-HSD2 expression and activity and are of potential relevance for glucocorticoid-mediated increased salt sensitivity.

Funding: SNF (Swiss National Foundation of Scientific Research)

TH-PO748

Sodium Induced Down Regulation of the Angiotensin II Receptor (AT-1) and CD39 in Endothelial Cells In Vitro Silvana L. Della Penna, Marijke M. Faas, Theo Borghuis, Harry Van Goor, Winston W. Bakker. *Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands.*

Background: High dietary salt intake is associated with hypertension, cardiovascular and renal risk. Endothelial injury leading to endothelial dysfunction plays a major role in these conditions.

To evaluate the mechanism of salt mediated damage, we focused on the endothelium protecting ecto-enzyme CD39 [i.e. ectonucleoside triphosphate diphosphohyrolase-1, (ENTPD-1), or ecto-apyrase] and on the angiotensin II receptor (AT-1). CD39 is sensitive to oxygen free radicals and possibly also to the pro-inflammatory action of angiotensin II.

Methods: Confluent human endothelial cells were co-cultured with human peripheral blood mononuclear cells ($1x10^6$ cells per well) using either medium (RPMI 1640) with a standard amount of NaCl (154.0 mMol/L) (low salt, LS), or with "high" NaCl (155.54 mMol/L) (HS). After 16 hours, cytospins of endothelial cells were prepared for flow cytometry or immunostained for AT-1 and CD39.

In another set of experiments PBMC from HS and LS cultures were isolated after 16 hrs of co-culture with endothelial cells and subsequently labeled with dichlorofluorescein diacetate for detection of O_2^- by flow cytometry.

Results: Endothelial cells cultured in HS medium show a significant decrease of CD39 expression as compared with control cultures, as demonstrated by both immunostaining (P<0.05) and flow cytometry (P<0.05). Endothelial AT-1 receptor expression also showed down regulation in the presence of HS (P<0.01), whereas HS showed enhanced O_2^- production in PBMC as compared with PBMC from LS cultures.(P<0.01)

Conclusions: The present data indicate that a slight increase of salt is able to induce impairment of an important endothelial anti-inflammatory ecto-enzyme (CD39) as well as down regulation of the AT-1 receptor in endothelial cells in vitro. These alterations may be mediated by toxic oxygen radicals produced by PBMC. Down regulation of endothelial AT-1 by salt in vitro may be in line with down regulation of the RAAS, known to occur after salt loading in vivo.

TH-PO749

Salt-Sensitivity of Blood Pressure in Mice with Targeted Knockout of the Insulin Receptor in Thick Ascending Limb through Collecting Duct Lijun Li, Radha Mayuri Garikepati, Karishma Sitapara, Carolyn M. Ecelbarger. *Department of Medicine, Georgetown University, Washington, DC.*

Background: Previously we showed reduced expression and phosphorylation of renal insulin receptors (IRs) in kidneys of insulin-resistant rats. We also demonstrated that targeted knockout of IR from the kidney of mice using Ksp-cadherin-targeted Cre recombinase led to modestly increased systolic blood pressure (BP) and reduced urine nitrates plus nitrites (UNOx) excretion. We hypothesized IR signaling might play a role in BP homeostasis and sensitivity to dietary NaCl.

Methods: To address this, young male KO and wild-type (WT) littermates (~4-months old) were instrumented with radiotransmitters to measure BP and heart rate and transitioned through a 4-week regimen that included 1-week periods on:the following diets: 1) medium-NaCl (MS, 0.5%); 2) low-NaCl (LS, 0.085%); 3) high-salt (HS, 5%) and HS plus the anti-oxidant Tempol (3 mM) in the drinking water. Urine was collected for 24 hours at the end of each week and UNOx measured.

Results: There were no differences in initial or final body weight between genotypes. Under low-NaCl diet (0.085%), there were no significant differences in systolic, diastolic, or MAP. Systolic BPs were as follows (mm Hg): 117 ± 3 (WT) versus 109 ± 7 (KO, p = 0.33, n = 7/genotype). The switch from low- to high-NaCl diet did not affect systolic BP in the WT mice (delta SBP, mm Hg) -2 ± 3, but increased it on average 16 ± 8 in the KO (p < 0.05) in the KO. Tempol led to a small decrease in systolic BP in both genotypes which was not significantly different: -2.7 ± 5.2 (WT) versus -4.4 ± 6.7 (KO, p = 0.84). UNOx

increased approximately 3-fold with HS and was not affected by Tempol. The area under the curve for the rise in UNOx was 20% reduced in the KO mice. Heart rate was slightly reduced in the KO mice, an effect that was significant in the MS period (p= 0.015).

Conclusions: Overall, KO mice show greater BP sensitivity to salt with some blunting of the potentially protective rise in NOx production. The fact that Tempol had little affect on responses to HS diet suggests that factors in addition to oxidative stress, are likely determinant(s) of enhanced salt sensitivity.

Funding: NIDDK Support, Private Foundation Support

TH-PO750

Sodium-Sensitivity Correlates with Differential Regulation of Peripheral Blood MicroRNAs Sophie Domhan,[1] Claudia Sommerer,[1] Felix J. Frey,[3] Martin G. Zeier,[1] Amir Abdollahi.[2] *[1]Nephrology, University of Heidelberg Medical School, Germany; [2]Radiation Oncology, University of Heidelberg Medical School, Germany; [3]Nephrology, Inselspital, University Hospital of Berne, Switzerland.*

Background: Sodium-sensitivity is an important risk factor for cardiovascular events and is associated with increased morbidity and mortality. MicroRNAs (miRs) are considered masterregulators of transcriptome. We aimed to identify the molecular determinants of sodium response and predictors of sodium sensitivity using the blood transcriptome as the sentinel organ.

Methods: Whole blood total RNA including miRs were collected during high (150 mmol/d) and low (30 mmol/d) sodium diet using PAXgene tubes. 24 hour blood pressure monitoring was performed during each diet to assess for changes in blood pressure. MiR isolation was performed using Qiagen's PAXgene Blood miRNA Kit and the QIAcube system. RNA quality control and quantitation was performed using total- and small RNA Agilent Chips (Bioanalyzer, Agilent) and NanoDrop spectrophotometer. Genome-wide miR profiling was performed using Illumina's microRNA DASL assay. Clustering and statistics were performed using SUMO software package.

Results: 53 miRs were differentially regulated ($p<0.02$) after sodium-rich vs. sodium-low diet in n=6 patients. The prevailing effect of sodium rich diet was downregulation of miRs (35 down- vs. 15 upregulated miRs). Given the inhibitory function of miRs on post/transcriptional regulation, our data suggest a global activation of the trancriptome via downregulation of miRs in response to sodium. Of note, the miR200 family, recently attributed to be involved in the EMT process were found to be induced by sodium. Three patients demonstrated increased blood pressure (MAP) of at least 8 mmHg after sodium-rich vs. sodium-low diet and were therefore considered to be sodium-sensitive. We found 105 miRs to be differentially regulated between sodium-sensitive vs. salt-resistant patients ($p<0.05$). The regulation of candidate sodium responsive/sensitivity miRs are confirmed by qRT-PCR.

Conclusions: Our data demonstrate the feasibility of peripheral blood miRNAs as sentinel organ to detect sodium response/sensitivity.

TH-PO751

Epigenetic Modulation of Renal b-Adrenergic-WNK4 Pathway in Salt-Sensitive Hypertension Shengyu Mu, Toshiro Fujita. *Department of Nephrology and Endocrinology, University of Tokyo, Japan.*

Background: How high salt intake increases blood pressure is a key question in the study of hypertension. Salt-induced increases in renal sympathetic activity have been shown to induce sodium retention. However, the mechanism underlying the sympathetic control of renal sodium excretion remains unclear. We have reported that β_2 adrenergic receptor (β_2AR) stimulation down-regulated WNK4 transcription (to less than 50%) through epigenetic modulation (Mu S. et al. Nat Med 2011).

Methods: We injected Isoproterenol (ISO) to wild type mice by subcutaneous mini pumps. WNK4 mRNA expression were measured by Realtime-RT-PCR. Then we use the HAT p300 inhibitor curcumin (Cur) to see if reverse of histone acetylation could adjust the WNK4 expression and prevent the sympathetic activity induced salt-sensitive hypertension. Blood pressure were measured by radio-telemetery system.

Results: In former study, we revealed that β_2-AR stimulation suppressed the activity of histone-deacetylase 8 (HDAC8) and recruit glucocorticoid receptor (GR) binding to the negative GR responsive element (nGRE) in WNK4 promoter region, and lead to the transcriptional inhibition of WNK4 gene. And in vivo model, we clarified that suppressed WNK4 leads to activation of the Na^+-Cl^- co-transporter (NCC), which led to the development of salt-induced hypertension (SBP 180mmHg ±10mmHg). In the present study, based upon our finding, we investigated possible therapeutic role of the inhibition of histone acetylase (HAT) in salt-sensitivity by modulating histone acetylation. In isoproterenol infusion mice model, high-salt loading developed obvious salt-sensitive hypertension, as same in our former report, however, the treatment of p300 inhibitor-curcumin to these mice recovered the WNK4 expression in kidney and prevented the development of salt-sensitive hypertension.

Conclusions: Our results illustrate a novel role for another epigenetic modulation – HAT inhibition in the development of salt-induced hypertension and we provided an alternative therapeutic target of salt-sensitive hypertension.

TH-PO752

Renal Afferent Nerves: Key Element of a Tonic Sympatho-Inhibitory System? Tilmann Ditting, Wolfgang Freisinger, Sonja Heinlein, Kirsten Siegel, Christian Fiedler, Roland E. Schmieder, Karl F. Hilgers, Roland Veelken. *Dept. of Nephrology & Hypertension, University of Erlangen Nürnberg, Erlangen, Germany.*

Background: Renal innervation consists of sympathetic efferent and peptidergic afferent nerves. The latter express TRPV1-receptors. We recently found neurophysiological evidence for a functionally relevant intrarenal peptidergic innervation, exhibiting sympatho-inhibitory effects. Hence, we now tested the hypothesis that stimulation of renal afferent nerve activity (ARNA) with the TRPV1-agonist capsaicin inhibits efferent renal sympathetic nerve activity (RSNA).

Methods: Eight methohexital anesthetized male Sprague-Dawley rats were instrumented as follows: arterial and venous catheters for recording of blood pressure (BP) and heart rate (HR) and drug administration; left sided renal arterial catheter for selective intrarenal administration (IRA) of the TRPV1 agonist capsaicin (CAP 3.3, 6.6, and 10*10-7M; 10µl; after 15, 30, 45, and 60 minutes) to stimulate ARNA; right sided bipolar stainless steel electrode for continuous RSNA recording; Before and after IRA CAP increasing intravenous (IV) doses of the NK1-receptor blocker RP67580 was given. The NK2- and CGRP blockers MEN10376 and CGRP8-37 where also tested.

Results: IRA CAP decreased integrated RSNA from 65.4±13.0 µV*sec (baseline) to 12.8±3.2 µV*sec (minimum), $P<0.001$. This sustained RSNA inhibition reached its minimum within 70 minutes, and was not directly linked to the transient electrical ARNA response which is usually seen with IRA CAP. Suppressed RSNA was transiently but completely unmasked by systemic (IV) administration of the NK1-blocker (maximum 120.3±19.4 µV*sec; $P<0.001$). Furthermore NK1-blockade transiently increased baseline RSNA to similar levels in dose dependent manner. NK2- and CGRP blockers had no effect.

Conclusions: Our study provides direct evidence that the afferent renal nerves provide a tonically acting sympatho-inhibitory system which seems to be rather mediated by neurokinin release acting via NK1-receptor pathways, than by electrical ARNA effects on central sympathetic outflow. However, the exact site of NK1-mediated action (central, ganglionic, or other) remains to be further elucidated.

Funding: Government Support - Non-U.S.

TH-PO753

The Pivotal Role of Midkine on the Development of Hypertension in Endothelial Dysfunction Yuka Sato, Waichi Sato, Tomoki Kosugi, Hiroshi Kojima, Kayaho Maeda, Hiroshi Nagaya, Mayuko Hori, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

Background: The pathophysiology of hypertension includes the complex interaction of vascular effectors such as renin-angiotensin system (RAS), oxidative stress, nitric oxide (NO). The growth factor midkine (MK) is a multi-functional heparin-binding protein, and its biological activity affects in the progress of ischemic renal injury, diabetes and hypertension. We have previously demonstrated that MK induction by oxidative stress regulated the RAS through lung angiotensin converting enzyme (ACE), eventually leading to systemic hypertension and renal dysfunction. However, the regulatory mechanism of hypertension remains unclear. NO regulates intraglomerular pressure, and its reduction causes cadiovascular disease and chronic kidney disease. In the present study, we investigated the role of MK in the development of hypertension in endothelial dysfunction by NO reduction.

Methods: Wild-type mice ($Mdk^{+/+}$) and MK-deficient mice ($Mdk^{-/-}$) are treated with unilateral nephrectomy (UNx) and NO synthase inhibitor, L-NAME administration in a drinking water. They were measured systolic blood pressure (BP) and albuminuria at 1, 2 and 4 months, and sacrificed on 4 months for immunohistochemical and biochemical analyses.

Results: Systolic BP of both $Mdk^{+/+}$ and $Mdk^{-/-}$ were elevated by UNx compared to non-treatment, whereas $Mdk^{+/+}$ with UNx+L-NAME developed marked hypertension compared to $Mdk^{+/+}$ with UNx. Remarkably, $Mdk^{-/-}$ with UNx+L-NAME exhibited normotension. Proteinuria and glomerular sclerosis were also prominent in $Mdk^{+/+}$ mice with UNx+L-NAME. Lowering BP using hydralazine significantly blocked the development of proteinuria and glomerlar sclerosis. However, lung and serum ACE activities were not increased in both groups. Aorta relaxation test by magnus apparatus revealed that MK is markedly associated with endothelial- dependent relaxation.

Conclusions: These data suggest that in endothelial dysfunction, glomerular injury with proteinuria depends on hypertension whereas hypertension by NO blockade might involve MK-NO-cGMP pathway, but not the activation of RAS.

TH-PO754

Genetically Determined Low Nephron Number Is Associated with Hypertension Due to Chloride, but Not Sodium Retention Kerstin Benz,[1] Julia Schlote,[2] Nada Cordasic,[3] Christoph Daniel,[2] Christoph Kopp,[3] Andrea Hartner,[2] Karl F. Hilgers,[3] Jens Titze,[3] Kerstin U. Amann.[2] *[1]Pediatrics, University of Erlangen-Nürnberg, Erlangen, Germany, Erlangen, Germany; [2]Pathology, University of Erlangen- Nürnberg, Erlangen, Germany; [3]Nephrology and Hypertension, University of Erlangen- Nürnberg, Erlangen, Germany.*

Background: An association between low nephron number and development of hypertension in later life has been demonstrated. The underlying pathomechanisms are unknown. Reduced renal Na^+ and water clearance is regarded as one option. We tested the hypothesis that GDNF heterozygous (GDNF+/-) with 30% reduction of nephron number have increased tissue electrolyte and water content compared to wildtype (wt) mice.

Methods: 34 GDNF+/- and 35 wt mice (45-55 wks) received low salt (LSD,0.03%,tap water) or high salt (HSD,4% NaCl,0.9% saline) diet for 4 weeks. Blood pressure (bp) was continuously measured by telemetry (n=4 per group). At the end of the experiment, blood samples were taken, and tissue Na^+,K^+,Cl^- and water content in skin, bone, and total body weight were determined by chemical analysis.

Results: Body weight, dry weight, total body water content, relative skin and body Na^+ and K^+ content were not significantly different between the groups, and no differences in serum concentrations were found. In contrast and independent of the diet, skin and total body Cl^- content of the skin was higher in GDNF+/- than wt mice, resulting in an increased Cl^-space in GDNF+/- mice which is a surrogate parameter for extracellular volume retention. Additionally, GDNF+/- mice under HSD showed significantly higher daily urine excretion and albuminuria compared to wt mice with HSD. In parallel with tissue Cl^- accumulation, bp was higher in GDNF+/- than in wt mice fed LSD. HSD resulted in a more pronounced bp increase in GDNF+/- mice compared to wt mice.

Conclusions: We found that tissue Cl^- accumulation, but no differences in Na^+ and water balance, parallel the development of hypertension in GDNF+/- mice. These findings suggest that reduced renal Cl^- clearance parallels hypertension in mice with reduced nephron number.

TH-PO755

Neutrophil Gelatinase-Associated Lipocalin, a Novel Mineralocorticoid Biotarget in the Cardiovascular System Celine Latouche,[1] Soumaya El Moghrabi,[1] Ivan Hernandez,[2] Diego Alvarez de la Rosa,[2] Natalia Lopez-Andres,[4] Patrick Rossignol,[3] Faiez Zannad,[3] Nicolette E. Farman,[1] Frederic Jaisser.[1] *[1]U872 team 1, INSERM, Paris, France; [2]Department of Physiology and Institute of Biomedical Technologies, University of La Laguna, Tenerife, Spain; [3]Centre d'Investigations Cliniques- 9501, Nancy-Université, France, Nancy, France; [4]U961, INSERM, Nancy, France.*

Background: Mineralocorticoid receptor (MR) activation is deleterious to the cardiovascular system and MR antagonists improve morbidity and mortality of patients with heart failure. However mineralocorticoid signaling in the heart remains largely unkown.

Methods: we used genetically modified mouse models, pharmacological mineralocorticoid stress in rodents and cultured cells to address the implication of Neutrophil Gelatinase-Associated Lipocalin (NGAL also known as Lipocalin2)NGAL as a biomarker of MR activation in the cardiovascular system.

Results: Using a transcriptomic approach, we identified NGAL as the highest upregulated gene in the heart of transgenic mice with conditional MR overexpression targeted to the cardiomyocytes. We show that NGAL expression is directly controlled by mineralocorticoid activation in the cardiovascular system. In addition, using NGAL knock-out mice, we show that NGAL is crucial for the occurrence of extracellular matrix remodeling induced by mineralocorticoid excess in the heart. Moreover, in asymptomatic obese subjects prone to develop heart failure, circulating levels of NGAL-MMP9 complexes are correlated with plasma aldosterone and with serum levels of biomarkers of cardiovascular fibrosis.

Conclusions: CONCLUSION: we suggest that NGAL is potentially a novel MR biotarget and may provide a link between mineralocorticoid stress and cardiovascular remodeling in humans. Whether NGAL is also a biotarget of aldosterone/MR in the kidney is currently under investigation.

Funding: Government Support - Non-U.S.

TH-PO756

SIRT1 Activation Protects the Endothelial Dysfunction by Inhibiting E-Selectin and VCAM-1 Generation Hideyuki Negoro,[1] Atsuo Goto.[2] *[1]Medicine, Harvard Medical School, Boston, MA; [2]Nephrology, Japanese Red Cross Medical Center Hospital, Tokyo, Japan.*

Background: SIRT1 is a conserved NAD(+)-dependent deacetylase and possesses beneficial effects against aging-related diseases, but little information is available on a putative role of SIRT1 in endothelial and vascular homeostasis. Endothelial senescence causes endothelial dysfunction to promote atherosclerotic change and contribute to age-related vascular diseases. Cellular adhesion molecules, such as endothelial-leukocyte adhesion molecule-1 (E-selectin) and vascular cell adhesion molecule-1 (VCAM-1) play an important part in the progression of age-related vascular diseases.

Methods: We established an in vitro senescence model by prolonged culture of primary endothelial cells isolated from bovine aorta. The freshly isolated young endothelial cells gradually underwent senescence during 1 month of repetitive passages. We knocked down SIRT1 to evaluate the protein levels of LKB1, phosphorylated AMPK, E-selectin and VCAM-1 in the knocked down cells.

Results: It was observed that protein expressions of SIRT1 were decreased time dependently in the senescent endothelial cells. In contrast, the protein levels of LKB1, a serine/threonine kinase, the phosphorylation of its downstream target AMP-activated protein kinase (AMPK), E-selectin and VCAM-1 generation were dramatically increased in the senescent cells. On the other hand, resveratrol activated SIRT1 in the endothelial cells. SIRT1 activation with resveratrol inhibited the increase of LKB1, AMPK. At the same time, SIRT1 activation with resveratrol reduced E-selectin and VCAM-1 generation in the endothelial cells significantly. We knocked down SIRT1 and the protein levels of LKB1, phosphorylated AMPK, E-selectin and VCAM-1 elevated in the knocked down cells. The protein levels of LKB1, phosphorylated AMPK, E-selectin and VCAM-1 generation did not changed in the SIRT1 knocked down cells even if they were stimulated with resveratrol.

Conclusions: These findings indicate that activation of SIRT1 provides beneficial effects against the endothelial dysfunction to promote atherogenesis by inhibiting E-selectin and VCAM-1 generation.

Funding: Government Support - Non-U.S.

TH-PO757

AT1 Antagonism and Renin Inhibition in Mice: Pivotal Role of Targeting Angiotensin II for Nephroprotection Christian Krebs,[1] Christoph Fraune,[1] Alexander H. Danser,[2] Harry Van Goor,[3] Edzard Schwedhelm,[1] Rolf A. Stahl,[1] Ulrich Wenzel.[1] *[1]Medicine, University of Hamburg, Germany; [2]Division of Vascular Medicine and Pharmacology, Erasmus MC, Rotterdam, Netherlands; [3]Pathology and Medical Biology, University Medical Centre Groningen, Netherlands.*

Background: The role of the renin-angiotensin system in hypertensive renal injury involves multiple peptides and receptors. Exerting antipodal pathophysiological mechanisms, renin inhibition and blockade of the Ang II type 1 (AT1) receptor ameliorate renal damage.

Methods: We compared the direct renin inhibitor aliskiren with the AT1 antagonist losartan in mice of the FVB strain with chronic kidney disease (CKD) due to renal ablation. Blockade of the RAS increases renin due to inhibition of the Ang II mediated negative feedback loop. Thus renin expression was used to quantify the extent of RAS blockade. Doses were adjusted to equipotent and maximal inhibition of the RAS.

Results: 6-week treatment with either 500 mg/l drinking water losartan or 50 mg/kg body weight/day aliskiren significantly decreased albuminuria, NGAL excretion, glomerular damage, and transcription rates of markers of fibrosis like PAI-1 and fibronectin to a similar extent. An array analysis comparing renal gene expression of losartan and aliskiren treated mice evaluating >34,000 transcripts demonstrated regulation for 14 genes only, with maximal difference of a signal-log ratio of 2. No superior nehroprotection was found by combining losartan and aliskiren compared to monotherapies. Compared to plasma concentrations, aliskiren accumulated ~7 to 29-fold in heart, liver, lung, and spleen and ~156-fold in the kidney. After withdrawal, plasma concentrations dropped to zero within 24 hrs, whereas renal tissue concentrations declined slowly over several days. Withdrawal of aliskiren in mice with CKD revealed a significantly delayed re-increase in albuminuria compared to withdrawal of losartan.

Conclusions: This study demonstrates equieffective nephroprotection of renin inhibition and AT1 antagonism in mice with CKD without additional benefit of combination therapy. However, aliskiren offers advantages derived by its pharmacokinetics.

Funding: Government Support - Non-U.S.

TH-PO758

Renoprotective Effect of Rosuvastatin in Salt-Sensitive Hypertensive Rats Sun Moon Kim,[1] Seung Hee Yang,[2] Yon Su Kim.[2] *[1]Internal Medicine, Chungbuk National University Hospital, Cheongju, Republic of Korea; [2]Kidney Research Institute, Seoul National University, Seoul, Republic of Korea.*

Background: Hypertension is a significant risk factor for the development and progression of chronic kidney disease. Although recent clinical studies suggested that statins might have pleiotropic effects upon cardiovascular disease, the evidences regarding the benefit of statins in renal injury was not clear. We investigated whether statin treatment would attenuate renal damage in Dahl salt-sensitive (DS) hypertensive rat models which develop hypertension by high salt diet (8.0% NaCl).

Methods: DS rats were fed with high salt diet from 7 weeks and rosuvastatin (20mg/kg/day by gavage, n=6) or vehicle (n=6) was administered from 13 to 21 weeks of age. Body weight, blood pressure, urine protein and creatinine, serum BUN, creatinine, and cholesterol were measured. Glomerulosclerosis index (GSI), tubular lesion index (TLI) and expression of TGF- β1 and ET-1 were assessed from kidney tissue.

Results: DS rat given a high salt diet developed hypertension (systolic blood pressure, 216 ± 42 mmHg) (compared to rat given in normal salt diet; 150 ± 8 mmHg) and showed azotemia and proteinuria. Between rosuvastatin-treated group and vehicle treated group, rosuvastatin treatment had little effect on the blood pressure and the serum level of total, HDL-, LDL-cholesterol, urinary protein/urinary creatinine or serum creatinine compared to vehicle treatment. However, it significantly decreased blood urea nitrogen level (18.0 ± 2.7 in rosuvastatin-treated group vs. 30.5 ± 8.5 mg/dL in vehicle-treated group, p = 0.014) and protected kidney injury assessed by renal histology index (GSI, 1.13 ± 0.27 in rosuvastatin-treated group vs. 1.57 ± 0.33 in vehicle-treated group, p = 0.048; TLI, 0.67 ± 0.08 in rosuvastatin-treated group vs. 1.05 ± 0.15 in vehicle-treated group, p = 0.001). Immunohistochemical analysis showed decreased level of TGF-β1 expression in rosuvastatin-treated rats compared to vehicle-treated rats whereas it showed no difference in the level of ET-1 expression.

Conclusions: Our results suggest that rosuvastatin mitigate hypertensive renal injury independent from lipid lowering effect and its effect may involve TGF- β1.

TH-PO759

Pentoxifylline Inhibits Angiotensin II-Stimulated Proliferation of Rat Vascular Smooth Muscle Cells Sang Youb Han,[1] Kum Hyun Han,[1] Young Sun Kang,[2] Dae R. Cha.[2] *[1]Internal Medicine, Inje-Univ. Ilsan-Paik Hosp., Goyang; [2]Internal Medicine, Korea Univ. Ansan Hosp., Ansan, Republic of Korea.*

Background: Pentoxifylline (PTX) is a non-selective inhibitor of cyclic-3', 5'-phosphodiesterase, which raises intracellular cAMP. PTX has been known to have an effect against cell proliferation, inflammation and fibrosis in some experiments. However, little is known about whether PTX can prevent Ang II-induced proliferation of vascular smooth muscle cell (VSMC) which is an important role in hypertensive vascular damage. To evaluate this, we tested anti-proliferative effect and cell cycle regulation of PTX in the Ang II-stimulated VSMC.

Methods: Rat VSMCs were isolated from the thoracic aortas of male Sprague-Dawley rats. The VSMCs were treated with 1 uM of Ang II and various doses of PTX. The Proliferation was determined by the MTT assay. Cyclic 3',5' –AMP was measured by the cyclic AMP EIA kit. The expressions of mRNA and protein were analyzed using real-time PCR and Western blotting.

Results: 1. Ang II significantly proliferated VSMC (Control: 0.29±0.02 vs. Ang II: 0.40±0.05, p<0.01). PTX dose-dependently suppressed Ang II-induced cell proliferation (PTX 0.1mM: 0.32 ± 0.05; PTX 0.5mM: 0.29 ± 0.03; PTX 1mM: 0.26 ± 0.04; PTX 2mM: 0.25 ± 0.03, p <0.05).

2. Ang II inhibited cAMP generation (Control: 34.62 ± 0.59 vs. Ang II: 17.49 ± 3.30, p <0.05). PTX significantly restored cAMP level in Ang II-stimulated cells (PTX 1mM: 40.68 ± 0.49, PTX 2mM: 41.50 ± 1.78, p<0.05).

3. Ang II significantly upregulated mRNA and protein expression of cyclin D1 by 2 folds, and downregulated mRNA and protein expression of p21 by half. There were no changes of cyclin E, cyclin A, CDK2 and CDK4, and P27. PTX pretreatment prevented the changes of cyclin D1 and p21.

Conclusions: PTX attenuated proliferation in Ang II-stimulated VSMCs. The anti-proliferative effect of PTX was related to the increment of cAMP and partial regulation of cell cycle. Further study will be required to elucidate the role of PTX on hypertensive vessel wall.

Funding: Government Support - Non-U.S.

TH-PO760

Vitronectin-Binding PAI-1 Protects Against Cardiac Fibrosis through Interactions with Fibroblasts Jianyong Zhong,[1] Haichun Yang,[2] Valentina Kon,[1] Iekuni Ichikawa,[1] Agnes B. Fogo,[2] Ji Ma.[1] *[1]Pediatrics, Vanderbilt University; [2]Pathology, Vanderbilt University.*

Background: Plasminogen activator inhibitor-1 (PAI-1) is actively involved in fibrotic processes of multiple organs, with the net effects dependent on the tissue and the cellular composition. Our previous *in vivo* studies indicated that, while PAI-1 deletion or the protease inhibitory PAI-1 variant increased profibrotic responses in the mouse heart exposed to chronic angiotensin II (AII) infusion, the vitronectin (Vn)-binding PAI-1 variant rather decreased expression of cardiac myofibroblast markers. The aim of the present study was to elucidate how Vn-binding and protease inhibitory PAI-1 differentially impact cardiac fibroblast functions.

Methods: Cardiac fibroblasts were stimulated with AII for 48 hours. One of the human PAI-1 variants including, PAI-1AK (AK, retaining protease inhibitory effects, but not binding to Vn), PAI-1RR (RR, binding Vn normally, but no protease inhibitory activity), control PAI-1 (CPAI, retaining all known functions of native PAI-1) was added simultaneously.

Results: Supernatant plasminogen activator and plasmin activities were significantly decreased by AK and CPAI, variants that inhibit protease. These changes were associated with significantly increased supernatant fibronectin (CPAI 1253.4±96.7 and AK 1264.7±2.1 ng/$\times 10^4$ cells *vs.* AII 852.9±45.7 and non-AII control 696.9±78.2 ng/$\times 10^4$ cells; $p<0.05$, respectively). RR did not increase supernatant fibronectin (857.6±47.9 ng/$\times 10^4$ cells). Compared to the AK, cells treated with RR had more TUNEL-positive apoptosis (RR 3.13±0.49% *vs.* AK 1.70±0.35%, $p<0.05$), while there was no difference in cell proliferation among the groups. Among the PAI-1 variants, AK, the non-Vn-binding variant, significantly up-regulated the expression of integrin β3 subunit (AK 0.55±0.02 *vs.* RR 0.36±0.04 and CPAI 0.41±0.03, $p<0.05$).

Conclusions: Protease inhibitory PAI-1 promotes extracellular matrix accumulation, while PAI-1, through its Vn-binding pathway, down-regulates integrin β3 and promotes apoptosis in cardiac fibroblasts. We speculate that preserving the Vn-binding ability of PAI-1 may protect against cardiac fibrosis.

Funding: NIDDK Support

TH-PO761

Overexpression of Na+, K+-ATPase and Sodium Sensitive Hypertension Induced by Ovariectomy in Adult Rats Fernando Raul Ibarra,[1] Luis A. Di Ciano,[1] Elisabet Monica Oddo,[1] Pablo J. Azurmendi,[1] Jorge Toledo,[1] Elsa Zotta,[2] Federico Ochoa,[2] Elvira Arrizurieta.[1] *[1]Instituto A Lanari; [2]Faculty of Medicine, Buenos Aires University.*

Background: We have shown that ovariectomy in adult Wistar rats caused an activation of renal kallikrein-kinin system and a decrease in mean blood pressure (MBP) (Kidney Blood Press Res 2009). Now we tested whether Wistar rats ovariectomized (OVX) when 60 days old and studied at 150 days of life could have changes in Na^+ excretion (ENa^+) and in systemic hemodynamic.

Methods: To this purpose, intact (I) and OVX rats consuming either normal sodium diet (NS, 0.24% NaCl) or high sodium (HS, 1% NaCl, in drinking water) for 5 days were studied.

The expression of total Na^+, K^+-ATPase (NKA) alpha1 subunit and its phospho state at Ser 23 (PKC site) was determined by western blot in renal homogenates. Current concepts agree that when dephosphorylated renal NKA is more active. Beta actin served as control. Immunohistochemistry was employed to localise tissue NKA. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by clearance techniques.

Results: On NS diet, expression of total and dephosphorylated NKA was higher in renal medulla from OVX by 10 ± 1% (p <0.05 vs I). The increment was localised in the outer medulla of OVX rats. Under a NS diet, ENa^+, diuresis and MBP did not differ between I and OVX rats, while OVX rats showed a trend to increase RPF (I 3.63±0.29 vs OVX 4.81±0.39 ml/min/g kwt).

When exposed to a HS diet, NKA alpha1 subunit Ser 23 dephospho state decreased in I and OVX rats. But, in OVX rats, Ser 23 remained markedly more dephosphorylated than in I rats (50% higher in renal cortex and medulla, p<0.01, both). Under a HS diet, ENa^+ (mEq/g kwt /day)was lower in OVX than in I rats (3.9±0.1 vs 4.6±0.3, p<0.05) and MBP significantly increased in OVX to 135±9 vs 116±7 mmHg in I rats, p<0.05. Diuresis, GFR and RPF were not different between I and OVX rats under HS diet.

Conclusions: Ovariectomized adult rats have a higher total and dephosphorylated Na^+, K^+-ATPase alpha1 subunit and a lower sodium excretion under HS diet than I rats. Both, together, may contribute to develop sodium sensitive hypertension in rats deprived of ovarian hormones.

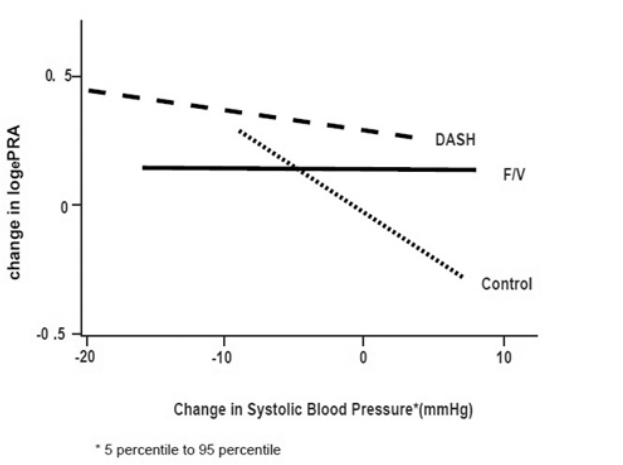
TH-PO762

The Effects of Dietary Patterns on Plasma Renin Activity: Results from the Dietary Approaches To Stop Hypertension (DASH) Trial Qi Chen,[1] Sharon Turban,[2] Edgar R. Miller,[1,3] Lawrence J. Appel.[1,3] *[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD; [3]Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, MD.*

Background: A diet rich in fruits, vegetables, and low-fat dairy products and reduced in saturated fat, total fat, and cholesterol (termed the "DASH" diet) significantly lowers blood pressure (BP). Previous studies have documented that certain therapies that lower BP increase plasma renin activity (PRA).

Methods: Using data from the DASH trial, we assessed the effects of dietary patterns on PRA and determined the relationship of change in PRA with change in BP on each diet. After eating a control diet for three weeks, participants were then randomized to receive for eight weeks: the control diet, a diet rich in fruits and vegetables (F/V), or the DASH diet. Baseline and follow-up levels of PRA were available in 381 participants.

Results: Compared to the control diet, the DASH diet increased PRA by 0.37 ng/mL/h (P=0.01). In multivariable linear regression analyses, there was an inverse association of PRA change with systolic BP change on the control diet (slope=-0.35, p=0.001), but PRA did not differ by BP change on the F/V (slope=-0.002, p=0.98) or DASH diet (slope=-0.08, p=0.32).



Conclusions: These data suggest that a blunted counter-regulatory response of the renin-angiotensin system is associated with the BP lowering effect of the F/V and DASH diets.

Funding: Other NIH Support - National Heart,Lung,and Blood Institute
National center for research resourc

TH-PO763

Dietary Salt and Potassium Intake Is Not Associated with Elevated Blood Pressure Levels in US Adults Shailendra Sharma,[1] Kim McFann,[1] Anna Jeanette Jovanovich,[1] Michel B. Chonchol,[1] Jessica B. Kendrick.[1,2] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Denver Health Medical Center, Denver, CO.*

Background: Current guidelines recommend reducing dietary salt intake and increasing dietary potassium intake to reduce blood pressure (BP) levels. The association of dietary salt and potassium intake with increased risk of BP levels is unclear.

Methods: The cross-sectional association between dietary sodium and potassium intake and BP levels was examined in 6985 adults 18 years of age or older with no prior history of hypertension who participated in the National Health and Nutrition Examination Survey (2001-2006). Dietary sodium and potassium intake was calculated from 24-hour dietary recall obtained by trained interviewers. Three BP measurements were collected from each participant. Multivariate logistic regression analysis was performed to evaluate whether high sodium and low potassium intake is independently associated with higher BP levels.

Results: The mean (SE) age of the participants was 45 (0.4) years. The mean (SE) sodium and potassium intake was 3520 ± 26 and 2761 ± 22 mg/day, respectively. In linear regression models there was no association between higher sodium or lower potassium intake with systolic BP (β = -0.000052 ± 0.00012, p = 0.67 and β = 0.000096 ± 0.00018, p = 0.59, respectively). In logistic regression models after adjustment for age, sex, race, diabetes and eGFR, there was no association between higher quartiles of sodium or lower potassium intake with the risk of a BP cutoff of >140/90 mmHg. We also examined the relationship of combinations of potassium and sodium intake with blood pressure cutoff of >140/90 mmHg. Median intake of potassium and sodium were used to determine high and low potassium and sodium intake. High potassium intake combined with low sodium intake was not protective for BP > 140/90 mmHg (p=0.13). Furthermore, high sodium intake combined with low potassium intake was not associated with an increased risk of BP > 140/90 mmHg (p=0.95).

Conclusions: In the US adult population without hypertension, increased dietary sodium or low potassium intake was not associated with elevated BP levels.

TH-PO764

Dietary Salt and Protein Intake and Obesity in Ireland Gemma M. Browne,[1] Joseph A. Eustace,[2] Ivan J. Perry.[1] *[1]Department of Epidemiology and Public Health, University College Cork, Cork, Ireland; [2]Department of Nephrology, Cork University Hospital, Cork, Ireland.*

Background: Obesity is linked to CKD through several established mechanisms including hypertension and salt sensitivity. Ongoing dietary intake of sodium and protein may contribute to progressive renal damage and confound above relationships. This study aims to quantify dietary sodium and protein intake in obese compared to non-obese subjects.

Methods: 599 community dwelling subjects including an occupational group and students, aged 18-81, using random and convenience sampling, participated in a cross-sectional study in Munster, Ireland. Subjects underwent blood pressure (BP) and anthropometric measures using a standardized protocol and performed one 24 hour urine collection. 488 subjects had valid analytes including urinary sodium and urinary urea nitrogen, with a complete collection based on *para*-amino benzoic acid appearance. Protein nitrogen appearance (PNA) was calculated (Maroni B, Mitch WE). The relationship of dietary intake with obesity was examined using multivariate logistic regression (STATA V11.0).

Results: Central obesity was significantly associated with dietary salt intake, urinary PNA, hypertension, age and gender (Table 1) compared to non obese subjects. When dietary associations of central obesity were explored in an adjusted model, salt intake (OR 1.13, p<0.001), urinary PNA (OR1.01, p=0.043), age (OR 1.06, p<0.001) and gender (OR 4.23, p<0.001) continued to be independently significant. Similar independent associations were present comparing subjects with BMI>30kg/m^2 compared to non obese subjects.

Dietary Salt Protein Intake, Hypertension & Central Obesity

Gender (N)	Men N=306		Women N=182	
Waist cm %	>102 (21%)	<102	>88 (31%)	<88
Salt gms/day mean(sd)	12.4 (4.7)	9.9 (4.0)	8.1 (3.1)	7.0 (2.7)
Urinary PNA gms/day mean(sd)	217 (52)	192 (53)	149 (40)	132 (40)
% BP >140/90 / Meds	65%	34.8%	66.7%	33.3%

Conclusions: Using validated methods, obesity is independently related to higher protein and salt intake. In addition to salt sensitivity, obese subjects have higher salt intake in association with higher protein intake. Appropriate dietary advice and salt restriction may be especially important in obese hypertensive subjects.

Funding: Government Support - Non-U.S.

TH-PO765

Association of Sweetened Beverage Intake and Incident Hypertension Is Similar between Sugar-Sweetened and Artificially-Sweetened Beverages Lisa J. Cohen, Gary C. Curhan, John P. Forman. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Consumption of sugar-sweetened beverages (SSBs) is associated with hypertension in cross-sectional studies. However, prospective data are limited.

Methods: We performed an analysis of originally non-hypertensive individuals in three large, prospective cohorts, the Nurses' Health Studies I (n=88,540) and II (n=97,991) and the Health Professionals' Follow-Up Study (n=37,360), to determine the relation between SSB and artificially sweetened beverage (ASB) consumption and the development of hypertension over time. Cox proportional hazards regression was used to calculate hazard ratios for incident hypertension based on type and quantity of beverage intake, and after adjusting for potential confounders. The association between fructose consumption from SSBs versus fructose from other sources and the risk of incident hypertension was also determined.

Results: Findings were similar across the cohorts. Higher SSB and ASB intake was associated with an increased risk of developing hypertension. In a pooled analysis of all three cohorts, participants who consumed at least one SSB daily had an adjusted HR for incident hypertension of 1.13 (95% CI, 1.09-1.17) compared with those who did not consume SSBs; for persons who drank at least one ASB daily, the adjusted HR was 1.14 (95% CI, 1.09-1.18). There was a significant interaction between carbonation and total beverage intake (p-interaction < 0.001 in NHS I, 0.03 in NHS II, and 0.02 in HPFS). In an analysis of fructose intake as a percentage of daily calories, higher fructose intake from SSBs was associated with increased hypertension risk in the NHS I and NHS II cohorts (p-trend=0.001 in both groups), while higher fructose intake from sources other than SSBs was associated with a decrease in hypertension risk in NHS II participants (p-trend=0.006).

Conclusions: Both SSBs and ASBs are each independently associated with an increased risk of incident hypertension after controlling for multiple potential confounders. The mechanisms that underlie these associations are unclear.

Funding: NIDDK Support

TH-PO766

Uric Acid Is Associated with Systemic Inflammation and Reduced MnSOD Expression in Endothelial Cells of Healthy Adults Diana I. Jalal,[1] Kristen L. Jablonski,[2] Kim McFann,[1] Michel B. Chonchol,[1] Douglas R. Seals.[2] *[1]Internal Medicine/Renal, University of Colorado Denver, Aurora, CO; [2]Department of Integrative Physiology, University of Colorado, Boulder, CO.*

Background: We previously reported that uric acid levels are not related to endothelial function in healthy adults. Yet, experimental data suggests uric acid may induce endothelial dysfunction and vascular inflammation in some clinical settings. To further understand our results, we explored the relation between uric acid and inflammation and oxidative stress systemically and in endothelial cells collected from study participants.

Methods: We examined the relation between uric acid levels and C-reactive protein (CRP) and oxidized- LDL in all participants (n=107) as continuous variables and according to uric acid quartiles (0-5.2, 5.3-6.1, 6.2-7, > 7.0 mg/dL). To evaluate the relation between uric acid and cellular inflammation and oxidative stress, immunostaining of endothelial cells was compared between the highest and the lowest uric acid quartiles (unpaired t-test with Welch correction). Immunofluorescence was performed on endothelial cells collected from the subjects' brachial arteries. The following markers were evaluated: NFκB p65 (n=19), NADPH oxidase p47^{phox} (n=13), nitrotyrosine (n=21), and MnSOD (n=12). To minimize the confounding effect of different staining sessions, values are reported as ratios of endothelial cell protein/human umbilical vein endothelial cell (HUVEC).

Results: CRP increased significantly with increased uric acid quartiles (P<0.005), and P value for the linear regression was 0.015. There was no correlation between uric acid and oxidized- LDL. The endothelial cells of participants with higher uric acid levels expressed 55% less MnSOD than the participants with the lower uric acid levels (intensity/HUVEC was 0.22±0.13 *vs* 0.49±0.12, P= 0.04). NFκB p56, NADPH oxidase p47^{phox}, and nitrotyrosine did not differ between the highest and the lowest uric acid quartiles.

Conclusions: In healthy adults, serum uric acid levels correlate with increased CRP and associate with reduced MnSOD expression in endothelial cells. These findings may have implications on cardiovascular risk for healthy adults.

Funding: NIDDK Support, Veterans Administration Support

TH-PO767

Combining Uric Acid with Lipoprotein a Could Predict the Atherosclerotic Renal Artery Stenosis in High Risk Patients Peng Xia,[1] Ling Qiu,[2] Limeng Chen,[1] Shuyang Zhang,[3] Xuemei Li,[1] Xuewang Li.[1] *[1]Nephrology Department, Peking Union Medical College Hospital, Beijing, China; [2]Department of Laboratory Medicine, Peking Union Medical College Hospital, Beijing, China; [3]Cardiology Department, Peking Union Medical College Hospital, Beijing, China.*

Background: This study aimed at exploring certain new risk factors of Atherosclerotic renal artery stenosis (ARAS) and establishing a possible tool which might facilitate the clinical decision making in diagnosing ARAS.

Methods: 190 patients highly suspected for ARAS who have received renal artery angiography in Peking Union Medical College Hospital from 2008 to 2011 are selected for analysis. 138 of all 190 patients also received coronary artery angiography and 89 of who

were diagnosed ARAS. The control group is 180 cases who received routine health check. The basic information and lab results such as uric acid (UA), serum lipids (lipoprotein a, total cholesterol, triacylglycerol, HDL and LDL), creatinine (Scr) and hsCRP are collected. Logistic regression analysis is used to identify possible correlations with ARAS and to establish a new tool for predicting ARAS in the high risk population.

Results: The levels of Scr, UA, Lp(a) and hsCRP of ARAS cases are significantly elevated compared to control cases. For high risk population and the patients received coronary artery angiography, there are no significant differences in the levels of Scr, lipids, UA and hsCRP between ARAS cases and non-ARAS cases. Logistic regression analysis shows that uric acid level >344μmol/L correlated to ARAS independently. Using the uric acid level >344μmol/L and lipoprotein a level >242mg/L as a predicting tool for ARAS in high risk population, the specificity is 96.0%, the positive likelihood ratio is 5.45, p=0.001 and the odds ratio is 6.78, 95%CI (1.90, 24.2), p=0.001.

Conclusions: The ARAS cases are experiencing lipids metabolic disorders and inflammatory reactions. In high risk population, the UA might be an independent risk factor of ARAS and combining UA with Lp(a) could predict the ARAS.

Funding: Government Support - Non-U.S.

TH-PO768

Uric Acid Is Not Associated with Blood Pressure in Adolescents with Type 1 Diabetes Jeffrey C. Sirota,[1] Diana I. Jalal,[1] Kim McFann,[1] Franziska K. Bishop,[2] David M. Maahs,[2] R. Paul Wadwa.[2] *[1]Division of Renal Diseases & Hypertension, University of Colorado Denver School of Medicine, Aurora, CO; [2]Barbara Davis Center for Childhood Diabetes, University of Colorado Denver School of Medicine, Aurora, CO.*

Background: Elevated uric acid is associated with increased blood pressure (BP), and treatment of hyperuricemia can improve BP in non-diabetic (non-DM) adolescents. In this study, we hypothesized that uric acid is associated with increased BP in adolescents with type 1 diabetes (T1D).

Methods: Data were collected for 256 youths with T1D (age=15.4±2.2yrs, 50% male, T1D duration 8.7±2.9yrs) and 78 non-DM controls (age=15.5±2.2yrs, 44% male). Cross-sectional association between uric acid and BP was examined by linear regression in unadjusted and adjusted models. Uric acid was log-transformed due to skewed distribution.

Results: T1D youth had higher hemoglobin A1c, waist circumference (WC), BMI-Z, total cholesterol, low density lipoprotein (LDL), & BP (Table 1). Mean uric acid was 4.6±0.8mg/dL in T1D subjects vs. 5.1±1.0mg/dL in controls (p=0.001). In unadjusted analysis, uric acid was significantly correlated with SBP in the entire cohort (R2=0.037, p<0.05), in T1D (R2=0.04, p<0.05), and in non-DM subjects (R2=0.16, p<0.05). After adjusting for age, sex, race, A1c, WC, BMI Z-score, high density lipoprotein (HDL), LDL, triglycerides (TG), creatinine, urinary albumin-creatinine ratio, & smoking status, uric acid was not associated with SBP. There was no correlation between uric acid & DBP.

Table 1

	non-DM (n=78)	T1D (n=256)	p
A1c	5.3±0.3	8.9±1.6	<0.05
WC (cm)	74.6±10.9	77.4±10.6	<0.05
BMI-Z	0.23±1.06	0.62±0.77	<0.01
total cholesterol (mg/dL)	147.2±27.4	158.0±34.7	<0.01
LDL (mg/dL)	81.9±22.4	89.5±27.1	<0.05
HDL (mg/dL)	48.7±9.2	51.2±10.4	0.07
TG (mg/dL)	82.9±40.8	86.3±50.3	0.80
uric acid (mg/dL)	5.1±1.0	4.6±0.8	<0.05
creatinine (mg/dL)	0.7±0.2	0.7±0.1	<0.05
SBP (mmHg)	108.6±8.4	113.2±8.4	<0.05
DBP (mmHg)	64.1±6.0	68.4±6.7	<0.05

Conclusions: Uric acid is weakly associated with SBP in adolescents with and without T1D. However, after adjustment for co-variates, there was no significant association between uric acid and SBP. Further work is needed to clarify the effects of uric acid in young people with T1D.

Funding: NIDDK Support, Private Foundation Support

TH-PO769

Association between Birth Weight and Blood Pressure in 9- to 10-Year Old Children Sandra Dis Steinthorsdottir,[1,2] Sigridur Birna Eliasdottir,[1,2] Olafur S. Indridason,[2] Runolfur Palsson,[1,2] Vidar O. Edvardsson.[1,2] *[1]Landspitali - The National University Hospital of Iceland; [2]University of Iceland.*

Background: Low birth weight has been associated with structural and functional changes in the vasculature, which may increase the risk of hypertension, cardiovascular disease, obesity and type 2 diabetes later in life. The aim of this study was to evaluate the association between birth weight and blood pressure (BP) in a cohort of healthy 9 to 10-year-old Icelandic school children.

Methods: Four seated BP measurements were performed in 1071 Icelandic children, aged 9-10 years. BP percentiles were calculated from an average of the four measurements. Height and weight were measured and information on birth weight was obtained from the Icelandic Birth Registry. Pearson´s correlation coefficient and multivariable linear regression was used for the analysis.

Results: Of 889 children with complete data, 452 were girls (50.8%). The mean BP in girls was 111/63 mm Hg and 112/64 mm Hg in boys (p=0.03). The prevalence of elevated BP was 13.2%. The mean birth weight of boys was 3753 ± 642 g and 3687 ± 588 g for girls (p=0.11). A significant negative correlation between birth weight and systolic BP percentile (r= -0.09, p=0.005) and diastolic BP percentile (r= -0.08, p=0.014) was observed. The relationship was stronger in girls (r= -0.09, p=0.005 vs -0.08, p=0.08). After controlling for body mass index (BMI), there was a significant correlation between birth weight and systolic BP percentile (beta= -0.16, p<0.001) and diastolic BP percentile (beta= -0.14, p=0.003) in girls, and systolic BP percentile (beta= - 0.11, p=0.016) in boys, for whom the association with diastolic BP percentile was of borderline significance (beta= -0.08, p=0.09). There was a direct correlation between birth weight and height (r=0.26, p<0.001), weight (r=0.21, p<0.001), BMI (r=0.14, p<0.001) and BMI percentile (r=0.19, p<0.001).

Conclusions: The results of our study suggest that low birth weight may be an important predictor of hypertension in children. Careful follow-up of BP may be indicated in these children as they may be at increased risk for future cardiovascular complications.

TH-PO770

High Prevalence of Obesity and White-Coat Hypertension in Children Evaluated by Ambulatory Blood Pressure Monitoring (ABPM) Jason M. Kurland,[1] M. Khurram Faizan,[2] Sharon W. Su,[2] Lance D. Dworkin.[1] *[1]Dept. of Medicine, Rhode Island Hospital, Providence, RI; [2]Dept. of Pediatrics, Hasbro Children's Hospital, Providence, RI.*

Background: Pediatric hypertension (HTN) is often secondary (2°) in etiology, as shown by U.S. and European cohorts in the 1980s. ABPM is a useful tool to confirm sustained HTN in children. Prior studies suggest certain ABPM variables- nocturnal dipping and blood pressure (BP) load- may distinguish primary (1°) from 2° HTN. Our study examines the epidemiology of pediatric HTN in an urban referral clinic and seeks ABPM endpoints able to differentiate 1° from 2° HTN in children.

Methods: Retrospective cohort study from 04/1999-09/2010, including ABPM data from all children referred to a pediatric nephrology clinic for evaluation of HTN first seen in a primary-care setting. HTN was defined as mean systolic (SBP) or diastolic BP (DBP) via ABPM as ≥95th percentile for age, sex, and height. All patients were naive to BP medications. 2004 NHBPEP Fourth Report guidelines were used to determine the adequacy of workup for 2° HTN.

Results: 293 patients were included. Mean age was 13.8 years, and 68% were male. Mean BMI was 27.5; 18% were overweight, and 52% obese. 217 patients (74%) were normotensive by ABPM, invoking white-coat HTN. 63 (22%) had 1° HTN, and 13 (4%) had 2° HTN. No significant differences between the 1° HTN and 2° HTN groups were found for the following variables: *(a) age, sex, height, weight, and BMI, (b) mean, SD, and peak for SBP and DBP, (c) nocturnal dip for SBP and DBP, (d) BP load at 90th, 95th, and 99th percentiles, (e) percentage of SBP and DBP values above 95th percentile, and (f) mean, SD, and nocturnal dip for heart rate.*

Conclusions: This study was limited by a small sample size and an unexpectedly low percentage of children with 2° HTN. Differences in ABPM parameters between 1° and 2° HTN may be subtle, requiring larger-scale trials to identify them. Our study demonstrates a strikingly high prevalence of overweight/obesity, which may predispose to 1° HTN. The frequency of white-coat HTN was also markedly higher than that cited from prior studies, reinforcing the value of ABPM as an initial screening tool for suspected HTN in children.

TH-PO771

Long-Term Follow-Up of Children with Essential Hypertension – Are We Minimizing End-Organ Injury? Isabel Roberti, Myriam Jean, Shefali Vyas. *Pediatric Nephrology and Kidney Transplantation, Saint Barnabas Medical Center, Livingston, NJ.*

Background: We have previously shown that essential hypertension (EH) is now the most common cause of HTN in children and it is associated at presentation with obesity in 50 % and end-organ injury in 27% of the cases.

The goal of therapy of children with EH is early lifestyle modification and avoidance of end-organ damage.

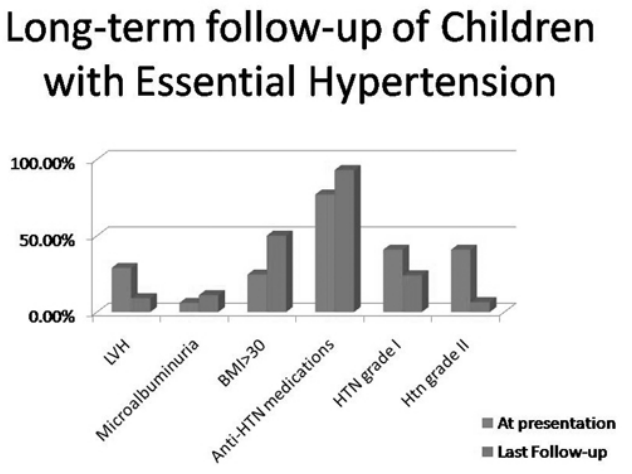
Methods: We reviewed all charts that had the diagnosis of "Essential Hypertension" in children seen at our outpatient office between 2004 and 2010 who had more than 1 year of follow-up. Secondary causes of HTN were excluded in all patients.

We reviewed birth data (gestational age and birth wt), family history (FH), degree of HTN, symptoms, SCr, microalbuminuria (MA), presence of LVH and BMI at presentation and at last follow-up. The impact of routine follow-up and therapy was reviewed including nutritional status change and end-organ involvement. All children received dietary advice regarding salt intake and ideal body weight.

Results: Of the 232 children with EH only 21% (N=49) were followed for at least 1yr (mean f/u = 3.3 yrs).

At presentation: Age: 4-19yrs (mean 13yrs); Ethnicity: 27 AA, 14C, 6 H; 60% male; 76% had + FH for EH; 16% ex-premature/LBW; 49% were symptomatic; 57% had hyperlipidemia.

HTN stages: pre-HTN=9, stage I=20, stage II=20 (41%).



Six of 49 (12%) required more than 1 anti-hypertensive medication. MA was only seen in stage II HTN. Among those with stage I or II HTN 19 (47.5%) were symptomatic. HTN grade significantly declined on f/u: only 3 children had stage II HTN.

Conclusions: The majority of children with EH had significant improvement in the HTN stage and LVH despite worsening BMI. The ability to keep a scheduled appointment and the adherence to life style/dietary changes among our children with EH were dismally poor. More resources are needed to educate these children and families with EH.

TH-PO772

Office Blood Pressure Monitoring (OBPM): A Possible New Tool for Evaluating Blood Pressure in Children and Adolescents Gianluigi Ardissino,[1] Patrizia Salice,[2] Silvia Ghiglia,[2] Pier Paolo Bassareo,[3] Francesca Tel,[1] Sara Testa.[1] *[1]Pediatric Nephrology Unit, Fondazione Ca' Granda Osp Maggiore Policlinico, Milano, Italy; [2]Pediatric Cardiology Unit, Fondazione Ca'Granda Osp Maggiore Policlinico, Milano, Italy; [3]Dpt Scienze Cardiovascolari e Neurologiche, Policlinico Universitario, Cagliari, Italy.*

Background: Recent data suggest that casual office BP measurement (CBP) in children may not be as sensitive as ABPM for detecting hypertensive patients. However ABPM is not feasible in toddlers and it can be difficult and/or misleading in hyperactive children or incompliant adolescents.

Methods: We have developed a method of BP measurement, OBPM: Office BP Measurement, alternative to ABPM for investigating BP in children. The present study compares the results obtained with OBPM and ABPM in 59 children (25F), mean age 11.8 ± 3.5 yrs, referred for suspected or confirmed hypertension to 3 centres.

Results: OBPM utilizes the same recorder and cuff as ABPM (Spacelab 90207) to perform 10 BP measurements in 1 hour just before the ABPM. The readings are introduced in a specific software (developed with FileMaker) that calculates the coefficient of variation (CV) of SBP and DBP, after excluding outlier values (measurements below the 5th and above the 95th of the recorded values). The CV provides an index of reliability of the calculated mean; we suggest to discard OBPMs with a CV >15%. The system finally calculates the mean of the remaining values and the z-score for age, gender and height according to the American Academy Reference values. The correlation between OBPM and ABPM was analyzed as Person's correlation coefficient. The table shows the findings obtained; OBPM and ABPM were performed on the same day.

	OBPM	CV%	24hABPM	r	p
SBP (mmHg)	121.6±14.8	6.3±3.1	120.8±14.3	0.815	<0.0001
DBP (mmHg)	72±11.3	4.4±2.2	71.5 ± 9.4	0.77	<0.0001

Conclusions: Preliminary analysis indicates that OBPM may represent a reliable and promising tool for investigating BP in children as an alternative to ABPM. The software for OBPM is available online at the following website: www.childproject.org

TH-PO773

Assessment of Cardiovascular and Renal Risk Factors in Pediatric Population during World Kidney Day in the Province of Chaco, Argentina Maria Eugenia V. Bianchi,[1] Dario Gomez,[3] Ricardo K. Tannuri,[2] Noelia Alejandra Dellamea,[2] Cecilia Abogado,[2] Ana Cusumano.[4] *[1]Argentina Northeast Kidney Foundation, Resistencia, Chaco, Argentina; [2]National Northeast University, Corrientes, Argentina; [3]Ministry of Health, Resistencia, Chaco, Argentina; [4]CEMIC, Buenos Aires, Argentina.*

Background: Chaco province, Argentina, has one of the highest rates in the nation of teenage pregnancy, low birth weight and infant mortality, with an ethnically diverse population of aboriginal and no aboriginal. Since 2008 and during the WKD celebration, anthropometric measurements and blood pressure (BP) were checked in children. Non aboriginal children were evaluated in the squares of the city and aboriginal ones in a domestic setting. Those with abnormal parameters were referred to medical assistance.

Methods: 18 students of the School of Medicine of the National Northeast University, were trained. Cuffs of different sizes were used with an anaeroid sphigmomanometer to measure BP. Data was analized following the WHO (2006) classification.

Results: 317 children were evaluated. 136 (42.9%) were male, mean age 9.4 yold, mode 13, ranging from 1 to 18 years. 127(47,7%) showed normal BP; 57 (21,4%) High Normal, 57 (21,4%) HT Stage 1, and 25 (9,4%) HT Stage II. The nutritional status of 266 (84%) showed: undernutrition in 13 (4,9%); 26 (9,8%) at risk of undernutrition, 123 (46,2%) normal, 59 (22,2%) at risk of overweight and 45 (16,9 %) with overweight.

Statiscally significant differences between non-Aboriginal and aboriginal children were found: Height: 37.3% of non-Aboriginal were above the 90 percentile, compared with 17.8% of Aboriginal ones.(p <0,005); normal weight was found in 29% versus 60.3%; overweight was detected mainly in the non-Aboriginal (27.4% versus 6.9%)

Conclusions: Cardiovascular and renal risk factors in pediatric population showed a high prevalence and a different pattern in the ethnic frame.

TH-PO774

The Beneficial Effect of Native Nephrectomy on Post-Transplant Hypertension Carrie Diamond, Kathleen M. Waybill, Halie Cook, Harold Yang. *Transplantation, PinnacleHealth, Harrisburg, PA.*

Background: Post-transplant hypertension occurs in >60% of renal transplant recipients, and is associated with cardiovascular complications and decreased allograft survival. While multiple factors may play a role (e.g. immunosuppressive medications, weight gain) the presence of atrophic native kidneys may be a significant contributing factor.

Methods: We performed a retrospective analysis of 16 renal transplant patients (ages 27-72) who underwent a second unilateral native nephrectomy(UNN2)[9 open/7 laparoscopic]. All patients had undergone a prior unilateral native nephrectomy. Five patients underwent UNN2 for severe hypertension; eleven patients required UNN2 for renal masses, polycystic kidney disease or urinary tract infections. We evaluated blood pressure, weight, creatinine, hematocrit, complications, number/dosages of antihypertensive medications at baseline, post-operative (2-6 weeks), and one year. Changes from baseline were evaluated with paired t-tests.

Results:

n=16	Baseline-Mean(SEM)	Post-Op-Mean(SEM)	One Year-Mean (SEM)
SBP(mmHg)	140.4(5.25)	123.6(4.62)*	128.3(4.15)*
DBP(mmHg)	83.1(3.15)	78.2(3.31)	76.7(3.48)*
MAP(mmHg)	102.2(3.24)	93.3(3.47)*	93.9(3.37)*
Weight(kg)	83.5(5.00)	82.0(5.11)	84.6(5.07)
Cr(mg/dl)	1.51(0.10)	1.38(0.10)	1.39(0.11)
Hct	38.7(1.17)	37.9(1.22)	40.7(1.03)
# Meds	3.2(0.37)	2.5(0.35)*	2.5(0.34)*

*p<0.05

In addition to significant reductions in number of medications, 12/16 patients had a >50% dosage reduction of at least one medication. Surgery was well tolerated, with mean length of stay: 5.9±1.63 days after open UNN2 and 2.7±0.29 days after laparoscopic UNN2. One complication occurred (wound infection) in a patient who had undergone open nephrectomy.

Conclusions: Current management of post-transplant hypertension focuses on use of antihypertensive medications. We have previously demonstrated sustained decreases in blood pressure and antihypertensive medications in post-transplant patients who had undergone simultaneous bilateral native nephrectomy. Coupled with similar beneficial results noted in this study,we conclude that surgical removal of native kidneys represents a safe and effective adjunct to medical therapy in the management of post-transplant hypertension.

TH-PO775

Blood Pressure Reduction According to Baseline Systolic BP Value and Diabetes Status in Hypertensive Outpatients: Interim Results of the Canadian ANCHOR Registry Paul Rene De Cotret,[1] Andrew W. Steele,[2] Martine Hubert.[3] *[1]Service de Néphrologie, Centre Hospitalier Universitaire de Quebec, QC, Canada; [2]Lakeridge Health Corporation, Oshawa, ON, Canada; [3]Novartis Pharmaceuticals Canada, Dorval, Canada.*

Background: ANCHOR (AliskireN Canadian HypertensiOn Registry) is a real-life Canadian non-interventional registry of outpatients in which physicians initiated aliskiren, the direct renin inhibitor as monotherapy or add-on, in order to achieve blood pressure control.

Methods: Patients were followed for one year after aliskiren initiation, with 2 visits at 3 months and 12 months. Efficacy and safety were assessed at each visit. An interim analysis was performed at mid-term. The main objective was to assess BP reductions after 3 months of use. One of the secondary objectives, presented in this abstract, was to measure BP changes from baseline to 12 months.

Results: At time of database lock (Aug 2010) for this interim analysis, 9716 patients were enrolled in the study; mean baseline characteristics (± SD) were: 52.1% males, age was 62.6 (12.9), 36.7% had diabetes, BMI was 31.5 (14.8), 76.4% were Caucasians, SBP (mmHg (± SD): 153.6 (15.9) and DBP: 87.6 (11.5). Baseline SBP in 6131 patients without diabetes was 154.7 (15.8) and in 3542 patients with diabetes 151.7 (16.0). At 12 months: mean (mmHg ± SD) systolic BP drop was -18.3 (16.8) in 576 patients with diabetes and -20.5 (18.4) in 830 patients without diabetes.

Table 1 below shows the mean SBP drop (in mmHg) at 12 months with an aliskiren-

based anti-hypertensive therapy according to baseline BP value and diabetes status.

Baseline SBP	N with No Diabetes	Mean SBPdrop	N with Diabetes	Mean SBPdrop
130-139	39	-5.8*	75	-8.9
140-150	213	-14.0	153	-13.2
151-160	250	-19.2	144	-19.1
161-170	166	-26.4	90	-26.0
171-180	75	-32.1	44	-30.3
>180	50	-46.4	33	-41.5

All p values for BP drop were <0.0001 except for *: p<0.004

Conclusions: In this registry, the mean SBP drop with an aliskiren-based anti-hypertensive therapy was comparable in 9716 patients with or without diabetes. In both subgroups the mean BP drop was the highest (> - 41 mmHg) in patients the highest baseline SBP (>180 mmHg). The treatment was generally well tolerated.

Funding: Pharmaceutical Company Support

TH-PO776

WKD 2011: Cardiovascular and Renal Risk Factors Profile in the General Population in Resistencia, Chaco, Argentina Maria Eugenia V. Bianchi,[2] Shyrley Lorena Basila Cosimi,[1] Facundo Manuel Ferrarini,[1] Dario Gomez,[4] Gabriela Mariel Audisio,[1] Ana M. Cusumano.[3] *[1]School of Medicine, Northeastern National University, Corrientes, Argentina; [2]Argentine Northeastern Kidney Foundation, Resistencia, Chaco, Argentina; [3]CEMIC, Buenos Aires, Argentina; [4]Ministry of Health, Resistencia, Chaco, Argentina.*

Background: Resistencia is the capital of the province of Chaco, Argentina, with an ethnically diverse population that is almost 600 thousand persons, and very few resources to conduct screening programs.

Methods: Last WKD, 431 students from the School of Medicine of Northeast National University were capacitated to obtain anthropometric measurements, glycaemia and blood pressure (BP) control to the citizens in five squares of the city during 8 hours of work. The results were given to the people and those with Stage two of Hypertension were referred to hospital care. VII Joint Comeetee definitions were adopted for hypertension and the ADA to define Diabetes Mellitus. Accucheck Roche was used as glucometer. Central obesity was determined by NIH set points. Data was analyzed by the students with EPI Info Version 3.1®

Results: 3.034 adults were monitored by students. The mean age was 44 (± 17), females 1597 (53%) CI 95% 50.8-54.4%. Normal BP was found in 1619 (53%) CI 95% 50.8-54.4%. BMI normal in 1221 (40%) CI 95% 38.5-42.0%. Central Obesity in 1117 (39,1%) CI 95% 37.3-41.0%. Glycaemia was determined in 1650 (54%).Statistically significant differences were found between sexes in: Height 1,73 m (± 0,07) for men, 1,61 m (± 0,06) for women; Diastolic Blood Pressure (DBP) 81,8 mmHg. (± 12,5) for men, 77,6 mmHg. (± 12) for women; Systolic Blood Pressure (SBP) 126,9 mmHg. (± 14,8) for men, 119,7 mmHg. (± 15,2) for women, BMI 27,2 Kg (± 4,3), for men, 26 Kg (± 5,3) for women, Weight 81,5 Kg (± 14,2) for men, 67,8 Kg (± 14) for women. Central Obesity was more common among females: 46,6%. Eight age groups were defined: glycaemia levels, SBP, DBP, weight, showed statistically significant differences. (p< 0,05)

Conclusions: WKD was a great opportunity to create standardized tables of height, weight, systolic and diastolic blood pressure in this population.

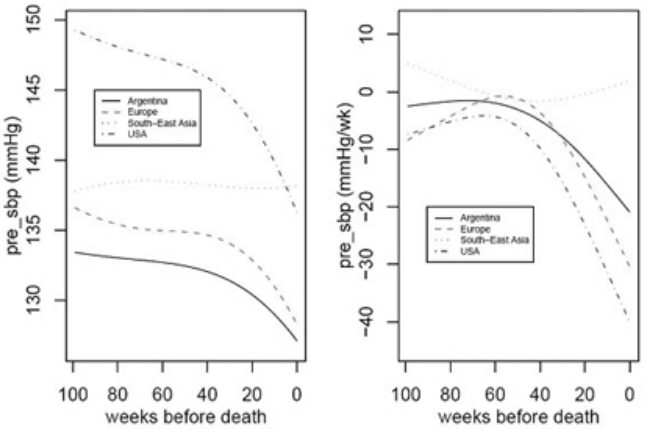
TH-PO777

Systolic Blood Pressure (SBP) Dynamics before Death in Chronic Hemodialysis (HD) Patients – Results of an International Study Daniele Marcelli,[1] Inga Bayh,[1] Aileen Grassmann,[1] Laura Scatizzi,[1] Len A. Usvyat,[2] Michael Etter,[3] Adrian Marcos Guinsburg,[4] Cristina Marelli,[4] Mathias Schaller,[6] Adam Tashman,[5] Yuedong Wang,[5] Nathan W. Levin,[2] Peter Kotanko.[2] *[1]FMC Europe, Bad Homburg, Germany; [2]RRI, NY, NY; [3]FMC Asia Pacific, Hong Kong, Hong Kong; [4]FMC Latin America, Buenos Aires, Argentina; [5]University of California - Santa Barbara, Santa Barbara, CA; [6]University of Cologne Medical Center, Germany.*

Background: Cardiovascular disease is leading cause of death in HD patients (pts). Recent studies in US HD pts showed that decline of SBP is a predictor of death (Li, 2006). We investigate if pattern before death holds for a globally diverse HD population.

Methods: HD data bases from FMC clinics in Europe, Asia, Latin America, RRI clinics in US, and KfH in Germany were queried. Pre-death SBP dynamics were analyzed by estimating the mean SBP level before death and its 1st derivative using quintic splines.

Results: 27807 HD pts from 23 countries were studied (Europe [N=12333; age 71.7, 59% males]; South-East Asia [N=1484; age 68; 53% males]; Argentina [N=10517; age 63.1; 58% males]; USA [N=3473; age 69.9; 56% males]). Pre-HD SBP levels [mean (SD); mmHg] before death were 122 (30); 136 (24); 126 (23); 134 (33); same order as above. In male pts from Europe, Argentina, and US, SBP levels dropped b-n 6 and 13 mmHg in the 2 years preceding death; no SBP change was seen in Asian pts (figure, left). SBP decline accelerated before death and was virtually identical in pts from all databases (figure, right).



The results were materially identical in females and pts from KfH (not shown).

Conclusions: This international study corroborates previous findings in cohorts from Europe and Argentina. Noteworthy, HD patients from South-East Asia show distinctly different SBP dynamics before death. The reasons for this finding are currently unknown.

TH-PO778

APrODiTe (Assessment of Blood Pressure Control and Target Organ Damage in Patients with Chronic Kidney Disease and Hypertension) – Interim Analysis Ran-Hui Cha,[1] Yon Su Kim.[2] *[1]Department of Internal Medicine, National Medical Center, Seoul, Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

Background: The loss of dipping in blood pressure (BP) and masked hypertension are related with target organ damages such as renal dysfunction and cardiovascular diseases.

Methods: APrODiTe is a nationwide multicenter cross-sectional study for Korean CKD stage 2, 3, and 4 patients. The primary aim is to find out the BP control pattern and the secondary aim is to identify the relationship between target organ damages and BP patterns.

Results: We analyzed data from 389 patients out of 1,600. Mean age was 57 year and 59.4% were males. Diabetic nephropathy was 21.1%. The distribution of each BP pattern was as below: sustained uncontrolled, 80.0%; masked, 6.8%; white coat, 10.4%; true controlled, 2.4%; Dipper, 43.6%; non-dipper, 44.1%; reverse dipper, 12.3%. Male gender, current smoking, and spot urine PCR higher than 25percentile (*p*) (100mg/g) were related with masked and sustained uncontrolled hypertension ($p = 0.059$, $p < 0.05$, $p = 0.075$, respectively). In multivariate analysis, female gender (OR = 0.467, p = 0.043) and spot urine PCR less than 25*p* (OR = 0.349, p = 0.007) were related with white coat hypertension, whereas male gender (OR = 2.103, p = 0.009) and spot urine PCR more than 25*p* (OR = 1.908, p = 0.032) with sustained uncontrolled hypertension. Diabetic nephropathy, higher serum creatinine, and lower estimated GFR were related with reverse dipper ($p < 0.05$, $p = 0.002$, and $p = 0.001$, respectively). In multivariate analysis, spot urine PCR less than 50*p* (304 mg/g) was related with dipper (OR = 0.619, p = 0.037), whereas diabetic nephropathy with reverse dipper (OR = 2.457, p = 0.013).

Conclusions: Apparently high proportion of CKD patients were treated inappropriately in Korea. And masked and sustained uncontrolled hypertension and reverse dipper were related with more profound target organ damages. Therefore, appropriate BP control in CKD patients is very important and ambulatory BP monitoring has a significant role to improve physicians' practices. We can understand the real practice in BP control for Korean CKD patients after additional data analysis.

TH-PO779

Clustering of Cardiovascular and Renal Risk Parameters in Non-Hypertensive Individuals George Thomas, Richard A. Fatica, Saul Nurko, Marc A. Pohl, Sankar D. Navaneethan, Titte Srinivas, Martin J. Schreiber, Emilio D. Poggio. *Nephrology & Hypertension, Cleveland Clinic, Cleveland, OH.*

Background: Clinical trial evidence suggests an increase in cardiovascular (CV) events beginning with a systolic blood pressure (SBP) of 115 mmHg and lower levels of GFR. We aimed to study early changes in CV and renal risk profile and their relationship with SBP in living kidney donors with no clinical diagnosis of cardiovascular or chronic kidney disease.

Methods: We studied 394 consecutive living kidney donors between Apr 1997 and Dec 2005 at our institution. Exclusion criteria were BP ≥140/90 mmHg (clinical hypertension), kidney disease, diabetes, CV disease, obesity, and proteinuria. Data collected included age, gender, race, body mass index (BMI), office blood pressure, fasting blood glucose (FBG), uric acid, lipid profile, and iothalamate glomerular filtration rate (iGFR). The study population was stratified into SBP <115 mmHg and SBP 115-139 mmHg. The relationships between risk factors and SBP were studied using regression analysis.

Results: 81% of the study population was Caucasian, with 59% women. Mean age was 41.0 ± 9.9 yrs. Compared to donors with SBP <115 mmHg, those with SBP 115-139 mmHg had significantly abnormal risk factor profile. Interestingly, iGFR was higher in

those with higher SBP. In regression analysis, BMI, FBG, and uric acid, even within the normal range, were independently associated with higher SBP.

Risk factors stratified by SBP < 115 mm Hg (n= 222) and SBP ≥ 115-139 mmHg (n=172)

Variable	SBP < 115 mmHg Mean (SD)	SBP ≥ 115-139 mmHg Mean (SD)	p value
Age	39.3 (8.6)	43.1 (9.7)	<0.001
BMI	25.6 (4.2)	28.0 (3.7)	<0.001
iGFR	104.7 (15.5)	109.3 (17.3)	0.006
eGFR (MDRD)	97.4 (19.5)	95.3 (17.2)	0.25
Total Cholesterol/HDL	3.7 (1.1)	4.0 (1.3)	0.005
LDL/HDL	2.2 (0.9)	2.5 (1.1)	0.008
Fasting blood glucose	84.7 (9.3)	89.2 (9.7)	<0.001
Uric Acid	4.7 (1.2)	5.3 (1.3)	<0.001

Conclusions: Increase in SBP, even in the non-hypertensive range, is associated with early derangement and clustering of risk parameters in otherwise healthy individuals. The higher iGFR observed in those with higher SBP needs further investigation. These results support primary prevention of cardiovascular disease at a much earlier phase.

TH-PO780

Optimal Blood Pressure Control and All Cause Mortality in a Primary Care Setting Zeeshan Khawaja. *Nephrology, Georgetown Univ.*

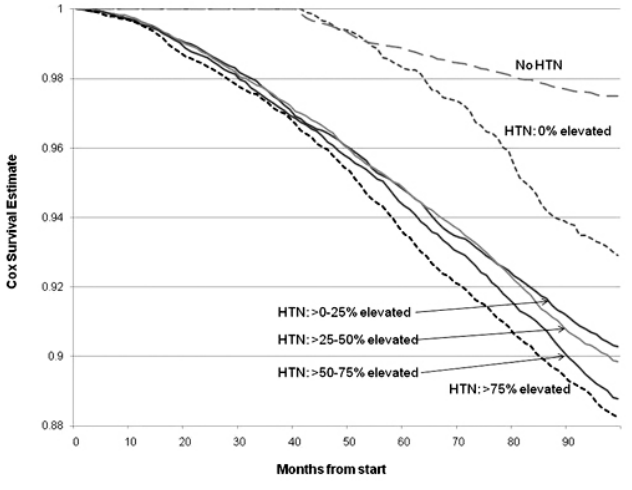
Background: Numerous controlled clinical trials have shown improvement in health outcomes with blood pressure control

Data from a meta-regression analysis of a large population have shown impressive improvement in outcomes for each 20/10 mm Hg reduction in BP for all age groups.

Methods: In the present study we assessed the impact of blood pressure control in 42,346 patients followed at the Washington D.C. VAMC , between January 2000 and December 2007. Electronic Medical Records were used for data gathering and data analysis. A blood pressure control initiative was initiated in 2000 and a battery of recommendations implemented in 2003.

Blood pressure control increased from 44% in the year 2000 to 79% by the end of 2007. In 42,346 patients with multiple BP measurements, systolic and diastolic BPs were averaged for a mean period of 98 months of follow up and accordingly they were allocated to one of 5 groups: G1:never hypertensive (N=4,459), G2: hypertensive always controlled (N=1,305) G3: BP elevated 1-25% of the time(N=8,160), G4: BP elevated 26-50% of the time(N=9,444), G5: BP elevated 51-75% of the time(N=8,045) and G6: BP elevated 76-100% of the time(N=8,045).Results were adjusted for age, sex, heart failure, diabetes mellitus, and BMI.

Results: During the follow up period 6,293 (14.9%) died. Of those 816 patients (10%) died in the Always Controlled group, 2,988 (15.5%) in the partially controlled group and 2,489 (16.7% patients died in the Not Controlled group(p<0.0001 for all comparisons). Cox proportional hazard analysis, adjusted for age, sex, heart failure, diabetes mellitus, and BMI demonstrated a relative risk of 1.24 (CI 1.14 –1.36) for the Partially Controlled group and RR 1.31(CI 1.20 –1.43) for the Not Controlled group compared to the Always Controlled group.



Conclusions: Sustained BP control resulted in the greatest reduction in mortality risk.

Funding: Veterans Administration Support

TH-PO781

Blood Pressure Measurement in a Peritoneal Dialysis Population. Which Method Is Best? Michelle M. O'Shaughnessy, Martin E. Durcan, Sinead Kinsella, Donal N. Reddan, Matthew D. Griffin, David Lappin. *Department of Nephrology, Galway University Hospitals, Galway, Ireland.*

Background: The optimal method of monitoring blood pressure (BP) in a peritoneal dialysis (PD) population is unclear. Ambulatory BP monitoring (ABPM) can refine cardiovascular prognosis but is inconvenient and costly. Home BP monitoring (HBPM) can be inaccurate in this population. The novel BpTRU device (VSM MedTech Ltd, Coquitlam, Canada) has not been studied.

Methods: A cross-sectional study in a single PD centre. All patients underwent office BP measurement (OBPM), BpTRU BP measurement (BpTRU-BPM), HBPM and ABPM over a 2-week period. Agreement between ABPM and the 3 comparator methods was determined.

Results: 17 patients (54.2±12.0 years, 70.6% male, 94.1% automated PD) were studied. Mean office SBP (126.4±16.9mmHg) and BpTRU SBP (123.8±13.7mmHg) closely approximated mean daytime ambulatory SBP (129.3±14.8mmHg), p=NS. Mean home SBP (143.8±15.0mmHg) over-estimated mean daytime ambulatory SBP by 14.2mmHg (95% CI 4.3 to 24.1mmHg, p=0.008). BpTRU SBP correlated well with daytime ambulatory SBP (r=0.49, p<0.05). Home SBP correlated poorly with daytime ambulatory SBP (r=0.24, p=0.37). Bland Altman plots of office SBP, BpTRU SBP, and home SBP, respectively, against daytime ambulatory SBP demonstrated poorest agreement between HBPM and ABPM. False-resistant hypertension was rare (n=1). Nocturnal non-dipping was prevalent (non-dipping, n=11; reverse-dipping, n=5; normal dipping, n=1).

Conclusions: BpTRU-BPM was more accurate than HBPM, with reference to ABPM, in this PD population. Standard OBPM was also superior to HBPM. We suggest two potential explanations for these findings. Firstly, PD patients are very familiar with medical procedures and may consequently experience minimal white-coat effect. Secondly, patients may not adhere to strict BP measurement technique when monitoring BP at home on a long-term basis. The high prevalence of nocturnal non-dipping observed in this PD population may relate to the predominance of automated PD over ambulatory PD. Larger studies are required to validate these findings. Whether BpTRU-BPM can to lead to improved patient outcomes remains to be determined.

TH-PO782

Hourly Ambulatory Blood Pressure Patterns in Patients with Intradialytic Hypertension Peter N. Van Buren, Bohyun Catherine Kim, Anand Srivastava, Robert D. Toto, Jula K. Inrig. *Nephrology, UT Southwestern Medical Center, Dallas, TX.*

Background: Hemodialysis (HD) patients with intradialytic hypertension (HTN), an increase in blood pressure (BP) from pre to post-HD, have higher average interdialytic BP compared to HD controls. This study's purpose is to compare hourly interdialytic BP slopes in intradialytic HTN patients and HD controls.

Methods: In this case-control study, inclusion criteria were prevalent adult HD patients with HTN (systolic BP [SBP] ≥140 mmHg pre-HD or ≥130 mmHg post-HD) during 6 screening HD treatments. Case subjects had SBP increases ≥10 mmHg from pre to post-HD in ≥4/6 treatments, and controls had decreases ≥10 mmHg in ≥4/6 treatments. A repeated measures mixed linear model analyzed interdialytic ambulatory BP slopes.

Results: Of 50 subjects (25 in each group), average age was 54.5 years, 80% were male, 38% African American, 62% Hispanic, and 86% diabetic. The ambulatory BPs are shown below.

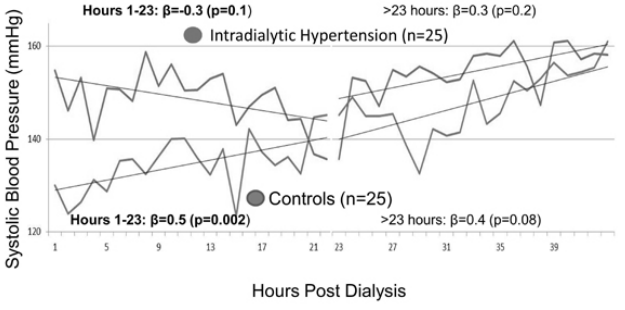
Blood Pressure Comparisons

ASBP (mmHg)	Intradialytic Hypertension	Control	p-value
44-Hr Average	155 (14)	142 (17)	0.005
Hours 1-23	153 (22)	138 (21)	0.0005
Hours > 23	156 (21)	151 (22)	0.2

ASBP=Ambulatory Systolic Blood Pressure

For the initial 23 hours post-HD there was an interaction between group and time (p=0.002). The slope for change in SBP per hour was +0.5 (p=0.002) for controls, but it was a non-signficant decrease in the intradialytic HTN group (β=-0.3, p=0.1).

Figure 1: Mixed Linear Model Analysis of Ambulatory Blood Pressure Slope in Intradialytic Hypertension Patients and Controls During the First 23 Hours Post-Hemodialysis and During the Remaining Interdialytic Time Period



There was a trend towards hourly BP increases in both groups during the remainder of the interdialytic period.

Conclusions: Interdialytic BP slope differs between intradialytic HTN patients and HD controls. The initial post-dialytic BP decrease for 23 hours in intradialytic HTN patients is not consistent with salt sensitive HTN. The increased ambulatory BP burden in intradialytic HTN patients remains to be fully explained, and mechanisms related to increased vasconstriction should be considered.

Funding: NIDDK Support, Other NIH Support - NIH NIDDK F32DK085965-O1A1 (PVB), NIH NIDDK K23 HL092297 (JI), NIH UL 1 RR024982

TH-PO783

Blood Volume Monitoring Characteristics during Intradialytic Hypertension Devasmita Choudhury,[1,2] Lynne M. Roetzer.[1] *[1]Medicine, Dallas VA Medical Center, Dallas, TX; [2]Medicine, University of Texas Medical Center, Dallas, TX.*

Background: Inadequate volume removal (VR) during dialysis can lead to elevated blood pressure (BP) and exacerbate volume load. Blood volume monitoring (BVM) can be used to assess rate of volume removal and intravascular volume refill during hemodialysis (HD). These characteristics were examined 71 chronic stable HD patients to determine differences in those with intradialytic normotension, hypertension (HTN), or hypotension.

Methods: Patients were grouped into high (H)BP, normal (N)BP, low (L) BP by changes (Δ) in BP from start to end of dialysis. An increase of ≥ 10mmhg systolic (sys) or diastolic (dias) BP was grouped as HBP. Sys BP changes within 10mmhg were considered NBP. A decrease of ≥ 10mmhg for sys BP during a HD session was grouped as LBP. ANOVA used for continuous variables. BVM was analyzed for vascular refill and drop in BVΔ slope >5%.

Results: Groups were similar in demographics - gender, DM, HTN, and CV disease, Rx time, bath type. Vascular refill rate was similar for HBP and NBP patient however with 20% fewer BVΔ (drops >5%) in HBP compared to LBP, table 1. This was present despite similar volume removal between HBP and LBP, p=.07. Both volume removal and highest ultrafiltration rate (hUFR) was greater for LBP group than NBP p=.002, p=.03 respectively.

Table1: Comparison of volume removal during intradialytic BP changes

	HBP (n=25)	LBP (n=32)	NBP (n=14)
Age (avg± SD)	67±9	65±9	66±15
VR (ml) (avg± SD)	2926±1039	3587±914	2372±1373
Highest UFR (ml) (avg± SD)	892±363	1082±371	757±400
Avg wt gain (kg) (avg± SD)	2.8±1.6	3.2±1.2	1.9±1.3
Refill (%)	84	71	92
slope Δ>5% (%)	52	72	57

Conclusions: These data suggest that patients with intradialytic systolic or diastolic HTN have increased rates of vascular refill with fewer drops in BV slope although they have no clinical evidence for volume retention. This suggests that volume remains an important contributor for intradialytic hypertension and volume removal should be given a trial in those with intradialytic hypertension.

TH-PO784

Should Every Hypertensive Patient Undergo Carotid Ultrasound? A Simple Statistical Method To Target Subgroups Merce Borras,[1] Javier Trujillano,[4] Jorge Roig,[1] Marisa Martin Conde,[1] Angels Betriu,[3] Adriana S. Dusso,[2] Elvira Fernandez.[1] *[1]Nephrology, Arnau de Vilanova Hospital, Lleida, Spain; [2]Experimental Nephrology, IRBLLeida, Lleida, Spain; [3]UDETMA, Arnau de Vilanova Hospital, Lleida, Spain; [4]Statistics, Lleida University, Lleida, Spain.*

Background: To identify the subgroups of hypertensive patients with high likelihood of carotid plaques(CP)

Methods: Hypertensive patients with carotid ultrasound (CU), CP was defined as Intima-media thickness > 1.1 mm. Demographics, blood and pulse pressure, antropometrics, family and smoking background were recorded.Serum glucose, lipids and C reactive protein as well as microalbuminuria and left ventricular hypertrophy (by echocardiography) was considered. Statistics: Classification tree CHAID (Chi-squared Automatic Interaction Detector) for the calculation of probability of developing CP.

Results: 610 hypertensive patients, mean age was 57,6 years, 62% were males, 23 % diabetics and 18,8% current smokers.The prevalence of CP was 54.7%. CHAID identified 10 decission rules.In hypertensive patients age< 64 years active smoking increased the likelihood of CP. In the subgroup of <43 years (11.9% plaque prevalence) (24% vs 2.9% ;p=0.013).In patients between 43 and 55 years (40.7% carotid plaques) (66.7% vs 29,3%; p=0.000); among non smokers those with pulse pressure >48.67 mmHg showed an increased probability of CP (39.5% vs 12.8%;p=0.014).In patients between 55 and 64 years (57.8% CP) (82.6% vs 54.5%;p=0.01); among non smokers, diabetic patients showed higher likelihood of CP (69.4% vs 48.8%;p=0.014).In patients >64 years (79.5% CP) those with a pulse pressure >65.33 mmHg showed an increased likelihood to develop CP (88.9% vs 67.5%;p=0.004).Using CHAID 52.5% of hypertensive patients would be eligible for CU and 80.2% of the patients would be well classified.

Conclusions: 1.The classification tree for the calculation of the probability to develop CP is a simple method to identify, according to age categories and smoking habit, the risk of hypertensive patients of having atheroesclerotic disease. 2.This method would allow us to select target population for CU if these results are confirmed in further studies.

TH-PO785

Gauging Central Hemodynamic Measure and Ambulatory Arterial Stiffness Index (AASI) To Assess the Subsequent Decline in Glomerular Filtration Rate (GFR) in Stage 3 and Stage 4 Chronic Kidney Disease (CKD) Rupesh Raina, George Thomas, Charbel A. Salem, Alaine E. McGarry-Gada, Martin J. Schreiber, Mohammad Rafey. *Dept. Nephrology and Hypertension, GUKI Institute., Cleveland Clinic Foundation, Cleveland, OH.*

Background: Historically, there has been significant emphasis on BP control to prevent progression of underlying CKD.However, the optimal BP target for patients with CKD remains unclear. We conducted this study to assess the association of central hemodynamic measurements with CKD progression.

Methods: We conducted a secondary data analysis using our preexisting CKD registry at Cleveland Clinic. Central hemodynamic assessment data and 24 hr ABPM pressure were obtained from our hypertension database. AASI was calculated as 1 minus the slope of diastolic on systolic BP in individual 24hr ABPM measure.

Results: We identified 62 patients (stage 3 and 4 CKD) from our CKD registry. The mean age of the study cohort was 72 ± 11.9 years with 54% females and 13% African Americans. The impact of central hemodynamic BP measures and 24 hr ABP on change in GFR is delineated in the table.

Central Hemodynamic Measures	Mean Change in eGFR using CKD-EPI equation	P value
Brachial BP >120/80mmHg	-1.4%	P>0.05
Central Systolic BP >125 mmHg	-2.3 %	P>0.05
Augumentation Pressure (AP) >16 mmHg	-4.2%	P=0.03
AASI index (all quartile) >0.5 U	-6.0%	P=0.01
Ambulatory Pulse pressure PP >50 mmHg	-4.1%	P=0.03

These findings persisted after adjustment for age, sex, active smoking, body mass index, race (White vs. African-American), DM, dipping status, hypertension, congestive heart failure, coronary artery disease, metabolic acidosis and alkalosis, hypertriglyceridemia, and HbA1c. A unique composite score consisting of AP, PP and AASI index was developed and demonstrated a mean decline in GFR of 8.1% at 3 years after adjusting for all variables.

Conclusions: Utilizing a hemodynamic composite score (using AP which estimates the effect of reflected wave form of systolic workload, AASI assessing arterial stiffness, and PP reflecting pulse volume and elasticity of vascular wall) may be more comprehensive in determining risk for CKD progression over time as compared to current applied BP targets and warrants further study.

TH-PO786

Does the BpTRU Device Improve the Diagnostic Accuracy of Office Based Blood Pressure Measurements? Cedric A.W. Edwards, Ankur Gupta, Swapnil Hiremath, Marcel Ruzicka. *Renal Hypertension Unit, University of Ottawa, ON, Canada.*

Background: Measurement of blood pressure (BP) in the office setting fails to identify patients with white coat hypertension (WH). 24 hour ambulatory BP monitoring (ABPM) is considered a gold standard to assess daytime BP. 24-hr ABPM has added costs including equipment and labour. BpTRU has shown promise to improve diagnostic accuracy of office BP readings, and hence, minimize the concern of WH.

Methods: In order to assess the diagnostic accuracy of BpTRU device in a tertiary care referral clinic, we conducted a retrospective analysis of adults with either diagnosed or suspected hypertension (HTN). We compared corresponding BP readings a) average of 3 resting readings taken by trained HTN clinic nurse using mercury sphygmomanomentry (RN), b) average of 5 readings obtained by BpTRU and c) average daytime readings from 24-hr ABPM. The clinic readings were obtained on the morning of ABPM recordings. The average Systolic BP (SBP) for each method was compared using Pearson's correlation and performing a Bland-Altman analysis. Amongst patients with WH (RN SBP > 140 mmHg but ABPM ≤ 135), the proportion of patients who were labelled as hypertensive by BpTRU (SBP > 140) were also calculated.

Results: Charts of 2000 consecutive patients from 2004-2010 were reviewed. 329 patients (mean age of 62 years with 49% males) fulfilled above criteria and were analyzed. Each patient data qualified for one entry only. The mean SBP (mmHg) for RN, BpTRU and ABPM was 143.87 ± 19.90, 136.27 ± 21.85, and 139.35 ± 14.24 respectively.

SBP correlations

Variable (SBP mmHg)	Variable(SBP mmHg)	Correlation
BpTRU	RN	0.74(P<0.0001)
ABPM	RN	0.56(P<0.0001)
ABPM	BpTRU	0.61(P<0.0001)

95 % of values of SBP (difference between ABPM and BpTRU) are between -33 to + 31 mmHg. Fifty-two patients (15.81%) had WH. The BpTRU SBP value in 57% of these patients was greater than 140.

Conclusions: Although there is a statistically significant correlation between SBP obtained by BpTRU and ABPM, the clinical utility of this is limited by the poor ability of BpTRU to correctly diagnose WH. Caution is to be used when relying solely on BpTRU readings to eliminate the diagnosis of WH.

TH-PO787

Ambulatory Blood Pressure Monitoring (ABPM) in the CKD Japan Cohort (CKD-JAC) Study Enyu Imai,[1] Satoshi Iimuro,[2] Kosaku Nitta,[3] Tsuyoshi Watanabe,[4] Seiichi Matsuo,[1] Tadao Akizawa,[5] Hirofumi Makino,[6] Yasuo Ohashi,[2] Akira Hishida.[7] *[1]Nagoya University; [2]University of Tokyo; [3]Tokyo Women's Medical University; [4]Fukushima Medical University; [5]Showa University; [6]Okayama University; [7]Yaizu City Hospital.*

Background: The CKD-JAC study was established to prospectively study the renal and cardiovascular outcomes in 2977 Japanese patients with CKD stage 3-5 who are visiting 17 outpatients clinics in Japan. Ambulatory Blood Pressure Monitoring (ABPM) was conducted in 1113 participants to study the impact of 24h BP on outcomes.

Methods: ABPM at 30-min interval was performed from September 2007 to April 2010. Data on medical history, medications, office BP and renal function were used from the data at registration. The following indicators related to BP were calculated from these data: 24h mean BP, daytime BP, nocturnal BP, degree of nocturnal BP fall, early-morning HTN, and morning surge. The criterion for HTN was 140/90mmHg for the office BP and 130/80 mmHg for the 24-hour mean BP.

Results: The data of 1,077 subjects was ultimately analyzed. Based on the 24-hour mean BP and office BP, the HTN patterns were classified as normal BP (37.5%), white-coat HTN (5.6%), masked HTN (30.8%), and persistent HTN (26.1%). Early-morning HTN accounted for 67.6%, and of this group, 41.6% were the morning surge type (with normal 24-hour mean BP). Nocturnal HTN accounted for 33%, and most of it was persistent HTN. Nocturnal BP fall patterns were classified as dipper (36.6%), extreme dipper (9.8%), non-dipper (37.7%), and riser (15.9%). CKD stages 4 and 5 patients had a higher comorbidity of non-dippers and riser. The percentage of non-dippers and risers was the highest (69.4%) in diabetes. Use of RAS inhibitor was associated with decreasing percentage of nocturnal hypertension, while use of diuretics was not.

Conclusions: A significant proportion of CKD population had masked HTN and early-morning HTN. The percentage of patients with non-dipper or riser was increased according to progression of CKD stages. The comorbidity of nocturnal hypertension is remarkable in patients with diabetic nephropathy.

Funding: Pharmaceutical Company Support

TH-PO788

Comparison of Heart Rate Variability between Patients on Kidney Transplantation, Hemodialysis, Peritoneal Dialysis Due to End Stage Renal Disease and Hypertensives in Korea

Joon Seok Oh, Joong Kyung Kim. *Division of Nephrology, Internal Medicine, Bong Seng Hospital, Dong-gu, Busan, Korea.*

Background: Heart rate variability (HRV) can be used to assess the effects of drug and other interventions including respiration, exercise, metabolic change and psychological or physical stress on cardiac autonomic tone. We investigate the autonomic nerve system activity by HRV in patients with kidney transplantation, hemodialysis, peritoneal dialysis.

Methods: We compared the pattern of cardiac sympathetic and parasympathetic activity through the time- and frequency-domain analysis of HRV with 24-hour Holter monitoring between 30 kidney transplanted subjects, 22 with hemodialysis, 20 with peritoneal dialysis and 34 control patients with hypertension. The subjects have been received renal replacement therapy at the Bongseng hospital between January 2006 and December 2010.

Results: All measures of kidney transplanted gorup were similar to those of control group. In peritoneal dialysis group, HRV index was decreased compared with other groups. In hemodialysis and peritoneal dialysis groups, normalized unit of low-frequency (LFnorm), ratio of low-frequency power to high-frequency power (LF/HF) were increased and normalized unit of high-frequency (HFnorm) was decreased compared with other groups.

The comparison of time-domain and frequency-domain HRV measures between groups

	HD	PD	KTP	Control	p value
HRV index	11.04 ± 5.58	9.21 ± 4.57	16.20 ± 4.57	17.29 ± 6.22	0.002
LF/HF	1.01 ± 1.02	0.85 ± 0.93	2.73 ± 2.48	2.16 ± 2.21	0.001
LF norm, nu	30.38 ± 19.99	25.94 ± 19.85	54.32 ± 19.93	47.46 ± 21.21	0.000
HF norm, nu	41.71 ± 15.71	40.83 ± 11.66	30.26 ± 15.54	33.46 ± 13.82	0.012

HRV index: Integral of the density distribution divided by the maximum of the density distribution, LF norm: LF in normalized units, HF norm: HF in normalized units

Conclusions: Autonomic tones in patients on hemodalysis and peritoneal dialysis are decreased compared with those in patients with kidney transplantation and hypertension. And parasympathetic tones in patients on hemodilaysis and peritoneal dialysis have the preponderance over sympathetic tones. Kidney transplantation may be a modality to improve HRV index in patients with ESRD.

TH-PO789

The Disturbed Circadian BP Rhythm in Chronic Kidney Disease Stage 1-3 Patients

Yan Qin, Lijun Mou, Xuemei Li, Xuewang Li. *Nephrology Department, Peking Union Medical College Hospital, Beijing, China.*

Background: The disturbed circadian rhythm of blood pressure(BP) was independent risk of CKD progression and CVD events. The mechanism of the changing of BP rhythm in CKD is not clear. The present research is to explore the circadian BP rhythm of the youth and middle age patients in CKD stage 1~3 and involved factors.

Methods: The patients underwent 24-hour ambulatory BP monitoring and collected the urine of daytime and nighttime synchronously. The urine was analyzed for Na, K, Cl ,Cr and albumin concentration.

Results: (1)62 patients in CKD stage 1~3 were enrolled,76.6% patients presented as Nondipper BP pattern.The night SBP,DBP and MAP in Nondipper group were higher significantly than the Dipper group.

(2)Both the 24hUP [(1.67±1.73)g/d v.s(3.64±3.00) g/d, P=0.014] and the night RUNa [(4.91±4.18)mmol/h v.s (6.84±3.81) mmol/h,P=0.011] in Nondipper group were significantly higher than those in Dipper group.

(3)Within the Nondipper group, the circadian rhythm of RUNa was reversed. Night RUNa was higher than day RUNa [(6.84±3.81) mmol/h v.s (6.26±3.55) mmol/h].

(4)Night SBP, DBP and MAP increased with the increase of night RUNa and 24hUP from the first to the third tertile significantly(P<0.050), even after adjusted by age, drugs and eGFR.

(5)In the linear regression equations, with the night/day ratio value of RUNa increasing by one, the night SBP, DBP and MAP increased by 2.51 mmHg, 2.54 mmHg and 2.44 mmHg respecitively.

(6)FENa increased significantly from 0.38±0.21 in the first tertile to 1.22±0.57 in the third tertile of night RUNa(P<0.001).

(7)The night RUNa and 24hUP were the risk factors of Nondipper BP pattern, the OR values were 2.37 and 1.42 respectively.

Conclusions: We concluded the disturbed diurnal BP rhythm in the youth and middle age patients in CKD stage 1-3 was common. Both of disturbed circadian rhythms of the RUNa and proteinuria were correlated with the elevation of the night BP and both of them were the risk factors of abnormal circadian BP rhythm. The increase of night RUNa was associated with the decreased ability of tubular sodium reabsorption.

TH-PO790

Low Plasma Renin Activity Levels Are Associated with Lower Mortality in Chronic Kidney Disease Patients

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Background: While the lack of suppression of plasma renin activity (PRA) in poorly controlled hypertension (HTN) has been described to have worse clinical outcomes, no studies to date have evaluated the prognostic implications of PRA in chronic kidney disease (CKD) patients. We sought to determine whether low or suppressed PRA conferred a protective effect on mortality in patients with CKD.

Methods: Longitudinal cohort study within a large ethnically diverse integrated healthsystem during 1/1/1998 thru 12/31/2008 of subjects age ≥ 18 with a minimum of 1 yr followup. Patients with measured PRA and estimated glomerular filtration rate (eGFR) below 60ml/min were included. Primary outcome was mortality rates compared across different levels of PRA. Comparisons were made based on population based quartiles of PRA using cox proportional hazards modeling adjusting for eGFR, age, gender, race, HTN, diabetes (DM), and charlson comorbidity index.

Results: Total of 785 patients were identified for inclusion in the study. Average age 65yrs, 43% men, 41% diabetics, 91% hypertensive, and mean followup was 3.8yrs for the cohort. Compared to the highest PRA quartile, adjusted hazards ratio (HR) for mortality at 2yrs were 1.16 (0.64-2.10 95%CI), 0.94 (0.51-1.76), and 0.55 (0.28-1.11) in the third, second, and lowest quartiles respectively. Linearity was observed where HR for mortality was 1.02 for every 1ng/ml/hr increase in PRA. When lowest PRA quartile (PRA<0.7ng/ml/hr) was compared against the rest of cohort, adjusted HR at 2yrs was 0.59 (0.36-0.96).

Adjusted HR for mortality

PRA quartiles	HR	95% CI
Q3 vs Q4 1yr	1.16	0.64-2.10
Q2 vs Q4 1yr	0.94	0.51-1.76
Q1 vs Q4 1yr	0.55	0.28-1.11
Q3 vs Q4 2yr	1.11	0.67-1.83
Q2 vs Q4 2yr	0.96	0.57-1.62
Q1 vs Q4 2yr	0.60	0.34-1.08

Conclusions: Therapy targeting the renin angiotensin aldosterone system is a cornerstone of CKD management. Our current study suggests that suppressed renin states may confer lower risk for mortality in CKD.

Funding: Pharmaceutical Company Support

TH-PO791

Antialbuminuric Response to Direct Renin Inhibition in Combination with HCTZ or Amlodipine in Patients with Hypertension

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Background: Microalbuminuria is commonly associated in patients with hypertension and diabetes. Aliskiren, a direct renin inhibitor, like ACEIs and ARBs reduces albuminuria and microalbuminuria in patients with hypertension. The purpose of this pooled analysis was to evaluate the effect of aliskiren in combination with hydrochlorothiazide (HCTZ) or amlodipine (AMLO) in reducing urinary albumin creatinine ratio (UACR) in patients with hypertension who do not have pre-existing renal disease (ie. albuminuria or chronic kidney disease).

Methods: Two separate pooled posthoc analyses were conducted in patients with hypertension (age=54 yrs; 51-56% males; BP=167/96 mmHg; BMI=34-35 kg/m2; 12-19% diabetes; 90-95% eGFR≥60 ml/min; 24-26% microalbuminuria) who were either randomized to aliskiren (300 mg) in combination with hydrochlorothiazide (25 mg; n=316) (pool 1) or amlodipine (10 mg; n=97) (pool 2) for 8-12 weeks of therapy.

Results: The response to aliskiren in combination with HCTZ or amlodipine demonstrated reductions in UACR in hypertensive patients without kidney disease.

	Pool 1		Pool 2	
Geometric means	Aliskiren/HCTZ (n=228)	Amlodipine (n=145)	Aliskiren/AMLO	Amlodipine (n=175)
Baseline (week 0)	1.1 mg/mmol	1.3 mg/mmol	1.36 mg/mmol	1.09 mg/mmol
Week 8/12	0.81 mg/mmol	1.29 mg/mmol	1.11 mg/mmol	1.25 mg/mmol
Δ baseline	-33%	-8%*	-16%	+12%†

*p<0.05 vs. Aliskiren/HCTZ; †p=0.06 vs Aliskiren/AMLO

Conclusions: In hypertensive patients with stage 1-2 kidney disease the treatment with aliskiren in combination with HCTZ or amlodipine reduced UACR. These results confirm the use of HCTZ or amlodipine with aliskiren will not only lead to further lowering of BP in hypertensive patients but will also help reduce urinary protein excretion.

TH-PO792

ACE Inhibitors (ACEI) Are Not More Effective Than Angiotensin Receptor Blockers (ARB) in Suppressing Plasma Aldosterone (pAldo) Levels in Hypertensive Patients (pts) Nabil J. Haddad, Udayan Y. Bhatt, Brad H. Rovin, Lee A. Hebert. *Internal Medicine/Nephrology, Ohio State University Medical Center, Columbus, OH.*

Background: Previous work suggests that ACEI are more effective than ARB in suppressing pAldo. However, those studies involved relatively small numbers of pts and/or used relatively low doses of ACEI or ARB.

Methods: This work involved relatively large numbers of pts who received chronic ACEI or ARB therapy and in usual dose. Although not a randomized comparison, the groups were well matched and we took into account ACEI/ARB dose intensity, co-morbidities, diuretic status, and 24-h intake of sodium and potassium assessed by 24-h urine collection, CKD-EPI eGFR, systolic blood pressure, and ACE genotype. The latter is important because the deletion (D) allele encodes for high ACE level, which might induce resistance to ACEI therapy.

Results: We studied 108 ACEI and 53 ARB treated pts receiving therapy for at least 3 months: Age (54.6 ± 1.4 SD vs 57.7 ± 2.1), percent male (55.6 vs 50.9), Caucasian (80.6 vs 77.4), African American (17.6 vs 17.0), hypertensive (83.3 vs 81.1), with diabetes mellitus, (35.2 vs 32.0), on diuretics (48.2 vs 64.2). eGFR was (53.8 ± 3 vs 51.8 ± 4.). None of the differences between the ACEI and ARB groups was significant, although there was a trend for greater diuretic use in the ARB group (p = 0.056). pAldo was not different between the ACEI and ARB groups by univariate analysis or by bivariate analysis adjusting for ACEI and ARB dose with stratification according to ACE genotype. The multivariate model adjusted for age, race, sex, CHF, diabetes, diuretics, 24-h urine Na and K, eGFR, SBP, and ACE genotype.

Conclusions: Our analysis suggests that ACEI are not better than ARB in suppressing pAldo.

Funding: Clinical Revenue Support

TH-PO793

Serum Potassium Level as a Predictor of Responsiveness to Eplerenone Add-On Therapy in Patients Taking Renin-Angiotensin System Blockers Noritaka Kawada,[1] Toshiki Moriyama,[2] Harumi Kitamura,[1] Tomonori Kimura,[1] Hiroki Omori,[1] Yoshitsugu Takabatake,[1] Masaru Horio,[1] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Div. of Nephrol, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Health Care Center, Osaka university, Suita, Osaka, Japan.*

Background: Renin-angiotensin system blockers are first-line antihypertensive agents, but antihypertensive monotherapy is often not sufficient to achieve appropriate blood pressure (BP) control for organ protection. This study determined whether addition of eplerenone could result in clinical benefits in patients already taking angiotensin-converting enzyme inhibitors (ACEis) or angiotensin receptor blockers (ARBs).

Methods: Twenty-five patients who were already taking ACEis or ARBs due to hypertension or chronic kidney disease were enrolled in this study. During the course of the study, patients were treated with a gradual increase of eplerenone to a final dose of 50 mg/day. BP, serum and urine osmolarity/sodium/potassium/ creatinine, estimated glomerular filtration rate (eGFR), and urinary protein/creatinine ratio were measured before and after the 4-month study period. Baseline plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were also measured in some patients.

Results: At baseline, there was no correlation between PAC and the calculated transtubular potassium (K) gradient (TTKG) or PRA in subjects who were taking ACEis or ARBs. However, PAC was inversely correlated with serum sodium (p=0.02) and osmolarity (p=0.05) and positively correlated with serum potassium level (p=0.008). The administration of 50 mg of eplerenone had no significant effects on BP, TTKG or proteinuria, possibly due to individual variability in the response to eplerenone. When the percent changes in proteinuria between basal levels and those at 4 months were calculated, there was an inverse correlation between the baseline serum potassium level and the percent change in proteinuria after eplerenone (p=0.03).

Conclusions: Serum potassium levels predict responsive to eplerenone add-on therapy in patients already taking renin-angiotensin system blockers.

Funding: Government Support - Non-U.S.

TH-PO794

Urinary Uromodulin Concentrations and Incident Hypertension: The Framingham Heart Study Conall M. O'Seaghdha, Shih-Jen Hwang, Caroline S. Fox. *NHLBI's Framingham Heart Study.*

Background: A common variant of the UMOD gene has been associated with extreme hypertension, independent of a primary defect in kidney function, in a recent genome-wide association study. The aim of the present study was to determine whether urinary uromodulin concentrations are associated with risk of hypertension in cross-sectional and prospective analyses.

Methods: Participants free of CKD were drawn from exam 6 of the Framingham Offspring Study (1995-1998), with follow-up at exam 8 (2005-2008) for prospective analyses (n=2948). Extreme hypertension was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg. In prospective analyses, participants with baseline hypertension, defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg were also excluded. Urinary uromodulin concentrations were related to risk of prevalent and incident hypertension and extreme hypertension using logistic regression in unadjusted- and multivariable-adjused analyses adjusted for age, sex and eGFR.

Results: In cross-sectional analyses, urinary uromodulin concentrations were similar between hypertension cases and controls (11.4 vs. 10.6 mg/dL; p=0.9). Similarly, baseline uromodulin concentrations were not statistically different between cases of incident hypertension (11.3 vs. 11.2 mg/dL; p=0.3) and incident extreme hypertension (11.2 vs. 13.9 mg/dL; p=1.0). Results were unchanged after adjustment for urinary creatinine, age, sex, or eGFR.

Conclusions: Although the UMOD gene has been associated with extreme hypertension, its gene product, uromodulin, is not associated with hypertension in the community.

Funding: Other NIH Support - NHLBI

TH-PO795

Effect of Lanosterol Synthase Polymorphisms on Blood Pressure and Endogenous Ouabain Levels in Two Different Clinical Settings Chiara Lanzani, Guido Gatti, Marco Simonini, Simona Pozzoli, Elisabetta Messaggio, Nunzia Casamassima, Simona Delli Carpini, Lorena Citterio, Paolo Manunta. *Chair of Nephrology, San Raffaele Scientific Institute, Milan, Italy.*

Background: Endogenous Ouabain (EO) may affect blood pressure and renal Na excretion through the modulation of the Na pump either as a Na transport system or a signal transduction triggering mechanism in renal tubular cell and in vascular smooth muscle cells. Lanosterol synthase (LSS) regulates the first step in the biosynthesis of cholesterol and steroid hormone, including EO. A polymorphism (rs2254524 V642L) in LSS gene affects mRNA expression in human kidney tissue and in transfected human cells. In this latter setting this polymorphism also affects protein expression, enzymatic activity and EO synthesis.

Methods: To investigate whether the effect of two polymorphism of LSS (rs914247 and rs2254524), on BP and EO levels in mild hypertensive patients in response to two different manouvers: acute Na load with 0.9% NaCl (Na Load) for 2 hrs, and low dietary Na intake (< 100 mEq/day, Low Na diet) for 30 days.

Results: Behaviours of EO plasma levels resulted influenced (p = 0.004) by LSS genotypes: carriers of the wild type variants decreased EO (LSS rs914247 from 259.5±15 to 221.5±19 pmol/L, and LSS rs2254524 from 281.1±16 to 242.2±20 pmol/L). However, mutant variants were associated to slight EO increase (rs914247 265.4±14 to 279.7 pmol/L and LSS rs2254524 269.9±16 to 284.2±20 pmol/L). Furthermore, pressure natriuresis relationship was steeper in those hypertensives carrying the mutated LSS together with ADD2 variants (p epistasis=0.006). Low Na diet: fall in systolic (-8,7±1,7 vs 3,0±1,5 p= 0.013), diastolic (-5,1±0,98 vs -1,4±0,94 mmHg) blood pressure and long term regulation of pressure-natriuresis (0,105±0,02 vs 0,015±0,02 mEq/mmHg/min, p=0.007) were affected by LSS rs2254524 mutated variant.

Conclusions: Our findings suggest that LSS variants regulate plasma EO levels after acute and chronic Na balance variations. Therefore, plasma EO may influence Na-K ATPase activity both at vascular and kidney levels, resulting in modification of blood pressure and urinary Na excretions.

Funding: Government Support - Non-U.S.

TH-PO796

Increased NO Activity during Statin Treatment in Healthy Humans Frank H. Christensen, Thomas Larsen, Jesper N. Bech, Erling B. Pedersen. *Department of Medical Research and Medicines, Holstebro Hospital, Holstebro, Denmark.*

Background: We investigated the effects of short term atorvastatin treatment on blood pressure (DBP and SBP), GFR and fractional excretion of sodium (FE-Na) during inhibition of nitric oxide synthase (NOS).

Methods: Twenty-six healthy men and women (18-37 years) were included in a randomised, placebo-controlled, double-blinded, cross-over study. All subjects attended 2 study days and were given standardized diet and either atorvastatin 80 mg per day or placebo 4 days prior to each examination day. ^{51}Chrom-EDTA clearance was used for GFR measurements and the NOS inhibitor, L-NMMA, were administered as a 4.5mg/kg bolus injection followed by 3.0 mg/kg/hour IV infusion for one hour. SBP, DBP and heart rate (HR) were repeatedly measured during the study day. Blood and urine samples were collected every 30 minutes during the 90 min baseline period, during L-NMMA infusion and 60 minutes after cessation of L-NMMA infusion. Differences are presented in means with standard deviation. Statistics were performed with t-test and paired t-test.

Results: Atorvastatin caused a significant reduction in total-cholesterol (3.7 vs. 3.1 mmol/l, p<0.001). Baseline levels of DBP (63±5 vs. 63±5 mmHg), SBP (112±8 vs.112±9 mmHg), HR (57±8 vs. 58±9/min), GFR (94±9 vs.96±9 ml/min/m^2) and FE-Na (1.28 vs. 1.35) were not different between groups. IV infusion of L-NMMA increased both DBP and SBP and decreased HR (p<0.001). From 15-60 min a stable blood pressure and HR was observed. There were no differences in change in SBP (4±3 vs. 4±3 mmHg), DBP (8±3 vs. 8±2 mmHg) and HR (-6±3 vs. -7±3/min) between groups. GFR decreased during L-NMMA infusion (p<0.001), but there were no differences between groups (-8±6 vs. -10±7%). FE-Na decreased during L-NMMA infusion (p<0.001) and this decreases was significantly higher during Atorvastatin treatment (-34 vs. -44%, p=0.007). There were no differences in 24 hour urine Na-excretion (106 vs. 114 mmol, p=0.32)

Conclusions: Short term Atorvastatin treatment significantly increased the reduction in FE-Na during NOS inhibition. Our results suggest that atorvastatin increases the bioavailability of renal NO and might be an explanation of the pleiotropic effects of statins.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO797

Patients Treated with the Angiogenesis Inhibitor Regorafenib Develop Hypertension with Rapidly Reversible Changes in Plasma Nitric Oxide and Endothelin-1 Nilka deJesus-Gonzalez,[1] Emily S. Robinson,[2] Radostin Penchev,[2] George Demetri,[3] Suzanne George,[3] Benjamin D. Humphreys.[2,3] *[1]Children's Hospital Boston; [2]Brigham and Women's Hospital; [3]Dana Farber Cancer Institute.*

Background: Hypertension (HTN) is a common and dose-limiting side effect of therapies that target VEGF signaling such as regorafenib. Understanding the mechanisms leading to HTN is important as such knowledge may lead to clinically useful biomarkers for predicting both toxicity and tumor response. We prospectively tested the hypothesis that regorafenib induces HTN by suppressing nitric oxide (NO) and increasing endothelin-1 (ET-1).

Methods: Plasma was collected from 32 subjects with gastrointestinal stromal tumor at baseline, 2, 4, and 6 weeks of therapy. Regorafenib was given on a 3-week on, 1-week off regimen, and plasma levels of NO, cyclic GMP (cGMP), a downstream effector of NO, and ET-1 were measured. Data analysis was by Wilcoxon and paired t-tests.

Results: The mean age was 55 years, 60% men, 84% white and 65% had prior HTN. At 2 weeks, regorafenib caused a rise in mean arterial pressure (MAP) with increased ET-1 and decreased NO. ET-1 and NO changes normalized after the 1-week washout period. No differences were observed for cGMP. After restarting regorafenib, ET-1 once again rose and NO fell (Table1).

Biomarkers evaluated at baseline and 2, 4, and 6 weeks after starting regorafenib therapy

Biomarker	baseline	2wks	4wks	6wks	*p(to baseline)
MAP,mmHg†	94(9)	97(12)*	94(11)	100(11)*	<0.05
NO,µmol/l††	26(21-35)	21(16-26)*	26(19-40)	13(10-20)*	<0.05
cGMP,pmol/ml††	150(128-172)	151(118-181)	174(73-210)	-	>0.05
ET-1,pg/ml,††	2.4(1.8-3.2)	3.4(2.2-4.7)*	2.8(2.3-3.5)	3.4(2.8-4.6)*	<0.05

†Mean (standard deviation); ††Median (Interquartile range)

Conclusions: These findings indicate the coordinated and reversible downregulation of the NO system and upregulation of ET-1 system by regorafenib, suggesting systemic vasoconstriction as a mechanism of HTN in these patients. This is the first prospective study evaluating both NO and ET-1 pathways as biomarkers of anti-angiogenic therapy-induced HTN in humans. Whether NO and ET-1 might predict the development of HTN or tumor response requires further study.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO798

Association between Sodium Intake and Hypertension Risk Varies Dependeing upon Levels of Biomarkers Indicating Endothelial Dysfunction John P. Forman,[1] Lieneke Scheven,[2] Paul E. de Jong,[2] Stephan J.L. Bakker,[2] Gary C. Curhan,[1] Ron T. Gansevoort.[2] *[1]Renal Div., Brigham and Women's Hospital, Boston, MA; [2]Dept. Nephrology, UMCG, Groningen, Netherlands.*

Background: The mechanisms underlying chronic sodium loading-related hypertension are unclear. A high sodium diet may first lead to vascular dysfunction, and then to hypertension if the high sodium diet is continued. We hypothesized that a higher sodium intake would be associated with increases in biomarkers of vascular endothelial dysfunction, specifically serum uric acid (SUA) and albuminuria (UAE), and that the association between sodium intake and risk of hypertension would vary according to levels of SUA and UAE.

Methods: We prospectively analyzed the associations of sodium intake with change in SUA, change in UAE, and hypertension incidence (defined by a systolic pressure ≥140 mmHg and/or a diastolic pressure ≥90 mmHg, or use of antihypertensive medications), during a median follow-up of 6.4 yr, among non-hypertensive participants of the PREVEND cohort (N=5571).

Results: After adjusting for multiple potential confounders, a higher sodium intake was significantly associated with increases in SUA and UAE. The relation between sodium intake and risk of incident hypertension varied significantly according to SUA and UAE. For each 1 gram higher sodium intake, the multivariable adjusted hazard ratio for developing hypertension was 0.98 (0.89-1.07) among those whose SUA was in the lowest tertile, and 1.10 (1.03-1.18) among those whose SUA was in the highest tertile. Similar analyses yielded adjusted hazard ratios of 0.99 (0.93-1.06) among participants whose UAE was <10 mg/d, and 1.18 (1.07-1.29) among those whose UAE was >15 mg/d.

Conclusions: Higher sodium intake was associated with increasing SUA and UAE, and was an independent risk factor for developing hypertension only among those with higher levels of SUA or UAE. A high sodium diet may lead to biological changes favoring the development of hypertension if the high sodium diet is continued.

Funding: Other NIH Support - NHLBI

TH-PO799

The Effects of Pregnancy, Pregnancy-Associated Hypertension and Gestational Diabetes on the Microvasculature Thao Vi Luong,[1] Alexander James Teare,[1] Tien Y. Wong,[2,3] Ryo Kawasaki,[2,4] Judith A. Savige.[1] *[1]University of Melbourne, Northern Hospital; [2]Centre for Eye Research, Australia; [3]Singapore Eye Institute, National University of Singapore, Singapore; [4]Department of Ophthalmology and Visual Science, Yamagata University, Japan.*

Background: The small vessels of the retina resemble placental vessels in size. This study evaluated the effect of pregnancy, pregnancy-associated hypertension and gestational diabetes on the microvasculature using retinal vessels as a model.

Methods: One hundred and 36 normal pregnant women, 23 with pregnancy-associated hypertension, and 44 with gestational diabetes provided clinical details and underwent retinal photography using a non-mydriatic camera (KOWA or CANON). Retinal vessel diameters were measured from digital fundus images by a trained grader using a computer-assisted method and summarised as the central retinal artery and vein equivalents.

Results: Arteriolar calibre did not change in normal pregnancies between the first trimester and immediately postnatally (138.8 ± 10.3 um, n=29, versus 140.3 ± 12.5 um, n=31 respectively, p=0.62), but was reduced in the third trimester in patients with pregnancy-associated hypertension alone (130.4 ± 6.5 um, n=9 versus 140.3 ± 12.5 um in normals, n=31, p=0.029), and in patients with pregnancy-associated hypertension plus diabetes (127.4 ± 14.7 um, n=6, p=0.031).

Venular calibre increased progressively in normal pregnancies between the first trimester and immediately postnatally (204.9 ± 19.5 um, n=29, versus 218.6 ± 22.0 um, n =33, p=0.012). In diabetic pregnancies, the venular calibre was normal in the first trimester (199.6 ± 10.9 um, n=3, versus 204.9 ± 19.5 um, n=29, p=0.65), but there was a trend to smaller vessels by the third trimester (201.7 ±15.7 um, n=26, versus 211.1 ± 20.4 um, n=31, p=0.06).

Conclusions: Systemic arterioles are narrowed in patients with pregnancy-associated hypertension alone or together with diabetes. Venules normally dilate throughout pregnancy but the dilatation is less in diabetics. The retinal vasculature represents a model to investigate the effect of treatments that improve placental blood flow in pregnancy-associated hypertension and gestational diabetes.

TH-PO800

Obstetrical Thrombotic Thrombocytopenic Purpura. A Retrospective Study Yahsou Delmas,[1] Sebastien Helou,[1] Viviane Guerin,[2] Pierre Chabanier,[3] Christian Combe.[1] *[1]Service de Néphrologie Transplantation Dialyse, CHU Bordeaux, France; [2]Service d'Hémostase Spécialisée, CHU Bordeaux, France; [3]Pôle Gynécologie-Obstétrique-et Biologie de la Reproduction, CHU Bordeaux, France.*

Background: Thrombotic thrombocytopenic purpura (TTP) caused by a deficiency of A-Disintegrin-And Metalloprotease-with-ThromboSpondin-type-1-domain 13 (AD13), is a rare thrombotic microangiopathy (TMA). During pregnancy, clinical and biological presentation can mimic other TMA particularly the HELLP syndrome. AD13 deficiency in pregnancy related TMA has been evaluated in this study.

Methods: We selected all hospitalized pregnant women with platelets count (Plt) <75 G/L in our hospital in 2008. In patients with no identified etiology of low Plt, plasma AD13 activity was measured by the full length method and an AD13 inhibitor was searched for by functional test and ELISA.

Results: There were 4,292 deliveries for 2008, 43 patients (pts) had Plt<75 G/L. 11 pts had a documented cause of low Plt as ITP, MYH9 syndrome. 6 pts were lost to follow-up. AD13 was measured in 26 patients. 4 pts had AD13<5% in peripartum: TTP pts had lowest Plt significantly below non AD13-deficient 22 women (23 vs 53 G/L, p=0,005). 3 primipara pts with initial diagnosis of HELLP had constitutive AD13 deficit (later confirmed on repeated AD13 dosages) and gave birth prematurely. Their children had intrauterine growth retardation and placental pathology showed major lesions of ischemia. The fourth patient had an episode of TTP on the 5th day post partum with significant anti-AD13 antibody and anti-nuclear antibody at 1:500. The 4 pts had spontaneous resolution of their TMA without any plasma therapy. 2 pts with constitutive defect lost their babies in the neonatal period. In subsequent pregnancies they received prophylactic plasma infusion, and gave birth to normal children.

Conclusions: This is the first description of systematic evaluation of AD13 deficiency in pregnant women with low Plt count. Obstetrical TTP incidence is probably underestimated; in our tertiary unit, it can be evaluated to ~1/1000 pregnancies. Identification of AD13 deficiency may be helpful for prophylactic plasma infusion during subsequent pregnancies.

TH-PO801

Acute High-Dose Intravenous Epoetin Does Not Increase Blood Pressure in Critically Ill Patients with Acute Kidney Injury Zoltan H. Endre,[1,2] Azrina Md Ralib,[1] John W. Pickering,[1] Tamas Major,[1] David A. Goodkin,[3] Suetonia Palmer.[1] *[1]Medicine, University of Otago Christchurch, Canterbury, New Zealand; [2]Nephrology, Prince of Wales Clinical School, Sydney, NSW, Australia; [3]Goodkin Biopharma Consulting, LLC, Bellevue, WA.*

Background: Erythropoiesis stimulating agents (ESA) correction of renal anemia increases blood pressure in as many as 35% of patients. It is uncertain whether this phenomenon is due to increased blood viscosity, increased blood volume, reduction of hypoxic vasodilation, and/or ESA-induced vasoconstriction. The Early Intervention in Acute Renal Failure (EARLYARF) trial provided an opportunity to assess whether IV epoetin induces immediate vasoconstriction.

Methods: A post-hoc analysis of blood pressure changes among 163 patients randomized to receive two doses 24 hours apart of IV epoetin (500 U/kg) or placebo. These intensive-care patients were enrolled following identification of acute kidney injury (AKI) by the urinary biomarkers γ-glutamyltranspeptidase and alkaline phosphatase. Mean arterial pressures (MAP) and norepinephrine equivalent dose (NED) were recorded hourly. NED was determined using equipotency conversion factors for doses of epinephrine, vasopressin, phenylephrine, or dopamine. The differences between maximum and baseline (Δ-MAP and Δ-NED) were determined 4, 24, and 72 hours after study drug administration.

Results: At baseline, MAP was 78±14 mmHg in the epoetin group and 81±15 mmHg in the placebo group (p=0.84). At 4-h after the first drug dose, there were no differences

between groups in Δ-MAP (6.5±13 vs 8.4±13 mmHg; p=0.36), in Δ-NED (-0.8±10 vs -0.1±7.7 µg/min; p=0.63), or in Δ-MAP adjusted for Δ-NED (p=0.14). Similarly, there were no differences after 24 or 72-h, or 4 or 24-h after the second dose. Hemoglobin concentration and hematocrit were unchanged. A subgroup analysis of patients with no vasopressor use (n=71) also showed no differences between epoetin and placebo.

Conclusions: Two high doses of epoetin did not increase blood pressure up to 72-h after administration. These data demonstrate that epoetin does not have an acute vasoconstrictor effect among critically ill patients with AKI.

Funding: Government Support - Non-U.S.

TH-PO802

Impact of Gender and Age on the Cardiac Autonomic Response to Angiotensin II in Healthy Humans

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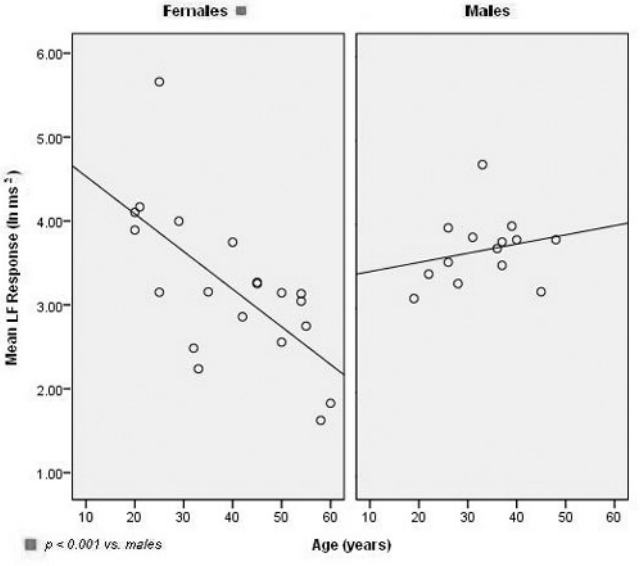
Background: Women are protected from cardiovascular disease (CVD) compared to men. Changes in cardiac autonomic tone such as sympathetic overactivity and vagal withdrawal result in altered heart rate variability (HRV), which when coupled with upregulation of the renin-angiotensin system (RAS) are associated with kidney and CVD, though the pathophysiology is unclear.

Methods: 36 healthy subjects (21 women, 15 men, age 38±2 yrs) were studied in high-salt state. HRV, calculated by spectral power analysis [low frequency (LF), sympathetic activity; high frequency (HF), vagal activity; and LF:HF, balance of autonomic tone], was recorded at baseline and during angiotensin II (AngII) infusion (3ng/kg/min x 30min,6ng/kg/min x 30min).

Results: Striking gender differences exist in the HRV response to AngII, with women maintaining overall autonomic tone (LF/HF 2.4±0.3; p=0.36 vs. baseline) while men exhibit increased sympathetic activity and profoundly decreased vagal activity (LF/HF 4.1±0.6; p=0.014 vs. baseline, p=0.018 vs. LF/HF female response). While gender and age remained predictors of HRV response on multivariate analysis (p=0.003 and p=0.042, respectively), LF and HF responses were increasingly sensitive to AngII with age solely in men (LF p<0.001; HF p=0.001 for gender-age interaction).



Conclusions: Women maintain baseline levels of cardiac autonomic tone in response to AngII. In contrast, men exhibit greater cardiac risk with exaggerated cardiac sympathetic activity in response to AngII, a response exacerbated by increasing age. Understanding the roles of gender and age in cardiac autonomic modulation may help guide novel strategies for high-risk populations, such as those with kidney disease.

Funding: Private Foundation Support

TH-PO803

Fibroblast Growth Factor 23 Levels Are Elevated in Children and Adults with Autosomal Dominant Polycystic Kidney Disease and Preserved Renal Function

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Background: Fibroblast growth factor 23 (FGF23) is risk factor for adverse outcomes in chronic kidney disease (CKD). Elevated FGF23 levels weres recently shown in adults with ADPKD and early stages of CKD. Cardiac manifestations of ADPKD, including increased left ventricular mass index, occur as early as childhood. Early intervention early may be more effective to slow progression of cardiac disease in ADPKD. We hypothesized that FGF23 levels are elevated in children and young adults before reduction of kidney function.

Methods: We compared FGF23 levels in 35 children and young adults with ADPKD and normal eGFR (age 17±4 years; mean eGFR 118±23 ml/min/1.73m^2), 33 adults with ADPKD and preserved kidney function (age 37±8 years; mean eGFR 87±18 ml/min/1.73m^2) and 9 adults with more advanced ADPKD (age 51±10 years; mean eGFR 34±7 ml/min/1.73m^2). Serum FGF23 levels were measured by ELISA (Kainos, Tokyo, Japan).

Results: Using the same assay, a previous study reported normal FGF23 level in healthy volunteers aged 20-83 years of 30±21 pg/ml. Mean FGF23 level was 37.3 ± 18.5 pg/ml in the 35 children and young adults with ADPKD , 39.9 ± 21.3 pg/ml in the 33 adults with ADPKD and preserved kidney function and 86.4 ± 61.4 pg/ml in the 9 adults with more advanced ADPKD. Twenty % of children and young adults with ADPKD had an FGF23 >50 pg/ml. In the adults with preserved kidney function, 36% had a level >50 pg/ml. Among adults with more advanced renal dysfunction, only one had an FGF23 level < 50 pg/ml.

Conclusions: FGF23 levels are increased early in ADPKD when kidney function is intact, and abnormally high levels of FGF23 are detectable in many children with ADPKD. Further research is necessary to determine the implications of early increases in FGF23 levels in children and adults with ADPKD and whether these increases contribute to progression of CKD and the developmenmt of cardiovacular disease.

Funding: NIDDK Support, Private Foundation Support

TH-PO804

The Relationship between Renal Volume and Renal Function in Autosomal Dominant Polycystic Kidney Disease

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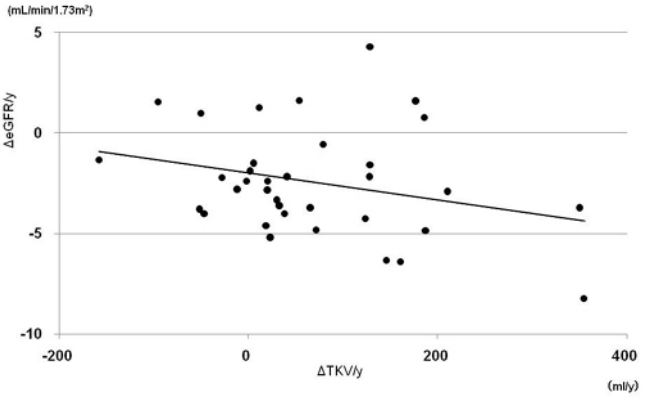
Background: In patients with autosomal dominant polycystic kidney disease (ADPKD), renal cysts grow exponentially. Since remaining renal parenchyma has a capacity to compensate for the loss of glomerular filtration, the glomerular filtration rate (GFR) may be sustained until the disease progresses. The purpose of this study was to determine if renal volumetric indices and clinical parameters are associated with renal function in Japanese patients with ADPKD.

Methods: In 73 ADPKD patients (28 men, 45 women), the associations of mean systolic blood pressure (SBP), mean diastolic blood pressure (DBP), estimated GFR (eGFR), the amount of proteinuria and albuminuria, body mass index (BMI), brachial-ankle pulse wave velocity (baPWV), ankle-brachial Index (ABI), and total kidney volume (TKV) were retrospectively analyzed.

Results: Multivariate linear regression analysis showed that eGFR was significantly and independently inversely correlated with patients' age, and BMI. The median change in eGFR per year (ΔeGFR/y) was -2.8 mL/min/1.73 m2/year. Multiple linear regression analysis showed that ΔeGFR/y was significantly and independently inversely correlated with the change in TKV per year (ΔTKV/y) (Figure1). Multiple linear regression analysis showed that ΔTKV/y was significantly related to initial TKV and the change in albuminuria per year (Δu-alb/y).

Conclusions: This study demonstrated a significant relationship between the change in renal function and the change in renal volume in Japanese ADPKD patients without renal insufficiency. It is possible that the volume measurements can be used as useful markers for disease progression in Japanese ADPKD patients.



TH-PO805

Baseline Renal Cysts Volume Predicts the Recombinant Human Erythropoietin Requirement in Autosomal Dominant Polycistic Disease

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Background: Prevalence of anaemia in patients with autosomal dominant polycystic kidney disease(ADPKD)increases according to the severity of chronic kidney disease(CKD). However,little is known about the relationship between kidney structure modification and recombinant human erythropoietin(rHu-EPO) requirement in these patients(pts).Aim of

this study was to evaluate the role of renal cysts and kidney size on rHu-EPO requirement in severe CKD and naive chronic hemodialysis(HD)patients.

Methods: A total of 43 pts with ADPKD and anemia treated with alfa-erythropietin (α-EPO)were enrolled(16 pts with CKD Stage 4 and 28 naive chronic HD pts),the total volume of the four largest cysts(cysts-Vol)and the mean antero-posterior renal diameter(AP) were prospectively followed-up for 18months with kidney ultrasound.

Results: Mean age was 65±13yrs.At baseline,AP was 19.4±2.1cm,cysts-Vol 407±369cm3.During the 18months follow-up,haemoglobin(Hb) was 10.8±0.7g/dl,α-EPO dose was 14403±7518UI/week,and α-EPO/Hb ratio was 1379±780.In fully adjusted model,baseline cysts Vol and AP predict EPO dose and EPO/Hb ratio and explain a large amount of variability.

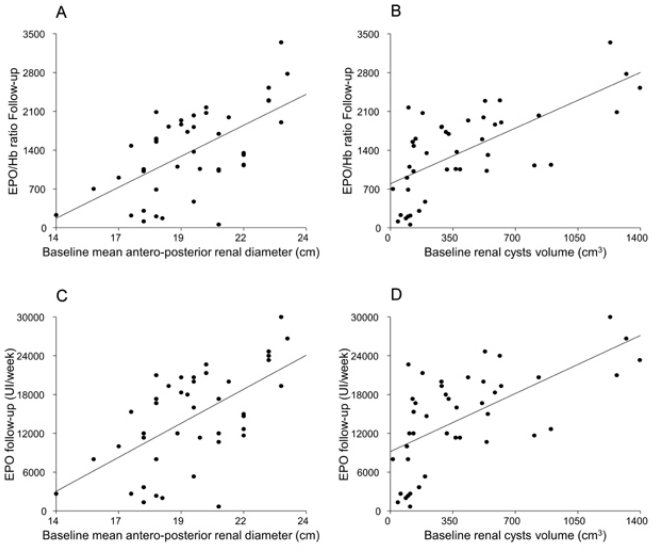


Table 1. Multivariate Analysis.

	Beta (95%CI)	P	R2 incr.
Dependent variable: EPO, UI/Week			
Cysts Vol (100cm3)	870 (250-1480)	<0.05	0.11
AP (cm)	1146 (68-2223)	<0.05	0.06
Model R2=0.46			
Dependent variable: α-EPO/Hb ratio			
Cysts Vol (cm3)	104 (43-165)	<0.05	0.14
AP (cm)	109 (3-215)	<0.05	0.05
Model R2=0.51			

Conclusions: Cysts volume is useful to predict prospectively the rHu-EPO requirement. This assumption is valid even in chronic dialysis patients, where the renal function is completely lost.

TH-PO806

Intravascular Treatment of 1,012 Patients with Symptomatic ADPKD and/or ADPLD Koki Mise, Yoshifumi Ubara, Keiichi Sumida, Rikako Hiramatsu, Tatsuya Suwabe, Kenmei Takaichi. *Nephrology, Toranomon Hospital, Tokyo, Japan.*

Background: Since the kidneys and liver of ADPKD patients are usually supplied by well-developed arteries, we have attempted to reduce the volume of enlarged kidneys and livers in these patients by transcatheter arterial embolization (TAE) using intravascular coils.

Methods: From 1996 to 2011, a total of 1,012 patients with ADPKD were treated for abdominal distension due to nephromegaly and/or hepatomegaly.

Results: Renal TAE was done for 711 patients with intractable enlarged kidneys. After TAE, nephrectomy was performed in 2 patients because of antibiotic-resistant renal cystic infection (unilateral in one patient and bilateral in the other). Nephrectomy was also performed for the affected kidney in 10 patients with renal cell carcinoma. The other patients had no TAE-related or renal complications, but complications of other organs (such as cancer and cardiovascular/cerebrovascular disease) led to death. Hepatic TAE was done in 301 patients with intractable symptomatic polycystic liver. After TAE, antibiotic-resistant hepatic cyst infection relapsed or developed newly in 30 patients. For these infections, cyst drainage was performed in 28 patients and partial hepatectomy was done in 2 patients. The prognosis of 15 patients with severe hepatic failure (total bilirubin >2.0 mg/dL) and intractable massive ascites was poor, but the other patients are doing well.

Conclusions: No major complications of TAE were encountered with treatment of either the kidneys or the liver. So far, it seems that TAE may be less effective in reducing total organ volume than surgical nephrectomy or hepatectomy. However, TAE is an option for patients with symptomatic polycystic liver and/or kidney who are in poor general condition and are not candidates for surgical treatment.

Funding: Private Foundation Support, Clinical Revenue Support

TH-PO807

Renal Denervation for Intractable Autosomal Dominant Polycystic Kidney Disease-Related Pain Marie C. Hogan,[1] Joanne Ryan,[1] James Glockner,[2] Stephen B. Erickson,[1] Dawn S. Milliner,[1] Claude Deschamps,[2] Vicente E. Torres.[1] *[1]Nephrology Div; [2]Radiology Dept; [3]Thoracic Surgery Div, Mayo Clinic, MN.*

Background: Kidney pain can be a severe & debilitating chronic problem in some individuals with ADPKD. Videothoracoscopic splanchnicectomy (VSPL) has anecdotally been used to manage ADPKD patients with intractable kidney pain.

Methods: We have performed VSPL in 15 ADPKD patients(11F, 4M) for chronic kidney pain in 13 [2 unilateral,11 bilateral procedures], a bilateral procedure for pain due to pancreatic cystic disease, & a unilateral procedure for liver pain; 11/15 enrolled in this study to evaluate its effectiveness in alleviating pain, opioid use, QOL, renal blood flow (RBF) [by MRI] & GFR. All had chronic pain >6 mo & were opioid dependent.

Results: All (mean age 38, range 20-51) reported relief of their pain (different/ less intense) immediately post op. Average hospital stay was 4d. Study participants have been followed a mean of 14 mo (range 1-24 mo). One developed severe chest wall pain post-op requiring nerve block & another developed orthostatic hypotension responsive to midodrine.

Improved pain treatment satisfaction occurred at mos 1 (n=9/11; p 0.002), 3 (n=8/10) & 12 (n=5/6) 24 (3/6) respectively compared to baseline. Physical ability improved at mo1(n=6/11; p =0.05), 3 (n=4/9) 12 (4/6) & 24mo(3/6). Depression improved in 5/11& 4/6 patients at 1 mo & 2 yrs. Opiates were discontinued at 1 mo in 4/11 & 3/6 at 2 yrs. Baseline aggregrate RBF (mean± SD) 302 ± 99 increased to 335 ±113 ml/min at 3 mo; (p; NS). Mean SBP (-4mmHg) & DBP (-6mmHg) improved over 2 years. GFR increased from 76 ±37ml/min (baseline) to 96 ±51ml/min/SA (n=7) at 3 mo (p; NS)

One had initial improvemnt at 3 mo but later underwent bilateral nephrectomies for recurrent pain. Another was lost to follow up after mo1. After initial response to VSPL for pancreatic pain that individual had recurrence & underwent pancreatectomy.

Conclusions: VSPL seems to be an effective palliative procedure for intractable visceral pain in some patients with ADPKD & may have a role in the stepwise approach for chronic pain management strategies in selected individuals with relatively preserved renal function.

Funding: Private Foundation Support

TH-PO808

A Quantitative Proteomic Study of Urinary Exosome Candidate Biomarkers in a Genetically Defined *PKD1* Cohort Marie C. Hogan,[1] Kenneth L. Johnson,[2] Douglas W. Mahoney,[3] Ann L. Oberg,[3] Peter C. Harris,[1] Christopher James Ward.[1] *[1]Nephrology; [2]Proteomics Ctr; [3]Biostatistics, Mayo Clinic, MN.*

Background: We have identified distinct subpopulations of urine exosome-like vesicles (ELVs) reproducibly isolated by gradient centrifugation (5-30% sucrose D_2O). One of these, (PKD-ELVs) is enriched in polycystin-1 (PC1), polycystin-2 (PC2)& fibrocystin/ polyductin (FCP). Analysis may reveal diagnostic PKD biomarkers.

Methods: PKD-ELVs were isolated from ~270mls 1st/ 2nd AM voids [7 controls (28±3 yrs);9 PKD cases (28± 6 yrs)] & proteins fractionated by SDS-PAGE, tryptic peptides id'ed & quantified by label-free LC-MS/MS. Phase 1 examined biomarker variability. Inter-individual variability was corrected by Loess method. Multivariate ANOVA (disease, gender, & interactions) was used to compare proteins between cases & controls. 5% FDR & p< 0.002 were used.

Results: On average we observed ↓of 27% of PC1, ↓27% PC2 & ↓25% FCP compared to controls. Using a p<0.002 cutoff, we identified 21 differentially regulated proteins in the PKD cohort: 9 up & 12 are down-regulated. PC1 peptides were consistently underrepresented in cases. Two Ig heavy chain proteins & haptoglobin were upregulated. SAHH-2 (IRBIT) which is down-regulated, is reported to modulate CFTR & BCe1-B, intracellular Ca by antagonizing IP3 receptor. HOMER1 a known PC1 interactor is consistently upregulated with1c isoform most highly expressed.

Selected Differentially Regulated Proteins in PKD-ELV Proteome

Protein	Uniprot Name	Fold Δ	p-value
Polycystin 1	PKD1_HUMAN	-2.0	0.015
Polycystin 2	PKD2_HUMAN	-1.6	0.086
Fibrocystin	PKHD1_HUMAN	-2.0	0.016
IRBit	SAHH2_HUMAN	-2.1	0.01
Solute Carrier family 12-1	S12A1_HUMAN	-2.8	0.008
Homer 1	HOME1_HUMAN	+3.4	0.002
Ig gamma-1 chain C region	IGHG1_HUMAN	+4.8	0.007
Phospholipid Scramblase 1	PLS1_HUMAN	+2.0	0.012

Conclusions: PC1, PC2 and FCP are ↓ in cases compared with controls, although NS in Phase I of this study. Inflammatory proteins also feature. Homer1 a known PC1 interactor is one of the few proteins that is increased in PKD. We are developing a ratiometric assay using a PC1Mab(7e12) /Homer1cMab to validate these findings if confirmed in our Phase II study (ongoing).

Funding: NIDDK Support, Private Foundation Support

TH-PO809

Modeling Vascular Lesions Associated with Autosomal Dominant Polycystic Kidney Disease Using Patient-Specifick iPSCs Kenji Osafune, Fumihiko Shiota. *Center for iPS Cell Research and Application (CiRA), Kyoto University.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent, potentially lethal, monogenic disorder, characterized by the development of multiple renal cysts and various extrarenal manifestations. Cardiovascular complications are the main cause of death in patients with ADPKD, including hypertension, intracranial aneurysms and dolichoectasias, thoracic aortic and cervicocephalic artery dissections, coronary artery aneurysms and valvular heart abnormalities. The pathogenesis of cardiovascular lesions as well as renal cyst formation remains largely unknown, and no therapeutic strategies have been established.

Methods: We generated induced pluripotent stem cells (iPSCs) from skin fibroblast samples from seven patients with ADPKD by trasducing four transcription factors, OCT4, SOX2, KLF4 and c-MYC or three factors, OCT4, SOX2 and KLF4. Then we differentiated the ADPKD-iPSCs into vascular endothelia and mural cells.

Results: We have obtained iPSCs from skin fibroblast samples from seven patients with ADPKD. These cells expanded robustly in culture and differentiated into vascular endothelia and mural cells in vitro. Using this differentiation system, we have identified several molecules whose expression levels were upregulated or downregulated in vascular cells differentiated from ADPKD-iPSCs as compared to those from normal Japanese iPSCs.

Conclusions: These results suggest that disease modeling using patient-specific iPSCs can be used for studying the mechanisms of vascular complications associated with ADPKD.

TH-PO810

Cysteamine Causes Angioendotheliomatosis by Stimulating Microvascular Endothelial Cell Proliferation Elena N. Levtchenko,[1] Mieke Dewerchin,[2] Martine T. Besouw.[1] *[1]Dept of Pediatric Nephrology/Laboratory for Pediatrics, KULeuven, Leuven, Belgium; [2]Vesalius Research Centre, KULeuven, Leuven, Belgium.*

Background: Cystinosis is an autosomal recessive disease caused by intralysosomal cystine accumulation. It initially causes renal Fanconi syndrome, progressing to end-stage renal disease by the age of 10 years if left untreated. At this moment, cysteamine is the only available treatment for cystinosis. Recently 8 patients were reported with muscular-skeletal weakness, skin striae and bruising-like lesions on elbows after administration of high doses of cysteamine. One patient died of cerebral ischemia. Skin biopsies of the elbow lesions showed vascular proliferation called angioendotheliomatosis. We aimed to study the mechanism of this severe complication.

Methods: Human dermal microvascular endothelial cells (HDMVEC, the cells involved in angioendotheliomatosis) were incubated with a range of cysteamine concentration (0-10 mM) during 6 or 24 hours in a 96-wells stadium. Cell viability was measured using WST-1, cell proliferation was measured by BrdU incorporation. Growth factors (VEGF: vascular endothelial growth factor, PlGF: placental growth factor, b-FGF: basic fibroblast growth factor, PDGF: platelet derived growth factor) were measured in supernatant medium after 6 and 24 hours of cysteamine exposure. All results represent mean of at least two independent experiments performed in triplicate. The paired student t-test was used for statistical analysis.

Results: HDMVEC viability increased by 135% ($p<0.01$) after 24 hours of cysteamine exposure (0-3.0 mM). Cell proliferation increased by 59% ($p<0.05$) and by 31% ($p<0.05$) after 6 and 24 hours of cysteamine exposure (0.03-1.0 mM) respectively. Cysteamine 0.03-0.3 mM stimulated VEGF production with 54-95% ($p<0.05$) while there was no differences in PlGF, b-FGF and PDGF concentrations.

Conclusions: Cysteamine can cause endothelial proliferation via stimulating VEGF production at concentrations described in patients' plasma (0.03-0.1 mM). We suggest that this mechanism underlies angioendotheliomatosis induced by cysteamine in cystinosis patients.

Funding: Private Foundation Support

TH-PO811

Biomarkers of Endothelial Dysfunction and Vascular Inflammation in ADPKD Jelena Klawitter,[1] Jost Klawitter,[1] Kyler Corby,[2] Uwe Christians,[1] Robert W. Schrier,[2] Berenice Y. Gitomer,[2] Michel B. Chonchol.[2] *[1]Anesthesiology, University of Colorado, Aurora, CO; [2]Renal Diseases and Hypertension, University of Colorado, Aurora, CO.*

Background: Cardiovascular disease (CVD) is the leading cause of premature mortality in autosomal dominant polycystic kidney disease (ADPKD). Recent evidence has implicated systemic endothelial dysfunction and vascular inflammation in the pathogenesis of ADPKD even before the loss of renal function.

Methods: Serum and urine samples from 61 ADPKD patients were analyzed. Among these, patients 15 had a total kidney volume (TKV) of < 800mL, 28 a TKV 800-1500mL and 18 a TKV > 1500mL. Multiplexing tandem mass spectrometry assays were used for quantitation of eighteen different lipid signaling molecules (HODEs, HETEs, HEPEs, resolvins) as well as asymmetric (ADMA) and symmetric dimethylarginine (SDMA). All markers were correlated with TKV, glomerular filtration rate (GFR) and cardiac left ventricular (LV) mass.

Results: Patients with higher kidney volume had significantly increased concentrations of pro-inflammatory hydroxyoctadecadienoic acids (HODEs). 9-HODE and 13-HODE increased from 25.3±11.3 and 21.4±9.9 ng/mL in patients with <800mL TKV to 43.1±14.7 and 37.6±13.3 ng/mL ($p<0.001$, respectively) in patients with TKV <1500mL. Urinary ADMA concentration significantly decreased with the higher TKV ($r=-0.378$, $p<0.001$), and also correlated positively with GFR ($r=0.385$, $p<0.001$). a significant 22% increase of ADMA between the patients with TKV <800mL and TKV >1500mL was observed. Its structural isomer SDMA followed the same trend, even showing a more pronounced increase between the two patient groups (1.4±0.3 vs. 2.3±1.1, $p<0.005$).

Conclusions: Increased serum levels of 9-HODE and 13-HODE in ADPKD patients with higher TKV suggest an increase in lipid peroxidation and oxidative stress. With both compounds shown to be implicated in the pathogenesis of atherosclerosis, their monitoring in ADPKD patients could be beneficial to minimize the resulting CVD.A disturbance in the clearance of ADMA and SDMA clearly points towards the role of endothelial dysfunction in ADPKD patients, and makes them to potential strong predictors of cardiovascular events

Funding: NIDDK Support, Private Foundation Support

TH-PO812

Methylation Cycle Intermediates in Autosomal Dominant Polycystic Kidney Disease Jelena Klawitter,[1] Jost Klawitter,[1] Kyler Corby,[1] Uwe Christians,[1] Robert W. Schrier,[2] Michel B. Chonchol,[2] Berenice Y. Gitomer.[2] *[1]Anesthesiology, University of Colorado, Aurora, CO; [2]Renal Diseases and Hypertension, University of Colorado, Aurora, CO.*

Background: An elevation of plasma total homocysteine (Hcy) has been identified as an independent risk factor for vascular disease in patients with reduced kidney function. Cardiovascular disease is the leading cause of death in autosomal dominant polycystic kidney disease (ADPKD).

Methods: Serum and urine samples from 61 ADPKD patients were analyzed. Among these, patients 15 had a total kidney volume (TKV) of < 800mL, 28 a TKV 800-1500mL and 18 a TKV > 1500mL. Tandem mass spectrometry assays were used to measure urinary aldosterone, methionine, Hcy, cysteine, adenosine, S-adenosylhomocysteine (SAH) and S-adensylmethionine (SAM). Results were correlated with TKV, glomerular filtration rate (GFR) and left ventricular (LV) mass.

Results: ADPKD patients with TKV > 1500mL had a significantly increased serum concentrations of Hcy (14.9±4.5 vs. 11.4±3.2 μM, $p<0.05$) and SAH (1.7±0.3 vs. 2.0±0.5 μM, $p<0.05$) compared to those with TKV <800mL. In urine the trend was reversed with lower concentrations of homocysteine in the patients with higher TKV (572.0±259.2 nmol/mg creatinine in patients with TKV <800mL vs. 413.4±162.2 nmol/mg creatinine with TKV >1500mL). The reduced excretion of Hcy was accompanied by reduced excretion of the methionine methylation pathways's end product, cysteine. While SAM remained unchanged, the concentration of SAH increased from 1.7±0.3 μM in serum of patients with TKV <800mL to 1.96±0.5 μM in those with TKV <1500mL ($p<0.05$). As a result the SAM:SAH methylation potential ratio decreased. In urine, the excretion of SAH was also significantly increased (17.5±7.6 vs. 22.9±9.3 nmol/mg creatinine, $p<0.05$).

Conclusions: Increased serum Hcy levels in ADPKD associated with increased TKV represents a valuable biomarker in ADPKD which can potentially be used to monitor cardiovascular diseaserisk in these patients. Reduced urinary Hcy suggests a disturbance in the renal excretion, which may account for the accumulation in the blood. No such inhibition was observed for the Hcy precursors, SAM or SAH.

Funding: Private Foundation Support

TH-PO813

Renal Phosphate Leak in Autosomal Dominant Polycystic Kidney Disease (ADPKD) Anna Jeanette Jovanovich,[1] Berenice Y. Gitomer,[1] Myles S. Wolf,[2] Xiang-Dong Yan,[1] Robert W. Schrier,[1] Michel B. Chonchol.[1] *[1]Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Nephrology, University of Miami Medical School, FL.*

Background: In a recent report of 100 ADPKD patients with chronic kidney disease (CKD) stages 1 - 2, serum phosphate levels were significantly lower compared to healthy volunteers and eGFR-matched CKD patients without ADPKD. We hypothesized that a renal phosphate leak is present in ADPKD compared to patients with CKD due to other etiologies and that these differences are detectable across the spectrum of kidney function.

Methods: Biochemical data on 697 ADPKD subjects were extracted from the University of Colorado ADPKD clinical database. Using a t-test we compared serum phosphate levels and 24-hour urinary phosphate excretion across the spectrum of eGFR to literature-reported levels from 3879 non-ADPKD participants in the Chronic Renal Insufficiency Cohort (CRIC) study.

Results: We examined serum phospahte and urinary phosphate excretion accross deciles of eGFR (eGFR 20-29, 30-39, 40 -49, 50 -59 and >60 ml/min/1.73m2). Serum phosphate levels were significantly lower accross the entire spectrum of renal function in the ADPKD participants compared to the eGFR matched CRIC participants. Urinary phosphate excretion levels were significantly higher in ADPKD participants with eGFR < 50 ml/min/1.73m2 (ADPKD vs CRIC) (eGFR 40-49, 800 vs 723 $p=0.0001$; eGFR 30-39, 745 vs 680 $p=0.0006$; eGFR 20-29, 726 vs 632, $p < 0.0001$) compared to eGFR-matched CRIC participants.

Conclusions: These data suggest that a renal phosphate leak occurs in ADPKD and is present across the spectrum of kidney function. Future studies are required to examine whether elevated levels of fibroblast growth factor 23 in ADPKD account for these findings.

Funding: NIDDK Support, Private Foundation Support

TH-PO814

Fractional Excretion of Phosphate Is Independently Associated with Renal Volume and Left Ventricular Mass Index in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) Berenice Y. Gitomer,[1] Kim McFann,[1] Myles S. Wolf,[2] Robert W. Schrier,[1] Michel B. Chonchol.[1] *[1]Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Nephrology, University of Miami Medical School, FL.*

Background: Small increases in serum phosphate within the normal range are associated with greater risk of all-cause mortality, cardiovascular events and kidney disease progression in patients with and without chronic kidney disease (CKD). While the precision for laboratory measurement of serum phosphorus is excellent (CVs < 3%), there is considerable diurnal variability in serum phosphate. This limitation may be overcome by concurrent measurements of serum phosphate and urinary phosphate excretion, to improve insight into phosphorus homeostasis in individual subjects. We hypothesized that higher urinary fractional excretion of phosphate (FEPO4) in patients with ADPKD would be associated with larger renal volume, a validated biomarker of more rapid progression of ADPKD, and increased left ventricular mass index (LVMI), a validated index of cardiovascular disease.

Methods: We measured FEPO4 in 683 adults with ADPKD who had available data on total kidney volume (TKV) measured by ultrasound. LVMI, measured by 2D-echocardiography, was available in 232 of these patients. Linear regression was used to assess the relationship between log transformed FEPO4 with log transformed TKV and LVMI.

Results: The mean (SD) age of the participants was 41(13) years and 61% were female. The mean (SD) estimated GFR, FEPO4, TKV and LVMI were 73 ± 36 ml/min, 26 ± 15%, 1630 ± 1284 ml^3, and 113 ± 37 g/m^2, respectively. Linear regression of lnTKV on lnFEPO4, adjusted for age, height, sex and hypertension, demonstrated a highly significant relationship ($\beta = 0.66 \pm 0.05$, $R^2 = 0.28$, $p < 0.0001$). In addition, FEPO4 was significantly related to lnLVMI ($\beta = 0.11 \pm 0.04$, $R^2 = 0.04$, $p = 0.0021$).

Conclusions: Abnormalities of phosphate homeostasis in ADPKD are associated with increased risk of progression of renal and cardiovascular disease. Future studies are necessary to whether disordered phosphate mechanism is a contributing cause or consequence of progression of renal and cardiac disease in ADPKD.

Funding: NIDDK Support, Private Foundation Support

TH-PO815

Achievement and Maintenance of Blood Pressure Targets in HALT:PKD Vicente E. Torres,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Ronald D. Perrone,[4] Dana C. Miskulin,[4] Theodore I. Steinman,[5] Franz Winklhofer,[6] William E. Braun,[7] Marie C. Hogan,[1] Frederic F. Rahbari-Oskoui,[3] Kaleab Z. Abebe,[8] James E. Bost,[8] Michael F. Flessner.[9] *[1]Mayo, Rochester, MN; [2]U CO, Denver, CO; [3]Emory U, Atlanta, GA; [4]Tufts U, Boston, MA; [5]Beth Israel, Boston, MA; [6]UKMC, Kansas City, KS; [7]Cleveland Clinic, Cleveland, OH; [8]U Pittsburgh, Pittsburgh, PA; [9]NIH/NIDDK HALT Study Grp.*

Background: HALT-PKD seeks to determine whether ACEI/ARB is superior to ACEI alone and low BP (<110/75) is superior to standard BP (120-130/70-80) in delaying cystic progression (CKD stage 1 or 2, Study A) and whether ACEI/ARB is superior to ACEI alone (BP target 110-130/70-80 mmHg) in slowing eGFR decline (CKD stage 3, Study B).

Methods: Stepwise dosing of lisinopril (LIS and telmisartan/placebo (P/T) (steps 1-4) followed by stepwise dosing of other agents (steps 5-10) is used to achieve BP targets. During 1/2006-5/2011, 516, 497, 464, 293, 141 A and 458, 446, 430, 253, 111 B patients completed 4, 12, 24, 36, 48-mth follow-up. BP control is assessed by home BP measurements.

Results:

	Mths	Study A Std	Study A Low*	Study B
Mean BP (mmHg)†	4	91.5±5.8	85.0±6.5	91.0±6.9
	12	91.7±6.3	83.7±6.7	91.5±5.8
	24	92.0±6.3	82.9±6.5	91.7±6.3
	36	92.2±6.3	82.2±6.1	91.5±6.2
	48	93.5±6.5	80.7±5.5	91.4±5.3
Step; LIS and P/T dose(mg)†	4	2.2, 15.0, 50.7	3.4, 24.0, 64.3	2.9, 18.2, 55.9
	12	2.2, 15.8, 50.9	3.7, 25.0, 66.2,	3.0, 18.7, 56.3
	24	2.3, 15.1, 51.0	3.9, 26.0, 65.8	3.2, 18.0, 56.1
	36	2.5, 16.1, 52.6	3.9, 26.0, 66.4	3.1, 17.5, 55.6
	48	2.4, 14.0, 51.3	4.2, 27.1, 68.3	3.0, 16.6, 53.8

† mean±SD. *Underlined: P<0.0001 compared to Study A standard.

Based on random regressions, significantly negative BP slopes in Study A low and significantly positive BP slopes in Study A standard result in increasing BP separation over time. Results are similar when only participants with complete data at each timepoint are included.

Conclusions: ACEI alone or ACEI/ARB achieve BP control and MAPs are within target in most subjects at 4-48 months of follow-up. Excellent and increasing separation in BP and doses of LIS and T/P between study A arms is achieved, without detectable differences in heart rate.

Funding: NIDDK Support

TH-PO816

Serum Uric Acid and Renal Disease Progression in Autosomal Dominant Polycystic Kidney Disease Imed Helal, Berenice Y. Gitomer, Kim McFann, Xiang-Dong Yan, Godela M. Brosnahan, Robert W. Schrier. *Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: Higher serum uric acid (SUA) levels are associated with an increased risk for cardiovascular disease. Increased SUA levels are also associated with hypertension, a strong risk factor for ADPKD progression. We examined the hypothesis that SUA levels correlate with progression of ADPKD.

Methods: This retrospective study included 716 ADPKD adults from the University of Colorado ADPKD registry. SUA was examined as gender specific quartiles - quartiles1 - 4 in women: <4.2, 4.3 - 5.1, 5.2-6.5 and >6.5 mg/dL; quartiles 1 - 4 in men :< 5.7, 5.71 - 6.8, 6.9 - 8.2 and >8.2 mg/dL. The study evaluated the association of baseline uric acid levels with ADPKD progression and age at end-stage renal disease (ESRD).

Results: In linear regression, SUA was significantly related to LnTKV (total kidney volume) ($\beta = 0.15 \pm 0.08$, $p < 0.0001$) and Ln TKV/BSA ($\beta = 0.13 \pm 0.01$, $p < 0.0001$). SUA was also significantly related to BMI (body mass index) ($\beta = 0.78 \pm 0.10$, $p < 0.0001$). Those who had SUA in the 3rd and 4th quartile were significantly older, had higher BMI, higher serum creatinine (adjusted for age and sex), lower creatinine clearances (adjusted for age and sex), higher TKV (adjusted for age and sex), higher TKV/BSA, and higher urinary protein than those in the 1st quartile and 2nd quartile. In a sub-group analysis, the 4th quartile had higher microalbumin excretion than those in the 1st quartile. History of hypertension was more prevalent among those ADPKD patients in the 3rd and 4th quartiles of SUA. 503 ADPKD patients had data available on age at ESRD or last known age without ESRD. There was a difference in survival curves among the 4 quartiles of SUA ($p < 0.0001$). Median survival to ESRD was 68 (58-81) years for the 1st Quartile, 70 (64- No Upper) for the 2nd Quartile, 61 (56-65) years for the 3rd Quartile, and 57 (54-60) years for the 4th Quartile. Survival time was shortest for those in the 4th Quartile.

Conclusions: Thus, SUA correlates with age, BMI, impaired kidney function, hypertension, proteinuria, kidney volume and ESRD in ADPKD patients.

Funding: NIDDK Support, Private Foundation Support

TH-PO817

The Effect of Everolimus Dose and Schedule on Renal Angiomyolipoma in Patients with Tuberous Sclerosis Complex John J. Bissler,[1] Elizabeth Jo Coombs,[1] Bradley P. Dixon,[1] David N. Franz.[2] *[1]Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Inhibition of the mTORC1 pathway in patients with tuberous sclerosis complex leads to a reduction of renal angiomyolipoma volume. The optimal dose and frequency of mTORC1 inhibitor for these patients is unknown. Determining the proper dosing is critical for patient care in order to maximize the angiomyolipoma treatment effect while minimizing patient toxicity. To begin to understand how to treat patients, we undertook a phase two non-randomized open label trial. Thirty patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis and renal angiomyolipoma(s) were evaluated. Renal angiomyolipoma volume was used as the primary endpoint, while safety was included among the secondary outcomes.

Methods: Patients received everolimus (RAD001) for one year, and were observed off drug for an additional year. We examined two different daily doses (5 or 10 mg) and three different weekly dose (30, 50, or 70 mg) regimens. Each treatment group contained a minimum of five patients.

Results: A total of 36 patients enrolled and a total of 30 completed the two-year study. The median age was 32 and there were 10 male and 26 female patients. The average reduction of the angiomyolipoma volume was approximately fifty percent at twelve months. Although once off drug, lesions demonstrated a range of responses from maintaining their reduction in volume to increasing back toward baseline size for both daily and weekly dosing. Grade three adverse events thought to be possibly, probably or definitely related to drug were infrequent, only three in the daily dosing and only one in the weekly dosing cohort.

Conclusions: Both weekly and daily dosing resulted in approximately a fifty percent reduction in angiomyolipoma volume. These results raise the possibility that lower doses, and different dosing schedules may be an option for patients not tolerating higher daily dosing.

Funding: Pharmaceutical Company Support

TH-PO818

Efficacy and Safety of mTOR Inhibitor for Early-Stage Autosomal Dominant Polycystic Kidney Disease Patients: A Meta-Analysis of Randomized Controlled Trials Qiang He, Chiayu Lin, Shunxian Ji, Jianghua Chen. *Kidney Disease Center, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

Background: The objective of this study is to conduct a meta-analysis of the randomized controlled trials (RCT) to make a profound review and an objective appraisal of the effectiveness and safety of the mammalian target of rapamycin (mTOR) inhibitor therapy in autosomal dominant polycystic kidney disease (ADPKD) patients.

Methods: RCTs regarding the mTOR inhibitor therapy in ADPKD patients are included. The data of studies and major outcomes include the changes of patients' Glomerular Filtration Rate (GFR), urinary protein, total kidney volume (TKV), cyst volume (CV), parenchymal volume (PV), blood pressure, lipid profile and the frequency of adverse events.

Results: Up to January 31st, 2011, 4 RCTs (568 patients) are included in this study. The meta-analysis made in this study indicates that mTOR inhibitor therapy group had smaller TKV than the control group, WMD of TKV after treatment is -302.08 (P=0.04), and also less increasing of TKV (WMD=-70.25, P<0.00001), CV (WMD =-35.91, P<0.00001) and PV (WMD =-34.84, P<0.00001). Also mTOR inhibitor treatment doesn't necessarily slow down the aggravation of renal function. Side effects like hyperlipidemia, myelosuppression, abnormal liver function, diarrhea, rash, aphthous stomatitis could occur during the mTOR inhibitor therapy, but the severities can be controlled by the appropriate use of drug.

Conclusions: Based on the current very limited clinical trials, mTOR inhibitor is a relatively safe drug to slow down the kidney volume growth in ADPKD patients, especially in the parenchymal tissues, but have limited impact on slowing down the decrease of renal function.

TH-PO819

Early Progression Markers in Autosomal Dominant Polycystic Kidney Disease. A Longitudinal Study in Patients with Normal GFR Pablo J. Azurmendi,[1] Adriana R. Fraga,[1] Marta G. Valdez,[3] Elvira Arrizurieta,[1] Rodolfo S. Martin.[1,2] *[1]Experimental Nephrology, Inst. de Invest. Méd. Alfredo Lanari, UBA., Buenos Aires, Argentina; [2]Hospital Universitario, Universidad Austral, Pilar, Buenos Aires, Argentina; [3]Metabolism, Inst. de Invest. Méd. Alfredo Lanari, UBA., Buenos Aires, Argentina.*

Background: It is well known that total renal volume (TRV) is a predictor of ADPKD progression. However, parameters other than TRV are also currently explored in early stages of the disease, when glomerular filtration rate (GFR) is still preserved. We have previously reported that both urinary monocyte chemoattractant protein-1 (MCP-1) and albuminuria, assessed as urinary albumin/creatinine (UACR), are candidates for early markers of progression. High UACR (>6.8 mg/gCr) was associated with both high levels of urine MCP-1 and carotid-intima media thickness as well, as compared with normal UACR (≤6.8).

To investigate whether there is an interaction among TRV, GFR and MCP-1 and a role for UACR as a predictor of disease severity, we performed a longitudinal study of 30±1 months in 32 young ADPKD patients (26±1 years old).

Methods: TRV was measured by ultrasound, urine MCP-1 by ELISA and GFR estimated by MDRD.

Results: TRV, GFR and urine MCP-1 baseline values were 415 ± 52.8 ml, 108 ± 3 ml/min/1.73m^2 and 152 ± 32 ng/gCr, respectively.

An association among the annual change in TRV, GFR and urine MCP-1 was found, independently of their basal values, UACR, age, sex or antihypertensive treatment. The annual change in TRV and urine MCP-1 was increased in high UACR (131 ± 33 ml and 108 ± 49%), as compared with normal UACR patients (48 ± 41 ml and - 5 ± 16 %, p<0.05, respectively). The GFR annual change was not different according to UACR and remained stable in patients treated with angiotensin converting enzyme inhibitors as compared with untreated normotensive subjects (3 ± 5 and -5 ± 2 ml/min/year, p<0.01).

Conclusions: Being MCP-1 and TRV markers of renal inflammatory and cystic component respectively, our results suggest an involvement of both processes in ADPKD progression, even when GFR is within normal limits. Besides this, slight increased rates of albuminuria could be also a predictor of worst prognosis.

Funding: Government Support - Non-U.S.

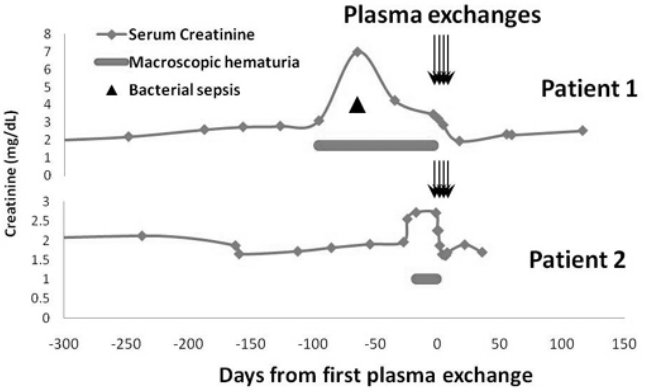
TH-PO820

Treatment of Complement Factor H-Related Protein 5 (CFHR5) Nephropathy with Plasma Exchange Daniel P. Gale,[1] Yiannis Athanasiou,[2] Michalis Zavros,[2] Constantinos Deltas,[3] Alkis Mikis Pierides,[2] H. Terence Cook,[1] Patrick Maxwell.[4] *[1]Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom; [2]Nephrology Department, Nicosia General Hospital, Nicosia, Cyprus; [3]Biological Sciences Department, University of Cyprus, Nicosia, Cyprus; [4]Division of Medicine, University College, London, United Kingdom.*

Background: CFHR5 nephropathy is a recently recognised autosomal dominant disease which is endemic in Cyprus (1). It is characterised by macroscopic hematuria with stepwise deteriorations in renal function during intercurrent infections and is associated with a heterozygous internal duplication mutation of the CFHR5 gene which results in the production of a larger protein that is detectable in the blood (1). Kidney biopsies show C3 glomerulonephritis, implying that complement alternative pathway dysregulation underlies the disease. Over 80% men (but <20% women) develop renal failure by age 50 (2). Disease can recur following renal transplantation, proving that it results from a defect of a circulating factor (3). This implies that correction of circulating complement regulation during acute flares might be effective.

Methods: 2 patients with CFHR5 nephropathy were treated with 4-7x3 liter plasma exchanges against fresh frozen donor plasma during episodes of macroscopic hematuria with persistently elevated serum creatinine above baseline.

Results: In both patients there was immediate cessation of macroscopic hematuria and rapid return of serum creatinine to the pre-flare baseline.



Conclusions: While further clinical studies are needed to determine if plasma exchange can alter the long term natural history of CFHR5 nephropathy, these cases provide initial evidence that manipulation of circulating complement regulators might be effective in this disease.

(1) Gale et al Lancet 2010
(2) Athanasiou et al CJASN 2011
(3) Vernon et al Am J Transpl 2011

Funding: Government Support - Non-U.S.

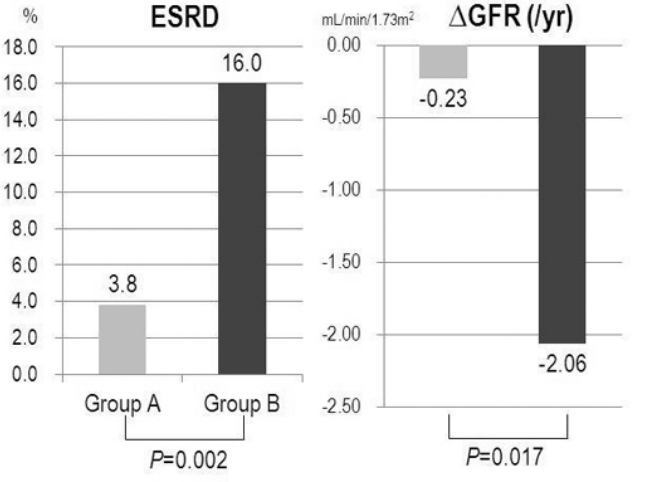
TH-PO821

Chronic Asymptomatic Pyuria Precede Overt Urinary Tract Infection and Renal Function Deterioration in ADPKD Jin Ho Hwang,[1] Young-Hwan Hwang,[2] Hayne C. Park,[1] Yu-Kyoung Yun,[1] Dong Ki Kim,[1] Kook-Hwan Oh,[1] Kwon Wook Joo,[1] Yon Su Kim,[1] Jin Suk Han,[1] Curie Ahn.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Internal Medicine, Eulji University School of Medicine, Seoul, Korea.*

Background: Urinary tract infection(UTI) is estimated to occur in 30%-50% of individuals with autosomal dominant polycystic kidney disease(ADPKD). However, the clinical manifestation and significance of asymptomatic pyuria(AP) in ADPKD are unknown.

Methods: We reviewed medical records of ADPKD patients who were registered to the ADPKD registry of Seoul National University Hospital from Aug 1999 to Aug 2010. We defined the AP as more than 5-9/HPF of urinary WBCs with no related symptom of overt UTI. AP was categorized into 4 groups depending on its duration and frequency: non-pyuria, transient, recurrent, and persistent pyuria. The former two group were combined into group A, and the latter two groups were combined into group B(chronic pyuria(CP) group). The prevalence of overt UTI and association with renal function decline were examined between these groups.

Results: With a mean follow-up of 66.2 months, 178(70.0%) out of 258 patients showed 688 episodes of AP and 55 episodes of UTI(6.6%). AP showed the incidence of 0.483 episodes/patient/year. Patients in group B showed female predominance, were more hypertensive(93.0% vs. 81.0%, *P*=0.01), and developed more ESRD(16.0% vs. 3.8%, *P*=0.002) compared to group A. Upper UTI occurred more frequently in group B patients(HR 4.5, 95% CI 1.69-12.02; *P*=0.003, when adjusted for gender and hypertension). The rate of decline in GFR(ΔGFR/yr) was significantly faster(-2.06 vs -0.23, *P*=0.017) in group B than group A.



Age and the CP were the independent factors associated with ΔGFR/yr.

Conclusions: CP may increase the risk of developing overt UTI and may be a contributing factor to renal function decline in ADPKD patients.

TH-PO822

Urinary N-Acetyl-β-D-Glucosaminidase as a Surrogate Marker for Renal Function in Autosomal Dominant Polycystic Kidney Disease Hayne C. Park,[1] Jin Ho Hwang,[1] Yu-Kyoung Yun,[2] Myeong-Ok Yoon,[3] Ah-Young Kang,[3] Jaeseok Yang,[1] Kook-Hwan Oh,[1] Jung-Woo Noh,[4] Young-Hwan Hwang,[5] Curie Ahn.[1] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Clinical Trials Center, Seoul National University Hospital, Seoul, Korea; [3]Transplantation Research Institute, Seoul National University Hospital, Seoul, Korea; [4]Department of Internal Medicine, Hallym University College of Medicine, Seoul, Korea; [5]Department of Internal Medicine, Eulji University College of Medicine, Seoul, Korea.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive renal cyst growth leading to end-stage renal disease. Because both serum creatinine (Cr) and total kidney volume (TKV) measurements are considered of limited use to monitor disease progression, we investigated urinary N-acetyl-β-glucosaminidase (NAG) as a useful surrogate marker in this prospective study.

Methods: From Apr 2010 to May 2011, a total of 163 patients were enrolled from SNUH ADPKD registry and 139 patients were followed up for 6months. GFR was estimated using MDRD equation and TKV was measured by modified ellipsoid method. We measured urinary NAG, β2-microglobulin, and neutrophil gelatinase-associated lipocalin (NGAL) and compared their predictive values for renal function.

Results: The mean age of subjects was 47 years. The baseline eGFR and TKV were 69.6 ± 20.0 mL/min/1.73m^2 and 1351.4 ± 977.2 mL, respectively. Log NAG/Cr was negatively correlated with both eGFR (r^2=0.169, p≤0.001) and TKV (r^2=0.043, p=0.01) at baseline. By using ROC curve analysis, AUC of NAG/Cr for decreased eGFR (<60mL/min/1.73m^2) (0.77, 95% CI [0.72-0.81]) was higher than those of NGAL (0.61, 95% CI [0.55-0.66]) and β2-microglobulin/Cr (0.71, 95% CI [0.66-0.76])(p<0.01 and p=0.012, respectively). Log NAG/Cr was also well correlated with the change in eGFR (r^2=0.051, p=0.005). The persistently high NAG/Cr (>4.3 IU/g) group showed renal function decline whereas the persistently low group revealed increased eGFR over 6months (-0.25 ± 17.6 vs. 9.4 ± 16.8 ml/min/1.73m^2 per year, p=0.005).

Conclusions: Urinary NAG may be a good and reliable biomarker to predict renal progression in ADPKD patients.

TH-PO823

Progression of Chronic Kidney Disease in Patients with Autosomal Dominant Polycystic Kidney Disease Nayara Panizo, Marian Goicoechea, Soledad Garcia de Vinuesa, David Arroyo, Claudia Yuste, Abraham Rincón, Caridad Ruiz Caro, Ursula Verdalles, Borja Quiroga, Jose Luno. *Nephrology, Hospital General Universitario Gregorio Marañón, Madrid, Spain.*

Background: The aim of this study was to analyze the factors influencing chronic kidney disease (CKD) progression in patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD).

Methods: We studied 101 patients (mean age 43 ±17.3, 43.56 % male) followed during a mean follow-up time of 77.9 ± 48 months from 1997 to 2010. The primary end point was : time to a 50% decrease of estimated glomerular filtration rate (eGFR) (CKD-EPI) since the first-time visit and/or time to initiation of renal replacement therapy, annual mean change of eGFR was also analysed . Clinical and demographic data, blood pressure, concomitant medications and analytical parameters were collected at each visit.

Kidney size by ultrasound was also recorded at baseline.

Results: Thirty-one patients achieved the primary end point after a mean time of 84.8 ± 39.1 months. Those patients who achieved the primary end point had higher SBP and DBP (p=0.017 and p=0.001), higher LDL-cholesterol (p=0.011), higher creatinine (p=0.006), higher uricemia (p=0.041), more severe proteinuria (p=0.033) and greater kidney size (p=0.05). The mean annual eGFR change was of – 3.52 ± 7.3 ml/min/1.73 m^2. Forty nine patients had a rapid decline renal function: Group A (higher than -3.52 ml/min/1.73 m^2) and 52 patients had a lower renal disease progression: Group B (< -3.2 ml/min/1.73 m^2). Adjusted Cox regression analysis showed that higher SBP and younger age at the first visit were independent variables for poorer renal outcome (p= 0.026).

Conclusions: Initial kidney function, proteinuria, renal size, hypercholesterolemia, hiperuricemia and SBP are the factors that influencing CKD progression in ADPKD. SBP and younger age are the only factors that maintain their independent predictive value in multivariant analysis.

TH-PO824

Progressive Nephromegaly Emerges during Adolescence in Children with ADPKD John F.S. Crocker, Philip D. Acott. *Nephrology, IWK Health Centre, Halifax, NS, Canada.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) has a major impact on the cost of health care. We previously described a clinical phenotype of children with ADPKD including variations of intracranial lesions (MRI angiography) in the subgroup with headaches, stone formation risk factors (i.e. glycinuria), hypertension, proteinuria, and dyslipidemia. This study evaluates serial renal imaging of ADPKD children followed from referral through adolescence.

Methods: We have been referred >300 children over a twenty year period with a family history of ADPKD. Parental and family concerns trigger the referral, especially if a family member with ADPKD had RRT or progressive renal failure.

Results: Sixty-four children of this cohort had radiographic cystic features with ultrasound consistent with ADPKD. Serial ultrasound assessments identified a group of 15 children with evolving significant nephromegaly (kidney size > 95th percentile expected for age, gender, weight and height) during adolescent development. In this sub-group during puberty, six had unilateral (left = 4; right =2) nephromegaly (female = 3; male = 3) and nine patients had symmetrical rapid renal growth (female = 6; male = 3). Many of these children had serial follow up for > 10 years. All children with nephromegaly, whether hypertensive or not, were placed on an ACE inhibitor (ACEi), as we suspect the emergence of nephromegaly during adolescence may influence their risk of renal decline during adulthood. Three of these treated children had reversal of renal size to normal limits. The sub optimal response to ACEi may reflect ACEi dosing, adolescent drug adherence, or ineffective ACEi effect once nephromegaly is present in the ADPKD adolescent population. Many of the children who entered the special program are now young adults and have stable cystic disease.

Conclusions: ADPKD is a disease which may be diagnosed clinically by cyst formation in young children, 15% of which have rapid renal growth and nephromegaly during puberty. It is possible this is a key marker of the cohort at risk of renal decline as adults. Further follow up and evaluation of the adolescent phenotype of ADPKD may sort out which group might benefit from early medical intervention.

TH-PO825

Renal Functional Involvement in Bardet-Biedl Syndrome – Largest Ever National Survey from England and Wales David Goldsmith, Mark Jenkins, Shehla Mohammed, Barbara McGowan. *Renal and Genetics Departments, Guy's and St Thomas' Hospitals, London, United Kingdom.*

Background: Bardet–Biedl syndrome (BBS) is a rare ciliopathic autosomal recessive disorder that affects many body systems. BBS has a prevalence of 1 in 140,000 to 1 in 160,000 newborns It is a pleiotropic disorder with variable expressivity and a wide clinical variability observed within and between families. Renal dysfunction is a major cause of morbidity and mortality. In 2009 the National Commissioning Group for Chronic Conditions set up a national clinical service to provide holistic, organized care for these patients.

Methods: This national service started in 2010, and has two adult centres – in London and Birmingham. There are very few systematic reports of kidney involvement in BBS, so we have compiled data from these centres for all patients seen in 2010-2011 to produce the largest single reported series with special reference to renal functional involvement and urinalysis findings.

Results: 111 subjects (63 men, 48 women) with a mean age of 33 years (range 15 to 56 years) attended the clinics. Three patient were on regular hemodialysis, one on peritoneal dialysis, and five others had functioning renal transplants. Blood samples for renal function were obtained from 98 patients. Plasma creatinine values were 44 to 803 umol/L, and using the 4 variable MDRD formula the eGFR range was 11 to > 90 mls/min. The mean GFR was 83 mls/min with 44 subjects having an MDRD eGFR > 90 mls/min. Significant proteinuria (urinary spot ACR/PCR) was evident in 19.4% of subjects, but only 7 subjects had a PCR > 10 (maximum recorded protein loss was 82 mg/mmoL). Microscopic haematuria was seen in < 10% of subjects. Mean BP was 140/83 mm Hg; 38% of patients had a SBP > 140 mm Hg and 26% had a DBP > 90 mm Hg. Obesity was common with the mean BMI for these subjects was 37 kg/m2.

Conclusions: This is the largest reported series featuring kidney involvement in BBS. About half of the subjects had evidence of kidney disease (within the limitations of single measurements of blood and urine) – around 20% of the patients had significant reduction of GFR, had a transplant, or were on dialysis. Neither heavy proteinuria nor haematuria was a feature of BBS.

Funding: Government Support - Non-U.S.

TH-PO826

What's Lurking in Autosomal Dominant Polycystic Kidneys below Detection by Current Magnetic Resonance Imaging (MR)? Sumanth Mulamalla,[1] Larry Cook,[2] Connor J. Grantham,[1] Jared J. Grantham.[1] *[1]Kidney Institute, Kansas Univ Medical Center, Kansas City, KS; [2]Radiology, Kansas Univ Medical Center, Kansas City, KS.*

Background: Cyst formation and growth are targets in the development of new drugs for the treatment of ADPKD. The CRISP MR study established that sustained cyst growth accounted for the impressive increase in kidney size and suggested that new cysts appeared in adults. A recent analysis of individual cyst growth rates suggested that exponential cyst growth beginning in the fetus could be the deciding factor leading to renal enlargement and declining GFR.MR scans can detect cysts >2mm in outer diameter (OD) so the appearance of new cysts in sequential exams could be due to small cysts growing above the detection limit rather than due to the formation of new cysts in normal-sized renal tubules.

Methods: To determine the number of cysts below the MR detection limit in ADPKD we measured the mean diameter (um) of cysts in random histologic sections of nephrectomy specimens from 6 adult patients with early (CKD <3; n=4 cases, 278 cysts) and more advanced pre-dialysis stages (CKD >3; n=2 cases, 134 cysts). A cyst had to exhibit an epithelial lining with an OD >200 um to avoid mis-labeling dilated tubules and empty glomerular capsules.

Results: In early stage,cyst OD was <2000 um in 91.4 % and would have gone undetected by MR;OD was <1000 um in 76.2 %. In later stage 87.3 % had OD <2000 um; in 64.9 % OD was <1000 um, similar to the early stage. So when did the invisible cysts develop? Assuming (early stage mean age 37y) that most cysts derived from collecting ducts as large as 100 um OD and grew at 17 %/y (CJASN 5:889,2010), cysts formed a few weeks after birth could have achieved diameters of 200, 400, 800 and 1000 um in 12, 25, 37 and 41 y, respectively; and 12 more y to reach MR detection limit of 2000 um.

Conclusions: 1. More renal cysts develop in ADPKD than meet the eye by conventional imaging; 2. After a period of extraordinary fetal cyst formation and growth, new cysts appear to form in renal tubules for several years thereafter; 3. Drugs targeting post-partum cyst formation may be beneficial if administered early in the course of the disease.

Funding: NIDDK Support

TH-PO827

Magnetic Resonance Imaging (MRI) to Non-Invasively Measure ARPKD Kidney Disease Progression Lan Lu, Christopher A. Flask, Bernadette O. Erokwu, Katherine M. Dell. *Depts of Radiology, Biomedical Engineering, Pediatrics and the CWRU Center for the Study of Kidney Disease and Biology, Case Western Reserve University, Cleveland, OH.*

Background: Autosomal Recessive Polycystic Kidney Disease (ARPKD) results in kidney failure in 40-50% of children. While some therapies have shown promise in animal models, the lack of methods to monitor kidney disease progression in ARPKD patients has limited the development of therapeutic trials. Newer quantitative Diffusion Tensor Imaging Magnetic Resonance Imaging (DTI-MRI) techniques have the potential to provide non-invasive assessments of kidney disease progression, but have not been studied in ARPKD. The objective of this study was to develop DTI-MRI methodologies to measure kidney disease progression in the PCK rat model of ARPKD.

Methods: Kidneys from 4 PCK rats were imaged serially at 2,4 & 6 mos of age using DTI-MRI. Apparent Diffusion Coefficient (ADC) maps were generated, then thresholded to differentiate cystic (higher ADC) from normal tissue. Total cystic area was defined as the cyst pixel number/total kidney pixels. Animals were sacrificed after the last imaging session at 6 mos and histologic scoring for cystic area (%cystic/total parenchyma) of hematoxylin stained paraffin-embedded sections was determined.

Results: Mean cystic area (%) increased at each time point studied (2 mos = 17±11; 4 mos= 36±9; 6 mos= 48±7). Average increase in cystic area over the 4 month study period was 8% per month (range =4-11%), with higher values seen in the 2-4 vs. 4-6 months intervals (10% vs. 6%). There was a strong correlation (r=0.67) between imaged cystic area and cystic area as assessed by histologic scoring at 6 months.

Conclusions: DTI-MRI provided non-invasive, quantitative measures of kidney disease severity in PCK rats at different time points in the disease. Increased cystic area was found at each successive time point, including the 4 to 6 mo interval, for which we have previously shown histologic progression without significant change in clinical parameters (e.g. kidney weight/body weight or serum creatinine). Further studies are necessary to determine if these methods can be used to monitor kidney disease progression in ARPKD patients.

Funding: NIDDK Support, Other NIH Support - NIH/CTSA

TH-PO828

Cyst Infection in ADPKD: A Critical Analysis of PET-CT Bruno E. Balbo,[1] Marcelo T. Sapienza,[2] Luiz F. Onuchic.[1] *[1]Nephrology, Univ. of Sao Paulo, São Paulo, Brazil; [2]Radiology, Univ. of Sao Paulo, São Paulo, Brazil.*

Background: Cyst infection (CI) remains a complex issue in ADPKD. Positron emission tomography (PET) has emerged as a promising tool for diagnosis.

Methods: A retrospective study was conducted in an ADPKD referral center, including 12 CI episodes in 9 patients (pts) during 12 months. The diagnosis was definite (microbiological) in 4 episodes and likely in 8 (fever, abdominal pain, C-reactive protein >50mg/L, absence of intracystic bleed and exclusion of other causes).

Results: Liver CI (LCI) occurred in 8 episodes (6 pts) and kidney CI (KCI) in 4 (4 pts). Pts with KCI were non-significantly younger and had a trend to lower creatinine clearance compared to LCI pts (21.5 vs 42.0 mL/min/1.73m^2). Four KCI and 2 LCI were associated with positive blood cultures and sepsis, whereas urine culture was positive in 1 KCI. Ciprofloxacin was the first-line antibiotic (ATB) in 8 episodes and association with a third-generation cephalosporin was used in severe presentations (9/12). Initial evaluation included 12 CTs and 1 MRI, suggestive of infection in only 4 cases (3 CTs and 1 MRI). PET-CT was performed in 9 episodes and PET Scan in 2. Total kidney volume (TKV) was non-significantly higher in pts with KCI than LCI while LCI pts showed a trend to higher total liver volume (TLV) than KCI individuals (5516 vs 2043 mL, P=0,063). Seven PET-CT and 2 PET Scan analyses were consistent with CI. Seven cases were submitted to invasive procedures and in 1 a follow-up PET-CT supported a prolonged ATB course. Two deaths occurred in pts with negative PET-CT in whom LCI was diagnosed by autopsy: a kidney Tx and immunosuppressed pt and a cirrhotic and hemodialytic pt. In both cases PET-CT was carried out on the third week of ATB while in the majority of the other cases it was performed within the first two weeks.

Conclusions: In our casuistic LCI included pts in earlier stages of CKD and outnumbered KCI. Our data suggest that increases in TLV and TKV may raise the risk of LCI and KCI, while the observed high reinfection rate supports that anatomical factors may play a role in CI. PET-CT, in turn, was associated with a high CI diagnostic sensitivity which apparently drops over time after initiation of ATB treatment.

Funding: Government Support - Non-U.S.

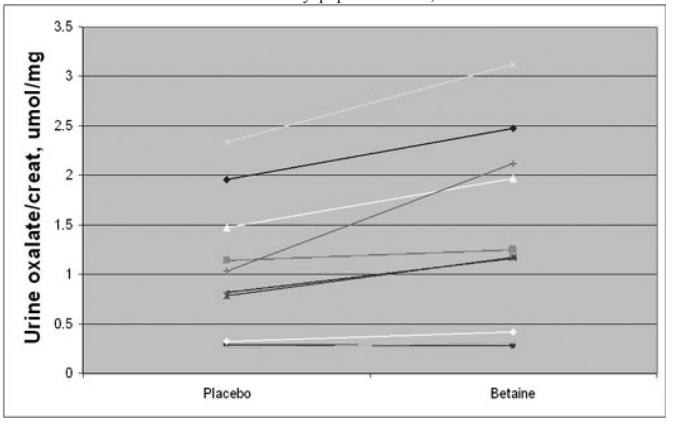
TH-PO829

Effect of Betaine on Urine Oxalate in Primary Hyperoxaluria, Type I Carla G. Monico,[1] Julie B. Olson,[1] Eric J. Bergstralh,[2] Raymond L. Heilman,[3] Dawn S. Milliner.[1] *[1]Division of Nephrology, Mayo Clinic, Rochester, MN; [2]Department of Health Sciences Research, Mayo Clinic, Rochester, MN; [3]Division of Nephrology, Mayo Clinic, Phoenix, AZ.*

Background: In primary hyperoxaluria, type I (PHI) deficiency of hepatic AGT enzyme results in overproduction of oxalate, hyperoxaluria, stones, and kidney failure. Pyridoxine (VB6) treatment reduces urine oxalate in patients (pts) with certain *AGXT* genotypes, suggesting a chaperone effect. Betaine partially corrected enzyme activity of the I244T mutation *in vitro* (Santana 2003). To ascertain effectiveness in PHI patients, we conducted a double blind, placebo controlled trial of betaine in children and adults with PHI who had missense mutations.

Methods: Subjects were randomly assigned oral betaine 10 gm (> 10 yrs old) or 6 gm (< 10 yrs old) or lactose placebo twice daily for 2 months, followed by a 2 month washout. Each then received the alternate study medication for 2 months. Usual medications continued throughout included VB6 (n= 9), neutral phosphate (n= 7), and citrate (n= 3). Two 24 hr urine collections were obtained at baseline, and during the eighth week of each study period. Uox was measured by oxalate oxidase.

Results: 10 of 15 enrolled PHI pts completed the study: 2 withdrew before initiation, 2 were noncompliant, in 1 symptoms led to withdrawal. Mean age was 20.1 yrs (median 18.5, range 6-43 y.o.). GFR was 79 ml/min/1.73m2 (median 92, range 39-134). Uox on betaine was 1.43 ± 0.97 umol/mg creat and differed from placebo 1.04 ± 0.71 by paired t test, p = 0.007. Betaine was well tolerated with dyspepsia in some, but no serious adverse effects.



Conclusions: Uox/creat was higher in pts receiving betaine compared with placebo. The mechanism is unclear and merits further investigation. Results suggest that caution is warranted in interpretation of *in vitro* chaperone effects.

Funding: NIDDK Support, Other NIH Support - ORDR, member of Rare Diseases Clinical Research Network, Pharmaceutical Company Support, Private Foundation Support

TH-PO830

Mutation Screening of the HALT PKD Population Jamie L. Sundsbak,[1] Christina M. Heyer,[1] Sandro Rossetti,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Vicente E. Torres,[1] Ronald D. Perrone,[4] Jared J. Grantham,[5] Theodore I. Steinman,[6] William E. Braun,[7] Kyong Tae Bae,[8] Kaleab Z. Abebe,[8] James E. Bost,[8] Peter C. Harris.[1] *[1]Mayo Clinic; [2]U Colorado; [3]Emory U; [4]Tufts U; [5]Kansas U; [6]Beth Israel Deaconess Med Ctr; [7]Cleveland Clinic; [8]U Pittsburgh Med Ctr.*

Background: The genetic characterization of the HALT-PKD population (1044 cases) provides a comprehensive view of the genetics of this disorder and how genotype correlates with phenotype

Methods: PKD1 and PKD2 were analyzed using conventional Sanger sequencing, plus MLPA to assay for large rearrangements.

Results: We have screened 804 families consisting of 916 subjects (73% PKD1, 14% PKD2, 13% No mutation detected). Mutations were identified in 700 families (84% PKD1, 16% PKD2). Truncating changes made up the largest functional mutation group in both PKD1 (68% truncating, 32% non truncating) and in PKD2 (89% truncating, 11% non truncating). Comparison with the ADPKD Mutation Database Version 2.1 showed 70% were novel.

Several patients with interesting mutation combinations were found: two PKD1 truncating (in cis), a PKD2 nonsense and PKD1 in-frame deletion, and combinations of two likely hypomorphic alleles in cis or trans.

As expected in age corrected data, PKD1 cases had more severe disease with lower eGFR ($p<0.001$) and larger total kidney volumes (TKV; $p<0.001$) than PKD2. Looking at extreme deciles for TKV, only 1/43 was PKD1 in the mild group but 45/45 in the severe group. More interestingly, in the PKD1 population, cases with truncating mutations had a lower eGFR than in-frame ones ($p<0.001$). In the smaller PKD1 population with TKV data, cases with truncating mutations tended to have larger kidneys but the difference was not significant ($p=0.161$). However, in the extreme deciles, 33/34 with the most severe disease had truncating mutations compared to 12/29 in the mildest group. This data indicates that a minority of PKD1 mutations are not fully penetrant.

Conclusions: The characterization of the mutations in this clinically well defined population will greatly aid molecular diagnostics of ADPKD and other genetics studies of this disorder

TH-PO831

Autosomal Dominant Polycystic Kidney Disease (ADPKD) Presentation Is Often Influenced by *PKD1* Hypomorphic Alleles Christina M. Heyer, Sandro Rossetti, Katharina Hopp, Jamie L. Sundsbak, Vicente E. Torres, Peter C. Harris. *Mayo Clinic, Rochester, MN.*

Background: ADPKD, a dominantly inherited kidney condition, is typically caused by single inactivating mutations to *PKD1* or *PKD2*, and on average results in end stage renal disease (ESRD) at 54y and 74y, respectively. However, we recently described *PKD1* hypomorphic alleles that modify the disease presentation. Homozygotes or compound heterozygotes had typical to infantile onset disease, while a hypomorph and inactivating mutation caused early onset ADPKD. The role of hypomorphic alleles has been proven by generating a knock-in mouse model of the R3277C variant. Homozygotes are viable with slowly progressive disease (similar to adult onset ADPKD), while compound heterozygotes with a null allele ($Pkd1^{R3277C/-}$) have early onset disease.

Methods: *PKD1* and *PKD2* were sequenced in all probands by direct Sanger sequencing followed by computational analysis and family segregation of potential mutations.

Results: Analysis of ADPKD families with unusually mild or severe disease has yielded further examples where *PKD1* hypomorphic alleles effect the disease presentation. Mild ADPKD, which seldom causes ESRD, was observed in three families where the only detected variant was IVS37-3 C>T. Allele L2816P is also likely incompletely penetrant as it was found to segregate in families with mild disease. Sometimes, more than one allele seems to be required to cause ADPKD. Examples are I3167F inherited in *cis* with R3277H, and N970K in *cis* with V609G. In utero onset ADPKD with enlarged kidneys was observed in three families when a truncating allele was inherited in *trans* with a likely hypomorphic allele. Examples of these hypomorphic alleles are S4189F, R4276W, and a novel splice site in exon 5. A hypomorph in *trans* with an inactivating allele may also result in early ESRD. In one family, the PKD1 variant G2452C was associated with just a few cysts in heterozygosity but ESRD at 25y when found with a truncating mutation.

Conclusions: Together with our previous data, these families illustrate the crucial role hypomorphic alleles play in influencing disease severity in ADPKD.

TH-PO832

PKD Mutational Analysis Using Next- Generation Sequencing in Czech Patients Jitka Stekrova,[1] Jana Reiterová,[2] Petr Lnenicka,[1] Vladimir Tesar.[2] *[1]Department of Biology and Human Genetics, 1st Medical Faculty and General Teaching Hospital, Prague, Czech Republic; [2]Department of Nephrology, 1st Medical Faculty and General Teaching Hospital, Prague, Czech Republic.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of inherited kidney disease that results in renal failure. ADPKD is a systemic disorder, with cysts and connective tissue abnormalities involving many organs. The disease is caused by mutations of *PKD1* and *PKD2* genes. In the PKD database 846 different variants of the PKD1 gene and 139 different variants of the PKD2 gene have been reported.

Methods: The direct detection of mutations in both *PKD* genes was performed within research projects in our laboratory. Next-generation sequencing method on GS Junior (Roche) analyzer was used. Mutational analysis was performed in relatives in ADPKD families with very-early onset of the disease to detect hypomorphic alleles probably inherited from healthy parents.

Results: The mutation analysis of *PKD1* gene has so far detected probably causal mutations in 57 families.42 mutations are unique for Czech population. Only the described nonsense mutation p.R4021X was detected in three nonrelated families and the described missense mutation p.E2771K was detected in two families; other mutations are unique for individual families; 42 mutations are unique for Czech population.

The mutation analysis of *PKD2* gene has so far detected probably causal mutations in 36 families. The frameshifting mutation c.203_204insC was identified in 9 families, nonsense mutation p.Q160X in 6 families. 14 mutations are unique for Czech population.

Conclusions: 42 new PKD1 mutations and 14 new PKD2 mutations were identified. Next- generation sequencing method is a promising method for mutational analysis of complicated genes.

Funding: Supported by the grant project VZ MSMT 0021620806

TH-PO833

Next-Generation Sequencing Technology for Research and Diagnosis of Ciliopathies Sophie Saunier,[1] Emilie Filhol,[1] Céline Huber,[2] Cecile Masson,[3] Frederic Tores,[3] Solenn Pruvost,[4] Mohammed Zarhrate,[4] Christine Bole-Feysot,[4] Patrick Nitschke,[3] Cecile Jeanpierre,[1] Isabelle Perrault,[2] Rémi Salomon,[1] Jean-Michel Rozet,[2] Valerie Cormier-Daire,[2] Tania Attié-Bitach,[2] Corinne Antignac.[1] *[1]INSERM U983, Hôpital Necker; [2]INSERM U781, Hôpital Necker; [3]Bioinformatic Platform, Université Paris Descartes; [4]Genomics Platform, Fondation Imagine, Paris, France.*

Background: Ciliopathies are genetically heterogeneous disorders caused by defects of ciliary function sharing a broad phenotypic spectrum: cystic kidney diseases including PKD or nephronophthisis (NPH), Joubert (JBTS), Meckel (MKS) and Bardet-Biel (BBS) syndromes, retinal dystrophies (Senior-Løken syndrome, SLS) and short rib-polydactyly diseases (SRP including asphyxiating thoracic dystrophy, ATD).

Methods: To identify novel disease genes, we performed exon-enriched next-generation sequencing of >1000 candidate genes using a customized Agilent SureSelect Target Enrichment library and the SOLID4 (Lifetech) technologies. Candidate genes were compiled from the Cilia Proteome and Cildb databases and by data mining of published cellular and animal models.

Results: We initially searched for mutations in a set of 25 patients for whom obvious candidate genes had been excluded and mapping data were available: 6 JBTS, 4 NPH, 5 JATD, 1 SRP-II, 5 SLS and 4 MKS. We confirmed the presence of BBS6 and BBS10 mutations in a JBTS case as an internal control. Pathogenic mutations were detected in known genes (TTC21B, NPHP4 and WDR35) in 4 families with NPH and situs inversus or JBTS. In addition, in 3 isolated NPH, JBTS and MKS cases, we found likely pathogenic homozygous or compound heterozygous mutations in 3 genes that have not previously been implicated in any ciliopathy. Functional studies of these mutations are under investigation.

Conclusions: To conclude, this method is a valuable and cost-saving approach to screen a large number of genes in ciliopathies. In addition to provide further insights into disease mechanisms, the analysis of numerous ciliary genes in patients with cystic kidney diseases and various extrarenal disorders will allow to explore the idea of mutational load as a potential predictor of clinical outcome.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO834

Whole Exome Resequencing Reveals Renal Transporter Mutations May Phenocopy Nephronophthisis-Related Ciliopathies Toby W. Hurd, Andrew Cluckey, Sivakumar Natarajan, Edgar Otto, Friedhelm Hildebrandt. *Pediatrics and Communicable Disease, University of Michigan, Ann Arbor, MI.*

Background: Nephronophthisis-related ciliopathies (NRCs) are the leading genetic cause of end-stage renal failure in the first three decades of life. To date, causative mutations in more than 18 different genes have been identified for NRCs, yet mutations in these genes can only explain approximately 30% of cases. To identify additional causative mutations for NRCs, we performed whole exome resequencing in eight families with apparent NRC phenotypes.

Methods: An Affymetrix Genome-Wide Human SNP Array 6.0 was utilized to identify 8 NRC families that displayed significant homozygosity and hence fit an autosomal recessive hypothesis for disease etiology. Exome capture was performed using the Nimblegen SeqCap EZ Human Exome Library v2.0 followed by resequencing on an Illumina HiSeq2000 platform.

Results: Of the 8 families, we identified mutations in known NRC genes in 3 of the families. These included a novel homozygous truncating mutation in NPHP4 in a patient with nephronophthisis; a previously described homozygous cryptic splice site mutation in BBS1 in a patient with Bardet-Biedl syndrome (BBS); a compound heterozygous mutation in ALMS1, mutations of which give rise to Almström syndrome, in a patient thought to have BBS. In addition, 1 patient who was described as having nephronophthisis based on the observation of renal cysts, was found to have a homozygous deletion in SLC4A1, a causative gene in distal renal tubular acidosis (dRTA). Re-inspection of ultrasound images in this patient revealed cysts surrounded by calcinosis, a hallmark of dRTA. Finally, in a patient with a BBS-like phenotype we found a homozygous missense mutation in the prostaglandin transporter, SLCO2A1, to which no disease has been associated so far.

Conclusions: We successfully identified by whole exome capture the causative mutations in 5 out of 8 patients with NCRs and enabled more accurate disease diagnoses than clinical data alone based on identification of the mutated gene. Moreover, we show that mutations of renal tubular transporter genes may represent phenocopies of NRCs.

Funding: Other NIH Support - ARRA, Private Foundation Support

TH-PO835

Renal Consequences of Megalin Deficiency in Humans Tina Storm,[1] Lisbeth Tranebjaerg,[4] Carina Anna Frykholm,[3] Tryggve Neveus,[5] Pierre J. Verroust,[2] Henrik Birn,[1] Erik I. Christensen,[1] Rikke Nielsen.[1] *[1]Institute of Biomedicine, Aarhus University, Aarhus, Denmark; [2]Institute de la Vision, INSERM, Paris, France; [3]Department of Audiology/Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden; [4]Department of Audiology, Bispebjerg Hospital, Copenhagen, Denmark; [5]Department of Women´s and Children´s Health, Uppsala University, Uppsala, Sweden.*

Background: Donnai-Barrow (DB)/facio-oculo-acoustico-renal (FOAR) syndrome is caused by mutations in the LRP2 gene encoding megalin. Patients are characterized by a number of abnormalities, including proteinuria. We had the opportunity to investigate a Swedish consanguineous family (Dr. Birgitta Sundelin, Sweden) with three children suspected to suffer from this syndrome. The purpose of the present study was to characterize the mutation and the role of megalin in human kidney function and development.

Methods: To study the renal phenotype of megalin deficieny in humans we used direct sequencing, *in silico* analysis, immnuhitochemestry, ELISA and immunoblotting.

Results: We identified a novel mutation of the donor splice site of *LRP2* exon 18 (c.2639+1G>A). *In silico* analyses predicted a premature termination codon likely to result in nonsense mediated decay of the transcript and immunohistochemistry confirmed the absence of megalin. Light microscopic analyses of two available biopsies revealed no gross alterations in kidney morphology. This included normal distribution and expression of the endocytic receptor cubilin.

Analyses of renal biopsies revealed that megalin ligands including retinol binding protein, albumin and α1-microglobulin were not detectable in proximal tubules. Urinary excretion of both megalin and cubilin ligands were detected. Quantitative measurements of the albumin excretion revealed microalbuminuria.

Conclusions: Our studies demonstrate that abrogation of megalin expression in man results in urinary loss of numerous filtered compounds including vitamin carriersas as observed in megalin knockout mice. Further, normal internalization of cubilin bound ligands is dependent on the presence of megalin, whereas normal kidney development is not. Finally, our study indicates that human filtration of albumin is in the micro-range.

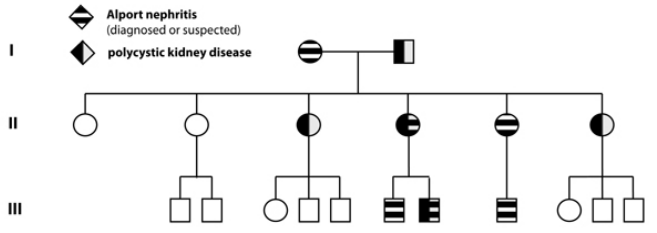
Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO836

Co-Occurrence of Alport and ADPKD – Early Progression to Renal Failure Kathy K.Y. Lee-Son,[1] Anna M. Lehman,[1] Mato P. Nagel,[2] Millan Patel,[1] Colin T. White.[1] *[1]BC Children's Hospital, Canada; [2]Nephrologische Praxis, Germany.*

Background: We describe a family with co-inheritance of ADPKD and Alport Syndome.

Methods:



Not all individuals in generation III have been screened for the two disorders.

The mother (II-4) was diagnosed with ESRD from pathology proven ADPKD at age 20 with transplant at 24. Two sons, conceived post-tx, were delivered prematurely. III-6 at 30 wk & 880g; III-7 at 32 wk & 1140g. Both had microscopic hematuria, bilateral echogenic kidneys in childhood & profound sensorineural hearing loss by age 14. III-6 progressed rapidly and was transplanted at age 16. III-7 had bilateral cortical cysts and presumptive diagnoses of PKD1 and Alport based on family history & phenotypic features. At age 18, he has reduced nuclear GFR [83ml/min/1.73m2] & heavy proteinuria on maximal ACE/ARB therapies.

Results: Genetic investigations of mom revealed 2 PKD1 gene variants of unknown significance: R1492H & I1610del. III-7 shared the I1610del, but not R1492H. Sequencing of her COL4A5 gene demonstrated a previously undescribed variant: c4447delG in exon 47, which leads to premature truncation of the protein. Biopsy of (III-6) showed a basket weave & absent IF for collagen α-3 and α-5, consistent with Alport Syndrome.

Conclusions: This family highlights the effects of multiple factors on the progression of renal disease. The mom's progression to ESRD in late adolescence from either Alport or PKD1 in isolation would be rare. We postulate the co-occurrence of both a glomerular and tubulocystic disease accelerated her renal deterioration. Although III-7 has both genetic conditions, his brother (III-6) with only Alport Syndrome progressed to ESRD much earlier than he. Given the similarities between both brothers through childhood, we postulate that the extreme prematurity & low birth wt of III-6 resulted in a diminished nephron endowment and a more rapid progression to ESRD despite his inheritance of only one genetic condition.

TH-PO837

COL4A3 Founder Mutation in a Novel Turkish-Cypriot Kindred Thomas Michael Connor,[1] Duriye Deren Oygar,[2] Daniel P. Gale,[1] Konstantinos Voskarides,[3] Constantinos Deltas,[3] Guy H. Neild,[1] Patrick Maxwell.[1] *[1]Division of Medicine, University College London, London, United Kingdom; [2]Nicosia State Hospital, Nicosia, North Cyprus, Cyprus; [3]Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus.*

Background: Mutations in COL4A3, the gene encoding the α3 chain of type IV collagen, are associated with Alport syndrome and Thin Basement Membrane Nephropathy (TBMN). Recent studies in the Greek-Cypriot population have highlighted a number of mutations in COL4A3. These mutations were associated with both TBMN and FSGS on biopsy, and with a high incidence of end-stage renal disease. These mutations were thought to originate from founder mutations in that population. We investigated a large Turkish-Cypriot pedigree from the Kyrenia district of North Cyprus that segregated microscopic hematuria, mild proteinuria, occasional renal cysts, and variable degrees of renal impairment. There were no extra-renal signs in any of the affected individuals.

Methods: DNA was isolated using the QIAamp DNA blood mini kit. SNP genotyping was performed using the Illumina 300K chip and linkage anlaysis with easyLINKAGE. Tetra-primer PCR was designed to confirm the presence of known mutations. Mutations were confirmed by direct sequencing.

Results: Linkage analysis gave a LOD score of 4.5 in association with a 0.5Mb region at 2q36-7. Testing for known mutations of COL4A3/4 demonstrated co-segregation with the G871C mutation in COL4A3.

Conclusions: This is the first description of familial nephropathy in the Turkish Cypriot population associated with mutations in COL4A3. This demonstrates that this mutation occurs in both Greek and Turkish-Cypriot populations and confirms that it is sufficient to cause end-stage renal disease. Haplotype analysis will allow us to more accurately determine the origin of the mutation.

Funding: Government Support - Non-U.S.

TH-PO838

Exonic Mutations Associated with Hereditary Renal Diseases Can Result in Major Alterations in the mRNA Felix Claverie-Martin, Marialbert Acosta-Herrera, F. Javier Gonzalez-Paredes, Elena Ramos-Trujillo. *Unidad de Investigacion, Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain.*

Background: The pathogenicity of missense and synonymous mutations is generally assumed to result from the predicted effect on the reading frame and protein function. However, it is now clear that an unexpectedly large fraction of exonic mutations could be pathogenic by affecting pre-mRNA splicing. The present study investigated the impact on splicing of missense and synonymous mutations previously found in the *CLDN16, SLC12A1, CLCNKB, PKD1* and *PKD2* genes of patients with familial hypomagnesemia with hypercalciuria, Bartter syndrome, and autosomal dominant polycystic kidney disease, respectively.

Methods: Bioinformatics analyses were used to predict the effect of mutations on mRNA splicing. The effect of mutations was tested experimentally by using splicing reporter minigene assays and site-directed mutagenesis. Analysis of RNA from transfected kidney-derived cell lines was performed by RT-PCR.

Results: Several mutations were predicted to disrupt pre-mRNA splicing by abolishing splice sites or creating new ones, or by inactivating or generating exonic splicing enhancers or silencers. We showed that these mutations induce different mRNA defects; *SLC12A1* mutation D648N leads to the incorporation of a truncated exon 14 in the mature mRNA, resulting in frameshift and predicted premature protein termination; *CLDN16* mutation G198A results in an mRNA that lacks exon 4; mutations R149L and L151F cause inclusion of a truncated exon 3 in the *CLDN16* mRNA; mutations D511V and L572L of *PKD2* result in skipping of exons 6 and 7, respectively; and *PKD1* mutation G109G leads to the incorporation of a truncated exon 3 in the mRNA.

Conclusions: Our results demonstrate that some renal disease-causing mutations, initially considered as missense or synonymous, induce splicing defects, resulting in major alterations in the mRNAs. We propose that these mutations are reclassified as splicing mutations. Assays for the effects of the mutation on mRNA splicing should be included in the routine analysis of these pathogenic mutations.

This work was supported by grants FIS PI071037 and FUNCIS PI17/09.

Funding: Government Support - Non-U.S.

TH-PO839

Structural-Functional Relationships of Fabry Nephropathy Behzad Najafian,[1] Marie-Claire Gubler,[2] Michael Mauer.[3] *[1]Pathology, University of Washington, Seattle; [2]Pathology, Université René Descartes, Paris, France; [3]Pediatrics, University of Minnesota, Minneapolis.*

Background: Renal failure is a major complication of Fabry disease (FD). In order to understand FD nephropathy progression mechanisms, we studied relationships between glomerular structure and renal function in FD patients across wide range of age and renal function.

Methods: Renal biopsies from 25 (male (M)/female (F)=14/11) FD patients were studied using electron microscopy stereology. Results were correlated with proteinuria, GFR and age.

Results: F were 14[8-63], median [range] and M were 19 [4-57] years old. M and F were not different for proteinuria, GFR, volume fraction of GL-3 inclusions per mesangial [Vv(Inc/Mes)], or endothelial cells [Vv(Inc/Endo)]. Volume fraction of GL-3 inclusions per podocytes [Vv(Inc/PC)] was greater in M (0.38±0.11) vs F (0.25±0.13), p=0.01. In all FD patients, there was a direct relationship between age and proteinuria (r=0.5, p=0.008), FPW (r=0.70, p=0.001), Vv(Inc/Mes) (r=0.64, p=0.001) and Vv(Inc/Endo) (r=0.67, p=0.00001). FPW was correlated with Vv(Inc/PC) (r=0.55, p=0.02). Vv(Inc/Mes) and Vv(Inc/Endo) were strongly correlated (r=0.96, p=0.00001). There was negative correlation between age and GFR (r=-0.60, p=0.02). Proteinuria was correlated with FPW (r=0.64, p=0.006), Vv(Inc/PC) (r=0.56, p=0.02), Vv(Inc/Endo) (r=0.57, p=0.01), and Vv(Inc/Mes) (r=0.56, p=0.02). None of the structural parameters were correlated with GFR. While there was no relationship between age and Vv(Inc/PC) (r=0.03, p=0.8) in the entire cohort, in young patients (age<20), Vv(Inc/PC), and not Vv(Inc/Endo) or Vv(Inc/Mes), was strongly correlated with age (r=0.78, p=0.001)

Conclusions: We demonstrated novel relationships between glomerular structure and proteinuria in FD patients across a wide range of age and renal function. Our studies indicate that structural-functional relationships of FD nephropathy may be different in different age groups, perhaps reflecting a difference in progression of various lesions n initial vs late stages of the disease.

Funding: Other NIH Support - grant, Pharmaceutical Company Support

TH-PO840

The Frequency of Fabry Disease with the α-Galactosidase A E66Q Variant in Japanese Dialysis Patients Yoko Kikumoto, Hitoshi Sugiyama, Hiroshi Morinaga, Ayu Ogawa, Masashi Kitagawa, Keiichi Takiue, Shinji Kitamura, Shigeru Akagi, Yohei Maeshima, Hirofumi Makino. *Okayama University, Japan.*

Background: Fabry disease (FD) is an X-linked disorder resulting in a deficiency in the α-galactosidase A (α-Gal) enzyme activity. FD is one of the causes of progressive renal dysfunction, but its diagnosis is often either delayed or missed. Its frequency has been reported to be much higher in dialysis patients, and recent newborn screenings have revealed a high incidence of later-onset FD mutations, including renal variants.

Methods: We first screened the α-Gal activity in the plasma of 892 Japanese hemodialysis (HD) patients. When the enzyme activity was below 6.4 nmol/mL/2hr, the test was defined as positive. Next, α-Gal activity was measured from dried blood spots via a fluorescence assay using 4-methylumbellifenyl. Then, online databases were searched to identify any previous screening studies for Japanese FD patients undergoing HD.

Results: One previously undiagnosed 70-year-old male patient was identified by this screening study. He had a low plasma α-Gal activity and was demonstrated to have an E66Q mutation in exon 2 of the α-Gal gene, and one of his daughters had the same mutation. The frequency of FD was 0.11% in all and 0.18% in male HD patients in this study. We further identified 5 previous studies and found a total of 4298 Japanese HD patients screened (2975 males and 1323 females), including those in our present study. Overall, the prevalence of FD was 0.50% in male (6 studies), and 0.23% in female HD patients (4 studies). Notably, the frequency of the E66Q variant was 33.3% (6 of 18 patients) of Japanese FD on HD. The classical manifestations of FD were not observed in any of these patients with E66Q; however, most of them had either cardiac or cerebrovascular involvement. Other mutations included M291I, Y365X, M296I, Q357X, A97V, G373D, Δ288A and Δ254V.

Conclusions: Our results demonstrate that the later-onset disease phenotype with the E66Q variant is more frequent in Japanese FD patients on HD than has been previously reported in other countries. Whether E66Q is a pathogenic mutation, or just a variation will need to be determined in further investigations.

TH-PO841

UMOD Mutations in the Chronic Kidney Disease Population in Taiwan Lim Lee Moay,[1] Chih-Chuan Yu,[3] Daw-Yang Hwang,[1] Chi-Chih Hung,[1] Shang-Jyh Hwang,[1,2] H.C. Chen.[1,2] *[1]Division of Nephrology, Department of Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [2]Faculty of Renal Care, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [3]Graduate Institute of Medicine, Collage of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.*

Background: Taiwan has the highest incidence and prevalence rate of end-stage renal disease (ESRD) and also high incidence and prevalence of chronic kidney disease (CKD) according to the USRDS report. The cause of this high incidence and prevalence are multi-factorials. Genetic factors are believed to be one of them. However, there is no data concerning the prevalence of medullary cystic kidney disease (MCKD) and UMOD gene mutation in CKD population in Taiwan.

Methods: We selected 160 patients, irrespective of family history, from the Kaohsiung Medical University Hospital CKD Care Program based on the criteria with uric acid level in the highest quartile in the CKD stage. For CKD stage 1 and 2, the criteria selected was uric acid higher than 8 mg/dL for male and 7.5 mg/dL for female. Since more than 90 percent of UMOD gene mutation was reported in the exon 4, we amplified this exon by polymerase chain reaction and direct sequencing. HEK293 cells were transient transfected with wild and mutant uromodulin. Immunofluorescence staining was used to study the uromodulin retention in the endoplastic reticulum(ER) and Western blot to study the cytosol cytochrome c expression.

Results: Two novel UMOD mutations, p.C41R and p.R142Q, were found. In vitro analysis showed that these two novel mutations caused retention of mutant uromodulin in the ER compared with wild type. Increased cytosol cytochrome c level in transfected HEK293 cells was also found.

Conclusions: The UMOD mutation contributed a relative small portion for the cause of CKD population in Taiwan. The p.C41R uromodulin caused greater ER retention than R142Q mutant, while the wild type showed the least retention. Both mutants showed increased cytosol cytochrome c, indicated the mutant uromodulin caused cell apoptosis. Our findings are comparable with other UMOD studies showing ER retention, and cell apoptosis may contribute to tubular cell death followed by interstitial fibrosis.

TH-PO842

How Nonsense Mutations in the COL4A4 Gene Cause Autosomal Recessive Alport Syndrome and Thin Basement Membrane Nephropathy Vanessa Sivakumar, Yanyan Wang, Mardhiah Binti Mohammad, Hayat Dagher, Judith A. Savige. *Department of Medicine, University of Melbourne, Melbourne, VIC, Australia.*

Background: Autosomal recessive Alport syndrome results from homozygous or compound heterozygous mutations in the *COL4A3* or *COL4A4* genes. Nonsense changes account for 10% of all mutations. In other collagen diseases, nonsense mutations have their effect through 'nonsense-mediated decay'. The aim of this study was to determine whether mutations in autosomal recessive Alport syndrome and Thin basement membrane nephropathy also resulted in nonsense-mediated decay.

Methods: Peripheral blood lymphocytes from a father and daughter with homozygous and heterozygous copies of S969X in *COL4A4* respectively were EBV-transformed. The resulting cell lines were cultured overnight at 37°C with 5% CO_2, with and without the addition of protein synthesis inhibitors (0.1 mg/ml cycloheximide, 0.1 mg/ml anisomycin). Levels of collagen IV α3, α4 and α5 mRNA were quantitated by real time PCR (7500 Real Time PCR System, Applied Biosystems), and compared with levels in untreated cells after normalisation using *GAPDH*. Levels of mRNA corresponding to pro- and antiapoptotic pathways (Casp 3, Bad and Bcl-2) were also quantitated.

Results: Levels of collagen IV α4 chain mRNA were increased after the addition of cycloheximide (p=0.025, p=0.012) or anisomycin (p=0.03, p=0.009) in both the homozygous and heterozygous cell lines. This is consistent with *COL4A4* nonsense mutations resulting in nonsense-mediated decay. There was no evidence of apoptosis.

Conclusions: Nonsense mutations in autosomal recessive Alport syndrome and Thin basement membrane nephropathy result in nonsense-mediated decay, but not in apoptosis. The use of therapeutic agents that inhibit nonsense-mediated decay in patients with autosomal recessive Alport syndrome may increase collagen IV α3 and α4 chains and ameliorate disease severity.

TH-PO843

Hypermethylation of the Mucin-Like Protocadherin (MUPCDH) Gene Promoter in Autosomal Dominant Polycystic Kidney Disease Jong Hoon Park, Yu Mi Woo, Eunji Lee. *Sookmyung Women's University, Seoul, Korea.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common human genetic disease and characterized by the formation of multiple fluid-filled cysts, which result in end-stage renal failure. However, cyst mechanism, early diagnosis and treatment for ADPKD are still not clear. Recently, it is reported that the alteration of epigenetic regulation as well as gene mutations may affect on pathogenesis.

Methods: To understand the epigenetic mechanism of pathogenesis of ADPKD, genome-wide methylation profile was compared with expression analysis by Methyl CpG Recovery Assay-Sequencing (MIRA-Seq). Based on this data, to validate the methylation status at CpG dinucleotides, HRM analysis and EpiTYPER was performed following bisulfite treatment. Furthermore, to confirm the effect on recovery of DNA methylation levels in ADPKD, DNMT inhibitors were treated into the immortalized human ADPKD cystic epithelial cells and changed mRNA level of MUPCDH was checked by qRT-PCR.

Results: As a result of genome-wide DNA methylation analysis, overall genome of ADPKD patients was hypermethylated compared with normal samples. Especially, promoter region of MUPCDH gene was significantly hypermethylated in ADPKD kidney tissue. As a result of HRM analysis, hypermethylation patterns was showed in the promoter region of ADPKD kidney tissues compared with normal control, ranging from upstream 1 kb to intron 1 region. As expected, hypermethylation in promoter region of MUPCDH was also observed in cystic patient kidney tissues compared with normal. On the contrary, its expression level was markedly decreased in ADPKD patients. Demethylation of MUPCDH gene promoter by treating DNMT inhibitor such as 5-aza-2'-dexoycytidine induced up-regulation of MUPCDH mRNA level.

Conclusions: In conclusion, MUPCDH promoter CpG islands were significantly hypermethylated in ADPKD patients and it is negatively correlated to its reduced expression level.

Funding: Government Support - Non-U.S.

TH-PO844

Correlation of Phenotype and *HOGA1* Variants in Obligate Heterozygotes from PHIII Families Carla G. Monico, Andrea G. Cogal, Julie B. Olson, Barbara M. Seide, Dawn S. Milliner. *Division of Nephrology, Mayo Clinic, Rochester, MN.*

Background: Recently, we described primary hyperoxaluria type III (PHIII), due to mutations in *HOGA1*, and identified hypercalciuria and hyperuricosuria in addition to hyperoxaluria in some patients. We also detected heterozygosity for *HOGA1* variants in 2 patients with mild hyperoxaluria and in 3/100 idiopathic calcium oxalate stone formers.

Methods: To further characterize the phenotype in *HOGA1* heterozygotes, we performed segregation analysis in all available first-degree relatives of PHIII probands. Analysis included 1 new and 9 previously ascertained unrelated PHIII families. Hyperoxaluria, hypercalciuria & hyperuricosuria were defined by age-appropriate normal reference ranges, based on 24-hour urine collections, completed on self-selected diets.

Results: Mild hyperoxaluria was observed in only 1 obligate heterozygote harboring 1 of the 2 most common (IVS700+5 G>T and c.944_946 del AGG) mutations. However, both hypercalciuria and hyperuricosuria were detected in some c.944_946 del AGG and IVS700+5 G>T heterozygotes. And both mild hyperoxaluria and hypercalciuria were observed in some R>C missense variant heterozygotes.

Association of mild or intermittent hyperoxaluria, hypercalciuria or hyperuricosuria in obligate heterozygotes with HOGA1 variants.

Family #	HOGA1 allele	↑ Uox	↑Uca	↑Uua	# FDR tested
1	c.944_946 del AGG	0/2	1/2	NA	2
2	c.944_946 del AGG	0/5	0/5	1/5	5
3	c.944_946 del AGG	0/1	0/1	NA	2
	IVS700+5 G>T	0/1	0/1	NA	
4	c. 209 G>C (R70P)	0/1	1/1	NA	2
	IVS700+5 G>T	0/1	1/1	NA	
5	c. 289 C>T (R97C)	1/1	1/1	NA	2
	c.944_946 del AGG	0/1	1/1	NA	
6	c. 769 T>G (C257G)	0/1	0/1	NA	1
7	c.944_946 del AGG	0/1	0/1	0/1	3
	c.907 C>T (R303C)	1/2	1/2	0/2	
8	IVS700+5 G>T	0/2	NA	NA	2
9	IVS700+5 G>T	0/4	0/4	NA	4
10	IVS700+5 G>T	1/2	0/2	1/2	2

FDR=first-degree relatives. NA=not available, Uox (urine oxalate), Uca (urine calcium), Uua (urine uric acid).

Conclusions: Detection of hyperoxaluria, hypercalciuria or hyperuricosuria in association with *HOGA1* heterozygosity was variable, even among first-degree relatives from the same family, suggesting an influence from other susceptibility factors, genetic or environmental. Diet-controlled studies and further characterization of the metabolic defect in PHIII are needed.

Funding: NIDDK SupportNIH ORDR, member Rare Disease Clinical Research Network

TH-PO845

Targeted Exome Sequencing and Homozygosity Mapping Identify Mutation of EMP2 as a Cause of Steroid Sensitive Nephrotic Syndrome Shazia Ashraf,[1] Virginia Vega-Warner,[1] Humphrey Fang,[1] Toby W. Hurd,[1] Sivakumar Natarajan,[1] Fatih Ozaltin,[2] Friedhelm Hildebrandt.[1,3] *[1]Department of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Department of Pediatrics, Hacettepe University, Ankara, Turkey; [3]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.*

Background: Idiopathic nephrotic syndrome is a common pediatric kidney disease 80% of all cases are steroid sensitive (SSNS). Whereas gene identification has furthered the understanding of pathomechanisms in steroid-resistant nephrotic syndrome (SRNS), disease mechanisms of SSNS remain unknown. To identify single-gene causes for SSNS, we combined homozygosity mapping, whole human exome capture and consecutive massively parallel re-sequencing.

Methods: In two siblings (of consanguineous parents) with SSNS, homozygosity mapping yielded 4 segments of homozygosity by descent with a cumulative genomic length of ~55 Mb. We performed whole human exome capture in one sibling using NimbleGen SeqCaP EZ Exome with consecutive massively parallel sequencing on an Illumina-Genome Analyzer II platform to identify the underlying disease-causing mutation.

Results: We identified a homozygous truncating mutation (p.Q62X) in the ***epithelial membrane protein 2*** (***EMP2***) gene as the first single-gene cause of SSNS. The mutation segregated with the affected status in this family and was absent from 86 Turkish healthy control individuals. EMP2, a tetraspan protein, has been shown to play a role in trafficking of molecules, which include integrins, major histocompatibility complex (MHC) class I molecules and glycosyl-phosphatidyl inositol (GPI)-anchored proteins. EMP2 is strongly expressed in human glomerular podocytes.

We are now screening ~600 unrelated SSNS patients for all coding exons of the *EMP2* gene to detect additional mutations in this gene.

Conclusions: We identified mutation of *EMP2* as causing SSNS. The identification of the first gene for *SSNS* presented here provides a first step towards our understanding of the pathomechanisms of SSNS.

Funding: Other NIH Support - Doris Duke Charitable Foundation

TH-PO846

Dominant Mutations Cluster in the Signal Peptide of *Renin* and Emphasize a Role for Renin in Erythropoiesis Bodo B. Beck,[1] Howard Trachtman,[2] Friedhelm Hildebrandt,[3] Matthias Tilmann Florian Wolf.[4] *[1]Institute of Human Genetics, University of Cologne, Germany; [2]Department of Pediatrics, Cohen Children's Medical Center, New Hyde Park, NY; [3]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [4]Pediatric Nephrology, University of Texas Southwestern Medical Center, Dallas, TX.*

Background: Renin is an important hormone regulating blood pressure and renal sodium reabsorption via the renin-aldosterone system. Homozygous or compound heterozygous *Renin (REN)* mutations cause renal tubular dysgenesis, which is characterized by death *in utero* due to renal failure and pulmonary hypoplasia. The phenotype resembles the fetopathy caused by angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) intake during pregnancy. Recently, heterozygous *REN* mutations were shown to result in early-onset hyperuricemia, anemia and chronic kidney failure. So far, only three different heterozygous *REN* mutations have been reported.

Methods: We performed mutation analysis of the *REN* gene in 39 kindreds with hyperuricemia and chronic kidney disease (CKD) previously tested negative for mutations in the *UMOD* and *HNF1*β genes.

Results: We identified a novel c.T28C (p.W10R) *REN* mutation in the signal sequence, affecting individuals over four generations. Patients carrying the novel *REN* mutation were characterized by significant anemia, hyperuricemia and CKD. We report the youngest patient with a heterozygous *REN* mutation so far, showing anemia, hyperuricemia and impaired renal function as early as 11 months of age. Documented anemia is severe in patients with heterozygous *REN* mutations and disproportional to the degree of renal impairment.

Conclusions: We highlight the possible mechanisms of heterozygous *REN* mutations causing anemia. Moreover all heterozygous *REN* mutations are localized in the signal sequence. We conclude that *REN* mutations are rare events in CKD patients and cause a clinical syndrome that resembles Uromodulin associated kidney disease. Screening of the *REN* gene should be considered for CKD patients with hyperuricemia and anemia, focusing on exon 1 sequencing, which encodes the signal peptide.

Funding: Government Support - Non-U.S.

TH-PO847

Whole Exome Capture Reveals Mutation of *ARHGDIA* as Causing Nephrotic Syndrome Pawaree Saisawat,[1] Virginia Vega-Warner,[1] Shazia Ashraf,[1] Yaacov Frishberg,[2] Toby W. Hurd,[1] Sivakumar Natarajan,[1] Friedhelm Hildebrandt.[1,3] *[1]Pediatrics, University of Michigan, Ann Arbor, MI; [2]Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel; [3]Howard Hughes Medical Institute.*

Background: Diffuse mesangial sclerosis (DMS) is a rare histologic variant of early-onset nephrotic syndrome (NS). In our multiethnic cohort of 71 DMS patients, 26 (37%) are caused by mutations in *WT1, NPHS1, NPHS2* or *PLCE1* while the majority of cases are still unsolved. To identify a new causative gene for DMS, we employed homozygosity mapping (Hildebrandt et al. *PLoS Genet* 5: e1000353, 2009) with consecutive whole exome capture (WEC) and massively parallel exon re-sequencing.

Methods: The parents of 3 siblings who were affected with early-onset NS that rapidly progressed to end-stage renal disease were first cousins of Ashkenazi Jewish background. Renal biopsy was carried out in one child and revealed DMS. *WT1, NPHS1, NPHS2* and *PLCE1* were screened and were mutation negative.

Results: Homozygosity mapping showed 5 homozygous candidate regions, confirming the history of consanguinity. We performed whole exome capture using the NimbleGen SeqCaP EZ Exome™ V2 protocol. Sequencing was performed using Illumina Genome Analyzer II. We detected 1 homozygous missense mutation (G173V) in *ARHGDIA* in an amino acid residue, which was consistently conserved to *S. cerevisiae*. G173V was predicted to be 'damaging' by PolyPhen score. The mutation was segregating in the family and was absent from the 1,000 genomes project. *ARHGDIA* encodes a GDP dissociation inhibitor (GDI), which regulates cycling between the GTP- and GDP-bound forms of the Rho family proteins. *Arhgdia*$^{-/-}$ mice develop massive proteinuria at 12 weeks of age and succumb to renal failure at an early age (*Oncogene* 18:5373, 1999). Kidney histology shows mesangial sclerosis and cystic tubular dilatation.

Conclusions: Because the *Arhgdia* loss-of-funcation mouse phenotype recapitulates the human DMS phenotype, our finding of *ARHGDIA* mutation very likely represents a new single-gene cause of early-onset NS.

Funding: Other NIH Support - Doris Duke Charitable Foundation

TH-PO848

INF2 Mutations as a Cause of Familial Versus Sporadic FSGS Elizabeth J. Brown,[1,2] Victoria Charoonratana,[1,2] Giulio Genovese,[2] Andrea Uscinski Knob,[2] Martin R. Pollak.[2] *[1]Medicine, Boston Children's Hospital, Boston, MA; [2]Medicine, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: Mutations in INF2, a member of the formin family of actin binding proteins, have been shown to cause autosomal dominant focal segmental glomerulosclerosis (FSGS). We previously described nine missense mutations in exons 2-4 of INF2 in 11/93 families with autosomal dominant inheritance of FSGS. We did not identify any disease-associated mutations outside of this region.

Methods: Genomic DNA was extracted from blood or saliva using standard methods. Using Sanger sequencing, we sequenced PCR amplified segments containing either exons 2-5 or exon 4 alone of INF2 in an additional 294 individuals with familial or sporadic FSGS. We sequenced exons 2-5 in all available family members of an individual in whom we found a variant. Clinical information was obtained from questionnaires and phone discussions with participants and/or their doctors.

Results: We identified 9 missense mutations in INF2 in 10 additional families with autosomal dominant FSGS. We did not find INF2 mutations in individuals with sporadic FSGS. Several of the mutations have been published previously (R214C, R218Q, R218W) while others are novel (H158D, G73S, V181G, R177C, C151R, L81P). All of the mutations are possibly or probably damaging by Polyphen2 analysis. All of the mutations segregate with disease. There are multiple individuals with end-stage renal disease and renal transplants within this new cohort, however, the penetrance is variable. In the H158D family, all 6 individuals in our study carry the mutation and have manifested ESRD. Whereas, in the R214C family, several individuals carrying the mutation are unaffected. Proteinuria was diagnosed in most of this cohort with INF2 mutations in their teens or 20s with ESRD developing in the third or fourth decade of life. No recurrent disease has been noted in renal transplant recipients.

Conclusions: We and others have shown that INF2 mutations are a significant cause of autosomal dominant FSGS. INF2 mutations do not appear to common in individuals with sporadic FSGS suggesting a different pathologic mechanism for the two forms of the disease.

Funding: Other NIH Support - K12 Institutional grant from CHRC, Private Foundation Support

TH-PO849

New *INF2* Mutations in a Large Cohort with Sporadic and Hereditary FSGS and Further Evidence for Variable Expressivity Rasheed A. Gbadegesin,[1] Peter J. Lavin,[2] Gentzon Hall,[2] Alison Homstad,[1] Guanghong Wu,[2] Alison Byrd,[2] Michelle P. Winn.[2] *[1]Pediatrics and Center for Human Genetics, Duke University, Durham, NC; [2]Medicine and Center for Human Genetics, Duke University, Durham, NC.*

Background: Focal and segmental glomeruloscerosis (FSGS) is a major cause of end-stage kidney disease. Recent advances in molecular genetics have provided evidence that defects in the podocyte play a major role in the pathogenesis of FSGS. Mutations in inverted formin 2 (*INF2*) were recently identified as a cause of autosomal dominant (AD) FSGS.

Methods: To define the role of *INF2* mutations in familial and sporadic FSGS, we screened for *INF2* variants in a large cohort with FSGS. The study had a secondary objective of defining a rational approach for genetic screening in families with AD FSGS.

Results: We identified 248 individuals with FSGS. There were 31 (32.6%) individuals with idiopathic disease. The remainder of individuals were from 64 families, 15 (15.8%) individuals from autosomal recessive kindreds and 49 (51.6%) individuals from AD kindreds. We found missense mutations in *INF2* in 8/49 (16%) of families with AD FSGS. Three of the variants detected were novel and all of the mutations were confined to exon 4 of *INF2*. Mutations in exon 4 of *INF2* are responsible for 90% of all mutations reported in FSGS due to *INF2* mutations.

Conclusions: In conclusion, *INF2* mutations are responsible for 16% of all cases of AD FSGS and mutations are clustered in exons 4 and 2, therefore screening for mutations in *INF2* may represent a rapid, non-invasive and cost effective method for the diagnosis of AD FSGS.

Funding: NIDDK Support, Private Foundation Support

TH-PO850

Genetic Backgrounds in Patients with Glomerulopathy with Fibronectin Deposits Hiromi Ohtsubo,[1] Fusako Hashimoto,[1] Shingo Ishimori,[1] Takeshi Ninchoji,[1] Fu XueJun,[1] Yuya Hashimura,[1] Hiroshi Kaito,[1] Naoya Morisada,[1] Noriko Uesugi,[2] Kazumoto Iijima.[1] *[1]Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; [2]Institute of Basic Medical Sciences, University of Tsukuba, Japan.*

Background: Glomerulopathy with fibronectin deposits (GFND) is a rare autosomal dominant glomerular disease associated with the massive deposition of fibronectin, caused by mutations of fibronectin 1 gene (*FN1*). There are currently few reports about genetic backgrounds in patients with GFND, and little is known about the clinical course. Our purpose in this study is to clarify the genotypes of *FN1* and the clinical features of GFND patients.

Methods: This study was designed for GFND patients and their family members. All patients underwent renal biopsies and were definitely diagnosed as GFND by renal histological findings. Genomic DNA was isolated from peripheral blood leukocytes after informed consent, and *FN1* was analyzed by using PCR and direct sequencing method.

Results: Seven (1 male and 6 females) patients and twelve family members were enrolled in this study. There were 2 cases with clinical renal manifestations in family members; one had persistent proteinuria, and the other was end stage renal failure for unknown reasons. All of the 7 patients had heterozygous mutations of *FN1*; four of them had p.Y973C, which was previously reported located in hepalin-binding domain, and the other three had novel (p.W1925X, p.1472del, p.L1974P). One of the novel mutations, p.1472del, located in integlin-binding domain, which play major roles in assembly of FN as well as hepalin-binding domain. There were six family members with heterozygous mutations of *FN1*; two of them had clinical renal manifestations, and the other four didn't at all.

Conclusions: We could detect heterozygous mutations of *FN1* in all of the patients, including the affected family members. Three novel mutations could be detected in this study, and furthermore this is the first report that demonstrated GFND can develop by *FN1* mutation in integlin-binding domain. It is of much interest that some individuals with the same *FN1* mutations as patients showed no renal manifestations, despite of comparatively late age.

TH-PO851

Two Novel Homozygous SLC2A9 Mutations Cause Renal Hypouricemia Type 2 Dganit Dinour,[1] Nicola K. Gray,[2,3] Liat Ganon,[1] Andrew J.S. Knox,[4] Susan Campbell,[5] Lindsay Sawyer,[6] Daniel Landau,[7] Alan F. Wright,[5] Eliezer J. Holtzman.[1] *[1]Sheba Medical Center and Tel Aviv University, Israel; [2]University of Edinburgh, United Kingdom; [3]Centre for Reproductive Biology, Edinburgh, United Kingdom; [4]Institute for Genetics and Molecular Medicine, Trinity College Dublin, Ireland; [5]MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom; [6]School of Biological Sciences, University of Edinburg, United Kingdom; [7]Soroka University Medical Center, Israel.*

Background: Elevated serum uric acid is associated with gout, hypertension, cardiovascular and renal disease. Hereditary renal hypouricemia type 1 (RHUC1) is caused by mutations in the renal tubular uric acid transporter URAT1, and can be complicated by nephrolithiasis and exercise-induced acute renal failure. We have recently shown that loss-of-function homozygous mutations of another uric acid transporter, GLUT9, cause a severe type of hereditary renal hypouricemia with similar complications (RHUC1).

Methods: Two unrelated families with renal hypouricemia were clinically characterized. DNA was extracted and *SLC22A12* and *SLC2A9* coding for URAT1 and GLUT9, respectively, were sequenced. Transport studies in *Xenopus laevis* oocytes were utilized to evaluate the function of GLUT9 mutations found. A molecular modeling study was undertaken to structurally characterize the effects of these mutations.

Results: Two novel homozygous GLUT9 missense mutations were identified: R171C and T125M. Mean serum uric acid level of four homozygous subjects was 0.15 ± 0.06mg% and fractional excretion of uric acid was 89-150%. None of the affected subjects had nephrolithiasis, exercise-induced acute renal failure or any other complications. Transport assays revealed that both mutant proteins had a dramatically reduced ability to transport uric acid. Modeling showed that R171C and T125M mutations are located within the inner channel that transports uric acid between the cytoplasmic and extracellular regions.

Conclusions: This is the second report of renal hypouricemia caused by homozygous GLUT9 mutations. Our findings confirm the pivotal role of GLUT9 in uric acid transport and highlight the similarities and differences between RHUC1 and RHUC2.

Funding: Government Support - Non-U.S.

TH-PO852

Evaluation of an Interactive, Visual On-Line Tutorial To Improve Urine Microscopy Teaching Craig E. Gordon,[1] Jessica Gray,[2] John R. Heinrick,[2] Maya Fayfman,[2] Laurence H. Beck.[1] *[1]Renal Section, Department of Medicine, Boston University School of Medicine, Boston, MA; [2]Boston University School of Medicine, Boston, MA.*

Background: The evaluation of urine sediment is one of the oldest non-invasive clinical tools used to diagnose renal and systemic pathology. We developed an online module for urine microscopy training and evaluated its efficacy as a learning tool.

Methods: To evaluate the tutorial, we created a ten-item multiple-choice pre and post-tutorial examination comprising questions focused on image identification, fact, and case-based questions, as well as a curriculum assessment tool using a five-point Likert scale. We hypothesized that post-test results would be higher than pre-test outcomes and that performance on image-based questions would improve more than other item types. Data were analyzed using a two-sample T test.

Results: Second year medical students accessed the tutorial and pre- and post-examinations throughout the renal module of the Disease and Therapy course. Of 183 enrolled students, 30 (16%) completed both pre- and post-tests. Mean score on the pre-test was 47% [95% CI, 41-54%] and 63% [95% CI, 56-70%] on the post-test, p-value < 0.001. Post-tutorial score on image identification items increased by 26% ($p < 0.001$) but did not increase significantly for other question types. Nine students completed the curriculum assessment and reported improved understanding of urine microscopy and that the tutorial was a valuable educational tool which they would recommend to future enrollees.

Conclusions: This novel urine microscopy tutorial is an effective learning tool for second year medical students. Post-tutorial examination scores were higher in general, but significantly for questions requiring image identification. Student evaluations of the tutorial were largely positive. The generalizability of these findings is limited by few students completing the pre- and post-tutorial examinations and curriculum assessment. Despite these limitations, this online educational tool appears valuable for medical student education, and potentially for resident/fellow training, as well as a model tutorial for other image-based medical topics.

TH-PO853

Development of a Validated Nephrology Clinic Letter Fellow Competency Assessment John D. Mahan, David S. Hains, Hiren P. Patel. *Pediatric Nephrology, Nationwide Children's Hospital/OSU, Columbus, OH.*

Background: Competency assessment of nephrology fellows in training should promote acquisition of essential skills, be judged practical by faculty and be relevant to fellows. We developed and tested a Pediatric Nephrology Fellow Clinic Letter Competency Assessment (CLCA) of 8 items deemed essential for an effective clinic letter (by consensus of faculty of the Pediatric Nephrology Division) to address the ACGME competency for Interpersonal and Written Communications. Fellows compose their letters after clinic visits; supervising faculty may provide feedback on the letter before it is finalized. Each starting fellow receives a copy of the CLCA form. 3 letters are randomly chosen and assessed every 6 months/fellow and results discussed at each fellow's semi-annual review.

Methods: For this study, 3 faculty independently evaluated 36 randomly selected letters distributed over 24 months from the entire set of > 1000 letters constructed by the 5 fellows each year. CLCA was completed per letter by each faculty member and data analyzed for 1) inter-rater reliability (IRR) for each item and for the total score for each letter, 2) intra-class correlation (ICC) and 3) class differences by ANOVA and t tests.

Results: Faculty found the CLCA tool easy to use; specific comments were added for 40% of letters. IRR was acceptable for individual elements (0.41) and good for total score (0.58). ICC Coefficient for total score/letter was good at 0.637. CLCA total scores increased per yr of training (ANOVA, $p = 0.057$). Fellows in 3rd yr (mean 7.79+/-0.25) scored better than 1st yr (mean 7.19+/-0.6748), $p = 0.0291$, consistent with increasing competency over time.

Conclusions: The Pediatric Nephrology Fellow Clinic Letter Competency Assessment helped direct and measure increasing competency by fellows. The CLCA tool had strong validity, good inter-rater reliability, and could be used in adult and pediatric programs. Implementation of validated methods for competency assessment can assist fellowship program directors and faculty in developing important skills in trainees. Additional practical and useful competency assessment methods offer the opportunity to more intentionally direct fellow training.

TH-PO854

Case Based Debates- An Innovative Teaching Tool Arun Chawla, Kenar D. Jhaveri. *Nephrology, Hofstra North Shore LIJ School of Medicine, NY.*

Background: There has been a growing concern about the decline in interest in nephrology as a career amongst medical students and residents. At our institution we have continuously worked to develop unique ways of teaching nephrology, which are meant not only to attract medical students and residents to the field of nephrology but also contribute directly to promote learning and training of renal fellows. We hereby present another teaching tool which we have used to teach transplant pathology -"Case Based Debates".

Methods: A faculty member chooses a challenging case (transplant related) and shares brief history and labs with the fellows in advance. Fellows are equally divided into two teams. Each team has a faculty member (gold card/lifeline) who can be used, at the most, twice during the whole debate/game. Fellows sit together to discuss the case and formulate a differential diagnosis and come up with a diagnostic plan involving minimum number of tests leading them to most likely diagnosis. Investigations/questions (like most relevant

history questions, pertinent lab data, donor specific antibodies, viral serologies etc.) are rewarded with points according to their relevance and diagnostic importance. Using the least number and most relevant tests and thereby earning more points, the team that comes closest to correct diagnosis is then asked to predict the biopsy findings. Next, the pathology slides are shown which fetches bonus points to the team who reads them accurately.

Results: The above teaching tool has been an instant hit amongst the fellows and residents. It has also been well received, most participated and the most appreciated teaching tool at the "faculty development workshop" at our institution.

Conclusions: We hope to introduce this tool to teaching of not only transplant pathology but also to discuss and teach challenging cases involving fluid and electrolytes, acid-base imbalance and other aspects of nephrology. It makes learning fun and easy to understand. Also, we learn to use the most cost-effective diagnostic tests, which is an important aspect to understand with increasing economic burden of the healthcare on our economy.

TH-PO855

Paging Doctor Google! Google Searching as a Tool for Diagnosing Renal Disease Peter B. Schrier, Louis R. Spiegel, Kenar D. Jhaveri. *Nephrology, North Shore/LIJ & Hofstra Medical School.*

Background: Patients often approach their doctors with medical information they obtained from the Internet. Increasingly, medical students, residents, and attending physicians have been using the Internet as a tool for diagnosing and treating disease. We asked whether people with basic introductory medical knowledge (i.e. Internal Medicine residents) will be better at diagnosing renal diseases using the internet's most common search engine, Google.com, as an aid.

Methods: We created 100 pairings of common and uncommon renal diseases with keywords related to the features of the disease using a standard renal text as a guide. Nephrology attendings and fellows, and first and second year Internal Medicine Residents were given random selections of keyword parings and asked to identify the associated diseases. The residents were given the aid of the first ten results returned by the Google Search Engine with the input of any or all of the given keywords. The attendings and fellows were not allowed to use google. The diseases were divided in to two groups: common and rare, and the results were analyzed as a whole and separately as divided groups.

Results: Overall, the "Googlers" correctly diagnosed renal diseases less often than the nephrology attendings (72.2% vs. 84.7%, p<.001), but with the same frequency as the nephrology fellows (72.2% vs. 71.5%, p=.795). In a subgroup analysis of common diseases,the "Googlers" correctly diagnosed renal diseases less often than the attendings (76.6% vs. 90.5%, p<.001) and fellows (76.6% vs., 82.3%, p=.031). In a subgroup analysis of rare diseases, the "Googlers" correctly diagnosed renal diseases less often than the attendings (65.2% vs. 76.1%, p=.014), but more often than the fellows (65.2% vs. 56.2%, p=.029).

Conclusions: Google.com is a good tool to help medical residents diagnose renal diseases, but it does not take the place of a trained and experienced clinician. "Googling" a clinical question may be especially useful in the case of rare or "syndromic" diseases, but is likely to be less useful in diagnosing more common renal diseases.

TH-PO856

Further Validation of Peer Chart Audit To Assess Practice-Based Learning and Improvement in a Nephrology Fellow Continuity Clinic Setting Leslie F. Thomas,[1] Suzanne M. Norby.[2] *[1]Nephrology & Hypertension, Mayo Clinic, Phoenix, AZ; [2]Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

Background: Peer chart audit appears to be a valid method to assess the Practice-Based Learning and Improvement competency in a nephrology fellow continuity clinic setting, with overall data collection accuracy of 91% based on a validation study in our fellowship program in 2010. Accuracy appeared to be associated with the type of data abstracted and was similar for fellows in all 3 years of training. The purpose of this study was to compare accuracy of data collection in 2010 and 2011 and to re-evaluate the validity of peer chart audit in the 2011 fellow cohort.

Methods: Twelve nephrology fellows were instructed to review medical records of 10 consecutive non-dialysis patients with chronic kidney disease (CKD) stage 3, 4, or 5 cared for by another fellow. Five data points were assessed: whether a plan was documented for blood pressure (BP) > 130/80 mmHg; whether hemoglobin (Hgb) and parathyroid hormone (PTH) were measured during the prior 6 months or at the time of the visit; whether iron studies were checked if Hgb < 11 g/dL; and whether a total 25-hydroxyvitamin D2+3 (25[OH]D) level was checked if PTH > normal. Chart audits were then repeated by the program director. Results were compared to those from 2010.

Results: Of 120 patient records reviewed, 7 did not meet inclusion criteria and were excluded prior to analysis. Collections yielded 565 data points from 113 patient records. Overall accuracy did not differ between 2010 and 2011 fellow cohorts (86% vs 91%) nor among 1st year fellows (83% vs 95%), 2nd year fellows (91% vs 88%), and 3rd year fellows (85% vs 89%). Similar to 2010, Hgb data collection was more accurate than iron data collection (98% vs 84%; p=0.05), and PTH data collection was more accurate than 25[OH] D data collection (97% vs 81%; p=0.02). Eight fellows performed the audit in both years; accuracy of individual fellows did not differ significantly from year to year.

Conclusions: Peer chart audit yields valid results overall with 88% mean accuracy for both years combined. Accuracy differed depending on parameters assessed, confirming data from the previous study.

TH-PO857

All Are Not Created Equal: Categorization of Publication Entries on Applications Submitted to a Nephrology Fellowship Program Megan L. Krause,[1] Timothy R. Long,[2] Suzanne M. Norby.[3] *[1]Internal Medicine, Mayo Clinic, Rochester, MN; [2]Anesthesiology, Mayo Clinic, Rochester, MN; [3]Nephrology, Mayo Clinic, Rochester, MN.*

Background: Listing publications in the Electronic Residency Application Service (ERAS) is one way for trainees to distinguish themselves. The ERAS Publications category often includes a wide variety of projects that are not equivalent in academic merit, varying from peer-reviewed journal articles to residency morning report presentations. Since any academic product can be listed at the discretion of the individual applicant, an exaggerated list of total publications may result.

Methods: This study categorizes the type of publications listed by 183 applicants to a single Nephrology fellowship program.

Results: Of these, 155 (84.5%) entered at least one publication on their ERAS application. 64 applicants (41.3%) listed a total of 224 peer-reviewed journal articles (median=2; range 1-22 per applicant), 69 of which (30.8%) were first author. In addition, 13 applicants (8.4%) reported articles accepted but not yet published (range 1-5), and 38 (24.5%) included articles submitted but not yet accepted (median=2; range 1-6). Poster presentations were listed by 115 applicants (74.2%), with the majority (84.6%) presented at regional/national society meetings and the remainder displayed during institutional events. In contrast, oral presentations listed in ERAS were more likely to be delivered in an institutional venue (56.2%) such as journal club or noon conference rather than at regional/national meetings. "Other articles," such as student publications and ongoing projects, were listed by 17 applicants (11%).

Conclusions: The publications category in ERAS allows for heterogenous documentation, and similar number of total "publications" represents different levels of academic success among applicants. By highlighting quantity rather than quality, the current system for categorizing publications in ERAS obscures the ability of program directors to compare applicants based on academic productivity.

TH-PO858

Physician Role in Chronic Kidney Disease Patient Education: Perspectives from Nephrology Trainees Julie A. Wright, Melinda A. Coston, Talat Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt University Medical Center, Nashville, TN.*

Background: Chronic kidney disease (CKD) education is an important component of patient care, yet little is known about nephrologists' perspectives on their own role in the patient education process. We performed structured interviews of nephrology trainees to gain insight on this topic.

Methods: Interviews of nephrology fellows were performed at one academic institution. Through moderated sessions, participants were asked a series of open ended questions about the role of physicians in patient CKD education. Transcripts were analyzed using content analysis.

Results: Seven out of 16 nephrology fellows (47%) participated. Mean (SD) age was 31 (2) years, 29% were female, 29% were Black, 29% Asian, and 42% White. Participants emphasized disease education as one of the most important roles of the physician. Patient education was viewed as separate from 'taking care of patients' and 'patient management'. 47% (93/196) of all statements focused on barriers to providing education. Limited patient understanding of medical information was perceived as a barrier by every participant. Other barriers included physician time constraints, complexity of CKD diagnoses, and cultural differences, including differing levels of patient formal education attainment. Facilitators included support staff, face-to-face communication, and written materials. Only 8% of statements discussed expectations physicians had of patients to learn more about CKD, and mainly centered on expecting patients to ask questions on topics about which they were unclear. Participants stressed a need for more multi-disciplinary, multi-media approaches using direct (e.g. face-to-face, classroom) and indirect (internet, literacy sensitive handouts) mechanisms of communication, to optimize existing patient education.

Conclusions: Educating patients about CKD is complex and perceived as a very important role by nephrology trainees. Of high importance to trainees was development of concise, clear, and culturally sensitive communication aids for physicians to use during patient encounters to facilitate optimal CKD patient education.

Funding: NIDDK Support, Other NIH Support - T32 DK007569, Private Foundation Support

TH-PO859

Effect of Multidisciplinary Pre-Dialysis Education in Advanced Chronic Kidney Disease Patients Assessed by Propensity Matched Pair Analysis Eun Jin Cho,[1] Yun Kyu Oh,[2] Ki Young Na,[3] Ho Jun Chin,[3] Curie Ahn,[1] Kook-Hwan Oh.[1] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul national University Boramae Hospital; [3]Internal Medicine, Seoul National University Bundang Hospital.*

Background: The mortality and morbidity of end-stage renal failure patients remains high despite recent advances in pre-dialysis care. Previous studies suggested a positive effect on the patient survival and other outcomes for those receiving multidisciplinary pre-dialysis education (MPE). However above studies were limited by unmatched comparisons between the MPE recipients and non-recipients.

Methods: We performed a retrospective single center study, enrolling 1,218 consecutive pre-dialysis chronic kidney disease patients, between July 2007 and Feb 2008, and followed them up to 30 months. By using propensity score matching, we matched 149 recipient- and non-recipient pairs from 1,218 patients. The incidences of renal replacement therapy, mortality, cardiovascular event and infection were compared between MPE recipients and non-recipients.

Results: Renal replacement therapy was initiated in 62 and 64 patients in the recipients and non-recipients, respectively (P>0.05). The MPE reduced unplanned urgent dialysis (8.7% vs 24.2%, P<0.001), and shortened hospital days (2.16 vs 5.05 days/patient/year). MPE recipients had a better metabolic status at the time of initiating renal replacement therapy. Although no significant survival advantage from MPE was exhibited, MPE recipients had lower incidence of cardiovascular event (adjusted hazard ratio, 0.24; 95% CI, 0.08 to 0.78; P=0.017), and a tendency toward a lower infection rate (adjusted hazard ratio, 0.44; 95% CI, 0.17 to 1.11; P=0.083).

Conclusions: MPE was associated with better clinical outcomes in terms of urgent dialysis, cardiovascular events and infection.

TH-PO860

Health Literacy among Patients Referred to a Chronic Kidney Disease Clinic Dana Rizk,[1] Matt J. Glathar,[1] Rosalind M. Peters,[2] Jane S. Davis.[1] *[1]Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Nursing, Wayne State University, Detroit, MI.*

Background: In 2004 the Institute of Medicine Report highlighted that 90 million Americans have suboptimal literacy skills. Despite the high prevalence of chronic kidney disease (CKD) little is known about the health literacy level in that population or its impact on management and outcomes. The goal of our study was to determine the health literacy level among patients referred to the CKD clinic at our institution.

Methods: We recruited 37 consecutive patients on their first visit to the CKD clinic. All patients had prior follow-up by a nephrologist. We collected demographic and clinical data on each subject and administered a 34- item questionnaire assessing the patient's understanding of CKD, its symptoms, causes and risk factors for progression.

Results: The mean patient age was 58.6±15.5 years. Most patients were female (62.2%), white (54.1%), insured (97.3%) and high school graduates (83.7%). At referral 29.7%, 46% and 21.6% had stages III, IV and V CKD, respectively. Although 97.3% of patients acknowledged having CKD, only 43.2% could name its etiology. About 35.2% and 48.7% were unfamiliar with the terms "creatinine" and "glomerular filtration rate" respectively with no difference between those with <6 vs.>= 6 months of nephrology follow up. Although hypertension and diabetes were identified as modifiable risk factors for CKD, only 46% of hypertensives could name their target blood pressure and 40% of diabetics knew their target HgbA1C. Medication intake was significantly lower than prescribed (9.5± 4.8 vs. 12.5±5.6, p<0.0001). Most patients could identify some signs and symptoms of CKD like swelling and anemia, but were less likely to recognize uremic symptoms like anorexia, nausea and vomiting.

Conclusions: Despite a reasonable educational level and access to health care, patients referred to our clinic have a low level of CKD health literacy. Lower literacy has been related to worse outcomes in other chronic conditions. Whether educational interventions in the CKD clinic can improve health literacy remains to be seen.

Funding: Clinical Revenue Support

TH-PO861

Fatal Flaws in Education about Vein Protection for Patients with CKD Marcia R. Silver. *Div of Nephrology, CWRU at MetroHealth Medical Center, Cleveland, OH.*

Background: HD patients using catheters for vascular access (VA) have much higher mortality and costs than those using AVFs. Yet ~80% of CKD patients starting HD in the US use catheters.. Medicare's Fistula First Breakthrough Initiative Change Concept 2 calls for vein protection "at the first sign of kidney disease." The best vascular surgeon can't make a native vein AVF if there is no intact vein to use.

Methods: A large dialysis company provides free classes about RRT options for CKD patients. They invite participants to enroll for follow-up. Some participants provide email addresses. We used these email addresses to query educated CKD patients about their knowledge of vein protection.

Results: Invitations to participate in this research were sent to 1,161 patients by email with a link to the SurveyMonkey survey. Responses came from 182 participants from 34 states. Mean age of respondents was 61 (range 21-96). 53% were male; 12% African-American. 40% had Diabetes. 43% had Stage 4 CKD. 16% reported CKD-1-3. 33% had CKD-5 or RRT. 49% had been advised to protect their non-dominant arm from needle sticks. Only 14% had been advised to use the dorsa of their hands for needle sticks rather than arm veins. Of those protecting veins, almost all understood why. Most who tried to protect their veins found health care personnel honored their requests.

Conclusions: Frequent venipuncture and intravenous catheter use are common in the current practice of medicine in the US. Vein protection is critical to success in creating AVFs in CKD patients. In this large convenience sample of CKD patients who received education about CKD and RRT, we found only ~ half understood the importance of vein protection, and only ~15% were protecting veins in both arms by preferentially using the dorsa of the hands for venipunctures (what we call vein-saving technique.) It is likely that participants in this study were patients with higher socio-economic status and more than average engagement in self care-suggesting average rates of education about vein protection may be lower than those found in our study. This study shows we need improved education of patients and staff to achieve better AVF rates and reduced mortality and costs in ESRD patients.

Funding: Private Foundation Support

TH-PO862

Nutritional Intervention Program: An Important Tool for the Control of Hyperkalemia Carmen B. Tzanno-Martins,[1] Camila Machado de Barros,[2] Elzo R. Junior,[2] Bárbara Margareth Menardi Biavo.[2] *[1]Centro Integrado de Nefrologia; [2]Home Dialysis Center; [3]Universidade Federal do Rio Grande do Sul.*

Background: Nutritional intervention can prevent or control most metabolic disorders manifested in chronic kidney disease, including hyperkalemia, which is a risk factor for cardiac arrhythmias and sudden death. The purpose of this study was to evaluate the influence of knowledge about potassium before and after nutritional intervention in hemodialysis patients, four hours/session, three times/week.

Methods: This is a longitudinal, descriptive and primary data collection, which evaluated 61 elderly hemodialysis patients (> 60 years old), with serum potassium above 5.5 mg/dl. The nutritional intervention consisted of a lecture about hyperkalemia, with emphasis on restricting foods rich in potassium, interactive dynamics, games and distribution of educational materials. For assessment, a questionnaire of knowledge about potassium was applied before and after nutritional intervention.

Results: Of the 61 patients, 95% (n = 58) participated in the survey before and after nutritional intervention. Two patients (3.3%) were excluded, because one showed limited level of understanding both questionnaires and the other was absent on the day of sample collection. Of the 58 subjects, mean serum potassium of the last three months before nutritional intervention was 6.2 ± 0.5 mg/dL. After nutritional intervention, 49% of patients showed a decrease of serum potassium. The assessment of knowledge about potassium in the first analysis (pre nutritional intervention) showed that the highest percentage of individuals (55.7%) had less than 90% accuracy. In the second analysis (post nutritional intervention) 93.4% of patients scored higher than 90% of the questions.

Conclusions: The nutritional intervention proposal showed efficacy to increase potassium knowledge and provided benefits in short time. Because of this results we suggest keeping regular educational activities.

TH-PO863

Identifying Educational Needs of Nephrologists in the Management of ANCA-Associated Vasculitides Terry Glauser,[1] Chad Williamson,[1] Jennifer Garick,[1] Stuart M. Levine,[2] Mazi Abdolrasulnia.[1] *[1]CE Outcomes LLC, Birmingham, AL; [2]Department of Rheumatology, Johns Hopkins University, Baltimore, MD.*

Background: Sparse data exists in the current literature regarding practice patterns of physicians in managing patients with ANCA-associated vasculitides (AAV). This study was designed to identify current practice patterns and perceptions of nephrologists in managing these patients.

Methods: A case vignette survey was developed that allowed nephrologists to make diagnostic and treatment decisions for patients with limited and systemic Wegener's granulomatosis (WG) and with microscopic polyangiitis (MPA). The survey was distributed via email to U.S. nephrologists in November 2010, 44 responses were analyzed.

Results: Nephrologists had difficulty distinguishing different forms of AAV. When presented with a patient with clinical indications of MPA, 41% selected WG as the most likely diagnosis. Non-aggressive treatment goals were set, with only 16% considered remission as a goal for a 46-year-old man with limited WG and mild symptoms and likely capable of remission; most (65%) would try to improve symptoms or prevent disease progression. Selection of initial treatment for the patient varied: cyclophosphamide, 36%; nasal saline rinses/trimethoprim-sulfamethoxazole, 21%; nasal rinses/corticosteroids, 11%; oral corticosteroids/methotrexate, 11%. For a patient in remission after 3 months of oral cyclophosphamide, 58% would continue the regimen an additional 3 months before switching to azathioprine for maintenance; only 16% would switch after the initial 3 month regimen.

Regarding perceptions of management, nephrologists are most confident in managing active disease (75% very confident) and least confident in managing relapsed AAV (48% very confident) and differentially diagnosing forms of AAV (50% very confident).

Conclusions: This study identified perception and practice gaps in the management of patients with AAV by nephrologists, including lack of confidence in management, recognition of clinical differentiators, remission rates for therapy, and strategies to minimize cyclophosphamide exposure; all areas that can be addressed and improved through educational initiatives.

Funding: Pharmaceutical Company Support

TH-PO864

Organizational Communication Efforts for the Prevention of Catheter Related Blood Stream Infection from Hemodialysis Catheters Jaswinder S. Rattan,[1] Pavani Rangachari,[1, 2] P. J. Rissing,[1] Donna P. Goins,[1] Wanda Gillespie,[1] Lauren Hall,[1] N. Stanley Nahman.[1,3] *[1]Medicine, Georgia Health Sciences University, Augusta, GA; [2]Health Informatics, Georgia Health Sciences University, Augusta, GA; [3]Medicine, Charlie Norwood VAMC, Augusta, GA.*

Background: Catheter related blood stream infection (CRBSI) is a significant cause of morbidity and mortality (NEJM 355:2725, 2006). Use of CRBSI prevention practices (i.e., the "central line bundle") significantly reduces CRBSI. We previously showed that a lack of an organizational communication network for educating providers about CRBSI was associated with poor compliance with CRBSI prevention practices (CRBSI-PP) in the MICU (Q Manage Health Care, 19:330, 2010). To address these issues, a prospective study examining patterns of communication related to CRBSI-PP was initiated to measure compliance with bedside application of the CRBSI-PP, including the faculty and fellows in the Section of Nephrology. The educational effort included a required one hour didactic session reviewing the CDC recommended CRBSI-PP (the central line bundle) and successful completion of a hands-on session in the simulation center demonstrating application of the bundle.

Methods: The institution initiated the study in January 2011 and the present data reviewed after 15 weeks. Nephrology was educated in the CRBSI-PP in the 11th week.

Results: 100% of faculty (N=8) and fellows (N=6) completed the educational bundle. Compliance with application of the bundle for the 10 weeks prior (PRE) and 5 weeks after (POST) to the educational sessions was 37.5 vs 100 % for PRE vs POST, respectively. In addition, compliance with the 5th point of the bundle (daily documentation for continued use of the device) went from 0 to 100% following the educational intervention. These data indicate that a focused educational effort at improving communication about CRBSI improves compliance with the bundle.

Conclusions: Educational communication efforts result in higher compliance with CRBSI-PP by Nephrology faculty and fellows. We anticipate that this will result in a reduction in CRBSI associated with the placement of temporary hemodialysis catheters.

Funding: NIDDK Support

TH-PO865

Nephrology Advanced Practitioner Salary and Benefit Information – A Survey Barbara M. Weis. *Division of Renal Hypertension and Diseases, University of Colorado-Denver, Aurora, CO.*

Background: Nephrology Advanced Practitioner salary and detailed benefit information is limited. NPs and PAs function similarly in Nephrology. The purpose of this study was to compare and contrast the salaries/benefits of NPs and PAs as well as the responsibilities of their positions.

Methods: NKF's Council of Advanced Practitioners (CAP) in conjuction with the American Academy of Nephrology Physician Assistants (AANPA)conducted a salary and benefits survey of NPs, CNSs (clinical nurse specialists), and PAs from January 2010 to June 2010. A Zoomerang link was sent out to CAP and AANPA members via email with directions that this link could be shared with other nephrology APs who were not CAP nor AANPA members. The survey link was sent out monthly for 6 months. There were 276 responses (CAP membership as of 6/1/10 was 240 and was used a denominator since many APs overlapped between CAP and AANPA) for a response rate of 115%.

Results: Over 85% of the respondents were white females, between the age of 31 and 59, who worked full time; 82% held a Master's degree. The top three places that APs work are dialysis unit (71%), office (46%) and hospital (31%). Within the dialysis unit, most APs handled dialysis rounds and primary care issues although close to half (49%) were responsible for call at the dialysis units. Within the office, most APs ran CKD clinics (59%) and did hospital follow-up visits (44%). The average annual salary for all full-time APs was $83,800. There was NO correction between degree and salary. There was a very STRONG correlation between years of experience and salary. Most common benefits were malpractice insurance (93%), health insurance (96%), and paid CME (88%). The most important 'benefit' to APs was to feel valued at work and to have a good working relationship with their physician partners.

Conclusions: In conclusion, the survey showed similar average salary and benefits for APs, with regional variances. Salaries are on the lower end of the spectrum when compared with other specialties, due to one primary payor (Medicare). APs continue provide a cost effective opportunity for the medical community to provide CKD and ESRD patient care.

TH-PO866

A Prospective Multi-Centre Evaluation of Timing of Renal Replacement Therapy for Acute Kidney Injury in Critically Ill Patients in Canada Edward G. Clark,[1] James William Barton,[3] Josee Bouchard,[4] Jan O. Friedrich,[5] Michelle A. Hladunewich,[6] Matthew T. James,[7] Jean-Philippe Lafrance,[8] Adeera Levin,[9] Louise M. Moist,[10] Neesh I. Pannu,[11] D. E. Stollery,[12] Ron Wald,[13] Michael Walsh,[14] Sean M. Bagshaw.[2] *[1]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.*

Background: The optimal timing of initiation of renal replacement therapy (RRT) for acute kidney injury (AKI) is unknown. Defining current practice is necessary to properly design interventional trials. We sought to describe the current state of practice in Canada regarding the timing of initiation of RRT for AKI.

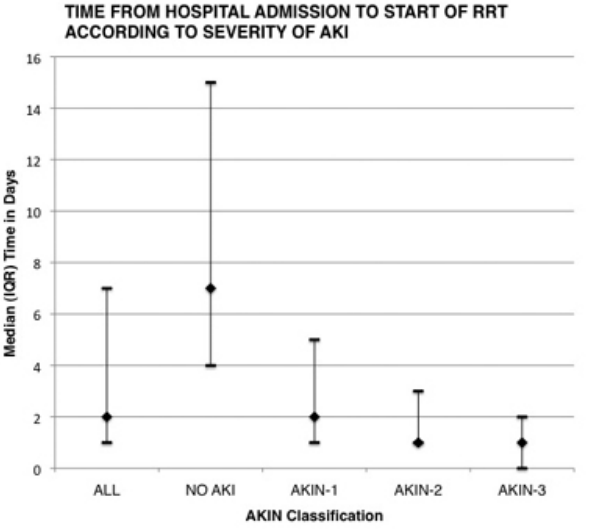
Methods: A prospective, observational study was undertaken at 10 intensive care units across Canada, enrolling 119 consecutive patients starting RRT for AKI. Data was collected regarding demographics, clinical and laboratory findings, as well as indications for, and timing of, RRT initiation.

Results: The mean (SD) age of patients starting RRT was 59 (15) and 34% were female.

Clinical and Laboratory Findings at Time of RRT Initiation

APACHE II Score, mean (SD)	27 (7)
Intubated / Receiving NIPPV, % (n)	83 (99)
Receiving Vasoactive Medication, % (n)	76 (90)
Loop Diuretic (Past 24h), % (n)	28 (33)
Serum Creatinine (umol/L), median (IQR)	322 (221-432)
Serum Urea (mmol/L), median (IQR)	20 (13-27)
Arterial pH, mean (SD)	7.25 (0.15)
Serum Potassium (mmol/L), mean (SD)	4.6 (1.0)

Median (IQR) time from hospital and ICU admission to the start of RRT was 2 days (1-7) and 1 day (0-2), respectively.



Conclusions: The ICU patients started on RRT in our study generally had advanced AKI, high illness severity, and received RRT early after hospital presentation. Our results describe the current state of practice with respect to the timing of initiation of RRT for AKI in Canada and should aid in the design of future interventional trials.

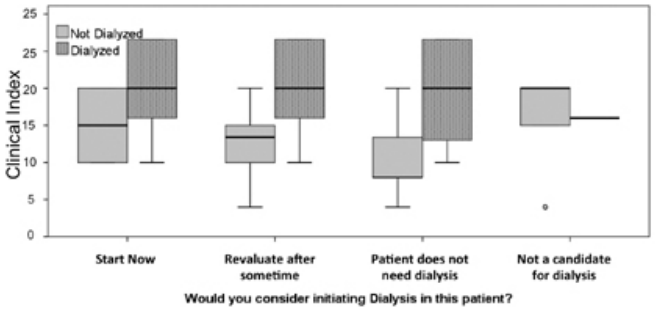
TH-PO867

Can Objective Criteria Improve Decisions for Dialysis Initiation in AKI? Etienne Macedo,[1] Josee Bouchard,[2] Jorge Cerda,[3] Ashita J. Tolwani,[4] Anjali Acharya,[5] Gary R. Cutter,[4] Ravindra L. Mehta.[6] *[1]University of Sao Paulo, Brazil; [2]Hôpital du Sacré-Coeur de Montréal, Canada; [3]Albany Medical College; [4]University of Alabama; [5]Jacobi Medical Center; [6]University of California San Diego.*

Background: Physician decisions vary widely on how criteria to initiate dialysis are applied. We hypothesized that an index based on clinical and lab data could characterize the need to support critically ill patients with renal replacement therapy (RRT) and correlate with physician decisions.

Methods: We utilized a web-based survey to present 12 real cases scenarios with pertinent clinical and lab data from 48 to 72 hs prior and up to the day of dialysis in actually dialyzed patients (n=6) or the day of peak creatinine in those patients that where not dialyzed (n=6). Physicians were asked whether they would dialyze immediately, reevaluate in some time, consider whether the patient does not need dialysis or would not benefit from dialysis. For each patient we calculated a clinical index (CI) each day. We analyzed the relationship of physician decision to start dialysis and the underlying CI on the day of dialysis or peak creatinine.

Results: There were a total of 206 questionnaires; 14 to 31 completed answers for each patient. Physician responses and actual events were frequently discordant (48%). Dialyzed patients had higher values of the CI. Within each category of physician choice, the CI correlated better with actual provision of dialysis than physician response. Among patients with lower CI value, physician choices correlated with the decision that the patient did not need dialysis.



Conclusions: Physicians decisions to initiate dialysis were influenced by the severity of illness, likelihood of benefit and logistic factors. An objective CI may provide further improvement in clinical decisions.

Funding: NIDDK Support

TH-PO868

Creatinine Clearance and Urine Sediment as Predictors of Need of Renal Replacement Therapy in Intensive Care Unit Patients with Acute Kidney Injury Radu R. Raducu,[1] Kolawole Omodayo Atandeyi,[1] Fredric O. Finkelstein.[1,2] *[1]Nephrology, Hospital of Saint Raphael, New Haven, CT; [2]Nephrology, Yale New Haven Hospital, New Haven, CT.*

Background: The patients who are admitted to ICU frequently develop AKI and are consulted by nephrologists. The study is evaluating the role of successive creatinine clearance measurements and urine sediment analysis as predictors of need of RRT during current hospitalization.

Methods: 57 patients were evaluated by nephrology department for AKI. The inclusion criteria were the following: age more 18 years, ICU location, nephrology consultation for AKI, strict monitoring of urine output through Foley catheters. A 2 hour creatinine clearance was measured on day of consult and at 24h and 48 h after nephrology consult. Urine sediment was evaluated on day of consult and an acute tubular necrosis score was devised. The patients were followed for the need of RRT during the entire hospitalization.

Results: 27 % of patients required RRT. In 95% of patients the renal injury was completely established at time of nephrology consult. In the five groups of patients (creatinine clearance at time of consult less than 2.5 mL/min, 2.5 to 5ml/min, 5 to 7.5 ml/min, 7.5 to 10 ml/min and more than 10 mL/min) the risk of requiring HD was 80%, 70%, 30%, 25% and 0%. If we consider the creatinine clearance at 48h the risk of requiring RRT was (in same groups) 100%, 80%, 50%, 0% and respectively 0%. ATN score was 5.5 in patients who didn't require RRT and 1.5 in patients who require HD (p-value= 0.23). The correlation coefficient between ATN score and creatinine clearance at time of consult was 0.135. The correlation coefficient between serum creatinine and need of RRT was 0.213.

Conclusions: At the time of nephrology consult the kidney injury is already completely established in the majority of patients with AKI and serial measurement of creatinine clearance is the best method to quantify the degree of injury, to evaluate the degree of recovery and to predict the need for RRT. Urine microscopy is not useful in predicting need for RRT (in fact kidneys with higher intrinsic function might have higher ATN score on urine analysis).

TH-PO869

Effect of Fluid Removal on Delivered Dose of Intermittent Hemodialysis (IHD) in Acute Kidney Injury (AKI) Rolando Claure-Del Granado,[1] Etienne Macedo,[2] Sharon Soroko,[1] Glenn M. Chertow,[3] Jonathan Himmelfarb,[4] Talat Alp Ikizler,[5] Emil P. Paganini,[6] Ravindra L. Mehta.[1] *[1]University of California San Diego; [2]University of Sao Paulo; [3]Stanford University School of Medicine; [4]Kidney Research Institute, University of Washington; [5]Vanderbilt University; [6]Cleveland Clinic Foundation.*

Background: Standard Kt/V (StdKt/V) is used to measure dialysis efficacy across different types of therapies of variable frequency in patients on chronic dialysis. In AKI, spKt/V or eKt/V is calculated for IHD. Previous equations to estimate StdKt/V were derived using a fixed-volume model. We evaluated the impact of fluid removal on StdKt/V. We hypothesized that not adjusting StdKt/V for fluid removal would underestimate delivered dose of IHD.

Methods: We analyzed data from 599 IHD sessions in 254 critically-ill patients with AKI from 5 centers included in the PICARD study. Delivered dose was calculated using spKt/V (Daugirdas equation), StdKt/V Leypoldt (Leypoldt JK et al. Semin Dial 17: 142-145; 2004) and using StdKt/V proposed by Daugirdas (Daugirdas JT et al. Kidney Int 77: 637-644; 2010) that includes effects of fluid removal. Patients with residual renal function were excluded. We compared the efficacy of 1 – 7 IHD sessions per week.

Results: A good correlation was found between spKt/V and Leypoldt StdKt/V (r^2 = 0.961; p < 0.001). StdKt/V was inversely proportional to the urea volume of distribution (r^2 = 0.017; p = 0.002). Higher frequency of IHD treatments per week was associated with increased delivered StdKt/V (median [IQR]): from 0.58 (0.47 – 0.67) for 1 treatment; 1.26 (0.92 – 1.47) for 2; 1.83 (1.50 – 2.15) for 3; 2.39 (1.91 – 2.84) for 4; 2.67 (2.17 – 3.19) for 5; 3.15 (2.60 – 3.97) for 6; and 3.77 (3.1 – 5.2) for 7 treatments per week respectively. Compared to Leypoldt StdKt/V, values of fluid removal adjusted Daugirdas StdKt/V equation were higher (2.0 IQR [1.5 – 2.7] vs. 1.9 IQR [1.4 – 2.5]; p = 0.001).

Conclusions: The use of StdKt/V Daugirdas et al equation provides an improved assessment of delivered dose of IHD adjusting for fluid removal. These results may inform the design of future studies of dialysis dose in AKI.

Funding: NIDDK Support

TH-PO870

Adverse Effects of Fluid Overload in Patients with Acute Kidney Injury Receiving Continuous Renal Replacement Therapy Min-Jee Han, Youn-Su Park, Jung-Ho Shin, Woojin Nam, Su Hyun Kim, Dong-Jin Oh, Suk-Hee Yu. *Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea.*

Background: Extensive fluid overload leads to acute physiologic changes such as metabolic imbalance of sodium and water, pulmonary edema and respiratory failure. In critically ill patients with acute kidney injury (AKI), mortality rates range from 50 to 70% and have usually been attributed to multiple complications related to AKI. We thus investigated whether fluid overload is associated with mortality in critically ill patients with AKI receiving continuous renal replacement therapy(CRRT).

Methods: We retrospectively reviewed 100 patients with AKI treated with CRRT at Chung-Ang University Hospital between April 2005 and December 2010. The definition of fluid accumulation is still subject to debate, but we defined fluid accumulation as more than 4L as a sum of daily fluid balance over a period initiating at CRRT.

Results: Of the 100 enrolled patients, 70 were assigned to a fluid overload group and 30 to a fluid restriction group; there were no significant differences between the groups with respect to base-line characteristics. The most common cause of AKI was septic shock (44%), followed by cardiogenic shock (19%). In-hospital mortality was 81.4% in the fluid overload group compared with 66.7% in the fluid restriction group (P=0.005). The mean cumulative fluid balance was 12.7±8.5 L in the fluid overload group and 0.9±3.6 L in the fluid restriction group (P<0.001). During the interval from admission to the initiation of CRRT, the fluid overload group showed significantly higher incidence of mechanical ventilation apply (85.7% vs. 66.7%, P<0.001), and higher use of inotropics (88.6% vs. 66.7%, P<0.001) than the fluid restriction group.

Conclusions: In critically ill patients with AKI, fluid accumulation poses a higher risk of death. Our data supported the concept that fluid accumulation is at least partially responsible for a poor outcome in patients with AKI and defends the strategy of attempting to achieve fluid restriction if tolerated hemodynamically. Thus carefully verification of the daily fluid balance is a great help to decrease mortality for critically ill patients with AKI.

TH-PO871

Phosphate Balance in Critically Ill Patients on Continuous Venovenous Hemofiltration Shilpa Sharma, Sushrut S. Waikar. *Brigham & Womens' Hospital, Boston.*

Background: Hypophosphatemia is a frequent complication during continuous renal replacement therapy (CRRT), and may contribute to poor patient outcomes due to phosphate's critical role in energy metabolism in every organ system. We sought to quantify phosphate clearance during CVVH in our institution.

Methods: By adding a T-connector to the effluent line, approx. 1% of the 24 hr effluent volume was diverted to a collection bag. Estimated phosphate removal was calculated by multiplying the total effluent volume with concentration of phosphate in the effluent fraction. Results were verified by comparison to 4 hr complete collections in a subset of enrolled patients.

Results: Seventy-eight 24-hr effluent collections and thirteen 24-hr urine collections were performed on 25 patients. Most patients were anuric and received intravenous or oral phosphate.

Patient	CVVH days	Serum phos range(mg/dl)	Net phos removed(mg)	Net Infused phos(mg)	Net Phos Balance(mg)
1	4	2.3-3.4	5400.7	2574.8	-2825.9
2	6	2.2-3.8	11780.6	4322	-7458.6
3	7	2- 4.3	15684.9	1901.6	-13783.3
4	2	2.8-3.1	5273	558	-4715
5	7	2.2-6.8	8217.22	2116	-6101.22
6	2	1.7	5661.1	1088	-4573.1
7	2	2.4-2.6	6040.7	798	-5242.7
8	2	3.2-3.5	2427.3	720	-1707.3
9	4	2.6-3.2	4091.12	1236.8	-2854.32
10	2	2.2-2.5	1447.08	465	-982.08
11	6	2.3-2.7	5637.18	2334	-3303.18
12	1	3	841.47	324	-517.47
13	2	2.7-4.7	3223.74	0	-3223.74
14	4	3-6.5	8062.42	143	-7919.42
15	1	3.8	2590.9	0	-2590.9
16	1	1.8	1821.6	624	-1197.6
17	3	2.5-3.3	2638.19	310	-2328.19
18	2	2.9-3.4	2482.74	310	-2172.74
19	1	3.1	1699.27	0	-1699.27
20	7	2.8-4.9	11100.5	2665.38	-8435.12
21	7	2.8	9933.1	620	-9313.1
22	1	2.8	2142.19	561	-1581.19
23	1	6.3	2264.85	0	-2264.85
24	2	2.6-2.9	1704.44	0	-1704.44
25	1	2.8	1588.08	518.4	-1069.68

Conclusions: CVVH results in a negative phosphate balance despite protocol-driven phosphate repletion strategies. Substantial amounts of phosphate may be cleared by CVVH before overt hypophosphatemia develops. Further study is warranted to determine

the physiologic consequences of CVVH-induced phosphate depletion on bone health, energy metabolism, 2,3 biphosphoglycerate levels and subsequent oxygen delivery to peripheral tissues.

Funding: NIDDK Support

TH-PO872

Plasma IL-10 Level and Monocyte HLA-DR Expression as Predictors in Critically Ill Patients Undergoing Continuous Renal Replacement Therapy

Chen Yu. *Nephrology, East Hospital, Shanghai, China.*

Background: To determine whether interleukin 10 (IL-10) level and monocyte expression of antigen-DR (HLA-DR) are predictors of infection and prognosis in critically ill patients undergoing continuous renal replacement therapy (CRRT).

Methods: A total of 43 critically ill patients undergoing continuous veno-venous hemofiltration (CVVH) were recruited from the intensive care unit (ICU). Anti-coagulated blood was obtained on the 1st, 4th, and 7th days after taken CRRT, and ELISA and flow cytometry were performed to determine serum IL-10 level and HLA-DR expression on the surface of $CD14^+$ monocytes, respectively.

Results: (1) Eighteen patients had no infection and negative cultures (Group 1) during the study; 19 patients had infection and positive cultures (Group 2) at entry; 6 patients had no infection (Group 3) at entry but became infected within 2 weeks after beginning CVVH; 7 patients died. (2) The IL-10 level was higher in patients than in healthy subjects ($P<0.001$), rapidly decreased in Group 1 after treatment ($P<0.05$), was unchanged in the remaining patients, and was closely related to the APACHE II score and duration of hospitalization ($P<0.05$). (3) Monocyte HLA-DR expression was lower in patients than in healthy individuals ($P<0.01$). After CVVH, most patients with increased HLA-DR expression were uninfected. However, patients with unchanged or declining HLA-DR expression were infected or developed post-treatment infection. The patients who died had persistent and extremely low HLA-DR expression.

Conclusions: IL-10 is an indicator of disease severity, and persistently high IL-10 level predicts poor prognosis. Persistently low monocyte HLA-DR expression can be used to predict concomitant or future infection.

Funding: Government Support - Non-U.S.

TH-PO873

Rational Use of Polymethilmetacrylate Dialysis Membrane To Remove Serum Free Light Chains

Paolo Fabbrini, Andrea Stella, Mariarosa Viganò. *Clinica Nefrologica Ospedale San Gerardo, Università degli Studi di Milano Bicocca, Milano, Italy.*

Background: in vitro study showed that PMMA dialysis membrane can remove at least up to 2 grams of FLCs through an absorption mechanism, but in vivo application showed variable results and no study have investigated maximum adsorbent capacity and time before membrane saturation in a 4 hours dialysis treatment. Aim of this study was to measure sFLC removal kinetic through PMMA membrane in order to optimize its use in clinical practice.

Methods: we performed 24 hemodialysis sessions (dialisys lenght 4 hours, dialyzer PMMA BK 2.1 Toray inc) in 7 consecutive patients with dialisys dipendent ESRD associated to elevated sFLC levels (4 l e 3 k) In each treatment we measured sFLC hour removal to calculate sFLC plasma reduction rate of each hour and of the entire session. According to these results we then performed 10 dialysis sessions of 4 hours substituting PMMA dialyzer every 2 hours using a specifically design circuit that allowed dialyzer switch without stopping dialysis session. Per hour and total treatment sFLC removal rate was calculated.

Results: the 24 single dialyzer sessions resulted in an hourly average reduction rate of 12,3 % (between 5% and 17%) that was effective only for the first two hours of treatment indicating saturation of PMMA membrane adsorption capacity. Coherently, entire session average sFLC removal was 23% (between 3% and 76%). During 10 double dialyzer sessions we measured average sFLC total removal of 50.9%(between 33,3 and 69%, $p<0.05$ vs single dialyzer) with an hourly rate removal of 12,5% ($p>0.05$ vs single dialyzer). Double dialyzer treatments did not change heparin use and albumin depletion in comparison with single dialyzer treatments.

Conclusions: dialysis with PMMA BK 2.1 can efficiently remove sFLC through absorption mechanism that reach saturation after 2 hours of treatment. The use of a dedicated dialysis circuit with membrane substitution after 2 hours improved treatment efficacy up to values that are usually reported for high cut off treatments indicating that PMMA can be considered a valid and not expensive therapy in FLCs removal.

TH-PO874

Evaluation of Free Light Chain Removal by Various Blood Purification Methods

Kyoko Kanayama, Midori Hasegawa, Yukio Yuzawa. *Nephrology, Fujita Health University School of Medicine, Toyoake, Aichi, Japan.*

Background: We evaluated various blood purification methods other than plasma exchange to remove free light chains (FLCs).

Methods: [CLINICAL study]Two patients of IgG kappa multiple myeloma and two patients of IgG lambda multiple myeloma with acute renal failure were treated by hemodiafiltration(HDF) using protein leaking dialyzer PES210Dα™ or hemodialysis(HD) using PMMA dialyzer. [IN study vitro]Dialysis using HCO membrane Theralite2100™ or plasma separator Evacure1A20™, diafiltration using protein leaking dialyzer PES210Dα™ and adsorption using β2microglobulin adsorption column LixelleS-35™ were performed in an *in vitro* circuit.

Results: [CLINICAL study]Removal rate of kappa FLC was from 21.8% to 71.6% by HDF using PES210Dα™ and from 38.3% to 71.0% by HD using PMMA dialyzer. Removal rate of lambda FLC was from 48.5% to 53.1% by HDF using PES210Dα™ and from 29.6% to 45.6% using HD using PMMA dialyzer. There was blood residue after HD using PMMA dialyzer when serum levels of FLCs were high. [IN study vitro]The highest removal rate was obtained by Theralite2100™dialysis among the four blood purification methods. Albumin loss was also the greatest in Theralite2100™dialysis. The removal content of FLCs per 1g albumin loss was better in PES210Dα™ diafiltration. The removal rate of FLCs by Evacure1A20 ™dialysis was the third highest. Adsorption of FLCs by the β2 microglobulin adsorption column Lixelle S-35™ was confirmed.

Conclusions: Theralite2100™dialysis was the best in removal of FLCs. In countries where Theralite2100™ is not available, HDF using protein leaking dialyzer could become an alternative option.

TH-PO875

Myoglobin Removal in Rhabdomyolysis: Clinical Studies and a Mathematical Model

Paolo Fabbrini,[1] Richard T. Keir,[2] Andrea Stella,[1] Colin A. Hutchison.[3] *[1]Clinica Nefrologica H San Gerardo, Monza, Italy; [2]University of Warwick, United Kingdom; [3]Renal Unit, University Hospital Birmingham, United Kingdom.*

Background: Acute kidney injury secondary to high serum myoglobin levels is a frequent cause of morbidity and mortality for patients with rhabdomyolysis. Theoretically rapid removal of myoglobin by protein permeable dialyzers would improve clinical outcomes for these patients by reducing the tubules exposure to myoglobin. The purpose of this study was to determine the optimum strategies for the removal of myoglobin in rhabdomyolysis using a high cut-off dialyzer.

Methods: Myoglobin clearance rates were studied in four patients with the HCO 1100, these were compared with those for high flux dialyzers and the larger 2.1m high cut-off dialyzer (Theralite). A four compartment model was then used to simulate use of these different membranes on different treatment schedules: continuous venous-venous hemofiltration and 2 and 8 hours of HD (each for a period of 3 days).

Results: The median percentage reduction in serum myoglobin levels was 52% (range 35-89) with HCO-HD. This equated to a median clearance rate of 34mls/min (range 10-63). The mathematical model parameters were fitted to these patients data and then simulated for treatment regimes of 2 hours at a myoglobin clearance rate of 2.2 ml/min (high flux dialyzer), 2 + 8 hours at 70 ml/min (Theralite 2.1m dialyzer) and continuous treatment at 34 ml/min (CVVH using the HCO 110 dialyzer). The simulations demonstrated that use of either of the two HCO dialyzers on both HD and CVVH settings resulted in greatly reduced renal exposure to myoglobin . Of the treatment options over a 3 day window CVVH using the HCO 1100 dialyzer reduced the area under the curve by 87% compared with standard HD. Simulations of HD using the Theralite dialyzer revealed a reduction in the AuC of 39 and 72% for 2 and 8 hour treatments respectively. All four patients recovered renal function and became independent of dialysis.

Conclusions: In summary, the HCO dialyzers provide a rapid reduction in myoglobin levels in rhabdomyolysis, clinical studies are now required to determine if this translates to improved patient outcomes.

TH-PO876

Light Chain Removal by Means of Adsorption in the Extracorporeal Treatment of Myeloma-Induced Cast Nephropathy

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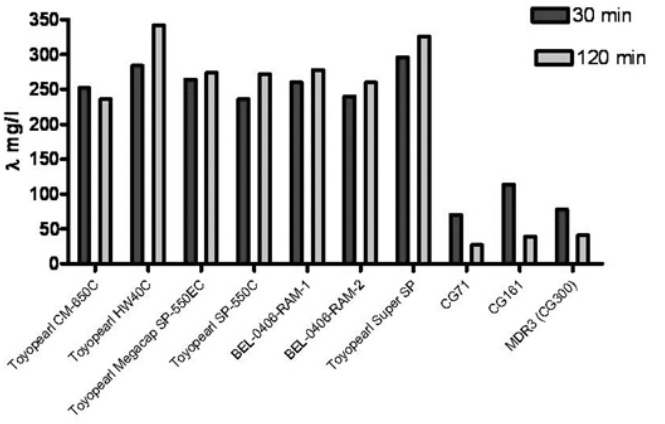
Background: The combination of chemotherapy with dialysis removal of light chains (LC) has been described as a new therapeutic strategy for cast nephropathy (CaN). However, due to their hign molecular weight (κ 12,000; λ 24,000 daltons), LC could also be adsorbed by resins. Coupled plasma-filtration adsorption (CPFA), presents as an ideal technique. A different efficacy, in terms of LC removal may be expected on the grounds of the sorbent resin used.

Methods: We performed an *in-vitro* study to identify the resin with the best adsorptive capacity, followed by an *in-vivo* study to verify the magnitude of LC removal. In some patients, a longitudinal evaluation was carried out to obtain information on the LC trend.

In vitro: serum from different patients was perfused onto different sorbent resins. LC after 30 and 120 minutes perfusion were compared. In vivo: 4-hour CPFA was performed in 10 CaN patients utilising the cartridge whose adsorptive capacity proved best. Apart from blood (start and end-treatment), pre- and post-resin plasma samples were taken every hour

Results: The MDR3 resin showed the best adsorptive capacities.



In vivo, the mean LC adsorption by the MDR3 cartridge was better for κ (28%) than for λ chains (22%). The pre-to-post treatment blood reduction ratio was 31% for κ and 26% for λ chains. In patients treated with at least 6 sessions, the LC concentration progressively decreased (κ 68±11% , λ 55±9% ; p=0.05)

Conclusions: Extracorporeal LC removal may be performed not only by diffusion but even by adsorption in 4-hour treatments. The effect of variables such as the resin volume in the cartridge, plasma flow, treatment time, are to be tested. The best schedule for associating chemotherapy with extracorporeal adsorption still needs investigation.

Funding: Pharmaceutical Company Support

TH-PO877

The Incidence of the Citrate Accumulation during Continuous Veno-Venous Haemodialysis with Regional Citrate Anticoagulation – A Monocentric Retrospective Study Dmytro Khadzhynov, Torsten Slowinski, Christin Baumann, Ina Lieker, Hans-Hellmut Neumayer, Harm Peters. *Department of Nephrology, Charite Campus Mitte, Charite Universitätsmedizin, Berlin, Germany.*

Background: Systemic citrate accumulation due to compromised citrate metabolization is a complication of a continuous renal replacement therapy (CRRT) with regional citrate anticoagulation (RCA). Impaired liver function and arterial hypoxia are described in literature as risk factors for citrate accumulation. Metabolic acidosis, increased total calcium/ionized calcium ratio (total-Ca/iCa>2.25) and increased demand for systemic calcium substitution are the common markers for citrate accumulation. The aim of the present study was to assess the incidence and clinical characteristics of the citrate accumulation on the basis of representative patient population.

Methods: The data from all ICU at our university hospital in 2010 was retrospectively analyzed. Weight adapted RCA for continuous veno-venous hemodialysis (CVVHD) was performed according to the protocol published by Morgera et al.

Results: 13 (3.6%) of 365 patients treated with citrate-based CVVHD (66.4±12.9 years old treated, 53.8% male, APACHE score 31.5±8.6) have developed the characteristics of citrate akkumulation. Six of 13 Patients (46%) had pre-existing liver dysfunction (mean S-bilirubin before treatment: 7.7 mg/dL). Elevated Ca substitution demand (up 123±18% compared to baseline rate), simultaneous decrease in systemic iCa concentration (decrease by 10% to 1.04±0.09 mmol/L) and the increase in total-Ca/iCa ratio (2.31±0.26 mmol/L) have been observed in all 13 patients. At the time when citrate accumulation was diagnosed all 13 patients developed severe lactic acidosis (pH 7.20±0.13, lactate 126±59 mg/dL, bicarbonate 15.0±3.5 mmol/L). Anticoagulation modality was immediately changed to heparin in all 13 patients. 12 of 13 patients died from the therapy-resistant shock at the ICU.

Conclusions: The incidence of citrate akkumulation was 3.6% of all CVVHD treatments based on RCA. Citrate akkumulation was found exclusively in patients with severe lactic acidosis in septic shock and was associated with poor prognosis (mortality> 90%).

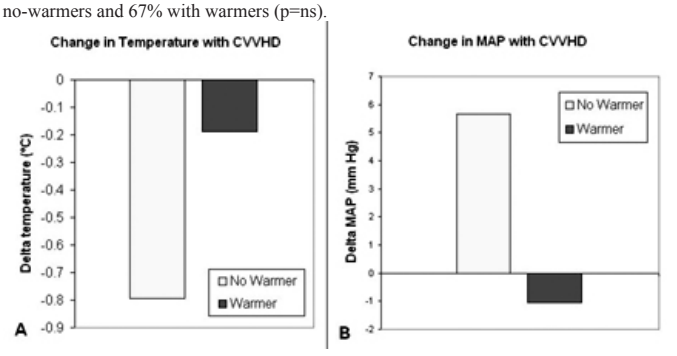
TH-PO878

Safety and Impact of CVVHD without Dialysate Warmers: A Difference of Opinion Swati Arora, Ayuk Eric Tabi, Leonardo P. Machado, Kalathil K. Sureshkumar, Rita L. McGill, Richard J. Marcus. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: Continuous veno-venous hemodialysis (CVVHD) is used to dialyze hemodynamically unstable patients. When the manufacturers of the device (NxStage®) had removed dialysate warmer from CVVHD circuit, intensive care unit (ICU) physicians expected adverse patient outcomes from the resulting hypothermia, but nephrologists expected cooled dialysate to be beneficial. This provided us a unique opportunity to study the impact of CVVHD with no warmers in critically ill patients.

Methods: We retrospectively reviewed the charts of patients who had CVVHD with or without warmers over a 6 month period. We calculated Sequential Organ Failure Assessment (SOFA) scores (range 0-24, higher the score, sicker the patient is), and collected core body temperature (cBT), mean arterial pressure (MAP), use of vasopressor drugs, ICU length of stay (LOS), and mortality.

Results: Thirty three patients without warmers had significantly lower cBT over the first 24 hours of CVVHD compared to 37 patients on warmers (35.4°C vs. 35.9°C, $P<0.05$). Fall in cBT from baseline was greater in the no-warmer group (0.79°C vs. 0.18°C, $p<0.05$), (figure 1A). Though there was a trend of higher MAP in patients with no warmers, absolute MAP during CVVHD did not differ between groups (77 mmHg vs. 73 mmHg, p=0.32), nor did ΔMAP from baseline (+5 mmHg vs. -1 mmHg, p=0.10), (figure 1B). SOFA scores did not significantly differ between no-warmer vs. warmer groups either at ICU admission (9±4 vs. 10±4) or 24 hours into CVVHD (12±3 vs. 12±4), nor did LOS (29±37 days vs. 29±31 days) or vasopressor use (1.3±1.0 vs. 1.2±1.3 agents). ICU mortality was 60% with no-warmers and 67% with warmers (p=ns).



Conclusions: Our study showed that CVVHD without dialysate warmer induced modest hypothermia. This did not adversely impact outcomes in critically ill patients.

TH-PO879

Pharmacokinetics of Ertapenem in Critically Ill Patients Receiving Continuous Venovenous Hemodialysis (CVVHD) or Hemodiafiltration (CVVHDF) Rachel F. Eyler,[1] A. Mary Vilay,[3] Michael Heung,[1] Melissa Pleva,[1] Kevin M. Sowinski,[2] Daryl Depestel,[1] Bruce A. Mueller.[1] *[1]University of Michigan, Ann Arbor, MI; [2]Purdue University, West Lafayette, IN; [3]University of New Mexico, Albuquerque, NM.*

Background: Ertapenem (E) is a broad spectrum carbapenem antibiotic indicated in many infections found in the ICU. No dosing recommendations exist for E in critically ill patients receiving continuous renal replacement therapy. The purpose of this study is to determine the pharmacokinetics (PK) of E in critically ill adults receiving CVVHD/F.

Methods: This study was approved by the U Michigan IRB, and was a prospective, open-label first dose PK study of E in critically ill adults receiving CVVHD or CVVHDF. One gram E was infused over 30 minutes. Effluent and pre-filter blood samples were collected at 1, 2, 4, 8, 12, 18 and 24 hours following E infusion. Samples were analyzed by HPLC-MS/MS. Non-compartmental methods were used to estimate PK parameters.

Results: Eight subjects ([mean ± SD] age 62 ± 16 years, weight 78.9 ± 19.8 kg) were enrolled. CVVHD/F was delivered at effluent rates of 38 ± 9.7 mL/kg/hr. The half-life, apparent volume of distribution at steady state, area under the concentration-time curve from 0-24 hours, and the E serum concentration at 24 hrs were 8.8 ± 3.2 hrs, 0.19 ± 0.060 L/kg, 710 ± 150 mcg/mL*hr, and 10 ± 4.0 mcg/mL, respectively. The total clearance (CVVHD/F and systemic clearance) was 21 ± 5.7 mL/min. The sieving coefficient was 0.21 ± 0.065 and CVVHD/F clearance was 10 ± 4.2 mL/min.

Conclusions: E half-life was twice as long as what is reported in normal volunteers. CVVHD/F was responsible for substantial E clearance in these subjects. The one gram E dose produced serum E concentrations above the MIC sensitive breakpoint of 2 mcg/mL for *Enterobacteriaceae* spp. for 100% of the dosing interval for all 8 patients.

Funding: Pharmaceutical Company Support

TH-PO880

Sulfamethoxazole and Trimethoprim Transmembrane Clearance during Modeled Continuous Hemofiltration A. Mary Vilay,[1] Jacob M. Kesner,[1] Renee-Claude Mercier,[1] Dean P. Argyres,[2] Scott E. Walker,[3] Craig S. Wong.[1] *[1]Univ. New Mexico; [2]VA Cooperative Studies Program; [3]Sunnybrook HSC.*

Background: Sulfamethoxazole (SMX) and trimethoprim (TMP) are administered concomitantly to treat a variety of infections. The physicochemical properties of SMX/TMP suggest they may be removed during continuous hemofiltration (CH). However, SMX/TMP removal during CH has not been systematically examined. The purpose of this study was to estimate SMX/TMP transmembrane clearance (CLtm) during modeled CH.

Methods: The invitro model consisted of 0.9 L heparin anticoagulated human blood continuously stirred at 37 °C. SMX/TMP was added to achieve concentrations of 160 ug/mL and 8 ug/mL respectively. Urea was added to serve as control. CH was performed with two commonly used hemofilters: HF1000 polysulfone (n=5) and M100 AN69 (n=5) hemofilters. Spent ultrafiltrate was recirculated back into blood to create a closed system. Ultrafiltration rates (Quf) of 1, 2, 3, and 6 L/h were investigated. At each Quf, prefilter blood and spent ultrafiltrate were collected and assayed for urea, SMX, and TMP. The concentration of solute in spent ultrafiltrate was divided by that in prefilter blood to calculate the extraction coefficient (E) of each solute. CLtm=E*Quf. Student's t-test was used to compare E between hemofilter types and ANOVA was used to compare E within each hemofilter type. P<0.05 was considered significant.

Results: Urea, the control solute, E was approximately 1 at all Quf rates studied.
SMX/TMP E and CLtm

Quf (L/h)	1	2	3	6	ANOVA p-value
SMX HF1000 E	0.39±0.03	0.38±0.03	0.40±0.03	0.40±0.03	0.71
SMX HF1000 CLtm (mL/min)	6±1	13±1	20±1	40±3	
SMX M100 E	0.31±0.03	0.33±0.05	0.32±0.02	0.33±0.04	0.74
SMX M100 CLtm (mL/min)	5±1	11±2	16±1	33±4	
t-test p-value for E	**0.01**	0.14	**0.01**	**0.02**	
TMP HF1000 E	0.62±0.05	0.58±0.04	0.64±0.05	0.64±0.05	0.19
TMP HF1000 CLtm (mL/min)	10±1	19±1	32±2	64±5	
TMP M100 E	0.67±0.07	0.75±0.12	0.74±0.07	0.73±0.10	0.57
TMP M100 CLtm (mL/min)	11±1	25±4	37±3	73±10	
t-test p-value for E	0.20	**0.02**	0.05	0.17	

Mean±SD

Conclusions: Substantial SMX/TMP CLtm was observed during CH with a HF1000 or M100 hemofilter at Quf between 1 and 6 L/h. Considering the CLtm observed and the nonrenal clearance reported to occur with SMX/TMP, SMX/TMP dosing adjustments during CH is required.

Funding: Other U.S. Government Support

TH-PO881

Piperacillin Clearance in Continuous Renal Replacement Therapy (CRRT) Predicts Failure To Reach Pharmacodynamic Targets Seth R. Bauer,[1] Peilin Wei,[2] Charbel A. Salem,[3] Joseph J. Groszek,[3] Maria E. Taylor,[2] Michael J. Connor,[4] Ashita J. Tolwani,[2] William Fissell.[3] *[1]Pharmacy, Cleveland Clinic, Cleveland, OH; [2]Nephrology, University of Alabama, Birmingham, AL; [3]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [4]Pulmonology and Critical Care, Emory University, Atlanta, GA.*

Background: Sepsis is the leading cause of death in acute kidney injury (AKI). Early appropriate antimicrobial therapy improves survival in sepsis, raising concerns about interactions between CRRT dose and pharmacodynamics in sepsis. In an IRB-approved multicenter study, we measured piperacillin levels in patients receiving CRRT. Drug levels were compared with pharmacodynamic (PD) goals, and we identified factors associated with failure to reach PD goals.

Methods: Inclusion: Patients with acute or chronic renal failure receiving CRRT. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, 30 minute post infusion peak, and second trough blood and effluent samples were drawn and stored on ice. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Traditional PK parameters (volume of distribution, elimination rate) were calculated. Logistic regression (JMP 9 for Windows) was used to test the association between PK and CRRT parameters and PD goals.

Results: 48 patients had data for analysis, of whom 36 had complete data. 11 patients had therapy interruptions between the peak and the second trough, and one had an error in sample collection. Pharmacokinetic parameters predicted attainment of PD goals of %t>MIC=64, as did total daily piperacillin dose. When total clearance was divided into CRRT and endogenous clearance, CRRT clearance was strongly and negatively predictive of achieving a PD goal of >50%T>MIC of 64 mcg/mL (OR 0.80 [95% CI 0.60-0.94] per mL/min of CRRT clearance).

Conclusions: CRRT significantly affects piperacillin PD. Higher CRRT clearance increases the risk of failing to meet PD goals. A larger multicenter study may permit more detailed analysis of the effect of PD on survival in CRRT.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO882

Piperacillin Pharmacodynamics Are Associated with Survival in Continuous Renal Replacement Therapy (CRRT) Charbel A. Salem,[1] Peilin Wei,[2] Maria E. Taylor,[2] Seth R. Bauer,[3] Michael J. Connor,[4] Joseph J. Groszek,[1] Ashita J. Tolwani,[2] William Fissell.[1] *[1]Nephrology and Hypertension, Cleveland Clinic, Cleveland; [2]Nephrology, University of Alabama, Birmingham, AL; [3]Pharmacy, Cleveland Clinic, Cleveland, OH; [4]Pulmonology and Critical Care Medicine, Emory University, Atlanta, GA.*

Background: Sepsis is the leading cause of death in acute kidney injury (AKI). Early appropriate antimicrobial therapy improves survival in sepsis, raising concerns about interactions between CRRT dose and pharmoacodynamics in sepsis. In an IRB-approved multicenter study, we measured piperacillin levels in patients receiving CRRT. We performed a logistic regression analysis to determine the impact of achieving phamacodynamic targets on survival in acute kidney injury.

Methods: Inclusion: Patients with acute or chronic renal failure receiving CRRT in the ICU. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, peak, and second trough blood and effluent samples were drawn. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Standard PD parameters were calculated from plasma levels. We a priori analyzed age and severity adjusted hospital survival for percentage time that free drug exceeded MIC = 50% (fT>MIC 50%) and 90% (fT>MIC 90%) at an MIC of 64 ug/ml using logistic regression (JMP 9 for Windows).

Results: 48 patients had data for analysis, of whom 33 had acute renal failure and complete data for analysis. Age- and severity-adjusted hospital survival was not associated with the fraction of time the MIC exceeded fT> MIC > 50% , but fT>MIC>90% was strongly associated with survival (OR 21.9 [2.1-665], p < 0.008).

Conclusions: Piperacillin pharmacodynamics appear to be associated with survival in patients treated with CRRT, but the association was only seen at a PD target of fT > MIC > 90%, rather than a more conventional PD target to fT > MIC > 50%. This suggests that survival in dialysis-dependent acute renal failure might be improved by tailored antibiotic dosing, but more study is needed.

Funding: NIDDK Support, Pharmaceutical Company Support

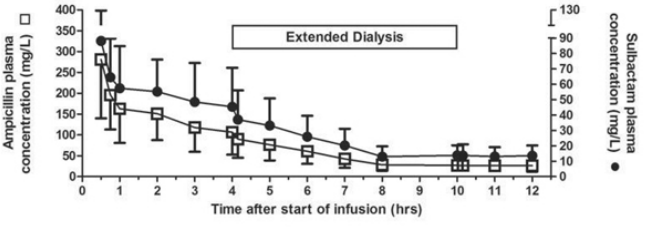
TH-PO883

Single and Multiple Dose Pharmacokinetics of Ampicillin/Sulbactam in Critically Ill Patients with Acute Kidney Injury Undergoing Extended Dialysis Johan M. Lorenzen, Jan T. Kielstein. *Nephrology, Hanover Medical School, Hannover, Germany.*

Background: The fixed antibacterial combination of ampicillin and sulbactam is frequently used for various infections. Intact kidneys eliminate ~ 60% of both substances. Patients on thrice weekly low flux haemodialysis exhibit an ampicillin half life of 2.3 h on and 17.4 h off dialysis. Despite its frequent use in intensive care units there are no dosing recommendations for patients with acute kidney injury (AKI) undergoing renal replacement therapy (RRT) available. Aim of this study was to evaluate pharmacokinetics of ampicillin/sulbactam in critically ill patients with AKI undergoing extended dialysis (ED) and to establish dosing recommendations for this treatment method.

Methods: Twelve critically ill patients with anuric AKI being treated with ED we enrolled in a prospective, open-label, observational pharmacokinetic study. Pharmacokinetics after a single dose of ampicillin/sulbactam (2g/1g) was obtained in twelve patients. Multiple dose pharmacokinetics after four days of twice daily ampicillin/sulbactam (2g/1g) was obtained in three patients.

Results: The mean dialyser clearance for ampicillin /sulbactam was 80.1 ± 7.7 / 83.3 ± 12.1 ml/min. The half life of ampicillin and sulbactam in patients with AKI undergoing ED was 2.8 ± 0.8 h and 3.5 ± 1.5 respectively.



There was no significant accumulation using a twice daily dose of 2g/1g ampicillin/sulbactam.

Conclusions: Our data suggest that patients treated with ED using a high-flux dialyzer (polysulphone, 1.3 m2; blood and dialysate flow, 160 ml/min; treatment time 480 min) a twice daily dosing schedule of 2g/1g ampicillin/sulbactam is necessary to avoid underdosing.

Funding: Pharmaceutical Company Support

TH-PO884

Clinical Data Imperfectly Predict Piperacillin Pharmacokinetics in Patients on Continuous Renal Replacement Therapy Peilin Wei,[1] Seth R. Bauer,[2] Charbel A. Salem,[3] Joseph J. Groszek,[3] Maria E. Taylor,[1] Michael J. Connor,[4] Ashita J. Tolwani,[1] William Fissell.[2] *[1]Nephrology, University of Alabama at Birmingham, AL; [2]Pharmacy, Cleveland Clinic, Cleveland, OH; [3]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [4]Nephrology and Pulmonology and Critical Care Medicine, Emory University, Atlanta, GA.*

Background: Sepsis is the leading cause of death in acute kidney injury (AKI). Early appropriate antimicrobial therapy improves survival in sepsis, but dose calculations depend on knowledge of pharmacokinetic (PK) parameters, particularly the volume of distribution and the clearance. In an IRB-approved multicenter study, we prospectively measured piperacillin levels in patients receiving CRRT in the ICU. PK were compared to anthropomorphic data and CRRT prescription.

Methods: Inclusion: Patients with acute or chronic renal failure receiving CRRT in the ICU. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, 30 minute post infusion peak, and second trough blood and effluent samples were drawn and stored on ice. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Linear regression (JMP 9 for Windows) was used to determine the associations between clinical data and PK parameters.

Results: 48 patients had data for analysis, of whom 35 had complete data. 12 patients had therapy interruptions between the peak and the second trough, and one had an error in sample collection. A multivariate linear regression model fitted Vd ($r^2 = 0.49$) and Ke ($r^2 = 0.30$) to patient and dialysis prescription data. Age ($p<0.01$) and weight gain since admission ($p<0.002$) were the only factors independently associated with Vd, whereas CRRT dose ($p<0.003$) was the only factor independently associated with Ke.

Conclusions: PK parameters needed to calculate piperacillin dose in patients receiving CRRT are imperfectly predicted by clinically available data. More information is needed to prospectively calculate optimal piperacillin doses for patients with AKI receiving CRRT.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO885

A Mathematical Model To Predict Two-Phase Calcium Supplementation in Continuous Venovenous Hemofiltration with Regional Citrate Anticoagulation Yin Zheng, Zhongye Xu, Feng Ding, Chuan-Ming Hao. *Nephrology, Huashan Hospital, Shanghai, China.*

Background: Calcium substitution is a determinant of the safety and efficacy of regional citrate anticoagulation (RCA) during continuous renal replacement therapy . We developed and clinically validated a mathematical model of two-phase calcium supplementation during continuous venovenous hemofiltration (CVVH).

Methods: Thirty-two critically ill patients who required CVVH treatment with citrate anticoagulation were enrolled in the study. A two-phase mathematical model using patients' clinical characteristics (body weight, Hct, serum proteins concentration) and related treatment parameters (Qpw, Qsub, Quf, Qcit) was proposed to predict the need for calcium supplementation. By measuring systemic and extracorporeal citrate and calcium concentrations repeatedly, two coefficients in the mathematical equation, namely, the proportion of filterable calcium and the correlation between the concentration of calcium and citrate were studied. The model was validated in patients receiving RCA-CVVH.

Results: The calcium supply during RCA-CVVH can be divided into two phases by reaching the steady-state of citrate. The two pivotal coefficients were solved.The filterable calcium accounted for 87 ± 1% of total calcium. The highest correlation was found between the increased bound calcium concentration and the citrate plasma level ($r = 0.70$, $p < 0.001$). Applied the modeling method to 15 patients' treatments, it was able to control the level of systemic and circuit ionized calcium at a safe and steady range in the setting of different treatment parameters. Afterwards, it had been validated in more treatments of RCA-CVVH in our institute. The incidence of hypercalcemia or hypocalcemia was reduced (4.9% vs 16.7%) with less frequency of ionized calcium monitoring. However, the model appeared less precise after 24 hours of treatment.

Conclusions: In this study, a methodology of a two-phase calcium supplementation during RCA-CVVH was developed. With the aid of the mathematical model, safer calcium supplementation can be performed with less frequency of calcium monitoring.

Funding: Government Support - Non-U.S.

TH-PO886

In Spite of Positive Charge on Polyethyleneimine, AN69 ST Membrane Does Not Tightly Adsorb Heparin during Continuous Renal Replacement Therapy Jun Seok Choi, Su-Kil Park, Jung-Sik Park. *Nephrology, Asan Medical Center, Seoul, Republic of Korea.*

Background: Owing to the positive charge of polyethyleneimine (PEI), AN69 ST membrane adsorbs heparin 600 IU/m2 at the priming with the mixture of heparin and normal saline (5000 IU/1L). More heparin attached to AN69 ST membrane may increase the longevity of filter life. Without continuous systemic administration of heparin during renal replacement therapy (RRT), washing with normal saline 1L for 5~7 minutes after priming could remove the bleeding risk due to not adsorbed heparin. We compared the effects of priming with different heparin doses (5000 IU/L in group A vs. 20000 IU/L in group B) on filter life span and systemic coagulation parameters in critically ill patients with acute kidney injury in this randomized cross-over study.

Methods: Different doses of heparin were randomly assigned to 30 patients (M:F = 22:8, median of age 70 (range, 50~96 years)) at the 1st and 2nd filter during RRT.

Results: There was no difference of median values in baseline hemoglobin (9.2 (7.1~14) g/dL vs. 9.4 (8.3~14.2) g/dL, p=NS), platelet count (123000 (37000~475000)/mm³ vs. 115000 (23000~485000)/mm³, p=NS), activated partial thromboplastin time (aPTT, 39.1 (27.2~84.8) sec vs. 36.0 (27.2~69.6) sec, p=NS), prothrombin time (PT, 1.19 (0.98~1.76) INR vs. 1.17 (0.97~1.86) INR, p=NS), collagen/epinephrine clotting time (205 (69~300) sec vs. 214 (42~300) sec, p=NS), APACHE II scores (24 (7~34) vs. 24 (10~39), p=NS) and filter life span (15 h 15 min (5 h 5 min~71 h 47 min) vs. 15 h 59 min (3 h 40 min~71 h 32 min), p=NS) between two groups. Compared with baseline value of aPTT, its prolongation did not appear in 30 minutes after starting RRT in group A (from 39.1 (27.2~84.8) sec to 38.7 (25.3~98.6) sec, p=NS). However, aPTT significantly increased in group B (from 36.0 (27.2~69.6) sec to 38.9 (29.7~86.8) sec, p=0.012) without clinical events.

Conclusions: Priming with the higher dose of heparin and washing did not reveal the beneficial effect on filter life but prolonged aPTT. It suggests that PEI does not strongly enough bind heparin and requires heparin-coated filter where heparin can be adhered more tightly.

TH-PO887

The Evodialyser™ Can Be Used as a Heparin Free Alternative for SLED in ICU Monica Doyle,[1] Iain R. Macleod,[2] Sean McArtney,[1] Sally Crofts,[3] Judith A. Joss,[3] Iain S. Henderson,[1] Samira Bell.[1] *[1]Department of Renal Medicine, Ninewells Hospital, Dundee, United Kingdom; [2]Department of Intesive Care Medicine, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; [3]Department of Intensive Care Medicine, Ninewells Hospital, Dundee, United Kingdom.*

Background: Sustained Low Efficiency Dialysis (SLED) has been increasing in popularity particularly in the care of critically ill patients within intensive care. Currently, most SLED treatments require the use of heparin to achieve prolonged dialysis times with slower blood pump speeds. Critically ill patients often have a bleeding tendency and thus a strategy to minimise or completely avoid systemic anti-coagulation would be clinically advantageous.

Evodialyser™ is a heparin impregnated dialysis membrane that has been developed to minimise the quantity of systemic heparin required for conventional intermittent haemodialysis.

We aimed to evaluate the use of the Evodialyser™ as a means of avoiding systemic heparinisation in intensive care patients with acute kidney injury (AKI) undergoing SLED.

Methods: We collated data prospectively in all patients in intensive care who required SLED from 01/07/2010 until 30/05/2011. The Evodialyser™ was used when the nephrologist felt it was indicated.

Results: During the study period 27 SLED treatments were carried out in 13 patients using the Evodialyser™. The median age of the study population was 71 years (IQR 65 – 77 years) The median APACHE II score was 26 (23-28) The indications for the use of the Evodialyser™ included coagulopathy, post major surgery and the use of Activated Protein C.

The standard treatment time was 8 hours with a mean time of 7 ± 2 hours achieved. 24 treatments (89%) were successful and achieved over 4 hours of SLED. Only 3 treatments were terminated at less than 4 hours and this was due to increased venous pressure in all cases. No systemic heparin was required in any patient. Ultrafiltration averaged 1.34 litres per session (Range 0-2L) and was achieved in all patients.

Conclusions: As far as we are aware, this is the first report of the use of the Evodialyser™ as an alternative means to avoid systemic heparinisation in critically ill patients undergoing SLED treatment in intensive care.

TH-PO888

Study on a Regional Citrate Anticoagulated Continuous Venovenous Hemodiafiltration Protocol with Variable Treatment Dose Ina Lieker, Dmytro Khadzhynov, Harm Peters, Hans-Hellmut Neumayer, Torsten Slowinski. *Department of Nephrology, Charite Universitätsmedizin, Berlin, Germany.*

Background: In order to add convective solute transport to a recently described citrate-CVVHD protocol we established a citrate anticoagulated CVVHDF protocol with variable treatment dose. To allow convective transport, we designed a new citrate-CVVHDF protocol with a postdilution hemofiltration dose.

Methods: Prospective observational study. A CVVHDF-based citrate anticoagulation protocol on the MultiFiltrate™ CRRT-device (Fresenius Medical Care, Germany (FMC)) using a 4% trisodiumcitrate solution, the dialysate fluid Ci-Ca™Dialysate K2 (FMC), and a continuous calciumchloride (91 mmol/L) infusion. For the filtration dose we used a standard bicarbonate-buffered substitution fluid (MultiBic™, FMC) in postdilution. 60 patients on ICU with acute kidney injury and need for RRT were included. For variable dose patients were divided in 3 groups according to their bodyweight.The initial flows for dialysate/substitution fluid in the groups were 1400/800, 1800/1000, and 2200/1200 mL/h, blood flow 80, 100, 120 mL/min, citrate flow 145, 175, 220 mL/h, and calcium flow 32, 45, 57 mL/min. Citrate flow was adjusted to postfilter ionized calcium measurements, target range 0.25-0.35 mmol/l, Calcium flow was adjusted to patients systemic iCa. Treatment time was limited to 72 hours.

Results: 49 of 60 patients reached the maximum treatment time of 72 h without clotting. Acidbase control: mean [95%CI] pH; st-bicarbonate at 48 h: 7.42 [7.38-7.44]; 24.6 [21.3-27.8] mmol/L, at 72 h: 7.39 [7.35- 7.43]; 25.0 [21.3-27.6] mmol/L. Electrolyte control: mean [95% CI] s-sodium; s-potassium at 48 h: 139 [137- 141]; 4.8 [4.4-5.2] mmol/L, at 72 h: 139 [137-142]; 4.9 [4.4-5.4] mmol/L. Mean [95%CI] treatment dose was 41 [37-46] mL/kg/h, mean [95%CI] s-urea at 48 h: 51 [37-66] mg/dL, at 72 h: 50 [35-65] mg/dL.

Conclusions: The citrate-CVVHDF protocol allows combined hemodialysis and -filtration in CRRT with variable treatment dose and with the advantages of regional citrate anticoagulation. In this study in 60 patients filter run-time in citrate-CVVHDF was remarkable and metabolic control excellent.

TH-PO889

Prescribing for Regional Citrate Anticoagulated Continuous Venovenous Hemodiafiltration (CVVHDF) Michele Giuseppe Messa,[1] Vittorio Ortalda,[1] Antonia Fabris,[1] Saulle Mazzolini,[2] Antonio Lupo.[1] *[1]Università Verona, Italy; [2]AOU Udine, Italy.*

Background: Although the use of regional citrate anticoagulation (RCA) for continuous renal replacement therapy (CRRT) continues to grow, current approaches have many drawbacks, including the use of unphysiologic, concentrated citrate solutions and the requirement of infusion pumps external to the CRRT device. For the study purpose we used a diluted citrate anticoagulant solution (sodium citrate12 mmol/l) in combination with a CRRT device which has an integrated pump specifically designed for the infusion of fluids proximal to the blood pump. Aim of the study was to identify the primary factors influencing calcium balance at different target citrate doses during CVVHDF therapy

Methods: In 17 acute kidney injury (AKI) pts, RCA was performed with different blood (Qb), citrate (Q_{Ci}), and dialysate (Qd) flow rates at target pre-filter blood citrate concentrations of 3, 4, 5 mmol/l_Qb. Postfilter ionized calcium (iCa_{post}) was measured for each Q_{Ci}, while total calcium in the effluent (Ca_e) was measured for each of the resulting flow combinations. Systemic haemoglobin (Hb), hematocrit (Htc), serum total calcium (sCa), total protein (sProt) and albumin (sAlb) concentration were also measured before starting each study procedure. Calcium mass transfer rate (MTRCa) was estimated from the product of Ca_e and the effluent flow rate. Based on multivariate regression analysis (MVRA), equations providing predicted values of iCa_{post} and Ca_e were developed

Results: MVRA indicated iCa_{post} was influenced by Q_{Ci} ($p < .0001$), sProt ($p < .001$) and sCa ($p < .0001$). MVRA also demonstrated that calcium in the effluent (Ca_e) depends largely on Q_{Ci} ($p < .0001$), Qd ($p < .0001$), sCa ($p < .0001$) and Qb ($p < .0001$). A significant correlation between observed and predicted values was determined for both iCa_{post} and Ca_e.

Conclusions: Our results characterize the important treatment-related factors influencing calcium balance during RCA with a diluted citrate anticoagulant solution. These data also suggest the possibility for development of a model in which calcium parameters are predicted based on these treatment-related factors.

TH-PO890

Regional Citrate Anticoagulation during Continuous Veno-Venous Hemodialysis: Two-Year Single-Center Experience Dmytro Khadzhynov, Christin Baumann, Torsten Slowinski, Ina Lieker, Hans-Hellmut Neumayer, Harm Peters. *Department of Nephrology, Charite Campus Mitte, Charite Universitätsmedizin, Berlin, Germany.*

Background: Regional citrate anticoagulation(RCA) has been shown to be a safe and effective form of anticoagulation for continuous renal replacement therapy(CRRT) in patients with high risk of bleeding. More over, it was recently assumed that the citrate application can bring a benefit in survival. We report a two-year single-center experience of renal replacement therapy based on a continuous veno-venous hemodialysis(CVVHD) with regional citrate anticoagulation.

Methods: Results of CRRT conducted in years 2008 and 2009 at 6 intensive care units at our university clinic are retrospectively analysed. CVVHD with RCA is a modality of choice at our campus and is initially used in the majority of cases, regardless of the patients bleeding risk and/or liver function. Collected data included demographic features, dialysis circuit life-time, overall mortality at ICU discharge.

Results: We detected 703 patients(in average 67.6±12.4 year old, 64.6% male, average APACHE score 26.2±8.5) treated with CVVHD with RCA from 01.01.2008 till 31.12.2009. Mean uncensored filter life-time was 66.4±42.5 hrs. CVVHD was performed in 181 patients (63.4±43.5 hrs) at general anaesthesiology ICU, 252 patients (66.0±42.1 hrs) at cardio-surgery ICU, 62 patients (76.3±41.1 hrs) at general surgery ICU, 68 patient (67.5±42.5 hrs) at cardiology ICU, 23 patients (77.5±46,8 hrs) at neurological ICU and 117 patients (65.9±42.4 hrs) at infection ICU. The incidence of citrate accumulation was 3.7% of all CVVHD treatments based on citrate anticoagulation. Renal recovery in patients treated with CVVHD based on RCA was 60,1% at ICU discharge. Overall mortality of the patients treated with citrate based CVVHD was 36.4% at ICU discharge.

Conclusions: Protocol of RCA for CVVHD was used as a standard modality of CRRT, regardless of the patients bleeding predisposition. The modality of CRRT was applied with equal success at 6 differently orientated ICUs of our university hospital. This approach allows reaching perfect regional extracorporeal anticoagulation efficacy with an adequate metabolic control.

TH-PO891

Digoxin Intoxication in Acute or Chronic Kidney Failure – Elimination of Digoxin Bound to Fab-Fragments (Digifab®) with High Cut-Off Filter Dialysis Susanne V. Fleig, Roland Schmitt, Jan T. Kielstein, Bernhard M.W. Schmidt. *Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: The cardiac glycoside digoxin is renally eliminated and not dialyzable. In cases of symptomatic intoxication, serum digoxin can be bound by anti-digoxin-antibody-fragments (Digoxin Immune Fab) and thereby inactivated; digoxin-antibody-complexes are then renally excreted. Digifab® (Digoxin Immune Fab) has a molecular size of 46kDa and does not pass normal dialysis filters. In dialysis-dependent patients, digoxin is set free again after degradation of Fab-fragments and symptoms of intoxication may reoccur.

Methods: We report two cases of dialysis-dependent patients (one with end- stage renal disease, one with acute postoperative renal failure) with symptomatic digoxin intoxication.

Results: Both had been given Digoxin Immune Fab (digifab®), which led to relief of symptoms for several hours. Yet, symptoms reappeared as the agent could not be excreted and was set free again after degradation of the antibody fragments.Six hours after a second dose of Digoxin Immune Fab (digifab®), we performed extended dialysis (Genius® singlepass dialysis system (Fresenius Medical Care)) with high cut-off dialyzers (Theralite®, HCO1100®, both for removal of plasma components with a molecular weight up to 45kDa). This way, we were able to eliminate fab-fragment bound digoxin. After dialysis with these filters, symptoms did not recur in both patients, and serum digoxin levels remained low (Digoxin levels: 4.72 nmol/l and 5.0 nmol/l before treatment, 2.08 nmol/l and 2.01 nmol/l directly after dialysis and 2.66 nmol/l and 2.50 nmol/l 12 hours after dialysis).

Conclusions: We show that dialysis with high cut-off filters can eliminate fab-fragment bound digoxin in patients with symptomatic digoxin intoxication and severely impaired kidney function.

TH-PO892

Path Batch Hemodialysis (PBH): A Safe and Efficient Method for Acute Kidney Injury (AKI) Cancer Patients in the Intensive Care Unit (ICU) Veronica T. Costa e Silva,[1] Ana Paula Leandro Oliveira,[1] Henrique Palomba,[1] Ludhmila Abrahão Hajjar,[2] Elerson Costalonga,[1] James Hung,[1] Luciane Oikawa,[1] Juliana Silva Bezerra,[1] Emmanuel A. Burdmann,[1] Luis Yu.[1] *[1]Nephrology Division, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Intensive Care Unit Department, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Background: The adequacy and safety of BPH have not been studied in ICU cancer patients (pcts).

Methods: We prospectively analyzed all PBH performed in AKI adult cancer pcts in the Sao Paulo State Cancer Institute ICU from June 2010 to May 2011.

Results: A total of 242 PBH were performed in 76 AKI pcts. Pcts' characteristics were age 61 ± 14 y, 60.5% male, 17.8% on vasopressors and 12.4% on mechanical ventilation. Most (82.9%) patients had solid cancer (genitourinary tract 38.2%, gastrointestinal tract 11.8% and gynecologic system 11.8%). The most important AKI etiologic factors were sepsis (48.7%), obstructive uropathy (24.7%) and surgery (21.1%). General ICU mortality was 50.7%. Venous access was temporary catheter in 97.9% (58% femoral and 41% internal jugular veins), high-flux polysulphone membrane in all filters (FS80 in 63.2%) and median blood and dialysate flow 250 (200 – 300) mL/min. Systemic anticoagulation was not used in 69.4% due to heparin contraindications. Median pre and post serum urea levels were 154 (109 – 199) and 62 (47 – 89), respectively. Urea reduction rate > 55% was observed in 62% of dialysis. The prescribed UF was 1500ml (1000 – 2000), which was attained in 66.2% of the procedures. The prescribed dialysis time was 240 (240 – 315) min, which was attained in 77.7% of dialysis. The main complications were hypotension (mean blood pressure <70 mmHg) in 27.3% (8.3% required dialysis interruption), prescribed blood flow reduction in 7.9%, lines reversion in 16.9% and system coagulation causing dialysis interruption in 16%. Clotting was associated with the need for decreasing blood flow (OR 6.3/CI 2.3 – 17.3) and heparin use (OR 0.31/IC 0.1 – 0.9).

Conclusions: In conclusion, PBH seems to be a safe and efficient alternative for dialysis in AKI ICU cancer pcts.

TH-PO893

Application of Plasmadiafiltration in Porcine Sepsis Models Induced by Laparoscopic Cecal Ligation and Puncture Jun Xue. *Nephrology, Huashan Hospital, Fudan University, Shanghai, China.*

Background: To study whether plasmadiafiltration (PDF) can reduce the circulating levels of critical inflammatory macromolecules, thus improve hemodynamics and increase survival time after establishing a porcine sepsis model by laparoscopic cecal ligation and puncture (CLP).

Methods: Twelve 80-day and 36-kilogram domestic male swine which fitted the diagnostic criteria of sepsis induced by laparoscopic CLP, were randomly treated either by PDF or by normal saline. PDF was performed with a selective filter with molecular weight cut-off (MWCO) of 60-70KD. The circulating levels of TNF-α trimer and high mobility group box 1(HMGB1), blood pressure and pulmonary arterial wedge pressure (PAWP) averaged cardiac output were assessed.

Results: Total of the 12 swine were successfully made as sepsis models. The odds ratios of PDF to reduce the circulating levels of TNF-α trimer and HMGB1 were 1.97(95%CI, 1.64-2.51, *P*=0.012), and 1.97(95% CI, 1.67-2.46, *P*=0.007), respectively. The odds ratios of PDF to improve systolic pressure and PAWP averaged cardiac ouput were 1.07(95%CI, 1.00-2.59, *P*=0.001) and 6.34(95% CI, 2.89-25.3, P=0.032), respectively. A linear relation was found between diastolic pressure and TNF-α trimers through multiple linear regression analysis (*P*=0.032).We also found a linear relation between PAWP averaged cardiac ouput and TNF-α trimer (*P*=0.043). The mean survival time was 36.3 hours for PDF group and 31.5 hours for control. In PDF group the risk ratio of death of all-cause was 0.11 (*P*=0.046), as compared with control.

Conclusions: We can successfully make porcine CLP sepsis models through laparoscope. PDF can reduce the circulating levels of critical inflammatory macromolecules, therefore improve the hemodynamics and increase survival time in animal sepsis models.

Funding: Government Support - Non-U.S.

TH-PO894

Membrane vs. Centrifuge Based Therapeutic Plasma Exchange – A Clinical Cross-Over Comparison Jan T. Kielstein, Carsten Hafer, Ansgar Reising, Bernhard M.W. Schmidt. *Department of Hyertension and Nephrology, Medical School Hannover, Hannover, Germany.*

Background: Therapeutic plasma exchange (TPE) is either performed using a centrifugation device (cTPE), a method preferred by hematology or blood bank-based physicians or by using a highly permeable filter with standard hemodialysis equipment (mTPE), a method preferred by nephrology-based physicians. The aim of the study was to perform the first direct comparison of these two techniques.

Methods: We performed a prospective, cross-over study comparing mTPE using the OctoNova device using the Plasmaflo OP 05W (Asahi Kasei Medical, Japan) with cTPE using the Spectra Optia (Caridian BCT). Seventeen patients that underwent TPE against albumin based substitution fluid were randomized to start either with cTPE or mTPE. The second treatment was done in a cross over design. We did choose this approach to allow

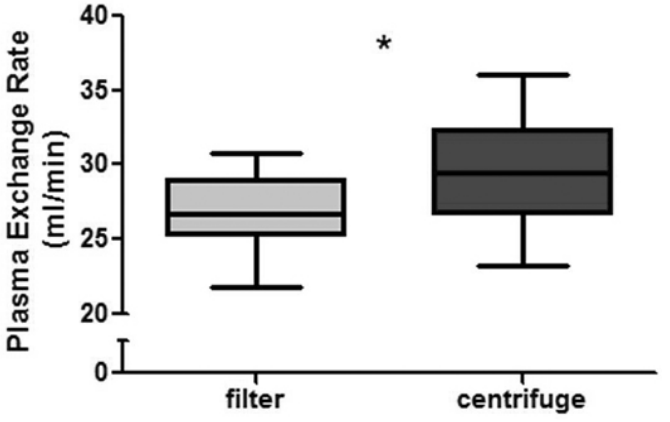
the measurement of the total removed immunoglobulines in the waste bag. We also took samples for complete blood count, immunoglobulines and fibrinogen before and after treatment and recorded the treatment time without any adverse effects on parameters measured in the complete blood counts.

Results: While there was no difference in the reduction rate as well as the absolute amount of immunoglobolines removed by the different techniques, cTPE allowed a faster exchange/treatment time (plasmavolume/treatment time).

Comparison of cTPE and mTPE

Parameter	unit	mTPE	cTPE
Pre-treatment IgG	g/L	6.05 ± 2.65	6.49 ± 2.84
Post-treatment IgG	g/L	2.15 ± 0.79	2.35 ± 1.15
Pre-treatment IgM	g/L	0.76 ± 0.34	0.81 ± 0.40
Post-treatment IgM	g/L	0.38 ± 0.13	0.44 ± 0.15
Total IgG in waste bag	g	12.31 ± 5.20	11.91 ± 6.29
Total IgM in waste bag	g	1.27 ± 0.57	1.50 ± 0.84

Data presented as mean ± SD



Conclusions: Centrifuge based techniques allow faster clearance of plasma from marker substances like IgG and IgM without any adverse events.

TH-PO895

Can MARS Be a Solution for Type 1 Hepatorenal Syndrome? Siba Kallab, Laurence Lavayssiere, Nadine Bassil. *Nephrology-Dialysis-Transplantation, CHU Rangueil, Toulouse, France.*

Background: Hepatorenal syndrome (HRS) is the development of acute renal failure due to excess splanchnic and systemic vasodilation in patients with severe liver failure. The molecular adsorbent recirculation system (MARS) is a liver support system based on albumin dialysis which can potentially reduce systemic vasodilatation by removing protein-bound vasodilators.

The aim of this study was to determine whether the MARS technique is able to improve renal function in patients with type 1 HRS.

Methods: A four-year retrospective study (2006-2010) was carried out in a specialized nephrology intensive-care unit (ICU) .The study group comprised 32 patients who had type 1 HRS and who underwent MARS treatment. Our endpoint was the complete, partial and the absence of renal recovery at 28 days after the diagnosis of type 1 HRS. We considered complete renal recovery (CRR) as a serum-creatinine level less than 133 µmol/L after treatment. Partial renal recovery (PRR) was defined as a 10% decrease in baseline serum creatinine level. We considered non-responders as all patients who required hemodialysis (HD). We made a one month follows up to assess renal recovery for the patients who received during the study period a liver transplantation .

Results: On admission to the ICU, the median MELD and SOFA score were 36 and 13 respectively ; 52 % had F score for RIFLE criteria .There was no significant difference between 24-h urine volume prior to and after MARS treatment ($p=0.7$).Thirteen of the 32 (40.6%) patients survived at 28 days. Of these, 5 patients (15.7%) had CRR, 5 other patients had PRR (15.7%) and in the 3 last patients kidney function did not recover. All the patients who died (19/32) required HD . Six patients had received a liver transplant during the period of study. Only one of these died due to severe hemorrhage during the transplantation while, in a one month follows up, 2 patients had CRR, 2 other patients had (PRR) and one patient required chronic hemodialysis.

Conclusions: In the present study, MARS therapy did not allow improving renal function in patients with type 1 HRS. MARS was a *bridge* to liver transplantation in two patients who had a complete renal recovery at a one month follows up.

Funding: Government Support - Non-U.S.

TH-PO896

Intraoperative Slow Low-Efficiency Dialysis during Emergency Surgery in Critically Ill Patients Joanna Matuszkiewicz-Rowinska, Grzegorz Ostrowski, Mariusz Mieczkowski, Pawel Kulicki, Tadeusz Grochowiecki, Waldemar Patkowski, Krzysztof Dudek, Bohdan Solonynko. *Medical University of Warsaw, Warsaw, Poland.*

Background: There is some positive experience with intraoperative hemodialysis in cardiac and liver transplant surgery, however data concerning other surgical procedures are scarse.In this study we present our experience with intraoperative slow low-efficiency dialysis (SLED) performed during emergency laparotomy.

Methods: In all cases SLED was chosen since it remains the method of choice in hemodynamically unstable critically ill patients in our center. The Genius® single-pass system was used, with blood flow 160, and dialysate flow 80 ml/min.

Results: Intraoperative SLED was performed in four men, aged 30-59 years. The diagnoses were as follows: reoperation due to massive hemorrhage after bilateral adrenalectomy, intestinal ischemia after abdominal aortic stent-graft placement, multiorgan injury after traffic accident, and liver retransplantation. All presented pts were hemodynamically unstable, 3 of them were given catecholamines. One of the pts had chronic kidney disease Stage 3, and the remaining 3 – acute kidney injury; in 3 pts anuria was present. The indications for emergency dialysis were: severe uncompensated lactic acidosis (3 pts) with tissue necrosis (2 pts), life-threatening hyperkalemia (3 pts) with need for RBC transfusion (3 pts), and massive overhydration (1 pt). In all cases SLED was well tolerate and successfully perfomed for 12 hours. Although only one pt received heparine, and 3 pts were given only saline flushes no filter circuit clotting was observed. The pt with intestinal necrosis died in 14 hours later, in the remaining 3 dialysotherapy was continued, however all of them died due to multiorgan failure.

Conclusions: In conclusion, our data suggest that intraoperative SLED is a safe procedure, and can be used in hemodynamically unstable pts with life- threatening fluid, electrolyte and acid-base disorders who need emergency surgery

TH-PO897

Pediatric CRRT for Severe Hyperosmolarity and AKI Timothy E. Bunchman. *Pediatric Nephrology, VCU School of Medicine, Richmond, VA.*

Background: Renal replacement therapy (RRT) for AKI with associated severe hyperosmolality may result in dialysis disequilibrium, cerebral edema or death. This is in part due to rapid changes in osmols related to the "normal" osmlarity of the dialysate as well as the volume of the dialysis solution per a RRT prescription. The osmolality of the dialysate in hemodialysis (HD) can be adjusted only within constraints of the conductivity of the HD machine, but the dialysate of Peritoneal Dialysis (PD) and CVVHD can be adjusted as needed. Further PD and CVVHD have an advantage over HD as a relative "inefficient" mode of clearance.

Methods: Two teens presented within 2 weeks of each other with new onset diabetes (glucose 550 mg/dl and 1300 mg/dl), oliguric AKI, with severe metabolic acidosis (pH 7.02) in one and severe hypernatremia (uncorrected Na of 175 mmol/L) in the other, each with neurologic deterioration and coma. The measured osmolality in the first child was 380 (also had received mannitol) while the second child was 488 mOsm/kg.

CVVHD was begun in both with NaCl added to the dialysate to minimize the osmolar gradient between the dialysate and the patient's osmols. In the first patient NaCl was added to make a total Na dialysate of 170 mmol/L (340 mOsm/l) in the second patient the total NaCl dialysate was 200 mmol/L (400 mOsm/L). Each patient had a purposeful low clearance prescription at 6-8 mls/kg/hour. Every 3 hours the total and calculated osmolality was measured and the Na supplementation in the dialysate was decreased by 10 mmol/L each time on an average of 2 times per day based upon an improvement in the child's osmolality. The hyperglycemia was treated per insulin protocol.

Results: In the first patient the pH was normalized slowly over 30 hours and the osmolality normalized over 60 hours when CVVHD was discontinued with renal recovery. In the second patient the osmolality normalized over 5 days when CVVHD was discontinued with renal recovery. Both teens had reversal of coma with a normal neurologic exam.

Conclusions: In conclusion, purposefully inefficient " hyperosmolar " CVVHD allows for a slow improvement in pH and a slow and safe normalization of the hyperosmolar with preservation of neurologic function in the setting of hyperosmolar AKI.

TH-PO898

Outcomes in Neonates on Renal Replacement Therapy; a Single Center Experience Shivanand S. Medar, Pamela S. Singer, James S. Killinger, Robert Woroniecki. *Department of Pediatrics, Children's Hospital at Montefiore, Bronx, NY.*

Background: Initiating dialysis in newborns is controversial. Data on outcomes following Renal Replacement Therapy (RRT) is inconsistent. Reports from Israel/Europe on outcomes of dialysis in newborns (0-28 days) show 75% mortality rate; North American Pediatric Renal Transplant Collaborative Studies (NAPRTCS) data (based on voluntary reporting) shows 24% mortality. We hypothesize that RRT in newborns is associated with high morbidity and mortality.

Methods: Records of patients who had RRT initiated before 28 days of age at our institution between January of 1997 and January 2011 were reviewed. Dialysis treatment during the study period was offered to all neonates and infants that required it, unless they had life-threatening co-morbidities. The RRT physicians and nurses, equipment,

methods and procedures associated with RRT delivery during this time period remained consistent. The end-point for time period on RRT treatment was death, January 2011 or first renal transplant (Tx).

Results: 19 neonates (age of initiation: 9.1±5.7d, birth weight: 3.0±0.58 kg, gestational age: 37.2±2.3wks) were identified. PD was administered in 13 (68%), HD in 4 (21%), CVVHDF in 5 (26%), and 4 (21%) had more than one modality. Indications for RTT: fluid overload in 10 (53%), acidosis in 3 (16%), genetic defect in 4 (21%). 3 (16%) had congenital heart defect, and 2 (11%) multi-organ failure. 9 (47%) had severe electrolyte abnormalities, 13 (68%) required inotropes, 15 (79%) multiple blood products, and 18 (95%) ventilatory support. 8 (42%) had peritonitis, 3 (16%) had cerebral vascular event and 1 (5%) had arterial hypertension. ICU length of stay was 32±33d. 6 patients survived to ICU discharge, with 1 requiring chronic RRT and none received a renal Tx. 13/19 (68%) subjects died during observation period.

Conclusions: Newborns on RRT have high mortality and morbidity and poor outlook for renal Tx. Our patient mortality rate is in concordance with European/Israeli published series and contrasts NAPRTCS registry data possibly due to selection bias. Nephrologists should report their center RRT outcomes to parents of newborns before initiating the treatment.

TH-PO899

Combined Hemodialysis and Plasma Exchange Is Safe and Faster as Compared to Sequential Treatment in Children Betti Schaefer,[1] Ranny Goldwasser,[1] Akos Ujszaszi,[2] Susanne Schaefer,[1] Karl Heinz Heckert,[1] Franz S. Schaefer,[1] Claus P. Schmitt.[1] *[1]Center for Pediatric and Adolescent Medicine, Heidelberg; [2]Institute of Pathophysiology, Semmelweis University, Budapest.*

Background: Patients with immune-mediated kidney disease and liver failure often require plasma exchange (PE) and hemodialysis (HD). Combining both methods, i. e. connecting the PE and HD circuit in series, should allow for a more efficient treatment. The outcome has not yet been evaluated.

Methods: 15 out of 46 children (7.8-38.5 kg) were treated with combined (c) PE/HD, nine alternately with both c and sequential (s) PE/HD (9.5-80.9 kg), and 22 (5-75 kg) with sPE/HD only. Treatment modalities, efficacy, anticoagulation and clinical findings were analyzed retrospectively.

Results: Mean treatment duration was 3.9±2.2h per session for cPE/HD and 5.8±1.6h with sequential therapy (HD 3.8±1.6h, PE 2.2±0.6h; p<0.001). Dialysate flow was 490±201 with cPE/HD and 324±172 ml/min/m² with sPE/HD (p<0.01). PE/HD filter size per m² BSA and blood flow rates were similar (cPE/HD 112±44, sPE 92±22, sHD 111±28 ml/min/m²; all p=n.s.). Initial bolus of heparin consisted of 999±729 for cPE/HD, 389±475 for sPE and 305±457 IU/m² for sHD (p=n.s. for cumulative dose). The dose of continuous and total heparin infusion and Activated Clotting Time were similar, as was the cumulative amount of citrate and calcium chloride infused in children treated with either method (n=16). Dialysis efficacy (creatinine, phosphate, urea and bilirubin removal) and ultrafiltration rates were comparable (cPE/HD 821±648 vs. sPE/HD 925±528 ml/m², p=n.s.). The decrease in INR was comparable in patients with liver failure. Both methods were well tolerated. Blood leakage and hemolysis occurred in 8 out of 89 cPE/HD sessions (9%) and in 4 out of 111 sPE/HD sessions (4%), hypothermia in one sPE/HD session (1%).

Conclusions: PE/HD performed in series within one session is safe and allows for a more rapid purification as compared to sequential therapy. This should be beneficial in patients with severe diseases and reduce work load. Careful dialysator pressure control, however, is required to prevent hemolysis and capillary leaks.

TH-PO900

Two Year Study of Bone Metabolism with Acetate-Free Bicarbonate Dialysate Buffered by Citric Acid Junji Uchino. *Mihama Hospital, Chiba, Japan.*

Background: To correct metabolic acidosis, sodium bicarbonate was added to the dialysate to achieve a blood plasma bicarbonate concentration of 24 mmol/L prior to maintenance dialysis. It has been reported that comparison of bone biopsy findings between before and after the correction of metabolic acidosis showed inhibition of secondary hyperparathyroidism and stimulation of the turnover of hypoplastic bone.

Methods: To investigate the effects of acetate-free bicarbonate dialysate (Carbostar Citric Acid 2 containing 35 mmol/L of bicarbonate; CB) on bone metabolism in patients on maintenance dialysis.

Subjects: Two-hundred and ninety eight of maintenance dialysis patients were studied. Their age was 66.5±11.7 and the duration of dialysis was 9.4±8.1 years.

The patients were classified into low (i-PTH < 60 pg/mL), normal (i-PTH 60-180 pg/mL) and high (i-PTH > 180 pg/mL) PTH groups based on measurement of intact PTH during the previous year of treatment with acetic acid dialysate (Kindaly solution AF-2P containing 8 mmol/L of acetate and 30 mmol/L of bicarbonate: KP). Then i-PTH and Ca levels were compared between before and after CB. Statistical analysis was performed using Wilcoxon's signed rank sum test and significance was set at $P < 0.05$.

Results: The median plasma bicarbonate concentration before dialysis (21.7 mmol/L [min.16.7, max. 23.9]) was increased to 24.1 mmol/L (min. 20.7, max. 26.8) at 22 months after changing the dialysate. Nineteen months after changing from KP to CB, intact PTH was increased to 68.0 pg/mL (26.0, 380.0) from 24.5 pg/mL (6.0, 58.0) in the low PTH group, to 183.5 pg/mL (6.0, 382.0) from 102.0 pg/mL (61.0, 180.0) in the normal PTH group, and to 245.5 pg/mL (134.0, 454.0) from 238.5 pg/mL (155.0, 793.0) in the high PTH group, resulting in near-normal values for the low and high PTH groups. During treatment with KP, plasma Ca levels were low before dialysis in the normal group compared with the low and high PTH groups. During treatment with CB, pre- and post-dialysis plasma Ca levels were similar in the high PTH group.

Conclusions: Adding sodium bicarbonate to the dialysate through use of CB may correct bone turnover.

Funding: Private Foundation Support

TH-PO901

Hypophosphatemia and Phosphate Supplementation during Continuous Renal Replacement Therapy (CRRT) in Adult Young Hye Song,[1] Kyoung Hee Jeong,[1] Hyun Jeong Jeong,[1] Young-Il Jo.[1,2] *[1]Dialysis Center, Konkuk University Hospital, Seoul, Republic of Korea; [2]Nephrology, Konkuk University Medical Center, Seoul, Republic of Korea.*

Background: Hypophosphatemia is a common complication of CRRT. However, there are a few studies in critically ill adults undergoing CRRT in which phosphate was added to the replacement and dialysate solutions. The objectives of this prospective study were to evaluate the incidence of hypophosphatemia during CRRT and the efficacy and safety of phosphate supplementation in critically ill adults undergoing CRRT.

Methods: Adult patients who admitted to the ICU and undergoing CRRT for at least 48 hours were recruited in this prospective randomized two-arm comparative study. All patients were randomly assigned to the P-15.0 group and P-22.5 group. If hypophosphatemia was detected during CRRT, we added phosphate 15.0 mEq or 22.5 mEq to both the replacement solution (5L) and the dialysate solution (5L) in the P-15.0 or P-22.5 group, respectively. The phosphate, calcium and potassium levels were recorded before CRRT and every 24 hours after starting CRRT.

Results: A total of 29 adult patients were enrolled (P-15.0 group, n=16; P-22.5 group, n=13). The mean levels of serum P at the beginning of of CRRT was 4.9±1.5 mg/dL. During CRRT, 24 patients (82.7%) were found to have hypophosphatemia (mean levels of serum P, 2.2±0.3 mg/dL) which occurred at 52.0±39.2 hrs following initiation of CRRT. After adding of phosphate to the replacement and dialysate solutions, serum P levels for the P-15.0 and P-22.5 group were 4.1±1.2 mg/dL and 4.6±0.8 mg/dL, respectively ($p>0.05$). Following phosphate addition, the times of restoration to normal values of serum P levels for the P-15.0 and P-22.5 groups were 37.7±18.1 hrs and 26.7±8.0 hours, respectively ($p=0.030$). Except one patient (3.5%), intravenous phosphate supplementation was not needed. No serious adverse effects of phosphate supplementation during CRRT have been observed.

Conclusions: These results indicated that the incidence of hypophosphatemia in critically ill adults undergoing CRRT is very high and the phosphate addition to replacement and dialysate solutions is safe and effective on correcting hypophosphatemia during CRRT.

TH-PO902

The Effect of Continuous Veno-Venous Haemodiafiltration on Monocyte Function in Canine Model for Multiple Organ Dysfunction Syndrome Chen Ji Hong. *Department of Nephrology, First Affiliated Hospital of Xinjiang Medical University, Urumqi, XinJiang Province, China.*

Background: Continuous veno-venous haemodiafiltration (CVVHDF) has gained wide acceptance for the treatment of multiple organ dysfunction syndrome (MODS) in intensive care. This study evaluated specific effects of CVVHDF on the monocyte counts, apoptosis rate, expression rate of DLA-DR, secretary function in Canine model for MODS.

Methods: 12 beagle dogs were subjected to hemorrhagic shock plus resuscitation and endotoxiemia to set up MODS model. Shock was produced according to the method of Wiggers. Then the animals were resuscitated by infusion of Ringer's solution at twice the volume of shed blood. After 12 hours of the resuscitation, Escherichia coli endotoxin (LPS) was dropped in a dose of 1.5 mg/kg in 500 ml of normal saline for 12 hours. Then 12 dogs were randomly divided into two groups: CVVHDF group and MODS group. Measurements of variables were obtained at baseline(T_1),before LPS injection(T_2) and 0h(T_3),3h(T_4),6h(T_5),9h(T_6),12h(T_7),15h(T_8) and 24h(T_9) after LPS injection was finished.

Results: 1 After treatment of CVVHDF, MAP and heart rate remained stable, with decreased body temperature, increased urine output, decreased total number of white blood cell and improved lung and renal function . 2 Early and late monocyte apoptosis rate in CVVHDF group was significantly lower than the MODS group, and the number of monocyte was significantly increased ($p<0.01$). 3 The expression of CD_{14}^+ DLA-DR in CVVHDF group was significantly higher than in MODS group ($p<0.01$). CVVHDF facilitated monocyte antigen-presenting function in the inhibitory state to recover gradually. 4 CVVHDF improved the secretion of IL-1β and IL-4 by monocytes, upregulated secretary strength of the anti-inflammatory factor IL-4 thus facilitating the gradual recovery of secretary function of monotyes in inhibitory state($p<0.01$).

Conclusions: Our results suggested that treatment with CVVHDF effectively removed cytokines from the circulation, reduced monocyte apoptosis, increased number of monocyte, and improved the monocyte function of presentation and secretory. Thus, it facilitated reconstruction of immune homeostasis.

Funding: Government Support - Non-U.S.

TH-PO903

Management and Practice of CRRT in ICU: A Survey of Italian Nurses Flavio Basso,[1] Mariangela Mattifogo,[1] Dinna N. Cruz,[1] Nathan W. Levin,[2] Zaccaria Ricci,[3] Alessandra Brendolan,[1] Federico Nalesso,[1] Francesco Garzotto,[1] Claudio Ronco.[1] *[1]Nephrology Dialysis and Transplantation, San Bortolo Hospital, Vicenza, Italy; [2]Renal Research Institute, New York; [3]Cardiosurgery, Bambino Gesù Hospital, Rome.*

Background: Nursing in continuous renal replacement therapy (CRRT) requires specific expertise for its correct implementation. The nurses' role is fundamental in the prevention and management of adverse events.

Our aim was to explore nursing practice in Italy in CRRT management in order to identify strategies to improve treatment quality.

Methods: During the course on "Nursing skill and practice for adequate CRRT" (Vicenza 2010), participants were surveyed about CRRT management, including the complications leading to loss of the circuit and the most important limitations for CRRT.

Results: The questionnaires were correctly filled out by 113 of 126 Italian nurse participants (89.7%).

CRRT was managed by either ICU nurses (42.8%) or nephrology nurses (49.6%).

The most frequent cause for discontinuation of treatment was circuit loss (59%), then high circuit pressures (28%), and problems such as hypotension (13%). 78.5% of nurses often/always performed coagulation tests q6hr, while 21.5% never did any.

Nurses indicated as limitations of CRRT the absence of comprehensive training (38%), frequent filter clotting (32%) and lack of a checklist (24%).

When an alarm is triggered, 86% of nurses identified the cause immediately and resolved it while 9% performed a full system check. Of note, 5% muted the alarm without investigation of the cause. This dangerous practice is a further demonstration of the need for better training of CRRT for nursing staff.

Conclusions: The results of the survey indicate that more careful training in the correct methods for CRRT is required. Such training would bring direct benefits to critically ill patients undergoing this procedure. Periodic surveys on nursing practice could identify potential areas for improvement.

TH-PO904

Renal Failure Requiring Dialysis after Slow Continuous Ultrafiltration in Patients with Advanced Heart Failure Edgard I. Wehbe,[1] Maria M. Patarroyo,[2] Jonathan J. Taliercio,[1] W.H. Wilson Tang,[2] Sevag Demirjian.[1] *[1]Nephrology and Hypertension, Glickman Urological and Kidney Institute; [2]Cardiology, Cleveland Clinic.*

Background: Slow continuous ultrafiltration (SCUF) has been increasingly utilized in patients with acute decompensated heart failure (ADHF). However, a subsets of patients develop worsening renal function requiring dialysis. We aim to describe the incidence and outcome of those patients.

Methods: Our cohort consisted of 63 patients who underwent SCUF as initial modality of ultrafiltration in patients with ADHF from 2004 till 2009. The cohort was divided in 2 groups; those who needed only SCUF and those who transitioned to continuous venovenous (CVVHD) or intermittent (IHD) hemodialysis.

Results: Out of 63 patients (mean age 59+/-11, 76% male) 37(59%)transitioned to CVVHD or IHD. There were no differences in demographics, comorbidites, medications and ejection fraction between the 2 groups. Patients who required transition had a higher creatinine at baseline(1.44+/-0.43 vs 1.87+/-0.76 P= 0.0135), at SCUF initiation and after 48 h(1.7+/-0.7vs 2.9+/-0.9 P=<0.001), lower systolic blood pressure at baseline and at time of initiation of SCUF(112+/-2 vs 101+/-2 P=0.003), lower systemic vascular resistance at baseline and at time of initiation of SCUF(1189+/-494 vs 826+/-229 P=0.008). There was no difference in mean pulmonary arterial pressure, central venous pressure, cardiac index/output, pulmonary capillary wedge pressure and pulmonary vascular resistant. Table 1 summarizes the clinical outcomes.

Conclusions: This study shows that a large number of patients required dialysis support after SCUF. Those patients had a higher creatinine and lower systolic blood pressure at baseline and at time of SCUF initiation. Transition to dialysis was associated with very high one year rate of mortality.

	SCUF only n= 26(41%)	Convert from SCUF to CVVHD or IHD n= 37(59%)	P value
Hospital stay in days	16+/-11	28+/-18	0.0093
Time on SCUF in days	2.3+/-1	3.5+/-2	0.06
Discharged from the hospital on dialysis	0(0%)	9(24%)	<0.001
Death at 30 days since SCUF start date	1(3.8%)	23(62%)	<0.001
Death at 90 days since SCUF start date	4(15%)	30(81%)	<0.001
Death at one year since SCUF start date	9(34%)	35(94%)	<0.001

TH-PO905

Weigth Gain after Renal Transplantation: Combined Effects of Nutritional Factors and Lower Physical Activity Eva Corpeleijn,[2] Dorien M. Zelle,[1] Willem Van Son,[1] Gerjan Navis,[1] Stephan J.L. Bakker.[1] *[1]Kidney Center, University Medical Center Groningen, University of Groningen, Netherlands; [2]Dept of Epidemiology, University Medical Center Groningen, University of Groningen.*

Background: Weight gain after renal transplantation is related to increased cardiovascular risk and decreased graft survival. Our aim was to identify lifestyle factors related to gain in fat mass, and its association with cardio-metabolic risk factors at one year after renal transplantation.

Methods: At 6 weeks and 3, 6 and 12 months after transplantation, post-transplant weight gain, changes in body composition (bio-electrical impedance, Biostat), cardio-metabolic risk factors, renal function, nutritional intake (24h recall and interviews; 24h urine collections) and physical activity (SenseWear diaxial accelerometer; SQUASH questionnaire) were assessed.

Results: From 29 participants, 26 (48% men, age 51 ± 12 yrs) completed the measurements. At 12 months, patients had gained on average 5.7 ± 5.0 kg (range -2 to + 20 kg) in weight, mainly fat tissue. Gain in body fat was positively associated with serum total cholesterol (r=0.46, P=0.02) and triglycerides (r=0.51, P=0.01) at 12 months. In the patients who gained most in body fat (>3% fat gain, N=13), cardiovascular risk factors at 12 months were higher, i.e. LDL-cholesterol (+ 0.8 mmol/l, P=0.02), total cholesterol (+1.2 mmol/l. P=0.006), and triglycerides (+1.0 mmol/l, P=0.03) compared to those who remained weight stable (<3% fat gain, N=13). Immunosuppressive therapy, renal function, random glucose and HbA1c were comparable. Those who gained in body fat showed lower daily physical activity (-39%, P=0.04) and walked fewer steps per day (-33%, P=0.01). In addition, body fat gain was related to a 30% lower intake in vegetables (P=0.04) and a 20% higher consumption of mono and disaccharides (P=0.02), mainly due to the consumption of energy-rich drinks and sugared dairy (P=0.05).

Conclusions: Daily physical activity, vegetable intake and high consumption of energy-rich drinks and dairy may provide targets for lifestyle intervention to prevent weight gain and improve long term cardiovascular and renal outcomes.

TH-PO906

Treating Post Transplant Anaemia with EPO Improves Quality of Life Taryn Pile,[1] Martin J. Raftery,[2] Magdi Yaqoob.[1] *[1]Translational Medicine and Therapeutics, Queen Mary College, London, United Kingdom; [2]Department of Kidney Medicine and Transplantation, Barts and the London NHS Trust, United Kingdom.*

Background: Anaemia affects 30-45% of renal transplant recipients. Treatment with ESAs has not been well studied and the effects of long term treatment are not known.

Methods: An exploratory study to assess the effect of treatment with Epoetin beta (EB) on renal progression in anaemic renal transplant recipients was performed. The effect on Health Quality of Life (HQOL) and Left Ventricular Hypertrophy was also assessed. The treatment arm received EB to achieve a target of 12-13.5g/dL. The No Treatment group (NT) was treated with EB if the Hb fell below 9g/dL. The primary end-points were progression of CKD, blood pressure and proteinuria. Secondary end-points were HQOL assessed by SF-36 and Left Ventricular Hypertrophy assessed by LVMI.

Results: 55 patients were recruited (NT N= 27, EB N=28) with a median of 23.34 months follow-up. At the end of the study the Hb was significantly higher in the EB group (EB: 12.3 ± 0.18 vs. NT: 9.99 ± 0.22 g/dL, $P < 0.0001$). There was no significant difference in delince of eGFR, PCR or blood pressure between the 2 groups throughout the study. Similarly LVMI was not significantly different.

Conclusions: However, an improvement was seen in Vitality (Baseline: 39.81 ± 2.55 vs. End of Study: 44.54 ± 2.66, $P = 0.03$), Physical Function (Baseline: 42.04 ± 2.28 vs. End of Study: 48.29 ± 2.62, $P = 0.01$) Domains as well as Physical Component Summary (Baseline: 38.76 ± 2.94 vs. End of Study: 44.69 ± 2.81, $P = 0.002$) in the EB group. There was also a small , but significant improvement in the Physical Function Domain in the NT group (Baseline: 39.27 ± 2.21 vs. End of Study; 42.52 ± 2.48 , $P = 0.03$). A trend to improvement in change from baseline was seen in the EB group when compared to the NT group. A significant improvement was seen in the Vitality domain (NT 3.12 (-3.1;6.24) vs. EB: 6.25 (3.12,12.5); $P = 0.02$).

Anaemic renal transplant patients treated with EB benefit from improved Vitality in this small exploratory RCT. A large, multi-centred RCT is warranted to study quality of life benefits in of long-term ESA treatment in PTA.

Funding: Pharmaceutical Company Support

TH-PO907

Residence Location and Likelihood of Transplantation among Pediatric Patients with End-Stage Renal Disease Susan M. Samuel,[1] Brenda Hemmelgarn,[1] Bethany J. Foster,[2] R. Todd Alexander,[3] Andrea Soo,[1] Alberto Nettel-Aguirre,[1] Marcello Tonelli.[3] *[1]University of Calgary, Calgary, Canada; [2]McGill University, Montreal, Canada; [3]University of Alberta, Edmonton, Canada.*

Background: Due to Canada's large size, many pediatric end-stage renal disease (ESRD) patients reside far from a pediatric renal care centre. It is unknown whether this potentially reversible geographical barrier affects likelihood of kidney transplant.

Methods: Population-based retrospective cohort study using data from national ESRD registry. Children (age 0-18) initiating renal replacement therapy between 1992-2007 were followed until death or last contact. Primary outcome was kidney transplant (living or deceased donor). Distances between nearest pediatric transplant centre and each patients' residence were calculated using geographic information software, and categorized as: <50 km, 50.0-149.9 km, 150-299.9 km, and ≥300 km. Cox proportional hazards models were used to compare likelihood of transplant by distance category, adjusting for gender, age, socioeconomic status and primary disease. Separate models were used for whites and non-whites due to a significant interaction between ethnicity and distance.

Results: 728 patients were included (52.2% males; 62.5% white). 38.5% lived <50 km, 20.1% lived 50-149.9 km, 14.3% lived 150-299.9 km and 27.2% lived ≥ 300 km away from a pediatric transplant center. Among whites, compared to those living <50 km, patients living between 150-299.9 km and ≥300 km from a transplant centre were less likely to receive a deceased donor transplant (adjusted hazard ratios [95% CI]: 0.55[0.33-0.92] and 0.61[0.39-0.94] respectively). There were no differences in likelihood of living donor transplant by distance to care centre among whites. Among non-whites there was no association between distance to a transplant centre and likelihood of transplant for both living and deceased donor transplants.

Conclusions: White pediatric ESRD patients living ≥150 km away from a transplant centre are less likely to receive deceased donor transplant. Further evaluation is necessary to determine barriers in access to deceased donor transplant among remote dwelling patients.

Funding: Private Foundation Support

TH-PO908

Risk Factors for Efficacy Failure with Tacrolimus-Based Immunosuppression after Kidney Transplantation – The OSAKA Study Bernhard Banas,[1] Laetitia Albano,[2] Lionel P.E. Rostaing.[3] *[1]University Medical Center Regensburg, Germany; [2]Centre Hospitalier Universitaire de Nice, France; [3]Toulouse University Hospital, France.*

Background: OSAKA is one of the largest clinical trials ever conducted in *de novo* kidney transplantation (n=1,251). This allows an analysis of donor and recipient risk factors that impact on the risk of efficacy failure on tacrolimus (Tac)-based immunosuppression.

Methods: Adult kidney recipients were randomized 1:1:1:1 to starting doses of Tac immediate release (BID) 0.2mg/kg/day (Arm 1), Tac prolonged release (QD) 0.2mg/kg/day (Arm 2), Tac QD 0.3mg/kg/day (Arm 3), all with MMF + corticosteroids (CS) for 24 weeks, or Tac QD 0.2mg/kg/day + MMF + basiliximab + CS (perioperative bolus only) (Arm 4). The primary composite endpoint was efficacy failure rate (graft loss, biopsy confirmed acute rejection, graft dysfunction [eGFR (MDRD) <40mL/min/1.73m^2] at 24 weeks). Logistic regression (LR) and a simplified Classification And Regression Tree (CART) method were used to quantify risk factors.

Results: Efficacy failure rates as defined (PPS) were 40.6% (Arm 1), 42.2% (Arm 2), 44.2% (Arm 3), and 48.2% (Arm 4). LR analysis identified donor age, female donor, donor death, and underlying focal segmental glomerulosclerosis (FSGS) as the most relevant risk factors, with donor age the most statistically significant factor. Each additional year of donor age increased the odds of efficacy failure by more than 4% on average. FSGS doubled the risk, a female donor increased the risk by nearly 28%, each point of mismatch by almost 10%, and a cadaveric donor by nearly 50%. CART confirmed that patients with the highest risk were those with a donor age over 61 years, and identified more than four mismatches and a female donor as additional risk factors.

Conclusions: With Tac-based therapy, donor age was the overriding risk factor for the composite endpoint of efficacy failure (as well as its single components).

		Wald test	
Risk factor	Odds ratio (95% CI)	Parameter estimate	P-value (chi-square)
Donor age	1.045 (1.034; 1.057)	0.0442	<0.0001
Female donor	1.276 (0.996; 1.634)	0.2434	0.0537
Deceased donor	1.496 (0.987; 2.265)	0.4025	0.0574
Underlying FSGS	1.921 (0.897; 4.112)	0.6528	0.0928

Funding: Pharmaceutical Company Support

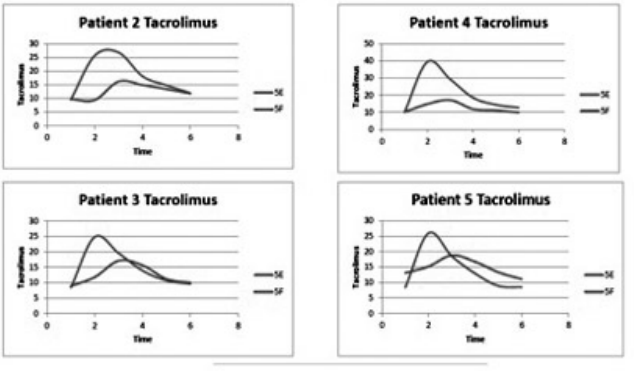
TH-PO909

Concomitant Food Ingestion Decreases Tacrolimus Absorption Well Below FDA Standards for Approved Generics Linda Awdishu,[1] Son Ho,[1] Amol Shah,[2] Robert W. Steiner.[2] *[1]Clinical Pharmacy, UCSD Skaggs School of Pharmacy, La Jolla, CA; [2]Medicine, Division of Nephrology, UCSD Medical Center, San Diego, CA.*

Background: The predictability and degree of diurnal tacrolimus (TAC) exposure in chronic renal transplant recipients (CRTRs) has received increased attention due to the availability of generic alternatives. Concern about TAC exposure is appropriate considering the disappointing increase in long term kidney transplant survival in the current "TAC decade."

Methods: TAC area under the curve (AUC0-12) was estimated in 15 CRTRs taking TAC (as Prograf), MMF, and prednisone. TAC was taken under direct observation simultaneously with a 700 kcal breakfast and on a second day 1 hour before eating. TAC concentrations were measured at 0, 1, 2, 4, 6 and 8 hours.

Results: Absorption profiles with and without food in 4 patients are shown in Figure 1.



Fasting curves were relatively uniform with robust early peaks. The fasting state AUC0-12 ranged from 120.05 to 249.98 ng*mL/hr. The fed state AUC0-12 ranged from 56.83 to 199.93 ng*mL/hr. The ratio of the fed to fasting state AUC ranged from 38 to 111%. We calculated the natural logarithm of the difference in peak concentration (Cmax), time to peak concentration (Tmax), and AUC between fasting and fed states. The difference in lnCmax was 0.24 ±0.27 ng/mL (p<0.001), Tmax was -0.93 ± 1.14 hr (p=0.008) and lnAUC0-12 was 0.24 ± 32 ng*mL/hr (p=0.005). Importantly, drug concentrations at 8 hrs post dose was similar between fed and fasting states demonstrating the importance of Cmax for drug exposure.

Conclusions: Ingestion of TAC with food reduces exposure well beyond any effect of an FDA approved generic when taken under the same circumstances. The food effect may be responsible for suboptimal immunosuppression and graft survival in some adherent patients. Such underexposure will not be detected by 12 hour trough TAC levels.

Funding: Other NIH Support - T32 training grant for student summer research, Pharmaceutical Company Support

TH-PO910

Predialysis Nephrology Care, Early Transplant Assessment and Patient Satisfaction with Physician Interaction Nancy G. Kutner,[1] Rebecca H. Zhang,[1] Kirsten L. Johansen.[1,2] *[1]USRDS Rehabilitation/QoL Special Studies Center, Emory University, Atlanta, GA; [2]Nephrology Section, San Francisco VA Medical Center, San Francisco, CA.*

Background: Satisfaction with physician interaction is a key component of dialysis patients' satisfaction with their care. We hypothesized that whether patients receive predialysis nephrology care and have early assessment for transplantation may influence their satisfaction.

Methods: In phone interviews the Comprehensive Dialysis Study (CDS) surveyed 1473 incident HD patients and 169 incident PD patients aged >18 from 296 randomly selected clinics throughout the US. Thinking about the kidney doctor they saw most often, respondents rated "the amount of time your kidney doctor spends with you" and "your kidney doctor's explanations of medical procedures and tests" [Medical Outcomes Study items].

Results: Although most respondents rated their interaction with their kidney doctor as good to excellent (76% for time spent, 83% for procedure/test explanations), in a linear regression analysis with adjustment for patient clustering in clinics, patients on HD, Hispanics, and patients who lacked predialysis nephrology care were significantly less satisfied with both aspects of physician interaction, and patients with less than high school education were less satisfied with their doctor's explanation of procedures/tests. No age, gender, or race differences were significant. The ESRD Medical Evidence Report indicated that 245 CDS participants had not been assessed for transplantation. Compared to those reported informed of transplant options, patients who had not been assessed were less satisfied with the time their doctor spent with them ($p < 0.05$) and with their doctor's explanations of procedures and tests ($p < 0.05$).

Conclusions: Congruence between expectations and perceived experience influences satisfaction. Patients who lack predialysis nephrology care may expect more time and information from their physicians, especially with regard to provider/patient communication related to kidney transplant options.

Funding: NIDDK Support

TH-PO911

Evaluation of the Living Kidney Donor Programme in the United Kingdom Cheralathan Arunachalam, Mila Garrues, Fiona Biggins, Aimun Ahmed. *Department of Renal Medicine, Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom.*

Background: Living kidney donation (LKD) has increased in the UK by 12% (1037 donors) in 2010 which is 38% of the total UK kidney transplant activity. There has been growing evidence and evolving guidelines for LKD. However there are still inconsistencies in practice between renal units. We aim here to study LKD assessment across the UK centres.

Methods: A structured questionnaire relating to LKD assessment was sent by post to 74 renal units across the UK between October to December 2010

Results: 19 of 23 (83%) transplant units (TU) and 21 of 51 (42%) non transplant units (NTU) responded. 12 TU perform both ABO and HLA incompatible transplantation. 4 TU perform ABO incompatible transplantation only and 3 TU do not perform either. 85% of the units are without a set upper age limit for donors, where as 15% of units excluded donors older than 70 years. 83% of the units have no lower age limit. 22% of units accept donors with BMI up to 35 and 10% of centres did not have upper limit for BMI. 27% of centres exclude hypertensive donors on more than one antihypertensive drug, 60% of units exclude donors if they are taking more than 2 drugs. 65% of centres rely on urine PCR to assess proteinuria and 27% of units perform 24 hour urine collection for protein estimation.

60% of centres perform CT angiography to assess renal vessels while 40% use MR angiography. 78% of centres perform split kidney function routinely where as 21% use this test only if there is a discrepancy in kidney size noted from other imaging studies. Removal of recipients who have a live donor from the deceased donor list is also inconsistent, 50% of centres suspend the recipient when theatre date has been set

Conclusions: This survey provides insight into contemporary UK practice for LKD assessment. We demonstrate significant variability regarding acceptance of donors based on age, BMI, and hypertension. Investigations used for LKD assessment also differ markedly within the UK. Unified and clear guidelines are needed as well as strong evidence on the most appropriate investigations to assist clinical decision-making in live donor assessment.

TH-PO912

Conversion to LCP-Tacro™ Tablets Once-Daily from Prograf® Capsules Twice-Daily in Kidney Transplant Patients: Central Pathology Results from a Phase III, Open-Label, Multicenter Trial Suphamai Bunnapradist,[1] Klemens Budde,[2] Shamkant P. Mulgaonkar,[3] Lionel P.E. Rostaing.[4] *[1]UCLA; [2]Charite Universitatsmedizin; [3]St. Barnabas Med. Ctr.; [4]CHU Rangueil.*

Background: Phase II studies of de novo and stable renal and liver recipients showed improved pharmacokinetics (PK) and a tendency toward fewer treatment failures and SAEs for extended-release tacrolimus tablets (LCP-Tacro) administered once-daily (qd) vs. immediate release tacrolimus capsules (Prograf) administered twice-daily (bid). A Phase III study of stable renal transplant patients converted to LCP-Tacro qd from Prograf bid showed noninferiority for LCP-Tacro vs. Prograf. Here we report pathology results from centrally read, blinded biopsies.

Methods: Kidney transplant patients 3-60 mos. post-transplant taking Prograf bid with tacrolimus trough levels 4-15 ng/mL were randomized to LCP-Tacro qd or to maintain their Prograf regimen for 12 mos. Initial LCP-Tacro dose was 30% lower (15% for African Americans) than the pre-conversion Prograf daily dose. Subsequently, trough levels of 4-15 ng/mL were targeted in both drugs.

Results: The mITT groups (LCP-Tacro: n=162; Prograf: n=162) were similar in demographics and tacrolimus trough levels throughout the study; 35 biopsies were centrally read (23 LCP-Tacro, 12 Prograf). One LCP-Tacro patient had 1 biopsy-proven acute rejection (BPAR) episode vs. 7 BPARs in Prograf patients (5 Prograf patients with BPAR; 2 patients had 2 episodes) (p=0.21). Tacrolimus trough levels preceding each BPAR were in target range. All BPARs were mild; BANFF chronicity score (chronic glomerular damage+interstitial fibrosis+tubular fibrosis+vascular intimal thickening) tended to be greater in the Prograf group (LCP-Tacro=2; Prograf mean=3.6).

Conclusions: LCP-Tacro qd-based therapy was associated with a numerically lower number of BPARs and the data suggest a tendency for less severe histological changes. Whether a statistically significant lower incidence of BPAR will be seen in studies with a larger sample and whether LCP-Tacro is associated with improved long-term outcomes remains to be examined. These results and the previous PK results suggest LCP-Tacro may be an attractive alternative to Prograf.

Funding: Pharmaceutical Company Support

TH-PO913

Early Steroids Withdrawal Improves Hemodynamic Profile and Does Not Increase Acute Rejection Risk in Kidney Transplant Recipients Jorge Andrade-Sierra,[1,3] Enrique Rojas-Campos,[1] Ernesto Cardona,[3] Luis Alberto Evangelista-Carrillo,[2] Trinidad Orlando Lugo Lopez,[2] Abel Puentes Camacho,[2] Salvador Mendoza Cabrera,[2] Benjamin Gomez-Navarro,[2] Mario Sandoval Sandoval,[2] Alfonso M. Cueto-Manzano.[1] *[1]Medical Research Unit in Renal Diseases, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico; [2]Department of Nephrology and Organ Transplant Unit, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico; [3]Department of Physiology, CUCS. University of Guadalajara, Guadalajara, Jalisco, Mexico.*

Background: Early steroids withdrawal (ESW) improve cardiovascular profile but could increase the risk of acute rejection (AR). **Objective:** To compare the effect of ESW on metabolic profile and AR frequency

Methods: Open label, randomized clinical trial; 55 KTR, with PRA<20%, between Jul/10-Jun/11; 28 in the ESW-G / 27 in the control group (CG), maintenance was with TAC and MMF and PDN in C-G; ESW-G, had a daily steroid reduction (250/day1-0mg/day5). Metabolic and hemodynamic profile, eGFR and graft biopsy were done at baseline, 3,6 months. Comparisons between groups, were done with Mann-Whitney U and χ^2 tests.

Results: Main results are shown in Table. At 3 moths, ESW-G had 4 (14%) borderline, CG had 3 (11%) Banf IA rejections; at 6 months ESW-G 3 (11%) borderline, CG had 3 (11%) borderline and 2 (7%) Banf IA rejections.

Conclusions: ESW-G shows a better control of blood pressure and trend to show lower AR frequency and severity compared to CG.

	ESW-G		C-G	
	Baseline	Follow-up	Baseline	Follow-up
Receptor Age (years)	21 (19-26)		22 (18-33)	
Dialysis Time (months)	15 (12-23)		16 (8-24)	
HLA antigens matching	4 (3-5)		4 (3-5)	
Glucose (mg/dl)	85 (80-92)	83 (82-89)	88 (78-98)	86 (80-102)
Triglycerides (mg/dl)	134 (99-161)	132 (112-165)	154 (126-194)	166 (136-215)
Cholesterol (mg/dl)	160 (141-188)	138 (113-164)	174 (135-196)	157 (132-201)
HDL (mg/dl)	42 (37-53)	41 (32-50)	42 (37-48)	38(35-54)
LDL (mg/dl)	93 (77-106)	82 (59-101)	100 (78-113)	90 (71-102)
SBP mmHg	130 (129-141)	112 (110-118)	131 (121-140)	129 (124-132)*
DBP mmHg	86 (78-90)	72 (70-78)	80 (75-90)	86 (78-89) *
eGFR ml/min	6±3	85±25	6±5	74±14*
Acute rejection (%)		25%		45%

*p <0.05 vs same evaluation ESW group

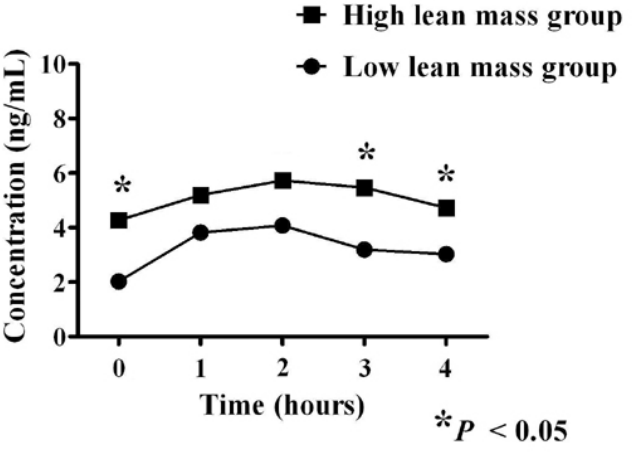
TH-PO914

Pharmacokinetics of Tacrolimus According to the Body Composition in Korean Kidney Transplant Recipients Seung Seok Han,[1] Curie Ahn,[1] Jin Suk Han,[1] Suhnggwon Kim,[1] Yon Su Kim.[1,2] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Clinical Research Center for End Stage Renal Disease, Korea.*

Background: Current dosing of calcineurin inhibitor after transplantation is based on the patient's body weight. However, kidney transplant recipients have a low correlation between body weight and body composition. Here, we evaluate the pharmacokinetics of tacrolimus according to the body composition in 17 Korean kidney recipients with stable graft function.

Methods: The body compositions such as lean and fat mass were calculated using the bioelectrical impedance analysis. Pharmacokinetic profiles were taken at 0, 1, 2, 3, and 4 hours after application of tacrolimus and compared between the low and the high level groups according to the median of body composition. The values of C_0, C_1, C_2, C_3, and C_4 were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

Results: The medians of body mass index (BMI) and body compositions were as follows: BMI, 25.0 kg/m^2 [interquartile range (IQR, 22.2–26.3)]; fat mass, 17.7 kg (IQR, 14.2–20.1); lean mass, 49.4 kg (IQR, 43.2–58.1). There were no statistical differences in pharmacokinetic profiles according to BMI. However, the concentrations (C_3 and C_4) in the high fat group were higher than the low fat group (*P*s = 0.060 and 0.034, respectively). The concentrations (C_0, C_2, C_3, and C_4) and the AUC were significantly different between the two groups of the lean mass (*P*s were 0.012, 0.068, 0.043, 0.021, and 0.027).



Other variables such as waist circumference, waist-hip ratio, and arm circumference did not differentiate the pharmacokinetic profiles of tacrolimus.

Conclusions: Taken together, these data provide the suggestion that the monitoring of the dose of tacrolimus should be based on the individual body compositions for further improvement of kidney transplant outcomes.

TH-PO915

Response to Cinacalcet in Renaltransplant Patients with Secondary Hyperparathyroidism and Hypercalcemia: What Is the Role of the Magnesium? Victor Martínez Jiménez.[1] *[1]Nephrology, Hospital Reina Sofia, Murcia, Spain; [2]Nephrology, Hospital Virgen Arrixaca, Murcia, Spain.*

Background: Cinacalcet is able to bind to the calcium-sensing receptor in parathyroid to modify it allosterically, being useful in controlling severe hyperparathyroidism with persistent hypercalcemia in renal transplant.

It is known that before correcting hypocalcemia is necessary to solve the deficit of magnesium, due to the hypomagnesemia would activate the calcium-sensing receptor. However, we found no studies evaluating the effect of magnesium on the hypercalcemia and the response of cinacalcet in these patients.

Objectives:

To evaluate the effectiveness of treatment with cinacalcet in renal transplant with severe hyperparathyroidism and hypercalcemia.

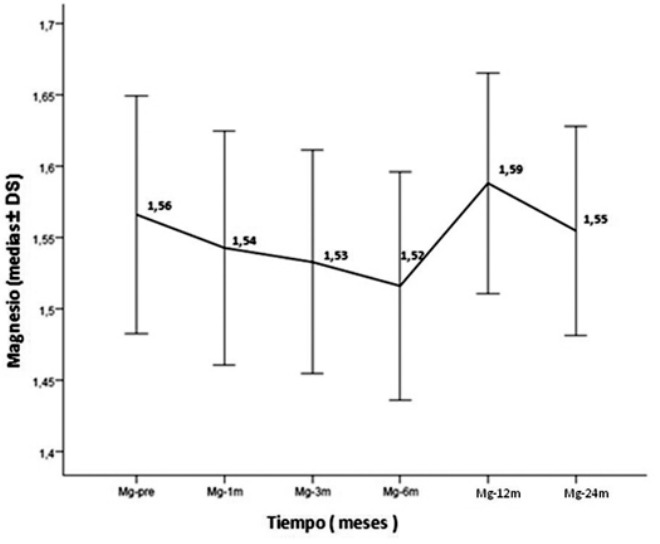
To study the role of magnesium in the response of cinacalcet.

Methods: -37 renal transplant with creatinine clearance>50 ml/min, Ca>10.5 mg/dl and PTH> 65 pg/ml have been studied for 3months to 4years

- Parameters to analyze: Ca, P, PTH and magnesium pretreatment with cinacalcet, 1month, 3months, 6months and annually. If the magnesium < 1.65 mg/dl iniciated oral magnesium tablets to maintain stable nivels of magnesium.

- Cinacalcet dose: 30-90 mg / day.

Results:



- Decreased calcium and PTH

- Increased phosphorus

- 14 patients (37.8%) had hypomagnesemia pretreatment, 13 of them were treated with oral magnesium.

- Patients with lower initial levels of magnesium (before starting treatment with cinacalcet) had, significantly, a greater decrease in PTH and calcium ($p < 0.05$), as shown on table 1.

Conclusions: Cinacalcet is effective in controlling hypercalcemia in renal transplant with secondary hyperparathyroidism.

Pretreatment hypomagnesemia seems to favor the response of cinacalcet.

In view of these results, should we try to correct magnesium deficiency in all cases or only in symptomatic patients?

Funding: Clinical Revenue Support

TH-PO916

ABO Incompatible Living Donor Kidney Transplantation(ABOi KT): Minimal Dose of Rituximab and Not a Routine but As-Needed Posttransplant Plasmapheresis Jin M. Kong,[1] Jeongmyung Ahn,[1] Byung Chang Kim,[2] Mi Young Jeon.[2] *[1]Nephrology, Maryknoll Hospital, Busan, Korea; [2]Pathology, Maryknoll Hospital, Busan, Korea.*

Background: ABOi KT is now an established procedure with outcome equivalent to ABO compatible KT. There are various protocols in different parts of the world, and the refinement of the protocol is necessary to improve outcome, safety and cost.

Methods: Since the initiation of ABOi KT in 2007 in our center, the protocol has been being evolved. Conventional dose(375mg/m2) of rituximab was used initially, the dose halved subsequently, and recently 100mg fixed dose was used. Pretransplant plasmapheresis aimed anti-ABO titer ≤8 on transplant day, but patients with higher(≥16) titer whose antibody could not be lowered to target on transplant day were also allowed to transplant. Posttransplant plasamperesis was not done routinely but as needed in patients with higher antibody titer or increase in creatinine while awaiting biopsy result during the critical period.

Results: A total of 36 ABOi KT was done since Feb. 2007. Median follow up was 17(0-52)months. Patient and graft survival is 100%. There was only 1 clinical acute AMR. Median(range) anti-ABO titer at initial, on transplant day and at 2 weeks was 64(8-1024), 2(1-16) and 2(1-16), respectively. The duration of the depletion of peripheral CD19+ cells among patients with different dose of rituximab seems not different. There was no CMV disease or BKV nephropathy. One patient developed progressive multifocal leukoencephalopathy with recovery of neurologic symptom by reduction of immunosuppressants.

Conclusions: ABOi KT with minimal dose of rituximab and restricted use of posttransplant plasmapheresis can be performed with excellent outcome, reduced cost and safety.

TH-PO917

Identification of Potential Criteria for Rituximab Responsiveness in Patients with Standard-Therapy Resistant Kidney Allograft Rejection Maximilian Ernst Daemmrich,[1] Jan U. Becker,[1] Verena Broecker,[1] Clemens L. Bockmeyer,[1] Wilfried Gwinner,[2] Anke Schwarz,[2] Cornelia Anneliese Blume.[2] *[1]Institute of Pathology, Medical School Hannover, Hannover, Lower Saxony, Germany; [2]Dept. of Nephrology and Hypertensiology, Medical School Hannover, Hannover, Lower Saxony, Germany.*

Background: Rituximab (anti-CD-20 antibody) is used in kidney transplant rejection refractory to standard therapy, although it carries a risk for serious complications and is not effective in all cases. In a retrospective study, we therefore aimed to identify clinical or histopathological criteria to predict Rituximab response.

Methods: 19 renal transplant recipients who received Rituximab (375 g/qm^2 body surface, 1-2 courses, 15 x combined with up to 5 courses of plasmapheresis) for therapy refractory rejection were included in the study. 10 lost their transplant function (non-responders), in 9, kidney grafts were rescued (responders). Clinical parameters and Banff components of the last biopsy prior to Rituximab were compared between both cohorts by Wilcoxon- or chi-square tests. Observation time after therapy was not different between groups (26±26 vs. 49±24 months; $p=0.07$).

Results: More responders had donor-specific antibodies (6/9 vs. 1/10; $p=0.01$). Responders had lower serum creatinine before therapy (175 vs. 291 µm/l; $p=0.003$). Banff glomerulitis ($p=0.014$) and C4d staining of peritubular capillaries (C4d ptc, $p=0.03$) was less severe in responders. Other clinical (recipient gender and age, number of HLA-mismatches, living or cadaveric donor, previous renal transplant, time after transplantation) and histopathological criteria (glomerular C4d staining, tubulointerstitial edema, cell type, ptc, peritubular and glomerular endothelial swelling) were not different between groups. Responders had a mean serum creatinine of 189 ± 45 µm/l at the end of the observation time.

Conclusions: In this retrospective analysis, lower serum creatinine before therapy, positive donor specific antibodies, less transplant glomerulitis and C4d staining of ptc were identified as potential criteria to predict Rituximab responsiveness. These criteria need validation in future prospective randomized studies.

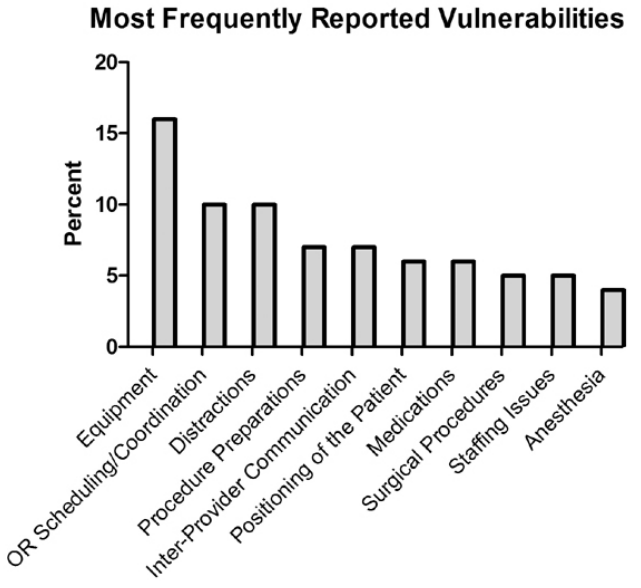
TH-PO918

Safety Issues Identified by Proactive Living Donor Kidney Transplant (LDKT) Safety Debriefing Daniela Ladner, Olivia A. Ross, Anton I. Skaro, Donna Woods, Vadim Lyuksemburg, Krutika Lakhoo, Renee Ziomek, Michael Abecassis, Jane Holl. *Northwestern University Transplant Outcomes Research Collaborative.*

Background: Little is known about the extent of safety vulnerabilities surrounding LDKT. Hospital incident reporting systems exist, but are known to vastly underestimate rates. To comprehensively assess vulnerabilities surrounding LDKT surgery, clinicians were asked to complete a debriefing survey.

Methods: A 28 question web-based debriefing was developed by an interdisciplinary team of patient safety experts and transplant clinicians. Comments were solicited on all safety related system or process issues. All clinicians in the LDKT surgery were asked to complete the anonymous survey immediately following the surgery. All incidents reported online or by phone to the hospital-wide reporting system for these LDKTs were reviewed.

Results: 210 debriefings were submitted on 83 LDKT procedures. Debriefings were completed by the entire clinical team: 40% by the surgical team, 37% by the nursing team, 16% by the anesthesia team, and 7% by others (e.g. lab tech, etc.). 213 vulnerabilities related to transplant systems and processes of care were reported. The most frequently reported included Equipment (e.g. broken surgical instruments), OR Scheduling/Coordination (e.g. avoidable delays), and Distractions (e.g. cell phones/pagers ringing).



In stark contrast, hospital-wide reporting systems identified just 22 reports for these LDKTs. 13 of these incidents were also captured by the safety debriefings.

Conclusions: Anonymous, short, proactive online debriefings can successfully elicit rich information on the safety risks related to LDKT surgery far beyond hospital wide reporting systems, which captured just 10% the number reported in the debriefings. This is supporting evidence that to date safety risks associated with LDKT are poorly described and vastly underestimated.

Funding: Private Foundation Support

TH-PO919

Incidence and Costs Associated with New-Onset Diabetes and Cardiovascular Events after Kidney Transplantation in a Commercially Insured Population

Suphamai Bunnapradist,[1] Schiffon L. Wong,[2] Brett Pinsky,[3] Fang Liu,[3] Cynthia Taylor,[3] Digisha Trivedi,[2] Tony Hebden.[2] *[1]Department of Medicine, UCLA, Los Angeles, CA; [2]Health Services, Bristol-Myers Squibb, Plainsboro, NJ; [3]Observational Research, OptumInsight, Eden Prairie, MN.*

Background: Kidney recipients are at increased risk of developing diabetes and cardiovascular (CV) events which adversely impact graft and patient survival. Our objectives were to describe the prevalence of pre-transplant diabetes and CV events; incidence of new-onset diabetes after transplant (NODAT) and CV events post-transplant; and associated costs in commercially insured kidney transplant recipients.

Methods: Adults with evidence of a kidney transplant (2004-2008) were identified using healthcare claims from a large U.S. managed care plan. "Incident" recipients (IR) had a transplant date within the study period. Medical claims with diagnoses for diabetes and CV events (stroke, coronary artery disease, revascularization, or myocardial infarction) were identified in the year prior to transplant and during the study period (earliest evidence of transplant until death, end of continuous enrollment, or 31Dec2009). Patient- and health plan-paid amounts for NODAT (costs for diabetes, insulin, or antihyperglycemic medication) and CV events (total costs) were assessed from NODAT onset or from CV event through the end of the study period.

Results: Among 1364 recipients, 25.7% had evidence of diabetes and 23.1% had a CV event in the year prior to transplant. Among patients without prior evidence of diabetes, 19.3% developed NODAT with a median (range) onset of 2.6 (0.0-59.1) months. 11.4% of IR without a prior CV event had an event post-transplant with median time to occurrence of 4.3 (0.0-53.9) months. Mean monthly costs associated with NODAT and post-transplant CV events were $953 and $21,439, respectively.

Conclusions: Kidney recipients have a substantial pre- and post-transplant prevalence of diabetes and CV events. Post-transplant onset of these morbidities is early and the associated costs are high. Strategies that reduce risk factors for diabetes and CV events may reduce the associated morbidity and costs of these conditions.

Funding: Pharmaceutical Company Support

TH-PO920

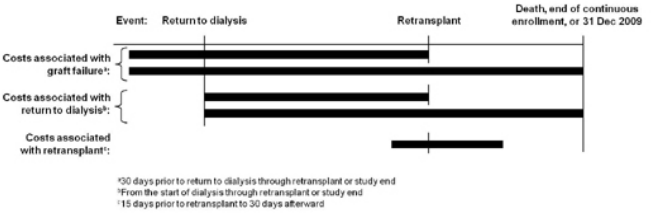
Incidence and Cost of Renal Graft Failure in Commercially Insured Kidney Transplant Recipients

Suphamai Bunnapradist,[1] Schiffon L. Wong,[2] Brett Pinsky,[3] Fang Liu,[3] Cynthia Taylor,[3] Digisha Trivedi,[2] Tony Hebden.[2] *[1]Department of Medicine, UCLA, Los Angeles, CA; [2]Health Services, Bristol-Myers Squibb, Plainsboro, NJ; [3]Observational Research, OptumInsight, Eden Prairie, MN.*

Background: The USRDS Annual Data Report cites a renal graft failure rate of 6.5 per 100 patient years in 2008 but little is known about the impact of graft failure on costs among commercially insured patients.

Methods: Adults with evidence of kidney transplant (2004-2008) were identified from US managed care healthcare claims. "Incident" recipients (IR) had a transplant date within the study period. "Prevalent" recipients (PR) had evidence of a transplant but an unknown transplant date. Graft failure was the first of retransplant or return to dialysis (2 or more dialysis visits in a 30 day period for 2 consecutive 30 day periods) and was identified from the earliest evidence of transplant until death, end of continuous enrollment, or 31Dec2009. Graft failure costs were patient and health plan-paid medical and pharmacy costs (Figure 1).

Timing of Cost Accrual for Graft Failure



*30 days prior to return to dialysis through retransplant or study end
†From the start of dialysis through retransplant or study end
‡15 days prior to retransplant to 30 days afterward

Results: Among 1364 IR, 11.8% had graft failure (10.9% returned to dialysis, 1.9% retransplant). Among 6753 PR, 8.4% had graft failure (8.2% returned to dialysis, 1.1% retransplant). Mean graft failure cost was $25,380/month. Return to dialysis was a major cost driver with a mean monthly cost of $16,867. Retransplant averaged $87,062. Median healthcare costs 3, 6, and 12 months post-transplant were $23,189 greater, $41,794 greater, and $55,789 greater, respectively, for patients with graft failure than for patients without failure in those time frames.

Conclusions: Graft failure occurred in 8-12% of these commercially insured kidney recipients and was associated with high costs for patients and commercial payers whether or not the transplant was paid for by the health plan. Strategies to mitigate graft failure may reduce these costs.

Funding: Pharmaceutical Company Support

TH-PO921

Incidence and Costs Associated with New-Onset Diabetes and Cardiovascular Events after Kidney Transplantation in Medicare Population

William Irish,[1] Schiffon L. Wong,[2] Debora Bowers,[1] Digisha Trivedi,[2] Tony Hebden.[2] *[1]CTI Clinical Trial and Consulting Services; [2]Bristol-Myers Squibb.*

Background: New-onset diabetes after transplantation (NODAT) and cardiovascular (CV) events increase the risk for late graft loss and patient death. The incidence and economic implications of these have not been fully explored.

Methods: Adult Medicare beneficiaries in the US Renal Data System who received kidney-only transplant (2001 - 2008) were eligible. Patients with evidence of diabetes or CV events (stroke, coronary artery disease, revascularization, or myocardial infarction) pre- and post-transplant based on ICD-9-CM diagnosis codes were identified. Patients with diabetes or CV disease prior to transplantation were subsequently excluded. Total Medicare costs (Institutional and Physician/Supplier claims) were calculated from date of first claim until permanent dialysis, retransplantation, death, end of continuous enrollment or followup. Costs are for the first year following the first claim (adjusted using the annual medical care component of the Consumer Price Index).

Results: Among 84,819 recipients, 38% had diabetes and 24% had CV disease prior to transplantation. NODAT occurred in 16,518 patients with a median onset of 4 months (range: <1-60 months) post-transplant. Incidence was 13.6 per 100 patient-years (PTY) (95% confidence interval [CI] = 13.4 to 13.8 per 100 PTY). Costs were categorized by diabetes-related complications. The most frequent complication was nephropathy (26.3%) with a median total one year cost of $1,205 while ketoacidosis (4.4%) was the most expensive (Median total one year cost=$10,079). Peripheral circulatory disorder occurred in 4.1% of NODAT patients and was the least expensive (Median total one year cost = $621). The incidence of CV events (n=12,863) was 7.5 per 100 PTY (95% CI=7.3-7.6 per 100 PTY) with median onset = 7.5; range: <1-60 months post-transplant. Total one year costs for CV events were $99,228.

Conclusions: NODAT and CV events may present early post-transplant and are associated with high healthcare costs. Strategies are needed to minimize the risk of diabetes and CV disease to lower their economic and clinical impact.

Funding: Pharmaceutical Company Support

TH-PO922

Kidney Disease Characteristics in Transplant Patients: Comparison between PREPARE and ANTICIPE Studies Laurent Juillard,[1] Eric Daugas,[2] Bertrand Dussol,[3] Patrick Henri,[4] Paul Stroumza,[5] Malik Touam,[6] Georges J. Mourad.[7] *[1]Nephrology, CHU Lyon, Lyon, France; [2]Nephrology, CHU Paris, Paris, France; [3]Nephrology, CHU Marseille, Marseille, France; [4]Nephrology, CHU Caen, Caen, France; [5]Nephrology, Clinique de la Residence du Parc, Marseille, France; [6]Nephrology, AURA Paris, Paris, France; [7]Nephrology, CHU Montpellier, Montpellier, France.*

Background: Comparing the chronic kidney disease (CKD) and its care in transplant patients (TP) and patients with CKD on native kidneys.

Methods: Comparison of characteristics of patients with CKD stage IIIB / IV based on the ANTICIPE and PREPARE studies. Prospective and observational studies. Collection during one week in TP for over one year and patients treated for CKD (eGFR < 60 ml/ min/1. 73m ²), respectively.

Results: 546 TP (373 stage IIIB, 173 stage IV) were compared to 1405 patients from PREPARE (659 stage IIIB and 746 stage IV). TP were younger (median 57 vs. 73, p <0.05) were less at vascular risk (BMI> 30, diabetes, smoking, dyslipidemia, p <0.05 for each) except for hypertension identically distributed (> 85% of patients). The occurrence of cardiovascular diseases was significantly lower in transplant patients and they receive fewer treatments for cardiovascular indications (RAS blockers, statins). Conversely, some of the complications associated with CKD are more common in TP: Anemia is more common, despite greater use of ESA (stage IV, Hb <12g/dL: 71 % vs 56%, p <0.05, ESA: 47% vs 35%, p <0.05), Only hyperparathyroidism is more frequent (stage IV, PTH> 150 ng/L: 47% vs. 39%, p <0.05), despite the most common therapeutic use of vitamin D. TP have more visits (monthly in stage IV: 47% vs 20%, p <0.05). In case of progression to stage V, the intention for transplantation is more common for transplant patients (60% vs 17%, p <0.05) including the pre-emptive transplantation.

Conclusions: Chronic renal transplant has its own characteristics including more frequent biological complications despite more frequent and closer monitoring of specific therapeutic targets, potentially more difficult to achieve.

Funding: Pharmaceutical Company Support

TH-PO923

myTRACKER – A Nordic Myfortic Observational Study Tracking the Gastro Intestinal (GI) Tolerability of Myfortic in Renal Transplant Recipients Converted to Myfortic Due to Cellcept Related GI Symptoms Sadollah Abedini, Morten Reier-Nilsen, Geir Nordbo, Kristian Heldal. *Medical Clinic, Vestfold Hospital Trust, Toensberg, Norway.*

Background: Clinical trails have demonstrated that *Myfortic* and *Cellcept* are equivalent in safety and efficacy but differences in occurrence of GI tolerability have not been examined in these trials. The primary objective was to evaluate if conversion from *Cellcept* to equivalent dose *myfortic* is associated with an improvement in patient-reported GI symptom during 3 months after the conversion. The secondary objectives were to assess compliance to treatment and overall safety and efficacy of *myfortic*.

Methods: Multi-centre, observational, non-interventional study in renal transplant recipients from Scandinavian centers converted to *myfortic* due to *Cellcept* related GI symptoms. The patients completed the Gastro Intestinal Symptom Rating Scale (GSRS) at baseline, 1 and 3 months after conversion. Treatment adherence was evaluated based on dosing information and from the Immunosuppressant Therapy Adherence Scale (ITAS). The assessment of safety and efficacy was based on collected adverse event data. Analysis, using descriptive statistics, was performed based on patient reported outcomes (GSRS) and Overall Treatment Effect (OTE) scales for GI symptoms.

Results: The study was terminated due to difficulty with patient recruitment and the presented data is based on 51 included patients (planned 150 patients), 33 males and 18 females with a mean age of 56 (13.3) years. The patients experienced about twice as many dose changes during the last 3 months before conversion while receiving *Cellcept* compared with the 3 months treatment with *myfortic* after conversion. After conversion to *myfortic* the patients experienced lower rate of abdominal pain (p<0.0001), constipation (p<0.076), diarrhea (p<0.0005), indigestion (p<0.0004) and reflux (p<0.0001) compared with baseline.

Conclusions: This study suggests that conversion to *myfortic* from *Cellcept* in kidney transplants results in improvements of GI related symptoms at 1 month and maintained at 3 months after conversion. Fewer dose-changes and lower occurrence of GI related symptoms are seen at equivalent therapeutic doses.

Funding: Pharmaceutical Company Support

TH-PO924

Comparisons of Enteric-Coated Mycophenolate Sodium and Mycophenolate Mofetil from the Mycophenolic Acid Observational Renal Transplant Registry Anthony J. Langone,[1] Richard W. Carson,[2] Fuad S. Shihab,[3] Oleh G. Pankewycz,[4] Anne Wiland,[5] Kevin M. McCague,[5] Laurence Chan.[6] *[1]Vanderbilt University; [2]Providence Sacred Heart Medical Center; [3]University of Utah; [4]Buffalo General Hospital; [5]Novartis; [6]University of Colorado.*

Background: The Mycophenolic Acid Observational Renal Transplant (MORE) Registry is a prospective, observational study of de novo renal transplant recipients (RTRs) receiving mycophenolic acid (MPA) therapy designed to determine effectiveness, tolerability and safety of enteric-coated mycophenolate sodium (EC-MPS) vs. mycophenolate mofetil (MMF)-based regimens based on local practice at 40 US sites.

Methods: Outcomes analyzed included: graft survival (GS), patient survival (PS), first biopsy-proven acute rejection (BPAR), mean serum creatinine (SCr), adverse event (AE) rates, and percentages of RTRs maintained on full MPA dose (1.44/2.0 g/day, EC-MPS or MMF respectively). Preliminary data from 901 patients (age 51.5, 64% male, 43% living donor transplant recipients) receiving tacrolimus were analyzed.

Results: Interim results at 1, 3, 6 and 12 months (613 EC-MPS/288 MMF patients) showed that more EC-MPS patients were maintained on full dose MPA (ECMPS/MMF: 79.2/71.2%, p=0.01; 68.6/55.6%, p<0.01; 52.7/43.0%, p=0.02; 47.3/39.4%, p=0.08). Actual mean MPA doses (SD) standardized to MMF dosing for EC-MPS/MMF were: 1853 (378)/1789 (441) mg, p=0.04; 1753 (465)/1648 (493) mg, p<0.01; 1590 (521)/1494 (529) mg, p=0.03; and 1532 (511)/1453 (522) mg, p=0.08 at months 1, 3, 6 and 12 posttransplant. Comparable 12-month clinical outcomes were achieved for effectiveness, tolerability and safety. There were no significant differences between EC-MPS and MMF treated RTRs in GS (96.9/97.6%, p=0.48), PS (99.3/98.4%, p=0.31), BPAR (9.6/8.0%, p=0.30), mean SCr (1.49/1.49 mg/dL, p=0.97) or reported incidence of AEs by organ system, infections or neoplasia.

Conclusions: The majority of renal transplant recipients who received tacrolimus as maintenance immunosuppression are maintained on full doses of MPA early posttransplant. Early significant dosing differences are seen between EC-MPS and MMF which may impact outcomes at later time points in this study.

Funding: Pharmaceutical Company Support

TH-PO925

Projection of Kidney Graft Survival Probabilities: An Evaluation of Methodologies Jiannong Liu, Yi Peng, Jon J. Snyder, Nicholas J. Salkowski, Ajay K. Israni, Kenneth E. Lamb, Bertram L. Kasiske. *SRTR, MMRF, Minneapolis, MN.*

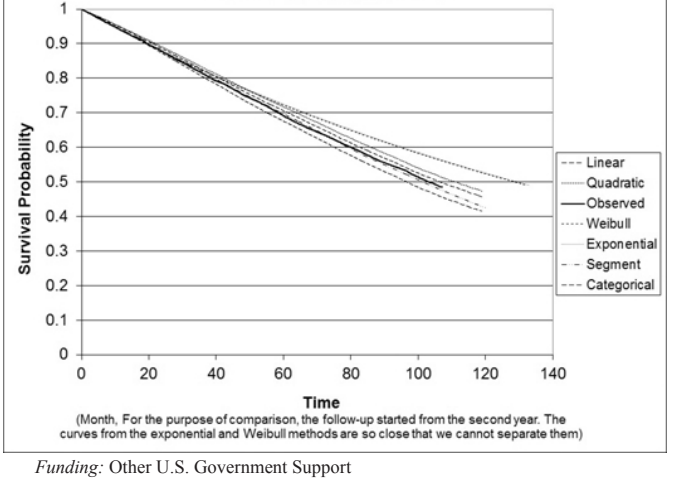
Background: Projected half-life is used to monitor post transplant patient and graft survival. A common method for projection, the exponential method, uses failure/death rate after the 1st year, assumes it is constant over time, and extrapolates it to the time when 50% of patients would be expected to lose the graft or die. Using the Scientific Registry of Transplant Recipients data, we evaluated this method and compared it with others.

Methods: Adult, deceased donor, 1st kidney transplant recipients in the US, 1991-2000, were included in the analysis. The outcome was all-cause graft failure. Observed and projected graft survivals (GSs) were calculated for the 2000 cohort. Patients were followed until the end of 2009 for observed GS. Data through 2002 were used to project GS. Methods for projecting GS included (1) exponential method, (2) Weibull method, (3) combining the 1991 cohort's GS (through 2002) and the temporal trend estimated from the 1991-2000 cohorts (using linear, quadratic, and categorical cohort year separately), and (4) segment method using the second-year death rate of the 2000 cohort, the third-year death rate of the 1999 cohort, and so on, to construct a piecewise survival curve for the 2000 cohort.

Results: Figure 1 displays the observed and projected GSs. For short-term projection, all methods work well. Long-term, the exponential and Weibull methods overestimate survival probability substantially; the segment and linear methods give the best estimates. For estimates of half-lives, the exponential and Weibull methods overestimated up to 32 months; estimate errors from the other methods were within 6 months and less than 2 months for the segment method.

Conclusions: The exponential method, currently widely used, can overestimate survival probability/half-life substantially; the segment and linear methods provide better estimates.

Figure 1. Projected and Observed Post Transplant Survival, 2000 Kidney Transplant Cohort



(Month, For the purpose of comparison, the follow-up started from the second year. The curves from the exponential and Weibull methods are so close that we cannot separate them)

Funding: Other U.S. Government Support

TH-PO926

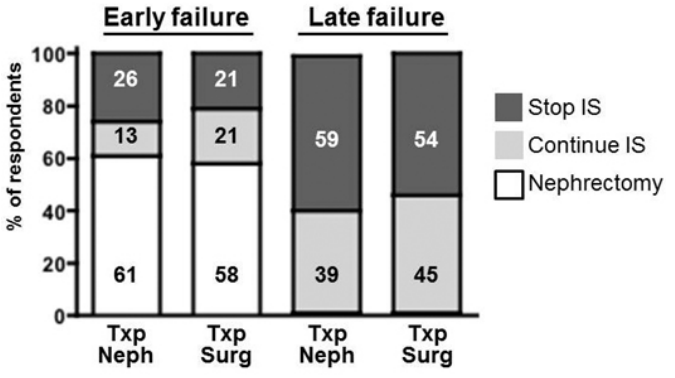
Time-to-Failure after Initial Transplantation Dictates Care for Repeat Kidney Transplantation Candidates Rowena B. Delos Santos,[1] Jordana Gaumond,[2] Roger Yuh,[1] Jennifer W. Leach,[1] Jagdeep Obhrai,[1] John M. Barry,[3] Mitchell Henry.[4] *[1]Dept Medicine, Division of Nephrology, Section of Transplant Medicine, OHSU, Portland, OR; [2]Dept Surgery, Portland VA Medical Center, Portland, OR; [3]Dept Surgery, Divisions of Urology and Abdominal Organ Transplantation, OHSU, Portland, OR; [4]Dept Surgery, Division of Transplantation, Ohio State University, Columbus, OH.*

Background: One in six patients on the waiting list for kidney transplantation in the US has received a prior kidney transplant. There are 3 strategies for managing asymptomatic patients with failed kidney transplants: elective transplant nephrectomy (N-TXP), discontinuance of immunosuppression (IS), and IS without N-TXP until the next transplant. We surveyed transplant nephrologists and surgeons to examine practice patterns or management of repeat transplant candidates.

Methods: We developed and validated internet based survey and sent it to transplant nephrologists and surgeons at all active US adult kidney transplant centers.

Results:

Figure 1 Management of Early and Late Allograft Failure for Transplant Nephrologists and Surgeons



More than 60% of centers responded. Management strategy depended on time of graft loss (< 6 months post-transplant or >10 years post-transplant). For early graft loss, most transplant nephrologists and surgeons chose N-TXP or discontinuance of IS. For late graft loss, most transplant nephrologists and surgeons chose discontinuing IS or IS without N-TXP. Surprisingly, neither patient-specific factors (co-morbidities or HLA-antigen sensitization), nor center-related factors (center size, academic affiliation, and location) influenced management. There was significant intra-center variability in management.

Conclusions: Time to graft failure was the primary factor for management of candidates awaiting a second kidney transplant. Provider-specific factors, rather than patient- or center-specific factors determined care strategy.

Funding: Private Foundation Support

TH-PO927

Urinary NGAL in Early Post-Transplant Period Predicts Long-Term Graft Function after Renal Transplant Hye Min Choi, Sung Yoon Lim, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Nephrology, Korea University Hospital, Seoul, Republic of Korea.*

Background: Graft function in early post-transplant period is known to affect long-term graft outcome, and several recent reports demonstrated the usefulness of urinary NGAL or IL-18 in predicting delayed graft function (DGF) with subsequent poorer graft outcome. However, the usefulness of urinary biomarkers in transplant patients with immediate graft function (IGF) or slow graft function (SGF) for predicting long-term graft outcome has never been assessed.

Methods: This was a single center, prospective observational cohort study of renal transplant patients (n=60, 33 deceased donor, 27 living donor). Only patients with IGF (n=43) or SGF (n=14) were enrolled to test whether urinary NGAL or liver type fatty acid binding protein (L-FABP) might be useful as biomarkers for predicting long-term graft outcome. Urine samples obtained at 0 hr, day 2 and day 6 were used for measurement of NGAL, L-FABP by ELISA. We examined long-term graft outcome by eGFR at 1yr after transplant.

Results: NGAL level on day 2, but not L-FABP or serum creatinine, was significantly higher in the SGF compared to IGF group. When patients were divided into 2 groups according to NGAL level on day 2, patients with higher NGAL level on day 2 were associated with significantly lower 1yr eGFR. In multivariate logistic regression analysis, day 2 urine NGAL was a significant, independent factor for predicting poor long-term outcome (1yr eGFR<55ml/min/1.73m^2). ROC curve analysis showed the ability of urine NGAL on day 2 for predicting lower 1yr eGFR was moderately accurate (AUC 0.742, cut-off value 86.7ng/ml with sensitivity 83.3% and specificity 67.0%).

Conclusions: We conclude that urinary NGAL at early post-transplant period is a useful predictor of long-term graft function even in patients with relatively good early graft function.

TH-PO928

Progression of Aortic Calcifications and Their Impact on Renal Function after Kidney Transplantation Carlo Maria Alfieri, Maria Daniela Croci, Brigida Brezzi, Francesco Barretta, Maria Teresa Gandolfo, Maria Meneghini, Manuela Curreri, Maria Pia Rastaldi, Piergiorgio Messa. *Nephrology and Dialysis, IRCCS Fondazione Ca' Granda Ospedale Policlinico, Milan, Italy.*

Background: Vascular calcifications (VCs) progress even after a well functioning kidney transplant (KTx).The main related factors and which impact, if any, VCs could have on the graft outcome is still unclear. The aims of our study were to evaluate: a) the factors associated with VC progression in the first year after KTx; b) VC impact on graft outcome over a 2-year follow-up period.

Methods: Abdominal aortic calcification index (ACI), FGF23, OPG, Fetuin, mineral metabolism (MM) parameters, were evaluated in 95 KTx pts (transplanted between 2006 -2008) at the 1st and at 12th months after KTx. All patients were followed-up over a 2-year period. Statistics: univariate and multivariate analyses (values: mean ± sd).

Results: In the overall cohort, mean ACI increased from 4.85 ± 5.84 (1st mo) to 5.23 ± 6.12 (12th mo) (p = 0.01). However, ACI increased (8.08±5.86 à10.25±5.60; p=0.0001) in 25% (Progr +), while it remained stable or improved (3.76±5.45 à 3.53±5.33; p ns) in 75% pts (Progr-). Progr + Pts were older (53±9 vs 46±11 yrs, p=0.01), had lower eGFR (MDRD: 50±15 vs 58±16 ml/min, p = 0.03), higher Ca (10.4±0.76 vs 9.93±0.65 mg/dl, p=0.002) and OPG (6±1.37 vs 4.8±1.55, p 0.002) levels at the 12th month, without any other significant differenc, including cumulative steroid doses.

In the Logistic RA, only serum Ca (p=0.004, OR 3.56) and OPG (p=0.01, OR 1.75) were significantly and positively associated with ACI progression. Over the 2-year follow-up, 3 patients died (2 Progr + , 1 Progr-) and 1 pt (Progr -) restarted dialysis. At the end of follow-up no significant difference was found in blood pressure control, in eGFR and urinary protein between the 2 groups

Conclusions: VCs progress even in the early post-KTx period. Higher levels of Ca and OPG seem to be associated with an increase in VCs . However, no major impact of VC progression on graft function was evident, at least over a 2-year follow-up. Studies on larger cohorts and over a longer period are needed for a definitve conclusion on this issue.

TH-PO929

The Burden of Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD) in Successful Renal Transplant Recipients: The ABC-Heart Study Sinead Kinsella, Joseph A. Eustace. *Department of Renal Medicine, Cork University Hospital, Cork, Ireland.*

Background: The burden of Chronic Kidney Disease Mineral Bone Disorder (CKD-MBD) in successful kidney transplant recipients is poorly defined.

Methods: We conducted a prospective observational study to quantify the presence and severity of CKD-MBD in first renal allograft recipients (eGFR > 30ml/min/1.73m^2). Patients underwent routine lab tests, iPTH, 25 OH Vitamin D, lumbar X-ray, DXA scan (Lunar IDXA), qCT of lumbar spine, pulse wave velocity measurement and measures of bone turnover (serum BAP and TRAC5B, urine NTx).

Results: 89 patients with mean age of 46.8 yrs, median dialysis and transplant vintage of 2.3 and 2.6 yrs were studied. Mean eGFR and iPTH was 54ml/min/1.73m^2 and 100ng/ml. 82% had iPTH > 65ng/ml, which was higher at lower eGFR. iPTH levels were similarly high in 8 pts with history of parathyroidectomy. iPTH correlated with total serum calcium (r=0.35, p<0.001) and phosphate (r= -0.43, p<0.001). The mean 25-OH Vitamin D level was 42nmol/l, 71% were Vitamin D deficient (<50nmol/l). Excluding 5 active vitamin D treated subjects, 25 OH Vitamin D was significantly associated with iPTH in normocalcemic but not in hypercalcemic subjects. 38% were hypercalcemic; spot urine calcium creatinine ratio (mean 0.16 mmol/mmol) was not associated with serum calcium but was increased in a dose dependant fashion in 9 subjects on cinacalcet. 22/69 pts had a venous pH <7.33. 25% of patients had osteoporosis, iPTH significantly correlated with bone density using DXA, especially at radial site, and with qCT at lumbar cortical sites. Fourteen patients suffered a post transplant low impact peripheral fracture. Forty-eight percent of subjects had evidence of aortic calcification which was higher in those with past cardiovascular history. Overall 97% had at least one element of CKD-MBD present.

Conclusions: The burden of CKD-MBD in successful renal allograft recipients is substantial and may contribute to their long term morbidity.

TH-PO930

Cholecalciferol Supplementation Reduces Proteinuria after Kidney Transplantation Ines Aires, Manuel A. Ferreira, Fernando Barbosa Nolasco. *Nephrology, Hospital Curry Cabral, Lisbon, Portugal.*

Background: Reduced native vitamin D (25-OH vit.D) levels are frequently found in chronic renal failure patients (pts.), but less is known in kidney transplant (KTx) population.

Methods: We prospectively evaluated the effects of 6 month cholecalciferol supplementation, in 124 KTx pts, 84 men, mean age 53.4±14.1 years, 29 diabetic, with a mean post transplant follow up of 79.3±63 months. All pts were naïve to 25-OH vit.D therapy. Immunosuppression regimen was tacrolimus in 74% and sirolimus in 21% pts.

Pts. were supplemented accordingly to basal (T0) calcidiol serum levels (ng/mL): deficiency (<15), insufficiency (15 to 30) and normal (>30).

Wilcoxon paired and Anova tests were used.

Results: At T0, 25-OH vit.D levels were 14.6±7.8 ng/mL. 58.9% pts had deficiency and 30.6% had 25-OH vit.D insufficiency. Mean plasma creatinine was 1.44±0.6 mg/dL.

At 6 months (T6), 64 pts. completed daily 25-OH vit.D supplementation (median dose 2664 UI). Mean calcidiol serum levels increased to 30.5±12.3 ($p< 0.001$). Only 6.2% remained in deficiency, and 46.9 % pts achieved normal serum levels (vs 10.5% at T0).

Proteinuria was significantly reduced from 0.931±1.48 g/dL (T0) to 0.685±0.66 g/dL (T6), p=0.03.After supplementation proteinuria levels were inversely correlated with 25-OH vit.D levels (r=-0.30: p=0.01).

Intact PTH decreased from 146.2±130 pg/mL (T0) to 118±112 pg/mL (T6), p=NS. No differences were observed in serum calcium, phosphorous and plasma creatinine. 25-OH vit.D supplementation was well tolerated, and no adverse events were reported.

Conclusions: Accordingly to our results, native vitamin D deficiency is highly prevalent among KTx pts. Oral supplementation with cholecalciferol is efficient, cheap and safe in the correction of calcidiol serum levels and leads to significant reduction in proteinuria.

Larger and longer randomized controlled studies are needed to confirm these relevant protective effects of cholecalciferol in kidney allograft survival.

TH-PO931

Should We Maintain Cinacalcet after Kidney Transplantation in Patients Receiving It on Dialysis Raphael Pereira Paschoalin, Jose-Vicente Torregrosa, Xoana Barros Freiria, Carlos Durán Rebolledo, Josep Maria Campistol Plana. *Nephrology Department, Hospital Clinic, Barcelona, Spain.*

Background: Nowadays, the treatment of secondary hyperparathyroidism (SHPT) with cinacalcet in patients that are on waiting list for kidney transplant (KT) is habitual. However, it is not clear if the treatment should be maintained after KT, and if so, which factors may define it.

The aim of this study was to evaluate the follow-up of KT recipients who discontinued cinacalcet just before the transplant.

Methods: Single center retrospective observational study. Enrollment began in June 2005 and ended in December 2010. 114 patients (75 men) were engaged. They were receiving cinacalcet on dialysis before KT. Median age: 51.3 years (22.5 to 78.5). The time on dialysis was 8.6± 8.4 years. All of them had three months follow-up and 92 completed one year of follow-up after KT. Criteria to reintroduce cinacalcet was serum calcium ≥ 10.5 mg/dL. We assess the factors involved in the reintroduction of cinacalcet after transplant (age, gender, time on dialysis, cinacalcet dose before KT, Ca / P / alkaline phosphatases / PTH at surgery, immunosuppression and renal function after KT. After KT biochemical parameters were measured at day 7, 15, 30, 60, 90, 180 and 365. Statistical analysis was made with SPSS-15.

Results: At three months 14 patients needed reintroduction of Cinacalcet and at the end of first post KT year, 20 patients (21.7%; group 1) were on cinacalcet treatment. Ninety-four patients did not need Cinacalcet reintroduction (group 2). Time on dialysis (131 ± 94 months and 97 ± 102 months; $p = 0.0267$) and the dose of cinacalcet before the KT ≥ 60mg per day (45 [30-120] y 30 [30-60] mg per day; p=0.039) were the only significant factors related to the reintroduction of cinacalcet.

Conclusions: The dose of cinacalcet before the transplant and the time on dialysis seems to be the most important factors related to the reintroduction of the drug during the first year after KT.

TH-PO932

Periodic Limb Movements in Sleep Are Associated with Serum Parathyroid Hormone in Kidney Transplant Recipients Zoltán Kiss,[1] Anett Lindner,[2] Rezso Zoller,[3] Katalin Fornadi,[2,3] Alpar S. Lazar,[3,4] Maria Eszter Czira,[3] Andrea Dunai,[3] Orsolya Agnes Veber,[3] András Szentkirályi,[3,5] Marta Novak,[3,6] Miklos Z. Molnar,[7,8] Istvan Mucsi.[3,8,9] *[1]Medical Affairs, Amgen Limited, Budapest, Hungary; [2]Dept. of Neurology, Semmelweis University, Budapest, Hungary; [3]Institute of Behavioural Science, Semmelweis University, Budapest, Hungary; [4]Surrey Sleep Research Centre, Faculty of Health and Medicinal Science, University of Surrey, Guilford, United Kingdom; [5]Institute of Epidemiology and Social Medicine, University of Muenster, Germany; [6]Dept. of Psychiatry, University Health Network, University of Toronto, Canada; [7]Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; [8]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [9]Dept. of Medicine, Division of Nephrology, McGill University Health Centre, Montreal, QC, Canada.*

Background: Several reports suggested an association between Restless Legs Syndrome (RLS) versus serum iPTH concentration. No data has been published about iPTH and Periodic Limb Movements in Sleep (PLMS). We tested the hypothesis whether serum iPTH is associated with PLMS in kidney transplant recipients.

Methods: Data were obtained from 100 stable, prevalent kidney transplant recipients (Tx) (randomly selected from more than 1200 Tx patients followed at one outpatient transplant clinic) in a cross-sectional survey. The patients underwent a standard, one-night supervised polysomnography.

Results: The prevalence of PLMS (defined as PLMS-index > 5/hour) was 52% and prevalence of severe PLMS (PLMS-index > 25/hour) was 16% . Patients with severe PLMS had higher iPTH (median [IQR} 104[134] vs 59[53] pg/ml). The PLMS-index was significantly and positively correlated with serum iPTH (rho 0.327, p=0.019). This association remained significant after adjusting for age, gender, eGFR and co-morbidity.

Conclusions: In our research, severe PLMS was associated significantly with higher iPTH level and the PLMS-index was positively correlated with serum iPTH level even after statistical adjustment for several covariables.

Funding: Government Support - Non-U.S.

TH-PO933

Vitamin D Therapy Post Renal Transplantation and a Potential Pothole Gauri Bhutani,[1] Horace J. Spencer,[3] Sundararaman Swaminathan.[2] *[1]Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; [2]Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR; [3]Biostatistics, College of Public Health, UAMS, Little Rock, AE.*

Background: 1,25(OH)2 Vitamin D3 has been shown to directly decrease IFN-y production in T cells by its action on two IFN-y promoter elements. Impaired viral specific immune response has been noted in patients developing reactivation of the BK virus. CD 4+ T cell related IFN production is thought to be the key factor in keeping this virus in check.

Methods: A case-control study was undertaken with an adult post renal transplant patient population. All patients were transplanted at our center between 2007 to 2009 (n=180). Cases included patients with new reactivation of BK virus post renal transplantation - patients with viruria, viremia or nephropathy (cases, n=29). Controls were patients with no known reactivation of the BK virus (controls, n=151). Percentage of Calcitriol and Ergocalciferol use was compared between cases and controls. Chi square and T test analysis were amongst the methods used to evaluate the statistical significance of the difference in percentage of Vitamin D use.

Results: Vitamin D use (Calcitriol and/or Ergocalciferol) was 63.35% in all patients with at least one year follow up (n = 131). No significant difference was noted in the 25 (OH) Vitamin D3 levels between cases (18.75) and controls (19.66), $p = 0.733$. Cases were found to have a higher percentage of Vitamin D use. The difference in percentage of use between cases and controls with at least one year follow up (n = 102) was, however, not statistically significant. This was consistent for Calcitriol use alone (27.59 vs 19.06%, $p = 0.736$), Ergocalciferol use alone (24.14 vs 15.02%, $p = 0.358$) and Calcitriol and/or Ergocalciferol use (68.97 vs 59.80%, $p = 0.397$). No significant difference was also noted in BK virus reactivation between patients on vitamin D (n= 102) and patients not on vitamin D (n = 80), 20.69% vs 12.5% (p= 0.169).

Conclusions: Our study indicates likely safety of vitamin D use in renal transplant patients in relation to reactivation of latent BK virus and perhaps other similar viral infections as well.

TH-PO934

Comparison of Estimated Glomerular Filtration Rate (eGFR) Calculations to Measured GFR in Kidney Transplant Recipients V. Ram Peddi, Maria A. Miguel, Kimi Ueda Stevenson. *California Pacific Medical Center, San Francisco, CA.*

Background: No clear consensus exists regarding the most accurate calculation to estimate GFR in renal transplant recipients (RTRs). The two most frequently used equations are the Modification of Diet in Renal Disease (MDRD) and Cockroft-Gault equations. However, neither of these has demonstrated consistency or accuracy in RTRs and each calculation has limitations that preclude their use.

Methods: Radionuclear GFR was performed using radiopharmaceutical I-125 agent (Glofil®) in 13 stable RTRs between 3 and 12 months post-transplantation. Additionally, blood draws for BUN, creatinine, and albumin were obtained at the same time as the radionuclear GFR for use in the various eGFR equations: Cockroft-Gault, MDRD (Levey), abbreviated MDRD, CKD-EPI, Nankivell and Jelliffe. Statistical comparisons were made between the measured radionuclear GFR and the eGFR calculations.

Results: The mean age was 55y. 6 of the 13 RTRs (46%) were ≥ 60 years. 9 (69.2%) were male. One was African American. 25 radionuclear GFR measurements were available for analysis. 12 pts had 2 measurements each and 1 pt had 1 measurement. 6 measurements (in 3 patients) corresponded with Chronic Kidney Disease (CKD) Stage III (CrCl 30-59ml/min); 15 corresponded with CKD Stage II (60-90ml/min); and 4 corresponded with CKD Stage I (>90ml/min).

	Mean	Median	Standard Deviation	Standard Error	p value*
Radionuclear study	75.1	77	21.3	4.3	-
Cockroft-Gault IBW**	105.8	109.2	42.0	8.4	<0.01
Cockroft-Gault ABW***	135.2	127.7	56.3	11.3	<0.001
Abbreviated MDRD	56.8	58.1	15.2	3.0	<0.001
MDRD	56.2	54.6	15.1	3.0	<0.001
CKD-EPI	57.3	56.6	16.3	3.3	<0.001
Nankivell	72.6	74.9	13.5	2.7	NS
Jelliffe	74.3	77.5	11.1	2.2	NS

*paired t-test; **IBW-ideal body weight; ***ABW-absolute body weight

Conclusions: The Nankivell and Jelliffe equations for estimating GFR produced values that were closest to the measured GFR in stable RTRs. The Cockroft-Gault equation tended to overestimate whereas both MDRD equations and CKD-EPI underestimated the GFR. This demonstrates that the most frequently used equations in RTRs (MDRD and Cockroft-Gault) may not be the most ideal for use in drug dosing in kidney transplant recipients.

TH-PO935

Renal Function Is Associated with Red Cell Distribution Width Independent of Iron Deficiency and Nutritional Status in Kidney Transplant Recipients

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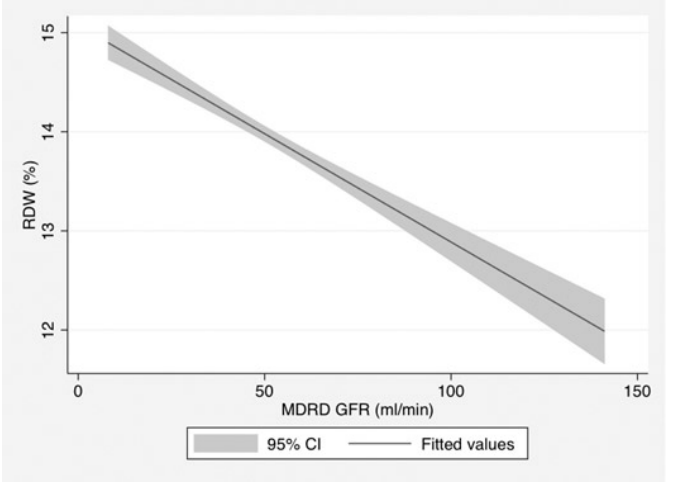
Background: Red cell distribution width (RDW) is a measure of heterogeneity in the size of circulating erythrocytes, which reflects iron deficiency, inflammation and nutritional status, predicts mortality in various patient populations. Impaired renal function is also a predictor of mortality and is associated with inflammation and protein-energy wasting. We assess if renal function is associated with RDW independent of iron deficiency, inflammation and nutritional status in stable kidney transplant (KT) recipients.

Methods: We examined the association of RDW with eGFR in a cohort of 807 prevalent KT recipients not receiving erythropoietin stimulating agents. Associations were examined in regression models adjusted for age, sex, Charlson Comorbidity Index, blood hemoglobin, percentage of hypochromic reticulocytes, soluble transferrin receptor, serum ferritin and transferrin, serum CRP and albumin, PO4 and iPTH and the use of immune suppressants.

Results: Lower eGFR was associated with significantly higher RDW (r=-0.399, -<0.001).



This association remained highly significant even after multivariable adjustments (beta=-0.178, p<0.001). The results were consistent in subgroups of patients with different levels of kidney function and various sensitivity analyses.

Conclusions: Lower eGFR is associated with higher RDW, a predictor of mortality, independent of comorbidity, iron deficiency, inflammation and nutritional status in kidney transplant recipients. Further studies need to determine the mechanisms linking impaired renal function to increased RDW and to increased mortality.

Funding: Government Support - Non-U.S.

TH-PO936

Estimating Glomerular Filtration Rate in Renal Transplantation: A Comparison between Serum Creatinine and Cystatin C-Based Equations

Jorge Malheiro, Isabel Fonseca, Manuela Almeida, La Salete Martins, Antonio Andresen Henriques, António Manuel Nunes Cabrita. *Nephrology Unit, CH Porto, Porto, Portugal.*

Background: Accurate monitoring of estimated Glomerular Filtration Rate (eGFR) is essential for an optimal management of kidney transplant (KT) patients. eGFR is often calculated using creatinine-based (SCRb) equations. Alternatively, cystatin C-based (CYSb) eGFR equations have been developed, but their exactness is still uncertain.

Methods: Creatinine Clearance (CrCl) in a 24-hours urine collection was the reference test. eGFR was calculated using CYSb equations (Le Bricon, Stevens) and SCRb equations [Cockcroft-Gault (CG), abbreviated MDRD (aMDRD)]. After evaluating randomly selected results of 215 KT patients with stable renal function and over 1 year post-transplant (PT), 173 were included. We excluded patients in the first and tenth deciles (in each gender) of 24-hours urine creatinine excretion divided by weight to deal with inaccurate urine collections. Bias, precision and accuracy of each equation were determined. Kappa statistics evaluated the concordance between the reference test and eGFR formulas in categorizing patients' renal function (positive test: <60 ml/min).

Results: Patients (108 males) had a mean age of 48.6 years and a median PT time of 6.8 years. Mean CrCl was 69.3 (range: 32 – 105) ml/min. The CYSb equations estimates (Le Bricon, Stevens) had the highest accuracy (83.8% and 87.9% within 30% of CrCl result, respectively). Le Bricon, Stevens and aMDRD precision was similar (13.5 ml/min) and much better than CG (22.5 ml/min). The lowest bias was seen in Le Bricon (-1.2 ml/min), followed by CG, Stevens and aMDRD (-2.6, -9.5, -16.5 ml/min, respectively). Kappa coefficient was higher in CYSb equations (0.53) in contrast with CG (0.48) and aMDRD (0.40). Stevens had a high sensitivity (90.8%) and low specificity (66.7%) and, conversely, Le Bricon had 64.6% sensitivity and 87.0% specificity for identifying patients with CrCl <60 ml/min.

Conclusions: CYSb equations showed the best accuracy and a low bias. Stevens performed better than Le Bricon in identifying patients with a lower CrCl. The inverse was observed in patients with mild or no kidney graft dysfunction.

TH-PO937

Renal Transplant Recipients Are at High Risk To Develop Precancerous Lesions of the Cervix

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Background: Immunosuppressive regimens improve survival of renal transplant recipients (RTR);however, a long-term complication is increased risk of developing cancer. Human papillomavirus (HPV) associated cancers of the genital tract are of particular interest, since they are sexually transmitted and in theory preventable. Cervical cancer rates are high in RTR but the data on the natural history of HPV infection in RTR are limited. Therefore, we investigate HPV and the development of cervical neoplasia.

Methods: A review was conducted in Medline database for articles in English investigating cervical HPV infection in RTR. Eligible studies included those that examined HPV infection by PCR testing and/or obatined cervical biopsies. Three reviewers extracted the data and compared rates to the general population.

Results: Out of 157 relevant citations, 12 met our inclusion criteria: 4 prospective studies included 231 RTR, 26.9% became HPV (+) and 13.2% that went for biopsy had a severe dysplasia. Six cross-sectional studies examined 187 RTR, 21.9% were HPV(+) and 16.7% had a high grade lesion. Two retrospective studies included 276 patients, only 25 RTR were sent for biopsy with 32% having severe dysplasia. The average duration of immunosuppression was 3.21 years to diagnose HPV or dysplasia.

Type of Study	No. of Studies	Duration of Immuno Suppression (yrs)	Frequency of HPV DNA (+) (PCR)	Frequency of Cervical Abnormalities among Patients sent for Biopsy	
				HPV or Mild Dysplasia	High Grade Dysplasia
Prospective Studies Total RTR=231	4	3.08	29.4% (68/231)	71.43% (30/42)	13.2% (7/53)
Cross-sectional studies Total RTR= 187	6	3.95	21.9% (41/187)	47.4% (54/114)	16.7% (17/102)
Retrospective Total RTR=276	2	2.6	Not Done	60% (15/25)	32% (8/25)
General Population Estimates (Peak Incidence) Am J Ob Gyn 2004;191: 105	N/A	N/A	10-33%	0.5%	0.8%

Conclusions: Studies on HPV and premalignant lesions of the cervix in RTR are limited, however, in our review we examined a large cohort. At about 3.1 years of immunosuppression 20-30% of RTR were HPV (+), rates similar to the general population. However, 13-32% of RTR biopsied had high grade dysplasias, while the general population rate is only 0.8%. Cervical cancer screening and prevention is important in RTR. Counseling should be aimed at limiting risky sexual behaviors post transplantation

TH-PO938

HLA-DR4 Protects Transplant Recipients from Cancer Mortality

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Background: Long term immunosuppression is associated with increased risk of malignancy. This is due in part to impaired immune surveillance and attenuation of the host response to certain oncogenic viruses. HLA DR4 is a key molecule in the presentation of tumour and viral antigens which trigger the development of activated T lymphocytes with potent anti-tumour action.

Methods: We investigated whether the presence of HLA-DR4 in renal transplant recipients conferred a survival benefit by reducing death from malignancy.

All patients receiving first deceased donor kidney transplants in Belfast between May 1986 and April 2005 had their clinical data recorded prospectively. Calcineurin inhibitors were introduced as part of the immunosuppressive regimen in 1989 and prior to this, all patients received dual immunosuppression with corticosteroids and azathioprine. All 707 recipients had their serologically defined HLA DR status recorded.

Survival analysis was performed using Kaplan-Meier and Cox-Regression plots.

Results: Renal transplant recipients with HLA DR4 were significantly less likely to die from malignant causes than those without a DR4 antigen (p = 0.037). This relationship persisted after correction for transplant recipient age.

There was no difference in overall mortality or death from infective causes between the two groups.

Conclusions: This study suggests that possession of an HLA-DR4 antigen provides significant protection from death due to malignant disease in renal transplant recipients. If this finding is confirmed in replication studies, then the presence or absence of a recipient HLA-DR4 antigen could help nephrologists stratify patients for future risk of cancer death and influence the choice of immunosuppression regimens.

TH-PO939

Ionizing Radiation Exposure from Medical Imaging in Renal Transplant Recipients Kim N. Nguyen, Francis L. Weng, Anup M. Patel. *Renal and Pancreas Transplant Division, Saint Barnabas Medical Center, Livingston, NJ.*

Background: Ionizing radiation and immunosuppression are risk factors for malignancies. Occupational radiation exposure is limited to 100 millisieverts (mSv) every 5 years and 50mSv in any single year. We analyzed the radiation exposure from medical imaging in renal transplant recipients (who have a higher risk of malignancy) from the time of transplant evaluation up to 3 months post-transplant.

Methods: We retrospectively analyzed 172 patients who received their first renal transplant during 2008. We determined types and numbers of medical imaging procedures from initial evaluation to three months post-transplant. For pre-transplant exposure, only imaging required for placement and maintenance on the transplant list were included. Estimates of radiation exposure for each procedure type were obtained from published literature.

Results:

mSv	Pre-Transplant	Post-Transplant	Total (n=172)
0-20	78 (45.3%)	110 (63.9%)	45 (26.1%)
>20-50	50 (29.1%)	44 (25.6%)	50 (29.1%)
>50-100	35 (20.4%)	16 (9.3%)	50 (29.1%)
>100	9 (5.2%)	2 (1.2%)	27 (15.7%)

An annual exposure (mSv/year) of 0-3, >3-20, >20-50, >50-100, >100, occurred in 10 (5.8%), 75 (43.6%), 65 (37.8%), 17 (9.9%), and 5 (2.9%) patients, respectively.

Multivariate Analysis of Factors Associated with Radiation Exposure >50mSv

		OR	95% CI	P value
Black race (vs. non-Black)		1.9	0.9-4.2	0.09
DGF (vs. no DGF)		3.6	1.3-10.3	0.01
Charlson Co-Morbidity Index (vs. 2-3)				0.01
	4-5	2.9	1.2-6.7	
	6-8	3.8	1.3-11.0	
Total Years of Follow-up (vs. </=1 year)				<0.001
	>1 to 2 years	1.6	0.5-4.7	
	>2 to 4 years	4.7	1.6-13.7	
	>4 years	11.2	4.1-30.9	

The tests responsible for the most exposure were nuclear stress tests (50.5% of total mSv) and CT scans of the abdomen/pelvis (22.7%).

Conclusions: Renal transplant recipients are exposed to significant amounts of ionizing radiation from medical imaging during their pre-transplant work-up and early post-transplant care. Strategies to reduce exposure, such as reducing the frequency of screening nuclear stress tests and following established guidelines for performance of CT scanning, should be considered.

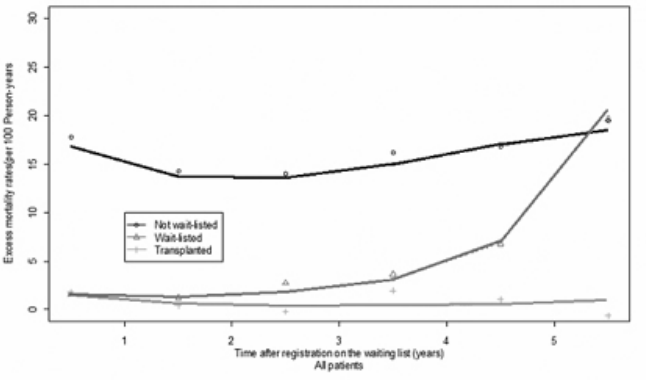
TH-PO940

Should We Change Our Renal Allograft Allocation Policy? Emmanuel Villar,[1] Liacine Bouaoun,[3] Cécile Couchoud,[2] Rene Ecochard.[3] *[1]Nephrology, Hospices Civils de Lyon, Pierre Benite, Rhone, France; [2]Registre REIN, Agence de la Biomedecine, La Plaine St Denis, Seine St Denis, France; [3]Biostatistics, Hospices Civils de Lyon, Lyon, Rhone, France.*

Background: Our aim was to quantify the evolution with time of excess mortality in end stage renal disease (ESRD) patients registered on renal transplant waiting list compared with non-transplant candidates and transplant patients.

Methods: We included all incident ESRD patients from 2002 and 2009 registered in the French REIN Registry. Excess mortality was computed each year after first dialysis in patients at risk stratified as follow: patients registered on renal transplant waitlist and never transplanted over study period (waitlist group), transplanted patients (transplant group), and patients never registered (dialysis group). Excess mortality was computed against French general population using relative survival method. Comparisons were adjusted for patients' characteristics.

Results: The study included 21 079 incident ESRD patients. There was no characteristic difference between waitlist and transplant patients. Dialysis patients were older and had more comorbidities. Variations of excess mortality were:



In waitlist group, excess mortality increased by 45% (p=0.0005) per year. Delay from first dialysis to registration (p=0.0004), age >65 (p=0.008), original nephropathy as diabetes or vascular cause of ESRD (p=0.028) and number of comorbidities (p=0.035) were independent predictors of excess mortality for patients registered on waiting list and not yet transplanted.

Conclusions: Our study quantifies annual increase of excess mortality while waiting for renal transplant. Results raised the question about priority of access to transplant in ESRD patients with comorbidities and/or older, considering as well access equity for all patients in the setting of organ shortage. These urge us to screen early high risk patients for transplantation.

TH-PO941

Listing Pre Dialysis Patients for Transplantation Increases Preemptive and Early Transplantation Post Starting Dialysis Wendy Brown, Damien Ashby, Megan Griffith. *Renal Unit, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Transplantation provides excellent outcomes for patients with ESRD and preemptive transplantation gives better outcomes compared to transplantation after commencement of dialysis. Planned living donor (LD) transplantation has increased rates of preemptive transplantation and UK guidelines recommend that people with advanced CKD (GFR<15mls/min) are activated for transplantation within 6 months of predicted need to start dialysis. We examined the effect of pre dialysis listing on LD and deceased donor (DD) transplantation.

Aim: to investigate the outcome of pre dialysis patients listed for transplantation

Methods: All patients in our low clearance clinics, anticipated to require dialysis in the next 6 months and deemed fit for transplantation were offered work up and listing for transplantation. The outcomes of those patients listed from Jan 2006-April 2011 were analysed

Results: 295 pre dialysis patients were worked up and listed on the national kidney allocation register. Table 1 summarises patient outcomes.

Outcomes	n	%	Median time to outcome in months
LD	66	22.5	2.6 [1.0-6.5]
DD	24	8.1	6.5 [2.6-11.9]
SPK	21	7.1	3.9 [1.6-8.8]
Dialysis	96	32.5	5.4 [3.9-17.9]
RRT not started	88	29.8	

In addition 10 of the patients who started dialysis subsequently received a transplant within 90 days. Therefore 55% (121/217) patients requiring RRT were transplanted before or within the first 90 days of starting dialysis. A small number of patients (3) not transplanted still did not require dialysis at 3 years. 15 patients were suspended due to medical reasons or stabilisation of CKD. 2 patients died.

Conclusions: Pre dialysis workup for renal transplantation successfully enables significant numbers of patients to receive both LD and DD preemptive renal transplants, and facilitates early transplantation post starting dialysis. However, predicting the time until dialysis will be required is difficult in some patients and better measures of this are required to optimize timing of transplantation and fair allocation of a scarce resource.

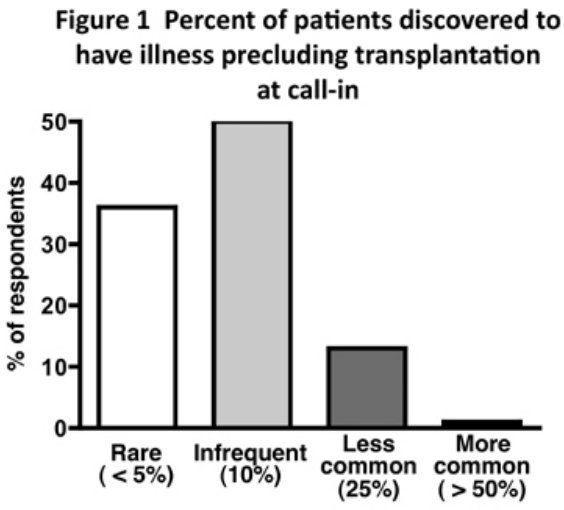
TH-PO942

Managing the Kidney Transplant Waitlist: 10 Years Later, More Patients and More Challenges Rowena B. Delos Santos, Jennifer W. Leach, Roger Yuh, Raghav Wusirika, Jagdeep Obhrai, Douglas J. Norman. *Dept Medicine, Division of Nephrology, Section of Transplant, Oregon Health Science University, Portland, OR.*

Background: The waitlist for kidney transplant (txp) has increased by 40% in the last 10 years. Waitlist management can impact cold-ischemic time at call-in for transplantation and overall cost of txp. However, no guidelines exist for waitlist care and little data exist on current practice.

Methods: To determine how care evolved since the 2002 national survey of waitlist management, we conducted an internet-based survey approximating the questions asked in the first assessment.

Results:



Respondents were asked to indicate what percent of patients called-in for transplant have problems that preclude transplantation.

This data reflects responses from a third of centers in the US. More centers follow patients after listing than in 2002 (96% vs 71%), and 2/3 risk stratify patients for follow-up frequency, up from 1/3. Compared to the previous survey that found that physicians frequently reported interval illness, patients are now more likely to be responsible for illness reporting. This change is associated with poor reporting efficiency at 70% of centers. An important measure of waitlist management efficiency is the detection of illness at call-in that prevents txp. At 64% of centers, >10% of patients called-in for a graft have problems preventing txp, up from 16% of centers. Timely illness reporting is uniformly correlated with dialysis staff involvement in illness reporting and low rates of cancellation of txp for medical illness.

Conclusions: More centers follow patients after listing now than in 2002. Despite this, interval illness is poorly reported and is associated with high rates of detection of illness preventing txp at call-in. Systems that improve timely reporting of illness may decrease cancellation of txp for medical illness discovered at the time of call-in.

Funding: Private Foundation Support

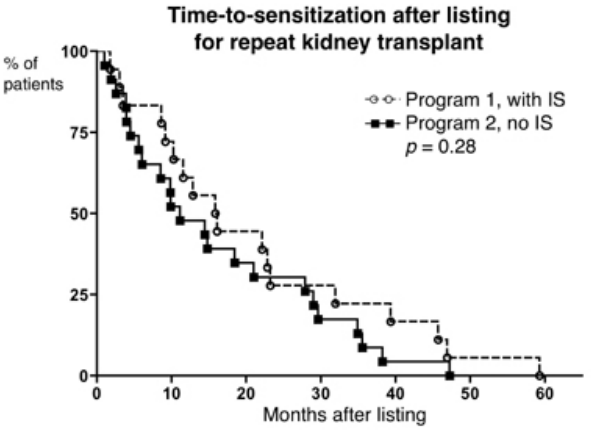
TH-PO943

Comparative Efficacy of Treatment Options for Patients Awaiting Repeat Kidney Transplantation Feroz Aziz, Rowena B. Delos Santos, Jagdeep Obhrai, Douglas J. Norman. *Medicine, Nephrology, Transplant Medicine, Oregon Health & Science University, Portland, OR.*

Background: Care of patients awaiting repeat renal transplantation can be complicated by sensitization stimulated by the failed transplant or by graft intolerance syndrome (GIS, rejection of the failed transplant). Early failure of renal transplants is usually treated either with elective transplant nephrectomy (N-TXP) or stopping immunosuppression (IS) without N-TXP. For late graft failure, most clinicians stop IS or continue some IS until the next transplant.

Methods: To determine whether there was a benefit for elective N-TXP for early transplant failure and IS for late failure, we retrospectively analyzed outcomes for waitlisted patients with a history of a prior transplant at two programs with similar patient populations but different treatment strategies. Program #1 (P#1) did elective N-TXP for all patients with early graft loss (< 2 years) and continued IS (prednisone + antiproliferative drug) for patients with late graft loss (≥ 2 years). Program #2 (P#2) discontinued IS in all patients with a failed graft while they awaited repeat transplantation.

Results: P#1 had 10/11 patients get N-TXP, but most P#2 patients with early graft loss ultimately required N-TXP (8/12). For patients with late graft lost, there was no difference in rates of N-TXP (p=0.12), sensitization (p=0.09), or time-to-sensitization at the 2 centers.



Immunosuppression (IS) does not impact time-to-sensitization for patients awaiting repeat kidney transplantation.

Conclusions: Our data indicate that N-TXP may be inevitable for patients with early transplant failure and support elective N-TXP for these patients. For patients with late graft loss, continuation of IS does not prevent sensitization or the need for N-TXP.

TH-PO944

Outcome after Late Conversion to Sirolimus Depends on Proteinuria and Renal Function Klemens Budde,[1] Wolfgang Arns,[2] Marcel Naik,[2] Fritz Diekmann,[2] Michael Fischereder,[2] Jan Gossmann,[2] Wilfried Gwinner,[2] Nils Heyne,[2] Jan Steffen Jürgensen,[2] Katharina M. Pressmar,[2] Christian Morath,[2] Frank Eitner.[2] *[1]Nephrology, Charité, Berlin, Germany; [2]German Sirolimus Study Group.*

Background: To better define predictors for a favourable outcome after late conversion to sirolimus (SRL)

Methods: We investigated all renal allograft recipients converted to SRL between 11.1.2000 and 12.12.2008 from 10 german transplant (Tx) centers. All data were retrospectively entered into a large national database.

Results: In total 726 pts started SRL after a median of 55 months (Mo) after Tx with a median follow-up of 24 Mo. In 75% it was their first Tx, 10% had received a combined Tx and 33% had experienced at least one rejection episode before conversion. Reasons for conversion included 26.3% CNI toxicity, 22.9% malignancy, and 17.6% chronic allograft nephropathy (CAN).

During the observation period 53 pts died, and 134 pts returned to dialysis. Overall patient and graft survival (including death) at 1, 3 and 5 yrs after conversion was 96%, 90.8%, 82.4% and 86.5%, 71.8%, and 57.7% respectively. In 33% renal function (GFR) improved and in 28% GFR deteriorated after conversion, resulting in overall stable GFR in the first year after conversion (40.4±18.4 before vs. 41.2±20.4ml/min after conversion; p=ns). Pts, who survived with a functioning graft had better GFR (42.8±19.5 vs. 27.7±13.8ml/min; p<0.001) and less frequent proteinuria >400mg/l (39.7% vs. 60.3%; p<0.001) at the time of conversion. Age, gender, time after Tx and previous rejection episodes did not differ between survivors and non-survivors. Pts with proteinuria >400mg/l, poor (<40ml/min) GFR, and CAN had significantly (p<0.001) inferior 5 year graft survival (75.1% vs. 88.9%, 55.0% vs. 75.7% , and 38.9% vs. 62.9%, respectively). Pts with malignancy had better graft survival (89.1% vs 60.7%; p<0,001), while pts with CNI toxicity had similar overall graft survival (64.7% vs. 68.4%; p=ns).

Conclusions: Conversion to SRL is a valid option for patients with malignancy and CNI toxicity, especially for those patients with adequate renal function and no severe proteinuria.

Funding: Pharmaceutical Company Support

TH-PO945

Long-Term Experience with Everolimus in Kidney Transplantation in the United States Diane M. Cibrik,[1] William Irish.[2] *[1]University of Michigan Health System; [2]CTI Clinical Trial and Consulting Services.*

Background: Limited long-term data exist on US kidney transplant patients who have received everolimus (Zortress®) at time of transplantation.

Methods: Using data from the UNOS/OPTN database, we described outcomes among adult patients who received a kidney transplant between 1998 and 2007 and everolimus maintenance immunosuppression at time of discharge. Outcomes included acute rejection (AR), new onset diabetes after transplant (NODAT), primary graft failure and serum creatinine (SCr, mg/dL). We included single organ, first-time transplants between 1998 and 2007 as a reference group. Survival rates and associated 95% confidence intervals (CIs) were estimated using the Life Table method. The complimentary log-log model was used to estimate the rates of AR and NODAT.

Results: A total of 392 patients received everolimus maintenance immunosuppression at time of discharge. Mean age was 47.5 years, 21% were Black and 65% males. Median followup was 3 years. Unadjusted primary graft survival at 3 and 5 years post-transplantation

was 87.2% (95% CI: 82.5-90.7%) and 77.4% (95% CI: 70.8-82.7%), respectively, in the everolimus group and 82.7% (95% CI = 82.4-82.9%) and 72.6% (95% CI = 72.2-72.9%) in the reference group. Primary graft survival rates stratified by living versus deceased donors are provided in Table 1. Cumulative incidence of AR and NODAT at 3-years post-transplant with everolimus were 11% and 8.5%, respectively, and 5% and 9% in the reference group. Mean SCr at 3 years post-transplant was 1.8 with everolimus versus 1.6 in the reference group.

Table 1: Life Table Primary Graft Survival Post-Transplantation

	Everolimus		UNOS/OPTN Reference	
Time Post-Transplant	Living Donor (n=199)	Deceased Donor (n=193)	Living Donor (n=49,066)	Deceased Donor (n=72,199)
1 Year	95.6%±1.5%	92.3%±2.0%	95.1%±0.1%	89.4%±0.1%
3 Years	90.3%±2.6%	87.2%±2.8%	88.5%±0.2%	79.7%±0.2%
5 Years	83.0%±3.9%	72.0%±4.5%	80.3%±0.2%	67.3%±0.2%
Log-rank test	p=0.1033		p<0.0001	

Conclusions: Incidence of AR and NODAT was slightly higher in the everolimus-treated patients. Primary graft survival at 3 and 5 years post-transplantation seem to favor everolimus. This favorable effect was more notable in recipients of deceased donor renal transplants.

Funding: Pharmaceutical Company Support

TH-PO946

Determinants of Successful Use of Sirolimus (SRL) in Renal Transplantation (Tx) Wilfried Gwinner,[1] Wolfgang Arns,[2] Klemens Budde,[3] Fritz Diekmann,[3] Frank Eitner,[4] Michael Fischereder,[5] Jan Gossmann,[6] Nils Heyne,[7] Marcel Naik,[3] Christian Morath,[8] Katharina M. Pressmar,[9] Jan Steffen Jürgensen.[3] *[1]Medical School Hannover, Germany; [2]Klinikum Koeln-Merheim, Germany; [3]Charite, Berlin, Germany; [4]University Aachen, Germany; [5]LMU, Munich, Germany; [6]University Frankfurt, Germany; [7]University Tuebingen, Germany; [8]University of Heidelberg, Germany; [9]University Erlangen-Nuernberg, Germany.*

Background: The German SRL Study Group has established a database among 10 German Tx-centres to better define indications, contraindications, adverse events & outcomes for SRL therapy in renal Tx patients (pts).

Methods: Included are 726 pts with conversion to SRL 3 months post-Tx or later in 2000-2008 (total observation 1582 patient-years, median 22.4 months).

Results: 462 males and 264 females (age 50±13) were put on SRL 80±74 (range 3-343) months post-Tx. SRL was initiated because of malignancies (23%), CNI side effects (26%), chronic allograft nephropathy (18%), creeping creatinine (23%), or a study protocol (11%). S-creatinine at SRL initiation was 2.2±1.0 mg/dl, proteinuria was 349±755 mg/l (range 1-5480). Before, 85% pts had CNI's, after SRL initiation 69%. SRL was terminated in 328 pts (45%), mostly early (1st year: 184; 2nd: 64; 3rd: 36; 4th: 17; 5th: 11). Main causes were renal impairment (52%; GFR decline, gross proteinuria, rejection, return to dialysis), infections & pulmonary complications (20%), musculoskeletal & skin problems (7%), gastrointestinal & metabolic complications (4%), neurological or psychiatric symptoms (1%), disturbed wound healing/before elective surgery (4%), patient's request (18%). Graft function at SRL initiation was slightly better in pts who continued on SRL (41±19 vs. 36±20 ml/min; p<0.001). The major difference between pts with and without continued SRL therapy was the degree of proteinuria at SRL initiation (means: 192 vs. 534 mg/l, medians: 75 vs. 133 mg/l, range 1-2473 vs. 5-5480; p<0.0001). Further, multivariate analysis revealed age, time of SRL initiation after Tx, and cause for SRL initiation as significant variables.

Conclusions: Absence of significant proteinuria is the major determinant for successful conversion to SRL.

Funding: Pharmaceutical Company Support

TH-PO947

Management of Rapamycin-Associated Side Effects in Kidney Transplant Recipients Heloise Cardinal,[1] Raymond Dandavino,[2] Marie-Josee Hebert,[1] Suzon Collette,[2] Catherine Girardin,[1] Anne Boucher.[2] *[1]Nephrology, Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada; [2]Nephrology, Hopital Maisonneuve Rosemont, Montreal, QC, Canada.*

Background: Rapamycin-based immunosuppression may preserve kidney graft function. The side effects of rapamycin can limit its usefulness, but their management and evolution are rarely reported in clinical trials.

Methods: We performed a retrospective cohort study on KTR who received a single kidney graft before December 31st, 2008 and who received rapamycin after the 1st post-transplant month to replace or use in combination with low-dose calcineurin-inhibitors (CNI). We determined the incidence, management and evolution of rapamycin-related side effects.

Results: Amongst the 301 patients studied, new or worsened dyslipidemia occurred in 56%. Decreased rapamycin dose, diet and lipid-lowering drugs led to successful control in 98%. Cytopenia was observed in 40%. Decreased rapamycin dose, supplementing with iron or erythropoietin for anemia, folic acid for leucopenia, as well as the withdrawal of other cytopenic agents resulted in acceptable control in 94%. Peripheral edema was observed in 35%. Although decreased rapamycin dose, salt and water restriction, and diuretics were used, 20% of subjects with peripheral edema had to discontinue rapamycin. New or worsened proteinuria occurred in 26%. Despite decreased rapamycin dose, salt and water restriction, and renin-angiotensin inhibitors, 27% had to discontinue rapamycin. Acne developed or worsened in 25%. Decreased rapamycin or prednisone dose, topical or oral antibiotic therapy resulted in acceptable control in 86%. Rapamycin was discontinued for related side effects in 135 subjects (45%). Increased body mass index (OR per 10 point increase: 1.80, 95% CI 1.09, 2.96) was associated with discontinuation, and there was a trend for older subjects (OR per 10-year increase: 1.19, 95% CI 1.00, 1.43) to be more likely to stop rapamycin.

Conclusions: Successful control of dyslipidemia, cytopenia and acne can usually be achieved without discontinuing rapamycin, whereas proteinuria and edema are harder to manage. Leaner, and perhaps younger patients are less likely to discontinue rapamycin due to side effects.

Funding: Pharmaceutical Company Support

TH-PO948

Race Is Associated with New Onset Hypertension and Diabetes after Living Kidney Donation Brian Boyarsky, Kyle Van Arendonk, Neha Deshpande, Nathan James, Robert Avery Montgomery, Dorry L. Segev. *Johns Hopkins.*

Background: While living kidney donation is generally considered safe, long-term development of co-morbidities is not well characterized. The goals of this study were to quantify new onset hypertension (HTN) and diabetes after living kidney donation and to explore risk factors associated with their development.

Methods: We surveyed 460 patients who donated a kidney at our center between 1984-2011. Median follow-up time was 4.4 years (IQR 2.1-7.9). Participants were asked to report new onset diagnoses of HTN and diabetes since donation, as well as all current medications. Multivariate logistic regression was used to examine the independent association of donor characteristics with HTN; because of low event rate, Fisher's exact test was used for diabetes.

Table 1. Multivariable models of associations between various donor factors and post-donation HTN

	Development of HTN		Requirement for anti-HTN therapy	
Factor	OR (95% CI)	p-value	OR (95% CI)	p-value
Black race	2.28 (1.04-5.47)	0.04	3.50 (1.41-8.67)	0.007
Age ≥ 50 at donation	1.80 (1.02-3.17)	0.04	2.91 (1.48-5.72)	0.002
Female sex	0.99 (0.56-1.74)	0.9	1.08 (0.56-2.05)	0.8
HS° education or less	1.42 (0.81-2.51)	0.2	1.12 (0.58-2.15)	0.7
Ever smoked*	0.64 (0.37-1.12)	0.1	0.54 (0.29-1.02)	0.06

° HS: high school; *Smoked ≥5 packs of cigarettes in life

Results: Of black living donors, 20.9% developed HTN and 7.0% developed diabetes. Conversely, of non-black donors, 12.7% developed HTN and 1.2% developed diabetes. Black race was significantly associated with new onset diagnoses of hypertension (p=0.04) and diabetes (p=0.03) in comparison to non-black race. After adjusting for multiple donor characteristics, black race was independently associated with new onset HTN (OR 2.28 95%CI 1.04-5.47) & HTN requiring pharmacologic therapy (OR 3.50; 1.41-8.67).

Conclusions: Black donors are at a greater risk for developing new onset HTN and diabetes after living kidney donation. Our findings through primary data collection in a large cohort of donors confirm previous suggestions of this effect made using administrative claims data.

TH-PO949

Patterns of Physician Visits before and after Living Kidney Donation Brian Boyarsky, Kyle Van Arendonk, Neha Deshpande, Nathan James, Robert Avery Montgomery, Dorry L. Segev. *Johns Hopkins.*

Background: Few transplant centers follow their donors on a long-term basis, leaving much of the role of follow-up care in the hands of primary care physicians (PCPs). Annual physician visits are generally recommended post-donation. The objective of this study was to describe the frequency of PCP and nephrologist visits among living kidney donors before and after kidney donation.

Methods: We surveyed 460 patients who donated a kidney at our center between 1984-2011. Median follow-up was 4.4 years (IQR 2.1-7.9). Frequency of visits to PCPs and nephrologists pre- and post- donation was categorized as more than annually, annually, less than annually, or not at all. Multivariate logistic regression was used to examine the independent associations of donor characteristics with seeing a PCP less than annually post-donation, with seeing a nephrologist post-donation, and with an increase in frequency of PCP visits after donation.

Results: Table 1. Frequency of visits to PCP and nephrologist pre-and post-donation

	PCP pre-donation	PCP post-donation	Nephrologist post-donation
>1x per year	18.0%	28.3%	5.0%
1x per year	62.7%	51.5%	5.0%
<1x per year	15.2%	10.7%	3.3%
Not at all	4.1%	9.6%	86.7%

After kidney donation, 20.3% reported seeing a PCP less than annually. In an adjusted model, men (OR 2.30 95%CI 1.43-3.69), donors with less education (OR 1.87; 1.14-3.06) and younger donors (OR 1.73; 1.05-2.85) were more likely to report seeing a PCP less than annually. 10.0% required nephrologist visits at least annually post-donation. Older donors were more likely (OR 1.84; 1.04-3.25) to have seen a nephrologist post-donation. Compared to before donation, 17.4% reported an increase in frequency of visits post-donation, 11.1% a decrease, and 71.5% no change. Males (OR 1.70; 1.04-2.78) and those ≥50 years old at donation (OR 1.59; 0.97-2.63) were most likely to increase PCP visit frequency after donation.

Conclusions: 20.3% of donors still saw a PCP less than the recommended frequency of once per year. Male sex, younger age, and lower education were associated with inadequate follow-up. Older donors were more likely to report seeing a nephrologist post-donation.

TH-PO950

Establishing an Incident Cohort of U.S. Living Kidney Donors by Direct Recruitment: The Kidney HALO Study Molly McGovern, Gary C. Curhan, Julie Lin. *Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: Although recent publications have contributed important new information about long-term outcomes in living kidney donors (LKD), almost all have been retrospective studies, which are subject to survival bias. We performed a pilot investigation to assess participant interest as well as the feasibility of direct recruitment of incident U.S. LKD for a new longitudinal cohort study.

Methods: In collaboration with the U.S. Health Research Services Administration, we obtained access to the mandatory registry of LKD to assess the feasibility and interest in their participation in a longitudinal cohort study (**K**idney **H**ealth **A**fter **L**iving **O**rgan donation or K-HALO) using a brief questionnaire. With a target 50% participation rate, K-HALO aims to recruit 6000 incident LKD participants over 2 years.

Results: In August 2010, we selected 405 consecutive LKD who donated at either 6 or 12 months earlier and sent each a questionnaire to assess interest in a proposed national living donor study. Addresses were invalid for 28 donors and 5 informed us they were liver donors. Of the remaining 372 donors, 210 (57%) completed the survey, 20 (5%) declined and 142 (38%) did not respond after three mailings. Of the 210 respondents, median age was 42 years, 143 (68%) were women, 153 (73%) were White, 15 (7%) were Black, 31 (15%) were Hispanic, 10 (5%) were Asian/other. This is representative of U.S. LKD according to 2009 UNOS/OPTN data (median age 41 years, 61% women, 69% White, 12% Black, 14% Hispanic, and 5% Asian/others). In addition, 198 (94%) were willing to participating in a longitudinal cohort study with biennial questionnaires, and 95% were interested in research on how dietary and lifestyle factors are related to continued health and quality of life after donation.

Conclusions: Direct recruitment of incident living kidney donors into a nationally representative and contemporary longitudinal cohort is feasible. Living kidney donors are interested in participating in research on dietary and lifestyle factors related to health outcomes as well as quality of life data after donation.

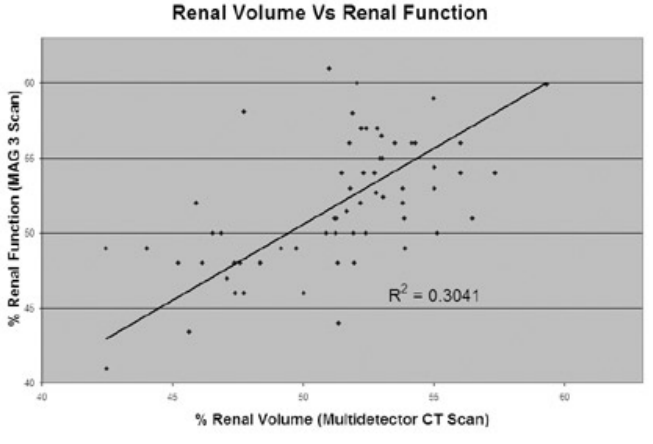
TH-PO951

Correlation between Relative Computed Tomography Based Renal Volumes and Split Renal Function in Living Kidney Donors Alejandro Diez, Tim E. Taber, Muhammad Ahmad Mujtaba, Muhammad S. Yaqub, John A. Powelson, Chandru Sundaram, Asif A. Sharfuddin. *Indiana University School of Medicine, Indianapolis, IN.*

Background: Multi-detector computerized tomography with 3-dimensional reconstruction (MDCT) provides a reliable method to calculate renal volumes. Technetium-99m mercaptoacetyltriglycine (MAG3) split function scans are used to determine the relative contribution of each kidney to a patient's overall renal function. Whereas MDCT provides morphologic information about each kidney and MAG3 split function testing provides functional information, data suggest that there might be correlation between both tests. Herein we perform a head-to-head comparison of both techniques.

Methods: Single center retrospective review of patients > 18 years of age who presented for living kidney donor evaluation. Patients with both a MDCT and a MAG3 performed were included. Each kidney's relative volume was calculated as a percentage of the sum of the total renal volume. The relative volume of each kidney was then compared to the percent renal uptake on MAG3 of each kidney.

Results: 340 charts were reviewed, of which 64 patients fit the inclusion criteria. The average total renal volume was 364.9 ml (Left: 187.06 ml [SD: 54.78], Right: 177.84 ml [SD: 49.76]). The relative left kidney size was larger than the right (Left: 51.15%, Right: 48.85%) (P=0.0049). The average split renal function was higher on the left kidney than the right (Left: 51.52%, Right: 48.23%). Calculation of Pearson's correlation coefficient was 0.3041.



Conclusions: Although the data provided by each technique is useful, there is insufficient correlation between both techniques. In select cases data from both tests may still be equivocal. Factors other than renal volume and function, such as vasculature, may be the deciding factor of which kidney to select for donation.

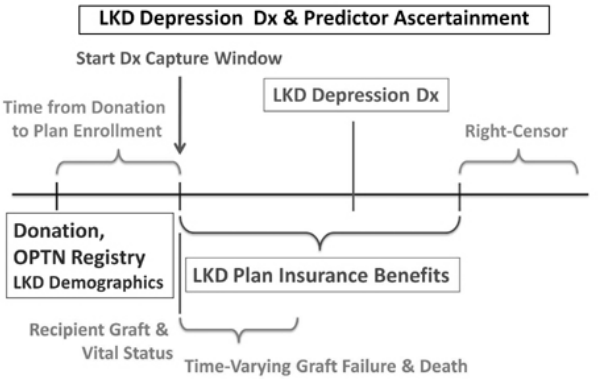
TH-PO952

Associations of Recipient Outcomes with Depression Diagnoses after Living Kidney Donation Krista L. Lentine,[1] Mark Schnitzler,[1] Daniel C. Brennan.[2] *[1]Saint Louis Univ; [2]Washington Univ.*

Background: We linked Organ Procurement & Transplantation Network (OPTN) registry data for a national sample of prior living kidney donors (LKD) with billing claims of a private insurer to study post-donation depression diagnoses (dx).

Methods: Participants (n=4,650) had OPTN records of donating in 1987-2007 and post-nephrectomy benefits at some point in the available claims (2000-2007). 81.2% of LKD were biologically related to their recipient, 7.6% were spouses, and 11.2% were non-spousal unrelated. Depression dx were ascertained from ICD-9-CM codes (296.2, 296.3, 311) on claims. Risk time began at start of post-donation benefits in the plan (Figure). We estimated associations of recipient graft failure and death with LKD depression by time-dependent Cox's regression. Depression dx rates in LKD were also compared with non-donors after 1-to-1 matching with general insurance beneficiaries by gender and age at benefits start.

Results: The median times from donation to start and end of captured benefits were 4.9 and 7.7 yrs, respectively. The cumulative frequency of depression dx after benefits start was 4.2% at 1yr and 11.5% at 5yr. After adjustment for baseline demographics, recipient graft failure and death were each associated with 2-to-3 times the relative risk of subsequent depression dx among non-spousal unrelated LKD (Table). There were trends towards increased depression risk after adverse recipient events in spousal LKD, but no associations in related LKD. Other correlates of LKD depression dx included female gender and white race. In matched-pairs comparison, the depression dx rate in LKD vs controls was 5.0 vs 7.1 per 100-PY (rate ratio 0.70, 95% CI 0.61-0.80).



Associations of Baseline LKD Factors & Recipient Outcomes with LKD Depression Dx

	Model 1 –Recipient Graft Failure as Predictor	Model 2 –Recipient Death as Predictor
	aHR (95% CI) for LKD Depression Dx	aHR (95% CI) for LKD Depression Dx
Time-Varying Recipient Event, by Relationship *		
If Recipient Biologically Related	1.06 (0.76–1.49)	1.11 (0.84–1.45)
If Recipient Spouse	2.04 (0.76–5.50)	2.00 (0.86–4.64)
If Recipient Not Biologically Related or Spouse	**3.30 (1.49–7.34)†**	**2.23 (1.11–4.48)†**
Donor Demographic Traits		
Female Gender	**2.44 (1.91–3.11)†**	**2.43 (1.90–3.10)†**
White, Non-Hispanic	**2.11 (1.53–2.92)†**	**2.12 (1.53–2.94)†**

†P<0.05

Conclusions: Recipient death and graft failure predicted depression dx risk among unrelated LKD. Informed consent and post-donation care should consider the impact of recipient outcomes on the LKD's psychological health.

Funding: NIDDK Support

TH-PO953

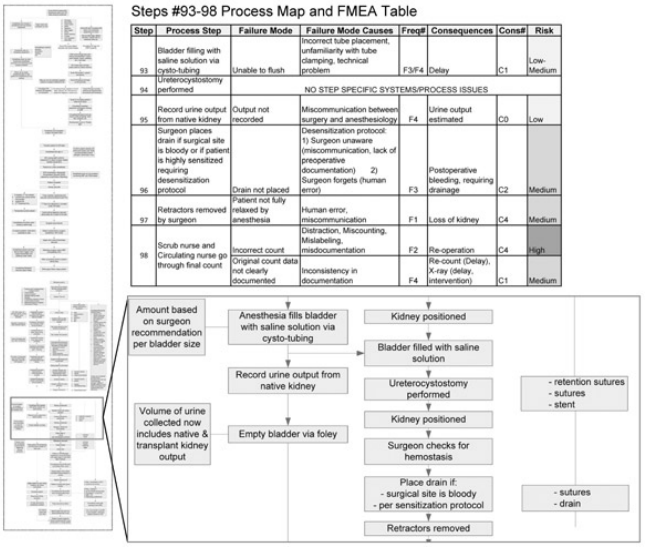
Knowing the Enemy – The First Step to Improving Safety in Living Donor Kidney Transplantation Daniela Ladner, Olivia A. Ross, Anton I. Skaro, Donna Woods, Vadim Lyuksemburg, Krutika Lakhoo, Anna Torricelli, Renee Ziomek, Gwen McNatt, Michael Abecassis, Jane Holl. *Northwestern University Transplant Outcomes Research Collaborative (NUTORC), Chicago, IL.*

Background: Safety in living donor kidney transplantation (LDKT) is essential as it involves not only a recipient but also a healthy donor. To assess LDKT proactively, rather than reactively after an adverse event has occurred, we performed a failure modes effects analysis (FMEA), a systematic methodology developed by industrial engineering.

Methods: FMEAs were performed for analysis of LDKT surgery. Safety experts led a series of FMEAs with all clinicians (surgeons, anesthesiologists, technicians, nurses, etc) involved in the LDKT surgery. Process maps were created, documenting each step in the

LDKT surgery. Then potential pitfalls (failure modes) were identified together with the potential causes, the frequencies, and the severity of the consequences. Ultimately pitfalls were categorized as low, medium, and high safety risk based on the frequency and severity of consequence (risk binning).

Results: 10 FMEAs were conducted involving a total of 17 clinicians. A total of 115 process steps were identified for LDKT surgery. Based on the results of the FMEA, 6 failure modes were classified as high risk, 44 medium risk, and 35 low risk. Predominant failure mode causes were communication, distraction, documentation errors, and protocol violations. Figure 1 highlights 6 steps of the process to provide an example of the LDKT procedure process map and the corresponding FMEA results (Figure 1).



Conclusions: FMEA of LDKT demonstrated a broad array of vulnerabilities in the systems and processes of care. The proactive analysis and systems and process improvement of LDKT is paramount to improving safety for donors and recipients alike.

Funding: Private Foundation Support

TH-PO954

Post Nephrectomy Kidney Function in Living Kidney Donors Imran Sajjad,[1] Molly McGovern,[1] Stefan Tullius,[2] Sayeed Khan Malek,[2] Julie Lin.[1] *[1]Renal Division, Brigham and Women's Hospital; [2]Transplantation Surgery, Brigham and Women's Hospital, Boston, MA.*

Background: Few published data are available on change in kidney function after living donor (LD) nephrectomy.

Methods: We analyzed 169 LD's who donated between 2006 and 2010 at a single U.S. transplant center. Plasma creatinine (pCr) at 1 year post-nephrectomy was the primary outcome. We compared donors who had ≥25% increase in pCr vs. those with <25% increase in pCr at 1 year and constructed a logistic regression model to examine associations between this outcome and pre-donation characteristics.

Results: Demographic and clinical characteristics of those with ≥25% (78%) vs. those with <25% increase in pCr at 1 year are shown in **Table1**. In addition, 27% had ≥50% and 31% had ≥0.4 mg/dl increase in pCr 1 year after nephrectomy. On univariate analyses, donor age, sex, race, MAP, and fasting glucose were associated with higher pCr ($p < 0.20$) and were selected for the MV model. In logistic regression, each 1 mmHg increase in MAP at time of donation was significantly associated with a ≥25% increase in pCr at 1 year [OR=1.06, 95% CI 1.01-1.11].

Conclusions: One year after nephrectomy, a substantial proportion of healthy kidney donors have persistently lower renal function. Longer term follow-up in LD's is needed to assess the time course of renal function recovery. Higher pre-donation MAP is associated with a ≥25% increase in pCr at 1 year after donor nephrectomy.

Table 1: Demographic and Clinical Characteristics of the LD Study Population

	<25% increase in pCr at 1 year (n=38)	≥25% increase in pCr at 1 year (n=131)	p-value
Age (years)	43 (34, 49)	45 (38, 54)	0.07
Male (%)	26	50	0.02
Caucasian (%)	89	79	0.23
Current smoker (%)	11	9	0.74
Past smoker (%)	56	50	0.80
BMI (kg/m2)	25.2 (22.2, 30.8)	26 (23.9, 29.1)	0.54
AER ≥5mcg/min (%)	18	17	0.81
MAP (mmHg)	85 (77, 91)	90 (83, 95)	0.003
Fasting glucose (mg/dl)	84 (81, 91)	87 (78, 93)	0.21
Baseline pCr (mg/dl)	0.81 (0.80, 1.00)	0.88 (0.74, 1.00)	0.66
pCr on POD # 1 (mg/dl)	1.10 (1.00, 1.20)	1.30 (1.03, 1.60)	0.14
pCr at 1 year (mg/dl)	1.00 (0.96, 1.10)	1.30 (1.10, 1.49)	n/a

Results expressed as median (25th and 75th percentile range) or %. MAP= Mean arterial pressure, pCr=plasma creatinine.

TH-PO955

Living Donor Kidney Transplants: Trends in Results throughout 20 Years of Experience Carlos R. Chiurchiu, Javier De Arteaga, Walter Guillermo Douthat, Jorge Luis De la Fuente, Pablo U. Massari. *Renal Service, Kidney Transplant Program and Postgraduate School of Nephrology, Hospital Privado-Centro Médico de Córdoba and Catholic University, Córdoba, Argentina.*

Background: Since the late nineties changes in immunossupressive protocols, introducing of living unrelated donors and Banff scoring might have influenced results in living kidney donors transplantation (LKT).

Methods: Our objetive was to study the impact of these changes altogether in the results of a cohort of LKT transplanted between 1988 up to 2007 (n:308). Results were analyzed in two cohorts according decade of transplantation: first decade 88' to 97' (n:121) and second decade 98' to 07' (n:187).

Results: Donor and recipient age increased from 39.9±12.8 to 44.8±12.2 ($p<0.001$) and from 30.5±12.4 to 35.5±15.2 ($p< 0.002$) respectively. HLA missmatch number increased from 1.9±1.2 to 2.4±1.2 ($p<0.001$). Months in dialysis also changed from 21.1±22.9 to 29.3±32.6 ($p<0.01$). Five years graft survival improved from 77% to 88% (logrank $p <0.05$), and patient survival from 91% to 97% (logrank $p<0.03$) between first and second cohort. Incidence of acute rejection (AR) decreased from 47.1% to 20.3% ($p<0.0001$). Delayed graft function was present in 26.4% and 10.2% of patients in first and second cohort respectively ($p<0.0001$). Urologic complications declined from 27.2% to 10.1% ($p<0.001$) and vascular complications remained unchanged 23,9% to 19.2% (p:n.s.) across decades. Multivariate analysis shows that only absence of AR was an independent risk factor for graft survival (OR: 0.46, 95%CI: 0.26-0.79, $p<0.005$). Worst patient survival was associated with patient's age (OR: 1.07, 95%CI: 1.03-1.12, $p<0.001$) and transplant in first decade (OR: 5.76 95%CI: 1.61-19.70, $p<0.001$).

Conclusions: Improvements across decades in patient care and immunosuppressive protocols have improved outcomes in LKD despite transplantation of patients with increasing risk factors and HLA missmatching.

TH-PO956

Comparison of Two Nuclear Scan Tracer Methods for Accurate Assessment of Kidney Donor Glomerular Filtration Rate Anna C. Porter, Nidhi Aggarwal, Ignatius Yun-Sang Tang, Sanjeev Akkina. *Nephrology/Transplant Surgery, University of Illinois at Chicago, IL.*

Background: Accurate assessment of the glomerular filtration rate (GFR) is essential to assess candidacy for kidney donation, but is often difficult to do, especially in obese donors. Collection of 24 hour urine specimens is often unreliable. Though it is considered the best method for donor GFR evaluation, iothalamate clearance measurement availability is limited. Nuclear scans are often used due to availability and perceived accuracy. However, the tracer used may influence the accuracy and therefore effectiveness for kidney donor evaluation. We assessed how two radioactive tracers correlate to commonly used GFR estimation equations which have been validated against iothalamate clearance studies in order to compare different nuclear tracer GFR measurement methods for GFR evaluation of obese donors.

Methods: GFR measurements (mGFR) from nuclear scans conducted between 2009-September 2010 using mercapto-acetyl triglycine (MAG3) and September 2010-present using Tc-99m-diethylenetriaminepentaacetic acid (DTPA) were compared to the Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (eGFR). We assessed bias (eGFR-mGFR), precision (R2 of regression), and accuracy (percent within 30% of mGFR).

Results: Average age, BMI, gender and ethnicity makeup were similar between the two groups. The DTPA scan had better precision with the Cockcroft-Gault, MDRD, and CKD-EPI equations compared to the MAG3. Accuracy and bias were statistically similar.

	MAG3	DTPA	p-value
Age	38.2±11.2	37.3±13.8	0.71
Male	44%	56%	0.26
BMI	30.9±5.8	33.5±8.0	0.11
Ethnicity			0.66
White	23%	33%	
Black	43%	41%	
Hispanic	26%	22%	
Asian/Other	7%	4%	
Cockroft-Gault			
R2	0.102	0.391	
% within 30%	64%	74%	0.31
Bias	19.2±22.6	12.7±17.3	0.16
MDRD			
R2	0.025	0.23	
% within 30%	70%	81%	0.20
Bias	2.3±27.1	-6.8±19.6	0.10
CKD-EPI			
R2	0.03	0.20	
% within 30%	70%	70%	0.93
Bias	-1.8±26.7	-10.9±20.8	0.09

Conclusions: In obese donors, the DTPA tracer offers improved precision while accuracy and bias were similar or slightly better and should be the preferred method if iothalamate clearance is unavailable at the transplant center.

TH-PO957

Structural and Mechanical Properties of Large Arteries after Kidney Transplantation: Major Impact of Donor Age and Living Donor Kidney Transplantation Michel Delahousse,[1] Alexandre Karras.[2] *[1]Nephrology and Transplantation, Foch Hospital, Suresnes, France; [2]Nephrology, Georges Pompidou European Hospital, Paris, France.*

Background: Damage to large arteries in ESRD patients is characterized by an outward remodeling of the carotid artery leading to an increased circumferential wall stress (CWS) and by increased aortic and carotid stiffness.

Methods: We measured carotid-femoral pulse wave velocity (PWV), aortic pressure and carotid remodeling and stiffness parameters at three months (M3) and one year (M12) post-transplantation in 77 consecutive kidney recipients (57 cadaveric kidney and 20 living donor kidney recipients).

Results: PWV decreased (10.8 + 2.5 vs 11.6 + 2.7 m/s, p = 0.015) independently of mean aortic pressure. CWS decreased (67.6 + 16 vs 71.4 + 16, p = 0.03) as a result of a decrease in carotid internal diameter. PWV change (PWVM12-PWVM3) correlated with mean aortic pressure change(MAP) (R=0.348, p = 0.003), donor age (R-0.308, p=0.008), living donor (R= -0.330, p = 0.004), plasma phosphate concentration at one month (R=0,309, p = 0,008, body mass index (BMI) change (R=0.310, P=0.006), M3 PWV (R=-0.260, p = 0.026), but not with isotopic GFR. MAP, donor age, living donor and BMI change remained independently related to PWV change in multivariate analysis.

In deceased donor kidney recipients, donor age was the main determinant of isobaric PWV change (improvement with young donors), confirming our previous results in an independent cohort of kidney recipients (J Am Soc Nephrol 19: 798 –805, 2008).

In living donor kidney recipients, the improvement of aortic stiffness, carotid remodeling and stiffness parameters was remarkable and highly significant.

Arterial parameter	M3	M12	P
Aortic stiffness (m/s)	11.4±3.4	9.8±1.9	0.002
Carotid stiffness (m/s)	6.8±1.5	5.9±1.3	0.005
Young elastic modulus (kPa)	520±224	352±126	0.003
Carotid internal diameter (mm)	6.44±1.03	6.22±0.94	0.039
Carotid IMT ([mum])	608±128	628±137	0.192
CWS (kPa)	76.2±16.3	65.4±14.8	0.007

Conclusions: Damage to large arteries can reverse after kidney transplantation. Donor age (in cadaveric kidney recipients) and living donor kidney transplantation are the main determinants of this improvement.

TH-PO958

Changes in Soluble Tumor Necrosis Factor Receptor-2 (sTNFR-2) after Living Donor Nephrectomy Molly McGovern, Julie A. Berkley, Sushrut S. Waikar, Julie Lin. *Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: Higher plasma sTNFR-2 levels have been strongly associated with subsequent kidney function decline in people with well preserved kidney function. Animal studies suggest sTNFR-2 is primarily renally cleared, but no studies in humans currently exist.

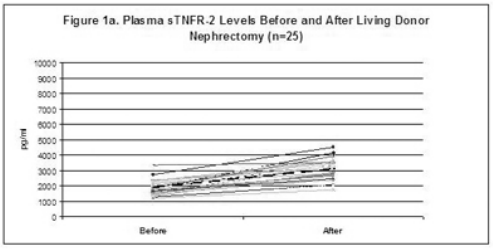
Methods: We studied 25 consecutive living kidney donors (LKD) at our center who had measurements of sTNFR-2 before and 24 hours post-nephrectomy as well as 5 patients who underwent CABG surgery with plasma collected prior to surgery and 24 hours post-op. sTNFR-2 was measured by an ELISA assay from R & D Systems using a quantitative sandwich enzyme immunoassay technique. Directly measured CV was 5% in blinded split samples.

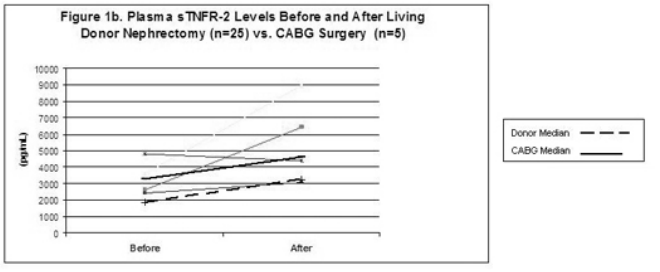
Results: Clinical characteristics for both groups are summarized in Table 1.

	LKD (n=25)	CABG (n=5)
Age (median and range) (years)	51 [27, 67]	79 [63, 84]
Sex (M/F)	48%/52%	40%/60%
White	96%	100 %
Non-white	4%	0%
Median pre-PCr (mg/dL)	0.81	1.03
Median post-PCr (mg/dL)	1.31	1.01
Median pre-sTNFR-2 (pg/ml)	1848	3285
Median post-sTNFR-2(pg/ml)	3293	4621
Median change sTNFR-2 (pg/ml)	1212	1336

pCr = plasma creatinine

Our preliminary data show that sTNFR-2 consistently increases after donor nephrectomy in patients (Figure 1a).





The CABG patients had a higher baseline level of sTNFR-2 (median 3285 vs. 1848 in LKD) that also increased post-operatively (median change 1336 vs. 1212 in LKD) (Figure 1b).

Conclusions: Plasma sTNFR-2 levels are universally increased after living donor nephrectomy; however, this phenomenon is also seen in post-CABG patients. We therefore conclude that increases in sTNFR-2 after donor nephrectomy do not primarily reflect loss of glomerular filtration rate.

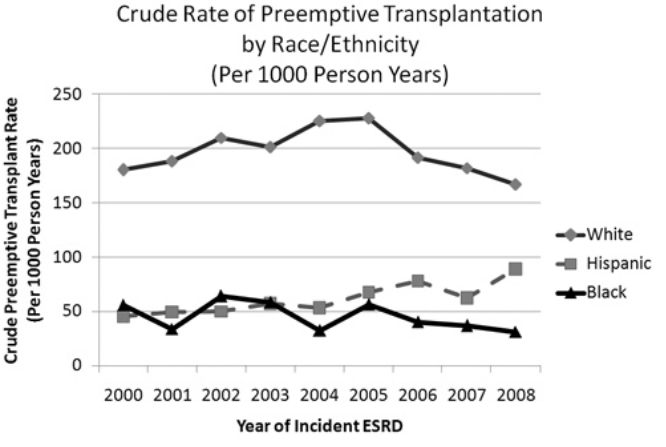
TH-PO959

Racial Disparities in Access to Preemptive Kidney Transplantation among the National Pediatric ESRD Population Rachel E. Patzer, Sandra Amaral, Nancy G. Kutner, William M. McClellan. *School of Medicine, Emory University, Atlanta, GA.*

Background: Kidney transplantation is the preferred treatment for pediatric End Stage Renal Disease (ESRD), and preemptive transplant may reduce morbidity and mortality. It is unknown how race and poverty impact access to preemptive transplant in the pediatric ESRD population.

Methods: We examined the incidence rate of preemptive renal transplant by race/ethnicity among the national pediatric (< 21 yrs) ESRD population using United States Renal Data System data from 2000-2008. Annual rates were calculated as incident preemptive transplant count divided by ESRD patient years. Rate Ratios for preemptive transplant by race/ethnicity were calculated using adjusted generalized linear Poisson models. We considered neighborhood poverty and health insurance as socioeconomic status (SES) measures.

Results: Among 10,855 pediatric ESRD patients (pts) from 2000-2008, a total of 1,287 patients (11.9%) had a start date of ESRD equivalent to their transplant date and no history of dialysis. The average annual rate of preemptive transplant was higher among whites (202/1000 person-years[PY]) than Hispanics (59/1000 PY) and blacks (48/1000 PY). Racial differences were evident in the type of preemptive transplant received, where more white pts had a living donor (78.8%) vs. Hispanics (57.3%) and blacks (48.8%)(p<0.0001). In adjusted analysis, Hispanics had a 50% (95% CI: 0.41-0.61) and blacks a 56% (95% CI: 0.36-0.54) lower rate of preemptive transplant vs. whites.



The effect of race on preemptive transplant was not modified by SES, and racial differences persisted after adjustment for SES.

Conclusions: Pediatric racial minorities have a significantly lower incidence of preemptive transplantation, which was unexplained by SES. Further studies are needed to elucidate why minority groups with pediatric ESRD are less likely to receive early renal transplantation.

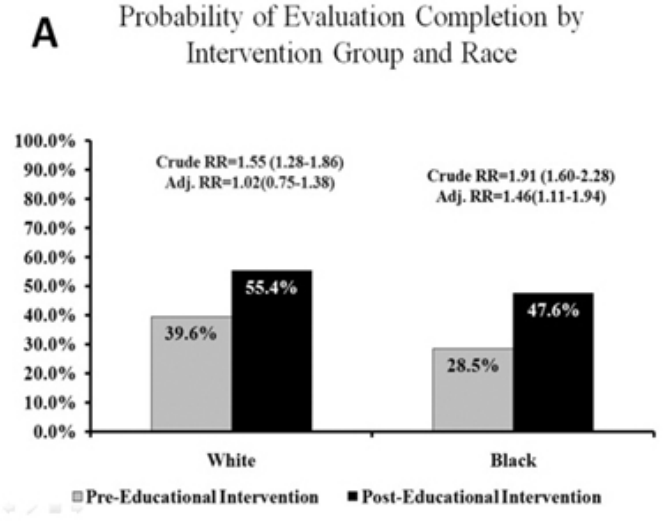
TH-PO960

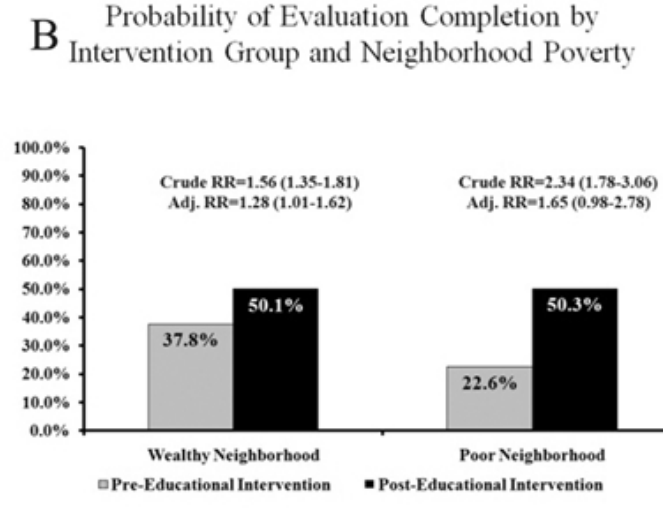
Reduced Racial and Socioeconomic Disparities in Kidney Transplant Evaluation Completion after Start of a Required Patient Education Program Rachel E. Patzer, Jennie P. Perryman, Stephen O. Pastan, Sandra Amaral, William M. McClellan. *School of Medicine, Emory University, Atlanta, GA.*

Background: In 2007, the Emory Transplant Center (ETC) kidney transplant program implemented a patient education class for End Stage Renal Disease (ESRD) patients referred for transplant. The purpose of this intervention was to improve patient awareness about the transplant process and to increase evaluation completion.

Methods: We examined one-year evaluation completion among ESRD patients referred 2005-2007. Patient data were abstracted from medical records and linked with the United States Renal Data System. Adjusted Risk Ratios (RR) by intervention group were calculated from binomial regression models adjusting for time trends. We also examined how the intervention impacted evaluation completion across levels of race and poverty (health insurance, education, employment, and neighborhood poverty by census tract).

Results: A total of 1,126 adult ESRD patients were examined in two transplant evaluation eras (75% pre- and 25% post-intervention). Evaluation completion at one year was higher among those in the post- vs. pre-intervention group (80.4% vs. 44.7%, p<0.0001), and was 38% higher (95% CI:1.12-1.71) after controlling for time trends. In crude and adjusted analyses, improvement in evaluation completion in the post- (vs. pre-) intervention group was greater among blacks (Panel A) and those living in poor neighborhoods (Panel B)(p for interaction<0.05).





Conclusions: The implementation of a required education program increased evaluation completion for all patients. The effect of the intervention was stronger among black and poor patients, resulting in reduced disparities in evaluation completion. These results suggest that standardizing transplant education may help reduce some of the racial and socioeconomic disparities observed in early stages of access to kidney transplantation.

TH-PO961

Potential of Kidney Transplantation in Undocumented Immigrants (UI) with End Stage Renal Disease Ellena A. Linden, George N. Coritsidis. *Nephrology, Elmhurst Hospital Center, Elmhurst, NY.*

Background: By virtue of our location in Queens, NY (the most ethnically diverse county in US) we have a large population of UI who are receiving dialysis. These treatments are covered by NY State Emergency Medicaid. Transplantation costs, however, are not covered under the same program. Our hypothesis is that UI have potential living kidney donors and that paying for the transplantation costs of those with available living donors is cost-effective.

Methods: We conducted a cross-sectional survey of UI at our outpatient dialysis unit. 50 patients qualified, 45 completed the questionnaire. We used our documented dialysis population as comparison group.

Results: Our UI are significantly younger and healthier than their documented counterparts.

	undocumented, n=45	documented, n=82	p value
average age, yrs (sem)	44 (2)	60 (1)	<0.005
DM, %	40	68	<0.005
CAD, %	7	33	<0.005
hospitalizations/yr/pt	0.9	1.0	ns
employed, %	51	6	<0.005

They are much more likely to be employed (51 vs 6%). The vast majority of our UI came to US to work and not to seek medical care. If transplanted, all UI who are employed will continue working. Of those who are unemployed, 82% state that they will seek work if transplanted. 60% were able to identify at least one potential donor. Among those with donors, the average number was 1.9. These donors are young and healthy. 64% of donors already reside in North America.

Conclusions: Life expectancy of an average 40-44 year old US dialysis patient is 8 years. Our UI are younger and healthier and will likely have a greater life expectancy. They have potential kidney donors. Paying for living donor transplantation for the UI would translate into savings of at least $342,000 per patient for NY State. Beyond these savings and improved quality of life, society will also benefit in that most of the patients would be willing and able to reenter the workforce. Lawmakers in New York State and other states that provide emergency medicaid coverage for UI should consider living-donor transplantation for those UI with available living donors. This strategy will significantly reduce the cost of caring for our undocumented patients.

TH-PO962

What Factors Influence Access to Renal Transplantation in Patients Undergoing In-Center Dialysis? Lina Mackelaite, Adam E. Gaweda, Zygimantas C. Alsauskas. *Division of Nephrology, University of Louisville, KY.*

Background: Patients with renal failure have a choice of different renal replacement therapies, including in-center hemodialysis (HD), peritoneal dialysis and renal transplantation. It is known that kidney transplant recipients live longer compared to patients on dialysis. Despite that only a small fraction of dialysis patients are on the transplant list.

Methods: We interviewed a total of 129 patients undergoing in-center hemodialysis. Data was obtained from standard questionnaires and CMS Medical Evidence Form 2728. Statistical analysis was performed using SPSS. We built a binary logistic regression model to evaluate predictors of being on the transplant list. We used Hosmer & Lemeshow model selection method to select the most influential predictors (with initial threshold $p \leq 0.3$ for the univariate models and $p \leq 0.05$ for the final multivariate model).

Results: We analyzed multiple factors, including age, education, weight, ethnicity, employment, discussing transplantation with nephrologist after initiation of RRT, dialysis vintage etc. In univariate analysis these factors where associated with higher likelihood of being on a transplant list: talking about transplantation with a nephrologist after initiation of HD(OR 22, P=0.003), presentation of different treatment options prior to starting HD(OR 2.8, p=0.05), knowing somebody with a transplant (OR 1.6, p = 0.27), knowing the benefits of kidney transplantation (OR 1.7, p=0.23). In multivariate analysis the only factor that was associated with being on a transplant list was talking about renal transplantation with nephrologist after initiation of RRT (OR 19, p=0.005).

Only 48 % of people with hemodialysis vintage of less than 1 year had discussion about renal transplantation compared to 73% of patients who were on dialysis for more than 1 year.

Conclusions: The most significant factor that determines access to renal transplantation is discussion about kidney transplantation with their nephrologist. Despite better post transplant patient survival with shorter dialysis vintage, discussion about renal transplantation happens in less than 50% of ESRD patients within the first year of initiation of dialysis.

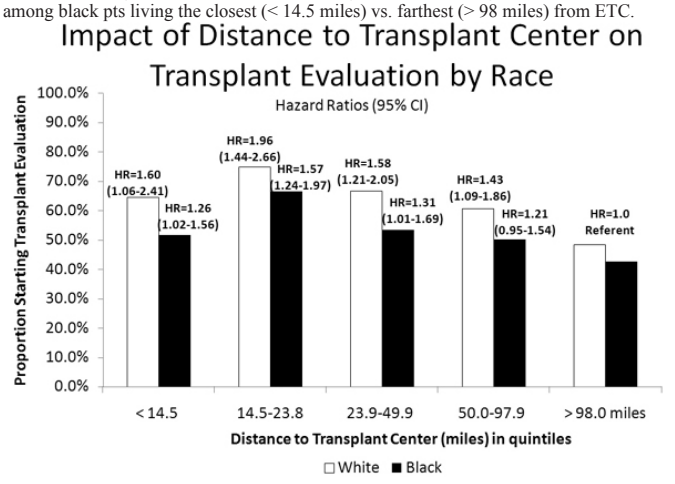
TH-PO963

Distance to Transplant Center Associated with Delayed Renal Transplant Evaluation in the Southeastern United States Rachel E. Patzer,[1] Sumit Mohan,[2] Richard Mutell,[3] Stephen O. Pastan,[1] Jennie P. Perryman,[1] Sandra Amaral,[1] Nancy G. Kutner,[1] William M. McClellan.[1] *[1]School of Medicine, Emory University, Atlanta, GA; [2]Department of Medicine, Columbia University, New York, NY; [3]DaVita Clinical Research, Minneapolis, MN.*

Background: Geographic variations in access to renal transplant have been reported but considered all prevalent ESRD patients, rather than only those referred for transplantation. Less is known about barriers to transplant evaluation, the first step in the transplant process.

Methods: We examined adult, ESRD patients (pts) referred to the Emory Transplant Center (ETC) kidney transplant program 2005-2007. Patient data were abstracted from medical records and linked with United States Renal Data System. Adjusted Cox models were used to examine the effect of distance on time from referral to start of the evaluation process by race.

Results: Among 2,228 ESRD pts referred to ETC, 55.3% (61.3% white vs. 52.1% black, p <0.0001) started the transplant evaluation. Mean age at referral was 51.1 years. Pts who started the evaluation (n=1,233) lived significantly closer to ETC than those who did not (35.3 vs. 51.8 miles) and the median distance to ETC was lower among black vs. white patients (27 vs. 53 miles) (p<0.0001). Blacks were less likely to start the evaluation than whites, regardless of distance to center (p for interaction = 0.7548). The rate of evaluation start was 60% higher (95% CI:1.06-2.41) among white and 26% higher (95% CI:1.02-1.56) among black pts living the closest (< 14.5 miles) vs. farthest (> 98 miles) from ETC.



Conclusions: Pts residing closer to ETC were more likely to initiate their renal transplant evaluation than those living farthest away. Distance to transplant center may be a potentially modifiable barrier to transplant evaluation, and barriers to transplant access may be mitigated by implementing outreach clinics.

TH-PO964

Effect of Diabetes Mellitus on Access to Renal Transplantation Bhanu K. Patibandla, Akshita Narra, Ranil N. Desilva, Alexander S. Goldfarb-Rumyantzev. *Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background: Previous studies have shown that diabetics with ESRD were less likely to receive a renal transplant. The goal of our study was to evaluate whether the decreased access to renal transplantation is mediated by increased co-morbidities and greater BMI.

Methods: Study cohort included all ESRD patients from January 2000 to September 2007 using data from the United States Renal Data System (USRDS). We analyzed two outcomes in Cox regression model: (1) likelihood of being placed on the waiting list for renal transplantation or transplanted without wait-listing (2) likelihood of receiving transplant in patients previously placed on the waiting list. Multivariate models were adjusted for age, sex, race, duration of pre-ESRD nephrology care, levels of albumin, hemoglobin and creatinine. In addition we performed subgroup analysis based on age, race, sex, co-morbidity index, and duration of nephrology care.

Results: We analyzed 721,521 patients (age of ESRD onset 63.6±15.3 years, 54.6% males, 64.7% white and 29.2% African American). When compared with non-diabetic population, diabetes was associated with reduced transplant access: both for wait listing/transplant [HR 0.87, p<0.001] and for transplant after being listed [HR 0.77, p<0.001]. When proportional hazard models were adjusted for BMI and co-morbidity index, the association changed dramatically, so that patients with diabetes had better access to transplantation, i.e., [HR 1.61 (p<0.001)] for wait listing/transplant and [HR 1.51 (p<0.001)] for transplant after being listed. This trend was the same in the subgroups studied.

Conclusions: Diabetics are less likely to be placed on the waiting list for kidney transplant; and once on the list are less likely to be transplanted. Our analysis suggests that effect is mediated by higher level of co-morbidity and greater BMI.

TH-PO965

Improving Access to Living Donor Kidney Transplantation Jonathan Reaney, Monica Monaghan, Aisling E. Courtney. *Department of Nephrology, Belfast City Hospital, Belfast, United Kingdom.*

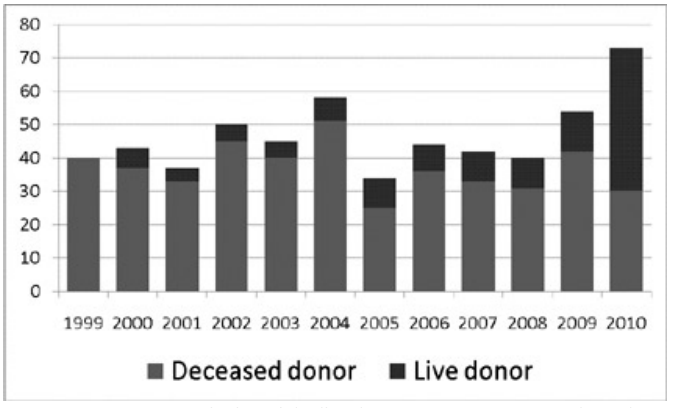
Background: Live donor transplantation is the optimal form of renal replacement therapy for the majority of patients with end-stage renal disease. There is considerable geographical variation in the opportunity for patients to access this treatment.

Methods: This study assesses the impact of reorganisation of services on the delivery of live donor transplantation in a single UK region.

Methods: Traditionally the assessment process for a potential live donor in Northern Ireland was long and cumbersome, requiring multiple attendances at hospital. It was customary for it to exceed 2 years. The number of live donor transplantations remained static at a maximum of 20% of all renal transplants per annum.

The donor pathway was reorganised with a one-day assessment combining all routine investigations and nephrology assessment. A formal multidisciplinary meeting was established weekly allowing a swift decision on suitability.

Results: The results were an increase in live donor transplant procedures from 9 to 48 per annum (an increase to 58% of all kidney transplants being live donor organs), a reduction in time to transplantation to five months from assessment with an substantial increase in pre-emptive transplantation especially in the paediatric population. This 400% increase in live donor transplants occurred over an 18 month period. Figure 1 Renal Transplantation in Northern Ireland.



Conclusions: Reorganisation of the live donor assessment process reduces donor fatigue and drop-out, reduces the time to transplantation, and increases the number of live donor transplant procedures. This can be achieved without a substantial increase in resources, providing opportunity for improved clinical outcomes and more economically viable renal replacement therapy programmes for populations with end-stage renal disease.

TH-PO966

Excellent Kidney Transplant Outcomes in a Safety Net County Hospital Giselle Kohler,[1] Merit E. Qviste,[3] John R. Hartono,[1] Christopher Y. Lu,[1] Nilum Rajora,[1] Meelie Debroy,[2] Doris L. Sweatt,[3] Miguel A. Vazquez.[1] *[1]Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, TX; [2]Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX; [3]Kidney Transplantation, Parkland Health and Hospital Systems, Dallas, TX.*

Background: Race and insurance status are important factors limiting access to kidney transplantation and associated with poor outcomes. The kidney transplant program at Parkland Health and Hospital Systems (PHHS) serves a predominantly indigent and minority population. PHHS is an integrated health care system that serves as primary teaching hospital for UT Southwestern Medical Center and, via a central campus and a network of primary health care clinics and specialty/subspecialty clinics, makes health care available to indigent patients in Dallas County.

Methods: We conducted a retrospective review of recent kidney transplants (2005-2010) performed at Parkland Hospital in Dallas, including racial/ethnic distribution of transplant recipients, insurance status and kidney transplant outcomes.

Results: Seventy-five percent of kidney transplant recipients during our study period were enrolled in Parkland Health Plus, which is a Parkland-based county assistance program to provide health care services and medications to indigent patients who do not have other means to cover health expenses.

n=26, (7/09-6/10)	Parkland	Region	US
White	7.7	34.5	44.9
African-American	38.5	27.8	32.3
Hispanic/Latino	46.2	32.4	15.2
Asian	7.7	3.7	6.2

	Parkland	Parkland	US	US
	1 Year (n=90)	3 Years (n=61)	1 Year	3 Years
Observed graft survival (%)	97.78	89.89	93.51	84.72
Expected graft survival (%)	(92.64)	(81.36)		

Conclusions: Efforts to allocate professional resources and multi-institutional commitments to provide integrated health care and assistance with health care costs, including access to medical services and assistance with medications can improve access to kidney transplantation and lead to superior transplant outcomes for patients who are under-insured or belong to underserved minority populations.

TH-PO967

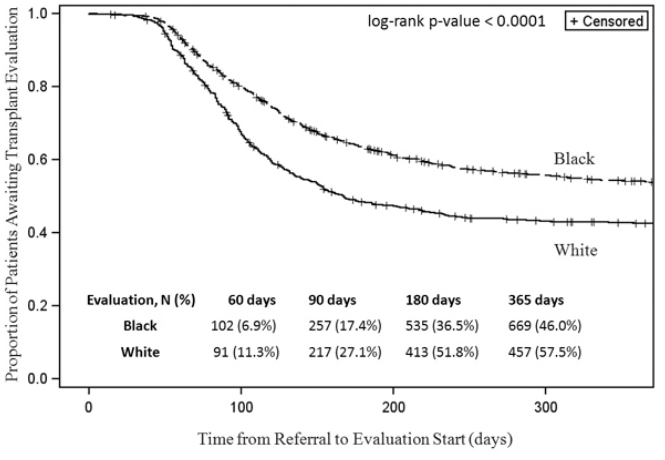
Race and Access to Renal Transplantation Evaluation in the Southeastern United States Justin D. Schrager,[1] Rachel E. Patzer,[1] Jennie P. Perryman,[3] Stephen O. Pastan,[3] Nancy G. Kutner,[3] William M. McClellan.[2] *[1]School of Medicine, Emory University, Atlanta, GA; [2]Emory Transplant Center, Atlanta, GA.*

Background: Disparities exist among End Stage Renal Disease (ESRD) patients in access to renal transplant. The effect of race and socioeconomic status (SES) on start of the kidney transplant evaluation following referral has not been thoroughly explored.

Methods: This study examined the effect of race on renal transplant evaluation, defined as the first scheduled on-site visit at the Emory Transplant Center (ETC), from 2005-2007 and followed through 2010. Patient characteristics were abstracted by chart review and linked with the United States Renal Data System and census tract poverty. Kaplan-Meier methods and Cox models were used to examine racial differences in access to evaluation. We used neighborhood poverty and health insurance as measures of SES.

Results: 2,291 prevalent ESRD patients were referred to ETC during the study period. Of these, 1015 (44.3%) never started the renal transplant evaluation process. Compared to patients who started the evaluation process, those who did not start were significantly more likely to be black (69.5% vs. 61.1%), black health insurance (17.7% vs. 14.5%) or have Medicaid (22.9% vs. 14.5%), to reside in the poorest census tracts (40.1% vs. 28.2%) and spend a longer time on dialysis prior to referral (314 days vs. 124 days). In crude Cox models, black (vs. white) patients were 28% less likely to start the evaluation at any given time during follow-up (HR=0.72; 95% CI: 0.65-0.81). After adjusting for demographic, clinical, and SES factors, this disparity persisted (HR=0.82, 95% CI: 0.73-0.93).

Conclusions: Racial disparities exist in renal transplant evaluation. Black patients were 18% less likely to start the evaluation process following referral, even after adjusting for clinical and SES characteristics.



TH-PO968

Access to Renal Transplantation at Nottingham University Hospitals Linda H. Bisset, Linda Evans, Catherine Byrne. *Nottingham Renal and Transplant Unit, Nottingham University Hospitals NHS Trust, City Campus, Nottingham, United Kingdom.*

Background: Early transplant referral and listing confers advantages in terms of life expectancy, quality of life, and graft survival. In the UK, equity to access and activation appears largely centre specific. Current recommendations are for pre-dialysis patients to be listed when eGFR <15. Previous work from our unit suggested fewer than expected patients were active on the renal transplant waiting list. We determined the timeline for referral and listing in our prevalent transplant waiting list population.

Methods: We retrospectively analysed all adult prevalent pre-dialysis (eGFR <15), dialysis (HD, PD) and failing transplant recipient patients to determine the timeline from first doctor meeting (FDM), and start of RRT to referral, assessment and transplant list activation.

Results: Of 691 prevalent patients, (193 pre-dialysis, 390 HD, 108 PD), 159 were listed for transplantation (25 pre-dialysis, 84 HD, 51 PD). Of those listed, 57% were male, 42% aged ≥50 and 9% had diabetes mellitus.

The table illustrates median times in the referral process. Only 8% HD and 24% PD patients were listed pre-dialysis, despite 74% and 88% patients having a FDM >90 days respectively. 13% of pre-dialysis patients were listed. Delays were identified in 21 patients, mainly relating to investigations. Within the cohort not listed, 37 patients (19% pre-dialysis) are being assessed and 19 have been referred (26% pre-dialysis). All patients not listed had an appropriate reason documented.

	Listed n= (%)	Referral to surgeon (wks)	Surgeon to list (wks)	Referral to list (wks)	Referred pre-dialysis n= (%)	Listed predialysis n= (%)	FDM > 90 days to RRT n= (%)
Pre-dialysis	25 (13%)	11	14.2	22	NA	NA	NA
HD	84 (22%)	10.8	9	21.8	20 (24%)	7 (8%)	62 (74%)
PD	51 (47%)	13	4	17.3	23 (45%)	12 (24%)	45 (88%)

Conclusions: Timeline for referral and listing is suboptimal. Data is retrospective and median times may be skewed due to historic long waits and many pre-dialysis patients were seen in advance of the need for listing. Delays were mainly related to cardiology investigations. Prospective audit shows significant improvements in the 18 week pathway over the last 12 months.

TH-PO969

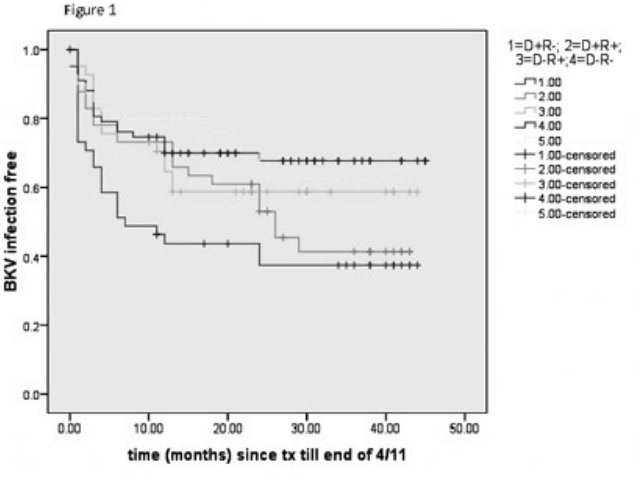
Combination of Pre-Transplant BKV Specific IgG Donor Positivity and Recipient Negativity Correlates with Post-Transplant BKV Infection Puneet Sood, Shamila Chaturi Senanayake, Kumar Sujeet, Christopher Johnson, Sundaram Hariharan. *Medical College of Wisconsin, Milwaukee, WI.*

Background: BKV infection can manifest as viremia/viruria which may lead to nephritis and graft failure. Risk factors for BKV infection have not been well defined. In our prospective study, we analyzed the effect of donor and recipient BKV specific antibody IgG status at time of transplant on the development of post-transplant BKV infection.

Methods: 240 patients were prospectively enrolled from July 2007 to July 2010 and followed until May2011. Baseline data on donor/recipient age, gender, race, cold ischemia time, donor source, PRA, induction therapy, HLA match, donor BKV immune status and prospective data on occurrence of rejection and quantitative BK viruria and viremia at 1,3,6 and 12 months was collected. Positive BKV specific antibody was defined as IgG EIA≥8 units. Study population was divided into D+R+/D+R-/D-R+/D-R- groups based on donor and recipient immune status. Occurrence of post transplant BKV infection defined by detection of BKV DNA in plasma or urine at the end of study period was used as endpoints. Kaplan-Meier survival curves were used to look at the difference between groups and Log-Rank test was used to test for significance.

Results: Patients were divided into 4 groups based on donor and recipient BKV specific positivity and negativity. Group 1(D+R-)n=41 pairs, group 2(D+R+)n=42 pairs, group 3(D-R+)n=41 pairs and group 4(D-R-)n=68 pairs. The demographic, transplant and post-transplant variables were similar in 4 groups. Recipients from group 1(D+R-) had the highest chance of developing post-transplant BKV infection (figure1), Log Rank p=0.009.

Conclusions: Pre-transplant BKV specific IgG antibody status in subjects with D+/R- is associated with a higher rate of post-transplant BKV infection and predominantly occurs in the early period.



TH-PO970

Risk Factors for BKV Infection after Renal Transplantation Puneet Sood, Shamila Chaturi Senanayake, Kumar Sujeet, Christopher Johnson, Sundaram Hariharan. *Medical College of Wisconsin, Milwaukee, WI.*

Background: BKV infection manifests as viremia or viruria which may progress to BKVN. Graft survival rates in patients with BKVN are worse than acute rejection and calcineurin inhibitor (CNI) toxicity. In our prospective study, we evaluated the risk factors for the occurrence of all BKV infections defined as any degree of viruria and/or viremia.

Methods: 240 patients were prospectively enrolled from July 2007 to July 2010 and followed until May 2011. Baseline data on donor/recipient age, gender, race, cold ischemia time, donor source, PRA, induction therapy, HLA match, donor BKV immune status and prospective data on the occurrence of rejection and quantitative BK viruria and viremia at 1, 3, 6 and 12 months was collected. Logistic regression modeling was used to ascertain risk factors for any level of BKV infection. T-test and Chi-sq test were used for continuous and categorical variables, respectively.

Results: Characteristics of BKV infected and non infected patients are summarized in table 1.

Risk factors	BKV infection (N=93)	No NKV infection (N=147)	P-value
Donor Source (LD/DD)	39/54	53/92	P=0.42
Donor age (>60/<60)	8/85	14/131	P=0.78
Donor race (AA/W+ others	11/82	17/127	P=0.99
Donor BKV specific IgG: EIA units ≥vs<8	37/47	52/67	P=0.96
Recipient age (>60/<60 yrs)	21/72	29/116	P=0.63
Recipient race (AA/W+ others)	13/80	48/99	**P=0.001**
Cold ischemia time (>14 /≤14 hrs)	30/60	51/84	P=0.49
HLA mismatch (>4/≤4)	40/51	75/66	P=0.17
PRA (>20%/<20%)	24/67	38/96	P=0.74
Induction therapy (Thymoglobulin/ IL-2R)	46/47	78/69	P=0.58
Acute rejection (Y/N)	18/75	30/117	P=0.84

African American recipients had lower risk of developing BKV infection compared to Caucasians and other races. This effect remained significant after controlling for other potential confounding variables (OR 0.38, 95% CI 0.16-0.84, P=0.018). Actuarial Kaplan-Meier curves for the occurrence of BKV infection was significantly more in Caucasians as opposed to African Americans.

Conclusions: African Americans had a lower incidence of BKV infection compared to Caucasians. This association with race needs further investigation in larger prospective studies.

TH-PO971

Low Physical Activity in Renal Transplant Recipients Is Associated with Mortality Independent of Markers of Subclinical Heart Failure and Cardiac Ischaemia Dorien M. Zelle,[1] Eva Corpeleijn,[1] Reinold O.B. Gans,[2] Gerjan Navis,[1] Stephan J.L. Bakker.[2] *[1]Kidney Center, University Medical Center Groningen, Netherlands; [2]Internal Medicine, UMCG.*

Background: We previously showed that low physical activity (PA) is a strong risk factor for mortality in renal transplant recipients (RTR). Although RTR with overt heart failure were excluded from our study, it was, not discerned whether this association could be driven by subclinical heart failure or cardiac ischaemia. We therefore investigated whether cardiac markers N-terminal pro-B-type natriuretic peptide (NT-proBNP) or hs-Troponin T (TNT) explain the association between PA and mortality in RTR.

Methods: Baseline assessments were done between 2001-2003. PA was assessed using validated questionnaires. Mortality was recorded until August 2007. NT-proBNP and TNT were measured on the Roche Modular E170.

Results: A total of 539 RTR (age 51 yrs,54% male) were studied. NT-proBNP and (r=-0.24,p=0.001) and TNT (r=-0.30,p=0.001) were inversely associated with PA. During follow-up for 5.3 years [4.7-5.7], 81 RTRs died (37 cardiovascular). In a Cox regression analysis, PA (HR=0.75 [95%CI 0.60-0.92], P=0.007) was associated with mortality, independent of age, sex, creatinine clearance and systolic blood pressure. In a further multivariate analysis, including NT-proBNP (1.51 [1.13-2.02], P=0.05) and TNT (1.42 [1.03-1.95, P= 0.03), PA (0.79 [0.64-0.98], P=0.04) remained independently associated with mortality. Similar results were found for cardiovascular mortality (PA 0.67 [0.49-0.92], P=0.01).

Conclusions: Only a small part of the association of low PA with mortality can be explained by markers of subclinical heart failure and cardiac ischaemia, while a much larger part is independent. This stresses the potential relevance of PA intervention programmes for improvement of survival in RTR.

TH-PO972

The Deterioration of Serum Sulfatide Level, a Novel Risk Factor of Cardiovascular Disease, Is Dramatically Improved by Kidney Transplantation in ESRD Patients Yuji Kamijo,[1] Makoto Harada,[2] Yasufumi Takahashi,[2] Makoto Higuchi.[2] *[1]Department of Metabolic Regulation, Shinshu University Graduate School of Medicine, Matsumoto, Nagano, Japan; [2]Department of Nephrology Internal Medicine, Shinshu University School of Medicine, Matsumoto, Nagano, Japan.*

Background: Sulfatide is a major component of glycosphingolipids in lipoproteins. Recently, we reported that a low serum level of sulfatide in hemodialysis patients is related to the high incidence of cardiovascular diseases. This earlier study suggests that the deterioration of serum sulfatide level would be a novel risk factor of cardiovascular disease. However, the serum kinetics of sulfatide in kidney disease patients and the function of endogenous serum sulfatide are still unclear.

Methods: To obtain novel knowledge concerning these issues, we investigated the serum kinetics of sulfatide in 5 adult kidney transplant recipients. We also analyzed the correlated factors influencing the serum sulfatide level, using multiple regression analysis.

Results: Kidney transplantation caused a dramatic increase of serum sulfatide without an alteration of its composition in all recipients in a time-dependent manner; however, the recovery speed was slower than that of the improvement of kidney function and the serum sulfatide reached a nearly normal level after 1 year. Multiple regression analysis showed that the significant correlated factor influencing the serum sulfatide level was log duration (time parameter) throughout the observation period, and the correlated factors detected in the stable phase were the decrease of serum concentration of malondialdehyde (an oxidative stress marker) as well as the elevation of platelet count.

Conclusions: The current study results demonstrate the deterioration of serum sulfatide level in ESRD patients is dramatically improved by kidney transplantation for the first time. The recovery of serum sulfatide might derive from the attenuation of systemic oxidative stress. The normal level of serum sulfatide in kidney transplant recipients might affect platelet function, and contribute to the reduction of cardiovascular disease incidence.

TH-PO973

Cardiovascular Risk and Stress Testing in Kidney Transplantation Rowena B. Delos Santos, Jagdeep Obhrai, Suzanne Watnick. *Division of Nephrology, Oregon Health and Science University, Portland, OR.*

Background: Kidney transplantation improves quality of life and life expectancy in the ESRD population. Cardiovascular disease is a significant cause of morbidity and mortality in this population. We aimed to identify baseline characteristics predictive of a positive stress test as well as cardiovascular outcomes in kidney transplant patients.

Methods: We conducted a retrospective review of 679 kidney transplant patients who underwent cardiac stress testing using exercise and pharmacological modalities with nuclear or echocardiography imaging. The composite outcome included: new MI, ACS, CVA, cardiac death, arrhythmias. We used logistic regression to evaluate eleven characteristics for their contribution to a positive stress test then at their contribution to cardiovascular outcomes along with a positive stress.

Results:

Multivariable logistic regression of predictive characteristics for positive stress and composite outcome

	Positive stress test		Composite outcome	
Predictor Variable	Odds ratio	p-value	Odds ratio	p-value
Age	1.1	< 0.001	1.0	< 0.001
BMI	1.0	0.02	1.0	0.8
Gender	1.4	0.09	0.9	0.9
Race	1.2	0.1	1.0	0.8
Smoking	1.1	0.6	0.9	0.6
MI/ACS	3.3	0.004	3.0	0.01
Diabetes	2.3	<0.001	1.9	0.02
METS	0.9	0.7	0.9	0.5
Hypertension	1.2	0.5	1.2	0.6
Dyslipidemia	1.1	0.6	1.0	0.9
Months on dialysis	1.0	0.02	1.0	0.1
Positive stress test	NA	NA	1.3	0.3

Of 679 patients, 158 had a positive stress test. The multivariable logistic regression showed age, prior cardiac disease, body mass index, diabetes and dialysis vintage were predictive of a positive stress test; metabolic equivalents were not. After adding a positive stress test to the model, only age, diabetes, and previous cardiac disease were significant.

Conclusions: Traditional and kidney specific risk factors are predictive of a positive stress test, but not necessarily cardiovascular outcomes. Some traditional predictors were not significant. Current recommendations for pre-operative testing may not be sufficient to predict cardiovascular outcomes post transplantation. Future studies could investigate methods of better identifying pre-kidney transplant patients who are at higher risk for post-transplant cardiovascular events.

Funding: Private Foundation Support

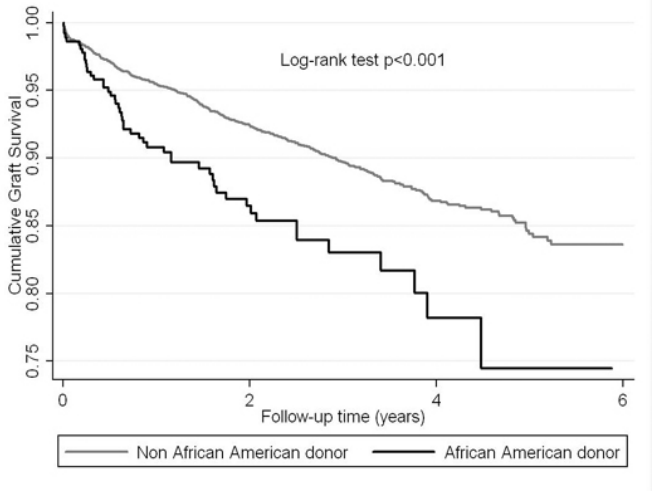
TH-PO974

African American Donor Race Is Associated with Increased All-Cause and Cardiovascular Mortality and Graft Loss, but Not with Delayed Graft Function Miklos Z. Molnar,[1,2] Csaba P. Kovesdy,[3] Suphamai Bunnapradist,[4] Elani Streja,[1] Mahesh Krishnan,[5] Allen R. Nissenson,[5] Istvan Mucsi,[2,6] Keith C. Norris,[4] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]Salem VA Medical Center, Salem, VA; [4]David Geffen School of Medicine at UCLA, Los Angeles, CA; [5]DaVita, Inc, Denver, CO; [6]McGill University, Montreal, QC, Canada.*

Background: In the last few years genetic factors were identified to explain the higher prevalence of end stage renal disease in African American (AA). We examined associations of donor race & post-transplant outcomes in a large national cohort of kidney transplant recipients.

Methods: Linking the 5-year patient data of a large dialysis organization to the *Scientific Registry of Transplant Recipients*, we identified 13692 hemodialysis patients who underwent kidney transplantation. Mortality or graft failure & delayed graft function (DGF) risks were estimated by Cox regression and logistic regression, respectively.

Results: Patients were 48±14 years old and included 39% women and 26% diabetics. AA donor race was associated with 39%, 80% & 30% higher all-cause mortality (1.39(1.09-1.78)), cardiovascular mortality (1.80(1.17-2.76)) & death-censored graft loss (1.30(1.03-1.64)), respectively over the 6-year observation period after adjusting for several relevant clinical & transplant-related variables. In non-AA recipients AA donor race was associated with significantly higher risk of graft loss.



In AA recipients AA donor race was not associated with significantly higher risk of outcomes. The risk of DGF did not show association with AA donor race.

Conclusions: AA donor race was associated with increased all-cause & cardiovascular mortality in non-AA recipients & graft loss, but not with DGF.

Funding: NIDDK Support

TH-PO975

Effects of Kidney Transplantation on Echocardiographic Abnormalities Ana Sofia Rocha, Nihil Chitalia, Helen Gregson, Rajan Sharma, Juan Carlos Kaski, Debasish Banerjee. *Renal & Transplantation and Cardiology Units, St Georges Hospital, London, United Kingdom.*

Background: Kidney transplantation (KT) decreases cardiovascular (CV) events in patients with chronic kidney disease, but reasons for this are unclear. Cardiac echocardiographic (ECHO) abnormalities are predictors of CV mortality, but scant data exist on long-term progression of these alterations after KT.

Methods: This study aimed to assess the progression of ECHO variables in KT recipients compared to patients who remained on the waiting list.

The study cohort included all patients on transplant waiting list with repeat ECHO at least one year apart; and all transplanted patients at least one year after KT, between January 2004 and December 2010. We excluded patients with previous AMI, valve heart disease and other structural cardiac abnormalities.

Results: We assessed 79 patients (57% male; age 55±11 [mean±SD] years; 27% diabetes); of these, 63 patients remained on waiting list (51% on dialysis) and 16 underwent KT. Of the latter, 12 (75%) were on dialysis for a median duration of 17±29 mo, and 4 (25%) had preemptive transplants.

On baseline ECHO, left ventricular (LV) hypertrophy [left ventricular mass index (LVMI) ≥110 g/m in females, ≥125 g/m² in males] using the Penn formula and indexed for height, was present in 54% of patients, left atrium (LA) enlargement (LA≥4 cm) in 41% and systolic dysfunction [fractional shortening (FS) ≤30%] in 23%.

Follow-up ECHOs (performed 35±20 mo after baseline ECHOs) on patients who remained on waiting list showed significant increases in LVMI (133±43 *vs.* 146±50 g/m, *P*=0.018) and LA diameter (3.8±0.6 *vs.* 4.1±0.8 cm, *P*=0.02), whereas in KT recipients, LVMI (135±48 *vs.* 136±43 g/m, *P*=0.9) and LA diameter (4.0±0.7 *vs.* 4.0±0.7 cm, *P*=0.8) did not change significantly. In KT patients, FS increased (32±6 *vs.* 41±10%, *P*=0.03), whilst it did not change in patients on the waiting list (37±8 *vs.* 36±9%, *P*=0.7).

Conclusions: These data suggest that kidney KT has a favorable impact on ECHO abnormalities by halting progression of LVH and LA enlargement, and improving systolic dysfunction. This may explain, at least in part, the reduced CV mortality reported in kidney transplant recipients.

TH-PO976

EFfect of Left Ventricular Dysfunction on the Development of Renal Transplantation. Case-Control Study with Paired Kidneys from the Same Donor Maria Moya,[1] M. Teresa Mora,[3] Esther Gonzalez Monte,[2] Laura Garcia-Puente Suarez,[2] Ana Huerta,[2] Manuel Praga,[2] Amado Andres.[2] *[1]Nephrology, H.U. La Princesa, Madrid, Spain; [2]Nephrology, H. U. 12 de Octubre, Madrid, Spain; [3]Nephrology, H. San Pedro de Alcantata, Caceres, Extremadura, Spain.*

Background: Heart failure secondary to ventricular dysfunction (VD) is relatively common in dialysis patients.This group of patients usually have a high surgical risk and it isn't clear whether they should be included in the renal transplantation waiting list.There is few data on the evolution of renal transplantation in this population.The aim of our study is to analyze a group of transplant patients with VD (EF≤50%) and asses the evolution of renal function and mortality after transplantation.

Methods: We performed a retrospective study analysing all patients with VD transplanted from January 2005-December 2010 in our medical center and compared them with a control group consisting of their partner transplants, who had received the contralateral kidney from the same donor.

Results: A total of 30 patients (40% male) 15p with VD and 15p without cardiac lesions were analyzed.Mean age was 54.6±15.7 years, 70% were a first transplant. Analyses carried out between the two groups are shown in the table.

	No ventricular injury (15p)	Ventricular dysfunction (15p)	p
Age (years)	55±16	54±16	NS
Delayed graft funtion (%)	33.3	53.3	NS
ATN days	4,5±4,1	9,7±7	0,01
Number of HD sessions	1,6 (r0-6)	3,4(r0-12)	NS
Kidney allograft loss (%)	6,6	13,3	NS
No primary function (%)	0	6,6	NS
Final follow-up sCr	1,3±0,5	2,1±1,9	0,01
Nr of admissions to hospital	1,5±1,4	3±2,6	0,04
Graft survival (%)	100	80	0,05
Patient survival (%)	93,4	80	NS
Follow-up time (months)	43±12	37±19	

In these patients (VD group) there is a tendency to a higher rate of delayed graft function, non-primary function and need of more hemodialysis sessions prior to renal allograft function. Graft survival is lower and there is overall worse final renal allograft function.

Conclusions: Despite the limited number of patients in the study, our data suggest that cardiac evaluation should be performed in all patients on hemodialysis, and the indication for kidney transplantation in patients with VD with an EF≤50% carefully evaluated.

TH-PO977

Arrhythmias and Sudden Cardiac Death Continue To Be Significant Events in Post-Kidney Transplant Patients Rowena B. Delos Santos,[1] Jagdeep Obhrai,[2] Suzanne Watnick.[1] *[1]Medicine, Division of Nephrology and Hypertension, OHSU, Portland, OR; [2]Medicine, Transplant Section, OHSU, Portland, OR.*

Background: Kidney transplant candidates undergo extensive cardiac evaluation for coronary artery disease. However, these tests do not detect the risk for arrhythmias and sudden cardiac death, which can be unrelated to coronary artery disease sequelae. We sought to investigate the rates and proportions of arrhythmias and sudden cardiac death in kidney transplant patients to compare them to those on dialysis.

Methods: We conducted a retrospective chart review of 679 post-kidney transplant patients from two transplant centers in Portland, OR. Specific outcomes of interest included death from cardiac cause, arrhythmias, acute coronary syndome, myocardial infarction and congestive heart failure episodes. Descriptive analyses were conducted and outcomes are expressed as percentages.

Results:

Cardiovascular outcomes post kidney transplant in 679 patients

Outcome	Total events N = 135 (%)
CHF	27 (20%)
ACS	16 (12%)
MI	17 (13%)
Arrhythmias	52 (38%)
Afib/Aflutter	32 (24%)
Vtach/Vfib	4 (3%)
PEA	4 (3%)
SVT/other rhythm	9 (6%)
Other rhythm nos	3 (2%)
Death CV cause	12 (9%)
Sudden death	7 (5%)
MI	2 (1%)
CHF	1 (1%)
Death non-CV cause	11 (8%)

A total of 135 events occurred in 679 post transplant patients. Of these events, 20 were arrhythmias that were not atrial fibrillation or atrial flutter while 7 events were sudden cardiac death. We found that of all these events, 38% were arrhythmias, 13% were MI, 12% were ACS, 20% were CHF. For causes of death, 9% were due to cardiovascular causes of death while 8% were due to non-cardiovascular causes of death. In comparison, rates for the ESRD population in general per USRDS data on causes of death include: arrhythmia/ arrest 26%, acute MI 5%, CHF 5% and CVA 4%.

Conclusions: Despite significant non-invasive and invasive cardiovascular testing prior to transplant, these patients have a large percentage of events that may not necessarily be related to coronary artery disease, which is similar to the ESRD population. Our cardiac evaluations may not be sufficient to predict other significant cardiac events.

Funding: Private Foundation Support

TH-PO978

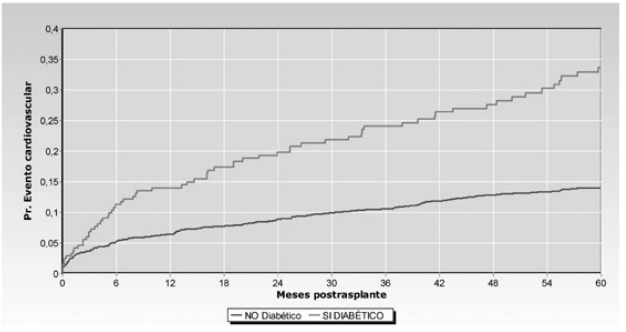
Pretransplant Diabetes Mellitus Doubles the Risk of Cardiovascular Events after Renal Transplantation. A Prospective Multicenter Study Jose M. Morales,[1] Robert Marcen-Letosa,[2] Amado Andres.[1] *[1]S. Nefrologia, H. 12 de Octubre, Madrid, Spain; [2]Grupo Forum Renal, Spain.*

Background: Cardiovascular disease (CD) is the main cause of death after transplantation. The aim of study was to investigate the influence of pretransplant diabetes (Pre-DM) on the presence of posttransplant cardiovascular events and survival figures after renal transplantation (RTx).

Methods: From a database of 2591 RTx patients performed in Spain during 2000-02 we prospectively analyzed the results at 5yr in patients with Pre-DM (n: 247, 9.5%) vs non-diabetic patients (n:2344, 90.4%)

Results: Pre-DM patients were significant older, males, with a higher body mass index and 30% of them had had higher pre-transplant CVE (30.4% vs 13% p 0.001 vs

non-diabetics). Immunosuppression was Steroids, Tac/CyA and MMF. No difference in acute rejection at 1yr (15% Pre-DM vs 16.7 % non-diabetic, p ns) and acute tubular necrosis (ATN) among groups. Graft survival at 5yr were lower in Pre-DM (uncensored death 63.8% vs 78%, censored death 77.8 vs 85% p<0.05). Patient death was the most frequent cause of graft loss in both groups (34.7% vs 27.8%) Patient survival was 78.5% vs 90% p<0.05) and CD and infections were the most frequent causes of death. Cox-regression model showed that donor age, patient age, ATN, pretransplant cardiovascular events, hepatitis C and pre-DM were independent risk factors for mortality. Pre-DM patients exhibited more major CVE during follow-up and the presence of diabetes doubled the risk of cardiovascular events (HR 2.03, 1.40-2.92% p<0.001).



Conclusions: RTx in patients with pre-DM offer acceptable results although showed lower survival figures at 5yr than non-diabetic patients. The presence of Pre-DM doubles the risk for posttransplant CD events. Therefore, pre and postransplant measures to improve CD especially in diabetic patients are mandatory

Funding: Pharmaceutical Company Support

TH-PO979

Rate of mGFR Decline Associates with Mortality in Transplant Kidney Recipient Olivier Moranne,[1] Lise Thibaudin,[2] Nicolas Maillard,[3] Christopher R. Mariat.[3] *[1]Nephrology & Public Health, Hospital, Nice, France; [2]Service de Néphrologie et Laboratoire d'Explorations Fonctionnelles Rénales, CHU de Saint-Etienne, France; [3]Nephrology CHU de Saint-Etienne, France.*

Background: Longitudinal declines in kidney function were found independently associated with increased all-cause mortality in adults and older with native kidney. Based on these observations, we wanted to test this hypothesis in transplant kidney recipient.

Methods: Out of 610 patients having received a kidney transplant between 1989 and 2000 at our institution, 488 (86%) were longitudinally screened for their GFR by performing urinary clearance of Inuline (mGFR) at one year post-transplant and then every 5 years. The mean follow-up was 12 ± 4 yrs with a total of 1,330 mGFR. Annual individual slopes in mGFR were calculated with joint modeling random effect and secondary divided into quartiles of mGFR decline. Crude and adjusted hazard ratios (HR) of quartiles of slope, for all cause mortality (i.e. death censored-graft loss and death occurring before or after starting dialysis) were analyzed into a Cox regression model.

Results: Baseline recipient characteristics were as follows : mean age 47±19 yrs, men 70%, diabetes 6%, first kidney transplant in 84%, preemptive transplantation in 7% ; donor age 38±14 yrs. After one year post-transplant: inulin clearance: 46±19 ml/min/1.73m2 and Uprot/creat > 300 mg/g in 20% of patients. 89% were treated with anticalcineurin inhibitor. During follow-up, 136 patients (28%) returned dialysis and 139 deceased (after returning dialysis for 30 of them). The mean [IQ] slope of mGFR for the whole population was -1.8 [-2.8;-0.5] mL/min/1.73m2/yr. HR of quartiles of mGFR slope for all cause mortality censored graft loss and after adjustment on mGFR (at one yr), sex, age recipient, diabetes, proteinuria, cohort period and dialysis duration, were: Q4: HR=2.7 [1.1;6.5] ; Q3 : HR=4.1 [1.9;8.5] ; Q2 : HR=3.5 [1.7;7.1] vs Q1). The association persisted with adjustment on other covariables and were stronger when the death was considered before and after dialysis.

Conclusions: mGFR slope after one year post-transplant is independently associated with all cause mortality risk.

TH-PO980

Left Ventricular Mass in South-East Asian Patients Awaiting Kidney Transplantation Nihil Chitalia, Ana Sofia Rocha, Dimitrios J. Poulikakos, Rajan Sharma, Debasish Banerjee. *Renal and Cardiology Units, St Georges Hospital, London, United Kingdom.*

Background: South-East Asian kidney transplant patients from UK have better survival when compared to other ethnic groups, the reason for which is unclear. Mortality in kidney transplant patients is predominantly cardiovascular, highlighting the possible importance of left ventricular hypertrophy. Ethnicity-related differences in left ventricular (LV) structure have been described in general population, but have not been investigated in patients with chronic kidney disease (CKD).

Methods: This study assessed differences in echocardiographic parameters in a multi-ethnic UK population of patients awaiting kidney transplantation.

The study cohort included 77 patients awaiting kidney transplantation. Left ventricular mass index (LVMI) was calculated using Penn formula and indexed for height. Patients with recent MI, major heart valve disease and other cardiac structural pathology were excluded.

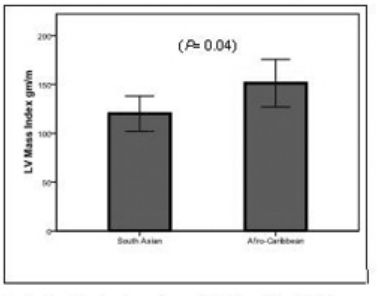
Results: Data were collected in 77 patients (55% male; age of 55±11 [mean±sd] years; 28% diabetes; 46% pre-dialysis, 39% haemodialysis and 15% peritoneal dialysis). 34 (44%) were Caucasian, 25 (32%) South-East Asians and 18 (23%) Afro-Caribbeans. Clinical and echocardiographic data are in table 1. South-east Asians had lower LVMI compared to Afro-Caribbeans (120±44 *v.* 151±50 g/m, *P*=0.04).

Table 1: Comparison of demographic, clinical and echocardiographic parameters across difference ethnic groups in patients on kidney transplant waiting list (n=77)

Variables	South Asian (n=25)	Caucasians (n=34)	Afro-Caribbean (n=20)	*P* value
Age (*years*)	57±11	56±10	51±12	0.24
Gender (*% males*)	48	59	65	0.49
Hypertension (%)	92	94	95	0.91
Diabetes (%)	44	12	30	0.02*
Systolic BP (*mmHg*)	142±17	138±21	147±15	0.38
Pulse pressure (*mmHg*)	65±16	55±16	51±24	0.08
BMI (*kg/m²*)	26±4	27±3	25±3	0.13
Left atrial diameter (*cm*)	3.68±0.63	4.00±0.68	3.98±0.61	0.20
IVS (*cm*)	0.97±0.20	1.08±0.16	1.13±0.21	0.02*
LVPW (*cm*)	0.95±0.16	1.02±0.13	1.06±0.20	0.06
LVEDD (*cm*)	4.66±0.59	4.94±0.53	5.00±0.65	0.10
LVM (*gm*)	187±75	230±63	252±91	0.01*
LVMI (*gm/m*)	120±44	136±38	151±50	0.84
FS (%)	35±7	35±6	36±8	0.50

BP, blood pressure; BMI, body mass index; IVS, intraventricular septum; LVPW, left ventricular posterior wall; LVEDD, left ventricular end-diastolic diameter; LVM, left ventricular mass; LVMI, left ventricular mass index; FS, fractional shortening.
* On ANOVA test, there were statically significant differences in prevalence of diabetes, IVS thickness and LVM between the groups.

Figure 1: LVMI in South-East Asians and Afro-Caribbeans.



South-East Asians have lower LVMI than Afro-Caribbeans (120±44 vs. 151±50 g/m, *P*=0.04)

Intraventricular septum was less thick in South-East Asians compared to Afro-Caribbeans (0.97±0.20 v. 1.13±0.21 cm); however the posterior wall thickness and LV diameter in diastole were not different between the two groups (table 1).

Conclusions: These data demonstrate and LVMI and LV wall thickness are lower in South-East Asian kidney transplant recipients. Less left ventricular hypertrophy may partially explain the improved survival in South-East Asian patients who receive kidney transplantation.

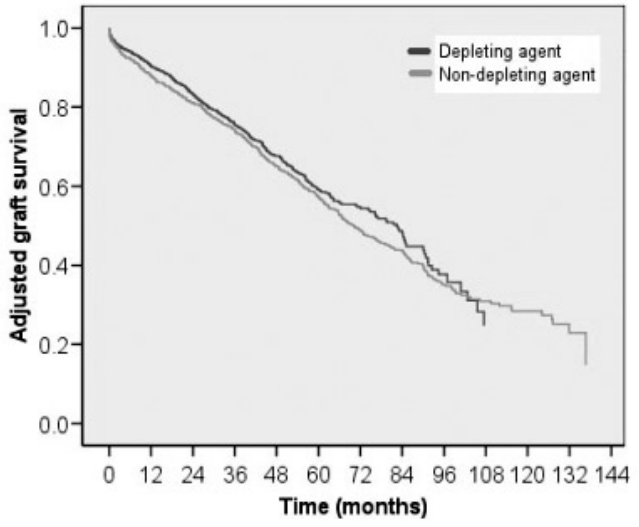
TH-PO981

Kidney Transplantation in Hepatitis C Positive Recipients: Does Type of Induction Influence Outcome? Kalathil K. Sureshkumar,[1] Ngoc L. Thai,[2] Richard J. Marcus.[1] *[1]Nephrolgy and Hypertension, Allegheny General Hospital; [2]Abdominal Trasnplantation, Allegheny General Hospital, Pittsburgh, PA.*

Background: Kidney transplantation in hepatitis C virus seropositive recipients (HCVR+) without advanced liver disease is associated with improved survival compared to staying on waiting-list. A concern for using depleting (versus non-depleting) induction agent for kidney transplantation in HCVR+ is the possibility that the associated enhanced immunusuppression might favor hepatitis C progression leading to adverse outcomes.

Methods: Using OPTN/UNOS STAR files, we identified HCVR+ who underwent deceased-donor kidney transplantation from either HCV seropositive or negative donors (HCVD+ and HCVD-) from 1998 to 2008 and received induction with either a depleting agent (rabbit-antithymocyte globulin or alemtuzumab) (n=1859) or a non-depleting agent (basiliximab or daclizumab) (n=1631). A multi-variate analysis was performed using a Cox regression model to evaluate the independent effects of the type of induction on graft and patient outcomes. Donor, recipient and transplant related covariates thought to affect the outcomes were included in the model.

Results: The median follow-up was 35.5 months (range 18.2 to 59.7 months). Adjusted graft survial for the two groups are shown in figure.



Compared to non-depleting induction, depleting agent induction was associated with neither inferior graft (HR 1.11, 95% CI 0.96-1.28, p=0.16) nor patient (HR 1.12, 95% CI 0.92-1.37, p=0.25) survival. HCVD+ status did not adversly affect either graft (HR 1.11, 95% CI 0.96-1.29, p=0.17) or patient (HR 1.15, 95% CI 0.93-1.42, p=0.2) outcomes.

Conclusions: In summary, our analysis supports the practice of transplanting HCVD+ kidneys into HCVR+ in order to alleviate waiting-list burden in deceased-donor kidney transplantation. Hepatitis C seropositivity should not influence the selection of induction agent.

TH-PO982

Despite More Advanced Kidney Disease, Simultaneous Liver-Kidney Transplant (SLK) Recipients Have Comparable Renal Outcomes in the Pre and Post MELD Era and Are Similar to Deceased Donor Kidney Transplants (DDKT) Mireille El Ters,[1] Ziad El-Zoghby,[1] Stephen C. Textor,[1,2] Fernando G. Cosio,[1,2] Charles B. Rosen.[2] *[1]Nephrology, Mayo Clinic; [2]William Von Liebig Transplant Center, Mayo Clinic.*

Background: Since the introduction of the Model for End-Stage Liver Disease (MELD) in 2002, SLK have been more widely performed in patients with advanced kidney disease raising concerns about possible worsening outcomes. The goal of this study was to compare SLK outcomes in the pre and post MELD era.

Methods: We reviewed clinical data of all 94 SLK adult recipients at our institution (n=35 pre & 59 post MELD). We compared 1) the SLK outcomes in the pre and post MELD era and 2) the outcomes of SLK with DDKT (n=267) in the post MELD era.

Results: The proportion of SLK among all liver transplants increased from 2.8% pre MELD to 7.8% post MELD (p<0.001). Post MELD recipients were slightly older (median age 56 vs. 53 years; p=0.16), had higher MELD score (median 26 vs. 20; p=0.1) and were more commonly on dialysis (68% vs. 62%; p=0.56) with longer dialysis time (22.6 vs. 19 weeks; p=0.85). Major indications for kidney transplantation differed: primary hyperoxalosis in the pre MELD era (31.4% vs. 13.6% post MELD; p =0.037) and acute tubular necrosis/hepato-renal syndrome in the post MELD era (25.4% vs. 8.6% pre MELD; p=0.045). During a median follow up of 4.5 years, 29 patients died (30.9%) and 36 (38%) lost their kidney graft. The most common cause of graft loss was death (n=21, 58.3%) followed by medical complications (6, 16.7%). There was a trend towards better patient and graft survival in the post MELD era.

	Year 1	Year 3	Year 5
Patient Survival*			
Pre MELD	82.3%	80%	71.2%
Post MELD	91.4%	88.3%	84.4%
Graft Survival**			
Pre MELD	77.2%	68.6%	65.6
Post MELD	87.9%	82.3%	71.7%

Pre/Post MELD comparasion: * p =0.56; ** p=0.75 (Log-Rank)

SLK and DDKT graft survival was similar (87.9% vs. 91.8% at 1 year; 82.8% vs. 82% at 3 years and 71.1% vs. 76.2% at 5 years, respectively).

Conclusions: Indications for kidney transplant in SLK recipients changed in the post MELD era however patient & graft outcomes remain comparable. Similarly, kidney graft survival is comparable between SLK and DDKT recipients.

TH-PO983

The Renal Outcome of Hepatorenal Syndrome after Liver Transplantation Yun Jung Oh,[1] Jung Pyo Lee,[2] Do Hyoung Kim,[1] Hyuk Yong Kwon,[1] Yun Kyu Oh,[2] Chun Soo Lim,[2] Yon Su Kim.[1] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea.*

Background: Hepatorenal syndrome (HRS) is a well-recognized serious complication of end-stage liver disease. Liver transplantation (LTx) is a treatment of choice for the patients. However, HRS is a risk factor for posttransplant chronic kidney disease and mortality. Here, we evaluated change of renal function after LTx and posttransplant recipients' survival in patients with HRS, and analyzed the risk factors for non-recovery of renal function.

Methods: Among 764 consecutive adult Korean patients underwent LTx in a cohort of a single Asian center from 1995 to 2009, a total of 76 patients, who were satisfied with HRS criteria of the International Ascites Club, were enrolled. Patients with prerenal failure, nephrotoxic renal failure, or parenchymal kidney disease or under age 18 were excluded.

Results: Mean age at LTx was 48±9.8 years and proportion of male was 73.7%. The most common etiology of liver disease leading to liver transplantation was HBV (67.1%), followed by alcoholic liver disease (7.9%) and HCV (3.9%). Pretransplant serum creatinine (sCr) level was 3.12±1.71 mg/dL. After LTx, renal function was significantly improved (sCr; at 1st month: 1.52±0.59, at 6th month: 1.63±0.41, at 12th month: 1.54±0.36 mg/dL). Early mortality of recipients with HRS was significantly higher, however, it was not significantly different thereafter compared to those without HRS (3rd month: 79.4% vs 93.1%, $P< 0.01$; 5th year: 83.7% vs. 77.7%, $P> 0.05$). Renal function of 32 patients (51.6%) had been recovered at posttransplant 1 year (sCr, 1.29±0.25 mg/dL). Early resolving of HRS within posttransplant 1 month did not predict the long-term patient survival. But the kidney fucntion at 1 year could predict the patient survival. A multivariate logistic regression analysis revealed that Child-Pugh Score was a significant risk factors for non-recovery of post-LTx renal function (P=0.024, OR 1.52, 95%CI 1.06-2.1).

Conclusions: Liver transplantation is the definitive treatment for HRS, but the non-recovery of kidney function at 1 year was associated with poor patient survival.

TH-PO984

How 'Overt' Is 'Occult Hepatitis C' in Hemodialysis and Kidney Transplant Patients? Seema Baid-Agrawal,[1] Petra Reinke,[1] Ralf Schindler,[1] Ulrich Frei,[1] Thomas Berg.[2] *[1]Dept of Nephrology and Medical Intensive Care, Campus Virchow Clinic, Charite Medical University, Berlin, Germany; [2]Division of Hepatology, University Clinic of Leipzig, Leipzig, Germany.*

Background: Our aim was to asses for the first time the prevalence of a newly defined entity called 'occult hepatitis C virus (HCV) infection', i.e. presence of HCV RNA in liver or peripheral blood mononuclear cells (PBMC) in absence of serum RNA, in large cohorts of chronic hemodialyis (CHD) patients and kidney transplant recipients (KTxR).

Methods: In this cross-sectional study, 421 CHD patients (Group 1), 418 KTxR (Group 2) and 2 control groups: 25 HCV-antibody (Ab)-positive patients with chronic hepatitis C (Group 3, positive controls) and 39 HCV-Ab-negative, HBsAg-negative healthy subjects (Group 4, negative controls) were enrolled. HCV RNA was tested in serum and PBMC using Versant TMA assay (Siemens Healthcare Diagnostics).

Results: CHD (Group 1): 405/421 patients were HCV-Ab-negative (Group 1a) and 16 were Ab-positive (Group 1b). The prevalence of HCV by positive serum RNA was 2.4% (10/421), of which 20% (2/10) were HCV-Ab-negative. Occult HCV was found in 2/405 (0.5%) HCV-Ab-negative patients.

KTxR (Group 2): 403/418 KTxR were HCV-Ab-negative (Group 2a) and 15 were Ab-positive (Group 2b). Prevalence of HCV by serum RNA in this group was 4.3% (18/418), of which 50% (9/18) were HCV-Ab-negative. Occult HCV was found in 2/403 (0.5%) HCV-Ab-negative patients.

	HCV RNA positive in		
	serum only	both serum and PBMC	PBMC only
Group 1a (N=405)	0	2 (0.5%)	2* (0.5 %)
Group 1b (N=16)	1 (6.3%)	7 (43.8%)	0
Group 2a (N=403)	3 (0.74%)	6 (1.5%)	2 (0.5%)
Group 2b (N=15)	0	9 (60%)	0
Group 3 (N=25)	1 (4%)	24 (96 %)	0
Group 4 (N=39)	0	0	0

* The test was weak-positive in both CHD patients and could not be confirmed by repeat testing.

Conclusions: Prevalence of occult HCV was low in both populations. Laborious and expensive testing for occult HCV is not required for screening and diagnosis of HCV infection in these patients. Instead, detection of HCV RNA in serum using ultrasensitive assays, and not HCV-Ab testing, should be the test of choice, as a significant proportion of HCV-Ab-negative infection was found in both groups, which would have gone undetected otherwise.

Funding: Private Foundation Support

TH-PO985

Antiviral Agents Has Improved the Clinical Outcomes in Renal Transplant Recipients with Chronic Hepatitis B Hyun Gyung Kim, Chul Woo Yang, Cheol Whee Park, Yong-Soo Kim. *Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: The introduction of antiviral agents has reduced complication associated with chronic hepatitis B virus (HBV) infection, which was one of the important causes of morbidity and mortality in renal transplant recipients (RTS) with chronic hepatitis B. However, the relationship between chronic HBV infection and clinical outcome after kidney transplantation (KT) remains controversial.

Methods: Sixty-nine RTR with chronic hepatitis B were included in this study. We compared retrospectively the baseline characteristics and clinical outcomes between the treatment group with prophylactic antiviral agents and the historical control group who received no prophylactic antiviral treatment. There were 25 patients in the treatment group and 44 in the historical control group

Results: There were no significant differences in baseline clinical parameters such as gender, age at KT, primary renal disease, the dialysis modality before KT and HLA mismatch number between the treatment and the control group. Nine patients (20.4%) in the control group received salvage treatment with antiviral agents after HBV reactivation. However, there were no significant differences in lamivudine resistance. The graft survival rate in the treatment group was significantly higher than that in the control group (5-year graft survival rate 81.8% vs. 55.9%, 10-year graft survival rate 81.8% vs. 34.2%, p=0.004). The treatment group also had better patient survival as compared to the control group (5-year patient survival rate 90.9% vs. 70.4%, 10-year patient survival rate 90.9% vs. 57.4%, p=0.014). In the control group, most common cause of graft loss was patient's death (51.6%, n=16) and the leading cause of death was fulminant hepatitis (66.6%, n=14/21), while there was no HBV related death in the treatment group.

Conclusions: Before the era of effective antiviral agents, renal transplant recipients with chronic hepatitis B showed a higher incidence of graft failure and HBV-related mortality. The development of antiviral agents had improved graft and patient survival in renal transplant recipients with chronic hepatitis B.

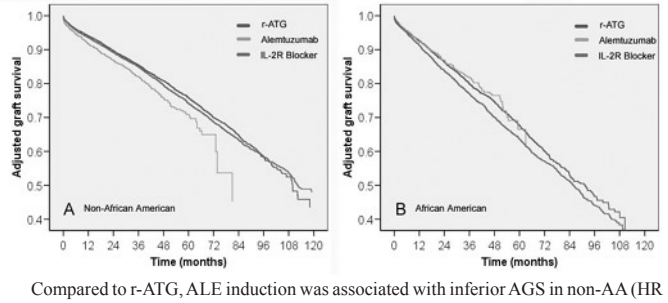
TH-PO986

Effect of Induction Agent on Post-Transplant Outcomes in Deceased Donor Kidney Transplant Recipients: Influence of Race Uzoamaka T. Nwaogwugwu, Kalathil K. Sureshkumar, Tina Y. Ko, Richard J. Marcus, Sabiha M. Hussain. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: The effect of induction agents on kidney transplant outcomes with respect to race is not well studied. We aimed to compare the outcomes of deceased donor kidney transplants (DDKT) in African American (AA) and non-AA recipients who underwent induction therapy with rabbit-antithymoglobulin (r-ATG), alemtuzumab (ALE) or IL-2 receptor blocker (IL-2B) and were maintained on a calcineurin inhibitor (CNI)/MMF based regimen with /without steroids.

Methods: Using OPTN/UNOS database, we identified patients (≥18 years) who underwent DDKT from January 2000 to December 2008 and received r-ATG (n=21,506), ALE (n=3476) or IL-2B (n=17869) and were maintained on CNI/MMF based regimen with /without steroids. Multivariate analysis was performed adjusting for steroid use and factors known to adversely impact graft outcome.

Results: The median follow up was 29.6 months (range 10.7-60.1 months). Adjusted graft survival (AGS) for AA and non-AA recipients are shown below.



Compared to r-ATG, ALE induction was associated with inferior AGS in non-AA (HR 1.29, 95% CI 1.14-1.46, p<0.001) but similar AGS in AA (HR 0.96, CI 0.80-1.14, p=0.61) recipients. AGS was similar with IL-2B induction in non-AA (HR 1.04, CI 0.98-1.1, p=0.18) but inferior in AA (HR 1.14, CI 0.80-1.14, p=0.002) recipients in comparison to r-ATG.

Adjusted patient survival was inferior with ALE (HR 1.27,CI 1.08-1.50, p=0.003) and IL-2B (HR 1.08, CI 1.0-1.2, p=0.04) inductions in non-AA but similar with ALE (HR 0.97, CI 0.75-1.26, p=0.82) and IL-2B (HR 1.07,CI 0.94-1.20, p=0.29) in AA recipients when compared to r-ATG .

Conclusions: Our study demonstrates racial differences in the outcomes following alemtuzumab induction in DDKT with inferior graft and patient survival in non AA but not in AA recipients.

TH-PO987

Residential Racial Composition Influences Outcomes Post Kidney Transplant Harini A. Chakkera,[1] Laura Szalacha,[2] Rudolph A. Rodriguez,[3] Brie N. Noble,[1] Ann M. O'Hare.[3] *[1]Mayo Clinic Arizona; [2]ASU; [3]VA Puget Sound Medical Center.*

Background: Dialysis patients living in predominantly black residential areas have less access to kidney transplant and inferior outcomes compared with patients living in other areas. Whether residential area racial composition is associated with outcomes after transplant is not known.

Methods: We used the USRDS and the 2000 US Census data to examine the association between the proportion of black residents in each patient's ZIP code of residence at the time of transplant with time to death and time to graft failure among patients who received their first kidney transplant between Jan 1, 2000 and Sept 30, 2005. ZIP codes were divided into 4 categories according to the percentage of all residents who were black: < 10%, 10 - 24.9%, 25 - 49.9% and ≥ 50%.

Results: Study cohort: 74,482 patients including 17,879 black and 51,442 white patients. Overall, 86% (n=7257) of black and 12% (n=972) of white transplant recipients lived in ZIP codes where 50% or more residents were black.

Transplant recipients living in ZIP codes with higher percentage of black residents were significantly more likely to have: higher BMI, hypertension, been treated with HD vs. PD pre-transplant, on average been on dialysis for longer pre-transplant, and less likely to be employed and be college educated, and were less likely to have received preemptive transplant and more likely to have received a deceased donor transplant.

Association of Percent of Black Residents in Neighborhood and Patient and Graft Survival

Proportion of black residents in the recipient's ZIP code	Unadjusted Patient Mortality *	Adjusted Patient Mortality * ^	Unadjusted Death Censored Graft Loss*	Adjusted Death Censored Graft Loss *^
< 10%	referent	referent	referent	referent
10- 24.9%	1.11 (1.04-1.17)	1.21 (1.07-1.36)	1.16 (1.11-1.22)	1.08 (0.96-1.20)
25- 49.9%	1.16 (1.09-1.25)	1.27 (1.10-1.49)	1.34 (1.27-1.42)	1.13 (1.01-1.27)
≥ 50%	1.29 (1.22-1.38)	1.29 (1.10-1.50)	1.54 (1.47-1.63)	1.14 (1.01-1.29)

* Hazard Ratio (95% CI) ^ adjusted for comprehensive set of recipient, transplant and donor variables

Conclusions: Patient and graft survival is worse for kidney transplant recipients living in predominantly black ZIP codes.

Funding: NIDDK Support

TH-PO988

African American Renal Transplant One Year Outcomes from the Mycophenolic Acid Observational Renal Transplant Registry Mohanram Narayanan,[1] Oleh G. Pankewycz,[2] Mohamed A. El-Ghoroury,[3] Fuad S. Shihab,[4] Anne Wiland,[5] Kevin M. McCague,[5] Laurence Chan.[6] *[1]Scott and White Healthcare; [2]Buffalo General Hospital; [3]St. Clair Specialty Physicians; [4]University of Utah; [5]Novartis; [6]University of Colorado.*

Background: The Mycophenolic Acid Observational Renal Transplant (MORE) Registry, a prospective study of de novo renal transplant recipients (RTRs) receiving mycophenolic acid (MPA) therapy, is designed to determine effectiveness, tolerability and safety of enteric-coated mycophenolate sodium (EC-MPS) vs mycophenolate mofetil (MMF) regimens.

Methods: Based on local practices at 40 US sites, outcomes analyzed included: graft survival (GS), patient survival (PS), first biopsy-proven acute rejection (BPAR), adverse event (AE) rates, serum creatinine (SCr) and proportion of RTRs maintained on full MPA dose (1.44/2.0 g/day, EC-MPS/MMF). A total of 217 African American-AA (149 EC-MPS/68 MMF) and 684 non-AA (464 EC-MPS/220 MMF) tacrolimus-treated RTRs were included.

Results: AA RTRs were less likely to receive a living donor (24 v. 49%) and more likely to experience DGF (21 v. 14%) than non-AA RTRs. Mean BMI was similar. At 1, 3, 6 and 12 months, more AA EC-MPS RTRs received full MPA dose (EC-MPS/MMF: 82.2/70.8%, p=0.07; 65.0/ 54.8%, p=0.21; 55.2/36.0%, p=0.05; 45.0/41.5%, p=0.71). Comparable 12-month effectiveness, tolerability and safety outcomes were achieved in both groups. Comparing EC-MPS to MMF in the AA RTRs, there was similar GS (94.5/97.9%, p=0.58), BPAR (14.5/13.1%, p=0.75), mean SCr (1.67/1.74 mg/dL, p= 0.77) and reported AEs by organ system, infections or neoplasia. PS (99.2/96.5%, p=0.04) was higher in the AA EC-MPS group. Similar outcomes were observed between EC-MPS and MMF in the non-AA RTRs. Comparing 12-month outcomes in AA to non-AA RTRs regardless of MPA type, BPAR (14.1/7.5%, p=0.01), GS (95.5/97.7%, p = 0.07) and SCr (1.67/1.43 mg/dL, p<0.01) were worse in the AA RTRs whereas PS (98.3/99.2%, p = 0.69) was similar.

Conclusions: More AA RTRs treated with EC-MPS were maintained on full doses of MPA. Despite this, AA RTRs exhibited higher BPAR, mean SCrs and worse GS than non-AAs which may impact clinical outcomes at later timepoints in this study.

Funding: Pharmaceutical Company Support

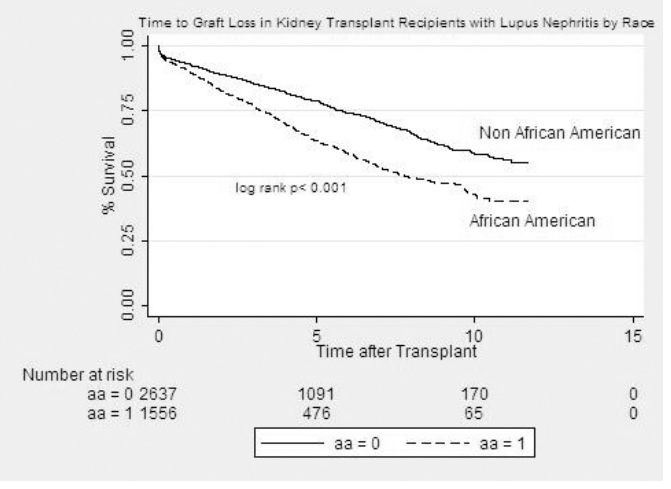
TH-PO989

Racial Differences in Allograft and Death Outcomes in Kidney Transplant Recipients with Lupus Nephritis: Analysis of United States Renal Data System Robert Nee,[1] Frank P. Hurst,[1] Lawrence Agodoa,[2] Kevin C. Abbott.[1] *[1]Department of Nephrology, Walter Reed Army Medical Center, Washington, DC; [2]NIDDK, National Institutes of Health, Bethesda, MD.*

Background: African Americans (AF) with lupus nephritis (LN) have an increased risk of graft loss compared to Caucasians in the kidney transplant (KT) population. Whether this disparity is greater than among KT patients without LN, or applies to death, has not been reported.

Methods: In a retrospective cohort of 150,118 patients first transplanted from January 1, 1995 to September 29, 2006, we identified 4,214 patients who had lupus nephritis as the primary cause of ESRD.

Results: In a Cox regression analysis, AF recipients (vs. non-AF) with LN had an increased risk of graft loss (adjusted hazard ratio [AHR] 1.41, 95% confidence interval [CI] 1.37-1.44). At 10 years, the allograft survival rate for AF was 42.9% (95% CI 38.5-47.3) as compared to 58.2% (95% CI 54.8-61.4) in non-AF. Furthermore, AF (vs. non-AF) with LN had an increased mortality rate (AHR 1.07, 95% CI 1.04-1.11). The disparity for graft loss among AF with LN was greater than among AF without LN (AHR 1.51 and 1.41, respectively; p <0.001) as well as for death (AHR 1.37 and 1.05, respectively; p< 0.001). There was significant interaction between AF race and LN for both outcomes. MMF was associated with lower risks for graft loss and death in the lupus cohort as compared to azathioprine, however this was not significant (AHR 0.89, 95% CI 0.51-1.59 and AHR 0.40, 95% CI 0.13-1.28, respectively).



Among KT recipients with ESRD due to lupus nephritis, AF are at increased risk for both graft loss and death as compared to the non-AF population.

Conclusions: The views expressed in this abstract are those of the authors and do not reflect the official policy of the National Institutes of Health, the Department of Defense, or the United States government

TH-PO990

Impact of Racial Differences in Kidney Transplant Recipients with Focal Segmental Glomerulosclerosis Adela D. Mattiazzi, Gabriel Contreras, Giselle Guerra, Warren L. Kupin, David Roth, Jochen Reiser. *Nephrology and Hypertension, University of Miami, FL.*

Background: The recipients of African Americans (AA) lineages have an overall increased risk for allograft failure. Thus, racial differences may impact allograft outcomes in different etiologies of End Stage Renal Disease including Focal Segmental Glomerulosclerosis (FSGS).

Methods: We assessed 10577 kidney transplant recipients with FSGS from the United Network for Organ Sharing files. We then evaluated if AA risk for allograft failure was independent of 1) Socio-demographic factors: donor and recipient age, gender and race-ethnicity; recipient education and insurance; donor-recipient race-ethnicity match; 2) Immunologic factors: donor type, panel reactive antibodies, ABO compatibility, HLA mismatch, pre-transplant dialysis, cytomegalovirus risk and delayed graft function (DGF); 3) Rejection. All hazard models were adjusted for transplant immunosuppression era.

Results: 6036 Caucasian (C), 3437 African (AA), and 1104 Hispanic (H) American recipients were followed for 4.84 ±3.64 years. AA and H were younger than C recipients (35, 33 and 41 years respectively) and received kidneys from younger donors (34, 33 and 37 years respectively). AA versus H and C received more kidneys from deceased donors (70, 53 and 52%) with higher two HLA loci mismatches for HLA-A (55, 44 and 37%), HLA-B (58, 52 and 39%) and HLA-DR (31, 27 and 22%). More AA versus H and C developed DGF (22, 13 and 14%) and rejection (42, 28 and 31%). 916 (27%) AA, 177 (16%) H, and 1001 (17%) C had allograft failure (p< 0.001). AA compared to C had a significantly increased hazard ratio (HR) (1.90 [95% confidence interval 1.74 to 2.08] p< 0.001) for allograft failure in the unadjusted analysis; however, their increased HR for allograft failure became non-significant (1.11 [0.79 to 1.55] p=0.56) after adjusting for transplant era, socio-demographic, immunologic differences, and rejection.

Conclusions: African Americans recipients with FSGS have a higher prevalence of risk factors for allograft failure as compared to other races.

TH-PO991

National Transplantation Pregnancy Registry (NTPR): Pregnancy Outcomes in 156 Female Kidney Recipients on Tacrolimus-Based Immunosuppression Serban Constantinescu,[1] Lisa Coscia,[2] Megan Clary,[2] Carolyn H. McGrory,[2] Michael J. Moritz,[3] Vincent T. Armenti.[2] *[1]Medicine, Temple University School of Medicine, Philadelphia, PA; [2]Surgery, Thomas Jefferson University, Philadelphia, PA; [3]Surgery, Lehigh Valley Health Network, Allentown, PA.*

Background: The purpose of this study is to describe pregnancy outcomes in 156 female kidney transplant recipients receiving tacrolimus-based immunosuppression who reported to the National Transplantation Pregnancy Registry (NTPR).

Methods: Data were collected via questionnaires, phone interviews, and medical records.

Results: There are 156 recipients who reported 250 pregnancies with 255 outcomes (including twins) to the NTPR. There was also mycophenolic acid (MPA) exposure during 61 of these pregnancies. Outcomes included: 179 (70%) livebirths, 66 (26%) spontaneous abortions, 4 therapeutic abortions, 5 stillbirths, and 1 ectopic pregnancy. The mean gestational age of the 179 liveborn was 35.3±3.5 wks and the mean birthweight was 2,487±824 g. There were 4 neonatal deaths. Maternal comorbid conditions during pregnancy included: 55% hypertension, 31% preeclampsia, 21% infections, 9% diabetes mellitus, and 2% acute rejections. Structural malformations were reported in 13 (7.3%) of the 179 livebirths and in 7 (25.9%) of 27 livebirths with MPA exposure. Microtia, an ear deformity, was present in 4 newborn with MPA exposure. There were 30 (49%) pregnancies with MPA exposure that resulted in spontaneous abortions. In October 2007 the FDA category label changed from C to D for MPA. The majority of recipients, 122 (78%) reported adequate kidney function at last follow-up. Only 10 recipients were on dialysis, 6 with reduced/poor function, 5 had died, and 13 were lost to follow-up.

Conclusions: Female kidney transplant recipients continue to report successful pregnancy outcomes. There is an increased incidence of spontaneous abortions and a pattern of birth defects reported with exposure to MPA during pregnancy. Pregnancies in female kidney transplant recipients are high-risk and appropriate counseling and close follow-up are warranted.

Funding: Pharmaceutical Company Support

TH-PO992

Ex Vivo Study of Transplacental Transfer of Tacrolimus in Renal Transplant Recipients Marion Jobard,[2] Noel Zahr,[3] Simasotchi Christelle,[1] Alexandra Benachi,[7] Marie-Catherine Lott,[2] Laetitia Croux,[2] Christophe M. Legendre,[4, 5, 6] Eric Thervet,[5, 6, 8] Sophie Gil.[1] *[1]Pharmacy, University Paris Sud 11, Chatenay Malabry, France; [2]Pharmacy, Beclere hospital, Clamart, France; [3]Pharmacology, Pitie Salpetriere APHP, Paris, France; [4]Renal Transplantation, Necker Hospital, Paris, France; [5]Universite Paris Descartes, Paris, France; [6]Fondation CENTAURE, Paris, France; [7]Obstetrical, Beclere Hospital, Clamart, France; [8]Nephrology, Hopital European Georges Pompidou, Paris, France.*

Background: Pregnancies in renal transplant recipients (RTR) are associated with an increased incidence of complications e.g. prematurity, intrauterine growth retardation and low birth weight. The role of foetal exposure to immunosuppressive drugs is possible but no information exists for quantitative transplacental transfer. The aim of the study was to evaluate the transplacental transfer of tacrolimus (TAC), using an ex vivo human cotyledon perfusion model.

Methods: Placentas from TAC pregnant RTR (TAC, n=4) and from untreated pregnant women with uncomplicated full-term pregnancies (control, n=6) were collected immediately after delivery. Cotyledons were perfused with TAC. Maternal perfusion was validated by quantification of antipyrine as internal control. Target TAC concentrations (80ng.mL-1) were tested to investigate possible modifications of TAC transport. Main transfer parameters including Fetal Transfer Rate (FTR, foetal to maternal concentrations ratio) and Clearance Index (CI, FTR ratio of tacrolimus vs. antipyrine) were assessed. Antipyrine and TAC concentrations were determined by HPLC and mass spectrometry respectively.

Results: In the TAC group, FTR of tacrolimus was 20.5±9.1% and the CI was 0.53±0.09. In the control group, these values were 19.6±4.8% and 0.62±0.09 respectively. Difference of transfer was not significant (p = 0.15).

Conclusions: TAC transfer through placenta exists, leading to foetal exposition. Transfer is similar in both groups but kinetics are different. By increasing patient numbers, We are studying the possible mechanisms for these differences. The demonstration of the transfer questions the role of foetal exposure in the related foetal complications.

TH-PO993

Predictors of Post Operative Renal Recovery in Orthotopic Liver Transplant (OLT) Patients Experiencing Renal Dysfunction (RD) Prior to Transplantation Jose I. Iglesias,[1] Sushil Mehandru,[1] John Davis,[1] Elliot Frank,[1] Jerrold S. Levine.[2] *[1]Department of Medicine, Division of Nephrology and Department of Surgery, Jersey Shore University Medical Center, Neptune, NJ; [2]Department of Medicine, Division of Nephrology, University of Illinois at Chicago, IL.*

Background: RD commonly occurs in patients with end-stage liver disease (ESLD) awaiting OLT. In patients with ESLD and RD, studies evaluating recovery of renal function post-OLT have yielded conflicting results. Employing the UNOS database we sought to evaluate factors predictive of renal recovery in OLT recipients with pre-OLT RD, transplanted from 1989-2005.

Methods: We defined pre-OLT RD as at least one of the following: serum creatinine (SCr) ≥2 mg/dL at time of registration, dialysis requirement at time of registration, or dialysis requirement at time of transplantation. Patients were excluded if SCr was ≤1.5 at time of transplant. Primary outcome was both recovery of renal function (SCr ≤1.5 mg/dL) at time of discharge and patient survival ≥29 days.

Results: There were 1997 cases of pre-OLT RD. Renal recovery occurred in 1016 cases (51%). Stepwise logistic regression analysis identified the following factors to be independently associated with renal recovery: higher estimated glomerular filtration rate (eGFR) at registration (p=0.01), higher eGFR at time of transplant (p=0.00001), presence of ascites at transplant (p=0.009), and use of thymoglobulin induction (p=0.049). The following risk factors were identified with persistent RD: liver graft dysfunction (p=0.00001), male sex (p=0.00001), higher body mass index (p=0.001), donor age (p=0.045), and tacrolimus use (p=0.039).

Conclusions: Among ESLD patients with pre-OLT RD, a greater eGFR at registration and/or transplant may indicate a greater renal reserve and be predictive of renal recovery. While tacrolimus use was associated with persistent renal dysfunction, thymoglobulin induction may be associated with renal recovery.

This work was supported in part by Dept. HHS contract 231-00-0115. The content is the responsibility of the authors and does not reflect the views or policies or imply endorsement by the Dept. HHS or the US Gov.

Funding: Other NIH Support - This work was supported in part by Dept. HHS contract 231-00-0115. The content is the responsibility of the authors and does not reflect the views or policies or imply endorsement by the Dept. HHS or the US Gov.

TH-PO994

Cellular Infiltrates and NF-kB Subunit Signaling in Kidney Allografts of Patients with Clinical Operational Tolerance Luis Eduardo Becker,[1] Matthias Schaier,[1] Lars Kihm,[1] Marie-Luise Gross-Weissmann,[2] Martin G. Zeier,[1] Christian Morath.[1] *[1]Nephrology, University of Heidelberg, Germany; [2]Pathology, University of Heidelberg, Germany.*

Background: NF-kB plays a potential role in allograft tolerance, by orchestrating onset, resolution of inflammation and T-reg differentiation through subunit c-Rel. Our aim was to characterize the cellular infiltrates and the expression of NF-kB1, c-Rel and its upstream regulators phosphoinositide 3-kinase (PI3K)/RAC-alpha serine-threonine kinase (Akt1) in allograft biopsies from patients with spontaneous clinical operational tolerance (COT).

Methods: Paraffin fixed kidney allograft biopsies from 28 patients with COT (n=4), acute interstitial rejection (IR) (n=12) and borderline changes (BC) (n=12) with eGFR (MDRD) >40ml/min at biopsy and no significant loss of allograft function in the following 12 months were used in the study. Cellular infiltrates and immunohistochemical expression of key proteins of the NF-kB pathway were evaluated using digital image analysis software. Results are given as percentage of area positively stained, positive cells/mm^2 or percentage of positive cells/infiltrating cells.

Results: Biopsies from subjects with COT exhibited comparable amount of cellular infiltrate to IR and BC (COT: 190±161; IR 291±230; BC 178±161 cells/mm^2), but reduced c-Rel expression in the infiltrates (COT: 1.7±1.4; IR: 8.7±6.4; BC: 5.9±5.8 cells/mm^2, p=0.03). In contrast, FOXP3 positive cells in the infiltrates were markedly increased in COT as compared to IR and BC (COT: 13±5.6%; IR: 3.2±2.2%; BC: 3.4±2%, % of positive cells, p<0.01). This was paralleled by a significantly lower tubular PI3K and c-Rel expression by COT compared to IR and BC (PI3K: COT: 3.1±0.27%; IR 7.5±3.2% and BC 7.4±3.2%, % of area positively stained, p=0.03) and (c-Rel: COT: 1.4±0.51%; IR 7.2±4.3% and BC 5±3%, p=0.009). HLA-DR tubular expression was significantly lower in COT compared to IR (COT: 0.23±0.1% and IR: 0.71±0.54%, p=0.02).

Conclusions: Though displaying significant infiltrates, allografts from COT patients show less PI3K and c-Rel expression but high FOXP3 positivity.

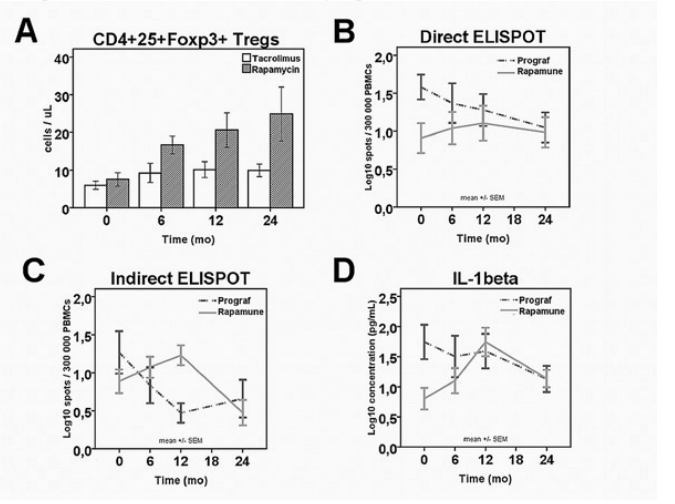
TH-PO995

Effects of Rapamycin Conversion on Cellular Immune Profile and Alloreactivity in Renal Transplant Recipients Sacha A. De Serres,[1] Monica Grafals,[1] Ciara N. Magee,[1] Luting Xu,[2] Usaila Ahmad,[1] Lorenzo G. Gallon,[2] Nader Najafian.[1] *[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Division of Nephrology, Northwestern University, Chicago, IL.*

Background: While rapamycin (Rapa) is known to expand regulatory T cells (Tregs), it has also been associated with inflammatory side effects. The aim of this study was to simultaneously characterize the Treg and donor-alloreactive T cell frequency and the cellular inflammatory profile in a cohort of renal transplant recipients enrolled in a randomized trial of conversion from tacrolimus (Tac) to Rapa at 12 months (mo) post transplant.

Methods: Blood was collected in 30 subjects (Rapa n=18; Tac n=12) at 0, 6, 12 and 24mo post randomization. T cell subset frequency was measured by flow cytometry, alloreactivity by the IFN-γ ELISPOT assay and cell culture supernatant cytokines by Luminex. Generalized estimating equations were used for analysis.

Results: Conversion to Rapa led to a sustained increase in CD4+25+Foxp3+ Tregs (Fig. A; p<0.01), but had no effect on the frequency of direct alloreactive T cells (Fig. B). Despite Treg expansion, there was a transient increase in indirect alloreactivity at 12mo, followed by a decrease at 24mo, a pattern mirrored by the cytokines IL-1B, IL-6 and TNF-α (Fig. C & D; all p<0.05). In contrast, subjects maintained on Tac displayed decreased direct and indirect alloreactive responses throughout the study (p≤0.01). The direct and indirect alloresponses were similar between the two groups at 24mo.



Conclusions: These data suggest that, despite Treg expansion, conversion to rapamycin results in a transient inflammatory response that concurs with increased indirect alloreactivity. We speculate that Tregs may initially be functionally impaired by the inflammatory milieu, but eventually succeed in attenuating the alloimmune response.

TH-PO996

Differential Impacts of Calcineurin and Mammalian Target of Rapamycin Inhibition on Alloreactive T Helper Cells Lorenzo G. Gallon, Giovanna La Monica, Luting Xu. *Medicine/Comprehensive Transplantation Center, Northwestern University, Chicago, IL.*

Background: Immunosuppressive drugs calcineurin inhibitor (tacrolimus,TAC) and mTOR inhibitor (sirolimus,SRL) affect naïve T cell differentiation and memory T cell expansion; however, their effects on the generation and expansion of different subpopulation of T helper cells are not fully elucidated.

Methods: Alloreactive CD4 T cells generated in a MLR were enriched and re-stimulated with autologous APCs with anti-CD3 plus TAC, SRL or the combination of TAC/SRL. At the end of culture, cells were restimulated and intracellular staining was used for detection of IFN-gamma, IL-17, and FOXP3 expression. Cytokine secretions from the supernatants were quantified with flowcytomix assay. Western blot was used for detection of DNA methyltransferase1 expression. SRL-derived Treg were tested for their suppressive activity by adding them in a new MLR. Thymidine incorporation and CFSE dilution technique were used as readouts.

Results: Alloreactive Th1/Th17 cells were differentially inhibited by TAC and SRL. Over 90% of the production of IFN-gamma and IL-17 and the percentage of intracellular expression of IFN-gamma (Th1) and IL-17 (Th17) were inhibited by TAC while SRL had only a moderate effect. FOXP3 expression (Tregs) was markedly increased in SRL treatment compared to TAC (average 3-fold increase). When used in combination, TAC at 2-5ng/ml with SRL at 2.5-10ng/ml achieved the maximal effect in inhibiting the productions of IFN-gamma and IL-17 while maintaining a high level of FOXP3 expression. When mimicking an inflammatory setting by adding cytokines (IL-1beta/IL-6/TNF-alpha) to the cell cultures, there was a marked decrease of SRL-induced FOXP3+Tregs. SRL-derived Tregs expressed normal surface markers, were anergic to allostimulations, and suppressed the proliferation of allogeneic effector T cells (Th1 and Th17). SRL significantly decreases DNMT1without affecting FOXO3/FOXP3 interaction thus maintaining a long-term FOXP3 expression by stabilizing FOXP3 transcription.

Conclusions: These findings can help to guide the clinical use of immunosuppressive drugs to promote Treg expansion while controlling Th1 and Th17 alloimmunity.

TH-PO997

DR^{high+}CD45RA^{-}-Tregs Disappear Excessively in Patients with Acute Kidney Rejection, Causing a Reduction in the Suppressive Activity of the Total Treg Pool Matthias Schaier,[1] Nicole Seissler,[1] Friederike Hug,[1] Martin G. Zeier,[1] Andrea Steinborn.[2] *[1]Department of Nephrology, University of Heidelberg, Germany; [2]Department of Gynaecology, University of Heidelberg, Germany.*

Background: Recent studies show that regulatory T cells (Tregs) play an essential role in tolerance induction after organ transplantation.

Methods: In order to examine whether there are differences in the composition of the total Treg cell pool between stable transplant patients and patients with biopsy proven rejection (BPR), we compared the percentages and the functional activity of the different Treg cell subsets (DR^{high+}CD45RA^{-}-Tregs, DR^{low+}CD45RA^{-}-Tregs, DR^{-}CD45RA^{-}-Tregs, DR^{-}CD45RA^{+}-Tregs). All parameters were determined during the three different periods of time after transplantation (G2: 0-30 days, G3: 31-1000 days, G4: >1000 days).

Results: Among 157 transplant patients, 38 patients suffered from BPR. Sorting and subsequent differential testing of all four Treg cell subsets revealed that the DR^{high+}CD45RA^{-}-Tregs, and to a slightly lower degree, the DR^{low+}CD45RA^{-}-Tregs had the highest suppressive activity within the total Treg pool. The significantly reduced suppressive activity of the total Treg cell pool obtained from transplant patients with BPR correlated both with a significantly reduced HLA-DR mean fluorescence intensity (MFI) of the DR^{+}CD45RA^{-}-Treg subset and a significantly reduced percentage of DR^{high+}CD45RA^{-}-Tregs within the total Treg pool. Therefore, it could be assumed that DR^{high+}CD45RA^{-}-Tregs potentially affect the suppressive activity of the total Treg pool and that the disappearance of this Treg subset gives a strong indication for acute rejection processes.

Conclusions: Therefore, both the monitoring of its percentage within the total Treg pool and the monitoring of the HLA-DR MFI of the DR^{+}CD45RA^{-}-Treg subset may be useful tools for the prediction of graft rejection.

Funding: Clinical Revenue Support

TH-PO998

Contrasting Effects of Immunosuppression on FOXP3+ Treg Biology in Liver and Kidney Allograft Recipients Tatiana Akimova,[1] Matthew H. Levine,[1] Ulf H. Beier,[1] Binita Kamath,[2] Elizabeth Rand,[1] Kevin E.C. Meyers,[1] Jens W. Goebel,[3] John Bucuvalas,[3] Wayne W. Hancock.[1] *[1]Children's Hospital of Philadelphia and University of Pennsylvania; [2]Toronto Hospital for Sick Children; [3]Cincinnati Children's Hospital Medical Center.*

Background: The effects of calcineurin inhibitor (CNI) therapy post-transplantation (Tx) on FOXP3+ Treg numbers and function are somewhat controversial.

Methods: We analyzed Treg numbers, FOXP3 methylation and suppressive function (SF) in 12 adults (8 liver, 4 kidney with serial sampling) and 46 children (38 liver, 8 kidney) on CNI or rapamycin (RPM)-based immunosuppression.

Results: In liver Tx recipients, CNI use led to decreased Treg numbers, viability and SF compared to use of RPM, but no significant differences were seen post-kidney Tx. In children with long-term grafts (8.5±0.6 y after Tx) and on CNI, Tregs of liver Tx recipients had more FOXP3 methylation ($p<0.05$) and 2-fold weaker SF but 23% more Tregs vs. children with kidney Tx. In mice, the liver can reportedly promote Tx tolerance, at least in part, by inducing T cells to become iTregs. Compared to thymic Tregs, iTregs can have increased FOXP3 methylation and suboptimal SF. We considered if clinical liver Tx might promote Treg conversion and thereby explain the greater higher number of Tregs, but weaker SF and increased FOXP3 methylation, in clinical liver vs. kidney Tx recipients. We found that murine and human liver samples contain 5-10% of Tregs within the intrahepatic lymphocyte population, whereas kidneys had no comparable resident component. Expression of the transcription factor, Helios, is reportedly a specific marker for thymic-derived nTregs, but no differences in Treg expression of Helios were found pre- or post-liver Tx, or between Tregs of kidney vs. liver Tx recipients. However, in studies of human and murine Tregs we noted that Helios was not specific to thymic nTregs and is best viewed as a marker of cell activation.

Conclusions: Tregs of kidney Tx recipients showed an unexpected greater resistance to CNI use than Tregs of liver Tx recipients. This may reflect CNI-linked impairment of the iTreg population developing within the liver and is the subject of ongoing studies in our lab.

Funding: NIDDK Support, Other NIH Support - NIAID

TH-PO999

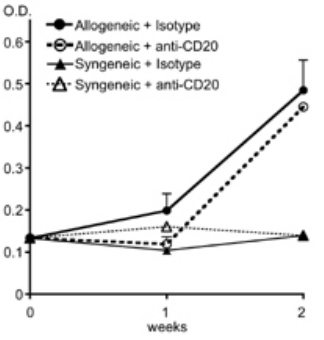
Allogeneic Transplantation Mitigates the Efficiency of B Cell Depletion with Anti-CD20 Antibody Lindsay A. Hilken, Clare E. Parker, Jagdeep Obhrai. *Medicine, Nephrology, Transplant Medicine, Oregon Health & Science University, Portland, OR.*

Background: Antibody-mediated rejection (AMR) in solid organ transplants is common, difficult to treat, and often results in irreversible organ dysfunction. Strategies to prevent AMR include B cell depletion (BCD) with monoclonal antibodies targeting CD20.

Methods: To examine the impact of BCD on the development of AMR, we treated mice with anti-CD20 antibody and then performed vascularized cardiac transplants.

Results: In mice that did not receive a transplant, CD20-targeted therapy rapidly and efficiently depleted naïve and germinal center B cells. Plasma cells (PC) in the spleen and bone marrow were also depleted but at a delayed tempo. Allogeneic transplantation reduced the efficiency of PC depletion and accelerated the repopulation of GC B cells. These recovering B cells produced high concentrations of donor-specific antibodies. Even when anti-CD20 antibody was given with sirolimus to block the T cell response, evidence of vascular antibody deposition (C4d deposition) and vasculitis was observed.

Allogeneic organ transplantation stimulated donor specific antibody expression in spite of anti-CD20 therapy



Mice received antibody (anti-CD20 or control IgG2a), and the next day received syngeneic or allogeneic heart transplants. Serum was collected from mice 1 and 2 two weeks after these treatments and was used to examine expression of donor specific antibody using a cellular ELISA. An O.D. of 0.6 equates to 4 μg/ml.

Conclusions: These data show that acute antigen challenge, such as an allogeneic transplantation, can facilitate the escape of responding B cells from BCD and that these B cells can cause the histological features of AMR. The limited efficacy of BCD, even in unsensitized animals, emphasizes the challenges of preventing AMR and highlights the need for a more detailed understanding of the processes that mediate B cell recovery after BCD.

Funding: Other NIH Support - NIAID

TH-PO1000

The Effects of Desensitization Treatment with IVIG and Rituximab on Blood Gene Expression Profiles by Microarrays Kwaku Marfo,[1] Yi Bao,[1] Robert Brent Calder,[2] Min Ling,[1] Enver Akalin.[1] *[1]Einstein/Montefiore Kidney Transplant Program; [2]Computational Genomics Facility, Albert Einstein College of Medicine, Bronx, NY.*

Background: We aimed to investigate the effects of intravenous immune globulin (IVIG) and rituximab desensitization treatment on kidney transplant rate and blood gene expression profiles by microrarrays.

Methods: We enrolled patients with PRA levels >50% and on the deceased-donor waiting list for >5 years. Patients received IVIG (2.0 g/kg) on day 0 and 30; and rituximab (375 mg/m^2) on day 15. The antibodies with mean fluorescence intensity (MFI) values > 5,000 were reported to UNET as unacceptable antigens. The gene expression profiles of blood samples collected in PAXGene were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: 40 of the 415 patients (10%) on the waiting list were eligible for desensitization treatment and 11 completed the treatment. While 15 of the remaining 29 patients (52%) received a transplant without therapy, only 2 of the 11 desensitized patients (18%) received transplant during a median follow-up of 217 days. While there were no statistically significant difference in demographics, desensitized patients had higher cPRA values (97% vs. 77%, $p=0.0005$) and more number of unacceptable antigens (39 vs. 10, $p=0.0001$). There was no significant change in the mean number of unacceptable antigens (39 ± 22 versus 39 ± 23) or reduction in the mean MFI values (11,333 ± 3,133 vs 11,289 ± 3,386). Analysis of genes chosen as significantly differentially expressed revealed downregulation of genes involved in B cells and immune system (CD79a, B and T lymphocyte associated transcript, B cell scaffold protein, CD22, CXCR5, fas apoptotic inhibitory protein). Gene set enrichment analysis using Pathogenesis Based Transcripts created by Edmonton Group demonstrated significant downregulation of B cell associated ($p=0.04$) and immunoglobulin transcripts ($p=0.03$).

Conclusions: Although, desensitization with IVIG and rituximab decreases the expression of B cell and immunoglobulin associated transcripts, it was not successful in increasing kidney transplant rate or in decreasing the number of unacceptable antigens.

TH-PO1001

De Novo Donor Specific Antibodies among Non-Desensitized Renal Transplant Patients Predicts Antibody Mediated Rejection but Does Not Predict Renal Function at 1 Year Praveen Kandula, Sanjiv Anand, Muhammad Ahmad Mujtaba, Asif A. Sharfuddin, Muhammad S. Yaqub, Tim E. Taber. *Nephrology, Indiana University School of Medicine, Indianapolis, IN.*

Background: Pre and post-transplant DSA is associated with poor kidney allograft survival in desensitized patients. The significance of routine monitoring of post-transplant de novo DSA in non-desensitized patients is not well known. We prospectively evaluated

renal transplant patients at our academic transplant center for the significance of DSA in predicting renal function at 1 year and AMR.

Methods: All patients undergoing renal transplant were offered to be enrolled in an ongoing prospective study monitoring DSA since January 2010. DSA testing was done with single antigen luminex bead assay (One lambda Inc, Lab Screen) antibody screening at 1, 2, 3 and 6 month post-transplant.

Results: In this ongoing study 140 patients have been enrolled so far. Twelve desensitized patients and 7 patients with no DSA data were excluded. Of the remaining 121 patients, 20 (16.5%) patients developed DSA. Overall mean baseline creatinine (at 1 month post-transplant) was 1.38 (±0.52) and at 1 year was 1.34 (±0.47). Mean age was 49 years (±14) and 43% were females. Twenty one (17%) patients had rejection episodes of which 19 were acute cellular rejection (ACR) and 2 were AMR. Among patients who developed DSA (n=20), 2 patients (10%) developed AMR whereas none of the patients without DSA developed AMR (p=0.02). There was no significant difference in the incidence of ACR among patients with DSA (p>0.05). Among those who developed DSA, baseline median creatinine was 1.29 (0.61-2.7) and at 1 year was 1.2 (0.6-4.1) which was not significantly different.

Conclusions: From our preliminary data development of post-transplant de novo DSA may be associated with higher incidence of AMR in non-desensitized kidney allograft recipient. However it is difficult to recommend routine monitoring of DSA in non-desensitized transplant recipients to predict AMR based on this study. DSA do not predict renal allograft function at 1 year. However larger studies with longer duration of follow up are needed to further evaluate the role of DSA.

TH-PO1002

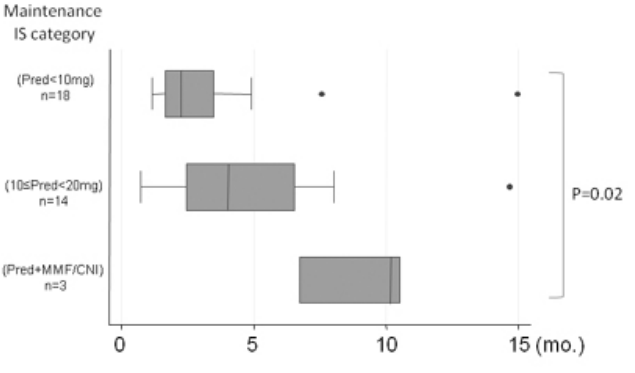
When De Novo DSA Appears after Reducing Immunosuppressants? Junichi Hoshino,[1,2] Hugo Kaneku,[2] Matthew J. Everly,[3] Paul I. Terasaki.[2,3] *[1]University of California Los Angeles, school of Public Health, Los Angeles, CA; [2]Terasaki Foundation Laboratory, Los Angeles, CA; [3]One Lambda Inc., Los Angeles, CA.*

Background: Weaning of immunosuppression (IS) is a common practice in transplant today. Based on the humoral theory, adequate HLA antibody monitoring is the most appropriate. However, the risk of de novo donor specific HLA antibody (DSA) after weaning is still unclear. Here we examine the duration of de novo DSA appearance after reducing IS.

Methods: Of the kidney transplantation patients (pts) from IKDRC-ITS, India under clonal deletion protocol, 72 pts, without pre–formed DSA, identical donor, nor splenectomy, were monitored presence of de novo DSA by LABScreen mixed/single antigen beads (one Lambda Inc.) for every month or every outpatient service. All pts had stable allograft functions and no DSA at the time of IS weaning. Positive DSA was defined as MFI >1,000.

Results: Within 72 pts (mean observation period, 12.4±8.1 months), 35 pts experienced de novo DSA. The half of them had class I DSA. To determine if the degree of reduction was important, we further evaluated patients into three groups: group 1 – 18 out of 26 pts on <10mg of prednisone(Pred) alone after weaning, Group 2 – 14 out of 24 pts on Pred ≥10mg after weaning, and Group 3– 3 out of 22 pts on Pred ≥10mg and MMF (or CNI). The mean time to de novo DSA was shorter according to IS levels (Group1, 3.3±3.2; Group 2, 4.8±3.6; and Group 3, 9.1±2.1 months, respectively) (p=0.02, Kruskal-Wallis).

Figure 1:Month after IS weaning to de novo DSA



Conclusions: This study highlights de novo DSA appears rapidly after weaning in low dose IS condition. Frequent HLA monitoring could be used to allow for early recognition of humoral activation when scheduled weaning is initiated.

TH-PO1003

Novel Risk Factors for Antibody-Mediated Rejection in Luminex Based Desensitization Strategies Maha A. Mohamed, David P. Foley, Brenda L. Muth, Vijay Vidyasagar, John D. Pirsch, Jose R. Torrealba, Dixon Kaufman, Arjang Djamali. *Medicine and Surgery, UW-SMPH, Madison, WI.*

Background: We sought to determine novel risk factors for acute rejection in Luminex-based desensitization strategies.

Methods: We performed a prospective analysis of 116 consecutive patients with a negative CDC crossmatch. All patients were desensitized based on pre-transplant immunodominant DSA (SAB-Luminex one-Lambda)(n=47, 12, 6, 16 and 35 in protocols D1, D2, D3, D4 and D5).

Desensitization Protocols

Protocol	Donor	iDSA	Induction	PE + IVIG	TAC + MPA
D1	Live	100-500	Simulect		Day -7
D2	Live	501-1000	Simulect	Days (-3, -1, 1, 3)	Day -7
D3	Live	1001-3000	Simulect	Days (-7, -5, -3, -1, 1, 3)	Day -7
D4	Deceased	500-1000	Thymo (5-7 mg/kg)		
D5	Deceased	1001-3000	Thymo (5-7 mg/kg)	Days (-1, 1, 3)	

Results: Mean peak PRA and DSA at transplant were 40% and 894 MFI respectively. There was a significant association between DSA, PRA and flow crossmatch (Fig 1)

Figure 1. There was a linear correlation between DSA, peak PRA and positive FXM

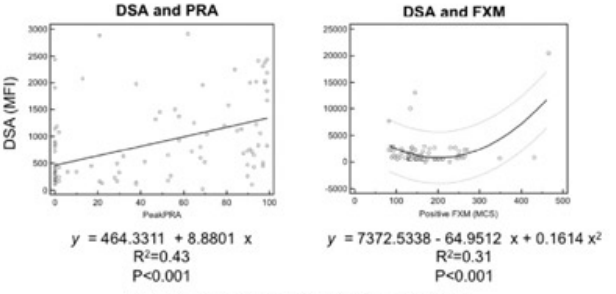
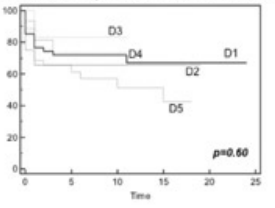


Figure 2. Acute Rejection after desensitization



One year patient and graft survival were 100%. Rates of any acute rejection (including indication and protocol biopsies), acute antibody-mediated rejection (AMR), acute cellular rejection (ACR) and acute mixed rejection were at 33%, 12%, 13%, 8% in the first year. These differences were not statistically different between protocols (Fig 2). Median time to rejection was 6 months. Uni and MV regression analyses including demographics, PRA, DSA, retransplant status, immunosuppression and desensitization protocols demonstrated that delta DSA at 3 months (HR 1.003, 95% CI 1.0001 to 1.0004, p=0.0002) and positive c4d staining (focal or diffuse) in post-reperfusion biopsy (HR 4.3, 95% CI 1.6 to 11.3, p=0.002) were the most important factors associated with AMR.

Conclusions: Delta DSA at 3M and C4d staining in post-reperfusion biopsy are novel risk factors for AMR in sensitized patients undergoing Luminex based preconditioning regimens.

Funding: NIDDK Support

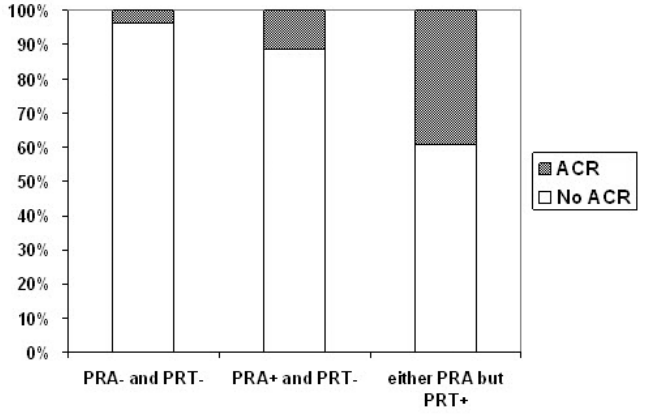
TH-PO1004

Pretransplant Cellular Allosensitization as a Predictor of Transplant Rejection Opas Traitanon, Titte Srinivas, Emilio D. Poggio. *Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

Background: Cellular alloreactivity is prevalent in dialysis patients and when present may relate to transplant rejection. It may occur independently from humoral allosensitization. Pre-transplant anti-donor alloreactivity has been shown to correlate with acute cellular rejection (ACR) but donor cells are often difficult to obtain. In this study, we evaluated recipient third party cellular alloreactivity as a surrogate for anti-donor cellular alloreactivity to predict ACR.

Methods: We prospectively studied 62 kidney transplant recipients in whom we tested peripheral blood mononuclear cells against donor when available (n=35), and a panel of third party allogeneic B cell stimulators (Panel of Reactive T cells or PRT assay) by IFN-γ ELISPOT assays at time of transplantation. The results were then correlated with ACR episodes.

Results: Mean age was 49±13 yo, 42% female, 30.6% AA. We found a correlation between anti-third party and anti-donor cellular alloreactivity (R^2=0.17, p=0.014). Percent reactivity to the panel expressed was higher in patients with ACR than in those with no ACR (53±8 vs 22±3%respectively, p=0.003). Significantly more patients with ACR were found to be PRT positive (defined as >40% reactivity to the panel) compared to those w/o ACR (78% vs 25%, p=0.002). PRT positivity (with either PRA results) predicted ACR in 39% of the recipients while PRA positivity only predicted it in 11%.(p=0.008)



Neither PRA or PRT can help predict acute humoral rejection. This result was independent of induction or immunosuppressive therapy.

Conclusions: Pretransplant third party cellular alloreactivity can serve as a surrogate of anti-donor cellular alloreactivity. PRT can complement the information obtained by PRA to predict ACR but not humoral rejection.

Funding: Other NIH Support - NIAID

TH-PO1005

Characteristics of Immune Profile in Renal Transplant Recipients with Long Term Allograft Acceptance Byung Ha Chung, Yu Ah Hong, Hyun Gyung Kim, In O Sun, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea.*

Background: We evaluated the immunologic profile in patients with stable allograft function during long term follow up compared to patients with deteriorated allograft function.

Methods: Twenty-four renal transplant recipients (RTR) who have showed stable allograft function for more than 10 years were included (Long term stable group (LS group). We compared the immunologic characteristics of these patients with age and post-transplant duration matched chronic rejection group (CR group). Patients with biopsy-proven acute rejection (AR, n=9), healthy group (HC, n=21) and end stage renal disease patients on hemodialysis (HD, n=23) was included as control group in this analysis as well.

Results: In effector T cell subset, the percentage of Th17 cell showed significant increase in the CR group compared to LS group ($P < 0.05$). The percentage of Th1 and Th2 cell did not differ significantly between LS and CR group ($P > 0.05$). In the chemokine receptor analysis, $CCR4^{-}CCR6^{+}$ T cell and $CCR4^{+}CCR6^{+}$ T cell was significantly increased in the AR group and the proportion of interleukin-17 producing in those cells was significantly increased in AR group as well (vs. all another groups, $P < 0.05$). The percentage of naïve T cell was increased in LS group compared with CR and AR group ($P < 0.05$, respectively) and the value of LS group was similar with that in HC group. In contrast, memory T cell (effector memory T cell (T_{EM}) and central memory T cell (T_{CM})) did not show significant differences between LS and CR group. In B cell subset, memory B cell was increased in LS group. Immature B cell did not differ significantly among LS, CR and AR group. The percentage of IL-10 producing immature B cell was increased in CR and AR group compared with LS group.

Conclusions: In renal transplant recipient who showed long term acceptance with minimal immune suppressant, the decrease of Th17 response was significantly dominant compared with patients with chronic rejection.

TH-PO1006

High Serum Soluble CD26 Levels in Acute Cellular Rejection in Recipients with Renal Transplant Sanjay Gupta,[1] Ankit Saxena,[2] Dipendra Kumar Mitra,[2] Amit K. Dinda,[2] Sandeep Guleria.[3] *[1]Nephrology, All India Institute of Medical Sciences, India; [2]Immunogenetics, AIIMS, India; [3]Surgery, AIIMS, India.*

Background: Cell mediated allograft rejection is one of the major causes of early renal graft dysfunction. Information regarding the presence of markers indicating activation of immune cells can help in early monitoring graft status. The T-cell activation marker, CD26 possesses dipeptidyl peptidase IV (DPP IV) enzymatic activity. Costimulatory efficacy and immunocompetence are associated with the enzymatic activity. Also CD26 is an important dipeptidly peptidases which is known to activate some of the important chemokine ligands (RANTES and CXCL10) that are involved in the recruitment of T cells in the rejected graft. Aim was to study any association of serum soluble CD26 levels with early clinical events of allograft viz acute cellular rejection (ACR), calcineurin inhibitor toxicity (CNI).

Methods: The serum sample was collected from the 44 renal transplant recipients: renal allograft dysfunction for the first time within first year n=22 and recipients with well functioning grafts (WFG)(n=22). Patients were on Tacrolimus (Tac), Mycophenolate Mofetil and Steroids. None had induction with antibodies. Allograft biopsies performed for all the graft dysfunction cases showed ACR n=11 (Banff Grade-IA-2, IIB-4, II-1, III-4) and CNI n=11. The serum soluble CD26 (sCD26) levels was analyzed by ELISA.

Results: Mean duration post transplant in ACR, CNI and WFG was 3.4±2.5, 4.8±3.4, 5.6±4.1 months respectively and mean tac levels in respective groups was 7.4±3.8, 9.1±5.7, 8.3±3.7 μg/L. Significantly high serum sCD26 levels were observed among patients with ACR (1.92±0.9 μg/ml) in comparison to the non-immunological graft dysfunction cases CNI toxicity (1.08±0.4 μg/ml (p=0.008) and WFG (1.05±0.3 μg/ml)(p=0.001). Tac levels did not correlate with sCD26 levels. All the 7 patients with sCD26 levels above 1.6μg/ml had ACR.

Conclusions: The high serum sCD26 levels are associated only with ACR suggesting T cell activation and not with CNI toxicity and well functioning graft. The sCD26 seems to be a promising biomarker to assess adequate immunosuppression in the early phase after kidney transplantation.

Funding: Government Support - Non-U.S.

TH-PO1007

Polyclonal Immunoglobulin Free Light Chains Provide a Novel insight into Immunosuppressant Use in Renal Transplant Recipients Shazia Shabir,[1] Anne Bevins,[2] Paul Cockwell,[1] Richard Borrows,[1] Colin A. Hutchison.[1] *[1]Renal Unit, University Hospital Birmingham, United Kingdom; [2]The Binding Site Group Ltd, Birmingham, United Kingdom.*

Background: Polyclonal free light chain (FLC) levels in part represent B-cell activity. The rapid clearance of FLC (2-6h) compared to immunoglobulins (Ig) (5-21 days) highlights a potential for FLCs to provide real-time monitoring of immune activity and dosing anti-proliferative medication. We investigated whether FLC levels were affected by routine immunosuppressants used in renal transplant recipients.

Methods: Serum samples were studied in two renal transplant populations: a cross-sectional prevalent cohort (n399) and an incident cohort (n40) with serial samples. Creatinine, polyclonal FLCs (κ+λ), Ig & cystatin C were measured, FLC production rates were calculated. Results were compared to a non-transplanted CKD cohort (n872).

Results: In the cross-sectional transplant cohort, total FLCs correlated with renal function, including eGFR (-0.537), cystatin C (0.594) and creatinine (0.531), all $p<0.001$. The median total FLC level(47.4mg/L, range 11.6-204.8) was lower than the CKD cohort, $p<0.001$ (62.5mg/L, 19.2-335.0), but stayed above the normal range ($p<0.001$). Patients receiving anti-proliferative therapy had lower total FLC (46.6mg/L, 11.6-262.0) than those not, $p<0.001$ (70.2mg/L, 23.2-431.0). Patients receiving Tacrolimus had lower FLC (45.0mg/L 11.6-366.0) than those on Cyclosporin, (53.0mg/L 12.3-431.0) p=0.002. There was no difference in FLCs between patients receiving prednisone or not (p=0.186). The intra-patient analysis showed a sharp decline in total FLC in 32/40 patients within 14 days of transplantation. FLC production was decreased at 2 weeks post transplant ($p<0.001$), and remained decreased over the 12month period ($p<0.001$). This was also true for IgG (2wk $p<0.001$, 12mth $p<0.001$) and IgA (2wk p=0.034, 12mth p=0.018). 26/32 patients had a gradual rise in FLC production between 2weeks and 12months as immunosuppression was reduced (p=0.001).

Conclusions: Polyclonal FLC levels vary with different immunosuppressant regimes and doses within the renal transplant population. Potentially FLC measurement could monitor immunosuppression in these patients.

TH-PO1008

In-Vitro Immunomodulatory Effect of Qu Mai (Dianthus Superbus) on Human Alloreactive T Cells Jessica A. Reid-Adam,[1] Nan Yang,[2] Ying Song,[2] Peter S. Heeger,[3] Xiumin Li.[2] *[1]Pediatric Nephrology, Mount Sinai School of Medicine, NY, NY; [2]Pediatric Allergy & Immunology, MSSM, NY, NY; [3]Nephrology, MSSM, NY, NY.*

Background: Immunosuppression for transplantation and autoimmunity is suboptimal, supporting the need for discovery of novel agents. We have been evaluating immune effects of traditional Chinese herbs.

Methods: We initially tested the effects of >50 herbs on human alloreactive T cell cytokine profiles using ELISPOT-based mixed lymphocyte cultures. This screening approach identified 4 candidate preparations that increased the IL-10/IFNγ ratio. Among these was Qu Mai (QM, Dianthus Superbus), an herb used to treat urinary tract disorders. We generated 3 fractions of QM with HPLC fingerprints and performed dose response curves for each fraction to assess effects on cytokine production in MLRs using PBMC from 12 normal volunteers. Flow cytometry was used to identify which cell types within the PBMC were altered by the therapy.

Results: Assays showed that 1 of the fractions (QM-AD, which contains flavonoid-rich compounds) exhibited a dose dependent inhibitory effect on alloinduced IFNγ (median decrease of 72% at 200 μg/ml) while enhancing IL-10 production 40 fold ($p<0.05$ vs. controls for each) and resulting in a marked change in IL-10:IFNγ ratios (0.2:1 in untreated MLR to 17:1 at 200 μg/ml, $p<0.05$). No effect on T cell apoptosis was observed. Flow cytometry and intracellular cytokine staining showed that in CD4 cells (including memory CD4 cells) QM-AD directly prevented production of IFNγ (mean 2.16% vs. 1.074% in QM-AD treated MLR), increased production of IL-10 (median 3.98% vs. 6.17% in QM-AD treated MLR), and induced CD4 CD25 Foxp3 Treg cells (mean 2.77% vs. 3.36% in QM-AD treated MLR).

Conclusions: Our data demonstrate that Chinese herbs, specifically QM-AD, contain compounds that favorably alter naïve and memory alloreactive T cell immunity toward an immune suppressive/Treg phenotype in vitro. These novel findings support the continued isolation and characterization of the QM-derived flavonoid compounds and testing them in in-vivo models as primary or adjuvant treatments of pathogenic immune responses, including autoimmunity and transplant rejection.

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TH-PO1009

PARVG Gene Polymorphism May Be Associated with Operational Renal Allograft Tolerance Eric Thervet,[2,3,4,7] Richard Danger,[4,5] Marie-Lise Grisoni,[6] Pierre Laurent-Puig,[7] Annaick Pallier,[4,5] Delphine Le Corre,[7] Christophe M. Legendre,[1,3,4] Sophie Brouard.[4,5] *[1]Renal Transplantation, Hopital Necker, Paris, France; [2]Nephrology, Hopital European Georges Pompidou, Paris, France; [3]Universite Paris Descartes, Paris, France; [4]Fondation Centaure, Paris, France; [5]ITERT, CHU Hotel DIeu, Nantes, France; [6]INSERM, INSERM UMR S937, Paris, France; [7]INSERM, INSERM UMR S775, Paris, France.*

Background: Drug-free operationally tolerant kidney recipients (TOL), with long term stable graft function and low-grade proteinuria in an immunosuppression-free environment, were characterized by a specific set of 55 genes with differential blood transcriptional expression compared to contrasted clinical situations and healthy volunteers. The aim of this study was to investigate whether these expressions could be influenced by genetic polymorphisms located in the corresponding genomic sequences and whether some of these single nucleotide polymorphisms (SNPs) could be associated with clinical status of kidney transplanted patients.

Methods: 1152 candidate tag SNPs spanning the 55 genes were genotyped using a Golden Gate Illlumina assay in a sample of 163 kidney transplant patients consisted in 11 TOL patients, 36 patients with antibody mediated chronic rejection defined by the last Banff classification (CR) and 116 patients with a stable graft function while under immunosuppressive treatment (STA). We then analyzed gene expression and clinical status according to the different SNPs.

Results: Among the genes demonstrating strong expression difference between TOL compared to CR & STA patients, PARVG, which is a member of a family of actin-binding proteins associated with focal contacts, stands out with two SNPs, (rs139144 and rs5764592) explaining about 15% of the gene expression variability. Linkage disequilibrium analysis of these two SNPs showed the rs139144-GG genotype was associated with decreased PARVG expression and tended to be more frequent in TOL (60%) than in CR&STA (28%) patients ($p = 0.068$).

Conclusions: These preliminary results that should be confirmed in a larger population open new perspective of regulation pathways and hypothesis in operational tolerance mechanism.

TH-PO1010

Interventions in Purine Metabolism toward Immunosuppression Oshri Naamani,[2] Yair Cohen,[2] Moshe Zlotnik,[1] Amos Douvdevani.[1,2] *[1]Department of Nephrology, Soroka Medical Center, Beer-Sheva, Israel; [2]Department of Clinical Biochemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel.*

Background: Purine metabolites have potent immunoregulatory roles. ATP, mainly by its P_2X_7 receptor promotes inflammation and increases lymphocyte proliferation. ATP is degraded to adenosine which can also support inflammation by its Gi-coupled A_1 receptor (A_1R) or suppress inflammation and cellular immunity by its Gs-coupled $A_{2A}R$. We reported that activation (preconditioning) of the A_1R upregulates the anti-inflammatory $A_{2A}R$.

Our aim is to modulate the purinergic network toward immunosuppression by upregulation of the antiinflammatory $A_{2A}R$, conversion of ATP to adenosine and maintaining high adenosine levels. In addition we apply an innovative antiinflammatory purine derivate to attenuate T cell activation.

Methods: Preconditioning was induced by injection of A_1R agonist (CCPA) at 24 h and 12 h before splenectomy. Splenocytes from preconditioned or control animals were stimulated with Con A, anti-CD3 or cultured in a mixed lymphocyte reaction in the presence of apyrase (ATPase), adenosine deaminase (ADA), ADA inhibitor (EHNA), inosine-monophosphate (IMP) alone or combined. T-cell activation was assessed by cell proliferation and interferon-gamma (IFN-gamma) secretion.

Results: A decrease in proliferation and IFN-gamma secretion of lymphocyte from preconditioned animals was observed. ATP depletion by apyrase reduced splenocytes proliferation (46%). Reduction of adenosine levels by ADA increased T-cell activation while elevation of adenosine by EHNA caused the opposite effect. Furthermore, we showed that IMP reduces T-cell activation in a dose dependent manner, and when combined with EHNA, T-cell proliferation was eliminated (95%) as effectively as with mycophenolic acid (CellCept®).

Conclusions: Elevation of $A_{2A}R$, ATP depletion and adenosine elevation effectively decelerate T-cell activation. In addition, for the first time IMP was shown to function as an independent immunosuppressant agent. We believe that deeper understanding of the immuno-modulatory mechanisms in which purine metabolites participate, can provide a basis for further therapeutic developments.

Funding: Private Foundation Support

TH-PO1011

Targeted Inhibition of Renal Rho Kinase: A Novel Approach To Reduce Macrophage Infiltration and Lymphangiogenesis in Acute Renal Allograft Rejection Fariba Poosti,[1] Saleh Yazdani,[2] Maria Emma Dolman,[4] Robbert J. Kok,[4] Jai Prakash,[3] Jacob Van den Born,[2] Jan-Luuk Hillebrands,[1] Harry Van Goor,[1] Martin H. De Borst.[2] *[1]Pathology & Medical Biology, UMCG, Groningen, Netherlands; [2]Nephrology, UMCG, Groningen, Netherlands; [3]Pharmacokinetics, UMCG, Groningen, Netherlands; [4]Pharmaceutics, Utrecht University, Netherlands.*

Background: Renal allograft rejection is associated with lymphangiogenesis, a process at least in part driven by inflammatory cell infiltration. RhoA is activated early in lymphangiogenesis and also plays a role in renal inflammation. We therefore investigated whether tubular cell-specific Rho kinase inhibition reduces lymphangiogenesis and macrophage influx in a rat model of kidney transplantation.

Methods: The Rho kinase inhibitor Y27632 was chemically bound to lysozyme (LZM), allowing selective uptake of the compound (Y27632-LZM) by proximal tubular cells upon its systemic administration. Renal allografting (Fisher->Lewis, n=12 per timepoint) was performed. The contralateral kidney was left in situ. Rats were not treated with immunosuppressive drugs to induce acute rejection. Animals were treated daily with Y27632-LZM (10 mg/kg equivalent to 278 µg/kg of free Y27632) or placebo until sacrifice at 1 or 4 d post-transplantation. Kidney sections were examined for macrophage influx (ED1) and lymphangiogenesis (Podoplanin).

Results: Y27632-LZM strongly reduced interstitial macrophage accumulation at day 1 (placebo 13.3±2.7; Y27632-LZM 10.8±7.1 macrophages/tubulo-interstitial field, $p<0.05$) and day 4 (177.6±62.1 vs 101.0±47.0, $p<0.05$) after allograft transplantation. Similarly, Y-27632-LZM reduced the numbers of lymph vessels at both day 1 (2.6±0.1 vs 2.0±0.2 lymph vessels/tubulo-interstitial field, $p<0.05$) and day 4 (4.2±0.4 vs 3.1±0.2, $p<0.05$) in allografts. Tubulo-interstitial macrophage and lymph vessel numbers were strongly correlated ($r^2=0.476$, $p<0.001$). Y27632-LZM did not affect blood pressure, suggesting local delivery of the compound.

Conclusions: Tubular cell-specific Rho kinase inhibition decreased renal lymph vessel numbers which may be secondary to reduced macrophage influx. Renal Rho kinase inhibition may be a valuable approach to treat allograft rejection.

TH-PO1012

Effect of Haemodialysis Membrane Exposure on Anti-HLA Antibody Formation in Incident Dialysis Patients Awaiting Renal Transplantation Claire Kennedy, Frank J. O Brien, Colm Magee, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background: Pre-emptive renal transplantation has superior outcomes to standard transplantation, particularly in terms of acute rejection rates. This suggests an immunological advantage for those patients who do not receive dialysis prior to transplantation.

In patients awaiting heart transplantation, left ventricular assist device have been associated with increased anti-HLA-antibody formation. New exposure to a 'foreign' haemodialysis membrane has not been compared to peritoneal dialysis (self) or no dialysis in terms of anti-HLA antibody formation. We assessed whether starting haemodialysis was associated with a subsequent increase in anti-HLA antibodies as opposed to starting peritoneal dialysis or not receiving any renal replacement therapy.

Methods: A retrospective cohort study of all patients who had been listed for a pre-emptive first renal transplant in Ireland was performed. Anti-HLA antibody formation was compared over time in patients who subsequently started haemodialysis, to those that started peritoneal dialysis and to those that started neither modality. Sensitizing events (pregnancies and blood transfusions) were also recorded.. We defined a significant increase in HLA antibodies as any increase in calculated PRA (determined by Luminex mehod) of 10%.

Results: 161 patients were included. Of these, 31 (19%) subsequently started haemodialysis (with a mean time on hemodialysis of 23 months), 18 (11%) started peritoneal dialysis (with a mean time of 14 months) and 112 (70%) started neither (with a mean follow-up time of 20 months). Baseline cPRAs were 19%, 29% and 24% respectively. There were no significant differences in age, gender, comorbidities, number of pregnancies or blood transfusions between the 3 groups. Rates of new anti-HLA antibody formation over time remained similar in all three groups: 22% of those who started haemodialysis, 16% of those who started peritoneal dialysis and 24% who started neither had a significant rise in cPRA..

Conclusions: Starting maintenance haemodialysis (and exposure to haemodialysis membranes) is not associated with an increase in production of anti-HLA antibodies.

TH-PO1013

Increased Interleukin-17 Producing Effector T Cells during Early Post-Transplant Period Byung Ha Chung, In O Sun, Hyun Gyung Kim, Yu Ah Hong, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea.*

Background: The change of precise immune profile during early post-transplant period has not been fully investigated. In present study, we investigated the response of immune cell subset during early post-transplant period in renal transplant recipients.

Methods: Twenty-nine living donor renal transplant recipients were enrolled in this study. We used triple immune suppressant composed of tacrolimus, mycophenolate mofetil and steroid and performed induction therapy by basilliximab in those patients.

We prospectively investigated the immune cell profile before and at 1 month after transplantation.

Results: The total lymphocyte counts did not differ significantly before and after transplantation ($P > 0.05$). The percentage of Th1 cell ($P < 0.05$) and Th2 cell ($P < 0.05$) in $CD4^+$ T cell significantly decreased after transplantation compared to before transplantation. In contrast, the percentage of Th17 cell did not reduce after KT compared to before KT ($P > 0.05$). The percentage of naïve T cell (T_{NAIVE}) and central memory T cell did not alter after KT ($P > 0.05$, respectively). In contrast, the percentage of effector memory T cell (T_{EM}) significantly decreased after KT compared to before KT ($P < 0.05$). However, the proportion of IL-17 producing cell out of T_{EM} was significantly increased after KT ($P < 0.05$). In $CD8^+$ T cell, the percentage of T_{EM} significantly decrease as like in CD4+ T cell ($P < 0.05$), and the proportion of IL-17 producing T cell in T_{EM} showed increasing pattern, even though it did not reach statistical significance. In B cell subset, all of memory B cell mature B cell, immature B cell in $CD19^+$ B cell did not show significant change after transplantation ($P > 0.05$, respectively).

Conclusions: In contrast with another helper T cell subset, Th17 cell response did not decrease and rather IL-17 producing T_{EM} cell increased after transplantation. It suggest that current immune suppressant is not enough to suppress allo-immune responses by Th17 cell during early transplant period.

TH-PO1014

Spleen Tyrosine Kinase Activation in Human and Experimental Acute Renal Allograft Rejection Sharmila Ramessur,[1,2] Frank Yuanfang Ma,[1,2] Jessica Ryan,[1,2] William Richard Mulley,[1,2] John Kanellis,[1,2] David J. Nikolic-Paterson.[1,2] *[1]Nephrology, Monash Mediacl Centre, Clayton, Victoria, Australia; [2]Medicine, Monash University, Clayton, Victoria, Australia.*

Background: Syk is an adapter molecule involved in B cell receptor and Fcγ-receptor signalling. Syk has also been implicated in neutrophil recruitment and platelet activation. These data suggest that Syk might play an important role in acute allograft rejection. To investigate this we examined Syk activation (phosphorylation of Tyr525/526 in the Syk activation loop) in human and experimental acute renal allograft rejection.

Methods: Three cases each of biopsy proven acute antibody-mediated rejection (AMR) and cell mediated rejection (CMR) of human renal allografts were analysed for p-Syk by immunostaining. A group of 5 Sprague-Dawley rats was immunised with Wistar spleen cells and three weeks later received an orthotopic Wistar renal allograft (one native kidney remained). Recipient rats were killed 7 days later.

Results: Normal human kidney shows no p-Syk immunostaining. In human AMR, p-Syk+ cells were prominent within glomerular capillary loops and in some interstitial areas. In CMR, many p-Syk+ cells were seen in the interstitium, with only small numbers of p-Syk+ cells in glomeruli. Most p-Syk+ cells appeared to be infiltrating leukocytes. In the rat model, all allografts showed severe renal arterial occlusion, thrombosis and areas of infarction with severe tubular necrosis. Other areas showed severe glomerulopathy, peritubular capillaritis and tubulitis. Rat allografts also exhibited rat IgG and C3 deposition, indicating elements of both AMR and CMR. Many infiltrating cells were stained for p-Syk. Double immunostaining identified Syk activation in both neutrophils and macrophages. Furthermore, phospho-p38 staining (a downstream Syk target) was evident in infiltrating leukocytes in a pattern similar to that of p-Syk, possibly indicating Syk-dependent leukocyte activation.

Conclusions: Prominent activation of Syk signalling is evident in infiltrating leukocytes in human and experimental allograft rejection. These findings suggest that Syk is a potential therapeutic target in acute renal allograft rejection.

Funding: Government Support - Non-U.S.

TH-PO1015

Comparison of Antibody Monitoring System with Single Antigen Luminex Assay in Renal Transplant Recipient Hyeonseok Hwang, Byung Ha Chung, Bumsoon Choi, Yong-Soo Kim, Suk Young Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: The antibody monitoring system (AMS) is a recently developed ELISA crossmatch assay to detect donor-specific anti-HLA IgG antibodies (DSA). This study was performed to compare the AMS with DSA detected by single- antigen Luminex panel reactive antibody assay in renal transplant recipients.

Methods: One hundred and one sera were screened from 71 patients on the waiting list for kidney transplantation for the presence of DSA. When anti-HLA Ab was detected by Luminex assay and the matched donor had the corresponding mismatched HLA antigen, it was considered to indicate DSA. The results of AMS and Luminex assay were compared.

Results: Twenty-nine (28.7%) sera were positive for DSA detected by Luminex assay. The DSA was directed against HLA class I Ag in 12 (11.9%) sera, against HLA class II Ag in 17 (16.8%) sera, and against both class I and class II Ag in 6 (5.9%) sera. AMS assay showed that the number of compatible sera with DSA was 79 (78.2%) and it was a significantly concordant ($k = 0.469$, $p < 0.001$). The sensitivity of the AMS assay for detection of DSA was 37.9%; the specificity was 97.2%; the positive predictive value was 84.6%, and the negative predictive value was 79.5%. Compared to complement dependent cytotoxic crossmatch test (CDC), AMS showed higher concordance rate than Luminex assay ($k = 0.541$ for AMS vs. $k = 0.458$ for Luminex). For flowcytometric crossmatch test, the concordance rate was similar between AMS and Luminex assay ($k = 0.432$ for AMS vs. $k = 0.436$ for Luminex). The estimated glomerular filtration rate at 12 months after transplantation was significantly lower in positive AMS patients than in negative AMS patients (46.8 ± 4.1 vs. 60.7 ± 25.4, $p = 0.009$), but the DSA positivity did not predict the lower glomerular filtration rate at 12 months (59.1 ± 25.0 vs. 59.2 ± 24.7, $p = 0.99$).

Conclusions: While AMS assay is less sensitive to detect DSA than Luminex assay, it is useful to predict relevant CDC crossmatch in positive DSA recipients and one-year graft function after transplantation.

Funding: Private Foundation Support

TH-PO1016

UGT2B7 -900 C>G Predicts Leucopenia in Pediatric Kidney Transplant Recipients David K. Hooper,[1] Barry L. Warshaw,[2] Tsuyoshi Fukuda,[1] Cassie L. Kirby,[1] Hiren P. Patel,[3] Deepa H. Chand,[4] Gina-Marie Barletta,[5] Scott K. Van Why,[6] Rene' G. VanDeVoorde,[1] Donald J. Weaver,[7] Lisa Martin,[1] Alexander A. Vinks,[1] Jens W. Goebel.[1] *[1]Cincinnati Children's Hospital; [2]Emory University; [3]Nationwide Children's Hospital; [4]Akron Children's Hospital; [5]Phoenix Children's Hospital; [6]Medical College of Wisconsin; [7]Levine Children's Hospital.*

Background: Mycophenolate Mofetil (MMF) causes leucopenia in a substantial proportion of kidney transplant (KT) recipients, prompting empiric dose reduction which can increase rejection risk. Single nucleotide polymorphisms (SNPs) in genes encoding uridinediphosphate glucuronosyltransferases (UGTs) and multi-drug resistance protein (MDR) have been associated with altered exposure to MMF.

Methods: A case-control gene association study was performed to determine whether UGTs and MDR1 SNPs would predict MMF-related leucopenia in pediatric KT pts. Pts were identified retrospectively and matched by race, center, induction therapy, steroid duration and age. Pts experiencing MMF-related leucopenia prompting dose reduction within the first year of KT were considered cases, whereas controls received full-dose MMF for 1 year following KT without leucopenia. Pts with lupus, liver disease, active nephrotic syndrome, alemtuzumab induction, and non-adherence were excluded. A paired t-test assuming an additive model was used to compare frequency of alleles between cases and controls.

Results: 54 of 225 (24%) pts qualified as cases, and 59 (26%) qualified as controls. 66 (29%) pts had transient leucopenia not requiring MMF dose change, 30 pts had other MMF-related side effects and 16 pts had leucopenia from other causes (e.g. cytomegalovirus). We enrolled 29 matched pairs for genetic analysis. The odds of UGT2B7 -900 G allele in cases were 2.4 times greater than in controls (p=0.03). SNPs at MDR1 3435, UGT1A9 -2152, and UGT1A9 -440 were not significantly associated with leucopenia (p =0.72, 0.16, 0.16 respectively).

Conclusions: MMF-related leucopenia prompting dose reduction was seen in 24% of pts. UGT 2B7 -900 C>G may be associated with an increased risk of leucopenia. Larger studies are needed to confirm this result.

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TH-PO1017

Racial Influence on ABCB1 Gene Expression in Peripheral Blood Mononuclear Cells in Stable Renal Transplant Recipients Kathleen M. Tornatore,[1,3] Daniel Brazeau,[1] Aijaz A. Gundroo,[3] Rocco C. Venuto.[3] *[1]Pharmacy, Pharmacy; [2]Pharmaceutics, School of Pharmacy; [3]Medicine, Erie County Medical Center; SUNY/Buffalo, Buffalo, NY.*

Background: Immunosuppressive therapy (IT) such as tacrolimus are influenced by p-glycoprotein (P-gp) which modulates cellular efflux of this drug. P-gp is present on peripheral mononuclear cells (PBMC) and is encoded by the ABCB1 gene. No data are available regarding the impact of race on ABCB1 gene expression in PBMCs post-transplant over IT dosing interval.

Methods: An observational study was completed in 20 African American (AA) and 11 Caucasian (C) stable renal transplant recipients (RTR) (ages 30-74 yrs) receiving tacrolimus (trough: 5 - 10 ng /ml), and enteric coated mycophenolate sodium. At time 0 (prior to IT) & 4, 8 and 12 hours after immunosuppression, PBMCs were collected for ABCB1 gene expression analysis by quantitative real-time-polymerase chain reaction (QRT-PCR). The target ABCB1 gene PCR product was cloned, and verified by sequencing. The cloned ABCB1 gene was used to establish standard curves (linear[K1] over 6 orders of magnitude; $r^2 = 0.996$) and assess PCR efficiencies. Total ABCB1 copies and normalized copies using Alien RNA were assessed.

Results: The normalized ($p<0.0006$) and non-normalized ($p< 0.0001$) ABCB1 gene expression was higher among Caucasians and at each time until 12 hours.

ABCB1 Gene Expression in PBMC

ABCB1 Copies x10 ^{6} Total RNA	0 hours	4 hours	8 hours	12 hours
African Americans	28.0±14.6	25±16.8	26±15.2	33.4±24.7
Caucasians	61.1±46.3	57.2±48.6	56.5±47.8	53.8±24.2
Post hoc Pairwise p values	0.003	0.004	0.006	0.08

Conclusions: A racial difference in ABCB1 gene expression was noted with greater expression in C than AA. These racial differences in ABCB1 gene expression may influence intracellular tacrolimus concentrations mediated by P-gp and affect clinical outcomes relative to African Americans and Caucasians.

Funding: Other NIH Support - ARRA funded R21 grant: NIH-R21 DK077325-01A1

TH-PO1018

B Cell Depletion Synergizes with ECDI-Fixed Rat Splenocyte Infusions To Induce Concordant Rat to Mouse Islet Xenotransplantation Tolerance
Shusen Wang, Taba Kheradmand, James Tasch, Jie Yang, Xun-Rong Luo. *Medicine, Northwestern University Feinberg school of Medicine, Chicago, IL.*

Background: Previously we demonstrated that infusions of ethylene carbodiimide (ECDI)-fixed donor splenocytes (SP) could only prolong concordant rat to mouse islet xenograft survival but fail to induce tolerance. The aim of this study was to determine whether combination of B cell depletion and rat ECDI-SP infusions could induce tolerance to islet xenografts in a rat-to-mouse transplant model.

Methods: Rat ECDI-SPs were infused i.v. to diabetic C57BL/6 (B6) mice at day -7 and day 1. 650 Lewis rat islets were transplanted into the renal subcapsular space of recipient B6 mice at day 0. Recipients were treated with 250 ug anti-CD20 mAb i.v. on day -10 and day 1.

Results: Mice receiving rat ECDI-SP infusions had prolonged islet xenograft survival (median of 48, range 27-61 days) compare with control group (median of 18, range 15-25, p=0.0026). In contrast, 100% of xenografts were indefinitely accepted (> 150 days post transplant) in mice treated simultaneously with anti-CD20 and rat ECDI-SP infusions (p=0.0018, compare with rat ECDI-SP infusion group). Moderate to high levels of mouse anti-rat antibodies could be detected in subtypes IgG1, IgG2a, IgG2b, and IgG3 in the serum at two weeks after infusion of rat ECDI-SP alone despite the observed graft prolongation. Conversely, with combination therapy, minimal levels of all subtypes of anti-rat IgG were detected in the serum, which correlated with indefinite graft survival. In long-term protected islet xenografts, histological examination revealed minimal deposition of IgG, IgM, or C3, in contrast to acutely rejected grafts. Furthermore, protected islet grafts harbored a significant number of Foxp3+ cells among peri-islet graft infiltrates, which was minimal in rejected islet xenografts. Furthermore, there was a significant decrease in spleen CD4+ effector memory T cell population (CD44highCD62LlowCD69low) in long-term tolerized mice compared with controls.

Conclusions: Simultaneously with anti-CD20 and donor ECDI-SP infusions may be a promising regimen for tolerance induction in xenogeneic islet transplantation.

Funding: NIDDK Support

TH-PO1019

Tolerance and Efficacy of Intravesical Bacille Calmette Guérin (BCG) Treatment in Non Muscle-Invasive Bladder Cancer after Renal Transplantation
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Background: Given the carcinogenicity of aristolochic acids, kidney recipients for end-stage AA nephropathy (AAN) benefit from a bilateral nephroureterectomy and regular cystoscopic examination in order to detect bladder tumors at an early stage. Intravesical instillations of BCG are the treatment of choice for these tumors, in spite of contra-indication due to immunosuppression. We evaluated the feasibility, efficacy and tolerance of BCG therapy in such kidney recipients.

Methods: According to guidelines for BCG usage, namely non-muscle invasive carcinoma of high grade (pT1G3) and/or carcinoma in situ (CIS), 7 patients were treated. Precautions were applied: double dosage of tacrolimus and prophylactic antituberculous treatment (isoniazid 150mg/d and rifampicin 300mg/d) at day -1, 0 and +1 of BCG instillation (Oncotice®) under ciprofloxacin prophylaxis. The tolerance to BCG was recorded clinically (pain, hematuria, fever). Regular follow-up with fluorescence cystoscopy (Hexvix®) was performed after 6 weekly cures, 3 maintenance cures and then every 3-6 months.

Results: So far, 6 patients are free of relapse since the beginning of BCG treatment with a follow-up period of 7-44 months. 5 patients had prior relapsed under intra-vesical Mitomycine C treatment. A 7th patient is just starting the treatment cycle. No change in renal function was observed. The tolerance was optimal, except in 1 patient who had to stop after 5 cures. No systemic tuberculous infection was observed.

Conclusions: We report here the successful treatment of non-muscle invasive bladder cancer with immunotherapy based on intravesical BCG in kidney recipients for end-stage AAN. Standardized conditions allowed the implementation of this treatment whose superiority to Mitomycin C needs to be validated in a larger cohort.

Funding: Clinical Revenue Support

TH-PO1020

Further Elucidation of Mechanisms Mediating Reduced Autoregulatory Tone in Diabetes
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Background: We demonstrated that diabetes altered connexin (Cx) expression in the juxtaglomerular apparatus, impairing purinergic tubuloglomerular feedback (TGF) signaling (Diabetologia in press).

Methods: Using 12 Zucker lean (ZL) and diabetic fatty (ZDF) rats with normal and high (6%) salt diet, renal hemodynamics and renin-angiotensin system (RAS) were characterized.

Results: On normal salt diet, glomerular filtration rate (GFR) was higher in ZDF than ZL rats (1.16±0.06 vs. 0.92±0.05 ml/min/g.kidney.wt (gkw), p<0.05). Autoregulatory index (AI) of GFR was worse in ZDF rats (0.92±0.10 vs. 0.01±0.11, p<0.01). Lithium clearance was lower in ZDF than ZL rats (0.26±0.01 vs. 0.32±0.01 ml/min/gkw, p<0.05). High salt diet for a week failed to alter blood pressure in both strains. However, salt load similarized GFR in 2 strains (1.05±0.06 (ZDF) vs. 0.95±0.05 ml/min/gkw (ZL)). AI of GFR was partly improved (0.47±0.12, p<0.05 vs. ZDF on normal salt) despite of restoration of lithium clearance (0.32±0.01 ml/min/gkw) in ZDF rats on high salt diet. The administration of 8-cyclopentyl-1,3-dipropylxanthine (0.3mg/kg iv.), a selective adenosine-1 receptor antagonist, but not GAP peptide for Cx40 (5mg ia.), to ZDF rats with high salt diet abolished an improvement in AI of GFR (to 0.94±0.10). Plasma angiotensin II was similar between 2 strains. Renal angiotensin II was higher in ZDF than ZL rats on normal salt diet (338±33 vs. 204±34 fmol/gkw, p<0.05), but the difference was disappeared by salt load (166±21 vs. 152±19 fmol/gkw).

Conclusions: Our results demonstrated an elevated renal AngII in ZDF rats. The present data implicate that in addition to Cx alterations, enhanced proximal reabsorption in early diabetes attenuates TGF, underlying glomerular hyperfiltration and RAS activation. Our findings suggest that high salt diet standardizes distal delivery in diabetes, suppressing RAS and improving GFR autoregulation and hyperfiltration via activation of adenosine-1 receptors. The present study may provide a basis for the applicability of glucose transport inhibitor for diabetic nephropathy.

Funding: Clinical Revenue Support

TH-PO1021

The Impact of Gender on Arterial Stiffness and the Renin Angiotensin System in Healthy Humans
Magdalena A. Sarna,[1] Jennifer M. MacRae,[1,2] Brenda Hemmelgarn,[1,2] Daniel A. Muruve,[1,2] Darlene Y. Sola,[1] Sofia B. Ahmed.[1,2] *[1]Department of Medicine, University of Calgary, Calgary, AB, Canada; [2]Alberta Kidney Disease Network, AB, Canada.*

Background: Women are protected compared to men in terms of kidney and cardiovascular (CV) disease and this protection may be mediated in part by gender differences in the renin-angiotensin-system (RAS). However, the impact of gender on RAS control of arterial stiffness, an important marker of future CV risk in both healthy and chronic kidney disease (CKD) populations is unknown.

Methods: Thirty-four healthy subjects (20 women, 14 men, age 35 ± 2 yr) were studied in high salt balance, a state of maximal RAS suppression. Arterial stiffness, measured as carotid-radial pulse wave velocity (PWV) and aortic augmentation index (AIX) by tonometry, circulating components of the RAS (plasma renin activity (PRA), angiotensin II (AngII), and aldosterone) and blood pressure (BP) were measured at baseline and in response to graded AngII infusion (3ng/kg/min x 30 minutes followed by 6ng/kg/min x 30 minutes), a well-accepted indirect measure of intrinsic vascular RAS activity. Post-menopausal women were excluded from the study. Women were studied in the same phase of the menstrual cycle.

Results: At baseline, women demonstrated similar PWV values compared to men (7.4 ± 0.2 m/s vs 8.0 ± 0.3 m/s, p=0.057), greater AIX values (12 ± 3 % vs 1.9 ± 3.0 %, p=0.017) and lower levels of circulating RAS components (PRA, p=0.012 vs men; AngII, p=0.041 vs men; aldosterone, p=0.031 vs men). Arterial stiffness increased in all subjects in response to AngII challenge (PWV, p<0.001; AIX, p<0.001 vs baseline, respectively). Though women demonstrated a greater arterial sensitivity to AngII challenge, this did not achieve statistical significance (ΔPWV: 1.8 ± 0.3 m/s vs 1.3±0.3 m/s, p=0.23; ΔAIX: 11.9 ± 3.2 % vs 11.3 ± 4.0 %, p=0.98).

Conclusions: Renin angiotensin system control of the vasculature does not appear to differ between healthy women and men. Future studies are required to determine the impact of gender on RAS control of arterial stiffness in patients at high risk of CV disease, such as those with CKD.

TH-PO1022

Protective Effect of AT1-Blockade on the Oxidative Stress and the Profibrogenic Response Produced in the Kidney of Normal Rats Fed with a High Salt Diet
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Background: Ang II and AQPs alter the renal ability to reabsorb water from the tubular fluid. Renin is co-localized with AQP-1 and AQP-2 in proximal tubules and collecting ducts, respectively, suggesting that renal Ang II may modulate AQPs expression in tubular cells. We evaluate the effects of the AT1-antagonist, losartan on oxidative stress, renal fibrogenesis and AQP-1 and AQP-2 expression in renal tissues from normal rats fed with a high sodium diet, an experimental model characterized by decreased circulating Ang II levels and increased renal Ang II.

Methods: Rats were fed during three weeks with: regular rat chow (NS), high-salt (8%) chow (HS), and NS and HS fed rats together with losartan (NS-L and HS-L) administration ($40mg.kg^{-1}$ in drinking water). We evaluated: systolic blood pressure (SBP); renal function; intrarenal levels of Ang II, TGF-β_1, α-smooth muscle actin (α-SMA), $p47^{phox}$ NADPH oxidase subunit and eNOS enzyme by immunohistochemistry; and AQP-1 and AQP-2 by western blot and immunohistochemistry.

Results: High salt increased SBP and induced overexpression of Ang II, TGF-β_1, α-SMA and p47^{phox} NADPH oxidase subunit, and decreased eNOS, AQP-1 and AQP-2. Losartan reduced SBP in NS and HS groups and exerted diuretic and natriuretic effects in HS. Losartan reduced fibrosis and oxidative stress markers, and restored eNOS expression and AQP-2 levels in HS-L group. Losartan upregulated AQP-1 immunoexpression, independently of dietary sodium, favouring sodium urinary concentration.

Conclusions: SBP reduction, together with increased natriuresis and diuresis, induced by AT1 blockade in HS fed animals, depend on intrarenal Ang II inhibition, when circulating Ang II is simultaneously decreased. Intrarenal Ang II, through AT1 receptor stimulation, may be responsible for oxidative stress, profibrogenic response and decrease of AQP-1 and AQP-2 in the kidneys of rats fed with a high salt diet.

TH-PO1023

Is Vascular Mineralocorticoid Receptor Involved in Cyclosporine A Nephrotoxicity? Jenny Lancon,[1] Jean-Philippe Bertocchio,[3] Soumaya El Moghrabi,[1] Guillaume Galmiche,[1] Jean-Paul Duong van Huyen,[2] Philippe Rieu,[3] Frederic Jaisser.[1] *[1]U872 Team 1, INSERM, Paris, France; [2]Laboratoire d'Anatomie Pathologique, Hôpital Européen Georges Pompidou, Université René Descartes, Paris, France; [3]Service de Néphrologie-Hémodialyse-Transplantation, Hôpital Maison Blanche, CHU de Reims, France.*

Background: Cyclosporine A (CsA) nephrotoxicity is one of its most frequent adverse effect but its physiopathology remains unclear. Pharmacological blockade of the Mineralocorticoid Receptor (MR) has been reported to prevent CsA nephrotoxicity in the rat by modulating the expression of vaso-active factors (Perez-Rojas et al. AJPRP 2005). We have recently shown that MR is expressed in the endothelium and the vascular smooth muscle of the renal vasculature (Nguyen Dinh Cat et al. FASEB J 2010) Moreover genetic manipulation of MR expression in the endothelium (Nguyen Dinh Cat et al. FASEB J 2010) or the smooth muscle (unpublished data) alters vascular function Our working hypothesis is that the activation of vascular MR plays a key role in CsA nephrotoxicity.

Methods: We studied the effect of the effect of pharmacological blocade of MR on acute CsA toxicity in the mousemale mice of the under low salt diet: control, Ctrl (vehicle), CsA (CsA 100mg/kg/d) and CsA+Can (CsA + canrenoate 30mg/kg/d in the drinking water)

Results: At day 7, 40% of the CsA mice were dead versus 0 in the CsA+Can mice ($p<0,05$). Kidney dysfunction induced by CsA is prevented with canrenoate Creatinine clearance, mL/min/100g; Ctrl: 1.15 +/- 0.13; CsA: 0.74 +/- 0.17; CsA+Can: 1.31 +/- 0.24, $p<0.05$. CsA-induced proximal tubular vacuolizations were partially prevented by canrenoate. The induction of BIP/GRP478, a marker of endoplasmic reticulum stress, was also blunted. Canrenoate prevented the increase in urinary renal excretion of NGAL, a biomarker of renal damage, observed in CsA.

Conclusions: In conclusion, we demonstrate that pharmacological MR antagonism has beneficial effects on survival and prevents histological and functional alterations in a mouse model of acute CsA nephrotoxicity. The implication of vascular MR is currently under investigation using genetically modified models.

Funding: Government Support - Non-U.S.

TH-PO1024

Urinary Albumin and Protein Excretion Are Associated with Increased Vascular Renin-Angiotensin System Activity in Healthy Humans David Donald McTavish Nicholl, Brenda Hemmelgarn, Tanvir Chowdhury Turin, Jennifer M. MacRae, Daniel A. Muruve, Darlene Y. Sola, Sofia B. Ahmed. *Medicine, University of Calgary, Calgary, AB, Canada.*

Background: Albuminuria and proteinuria, both linked to augmented renin angiotensin system (RAS) activity, are associated with adverse kidney and cardiovascular (CV) events. However, the relationship between urinary albumin excretion (UAE) and protein excretion (UPE) in the normal range and RAS activity is unclear. We examined the association between measures of UAE and UPE and the hemodynamic response to angiotensin II (AngII) challenge, a well-accepted indirect measure of RAS activity, in healthy individuals with normal UAE and UPE.

Methods: Forty subjects (15 men, 25 women; 38±2 yrs), were studied in high salt balance. All subjects had normal UAE (3.32±0.55 mg/day) and UPE (57±4 mg/day). Blood pressure (BP), arterial stiffness (aortic augmentation index (AIx) and carotid-radial pulse wave velocity (PWV_{cr})), and circulating RAS components were measured at baseline and in response to graded AngII infusion (3ng/kg/min x 30min followed by 6ng/kg/min x 30min). All studies were performed during the same phase of the menstrual cycle in women. The primary outcome was the hemodynamic response to AngII challenge at 30 and 60 minutes.

Results: UAE was associated with a blunted diastolic BP response to AngII infusion (30min, $r=-0.44$, $p=0.005$; 60min, $r=-0.23$, $p=0.2$), a relationship which remained even after adjustment for covariates (30min, $\beta=-0.72$, $p<0.001$; 60min, $\beta=-0.77$, $p=0.04$). Similar results were observed with UPE (30min, $r=-0.34$, $p=0.03$; 60min, $r=-0.53$, $p=0.001$), even after multivariate analysis (30min, $\beta=-0.076$, $p=0.02$; 60min, $\beta=-0.20$, $p<0.001$). Neither UAE nor UPE were associated with the systolic BP, AIx, PWV_{cr}, PRA, or aldosterone responses to AngII challenge.

Conclusions: Even in our healthy population with 'normal' urinary albumin and protein excretion, increased levels of albuminuria and proteinuria are independently associated with augmented vascular RAS activity which is known to be deleterious to both renal and cardiac function. Larger, prospective studies are required to determine whether albuminuria and proteinuria have a causal role in kidney and CV disease.

TH-PO1025

Autonomic Nervous System Activation during Intradialytic Hypertension Dvora Rubinger, Rebecca Backenroth, Dan Sapoznikov. *Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.*

Background: To define the relationship between heart rate (HR) and blood pressure during intradialytic hypertensive episodes (HD-HyE), continuous interbeat intervals (IBI) and systolic blood pressure (SBP) were monitored in 108 patients during 113 HD sessions.

Methods: HD-HyE, defined as an increase of at least 10 mmHg in SBP between the beginning and the end of dialysis, or hypertension resistant to ultrafiltration occurring during or immediately after dialysis, were detected in 62 sessions. SBP and IBI variability and baroreceptor sensitivity (BRS) in the low (LF) and high frequency (HF) ranges were assessed using complex demodulation method (CDM). LF and HF oscillations are believed to be representative of sympathetic and parasympathetic activation respectively.

Results: HD-HyE were associated with increased (↑, n=45) or decreased (↓, n=17) HR. Mean SBP, IBI and their variability, BRS and LF/HF ratio, representative of sympatho-vagal bala Table 1.

	↑HR			↓HR		
	Before	*During*	p	*Before*	*During*	p
SBP (mmHg)	139 (24)	158 (30)	0.001	141 (37)	159 (25)	0.001
IBI (msec)	868 (201)	822 (191)	0.001	848 (167)	917 (177)	0.001
LF SBP (mmHg)	1.71 (0.88)	2.28 (0.95)	0.001	2.02 (1.03)	1.81 (0.70)	NS
HF SBP (mmHg)	1.56 (0.86)	1.85 (0.87)	0.001	1.90 (1.24)*	1.77 (0.93)	NS
LF IBI (msec)	6.49 (7.10)	8.41 (7.08)	0.011	6.62 (4.47)	5.95 (5.21)	NS
HF IBI (msec)	6.43 (4.55)	6.31 (3.96)	NS	5.92 (4.16)	5.82 (3.98)	NS
LF BRS (msec/mmHg)	4.63 (3.62)	3.81 (2.70)	0.001	3.05 (2.46)	3.77 (2.48)	NS
HF BRS (msec/mmHg)	4.62 (2.97)	3.69 (3.34)	0.001	2.88 (3.45)*	3.55 (2.56)	NS
LF IBI/HF IBI	1.02 (0.35)	1.22 (0.58)	0.007	0.92 (0.70)*	1.06 (0.60)	NS

* $p<0.05$ vs. ↑HR *Before* nce, *before* and *during* HD-HyE were (median and interquartile range):

Conclusions: Maximal SBP was similar in both groups. In ↑ HR , HD-HyE were associated with increased SBP and IBI variability, suppressed BRS and enhanced LF/HF ratio, while in ↓ HR, there were no significant changes in the above parameters. Our data point to sympathetic overactivity as an important mechanism of ↑ HR and hypertension in a significant proportion of patients. In those with ↓ HR, sympathetic activity seems to be counterbalanced by vagal effects. The triggers of increased sympathetic activity during HD remain to be determined.

TH-PO1026

Reduced Aortic Relaxation Rate in Rats with Adenine-Induced Chronic Renal Failure Lisa Nguy,[1] Jaana Lundgren,[1] Holger Nilsson,[2] Gregor S. Guron.[1] *[1]Department of Molecular and Clinical Medicine, Institute of Medicine, Gothenburg, Sweden; [2]Department of Physiology, Institute of Neuroscience and Physiology, Gothenburg, Sweden.*

Background: The aim was to investigate vascular function in rats with adenine-induced chronic renal failure (A-CRF).

Methods: Male Sprague-Dawley rats received either chow containing adenine for 6-12 weeks (0.5% for 3 weeks, 0.3% for 2 weeks, 0.15% thereafter) or were pair-fed with an identical diet without adenine (controls). Systolic blood pressure (SBP) and plasma were analyzed, at 2, 4 and 6 weeks. Segments of thoracic aorta and mesenteric arteries (2nd order) were analyzed with wire myograph. Data are means±SEM.

Results: A-CRF rats showed a marked increase in serum creatinine (281±25 vs. 28±1 µmol/L, $P<0.05$) and parathyroid hormone levels were increased approximately 9-fold vs. controls ($P<0.0001$). SBP was significantly increased in A-CRF rats (at 6 weeks 164±2 vs. 131±2 mmHg, $P<0.05$). There were no significant differences between groups in EC50 values for norepinephrine/phenylephrine, acetylcholine (ACh) or sodium nitroprusside (SNP) in either the aorta or mesenteric arteries. The rate of relaxation was markedly decreased specifically in the aorta of A-CRF rats in response to wash in KCl precontracted vessels (0.06±0.01 vs. 0.21±0.02 mN/s, $P<0.0001$) and this abnormality was evident also in calcium-free buffer. In addition, aortas from rats with A-CRF showed reduced relaxation rates in response to different vasodilators, e.g. SNP, ACh and forskolin. Concentration-response curves for KCl and calcium showed an increased KCl sensitivity and an attenuated response to calcium in A-CRF aortas compared to controls. Aortas from A-CRF animals did not show any calcifications on von Kossa-stained sections.

Conclusions: Rats with A-CRF did not show an impaired sensitivity to ACh or SNP in the aorta or mesenteric arteries in spite of severe renal insufficiency. The major vascular abnormality in A-CRF rats was a reduced rate of relaxation specifically in the aorta, which was evident also in the absence of extracellular calcium. We speculate that a reduced aortic relaxation rate might contribute to aortic stiffness independently of calcifications in chronic renal failure.

Funding: Government Support - Non-U.S.

TH-PO1027

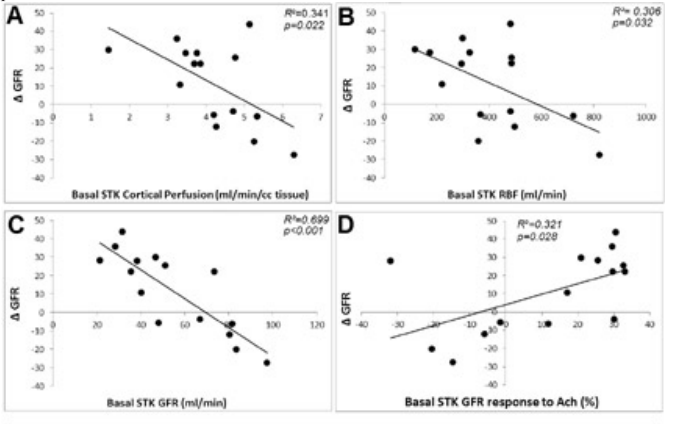
Basal Stenotic-Kidney Hemodynamics and Function Correlate with Response to Revascularization in Swine Renal Artery Stenosis (RAS) Alfonso Eirin,[1] Xiang-Yang Zhu,[1] James Krier,[1] John A. Crane,[1] Stephen C. Textor,[1] Amir Lerman,[2] Lilach O. Lerman.[1] *[1]Divisions of Nephrology and Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

Background: Percutaneous transluminal renal angioplasty (PTRA) can restore vessel patency in RAS, but selection of subjects likely to improve glomerular filtration rate (GFR) after revascularization is difficult. This study examined hemodynamic factors linked to improved renal function after PTRA in a swine model of RAS.

Methods: Pigs (40-55kg) after 6-weeks of hemodynamically significant RAS (60-99%) were studied immediately before and 4 weeks after technically successful PTRA and stenting (n=15) or sham (n=7). Stenotic kidney (STK) hemodynamics and function were evaluated by multidetector computerized tomography before and after challenge with the endothelium-dependent vasodilator and diuretic acetylcholine (Ach, 0.5 μg/kg/min IV). Response to PTRA was evaluated by the change in GFR (Δ GFR).

Results: Four weeks after PTRA blood pressure was normalized in all pigs, and single-kidney GFR increased in 9/15 (ΔGFR= 27.3±3.1 mL/min). A positive functional change in GFR to Ach (possibly reflecting preserved tubular response) directly correlated with ΔGFR (Figure).

Conclusions: The combination of preserved functional stenotic-kidney GFR response to Ach and lower basal GFR may prove to be a powerful and potentially clinically applicable predictor of benefit from revascularization in RAS.



In RAS pigs treated with percutaneous transluminal renal angioplasty (PTRA), ΔGFR correlates inversely with basal STK cortical perfusion (A), RBF (B), and GFR (C), but directly with GFR responses to Ach (D).

Funding: Other NIH Support - DK73608, DK77013, HL77131, HL085307, and RR018898.

TH-PO1028

Kruppel-Like Factor 4 Mediates High Phosphate-Induced Conversion of Vascular Smooth Muscle Cells into Osteoblast-Like Cells Tadashi Yoshida, Maho Yamashita, Matsuhiko Hayashi. *Apheresis and Dialysis Center, Keio University School of Medicine, Tokyo, Japan.*

Background: Cardiovascular complications are the leading cause of death in patients with chronic kidney disease. These patients often have vascular calcifications, which have been associated with hyperphosphatemia. Previous studies have shown that high phosphate-induced phenotypic switching of vascular smooth muscle cells into osteoblast-like cells plays an important role in the calcification process. In the present study, we determined if phosphorylated Elk-1 and Kruppel-like factor 4 (Klf4), which are critical regulators of smooth muscle marker gene expression, were involved in this process.

Methods: Cultured rat aortic smooth muscle cells were incubated in the medium with normal or high phosphate concentration (5 mmol/l) and were harvested for subsequent analyses.

Results: After the incubation with high phosphate medium for 10 days, severe calcification was observed by von Kossa staining. Expression of SM a-actin was decreased, whereas Runx2 expression was induced, as determined by real-time RT-PCR. In this culture system, Klf4 expression was markedly induced at mRNA and protein levels. However, phosphorylation of Elk-1 was undetectable at any time points examined. Furthermore, knockdown of Klf4 by siRNA attenuated high phosphate-induced repression of SM a-actin expression.

Conclusions: Results suggest that Klf4 mediates high phosphate-induced phenotypic switching of vascular smooth muscle cells into osteoblast-like cells.

Funding: Government Support - Non-U.S.

TH-PO1029

Candesartan Inhibits Toll-Like Receptor Expression in Human Renal Tubular Epithelial Cells Chen Yu. *Department of Nephrology, East Hospital, Shanghai, China.*

Background: Toll-like receptors (TLRs) play a key role in the innate immune system and are found to be crucial in inflammatory respond. Recent research found that TLR4 was upregulated in the inflammatory diseases, such as hypertension, arteriosclerosis and renal fibrosis. Angiotensin II is involved in inflammatory responds via Angiotensin II type-1 receptor, whereas Angiotensin II type-1 receptor blockers (ARB) exert anti-inflammatory effect. The aim of present study is to investigate whether candesarsan, an ARB, exerts its anti-inflammatory effect through Toll like receptors pathway.

Methods: Human renal tubular epithelial cells (HKC) were stimulated with LPS (200ng/ml) in the absence or presence of candesartan and TLR4 protein expression was measured by flow cytometry. Knocked down AT1R in HKC using AT1R siRNA and then tested AT1R expression induced by LPS. LPS and/or candesartan stimulated HKC for 0.5hrs, 1hrs and 3 hrs, and collected cells for phosphorylation of NF-kB using western blot. Stimulation with LPS and candesartan (10^{-5}mol/L,10^{-7}mlo/L,10^{-9}mol/L) and extracted RNA to test mRNA expression of inflammatory factors MCP-1 and RANTES.

Results: LPS increased TLR4 protein expression in HKC, which was inhibited by candesartan markedly. Silence of AT1R improved LPS-induced TLR4 expression. Pretreatment of HKC with candesartan significantly decreased LPS induced NF-kB activity and TLR4 expression ($P < 0.05$ vs. control) along with decrease in the mRNA expression of MCP-1 and RANTES in a concentration-related pattern.

Conclusions: LPS induced TLR4 expression, NF-kB activity and further inflammatory factors expression are inhibited by candesartan. Thus, we define a novel pathway by which candesartan could induce anti-inflammatory effects, that is through Toll like receptors pathway.

Key Words: Toll-like receptors (TLRs), LPS, Angiotensin II, ARB

Funding: Government Support - Non-U.S.

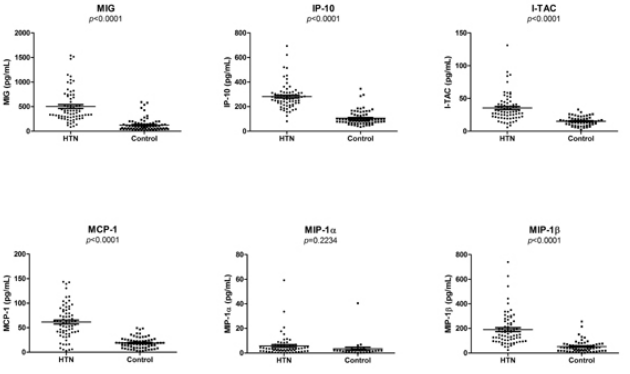
TH-PO1030

Hypertensive Nephrosclerosis Is Associated with Periarteriolar T Cell Infiltrates and Increased Level of Serum T Cell-Driven Chemokines Beom Jin Lim,[1] Youn Jong-Chan,[2] Hyeon Joo Jeong,[1] Sungha Park.[2] *[1]Department of Pathology, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Severance Cardiovascular Center, Yonsei University College of Medicine, Seoul, Korea.*

Background: The contribution of chemokines in primary hypertension has been recently proposed in animal models, however, their roles in human hypertension are not well known. We evaluated the infiltration of chemokine-producing T cells in renal tissue of hypertensive nephrosclerosis and circulating levels of chemokines in hypertensive patients.

Methods: We evaluated infilatration of T lymphocytes and their expression of chemokines in 10 hypertensive nephrosclerosis cases by immunohistochemistry and compared with normal control group. We measured circulating levels of 6 chemokines (MIG, IP-10, I-TAC, MCP-1, MIP-1α, MIP-1β) in 71 hypertensive patients (51.6±11.2 yrs, M:F=35:36) and in age, sex-matched 71 control subjects by cytometry bead array method. We also analyzed the expression of CXCR3 and CCR2 in peripheral blood mononuclear cells of hypertensive patients using multicolor flow cytometry.

Results: Periarteriolar inflammatory cells were increased in hypertensive nephrosclerosis and most of them are composed of T lymphocytes. Some of infiltrating cells express chemokines. Circulating chemokine levels were significantly higher in patients with hypertension than in control subjects. CXCR3 were mainly expressed in CD8 T cell subset, which co-expresses CX3CR1 as well.



Conclusions: Although this observation does not provide direct evidence of T cell function in human hypertension, increased T cells and chemokines in hypertensive patients suggest the role of T cell-associated imflammatory pathway. More detailed characterization of T cells, associated chemokines and the expression of chemokine receptors in renal tissue may offer a new insight in the pathophysiology of primary hypertension.

TH-PO1031

High Throughput Screening of Drugs That Inhibit WNK-OSR1/SPAK Signaling Cascade Takayasu Mori, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

Background: WNK kinases were identified as causative genes of pseudohypoaldosteronism type II (PHA II), a hereditary hypertensive disease with hyperkalemia and acidosis. We identified that WNKs form a signal cascade with OSR1/SPAK kinases and Slc12a transporters (NKCC1, NKCC2, and NCC). We also found that this signal cascade have a pivotal role in controlling vascular tone as well as renal NaCl excretion. Therefore, inhibiting this signal cascade could be a new type of antihypertensive drugs.

Methods: To explore this possibility, we adopted a strategy to inhibit the binding of WNKs with their substrates OSR1/SPAK since the binding domains are already known. Furthermore, we introduced a Fluorescence Correlation Spectroscopy (FCS) method to efficiently screen the inhibitors. FCS is a method to be able to measure fluctuation rate of a fluorescence-labeled single peptide. We labeled an RFxV/I motif of WNK4 with TAMRA, mixed it with the CCT domain of SPAK fused with GST, and could confirm the binding of these two molecules by FCS in 384-well plates. Using this newly developed system, we could screen chemical compounds to inhibit the binding.

Results: As a result of initial screening of 16,000 compounds owned by Tokyo Medical and Dental University Chemical Biology Screening Center, we found 10 different primary candidates. We then tested whether these compounds could inhibit the signals from WNKs to OSR1 in vivo in COS7 cells, and finally obtained three compounds showing the concentration-dependent inhibitory effect on endogenous OSR1 phosphorylation by WNKs.

Conclusions: These compounds could be promising seeds for new types of antihypertensive drugs, and the method we developed in this study could be applicable to any screenings for compounds that inhibit binding of two molecules.

Funding: Government Support - Non-U.S.

TH-PO1032

WNK-OSR1/SPAK-SLC12A Phosphorylation Cascade in the WNK1 (+/-) Mice Koichiro Susa,[1] Eisei Sohara,[1] Tatemitsu Rai,[1] Satomi Kita,[2] Takahiro Iwamoto,[2] Dario Alessi,[3] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [2]Department of Pharmacology, School of Medicine, Fukuoka University, Fukuoka, Japan; [3]MRC Protein Phosphorylation Unit, MSI/WTB Complex, University of Dundee, Dundee, United Kingdom.*

Background: We found that hypertension in pseudohypoaldosteronism type II (PHA II) caused by a WNK4 missense mutation was the result of activation of WNK-OSR1/SPAK-SLC12A phosphorylation cascade (Cell Metab 2007, J Cell Sci 2010). On the other hand, pathogenic effect of the large intronic deletions of WNK1 gene also observed in PHA II remains to be determined. To understand the physiological roles of WNK1 in vivo, WNK1(+/-) mice has been analyzed and was shown to have lower blood pressure compared with wild-type mice. However, detailed analyses in kidney and other organs of WNK1(+/-) mice have not been performed.

Methods: We analyzed WNK1(+/-) mice focusing on the status of WNK-OSR1/SPAK-SLC12A signal cascade.

Results: First, we confirmed by immunoblotting and real time PCR that WNK1 expression was reduced by half in brain, aorta, and kidney cortex. Blood pressure of WNK1(+/-) mice measured by tail-cuff method was not decreased compared with that of wild-type mice, even under low salt diet, which was not consistent with the previous report. We then evaluated the phosphorylation status of OSR1, SPAK, and SLC12A in kidney, and no significant decrease of the phosphorylation was observed in WNK1(+/-) mice. Furthermore, we mated WNK1(+/-) mice with WNK4(D561A/+) knockin mice, in which WNK-OSR1/SPAK-SLC12A cascade was constitutively activated. Even under the WNK4(D561A/+) background, the heterozygous deletion of WNK1 did not affect the phosphorylation of SLC12A. These results suggest that the contribution of WNK1 to the whole WNK kinase activity in kidney to stimulate SLC12A phosphorylation may be small. On the other hand, we observed significant decrease of NKCC1 phosphorylation in aorta, and blunted myogenic tone of mesenteric arteries (resistance vessel), in WNK1(+/-) mice.

Conclusions: WNK1 may have substantial roles in arteries, rather than in kidney, in terms of blood pressure regulation.

Funding: Government Support - Non-U.S.

TH-PO1033

Inhibition of Uricase Plus Physiological Amounts of Fructose and Glucose: A Model of Metabolic Syndrome and Glomerular Hypertension in Rats More Related to the Human Condition Tapia Edilia,[1] Magdalena Cristobal,[1] Fabiola Monroy,[1] Ursino Pacheco,[1] Takahiko Nakagawa,[2] Richard J. Johnson,[2] L. Gabriela Sanchez-Lozada.[1] *[1]Nephrology, INC Ignacio Chavez, Mexico City, DF, Mexico; [2]Renal Diseases & Hypertension, U Colorado, Aurora, CO.*

Background: A 60% fructose diet to rats is a model of metabolic syndrome (MS) and renal damage characterized by glomerular hypertension and renal vasoconstriction. Febuxostat treatment improved these alterations suggesting the participation of uric acid in these processes. The purpose of the present study was to evaluate whether murine uricase blockade with oxonic acid (OA, 750 mg/day/kg BW) concomitant to a combination of glucose-fructose (GF, ratio G 3.8%: F 7.2%) in drinking water might induce similar renal alterations.

Methods: We studied three groups of rats: Water+OA (n=4), FG+Veh (n=4) and FG+OA (n=6) for 8 wks. At the end of the study body weight (BW), fasting plasma triglycerides (TG), MAP (under anesthesia), GFR and glomerular pressure (PGC, by renal micropuncture) were evaluated.

Results:

Group	BW (gr)	TG (mg/dL/kg BW)*	MAP (mmHg)	GFR (mL/min)*	PGC (mmHg)
Water+OA	316±12	226±41	143±4.5	1.1±0.2	63±2
FG+Veh	334±6	284±15	130±10	0.7±0.1	53±4[1]
FG+OA	349±14	341±31	154±5.4	0.6±0.1	70±1[2]

*=p<0.05 linear trend; 1=p<0.05 vs W+OA; 2=p<0.05 vs FG+Veh

Conclusions: Inhibition of uricase concomitant to physiologic amounts of FG combination induced higher plasma TG and hypertension, lower GFR and higher PGC compared to OA or FG alone. Thus, this murine model is more representative to the human situation characterized by the lack of uricase and FG consumption. Moreover, these results support the synergistic role of uric acid on the development of the MS secondary to high carbohydrate consumption and the renal damage associated to it.

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

TH-PO1034

Functional Vascular Dysfunction Related to *COL4A1* Gene Mutation Emmanuelle M. Plaisier,[1] Sandrine Placier,[1] Jean-Claude Dussaule,[1] Migeon Tiffany,[1] Christos Chatziantoniou,[1] Gerard M. London,[2] Pierre M. Ronco,[1] Mohamad Zaidan.[1] *[1]INSERM UMRS 702, Paris, France; [2]Mahnes Hospital, Fleury Merogis, France.*

Background: HANAC (Hereditary Angiopathy, Nephropathy, Aneurysms, Cramps) is a multisystemic autosomal dominant syndrome related to mutations of *COL4A1* gene, that encodes the collagen IV component of basement membrane (BM). Vascular defects in HANAC associate a small vessel brain disease, cerebral aneurysms and Raynaud phenomena. This study further evaluated structural and functional vascular properties in HANAC patients and in mice bearing the *Col4a1* G495V mutation .

Methods: Non-invasive analysis of the arterial geometry and of the arterial and endothelial functions was performed in 4 HANAC patients from 2 families. Systemic and renal hemodynamic parameters of adult Col4a1^{G495V} mice and wild-type littermate were evaluated under basal conditions and after exposure to vasoactive agents.

Results: Compared to controls, HANAC patients showed a low intima/media thickness with elevation of the compliance and distensibility of the brachial artery. Very low basal flow, shear rate and shear stress were recorded, as well as a very low dilation response to shear stress that was corrected by exogenous nitrite oxide (NO) administration. Under basal conditions, both Col4a1$^{+/G495V}$ and Col4a1$^{G495V/G495V}$ mice showed a lower systolic and mean blood pressure compared to wild-type. Vasodilation response and decrease of the renal blood flow (RBF) after acetylcholine infusion was significantly lower in homozygous animals contrasting with a normal response to bradykinine. While the systemic blood pressure response to angiotensin II and norepinephrine was not affected in mutant mice, the decrease of RBF after angiotensin II exposure was significantly lower in homozygous females.

Conclusions: These data point to an essential structural and functional role for the collagen IV network of the vascular BM. Abnormal dilation response to acetylcholine in mice and to shear stress in patients suggest either a primary endothelial dysfunction with defect in NO production and/or a abnormal cross talk between endothelial cells and the adjacent vascular smooth muscle cells.

Funding: Government Support - Non-U.S.

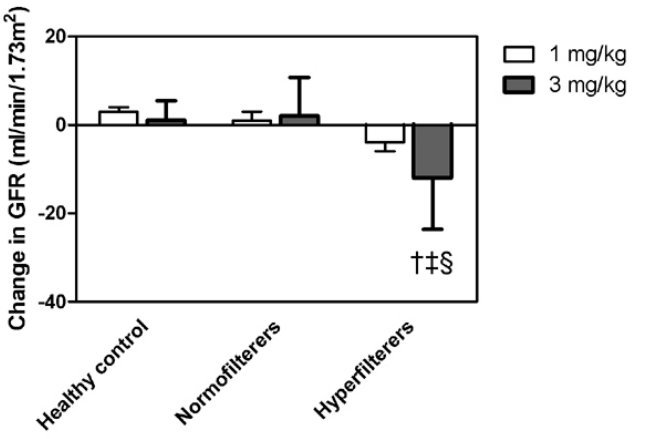
TH-PO1035

Hyperfiltration and the Effect of Nitric Oxide Inhibition on Renal Hemodynamic and Endothelial Function in Humans with Uncomplicated Type 1 Diabetes Mellitus David Cherney,[1] Heather N. Reich,[1] Shan Jiang,[1] Vesta S. Lai,[1] James W. Scholey.[1] *[1]Medicine, Division Nephrology, University of Toronto, University Health Network; [2]Pediatrics, Pediatric Endocrinology, University of Toronto, The Hospital for Sick Children.*

Background: Experimental models of diabetes mellitus (DM) have been associated with increased renal nitric oxide (NO) bioactivity, leading to hyperfiltration. Paradoxically, type 1 DM is also associated with impaired brachial flow mediated dilatation (FMD), suggesting an opposite suppression of systemic NO bioactivity. Our aim was to clarify the role of the NO system in the pathophysiology of early renal hyperfiltration and peripheral vascular dysfunction in patients with type 1 DM.

Methods: Renal hemodynamic function, FMD, urinary and circulating cGMP and NO levels were obtained before and after a graded IV infusion of L-LNMMA (1 mg/kg and 3 mg/kg) during clamped euglycemia in type 1 DM patients with renal hyperfiltration (n=15) or normofiltration (n=19). Healthy control subjects (n=20) underwent identical studies.

Results: Baseline characteristics were similar in the three groups. In response to L-NMMA, hyperfiltering subjects exhibited exaggerated declines in effective renal plasma flow (806±96 to 540±83 ml/minute/1.73 m^2) and GFR vs. normofiltering and healthy subjects (repeated measures ANOVA, p<0.05).



In contrast with renal hemodynamics, baseline FMD was lower in the hyperfiltering group (repeated measures ANOVA, $p<0.05$). Furthermore, FMD declined in healthy control and normofiltration groups in response to L-NMMA but did not change in the hyperfiltration group.

Conclusions: Renal hyperfiltration is associated with increased renal NO bioactivity and impaired endothelial function in patients with uncomplicated type 1 DM, suggesting that a paradoxical state of high renal and low systemic vascular NO bioactivity exists in humans.

Funding: Government Support - Non-U.S.

TH-PO1036

Altered Endothelial Glycocalyx in Albuminuria: A Potential Link between Albuminuria and Widespread Vascular Dysfunction

Andy Salmon,[1,2,3] Joane Ferguson,[1] James L. Burford,[3] Anush Gevorgyan,[3] David O. Bates,[1] Steve Harper,[1] Janos Peti-Peterdi.[3] *[1]Microvascular Research Laboratories, School of Physiology & Pharmacology, University of Bristol, United Kingdom; [2]Academic Renal Unit, School of Clinical Sciences, University of Bristol, United Kingdom; [3]Department of Physiology, University of Southern California.*

Background: Widespread vascular dysfunction occurs in albuminuria. Endothelial glycocalyx (GLX), a glycosaminoglycan/proteoglycan mesh lining all blood vessels, regulates glomerular permeability and extra-renal vascular function. We hypothesised that GLX is damaged in albuminuria, mediating widespread vascular dysfunction.

Methods: GLX and vascular function (permeability) were assessed in glomeruli & mesentery of rats (4-6mo old Munich-Wistar-Fromter (MWF)) with spontaneous FSGS & 10-fold albuminuria*.

*$p<0.05$ v non-albuminuric rats; ‡$p<0.05$ or ^{ns}not sig v baseline.

Results: In glomeruli of albuminuric MWF rats, albumin sieving coefficient (Θalb: multiphoton microscopy measurements *in vivo*) was elevated (0.0004 to 0.0009)*, ultrafiltration coefficient (L_p-area product: L_pA; isolated single glomerular measurements) was elevated 2.4-fold*, & GLX coverage (multiphoton microscopy of lectin (WGA) binding to GAG constituents of GLX *in vivo*) was reduced (87% to 25%)*.

In mesenteric microvessels of albuminuric MWF rats, hydraulic conductivity (L_p: single perfused microvessel measurements *in vivo*) was elevated 2.3-fold*, GLX depth was reduced (0.65 to 0.20μm)*, & GLX vessel coverage was reduced (89% to 25%)*.

In healthy rats, GLX removal with neuraminidase increased Θalb (0.0004 to 0.001)‡, reduced GLX coverage in glomeruli (83% to 31%)‡, & reduced mesentery vessel GLX coverage (89% to 15%)‡ & depth (0.65 to 0.17μm)‡.

However in albuminuric rats, GLX removal with neuraminidase was without effect (pre- and post-neuraminidase: Θalb (0.0009 to 0.0009)^{ns}; GLX coverage in glomeruli (39% to 32%)^{ns} & mesentery (25% to 16%)^{ns}).

Conclusions: Our results demonstrate functionally important loss of GLX in albuminuric animals.

Damaged GLX in albuminuria may contribute to the systemic vascular dysfunction/disease that characterises albuminuria, as well as the pathophysiology of glomerulosclerosis.

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

TH-PO1037

Adenosine Receptors Modulate Angiotensin II-Mediated Effects on Tubuloglomerular Feedback

Mattias Carlstrom, Christopher S. Wilcox, William J. Welch. *Dept. of Medicine, Georgetown University, Washington, DC.*

Background: Tubuloglomerular feedback (TGF) response is mediated via activation of A1 receptors, and modulated by A2 receptors on the renal afferent arteriole. Synergistic interaction between angiotensin II (Ang II) and adenosine has been described in the renal microvasculature; however, the underlying mechanism and its role in TGF regulation are unclear. We tested the hypothesis that adenosine A1 and A2 receptors modulate Ang II-mediated effects on TGF.

Methods: TGF responses were measured in adenosine A1 receptor knockout (A1-/-) and wildtype mice (A1+/+), as changes in proximal stop-flow pressure (ΔPSF) in response to increased perfusion of loop of Henle (0 to 35 nl/min). Maximal TGF responses were studied during perfusion with artificial tubular fluid (ATF) alone, or ATF supplemented with i) Ang II (10-9 M), ii) Ang II (10-9 M)+Tempol (10-3 M), or iv) Ang II (10-9 mol/L)+A2 receptor antagonist (10-7 M).

Results: TGF response (ΔPSF) during control (ATF alone) was 8.1±0.6 mmHg in A1+/+, whereas TGF responses were abolished in A1-/- mice. Perfusion with Ang II enhanced ΔPSF in A1+/+ (12.3±0.8 mmHg), whereas an inverse TGF response was observed in A1-/- mice (+3.5±0.5 mmHg). Co-treatment with superoxide dismutase-mimetic (Tempol) during Ang II perfusion normalized TGF responses in both A1+/+ (8.6±0.6 mmHg) and A1-/- mice (+0.6±0.8 mmHg). Simultaneous application of Ang II and A2 antagonist enhanced ΔPSF in A1+/+ (14.5±0.8 mmHg), and induced TGF response in A1-/- mice (3.5±0.8 mmHg).

Conclusions: Adenosine A1 receptors enhance, whereas A2 receptors attenuate Ang II-mediated effects on TGF responses. Mechanistically, Ang II-induced oxidative stress may increase adenosine formation, which during A1-deficiency causes A2 receptor-mediated dilatation.

Funding: NIDDK Support

TH-PO1038

Abstract Withdrawn

TH-PO1039

Non-Steroidal Anti-Inflammatory Drugs Modulate Vasa Recta Diameter

Teresa M. Kennedy-Lydon,[1] Carol Crawford,[1] Liam Sawbridge,[1] Robert J. Unwin,[2] Scott S.P. Wildman,[1] Claire M. Peppiatt-Wildman.[1] *[1]Royal veterinary College, London, United Kingdom; [2]UCL Medical School, London, United Kingdom.*

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are nephrotoxic and reduce medullary blood flow (MBF). NSAIDs inhibit cyclooxygenases (COX) and endogenous production of medullary prostaglandin E_2 (PGE_2). PGE_2 has been shown to attenuate the vasoconstrictor effects of endothelin-1 (ET-1) and angiotensin-II (Ang-II) at vasa recta. Here we investigate the responses of *in situ* vasa recta pericytes to (i) PGE_2 (ii) ET-1 and Ang-II plus and minus PGE_2 (iii) NSAIDs, and the location of medullary COX-1 and 2 in the kidney slice model.

Methods: Live kidney slices obtained from adult male Sprague-Dawley rats were secured in an open-bath chamber on the stage of an upright microscope and continuously superfused with oxygenated physiological saline solution. Real time images of *in situ* vasa recta were recorded and vasa recta diameter at pericyte and non-pericyte sites was measured off-line.

Results: Application of PGE_2 to live kidney slices evoked dilation of vasa recta specifically at pericyte sites. PGE_2 significantly attenuated pericyte-mediated constriction of vasa recta evoked by both ET-1 and Ang-II. Indomethacin a non-selective inhibitor of COX evoked a significantly greater constriction of vasa recta capillaries at pericyte sites than at non-pericyte sites. The COX-1 selective inhibitor SC-560 and COX-2 selective inhibitors meloxicam and celecoxib also evoked a significantly greater constriction at pericyte sites than at non-pericyte sites. COX-1 and -2 were identified in vasa recta endothelial cells by immunohistochemistry.

Conclusions: Data presented show (i) PGE_2 dilates vasa recta at pericyte sites and reverses the ET-1- and Ang-II-evoked constriction of vasa recta at pericytes, (ii) vasa recta endothelial cells are a source of COX-1 and -2, (iii) application of selective and non-selective COX inhibitors leads to pericyte-mediated constriction of vasa recta. These data re-iterate the key role of PGE_2 in regulation of medullary blood flow and reveal that attenuation of PGE_2 production causes pericyte-mediated constriction of vasa recta. Thus pericytes may be key in NSAID-evoked reduction in MBF.

TH-PO1040

Rapid, Dynamic Increases in Glomerular Permeability during Angiotensin-II (AII) Infusion in Rats. Reactive Oxygen Species (ROS) May Be Involved

Josefin Axelsson, Anna Rippe, Kristinn Sverrisson, Bengt Rippe. *Department of Nephrology, Lund University, Lund, Sweden.*

Background: This study was performed in order to investigate the dynamics and mechanisms of action of AII on glomerular permeability.

Methods: In anaesthetized Wistar rats (250-280g) the left ureter was cannulated for urine collection, while simultaneously blood access was achieved. Rats were continuously infused i.v. with AII [62 ng/min (Lo-AII; n=5) or 250 ng/min (M-AII; n=8) or 500 ng/min (Hi-AII; n=8)], respectively, and with polydisperse fluorescein isothiocyanate (FITC)-Ficoll-70/400 (mol.radius 13-90Å) and ^{51}Cr-EDTA for 2 h. Plasma and urine samples were taken at 5, 15, 30, 60 and 120 min of AII infusion, and analyzed by high performance size exclusion chromatography (HPSEC) for determination of glomerular sieving coefficients (θ) for Ficoll. GFR was also assessed (^{51}Cr-EDTA).

Results: In all AII groups there was a rapid (within 5 min), marked increase in glomerular permeability to Ficoll molecules >34Å in radius. θ for Ficoll of radius 70Å increased 20-fold (Lo-AII) up to 30-fold (Hi- and M-AII), respectively. For the Lo-AII group the permeability increase was reversible within 15-60 min, whereas in the M-AII and the Hi-AII groups there was only a partial reversibility within this time frame. Thus, a moderate, sustained increase in glomerular permeability remained even at 120 min. GFR was well maintained in the Lo-AII group, but (somewhat) decreased in the M-AII and Hi-

AII groups. The AII-receptor blocker, candesartan, completely abrogated the AII response. The ROS inhibitor, DMTU (dimethylthiourea), partly reduced the AII response.

Conclusions: AII infusion into rats caused a rapid, marked and partly reversible increase in glomerular permeability with a maximum at 5-15 min after start. AII blockade completely abrogated this response, while DMTU, a ROS-inhibitor, partly reduced it. The glomerular permeability increase occurring to Ficoll molecules >34Å in radius is compatible with an increase in the number of "large pores" of the glomerular filter. ROS may be partly involved in the permeability increase induced by AII.

Funding: Government Support - Non-U.S.

TH-PO1041

Charge Modified, Conformationally Intact Anionic Ficoll Is Retarded Relative to Neutral Ficoll across the Rat Glomerular Filtration Barrier *In Vivo* Josefin Axelsson,[1] Kristinn Sverrisson,[1] Anna Rippe,[1] William Fissell,[2] Bengt Rippe.[1] *[1]Department of Nephrology, Lund University, Lund, Sweden; [2]Biomedical Engineering, Cleveland Clinic, Cleveland, OH.*

Background: The glomerular filtration barrier (GFB) is commonly conceived as a negatively charged sieve. Recent studies, however, indicate that glomerular charge effects are small for anionic, carboxymethylated (CM) dextran *vs*. neutral dextran. Two studies assessing the glomerular sieving coefficients (θ) for CM-Ficoll *vs*. native Ficoll have actually demonstrated an "anomalous" behavior of these polysaccharides, i.e. a higher glomerular permeation of anionic than neutral Ficoll. The CM-Ficoll used in these studies showed a larger Stokes-Einstein radius (a_e) than neutral Ficoll. Hence, it was proposed that the introduction of negative charges in the Ficoll molecule had made it more extended and flexible, and thereby, more permeable.

Methods: Recently, a negatively charged fluorescein isothiocyanate (FITC) labeled CM-Ficoll was produced with a conformation identical to that of native FITC-Ficoll. Using these probes we determined their θ:s in anesthetized Wistar rats (259±2.5 g). After blood access had been achieved, the left ureter was cannulated for urine sampling. Either polysaccharide was continuously infused (i.v.) in parallel with a marker of glomerular filtration rate (GFR), while urine and plasma were collected. Assessment of FITC-Ficoll in plasma and urine was achieved by high performance size-exclusion chromatography (HPSEC).

Results: CM-Ficoll and native Ficoll had identical elugrams on the HPSEC. Diffusion of anionic Ficoll was significantly reduced compared to that of neutral Ficoll across the GFB for molecules of a_e ~ 20-35Å, while there were no charge effects for Ficoll of a_e = 35-80Å. The data are consistent with a charge effect present in "small pores", but not in "large pores", of the GFB and mimicked those obtained for anionic membranes *in vitro* for the same probes.

Conclusions: In conclusion, the GFB is negatively charged. However, the negative charge selectivity was found to be much less pronounced than previously demonstrated for sulphated *vs*. neutral dextran.

Funding: Government Support - Non-U.S.

TH-PO1042

Decrease in Retinal Arteriolar Caliber Is Associated with a Lower Renal Function in Normotensive and Never-Treated Hypertensive Subjects Vincent Daien,[2] Ryo Kawasaki,[3] Jean Ribstein,[1] Guilhem Du Cailar,[1] Max Villain,[2] Albert Mimran,[1] Pierre Fesler.[1] *[1]Department of Internal Medicine, Lapeyronie Hospital, Montpellier, France; [2]Department of Ophtalmology, Gui de Chauliac Hospital, Montepellier, France; [3]Retinal Vascular Imaging Centre, Centre for Eye Research Australia, Melbourne, Australia.*

Background: Microvascular change has been postulated to represent one of the key mechanisms of kidney aging. Retinal arteriolar narrowing has been used as a marker of the altered microcirculation. The primary objective was to assess the association between retinal arterial caliber and renal function in normotensive (NT; <140/90 mmHg) and never-treated hypertensive (HT) subjects.

Methods: Study subjects were 57 persons with NT and 48 persons with never-treated essential HT with serum creatinine (SCreat) < 130 µmol/L and without diabetes. Retinal arteriolar caliber was measured from fundus photographs using a computer-assisted program and summarized as central retinal artery equivalent. Glomerular filtration rate (eGFR) was estimated from the MAYO clinic quadratic equation.

Results: Mean age of study subjects was 48+/-13 (mean +/- SD), and 50% were women. Mean SCreat was 0.79+/-0.17 mg/dl and eGFR was 113.7 ml/min/1.73m², and there was no difference between persons with NT and HT. Mean retinal arteriolar caliber in persons with NT and HT were 147.6 +/- 12.7 µm and 135.1 +/- 10.9 µm, respectively (p=0.001). In the whole population, retinal arteriolar caliber was positively and significantly correlated to eGFR (univariate r^2=0.16; p = 0.001), even after adjustement for age, gender, mean arterial blood pressure, smoking, glycemia, body mass index, total cholesterol, triglycerides (model r^2 =0.49, p=0.0001). When replacing eGFR by 1/Screat and adding size and weight as predictive variables, the association between retinal arteriolar caliber and renal function remained significant (model r^2=0.22, p=0.0001).

Conclusions: In never-treated NT or HT subjects, a decrease in retinal arteriolar caliber is associated with a lower kidney function, independently of other potential determinants of retinal vascular changes. The mechanisms of this apparent common aging process remain to be documented.

TH-PO1043

Endothelial Deletion of Sirtuin 1 (SIRT1) in Mice Impairs Vasomotion, Angiogenesis and Accelerates Senescence Sandhya Xavier, Radovan Vasko, Jun Chen, Frank Fan Zhang, Alberto Nasjletti, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

Background: Stress-induced premature senescence (SIPS) of endothelial cells (EC) has emerged as a notable contributor to global endothelial cell dysfunction (ECD) in diverse diseases. SIRT1, a member of a family of histone deacetylases involved in metabolism and differentiation, has been implicated in cellular and organismal longevity. SIRT1 mutant mice carrying a deletion of exon 4 die perinatally and exhibit developmental defects of retina and heart.

Methods: To define the role of SIRT1 in vascular homeostasis, we generated mice with deletion of SIRT1 in vascular endothelial and endothelial progenitor cells by mating mice homozygous for the floxed SIRT1 allele with mice heterozygous for Cre-recombinase under the control of Tie2-promoter. From these matings $SIRT^{Flox/Flox}$ (WT-control), $SIRT^{Flox/WT}$ (Het-control), Tie2-Cre;$SIRT^{Flox/Flox}$ (KO) and Tie2-Cre;$SIRT^{Flox/WT}$ (Het) mice were obtained at Mendelian ratios.

Results: Loss of SIRT1 in the endothelium under basal conditions did not affect thriving, caused microalbuminuria or elevation of serum creatinine levels and no hypertension in mice aged 12 weeks or less was detected. Glucose tolerance was not impaired. However, when aortic rings from control, KO and Het mice were subject to acetylcholine-induced vasorelaxation, a mild reduction in vasorelaxation was observed in $SIRTendo^{-/-}$ mice, but not in controls, an indication of developing endothelial dysfunction. This was paralleled by accelerated endothelial senescence. In an *ex vivo* aortic ring assay, sprouting and microvessel formation were both inhibited in endothelial-deleted SIRT1 mutant mice, indicating that Sirt1 in the endothelium controls angiogenesis *ex vivo*.

Conclusions: We developed a model system to study consequences of the isolated defect in endothelial cell function. Under basal conditions, SIRT1 deficiency in endothelial cells did not lead to systemic hemodynamic abnormalities, but significantly accelerated endothelial senescence and impaired angiogenic competence and vasorelaxation at the early age.

Funding: NIDDK Support

TH-PO1044

Renal Responses to Administration of a Peroxynitrite Scavenger in Anesthetized Wild Type and Knockout Mice Lacking the Gene for Extracellular Superoxide Dismtase Dewan S. Majid, Alexander Castillo, Purnima Singh. *Physiology, Tulane University School of Medicine, New Orleans, LA.*

Background: Peroxynitrite ($OONO^-$) is continuously being produced in the body via interaction of nitric oxide and superoxide (O_2^-). However, its potential role in regulating cardiovascular and renal function is not yet clearly defined. In the present study, we assessed its regulatory role in the control of systemic arterial pressure (SAP) as well as renal hemodynamics and excretory function in mice.

Methods: $OONO^-$ scavenger, mercaptoethyl guanidine (MEG), was administered intravenously at an incremental doses (10, 30 and 50 µg/kg/min for 45 min each) in anesthetized wild type (C57BL6; n=6) as well as knockout mice lacking the gene for extracellular superoxide dismutase (ecSOD KO; n=4) which would have a higher level of $OONO^-$ due to enhanced O_2^- and NO interaction. SAP was recorded using a pressure transducer connected to a cannula placed in the left carotid artery. Renal blood flow (RBF) and glomerular filtration rate (GFR) were determined by PAH and inulin clearances respectively. A cannula was inserted into the urinary bladder for collection of urine.

Results: Infusion of MEG doses caused small but significant increases in SAP in both WT and ecSOD KO mice. The highest dose of MEG increased mean SAP from 93±3 to 99±4 mmHg in WT and from 81±5 to 87±5 mmHg in ecSOD KO mice. Although MEG did not cause significant changes in RBF (6.8±0.7 to 7.4±1.4 mL/min/g) or in GFR (0.96±0.12 to 1.01±0.14 mL/min/g) in WT mice but there was a marked decrease in GFR (1.00±0.17 to 0.77±0.12 mL/min/g; P<0.05) without appreciable change in RBF (5.9±0.9 to 5.6±0.8 mL/min/g) in ecSOD KO mice. MEG infusion increased urinary sodium excretion ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}) in both WT ($U_{Na}V$, 0.62±0.17 to 1.65±0.24 µmol/min/g ; FE_{Na}, 0.43±0.11 to 1.17±0.03%) and ecSOD KO mice ($U_{Na}V$, 0.77±0.19 to 1.42±0.21 µmol/min/g; FE_{Na}, 0.52±0.09 to 1.25±0.19%).

Conclusions: These results indicate that endogenous formation of $OONO^-$ contributes to arterial vasodilator tone systemically and provides a renoprotective role in maintaining GFR in the condition where dismutation of O_2^- is limited due to SOD deficiency.

Funding: Other NIH Support - NHLBI

TH-PO1045

Vitamin D Status, Arterial Stiffness, and the Renin Angiotensin System in Healthy Humans Ahmed Abdi Ali, David Donald McTavish Nicholl, Brenda Hemmelgarn, Jennifer M. MacRae, Darlene Y. Sola, Sofia B. Ahmed. *University of Calgary.*

Background: Vitamin D (VD) status is an increasingly notable predictor of kidney and cardiovascular (CV) risk. While the mechanism is unclear, VD appears to modulate RAS activity.We sought to clarify the influence of VD in modulating RAS control of arterial stiffness, an important parameter for the assessment of CV risk in both the healthy and CKD populations.

Methods: Forty-one normotensive, non-obese, healthy subjects (26 females and 15 males) were studied in a high salt balance, a state of maximal RAS suppression. Women

were studied in the same phase of their menstrual cycle. Arterial stiffness, expressed as aortic augmentation index (AIx) and brachial pulse wave velocity (PWV) was measured by tonometry at baseline and in response to AngII infusion (3ng/kg/min x 30 min then 6ng/kg/min x 30 min). The primary outcome was the effect of VD status on the arterial stiffness response to AngII challenge, a well-accepted marker of the intrinsic RAS activity of the vasculature.

Results: Results were analyzed according to serum 25(OH)-VD status: deficient (<50nmol/L, n=12), insufficient (50-80nmol/L, n= 15), and sufficient (>80nmol/L, n=14). Increasing 25(OH)-VD status was associated with improved arterial stiffness (AIx: VD deficient, 17.3±3.4%; VD insufficient, 11.3±4.0%; VD sufficient, -2.0±5.6%; p=0.015, p =0.009 for trend), though a similar relationship was not observed between VD status and PWV (p=0.5). As anticipated, all subjects demonstrated an increase in AIx (p<0.001) and PWV (p<0.001) in response to AngII challenge, though the AIx response did not differ by 25(OH)-VD status (AIx: VD deficient, 19.1±4.7%; VD insufficient, 22.2±5.3%, sufficient 12.5±4.3%; p=0.3). In contrast, the brachial PWV response to AngII differed according 25(OH)-VD status (ΔPWV: VD deficient, 2.6±1.1m/s; VD insufficient, 3.4±0.7m/s; VD sufficient, 1.4±0.3m/s; p=0.032).

Conclusions: Improved VD status is associated with reduced arterial stiffness in healthy humans, possibly through an RAS-dependent mechanism which appears to differ according to VD level and the vascular bed. Further studies are needed to clarify optimal VD status and the role of VD supplementation on CVD risk.

TH-PO1046

25-hydroxyvitamin D insufficiency, Which Can Be Improved with Rosuvastatin Treatment, Is Associated with Renal Endothelial Function

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Background: Vitamin D deficiency is considered as cardiovascular and renal risk factor. We tested the hypotheses whether vitamin D level is related to endothelial function of the renal vasculature. Since statin treatment is known to improve endothelial function, we analyzed the impact of statins on vitamin D levels and renal endothelial function.

Methods: In a double-blind, randomized study 31 hypercholesterolemic patients with at least vitamin D insufficiency (< 30 ng/ml) were randomly assigned to rosuvastatin (10 mg/d) and placebo for 6 weeks. Renal hemodynamics were determined by constant input clearance technique with p-aminohippurate (PAH) and inulin. Basal NOS activity of the renal vasculature, was assessed by measuring renal plasma flow (RPF) both before and after blockade of NOS with systemic infusion of N(G)-monomethyl-L-arginine (L-NMMA). In parallel, 25(OH)D was measured.

Results: Compared to placebo treatment, rosuvastatin increased 25(OH)D levels (21.6±4.0 vs 24.1±8.0 ng/ml, p=0.039). Moreover, the decrease in RPF in response to L-NMMA (an estimate of basal NO activity) was significantly more increased after 6-week therapy with rosuvastatin than with placebo (-94.8±70 vs. -68.2±32 ml/min, p=0.044), indicating increased basal NOS activity after 6 weeks of rosuvastatin treatment. The change of basal NO activity in the placebo phase treatment was correlated inversely with 25(OH) D (r=-0.385; p=0.027). Multiple regression analysis revealed that at baseline 25(OH)D, but not blood pressure and cholesterol levels, is an independent determinant of basal NO activity (β=-0.446, r=0.015). In contrast, no correlation was evident between 25(OH)D and basal NO activity after rosuvastatin treatment.

Conclusions: Thus, rosuvastatin may beneficially influence the impact of vitamin D insufficiency on renal endothelial function.

TH-PO1047

Ambulatory Arterial Stiffness Index (AASI) and All Cause Mortality

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Background: Ambulatory Arterial Stiffness Index (AASI) and pulse pressure (PP) are indices of arterial stiffness that can be computed from 24-hour ambulatory BP measure (ABPM). We investigated the association of AASI and PP with all-cause mortality.

Methods: AASI (1 minus the slope of diastolic on systolic BP in individual 24 hr ABPM) was calculated for182 pts. from 1994 through 1997. Data collected included demographic characteristics and treatment for hypertension (HTN) or diabetes mellitus (DM). Mortality data (date of death) was obtained from the Social Security death registry in February 2011.We applied Cox regression to relate mortality to AASI and PP while adjusting for sex, age, BMI, 24-hr ABPM, smoking, DM , chronic kidney disease (CKD) and cardiovascular disease (CVD).

Results: Mean age was 58 ±14 yrs, 33% were female and 22% were African-American. Mean BMI was 27.2 ± 4.5 kg/m^2, and treatment for HTN (58%) and DM (23%). Eighteen deaths occurred during the 15 year period.AASI mean score >0.51 U was associated with higher all cause mortality (16 /18, P =0.001).However this correlation was not seen with pulse pressure.

AASI Quartiles and PP Quartile and its Correlation with All Cause Mortality

AASI Quartiles	HRs (95% CIs)	24-Hour PP	HRs (95% CIs)
<0.39	0.81 (0.67, 0.99)	<45.8	0.63 (0.50, 0.78)
0.39–0.45	0.86 (0.72, 1.06)	45.8–50.2	0.91 (0.53, 1.55)
0.45–0.51	0.90 (0.74, 1.09)	50.2–55.6	0.92 (0.56, 1.16)
>0.51	1.47 (1.33, 1.85)*	>55.6	1.04 (0.83, 1.31)

HRs- Hazard Ratio:CIs Confidence Interval and * P<0.05

Patient with high AASI index (>0.51 U) were older (P < 0.003), more likely to be non white (P < 0.05), and had higher rates of smoking, DM, HTN, CVD, and CKD (P < 0.01).

Conclusions: AASI, a measure of the dynamic relation between diastolic and systolic blood pressure through the day, was a better predictor of all cause mortality compared to the 24-hr ABPM pressures, anthropometric characteristics, and / or cardiovascular risk factors. Based on the current data, coupling a physiological characterization of the 24 hr ABPM pressures with AASI- indices may improve their prognostic ability to evaluate vascular health.

TH-PO1048

Preservation of Cortical-Medullary Oxygen Gradients in Human Hypertension, Despite a Fall in Blood Flow with Age in Caucasians and Greater Medullary Hypoxia in African-Americans

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Background: Regulation of kidney cortical and medullary oxygenation as a function of age, ethnicity and regional blood flows in essential hypertension (HTN) remain poorly understood.

Methods: We studied 32 Caucasian (C) and 23 African-American (AA) non-diabetic subjects with treated HTN and normal GFR during 3 days with 150 mEq/d Na+ intake and ACE/ARB Rx. Single-kidney volumes and blood flow were measured using Multidetector CT. De-oxyhemoglobin levels (R2*) and cortical-medullary oxygen (O2) gradient (difference in R2* between superficial cortex and deep medullary segments) were measured by Blood Oxygen Level Dependent (BOLD) MRI at 3T before and after furosemide suppression of solute transport. On the first day, urinary (U) Na+, GFR, isoprostanes, microalbumin, protein and TGF-beta were also measured.

Results: Average Age was 58 (26-85) years. BP: 135±2 / 75±2 mmHg. Single kidney GFR: 49±2 ml/min/1.73m2.

	Cortex	Medulla	p-value
Tissue volume (cc/kidney)	97±2.5	54±2	p<.0001
Blood flow (ml/min)	354±15	71±3	p<.0001
Oxygenation (R2*/sec)	17.9±0.3	38.1±0.6	p<.0001

Cortical-Medullary Gradient averaged 20.1±0.6 /sec which fell to 12.1±0.6/sec after furosemide (p<.0001). Age was inversely related to GFR, diastolic BP, and isoprostane excretion. Cortical perfusion and blood flow fell with advancing age only in C with preserved cortical-medullary O2 gradients. Urinary microalbumin rose with SBP. Kidney injury markers including U protein and TGF-beta were directly related to SBP and initial Na+ excretion (R=0.546, p<.0001).

Conclusions: These data demonstrate preserved cortical-medullary O2 gradients and furosemide-suppressible O2 consumption in essential HTN, despite loss of cortical blood flow in C. Isoprostane differed between C and AA, whose levels rose with age and blood flow to medulla. Correlation between fibrogenic and oxidative biomarkers suggest that prior Na+ intake and microvascular dysfunction contribute to renal injury in essential HTN.

Funding: Other NIH Support - NHLBI

TH-PO1049

Effect of One Week Naproxcinod Treatment on Sodium Balance and Acute Natriuretic Effect of Furosemide: A Randomized Double-Blind Placebo and Naproxen-Controlled Trial in Healthy Volunteers

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Background: Naproxcinod (CIN) is a cyclooxygenase inhibiting nitric oxide (NO) donor developed for the treatment of osteoarthritis. The objective of the study was to evaluate the effects of CIN on sodium (Na) balance and response to furosemide (FUR).

Methods: 31 healthy male volunteers were randomized into three parallel groups: CIN 750 mg *bid*, naproxen (NAP) 500 mg *bid* or placebo (PLA) *bid* during eight days (D1-D8). 24h-Na and aldosterone excretions were measured from D -1 to D4. On D8, natriuresis, plasma renin activity (PRA) and glomerular filtration rate (GFR), using inulin clearances, were measured before and after 40 mg of intravenous FUR.

Results: On D-1, 24h Na excretion and aldosterone excretion were respectively 193 ± 34 mmol/24h and 5.2 ± 0.9 µg/24h for PLA, 174 ± 40 mmol/24h and 5.4 ± 0.7 µg/24h for CIN, 196 ± 54 mmol/24h and 6.5 ± 0.8 µg/24h for NAP. On D1, 24h Na excretion was 182 ± 49 mmol/24h for PLA, 147 ± 34 mmol/24h for CIN, 140± 45 mmol/24h for NAP. On D4, aldosterone excretion was 6.2 ± 1.0 µg/24h for PLA, 2.9 ± 0.5 µg/24h for CIN, 2.7 ± 0.4 µg/24h. Table 1 shows Na excretion, GFR and PRA before and after FUR on day 8.

Table 1

Day 8		FUR (Baseline)	FUR + 60'	FUR + 120'
Natriuresis (mmol/h)				
	PLA	38.2 ± 12.1	136.5 ± 34.7	53.5 ± 10.8
	CIN	32.7 ± 5.2	129.9 ± 17.5	54.7 ± 21.3
	NAP	27.2 ± 9.9	131.2 ± 19.1	54.8 ± 14.3
GFR (ml/min)				
	PLA	117 ± 36	105 ± 13	108 ± 55
	CIN	94 ± 22	94 ±20	79 ± 24
	NAP	103 ± 21	96 ± 28	79 ± 22
PRA (ng/ml/h)				
	PLA	0.82 ± 0.52	1.79 ± 0.91	2.72 ± 0.81
	CIN	0.32 ± 0.21	0.84 ± 0.36	1,37 ± 0.63
	NAP	0.35 ± 0.19	1.01 ± 0.49	1.91 ± 0.89

Values are means ± SD

Conclusions: CIN and NAP had some degree of Na retention (progressive decrease in 24h urinary aldosterone excretion) compared to PLA. After 8 days of treatment, no difference in Na excretions after FUR was detected between groups, but PRA response to FUR were slightly blunted in the CIN and NAP groups. The addition of NO moiety to naproxen does not seem to influence the Na balance or the natriuretic response to FUR compared to naproxen alone in healthy volunteers.

Funding: Pharmaceutical Company Support

TH-PO1050

The mTOR Inhibition Reduces the In Vitro Mineralization of Vascular Smooth Muscle Cells Jasmin Pruefer,[1] Mirjam Schuchardt,[1] Markus Tolle,[1] Matthias Höhne,[2] Markus van der Giet.[1] *[1]Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany; [2]Novartis Pharma AG, Nürnberg, Germany.*

Background: Vascular disease contributes to the high cardiovascular mortality among organ transplant recipients. The immunosuppressive regimes, necessary for preventing transplant rejection, have different side effects on the vascular system. The aim of this study was to investigate whether the mTOR inhibitor rapamycin (RPA) is also effective for the prevention of vascular calcification in an in vitro mineralization assay using vascular smooth muscle cells (VSMCs).

Methods: In vitro calcification in VSMCs were induced with calcification medium (CM: DMEM containing 4.5 g/L glucose supplemented with 15% FCS, 10 mmol/L sodium pyruvate, 50 µg/mL ascorbic acid, and 10 mmol/L ß-glycero phosphate) and dexamethasone (DEX, 100 nmol/L). Calcium deposition was quantified by O-cresolphthalein complexone method. Alkaline phosphatase (ALP) enzyme activity was measured by p-nitrophenol method.

Results: Cultivation of VSMCs in CM induced mineralization of VSMCs, which could be enhanced in the presence of DEX. The calcification was quantified by measuring the extracellular calcium content and visualized by Alizarin Red staining. Pretreatment with RPA could significantly and time-dependently decrease the mineralization of VSMCs by reducing extracellular calcium. For the precipitation of calcium phosphate, the activation of ALP is necessary. CM and +DEX led to a significant and time-dependent increase in ALP enzyme activity, which is significantly diminished by pretreatment with RPA.

Conclusions: In this study we were able to show that the mTOR inhibitor RPA diminished the mineralization of VSMCs in vitro. Therefore, it seems possible that RPA might be effective in the prevention of arteriosclerosis after organ transplantation that would contribute to a better cardiovascular outcome of these patients.

TH-PO1051

Reactive Oxygen Species Acutely Stimulate Renin Release in Mouse Juxtaglomerular Cells Mariela Mendez, Jeffrey L. Garvin. *Hypertension and Vascular Research. Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.*

Background: Renin and its enzymatic product Angiotensin I, are essential in the regulation of blood pressure. Chronically enhanced circulating renin induces hypertension and renal damage. Low levels of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, play a role in renal hemodynamics and tubular NaCl transport. Enhanced ROS in the kidney are involved in the development of hypertension and kidney disease. However, it is not known whether ROS affect renin release from juxtaglomerular (JG) cells.

Methods: We generated primary cultures of mouse Juxtaglomerular cells (JG) cells and measured the effect of H2O2 or superoxide on renin release to the media and total renin content.

Results: Treatment with H2O2 (1 hour) at 100 or 500 nM increased renin release by 101±43% ($p = 0.08$) and 166±47% ($p < 0.04$), respectively. In addition, decreasing endogenous hydrogen peroxide levels by treating JG cells with catalase (1mU/ml) decreased basal renin release by 45±9% ($n = 6$; $p < 0.05$). These data indicate that endogenously produced H2O2 tonically stimulates renin release. H2O2 peroxide had no effect on total renin content in JG cells ($n = 6$; p = n.s.) suggesting that H2O2 stimulates renin exocytosis. Superoxide produced by surrounding cells or endogenously by JG cells may influence renin release. Increasing extracellular superoxide by adding xanthine oxidase/hypoxanthine (XO/HY) increased renin release by 334±140% ($p < 0.03$; $n = 4$). In the presence of the superoxide scavenger tempol, enhancing extracellular superoxide did not stimulate renin release (56±30% *vs* control, n.s). Treating JG cells with tempol alone did not affect basal renin release ($n = 4$).

Conclusions: We concluded that the ROS, hydrogen peroxide and superoxide, stimulate renin release from mouse JG cells. These data suggest a novel and rapid pathway for the stimulation of renin release that may be involved in the development of hypertension and kidney damage during enhanced ROS production in the renal cortex.

Funding: Other NIH Support - NRSA to Mariela Mendez

FR-PO1052

The Ratio of alpha-1-Acid Glycoprotein to alpha-1B Glycoprotein in Urine Is an Early and Accurate Predictor of Acute Kidney Injury Joseph Alge,[1] Lakhmir S. Chawla,[4] James A. Tumlin,[5] John M. Arthur.[1,2] *[1]MUSC; [2]Ralph H Johnson VAMC; [3]Duke University; [4]George Washington University; [5]University of Tenessee Chattanooga.*

Background: Acute kidney injury (AKI) is a common complication of cardiac surgery. A biomarker that is diagnostic of AKI earlier in the course would facilitate intervention and improve patient outcomes. We identified early biomarkers of AKI using a mass spectrometry based proteomics approach.

Methods: Urine samples were obtained from patients shortly (mean 10 hours) after cardiac surgery. Four patients developed severe AKI (mean increase Cr 3.3 mg/dl), and 4 did not (mean increase 0.2). Proteomic analysis was done by liquid chromatography and tandem mass spectrometry. Proteins were identified with Mascot and validated with Scaffold. The exponentially modified protein abundance index (emPAI) was used for quantification. Candidate biomarkers were identified by comparison of emPAI values.

Results: We identified 227 high confidence proteins with >2 peptides (FDR <1%). The high abundance proteins albumin, lambda light chain, and kappa light chain did not differ in abundance between the two groups. Previously described AKI biomarkers alpha-1-acid glycoprotein (AGP-1), cystatin-C , hemopexin and NGAL were different between the groups. AGP-1 and Alpha-1B-glycoprotein (A1BG) changed in opposite directions with AKI. The mean emPAI value of AGP-1 was 0.33±0.06 in the AKI group and 0.15±0.04 in the non-AKI group (p=0.04). The mean emPAI value of A1BG was 0.06±0.02 in the AKI group and 0.19±0.03 in the non-AKI group (p=0.04). Furthermore, when the ratio of APG-1 to A1BG was calculated we found that it was able to predict AKI at this early time point with 100% accuracy. The segregation between the AGP-1:A1BG ratios for AKI and non-AKI patients was large. The mean difference in ratios between groups was over nine-fold and the smallest difference between individual members of the groups was greater than 2-fold.

Conclusions: The ratio AGP-1 to A1BG is a potential early marker of AKI. The use of a ratio of proteins which change in opposite directions enhances the sensitivity of the predictor and obviates the need for normalization.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1053

Biomarkers in Acute Kidney Injury: Should We Assess Them in Serum or Urine? Josee Bouchard,[1] Rakesh Malhotra,[2] Ashita J. Tolwani,[3] Ravindra L. Mehta.[2] *[1]Universite de Montreal, Canada; [2]University of California San Diego; [3]University of Alabama at Birmingham.*

Background: There is limited information on the value of serum vs. urine biomarkers in acute kidney injury (AKI) diagnosis. The aim of this study was to compare the predictive capacity of serum and urine biomarkers to diagnose AKI.

Methods: We conducted a prospective, multicenter observational study to evaluate the role of serum vs. urine neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C and osteopontin levels to diagnose AKI in 51 critically ill patients. AKI was defined according to the AKIN creatinine criterion. Samples were collected every 12 hours after intensive care unit (ICU) admission for a ≥48 hours and up to 10 days.

Results: From the 51 patients enrolled, 17 developed AKI on average 1.5 ± 1.7 days after ICU admission. The Area Under the Curve (AUC) of the different biomarkers to predict AKI are included in the table:

	in serum	in urine
NGAL	0.482 (0.343-0.622)	0.598 (0.489-0.707)
KIM-1	0.714 (0.613-0.814)	0.783 (0.680-0.886)
Cystatin C	0.650 (0.549-0.750)	0.710 (0.619-0.801)
Osteopontin	0.651 (0.540-0.762)	0.630 (0.510-0.749)

Conclusions: In our cohort, NGAL, KIM-1 and cystatin C offered a better discriminative performance in urine than in serum for AKI diagnosis. These results are helpful to plan further studies using biomarkers in AKI.

Funding: Pharmaceutical Company Support, Private Foundation Support, Clinical Revenue Support

FR-PO1054

MYH9 Gene Variant Is a Risk Factor for Acute Kidney Injury after Severe Trauma Michael G. Shashaty, Paul Lanken, Scarlett L. Bellamy, Sandra Kaplan, Jason Christie, Nuala J. Meyer. *University of Pennsylvania School of Medicine.*

Background: Acute kidney injury (AKI) is a source of substantial morbidity and mortality. Clinical factors incompletely explain AKI risk. Multiple studies have highlighted a locus at chromosome 22, encompassing the APOL1 and MYH9 genes, as strongly associated with chronic kidney disease (CKD). We hypothesized that these genetic variants may be associated with increased risk of AKI.

Methods: Patients admitted to our ICU with severe trauma were prospectively followed for AKI, defined by Acute Kidney Injury Network (AKIN) creatinine criteria. We tested the association of two candidate gene single nucleotide polymorphisms (SNPs) previously associated with CKD—rs4821480 (MYH9) and rs73885319 (APOL1)—with AKI. Taqman genotyping of genomic DNA was performed. As the APOL1 SNP is private to African populations, only African ancestry (AA) subjects were typed for this variant. Significance of odds ratios was determined with χ^2 testing assuming an additive model of genetic risk. Multivariable logistic regression was used to adjust for clinical confounders.

Results: Of 443 subjects enrolled, 112 (25.3%) developed AKI. The cohort was 46% African and 49% European ancestry. MYH9 rs4821480G was significantly associated with AKI (OR 1.39, 95% CI 1.03,1.88, p=0.030). There was no confounding of this association by sex, age, ancestry, baseline creatinine, hypertension, diabetes, or Injury Severity Score (OR 1.49, 95% CI 1.09,2.04, p=0.011). APOL1 rs73885319 did not show a significant association with AKI in the AA population (OR 1.40, 95% CI 0.78,2.49, p=0.256).

Conclusions: MYH9 rs4821480G was significantly associated with AKI after severe trauma, highlighting possible mechanistic overlap between AKI and CKD. The function of rs4821480, an intronic SNP, is not yet known, though MYH9 encodes a protein that may be involved in podocyte structure and function. An association of AKI with APOL1 rs73885319 could not be ruled out given the limited AA sample size. Genetic risk factors offer the potential to improve AKI risk stratification and better elucidate AKI pathogenesis.

Funding: Other NIH Support - P50-HL60290, P01-HL079063, K12-HL090021

FR-PO1055

Genetic Variation in SLC22A2 Organic Cation Transporter 2 (OCT2) Influences Cisplatin (CDDP)-Induced Nephrotoxicity in Cancer Patients Kazufumi Iwata,[1] Keiji Aizawa,[1] Saori Kamitsu,[1] Eiko Fukunaga,[1] Minoru Yoshida,[2] Akinobu Hamada,[3] Hideyuki Saito.[3] *[1]Pharmacy, Kumamoto Red Cross Hospital, Kumamoto, Japan; [2]Medical Oncology, Kumamoto Red Cross Hospital, Kumamoto, Japan; [3]Pharmacy, Kumamoto University Hospital, Kumamoto, Japan.*

Background: CDDP has been a mainstay for chemotherapy of multiple solid tumors. However, CDDP is clinically often complicated due to its dose-limiting nephrotoxicity. As the organic cation transporter SLC22A2/OCT2 is highly expressed in the basolateral membrane of proximal tubules, thereby being considered as a predominant transporter mediating active accumulation of CDDP in the kidney. Besides, single-nucleotide polymorphism (SNP) in the OCT2 808G>T (Ala270Ser) has been suggested to correlate with the reduced CDDP-induced nephrotoxicity. In this study, we explored the effect of 808G>T SNP in the OCT2 gene on the adverse events and the systemic exposure of CDDP in cancer patients.

Methods: We evaluated 53 patients with urothelial, lung, esophagus, head-neck, stomach and mesotheliomas carcinomas who had been treated with CDDP at a dose of over 60 mg/m^2. Genotyping was performed by using TaqMan SNP Genotyping Assays. The plasma concentration of CDDP was evaluated on day 3 and 6 after the treatment, as the free platinum level measured by ICP-MS. The toxicity grade was evaluated by CTCAE version 4.0 criteria.

Results: The number of patients who had the OCT2 808GG, GT and TT was 44, 9 and none, respectively. Differences in serum creatinine (SCr) levels between the baseline and cycle 1 in the patients with the GG and GT were increased by 1.43 and 1.19 folds, respectively. In the total treatment cycles, 12 patients (27%) with the GG experienced the grade2 SCr elevation, whereas the patients with GT showed no apparent toxicity. White blood cell and platelet levels showed no difference between the both groups. On day 3, the plasma concentrations of CDDP in the patients with the GG and GT were 75 and 63 ng/mL, and on day 6, those in GG and GT were 70 and 57 ng/mL, respectively.

Conclusions: In conclusion, 808G>T SNP in the OCT2 gene appeared to be associated significantly with the CDDP-induced nephrotoxicity, but not with the pharmacokinetic profile of CDDP.

Funding: Government Support - Non-U.S.

FR-PO1056

Natural History of Acute Kidney Injury in Intensive Care Unit Patients: The Multicenter International O'Brien Center Registry Josee Bouchard,[1] Jiandong Wei,[2] Sharon Soroko,[2] Anjali Acharya,[3] Jorge Cerda,[4] Elizabeth R. Maccariello,[5] Rajasekara Chakravarthi Madarasu,[6] Ashita J. Tolwani,[7] Xinling Liang,[8] Ping Fu,[9] Zhi-Hong Liu,[10] Ravindra L. Mehta.[2] *[1]U de Montreal; [2]UCSD; [3]Jacobi Medical Center; [4]Albany Medical College; [5]Rede d'Or; [6]CARE Hospitals; [7]UAB; [8]Guangdong General Hospital; [9]Sichuan Univ; [10]Nanjing Univ.*

Background: Although AKI is common and associated with increased mortality, there is a lack of multicenter prospective large databases on mild to severe AKI throughout the world.

Methods: We conducted a prospective observational study to determine the incidence of AKI in ICU patients in 13 countries using AKIN criteria and to characterize differences in etiology, clinical factors and process of care among patients.

Results: Between 2008 and 2011, 778 of 3879 critically ill patients (20%) had AKI during the first week of ICU admission, and 479 (62%) were enrolled in our registry. Mean age was 59±17 yrs, 69% were male, 66% were non-caucasian, 16% had CKD, 40% were mechanically ventilated, 26% on pressors, 42% on diuretics and 65% were oliguric. Mean SOFA score was 6.5±4.1. Pre-renal factors were the most common risk factors for AKI (64%). 16% required renal replacement therapy (RRT). Continuous RRT, intermittent hemodialysis and sustained low-efficiency dialysis were used in 51%, 26% and 20% of patients, respectively, while peritoneal dialysis was used in 3%. 15% of patients were dialysis-dependent at hospital discharge. Overall hospital mortality was 19% (33% in patients who required RRT vs. 17% in patients who did not require RRT;p=0.005). Independent risk factors for hospital mortality among AKI patients included use of pressors (OR 1.80; 95%CI 1.01-3.19;p=0.04) and mechanical ventilation (OR 5.75; 95%CI 2.86-

11.60;p<0.001) while the use of diuretics was associated with a protective effect on mortality (OR 0.43; 95%CI 0.23-0.80;p=0.008).

Conclusions: We describe an ongoing registry of AKI in ICU patients. AKI incidence was 20% and was associated with a 19% mortality rate. This multicenter multinational registry also provides a contemporary overview of clinical factors and management of AKI in ICU worldwide.

Funding: Private Foundation Support, Clinical Revenue Support

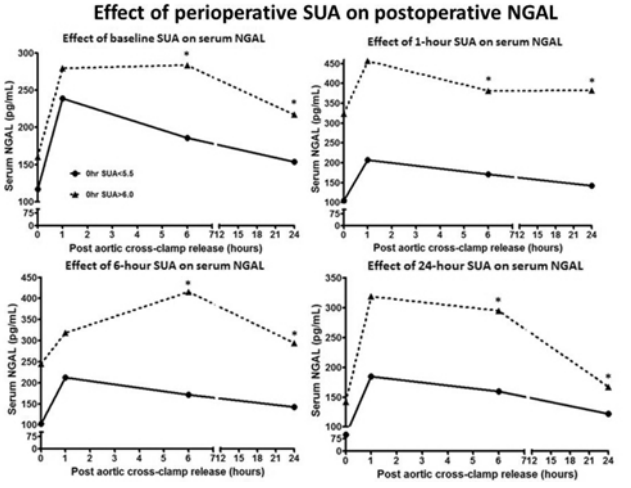
FR-PO1057

Perioperative Serum Uric Acid Affects Serum NGAL Concentrations A. Ahsan Ejaz,[1] Abdo Asmar,[1] Michiko Shimada,[2] Ganesh Kambhampati,[1] Noel I. Ejaz,[1] Amir Ahsan Arif,[1] Mourad Alsabbagh,[1] Richard J. Johnson.[3] *[1]Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, FL; [2]Division of Cardiology, Respiratory Medicine and Nephrology, Hirosaki University Graduate School of Medicine, Hirosaki, Aomori, Japan; [3]Division of Renal Diseases and Hypertension, University of Colorado Health Sciences Center, Denver, CO.*

Background: We have previously demonstrated that preoperative serum uric acid (SUA) is an independent risk factor for AKI and that SUA >6mg/dL was associated with a 4-fold increased risk factor for AKI in patients undergoing cardiac surgery. Since NGAL is a reliable biomarker of early AKI, we investigated the effect of SUA serum on serum NGAL concentrations in the same reported cohort of cardiac surgery patients.

Methods: SUA and NGAL were measured from stored, frozen serum samples obtained at 0, 1, 6 and 24 hours post aortic cross-clamp release in our previously reported cohort. The relationship of SUA and NGAL were investigated using appropriate statistical tests. At every time point of measurement of SUA, its effect on 0, 1, 6 and 24-hr NGAL were assessed.

Results: 37 patients were included for analyses. The effects of SUA>6mg/dL on NGAL were compared in relation to reference SUA<5.5mg/dL, the level above which uric acid manifests its pro-inflammatory effects. At any given time of SUA measurement, SUA>6mg/dL was associated with significantly elevated serum NGAL concentrations measured at 6 and 24hrs. SUA>6mg/dL was also associated with significantly elevated SCR values at postoperative days 1-5.



Conclusions: These data provides biomarker evidence of early renal parenchymal damage associated with elevated SUA in patients undergoing cardiac surgery.

FR-PO1058

Risk Factors and Etiology of Acute Kidney Injury in Intensive Care Unit Patients: The Multicenter International O'Brien Center Registry Josee Bouchard,[1] Jiandong Wei,[2] Sharon Soroko,[2] Sam Kuo,[2] Anjali Acharya,[3] Jorge Cerda,[4] Elizabeth R. Maccariello,[5] Rajasekara Chakravarthi Madarasu,[6] Ashita J. Tolwani,[7] Xinling Liang,[8] Ping Fu,[9] Zhi-Hong Liu,[10] Ravindra L. Mehta.[2] *[1]U de Montreal; [2]UCSD; [3]Jacobi Medical Center; [4]Albany Medical College; [5]Rede d'Or; [6]CARE Hospitals; [7]UAB; [8]Guangdong General Hospital; [9]Sichuan Univ; [10]Nanjing Univ.*

Background: AKI is frequent in ICU patients and is associated with increased mortality. However, there is a lack of information on the risk factors and etiologies of AKI across the world.

Methods: We conducted a prospective observational study to compare etiology of AKI and risk factors for AKI in ICU patients with and without AKI in 13 countries.

Results: Between 2008 and 2011, 778 of 3879 patients (20%) developed AKI during the first week of ICU admission. AKI patients were older (61±18 vs. 57±19 yrs;p<0.001) and had more comorbidities than non-AKI (diabetes 24% vs. 14%;p<0.001, obesity 30% vs. 24%;p=0.004, liver failure 11% vs. 5%;p<0.001, heart failure 19% vs. 9%;p<0.001 and CKD 20% vs. 11%;p<0.001). Mean patient age and AKI incidence were similar between developed and emerging countries. However, patients in emerging countries were less likely to be male (58% vs. 63%;p=0.004), obese (15% vs. 30%;p<0.001), and have liver failure (5% vs. 7%;p=0.001); they were more likely to have heart failure (14% vs. 10%;p<0.001) and CKD (20% vs. 12%;p=0.02). AKI etiologies in patients enrolled in our registry (n=479/778;62%), included acute tubular necrosis (ATN) (21%), glomerulonephritis (GN) (4%), interstitial nephritis (2%), and prerenal (64%). 65% had multifactorial etiologies. Sepsis was present at AKI diagnosis in 50%. ATN and prerenal were more frequent in developed countries (27 vs. 15%;p<0.001 and 76 vs. 52%;p<0.001), while GN was more common in emerging countries (7% vs. 0.5%;p=0.004).

Conclusions: We describe an ongoing multicenter multinational registry of AKI in ICU patients. Risk factors and etiologies of AKI differed considerably between emerging and developed countries. These comparisons are useful to establish preventive and therapeutic strategies to improve AKI outcomes.

Funding: Private Foundation Support, Clinical Revenue Support

FR-PO1059

Study on the Usefulness of Urinary Parameters in Early Detection of Acute Kidney Injury after Cardiac Surgery in Adults Katsuomi Matsui, Takeshi Sugaya, Takashi Yasuda, Kenjiro Kimura. *Department of Nephrology and Hypertension, Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.*

Background: Acute kidney injury (AKI) is a common complication after cardiac surgery. Urinary liver-type fatty acid-binding protein (L-FABP) reflects the presence of renal tubular injury. The aim of this study was to evaluate the utility of urinary L-FABP compared with other urinary parameters for the early detection of postoperative AKI among adult patients undergoing cardiac surgery.

Methods: Adult patients undergoing cardiac surgery in our hospital were eligible for enrollment. A total of 85 patients were prospectively studied from August 2009 to October 2010. Patients who depended on chronic dialysis support, patients undergoing emergency operation (operation performed within 24 hours after admission) and patients who died within the first 24 h after surgery were excluded from this study. Patients were divided into the AKI and non-AKI groups according to whether they developed AKI within 48 h after surgery. Postoperative AKI was defined according to AKIN criteria. Changes in various parameters, such as serum creatinine, urinary L-FABP, urinary neutrophil gelatinase-associated lipocalin, urinary albumin, and urinary *N*-acetyl-β-D-glucosaminidase, were evaluated. Urine and serum samples were obtained from each patient at the following time points: preoperative, immediately postoperative, and 3, 6, 18, 24, and 48 h postoperatively.

Results: The urinary L-FABP level was significantly higher in the AKI group than in the non-AKI group at every time point, while other parameters did not show such tendency. The parameter with the largest area under the curve at every time point for predicting the onset of AKI was urinary L-FABP. On multiple logistic regression analyses, the urinary L-FABP level pre-operation and within the first 6 h after cardiac surgery was significantly associated with the onset of AKI.

Conclusions: Our study suggests that urinary L-FABP was a useful biomarker for early detection of AKI and was an early good predictor of the onset of AKI.

Funding: Private Foundation Support

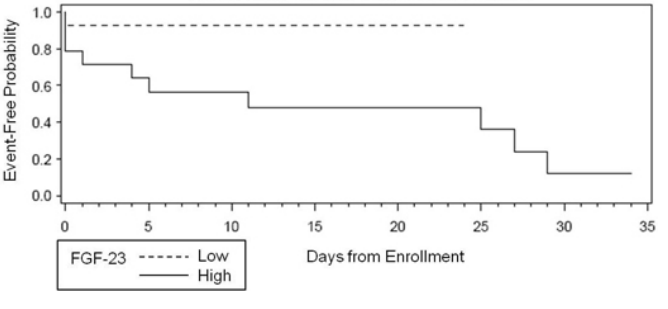
FR-PO1060

Fibroblast Growth Factor 23 as a Biomarker in Acute Kidney Injury David E. Leaf,[1] Myles S. Wolf,[2] Leonard Stern.[1] *[1]Department of Medicine, Division of Nephrology, Columbia University Medical Center, New York, NY; [2]Division of Nephrology and Hypertension, University of Miami, FL; [3].*

Background: Fibroblast growth factor 23 (FGF-23) regulates phosphorus and vitamin D homeostasis. Elevated levels are independently associated with increased mortality in patients with chronic kidney disease and ESRD. Whether FGF-23 levels are elevated and associated with adverse clinical outcomes in patients with acute kidney injury (AKI) has not been studied.

Methods: We recruited 28 participants with AKI and 30 controls from the medical intensive care unit and general hospital wards of Columbia University Medical Center. FGF-23 levels were measured at baseline and repeated 5 days later (Immunotopics ELISA assay). The combined clinical outcome was death or need for renal replacement therapy (RRT).

Results: FGF-23 levels were significantly higher among participants with AKI than controls [median(IQR) 1546 (253-2988) and 263 (87-577) RU/ml, respectively, p=0.003]. Among participants with AKI, baseline FGF-23 levels above versus below the median were associated with a significantly higher probability of death or need for RRT (log rank test, p=0.03).



Conclusions: FGF-23 levels are elevated in AKI and are associated with greater risk of death or need for RRT. Additional work is needed to determine potential mechanisms underlying these preliminary findings.

Funding: Private Foundation Support

FR-PO1061

Urinary IL-18 Is the Most Useful Early Predictive Biomarker of Contrast-Induced Nephropathy (CIN) on Chronic Kidney Disease (CKD) Stage 3 Patients in Comparison with NGAL and L-FABP Kosuke Inoue, Yoshiko Shimamura, Koji Ogata, Masayuki Ishihara, Toru Kagawa, Yoshio Terada. *Endocrinology, Metabolism and Nephrology, Kochi Medical School, Nankoku, Kochi-prefecture, Japan.*

Background: Contrast-induced nephropathy (CIN) is the important cause of hospital-acquired acute kidney injury (AKI) on Chronic kidney disease (CKD) patients. However, some urinary biomarkers for AKI were reported to be high in CKD patients. Thus, sensitivity and specificity of these urinary biomarkers are not determined for the diagnosis of AKI in CKD patients. This study was designed to investigate whether human urinary interleukin-18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid binding protein (L-FABP) are early predictive markers for CIN after coronary angiography in CKD patients.

Methods: 41 patients of CKD Stage 3 undergoing coronary angiography were enrolled. Urine samples were collected before, 3 h, 6 h, 24 h after coronary angiography and IL-18, NGAL, and L-FABP levels measured by using an ELISA kit. Urinary creatinine values were measured and the values of urinary biomarkers were corrected by the creatinine concentration because of urinary concentration. This study is in accordance with the Declaration of Helsinki (2002) and was approved by Kochi Medical School review boards. All patients provided written informed consent.

Results: eGFR (estimated glomerular filtration rate) decreased more than 10 ml/min in 14 patients (decreased eGFR group) and did not decrease in remaining 27 patients (non-decreased group) after coronary angiography. At 3 h, 6 h, and 24 h after the procedure, the ratio with the previous value of the urinary IL-18 and L-FABP were significantly increased in the decreased eGFR group, but not in the non-decreased group. In contrast, NGAL are rapidly increased in both group, however, no statistically significant difference of NGAL was observed between two groups. When we used uncorrected biomarker values by creatinine, the specificity and sensitivity were significantly decreased. Rather more, urinaly IL-18 was better than urinaly L-FABP on ROC analysis.

Conclusions: We conclude that urinary IL-18 could be early biomarkers of CIN in CKD Stage3 patients.

FR-PO1062

Urine Biomarkers of Aminoglycoside Nephrotoxicity in Children Zubaida Al-Ismaili,[1] Joseph V. Bonventre,[3] Prasad Devarajan,[2] Melissa Piccioni,[1] Venkata Sabbisetti,[3] Michael R. Bennett,[2] Qing Ma,[2] Michael Zappitelli.[1] *[1]McGill University, Canada; [2]Cincinnati Children's Hosp Med Center; [3]Brigham and Women's Hospital.*

Background: Aminoglycosides(AG) are commonly used in children but are nephrotoxic. Acute kidney injury(AKI) biomarkers and serum Cystatin C(CysC) have not been validated for AG-AKI. We hypothesized that AKI biomarkers are diagnostic of AG-AKI and the association is stronger when defining AKI by CysC.

Methods: We reported on 86 prospectively studied AG treatments(tx) in children on non-critical care units. Daily urine neutrophil gelatinase-associated lipocalin(NGAL), interleukin-18(IL-18) and kidney injury molecule-1(KIM-1) were measured. AKI was defined as 1)AKItrad("traditional": >50% or 27 umol/l rise from baseline in 3 months prior); 2)AKIfirst(same definition but baseline was SCr within 72hrs of AG start); 3)AKIcysc(like AKIfirst, but CysC change instead). We calculated area under the curve (AUC) of a)Peak biomarker levels and b)Tx day 1 or 2 levels to predict AKI by all 3 definitions. We calculated Spearman correlation between Peak biomarker levels and days with AKI.

Results: Mean[SD] age and tx duration were 8.3[4.9] yrs and 8.4[8.8] days; 51% were boys, 74% on oncology wards, 77% tobramycin. 53% developed AKItrad, 19% AKIfirst, 21% AKIcysc. AUC's to predict AKItrad using Peak NGAL, IL-18 and KIM-1 were: 0.53, 0.46, 0.63; to predict AKIfirst: 0.52, 0.44, 0.61; for AKIcysc: 0.51, 0.54, 0.52. Combined AUC's (logistic regression) to predict AKItrad, AKIfirst and AKIcysc including all 3 Peak biomarkers were: 0.61, 0.57, 0.68. AUC's to predict AKItrad using Tx days 1 or 2 NGAL, IL-18 and KIM-1 were: 0.53, 0.38, 0.54; to predict AKIfirst: 0.43, 0.35, 0.56; for AKIcysc: 0.23, 0.32, 0.52. The combined AUC's to predict AKItrad, AKIfirst and AKIcysc including all 3 Tx 1-2 biomarkers were: 0.55, 0.69, 0.79. Only Peak KIM-1 on treatment correlated with number of days with AKI ($r = 0.23$, $p = 0.03$).

Conclusions: Urine KIM-1 was more strongly associated with AG-AKI and AKI severity. Combining information from multiple biomarkers enhanced AG-AKI prediction. AKI-biomarker associations were strongest when defining AKI by CysC.

FR-PO1063

Biomarkers of Cisplatin and Ifosfamide Nephrotoxicity in Children Melissa Piccioni,[1] Zubaida Al-Ismaili,[1] Prasad Devarajan,[2] Joseph V. Bonventre,[3] Venkata Sabbisetti,[3] Michael R. Bennett,[2] Qing Ma,[2] Michael Zappitelli.[1] *[1]McGill University Health Centre, Canada; [2]Cincinnati Children's Hospital Medical Center; [3]Brigham and Women's Hospital.*

Background: Cisplatin (Cis) and Ifosfamide (Ifos) are nephrotoxic. Improving Acute kidney injury (AKI) diagnosis using new biomarkers may improve management of AKI and complications.

Methods: We prospectively collected serum creatinine (SCr), urine neutrophil gelatinase-associated lipocalin(NGAL), interleukin-18(IL-18) and kidney injury molecule-1(KIM-1) during 16 Cis and 21 Ifos treatments(Tx's) in children with cancer(mostly solid tumours) in an ongoing pilot study. We characterized biomarker increases on Tx and used Kruskal-Wallis test to evaluate rise. We defined AKI as >=50% or 27 umol/l SCr rise from baseline. We calculated area under the curve(AUC) for a)Peak biomarker concentration and b)Day 2 biomarker concentration to predict AKI during Tx (Cis and Ifos combined). We used logistic regression to calculate combined AUC for Peak and Day 2 biomarker concentration to predict AKI during Tx.

Results: Mean[SD] age was 9.8[4.3] yrs; 41% were boys; 31% had AKI on Cis and 48% on Ifos. All 3 biomarkers rose during the first 4 days of Cis and Ifos; only KIM-1 rise was statistically significant: Days 1 to 4 of Cis, median KIM-1 (pg/ml): 36.1, 52.1, 174.4, 208.2, respectively ($p=0.009$); Days 1 to 4 of Ifos, median KIM-1 (pg/ml): 94.3, 165.5, 196.2, 339.8, respectively ($p=0.002$). AUC's for Peak NGAL, IL-18 and KIM-1 to predict AKI were 0.58, 0.64, 0.71; when combining all 3 biomarkers, the AUC increased to 0.75. AUC's for Tx Day 2 NGAL, IL-18 and KIM-1 to predict AKI were 0.42, 0.37, 0.69; when combining all 3 biomarkers, the combined AUC increased to 0.76.

Conclusions: AKI during Cis and Ifos was common. All 3 biomarkers rose during Tx but only KIM-1 rose significantly and KIM-1 most strongly predicted AKI on treatment. Given previous literature on very early rise of NGAL and IL-18 with AKI, it may be necessary to collect these biomarkers very shortly after Cis or Ifos dosing to best evaluate their diagnostic value.

FR-PO1064

Risk Factors and Severity of Illness of Contrast-Induced Nephropathy in Elderly Patients with Diabetes Zhan Li,[1] Hua Wu.[2] *[1]Nephrology, Beijing Hospital; [2]Nephrology, Beijing Hospital.*

Background: To analyze the risk factors and the severity of contrast-induced nephropathy (CIN) in the elderly patients with diabetes during PCI.

Methods: We studied and analyzed retrospectively 269 elderly patients who were done coronary angiography and PCI from January 2007 to December 2009 in our hospital. The patients were divided into two groups according to developing CIN or not. The possible risk factors for CIN were compared between the two groups. Then, the patients with CIN were divided into the serume creatinine increasing <50% or ≥50% to determine the possible risk factors to aggravating CIN.

Results: 269 patients, the average age is 72.69±4.74 years(65~86 years); BMI 25.61±3.15 kg/m2. HbA1c 7.37±1.38%. The prevalence of hypertension was 80.7%, CKD was 29.7%, myocardial infarction was 24.2%. The incidence of DN was 25.7% and DR 21.9%. The mean eGFR of preoperation was 70.14±21.55ml/min and Scr was 83.90±34.71μmol/L. The mean dose of Iohexol was 176.83±71.75ml. 97 patients(36.1%) did the intravenous hydration peri- PCI.

The incidence of CIN was 9.3%(25/269). According to e-GFR: ≥90ml/min, 90-60ml/min, 60-30ml/min, <30ml/min, then the incidence of CIN for subgroup is 2.2%(1/45), 4.4%(6/135), 17.3%(14/81), 50%(4/8)respectively. The incidence of CKD, diabetic complication, preoperative e-GFR, dosage of contrast medium, serume creatinine concentration, ACEI and loop diuretic usage in CIN group were significantly higher than those in the non-CIN group($P<0.01$ or 0.05). Multivariate logistic gradual regressive analysis showed that loop diuretic usage, preoperative e-GFR(<60ml/min), dosage of Iohexol(≥200ml), CKD were independent risk factors of CIN. Their odds ratio(OR)was 6.07, 3.27, 3.26, 2.80, respectively, $P = 0.001, 0.024, 0.015, 0.048$, respectively. In analysis for severity of CIN, CKD, preoperative e-GFR, diabetic nephropathy and retinopathy,LVEF between two groups were significantly different($P<0.05$).

Conclusions: Loop diuretic use, preoperative GFR(<60ml/min), dosage of contrast medium (≥200ml), CKD are independent risk factors of CIN. CKD, preoperative GFR(<45ml/min), diabetic nephropathy and retinopathy have an effect on the severity of CIN.

FR-PO1065

Cystatin C as a More Precise Measure To Estimate Glomerular Filtration Rate in Critical Ill Patients Than the MDRD Formula Gisela Schieren,[1] Detlef Kindgen-Milles,[3] Ralf Westenfeld,[2] Lars C. Rump.[1] *[1]Nephrology, University Hospital Düsseldorf, Düsseldorf, Germany; [2]Cardiology and Pneumology, University Hospital Düsseldorf, Düsseldorf, Germany; [3]University Hospital Düsseldorf, Anesthesiology, University Hospital Düsseldorf, Germany.*

Background: Accurate knowledge of renal function on the intensive care unit (ICU) is not only of prognostic value, but also imperative for medical decisions such as drug dosing and begin of dialysis. In clinical practice creatinine and creatinine based formulae are commonly used to estimate glomerular filtration rate (GFR). Recently cystatin c based formulae were proposed as more accurate indicator of renal function in elderly, children or tumor patients. In ICU patients significant differences in creatinine- and cystatine C based

GFR estimates were seen, but accuracy of the different GFR estimates not compared to a reference method such as creatinine clearance (ECC) or inulin clearance.

Methods: Retrospectively we analyzed the accuracy of creatinine-based MDRD formula and cystatine c-based Larsson, Behring and Hoek formulae with ECC as reference method in ICU patients. Correlation between estimated GFR and ECC-based GFR was analyzed by Bland Altman statistics. When ECC was < 20 ml/min, mean urea and creatinine clearance was used as reference value as recommended (K/DOQI).

Results: 47 observations were recorded in 32 ICU patients. Mean difference between ECC and GFR estimates for MDRD formula was 22 ml/min (95%CI 15.8 - 28.9), for the cystatine C based formulae -3,07 ml/min (95%CI -9.47 - 3.33) [Behring], -0.82 ml/min (95%CI -7.22 - 5.58) [Larsson] and 2.19 ml/min (95%CI -4.07 - 8.45) [Hoek]. Bland altman analysis revealed significant overestimation of GFR by the MDRD formula (bias 22,4; p<0.001), but no statistical significant difference between reference method and cystatin C based GFR estimates. Limitation of the study was its retrospective character.

Conclusions: The MDRD formula significantly overestimates GFR in ICU patients and should be avoided, while cystatin c based formulae provide more accurate estimates of renal function.

FR-PO1066

The Epidemiology of Acute Hemodialysis in Pennsylvania, 2005-2007

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Background: Although the US Renal Data System provides detailed information about patients with end-stage renal disease (ESRD) undergoing chronic hemodialysis (HD), little is known about the epidemiology of acute HD. Here we describe the epidemiology of acute HD, including patient- and hospital-level predictors, in >3 million consecutive inpatient admissions.

Methods: We conducted a retrospective cohort analysis of all adult acute-care hospitalizations in Pennsylvania (PA) from October 2005 to December 2007 using data from the PA Health Care Cost Containment Council. We defined acute HD by the ICD-9-CM procedure code for HD in any field, excluding patients with diagnosis codes indicating imminent, current, or prior ESRD (including history of kidney transplant) and patients with procedure codes for peritoneal dialysis or kidney transplant. We descriptively summarized the characteristics of the acute HD patients and then, controlling for Charlson Comorbidity Index, used multivariable hierarchical logistic regression to identify patient- and hospital-level independent predictors of acute HD.

Results: Among 3,184,361 admissions of non-ESRD patients age ≥21 in PA in this period, 7960 patients had 8888 admissions in which they received acute HD, yielding an annual incidence of 4.3 admissions with acute HD per 10,000 non-ESRD PA residents age ≥21. Independent predictors of acute HD were:

	Adjusted odds ratio	95% confidence interval
Age <65 (per yr)	1.01	1.01-1.01
Age ≥65 (per yr)	0.96	0.96-0.97
Female	0.80	0.76-0.83
Black	1.42	1.33-1.51
Uninsured (vs. Medicare / commercial)	0.58	0.44-0.72
Uninsured (vs. Medicaid)	0.48	0.36-0.59
Primary diagnoses (top 3 by z-score)		
Acute / unspecified renal failure	54.3	50.4-58.4
Sepsis	20.4	18.7-22.3
Complication of device, implant, or graft	15.3	14.0-16.8
Admission to hospital with high-intensity end-of-life care	2.07	1.71-2.51

Conclusions: We have provided one of the first descriptions of the epidemiology of acute HD in a large US population of adults age ≥21. Receipt of acute HD varied by age, sex, race, insurance status, and hospital treatment intensity. Future research should elucidate reasons for such marked variations in provision of care thought to be necessary for patients with acute kidney failure.

Funding: NIDDK Support, Other NIH Support - T32 MH019986
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FR-PO1067

Fluid Balance and Acute Kidney Injury: A Prospective Observational Study

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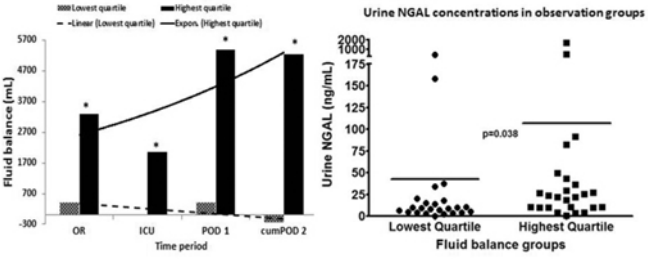
Background: It is unclear whether FB is the cause or result of AKI. We therefore performed a prospective, observational study to investigate this relationship.

Methods: Adult, non-transplant cardiovascular surgery patients were divided into quartiles based on FB status. Incidences of AKI, urine NGAL and IL-18, serum cytokine and SCr concentrations were compared between the lowest (Low-FB) and highest (High-FB) quartiles.

Results: 100 patients were analyzed. The major finding: High-FB group had a six-fold increased risk for AKI (adjusted OR 6.48, CI95%1.37-30.51, p=0.018) and was associated with higher urine NGAL (p=0.038) concentrations. An important observation was that positive FB occurred early in the intraoperative period and continued into the initial ICU period. Positive FB preceded the development of AKI. The High-FB group received more blood product transfusions (p=<0.001), medications (p<0.001) and fluids (p<0.001) and had longer duration of surgery (p=0.003) and time on cardiopulmonary bypass machine (p=0.018). Urine output was not different (p=0.483) between groups in the intraoperative period. Both groups demonstrated a 35-40% reduction in intraoperative MAP from baseline values, but significant differences between them were not observed (p=0.954). High-FB group had longer ICU-LOS (p=0.001) and hospital-LOS (p=0.048) but duration of mechanical ventilation were not different (p=0.650).

Figure 1. Emergence of FB and relationship with urine NGAL



Conclusions: Positive FB in the first 24-hours from initiation of surgery may be an independent risk factor for postoperative AKI.Positive FB is an excellent and simple predictive marker that precedes the rise in SCr.

FR-PO1068

Preoperative Low HDL Level Is Associated with Increased Risk of Acute Kidney Injury Postprocedure/Surgery for Peripheral Vascular Disease

Pradeep Arora,[1,2] Nauman Tahir,[1] Pooja Mahajan,[1] James W. Lohr,[1,2] Hassan H. Dosluoglu,[4] Nader Nader.[3] *[1]Department of Medicine, SUNY, Buffalo, NY; [2]Division of Nephrology, VAMC, Buffalo, NY; [3]Department of Anesthesiology, VAMC, Buffalo, NY; [4]Department of Surgery, VAMC, Buffalo, NY.*

Background: High-density lipoproteins (HDL) have been shown to reduce organ injury and mortality in animal models of shock via modulation of the expression of adhesion molecules and pro-inflammatory enzymes. However clinical studies are lacking.

Methods: We studied the association of HDL level and acute kidney injury (AKI) in patients, who have undergone lower extremity revascularization as the primary procedure at VA Western New York Healthcare System between January 1, 2001 and December 31, 2009. Patients with primary amputation or ESRD were excluded. All data were collected prospectively. Patients were divided in 2 groups; Group A included patients who had preoperative HDL level <40 mg/dl and group B who had HDL >40 mg/dl. Multivariate and propensity score analyses were performed to evaluate the association of AKI and low HDL level.

Results: Study population included 740 patients, 61% of patients had low HDL. Patients in group A were more likely to have hypertension, diabetes, high LDL level, and CKD compared with group B patients. Patients in Group A were significantly more likely to be on ACEI/ARB and statins preoperatively. 9.5% of patients developed AKI by AKIN criteria and 2.7% by RIFLE criteria. Multivariate logistic model results are shown in the table.

Mutiple Logistic Regression Analysis

Parameter	Odds Ratio	95% CI
HDL (low/high)	13.43	8.62-20.92
Age	0.96	0.95-097
CAD	1.24	0.76-2.04
CKD	2.81	1.65-4.77
DM	1.28	0.78-2.08
HTN	0.91	0.53-1.53

Propensity score analysis showed that patients with low HDL level had 40% higher odds of having AKI (OR 1.41 with 95% CI 1.14-1.73).

Conclusions: We conclude that low HDL level is associated with higher odds of AKI postoperatively. Although, low HDL was very common in this population, only 3% were on niacin. It needs to be determined if use of niacin preoperatively will decrease prevalence of AKI.

FR-PO1069

Acute Kidney Injury in Hospitalised Patients Is Under-Recognised and Under-Treated

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Background: Acute kidney injury (AKI) is associated with significant morbidity and mortality. In 2009, the National Confidential Enquiry into Patient Outcomes and Death (NCEPOD) identified significant failings in the recognition and management of hospitalised patients with AKI. Our aim was to explore the prevalence, recognition and quality of early management of level 1 patients with AKI in a London teaching hospital.

Methods: During a 7 day period in May 2011, electronic patient records of all level 1 medical and surgical patients were screened daily. AKI was defined by KDIGO criteria. Independent assessors reviewed the medical notes using similar criteria as the NCEPOD report.

Results: 99 out of 1379 level 1 patients (7.2%) had AKI (53% male; mean age 72.6 years; 64% medical and 36% surgical patients). 93% of these patients had risk factors for AKI (median number 2 [range 0-6]).

Data of 266 AKI patient-days were available. 22 were excluded due to limitations of care. In 59% of cases, AKI was recognised by the treating team. Of these, 83% had a documented management plan regarding AKI, 85% had an early senior review, and 54% had a urine dipstick recorded. Management was worse when AKI was not recognised by the treating medical team (Table 1). On 3 occasions, the independent assessors noticed potentially life threatening problems and intervened.

Table 1: Early management based on recognition of AKI

	Recognised AKI (n=144 AKI days)	Unrecognised AKI (n=100 AKI days)
Assessment of fluid status	49%	21%
Complete fluid balance chart	36%	25%
Discontinuation of nephrotoxics	45% (29/65)	13% (6/47)
Adherance with contrast nephropathy prevention guideline	40% (4/10)	50% (4/8)

Conclusions: AKI is prevalent among hospitalised non-critically ill patients. Only 60% of cases are recognised by the treating team. Even when recognised, a significant proportion of AKI patients do not receive appropriate simple early management. More education and awareness about AKI is necessary, especially among junior medical staff.

FR-PO1070

Acute Kidney Injury in Neonates Undergoing Complex Cardiac Surgery Is Associated with Adverse Outcomes Catherine Morgan,[1] Michael Zappitelli,[2] Charlene Robertson,[1] Gwen Alton,[1] Ari Joffe.[1] *[1]Pediatrics, University of Alberta, Edmonton, AB, Canada; [2]Pediatrics, McGill University, Montreal, QC, Canada.*

Background: Neonates represent one of the most fragile and largest pediatric groups having cardiac surgery (CS). Very little data on neonatal acute kidney injury (AKI) epidemiology and outcomes are available.

Methods: We evaluated prospective data on 248 neonates ≤6 weeks old who had biventricular cardiac repairs from Jan '02-Dec '09. We 1) calculated the CS-AKI rate (≥50% postoperative creatinine (Cr) rise), 2) evaluated pre-/intra-operative AKI risk factors (age, gender, preoperative ventilator days and inotrope use, gestation, weight percentile at surgery, preoperative Cr, bypass time (CPBt), surgical group, deep hypothermic circulatory arrest (DHCA)) using stepwise logistic regression and model area under the curve (AUC), 3) evaluated the effect of AKI on short-term outcomes (death, death-censored length of stay and ventilator days) using survival analysis and on long-term health care use using Poisson regression, and adjusting for severity of illness.

Results: Mean(SD) gestation, birth weight and surgery age were 38.7(2.0) weeks, 3.2(0.6) kg and 17(20) days. 63% had post-operative AKI. Independent (adjusted) AKI risk factors were pre-op Cr (higher SCr = lowest AKI risk), lower gestational age, younger surgical age, DHCA use, longer CPBt and surgical group. The clinical model's AUC for AKI prediction was 0.77. AKI was independently associated with longer time to extubation (adjusted HR 0.76, p=0.04) and longer time to ICU discharge (adjusted HR 0.75, p=0.04). Adjusted risk for mortality in infants with more severe AKI (a doubling of Cr) was higher than those without AKI (HR 2.9, p=0.02). AKI was independently associated with higher number of non-cardiology specialists seen at 2 year follow up (incident rate ratio 1.31 p=0.04).

Conclusions: CS-AKI is very common in neonates and is associated with adverse hospital outcomes and long-term health care use. In addition, preoperative and intraoperative clinical variables may provide a significant contribution to predictive modelling for neonatal AKI.

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FR-PO1071

Early Changes in Transgastric Renal Doppler Indexes Predict Acute Kidney Injury after Major Heart Surgery Giuseppe Regolisti,[1] Carola Cademartiri,[1] Simona Gualtieri,[1] Umberto Maggiore,[1] Aderville Cabassi,[1] Loredana Belli,[2] Sandra Pincolini,[2] Elena Cremaschi,[1] Alberto Caiazza,[1] Tiziano Gherli,[2] Enrico Fiaccadori.[1] *[1]Intern Med & Nephrology, Parma University, Parma, Italy; [2]Heart Surgery, Parma University, Parma, Italy.*

Background: We investigated if early changes in Doppler indexes reflecting intrarenal hemodynamics may predict acute kidney injury (AKI) after major heart surgery (MHS).

Methods: We studied renal hemodynamics by transgastric echo-Doppler in 60 consecutive pts (mean age 69.5 ys, range 30-88 ys; 41 m) undergoing MHS. Measurements were taken before (anesthesia induction), and after (at 2 and 4 hours) cardiopulmonary bypass (CPB) start. Resistance (RI) and pulsatility indexes (PI) were derived both at renal sinus at the level of the left renal artery trunk (***rs*RI** and ***rs*PI,** respectively), and intraparenchymally at the level of the interlobular/arcuate arteries (***intra*RI** and ***intra*PI**, respectively). Serum neutrophil gelatinase-associated lipocalin (NGAL) was measured at the same time points, plus at 24 hours after CPB start (Triage NGAL Test). Statistical analyses were based on linear mixed models for repeated measures and least-square regression.

Results: AKI developed in 27/60 pts (45%); two required dialysis. Systemic hemodynamic parameters(Swan-Ganz catheter) were similar in pts who did and who did not develop AKI. rsRI increased significantly at 2 and 4 hours in both groups (P=0.04), with higher absolute values in AKI pts (average difference between groups, P=0.03). intraRI values were also significantly higher at all time points in the AKI pts (P<0.001), although no clear-cut intraoperative increase was observed (mean rate of change P=0.91). rsPI and intraPI displayed similar changes, though with greater variability. Serum NGAL increased significantly in both groups (P<0.001) early after surgery, with significantly higher values in the AKI pts at all time points (average difference between groups, P<0.01). Within the whole patient population serum NGAL significantly correlated with rsRI, intraRI, and rsPI (P<0.05 for all).

Conclusions: Early intraoperative changes in renal Doppler indexes may help identify the pts with the highest risk of developing AKI after MHS.

FR-PO1072

Does Acute Kidney Injury Cause or Worsen Chronic Kidney Disease? Mark Dominic Uniacke,[1,2] Robert Lewis,[1] Scott Harris,[2] Paul J. Roderick.[2] *[1]Wessex Renal and Transplantation Service, Portsmouth, United Kingdom; [2]Public Health Sciences and Medical Statistics, University of Southampton, United Kingdom.*

Background: The impact of mild to moderate AKI on baseline renal function remains unknown. We have therefore studied AKI in hospitalized patients and explored its effect on the development of de novo CKD and progression of known CKD.

Methods: Prospective single centre observational study. Subjects were recruited over 17 months from Nov. 2009 to April 2011 from unselected admissions to a general hospital with a catchment population of 600,000. Two groups were recruited - Group 1: with previously normal kidney function developing AKI and Group 2: with background of CKD developing AKI. Baseline kidney function was identified from measurements of eGFR from the previous year. AKI was defined by elevations in serum creatinine from baseline using the AKIN criteria. Recovery of function is defined as a return to within 5mls/min of baseline eGFR. Hospital outcomes were recorded and function was reassessed after 6 months using the same laboratory. 6 month follow-up is ongoing and will finish in October 2011. Preliminary 6 month follow-up data for n=89 is presented here.

Results: 375 patients were recruited for follow up (Group1 n=189, Group 2 n=186). Mean age was 72 years (range 18-97, 53% male, 47% female). In hospital mortality was 4.2% in Group 1 and 9.2% in Group 2. At discharge failure to return to baseline function was seen in 54.9% of Group 1 and 34.3% of Group 2. Failure to recover was seen across all AKIN stages even mild AKIN stage 1. Preliminary data shows that after six months from AKI, recovery is not complete in 50% (21/42) of Group 1 and 27.6% (13/47) of Group 2. This failure to recover at six months is again seen across all AKIN stages. So far we have found a high readmission rate of 41.5% in both groups during follow up with 14% having at least one more AKI.

Conclusions: AKI across all AKIN stages appears to be failing to recover after six months in some patients and may be resulting in incident CKD and contributing to further irreversible loss in some existing CKD patients. These findings have important implications for clinical practice and population health.

Funding: Private Foundation Support

FR-PO1073

Hemoglobin and Acute Kidney Injury after Noncardiac Surgery Michael Walsh,[1] Philip J. Devereaux,[1] Amit X. Garg,[2] Daniel Sessler.[3] *[1]McMaster; [2]UWO; [3]Cleveland Clinic.*

Background: Over 200 million adults undergo noncardiac surgery annually and it is frequently complicated by acute kidney injury (AKI). We assessed the association between postoperative changes in hemoglobin (Hgb) and AKI.

Methods: All patients undergoing noncardiac surgery at the Cleveland Clinic between January 2005 and December 2009 with at least one preoperative and one postoperative creatinine within 7 days of surgery, a preoperative estimated glomerular filtration rate of >60 ml/min, and did not undergo a urologic procedure were eligible. AKI was defined as >1.5 fold increase or >0.3 g/dL increase in creatinine within 7 days. Change in Hgb was the difference in the preoperative value and lowest value within the first 24 hours of surgery. All associations were assessed using logistic regression adjusted for age, sex, Risk Stratification Index (a validated score for mortality), surgery type, preoperative blood pressure, baseline Hgb and intraoperative transfusions.

Results: 41,498 patients met the eligibility criteria. The mean (standard deviation) age was 56 (16) years, creatinine was 0.82 (0.18) mg/dL and preoperative Hgb was 13.1 (2.0) g/dL. AKI occurred in 2654 patients (6.4%). AKI was associated with 30 day mortality with an adjusted odds ratio of 2.73 (95% confidence interval (CI)2.24 to 3.34). The median change in Hgb was a 2.2 g/dL decrement. Both preoperative Hgb <12 g/dL and a drop in Hgb of ≥2 g/dL was associated with a graded increase in the risk of postoperative AKI (Table 1). These associations were consistent when AKI was defined by more marked changes in creatinine.

Table 1. Association between AKI and drop in hemoglobin.

	Adjusted Odds Ratio (95% CI)	p-value
Drop in Hemoglobin (g/dL)		
No Drop	Reference	
<1	1.15 (0.96-1.37)	0.12
1-1.99	1.04 (0.86-1.24)	0.23
2-2.99	1.40 (1.16-1.70)	<0.001
3-3.99	1.88 (1.54-2.22)	<0.001
≥4	2.70 (2.22-3.29)	<0.001
Baseline Hemoglobin (g/dL)		
≥12	Reference	
10-11.9	1.70 (1.50-1.92)	<0.001
8-9.99	2.32 (1.97-2.73)	<0.001
<8	2.40 (1.72-3.33)	<0.001

Conclusions: AKI is an independent risk factor for postoperative mortality. Preoperative Hgb and early decrements in Hgb are independent risk factors for AKI and may be modifiable.

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FR-PO1074

A Comparison of the Acute Kidney Injury Network and Kidney Disease: Improving Global Outcomes Criteria for Acute Kidney Injury in Critically Ill Patients Hibiki Shinjo,[1] Waichi Sato,[1] Tomoki Kosugi,[1] Hiroki Hayashi,[2] Shoichi Maruyama,[1] Enyu Imai,[1] Yukio Yuzawa,[2] Seiichi Matsuo.[1] *[1]Division of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Division of Nephrology, Fujita-Health University, Toyoake, Japan.*

Background: Acute kidney injury (AKI)-associated hospital mortality can strikingly increase in comparison with patients without AKI. In order to have a uniform standard for classify AKI, the AKI Network (AKIN) group has proposed modifications to the previous criteria referred to the RIFLE and suggested staging of AKI based on changes in creatinine (Cr) within 48 hours. In actual clinical practice, however, the peak of Cr may be missed. More recently, Kidney Disease: Improving Global Outcomes (KDIGO) group proposed a new definition for AKI, which is defined as an abrupt reduction within 7 days in kidney function. We evaluated the incidence of AKI and compared the ability of the AKIN (48h) and KDIGO (7d) criteria in predicting hospital mortality of intensive care unit (ICU) patients.

Methods: We performed a retrospective cohort study on 2582 patients admitted between June 2005 and May 2009 in an ICU of the Nagoya university hospital. Chronic kidney disease patients undergoing dialysis and renal transplant patients were excluded.

Results: The KDIGO and AKIN criteria were total incidences of AKI (38.5 vs. 29.6%). KDIGO criteria significantly increased the number of patient classified as AKI in all stage compared with AKIN criteria (stage 1; 24.7 vs. 20.7%, stage2; 6.2 vs. 3.4%, stage3; 7.6 vs. 5.5%). 238 patients (9.2%) that classified as no AKI by AKIN and AKI by KDIGO, were similar hospital mortality for Stage 1 by both criteria. In both criteria, AKI were associated with hospital mortality after adjusting for multiple covariates. And the odds ratio was elevated, according to the severity of AKI. The area under the receiver operator characteristic curve for hospital mortality estimated by KDIGO and AKIN criteria were 0.76 and 0.73.

Conclusions: Both criteria might be reliable for predicting severity and outcome of AKI. Particularly, 7d time window in KDIGO improve on the sensitivity of the AKI diagnosis and the ability in predicting hospital mortality.

FR-PO1075

The Epidemiology of Contrast-Induced Nephropathy in the Era of Hydration Protocols Corinne E.A. Balemans,[1] Jan A.J.G. van den Brand,[1] Louis J.M. Reichert,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [2]Nephrology, Rijnstate Hospital, Arnhem, Netherlands.*

Background: Acute renal failure can complicate the use of iodinated contrast media. This contrast-induced nephropathy(CIN) is associated with marked morbidity and mortality. Current guidelines advise identification of high risk patients and hydration as preventive measure. We evaluated the incidence of CIN and determined risk factors associated with CIN in patients receiving intravenous contrast and treated according to the guidelines.

Methods: All patients with an eGFR<60ml/min/1.73m^2 were seen at the outpatient clinic. Patients were stratified for the risk of CIN; high risk (HR) or low risk (LR) based on absolute GFR (MDRD x BSA/1.73 m^2) and the presence of risk factors; diabetes, peripheral arterial disease, heart failure, age, anemia and use of diuretics and/or NSAID's. HR patients were hydrated with isotonic saline 1000ml before and 1000ml after contrast exposure. Serum creatinine was measured 3-5 days later, CIN was defined as a ≥25% rise from baseline.

Results: We evaluated 1420 procedures performed in 1111 patients in the period from Sept 2007 until Dec 2010. Mean age was 71.7 years and there were 45% females. In 757 procedures (53%) patients were hydrated. We observed CIN after 37 procedures (2.6%). (table1) In multivariate analyses heart failure (Odds ratio (OR)=2.47 95%confidence interval(CI) 1.14-5.36 p=0.02) and repeated contrast (OR=1.87 CI 0.96-3.64 p=0.06) were found to be independent predictors of CIN. The mean interval between contrast exposures was 4.4 months.

Conclusions: The incidence of CIN in this population was low and renal insufficiency and diabetes were no longer identified as risk factor. This supports the efficacy of the current strategy.

We identified heart failure and repeated contrast as risk factors for CIN. This last finding requires validation, but might suggest that subclinical injury remained present after first contrast exposure.

Risk category (N) (Risk)	Incidence CIN
GFR ≥60 (426) (LR)	2.1%
GFR 45-60 <2 risk factors (310) (LR)	2.9%
GFR 45-60 ≥ 2 risk factors (355) (HR)	2.5%
GFR <45 (329) (HR)	3.0%

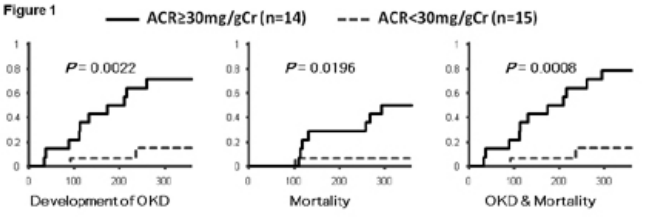
FR-PO1076

Development of Microalbuminuria Following Hematopoietic Stem Cell Transplantation Is Associated with Near-Term Loss in Renal Function and Mortality Taku Morito,[1,2] Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: Microalbuminuria is a risk factor for overt kidney disease (OKD), cardiovascular disease and mortality. Therefore, the presence of microalbuminuria could be a sign of the future OKD or death in the setting of hematopoietic stem cell transplantation (SCT).

Methods: A 1-year prospective cohort study was conducted in 29 patients (46.3±12.5 years) receiving allogeneic myeloablative SCT. The urinary albumin to creatinine ratio (ACR) was consecutively measured before conditioning therapy (baseline), at the time of SCT, and on days 7, 14 and 28 after SCT. Microlbuminuria was defined as ACR ≥30mg/gCr. OKD was defined as persistent loss in renal function, that is, eGFR <60 ml/min/1.73m^2. Cumulative incidence of OKD or mortality or both was assessed by the Kaplan-Meier analyses. Multivariate Cox regression analysis was used to calculate the HR of developing OKD and mortality for microalbuminuria on 1 month (day 28) after SCT. Patients with OKD at baseline and those who died by 1 month after SCT were excluded.

Results: The prevalence of microalbuminuria was 6.9% at baseline, and increased to 62.1% at the day of SCT. It varied among the time points; 62.1% on day 7, 48.3% on day 14 and 51.7% on 1 month, respectively. Cumulative incidence of OKD or mortality or both was significantly higher in the group with 'microalbuminuria on 1 month'.



In addition, the presence of 'microalbuminuria on 1 month' was significantly associated with either the development of OKD or death (adjusted HR 17.4; 95% CI 3.66 to 134.9, *P*=0.0001).

Conclusions: The presence of 'microalbuminuria on 1 month' is a promising predictor of near-term development of loss in renal function and mortality in the setting of SCT.

FR-PO1077

Study of Acute Kidney Injury (AKI) on Admission to the Emergency Assessment Unit (EAU) Nihal Y. Abosaif, Lee J. Dowson, Ewa Werpachowska, Rhys Lodwick. *Medicine, Royal Liverpool University Hospital, West Midlands, United Kingdom.*

Background: Mortality rates from AKI are increasing in hospitalised patients in the UK because of increased rates of sepsis and circulatory failure. The National Confidential Enquiry into Patient Outcome and Death (NCEPOD) report has shown that most patients admitted with AKI were not assessed properly especially by trainees. Onward referral to renal physicians was also delayed. In this study, we evaluated the incidence of AKI in our EAU and estimated the risk of death among patients admitted according to the definition of AKI. Also for new patients admitted to EAU, we aim at implementing a new risk assessment tool to score patients on admission for the risk of developing AKI and hence establish their level of care.

Methods: All patients admitted to the EAU with a serum creatinine(S.Cr) >100 mmol/L between April 2010 and October 2010 were screened. Patients with known chronic kidney disease (CKD) or unknown baseline Cr were excluded. 1530 patients were found to match the inclusion criteria from which 54 patients were randomised. Medical notes, laboratory results and radiological investigations were extracted and examined for the purpose of the study, 23 out of 54 patients died because of the original presentation or secondary to AKI per se. The characteristics of those patients who died (group D) were compared with those of survivors (group S). We also assessed compliance with NCEPOD guidelines.

Results: Group D was significantly older than group S. There was no significant difference as regards their demographic, clinical or laboratory parameters apart from admission S.Cr, eGFR, follow up Cr, S.urea, S.albumin and platelets that were significantly deranged in group D. The Hazard Ratio (HR) of recognition of AKI {HR 3.475 (CI; 0.3-35.3)}, its cause { HR 2.3 (CI; 0.25- 21.2), p<0.001}and management {HR 0.52 (CI; 0.05-5.12), p<0.001 } showed very highly significant bivariate correlation to group D.

Conclusions: The cohort with a poor outcome was correctly identified and managed according to NCEPOD guidance implying that further steps are required. Development of a new scoring system for diagnosis of AKI in high risk patients may help target interventions.

FR-PO1078

Nutritional Support Influences Outcomes in Intensive Care Unit (ICU) Patients with Acute Kidney Injury (AKI) Soo Young Yoon,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] Talat Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]Univ California San Diego, San Diego, CA; [2]Stanford Univ, Palo Alto, CA; [3]Univ Washington, Seattle, WA; [4]Vanderbilt Univ, Nashville, TN; [5]Cleveland Clinic Foundation, Cleveland, OH.*

Background: Nutritional support (NUTS) is commonly utilized to manage ICU patients however it is unclear whether enteral (EN) or parenteral nutrition (PN) influence outcomes in AKI. We assessed the pattern of NUTS and its relationship to outcomes in AKI patients enrolled in the PICARD study (KI, 2004, 66: 1613-1621). We hypothesized that NUTS would be associated with improved survival and shorter ICU duration.

Methods: We analyzed data from 615 of the 618 patients who stayed in ICU >48 hours. We assessed mode of nutrition (MON), duration, amount and timing for NUTS and examined the relationship to fluid overload >10% body weight (FL/BW), hospital mortality (HOM), ICU length of stay (LOS) and dialysis requirement at hospital discharge (DIAL) in survivors.

Results: Among 615 patients, 199 ate orally without any NUTS (Oral), 76 received no oral or NUTS (None), 183 received EN, 66 PN, and 91 both EN+PN. A subset of the NUTS patients had oral intake for part of their ICU stay (EN n=81; PN n=22; EN+PN n= 27).

	None	EN (+oral)	PN (+oral)	EN+PN (+oral)	Oral	p*	p**
#Pts	76	102 (81)	44 (22)	64 (27)	199		
%HOM	59.2	54.9 (27.2)	63.6 (36.4)	54.7 (25.9)	15.1	0.000	0.000
Mean LOS	10.2	24.8 (21.8)	21.4 (20.6)	32.8 (29.1)	8.8	0.000	0.000
%DIAL	32.9	31.4 (13.6)	36.4 (18.2)	31.3 (22.2)	18.1	0.004	0.039
%FL/BW	12.1	21.1 (15.5)	37.2 (27.3)	12.1 (23.1)	14.6	0.000	0.000

*including Oral; **excluding Oral

In Cox proportional hazard model, FL/BW (HR 14.928), no NUTS (HR 5.185 vs. Oral), and single NUTS mode (HR 1.899 in EN and 2.863 in PN vs. Oral) predicted mortality. Nutritional duration and calories were not independent predictors for mortality. Transitions in MON from any nutrition to oral eating were associated with improved survival (HR 0.160 for mortality vs.oral to any nutrition).

Conclusions: In patients with AKI in the ICU, requirement for NUTS is associated with worse outcomes. Patients who can eat have the best outcomes regardless of underlying severity of kidney disease and underlying co-morbidities.

Funding: NIDDK Support

FR-PO1079

Dialysis Requirement and Nutritional Support in Intensive Care Unit (ICU) Patients with Acute Kidney Injury (AKI) Soo Young Yoon,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] Talat Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]Univ California San Diego, San Diego, CA; [2]Stanford Univ, Palo Alto, CA; [3]Univ Washington, Seattle, WA; [4]Vanderbilt Univ, Nashville, TN; [5]Cleveland Clinic Foundation, Cleveland, OH.*

Background: Nutritional support (NUTS) is variably utilized in ICU patients with AKI due to concerns of solute load and fluid accumulation. We evaluated the influence of dialysis requirement (RRT) on the application of NUTS in AKI patients enrolled in the PICARD study (KI, 2004, 66:1613-1621). We hypothesized that RRT would allow more nutrition to be given and improve outcomes.

Methods: We analyzed data from 610 of 618 patients who stayed in ICU >48 hrs. We assessed the nutritional mode (Oral=eating; EN=enteral; PN=parenteral; EN+PN=both and None=no oral and no NUTS), duration (%nutrition days/ICU days), amount (delivered/prescribed calories) and the timing for NUTS start (days from ICU admission) and examined the relationship to fluid overload >10% body weight (FL/BW), hospital mortality (HOM) and ICU length of stay (LOS) in survivors in patients with RRT (n=397) or without dialysis (NoRRT n=213).

Results: There was no difference in the mode, duration, or timing of NUTS in RRT vs NoRRT, however RRT patients had a higher % of delivered calories.

	None	EN	PN	EN+PN	Oral	Total	p
NoRRT							
#Pts (%)	28 (13.1)	53 (24.9)	19 (8.9)	17 (8.0)	96 (45.1)	213	
%Duration	-	51.3	48.0	64.7	75.8	56.4	0.000
%Calories	-	81.8	57.5	73.6	-	-	0.025
Timing	-	4.8	4.9	2.7	1.1	2.7	0.000
LOS	6.1	19.9	22.6	23.2	7.8	13.2	0.000
%FL/BW	0.0	3.9	0.0	0.0	1.2	1.6	0.131
%HOM	40.7	35.8	36.8	23.5	7.3	22.6	0.000
RRT							
#Pts (%)**	47 (11.8)	126 (31.7)	47 (11.8)	74 (18.6)	103 (25.9)	397	
%Duration#	-	60.5	68.0	67.2	70.7	58.1	0.000
%Calories	-	84.7	71.7**	81.3	-	-	0.011
Timing#	-	4.0	4.4	3.8	1.2	3.2	0.002
LOS	12.7*	24.4*	20.5	33.6	9.6	20.4**	0.000
%FL/BW#	16.2	1.8	13.0	2.9	3.5	5.5	0.000
%HOM	69.8*	46.0	61.7	51.4*	22.3*	45.3**	0.000

Significance vs. NoRRT *p<0.05; **p<0.001; # NS

Conclusions: RRT requirement modifies the amount of calories provided by NUTS in ICU patients with AKI. Oral intake is associated with improved outcomes. Further studies are required to assess the effect combined Oral and NUTS in improving outcomes from AKI.

Funding: NIDDK Support

FR-PO1080

Age Modifies the Predictive Effect of AKIN Staging for Hospitalization Outcome in Elderly Patients Undergoing Major Surgery Chia-Ter Chao, Fan-Chi Chang, Vincent Wu. *Division of Nephrology, Department of Internal Medicine, National Taiwan University Hospital, Taiwan.*

Background: Acute kidney injury (AKI) occurs with increasing frequency in patients admitted to intensive care unit (ICU), and elderly patients are particularly vulnerable. AKIN (AKI network) classification is widely used to gauge severity of AKI, but has not been formally tested in geriatric patients for its efficacy.

Methods: We conduct a multicenter, prospective observational study, based on the NSARF (National Taiwan University Hospital Surgical ICU Associated Renal Failure) database. A total of 4964 elderly patients (defined as age more than 65) who developed AKI after admission for major surgeries between 1 Jan, 2002 and 31 Dec, 2008 were enrolled, divided into 3 categories according to age: younger-old (65-75), old (75-85) and oldest old (>85). Demographic profiles, comorbidities, types of surgery, and ICU treatment variables were all collected. We determined the predictive power of AKIN classification for 180-day in-hospital mortality after adjusting relevant factors.

Results: Overall, 49.2%, 41.4% and 9.4% of patients were each classified in younger old, old and oldest old group. In-hospital mortality increased stepwise from 15% to 16% and 21% as age rose. Age, AKIN stage (hazard ratio (HR) 1.707 for stage III, p<0.001), hypertension (HR 1.345, p<0.001), diabetes mellitus (HR 1.348, p<0.001) and treatment related factors (tracheostomy, Swan-Ghanz catheter use) were associated with higher risk of adverse outcome. Cox multivariate-adjusted analysis showed that patients with AKIN stage I and II had similar outcome across all age categories. In addition, younger-old patients with AKIN stage III had worse outcome than the oldest-old with stage I insult (HR 1.902 vs 1.742), while the old and the oldest-old with stage III insult denoted a higher mortality.

Conclusions: The finding of this study exemplifies the aging effect on utility of AKIN staging for AKI in predicting hospitalization outcome in geriatric population, especially during milder form of AKI.

FR-PO1081

Border-Crossers' Nephropathy: The Risk of Coming to America Nduka-Obi Ossai, Harold M. Szerlip. *Department of Medicine, University of Arizona College of Mdicine, Tucson, AZ.*

Background: The 380 mile border between Mexico and Arizona has become a prime entry point for persons wanting to enter the United States. Because of increased enforcement, these individuals usually attempt to cross the border in desolate areas where they may wander for days without food or water before being "rescued". The Summer of 2010 was one of the five hotest summers on record. During 2010, 232 individuals died trying to cross the into Arizona and 212,202 people were apprehended in the Tucson Sector. The University of Arizona Medical Center, South Campus is one of the closest tertiary care medical centers to the Mexican border. Detainees with medical problems are frequently brought to this campus. Herein we describe our experience with myoglobinuric acute kidney injury (AKI) in these border-crossers.

Methods: We reviewed the records of all individuals admitted between June 1-December 31, 2010 with AKI as defined by AKIN criteria who also had an elevation of CPK > 1000 IU/L. We recorded the age, gender, temperature, creatinine on presentation, CPK on presentation, urine output in first 24 hours, need for dialysis, length of stay and creatinine on discharge.

Results: During this time period 24 people were diagnosed with myolobinuric AKI. On presentation all patients were vigorously resuscitated with either 0.9% NaCl or a mixture of NaCl and NaHCO3. The mean age was 31.7 years (range 18-53). 21 were male and 3 female. They had wandered in the desert between 1and 8 days with a mean of 3.9 days. Five had stage 1 AKI, 7 stage 2 and 12 stage 3. Three patients required dialysis. Only 1 patient was oliguric and only 1 had a temperature > 100.6 on arrival. CPKs ranged between 1355 and 447,966 IU/L. Length of stay was between 2 and 17 days with a mean of 4.3 days. One patient was discharged on hemodialysis and 4 were discharged with creatinines >1.3 mg/dl.

Conclusions: This is the largest series of myoglobinuric AKI reported in border-crossers. The presumed etiology is excessive heat combined with volume depletion and stressful exercise. We have coined the term "border-crossers nephropathy" for this disorder. Many of these patients may develop CKD and require nephrologic care in their home country.

Funding: Clinical Revenue Support

FR-PO1082

Acute Kidney Injury, Length of Stay and In-hospital Mortality in Critically Ill Cirrhotic Patients: A Cohort Study Mário Raimundo, Maria Joao Melo, Ana Cortesao Costa, Antonio Gomes da Costa, Jose António Lopes. *Nephrology and Kidney Transplantation, Hospital de Santa Maria - CHLN, Lisbon, Portugal.*

Background: Recently, members of the Acute Dialysis Quality Initiative (ADQI) and the International Ascites Club (IAC) proposed the definition of AKI in cirrhosis as an increase in serum creatinine of >50% from baseline or a rise in serum creatinine of >26.4 mmol/l (>0.3 mg/dl) in <48 h. The aim of this study was to relate this classification to length of stay and in-hospital mortality in a cohort of critically ill cirrhotic patients.

Methods: One-hundred eighty-two cirrhotic patients [mean age: 56 (12.2) years; 105 Male; 171 Caucasian; Child-Pugh score: 9.3 (2.4); Model for End-stage Liver Disease

(MELD): 36.2 (7.6); and Sequential Organ failure Assessment (SOFA): 5.4 (3.5)] admitted to the Gastrenterology and Hepatology Intensive Care Unit (ICU) of the Hospital de Santa Maria (Lisbon, Portugal) between January 2003 and December 2005 were retrospectively evaluated.

Results: Forty-seven patients (25.8%) developed had AKI during ICU stay. Those patients had lengthened time of ICU (P<0.0001) and hospital stay (P=0.002), as well as higher in-hospital mortality as compared with patients with no AKI (63.8% versus 8.1%, P<0.0001). AKI was independently associated with increased in-hospital mortality (adjusted odds ratio 6.9, 95% confidence interval 2.4-20.2, P<0.0001). The area under the receiver operator characteristic curve for in-hospital mortality was 0.806 for AKI (P < 0.0001).

Conclusions: AKI was associated with increased length of stay and in-hospital mortality among critically ill cirrhotic patients, and it presented good discriminative power regarding in-hospital mortality.

FR-PO1083

Renal Function Estimation Equations Using Non-Steady State Serum Creatinine Perform Better among Patients with Low Baseline Renal Function Sevag Demirjian,[1] Brian R. Lane,[2] Jesse D. Schold,[3] Steve Campbell,[4] Emilio D. Poggio.[1] *[1]Nephrology, Cleveland Clinic, Cleveland, OH; [2]Urology, Spectrum Health and Michigan State University, Grand Rapids, MI; [3]Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH; [4]Urology, Cleveland Clinic, Cleveland, OH.*

Background: Estimation of glomerular filtration rate (eGFR) in subjects with acute kidney injury (AKI) is problematic due to the non-steady state of serum creatinine (sCr). We examined the agreement of sCr based estimation equations with measured GFR (iGFR) during the immediate post-operative course in subjects undergoing nephrectomy.

Methods: we measured GFR using I-iothalamate renal clearance within a week of nephrectomy procedure. Only subjects with post-operative sCr rise of 0.3 mg/dl or higher were included in the analysis. We compared iGFR with eGFR using Pearson's correlation (ρ), and concordance correlation coefficients (CCC) in subjects with or without baseline chronic kidney disease (CKD). CKD was defined as baseline eGFR < 60 mL/min/1.732. eGFR was calculated using MDRD 4-variable, CKD-EPI and Jelliffe estimation equations based on sCr drawn at the time of iGFR measurement.

Results: 69/90 subjects who underwent nephrectomy sustained post-operative sCr rise of 0.3 mg/dl or greater. Mean age was 61 ± 11, and 55/69 were male. Baseline sCr was 1.2 ± 0.4, and 25/69 (36%) had baseline CKD. The correlation of eGFR with iGFR was particularly strong among CKD patients.

Correlation of measured GFR with serum creatinine based estimation equations in all subjects, and per baseline chronic kidney disease:

	All		CKD		No CKD	
	ρ	CCC	ρ	CCC	ρ	CCC
MDRD*	0.81	0.75	0.86	0.75	0.78	0.72
CKD-EPI*	0.81	0.74	0.86	0.73	0.78	0.71
Jelliffe*	0.81	0.77	0.93	0.88	0.76	0.7

*Glomerular filtration rate estimation equations;ρ, Pearson's correlation coefficient; CCC, concordance correlation coefficient.

Conclusions: sCr based estimation equations, particularly Jelliffe's equation, correlate well with measured GFR in subjects with post-operative rise in sCr and baseline CKD. Whereas, all three estimation equations had modest correlation in subjects without baseline CKD.

Funding: Clinical Revenue Support

FR-PO1084

Red Blood Cell Distribution Width Is an Independent Predictor of Mortality in Acute Kidney Injury Patients Treated with Continuous Renal Replacement Therapy Hyung Jung Oh, Seung Jun Kim, Dong Eun Yoo, Mi Jung Lee, Dong Ho Shin, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Red blood cell distribution width (RDW) has been found to be independently associated with adverse outcomes in patients with heart failure and coronary heart disease. However, little is known on the relationship between RDW and clinical outcomes in acute kidney injury (AKI) patients treated with continuous renal replacement therapy (CRRT). This study was conducted to investigate whether RDW was associated with overall mortality in AKI patients treated with CRRT.

Methods: A total of 470 AKI patients, who were treated with CRRT at Yonsei University Health System, Seoul, Korea, were included. Patients were divided into 2 groups according to the RDW levels at CRRT initiation, and clinical and laboratory data, echocardiographic findings, and overall mortality at 28-day were compared between the two groups.

Results: RDW ranged from 11.7 to 28.0%, and 317 patients (67.5%) had RDW above the upper limit of normal (>14.5%). Patients with high RDW values had higher white blood cell (WBC) counts, and lower hemoglobin (Hb) and total cholesterol levels compared to patients with normal RDW values. In addition, there were significant correlations between RDW values and WBC counts, Hb levels, and total cholesterol concentrations. However, there were no significant differences in age, gender, SOFA score, eGFR, albumin, and echocardiographic parameters between the two groups. Patients with high RDW values exhibited significantly higher 28-day mortality rates than patients with low RDW levels in Kaplan-Meier analysis (p<0.01). Univariate Cox proportional hazard analysis revealed that baseline RDW levels, SOFA score, mean arterial pressure, and total cholesterol concentrations were associated with mortality. In multivariate analysis, RDW value at CRRT initiation was a significant independent predictor of 28-day overall mortality after adjusting for other risk factors.

Conclusions: The results of this study suggest that RDW could be an additive predictor for overall mortality in AKI patients on CRRT.

FR-PO1085

Analysis of Acute Kidney Injury and Its Prediction by Urinary Biomarkers in Adult Japanese ICU Patients Haruyo Ujike,[1] Yohei Maeshima,[1] Masaru Kinomura,[1] Kiyoshi Mori,[2] Daisuke Saito,[1] Hiroko Yamasaki,[1] Hiroyuki Watatani,[1] Norikazu Hinamoto,[1] Hitoshi Sugiyama,[1] Kazuwa Nakao,[2] Hiroshi Morimatsu,[3] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ. Graduate School of Medicine, Okayama, Japan; [2]Medicine and Clinical Science, Kyoto Univ. Graduate School of Medicine, Kyoto, Japan; [3]Anesthesiology, Okayama Univ. Med. School, Okayama, Japan.*

Background: Acute Kidney Injury (AKI) is frequently complicated in critically ill patients in intensive care unit (ICU), and associated with increased mortality. This study aims to determine the clinical characteristics of AKI and to evaluate urinary biomarkers to predict *de novo* onset of AKI.

Methods: We prospectively collected data of patients admitted to the ICU in the Okayama University Hospital (Nov. 2010-May 2011). Patients already in end-stage renal disease and receiving renal replacement therapy (RRT) were excluded. Urine was collected within 24 h of ICU admission (Day 1). Urinary levels of neutrophil gelatinase-associated lipocalin (NGAL), NAG and albumin were determined and were normalized to creatinine levels.

Results: Of the 86 patients, 23 (26.7%) developed AKI based on the AKIN criteria. The mean age was 65 in non-AKI and 60 in AKI group and the mean baseline eGFR was 72 and 74 ml/min/1.73m2, respectively. Surgical operation in the non-AKI (92%) and the AKI (74%) group was the leading cause of ICU admission. AKI was frequent in the recipients of liver transplantation (7 out of 9 developed AKI). In the AKI group, 2 patients (8.6%) required RRT and a patient died. Overall, the ratio of serum Cr at Day 3 to Day 1 after admission to ICU was positively correlated with urinary NGAL/Cr ratio (R=0.46, P=0.001) or NAG/Cr ratio (R=0.33, P=0.019). In 15 cases developing de novo AKI, the levels of urinary NGAL/Cr and NAG/Cr were significantly elevated compared with non-AKI group (126.2 vs. 40.3 μg/gCr, P=0.003; 58.9 vs. 13.8 U/gCr, P<0.0001, respectively), but urinary levels of albumin/Cr was not significantly elevated.

Conclusions: These findings suggest clinical significance of urinary NGAL and NAG as useful biomarkers superior to urinary albumin in predicting the onset of AKI in critically ill patients.

FR-PO1086

Low Risk of Contrast-Induced Nephrotoxicity after Coronary Angiography and Intervention in Modern Clinical Practice Jonas Spaak, Masih Khedri, Majid Kalani, Stefan H. Jacobson. *Clinical Sciences, Danderyd University Hospital, Karolinska Institutet, Stockholm, Sweden.*

Background: Many patients with kidney dysfunction are not provided invasive diagnostic and therapeutic interventions of concern for contrast-induced nephropathy (CIN), while 40% of all our patients with acute coronary syndrome (ACS) have at least moderately reduced kidney function (GFR<60 mL/min). However, clinical experience indicate that CIN is becoming less frequent than earlier reported. Understanding the clinical risk of CIN is crucial to provide correct treatment to patients with acute or chronic coronary heart disease and reduced kidney function.

Methods: We aimed to assess current incidence and predictors of CIN, and long-term kidney function after coronary angiography (CAG) and/or intervention (PCI) in a modern setting using low amounts of low-osmolality contrast agents.

Results: We retrospectively evaluated all 1227 patients whom during 2009 underwent CAG/PCI at our University Hospital Clinic. S-creatinine levels were assessed within 2-years around the procedure.

Data was available to evaluate CIN in 384 patients. Of these CIN occurred in 21 (5.5%). Of these, 6 patients suffered concomitant severe illness. Six patients (1.8%) of 332 with available follow up data developed persistent kidney injury. CIN was relatively less common in patients with reduced kidney function. GFR was significantly higher in the CIN group (85±31 vs. 69±20 mL/min, p=0.025), and the used contrast amount was significantly lower (86± 42 vs. 110±51, p=0.035).

Conclusions: Our main finding is that current incidence of CIN is lower than previously reported, also among patients with reduced kidney function. The inverse relationship between kidney function and CIN indicate that invasive procedures may be underutilized in these patients.

Funding: Private Foundation Support

FR-PO1087

Vancomycin-Induced Acute Kidney Injury (AKI): An Under-Recognized Issue with Increasing Incidence Preventable by Mandatory Drug and Serum Creatinine Monitoring during Porlonged Administration Talla A. Rousan, Omar S. Abu-Romeh, Ahmad Bilal, Kai Lau. *Medicine, University of Oklahoma, Oklahoma City, OK.*

Background: For decades, IV vancomycin has been used with remarkable efficacy to treat gram positive bacteria, notably methicillin-resistant staphalococcal (MRS) aureus (A) or epidermis (E). When levels are closely monitored & doses continually adjusted based on weights, clinical indications, ongoing & changing renal functions, chronic parenteral vancomycin is generally considered quite safe.

Methods: Recently, acute kidney injury appears to be on the rise, largely due to the increasing incidence of documented MRSA and MRSE and also due to the exponentially growing utilization of vancomcyin for both infections by bacteria with known sensitivity as well as infections like "Health Care Facility Acquired (HCFA)" pneumonia. In the latter scenario, the current Infectious Disease guideline recommends the empiric coverage of presumed MRSA with vacnomycin, hence its widespread prescriptions over the last few years.

Results: In the course of a month on renal consults covering 449 acute adult beds, we encountered & treated 7 cases of significant ARF that developed in patients treated with vancomycin. Based on serial drug levels, daily & cumulative administered doses, the temporal relationship, & the course of renal failure and recovery on stopping vancomycin, we excluded other confounding variables to establish vancomycin as the etiology. The estimated baseline creatinine clearance was 103 mL/min with an estimated nadir of 18.4 mL/min. All patients sustained AKI with a mean decline in clearance of 25 mL/min ($p < 0.01$) with a recovery time of 33 days. On average, patients received drug for 16 days prior to recognizing AKI. Mean cumulative dose was 34.5 g with a mean peak level of 78.3 mg/L.

Conclusions: We recognize the efficacy of vancomycin in treating different infections. Kidney function should be regularly monitored during treatment besides checking vancomycin trough levels for avoidance & early detection of renal failure. At the same time, subtle increments in creatinine values should not be taken lightly as they may reflect decline in kidney function requiring prompt dose adjustment.

Funding: Veterans Administration Support, Private Foundation Support, Clinical Revenue Support

FR-PO1088

Factors Affecting Timing of Dialysis Initiation in Acute Kidney Injury (AKI) in Intensive Care Unit (ICU) Charuhas V. Thakar,[1,2] Anthony Leonard,[1] James Rousseau.[1] [1]*Internal Medicine/Nephrology, University of Cincinnati, OH;* [2]*Medical Service/Renal Section, Cincinnati VA, Cincinnati, OH.*

Background: Although it is argued that early initiation of dialysis may be beneficial in AKI, it remains a subjective clinical decision based on multiple factors.

Methods: We conducted an online survey of U.S. and international nephrologists to study the factors affecting initiation of early dialysis in AKI in ICU. We studied how parameters used in determination of dialysis initiation influenced this decision across three case scenarios (predicted hospital mortality of < 10%, 10 – 30% and > 30% respectively).

For each case, 4 questions were asked about decision to initiate dialysis within 24 hours based on given clinical information; Q1 – subjective likelihood; Q2 – blood urea nitrogen (BUN) levels (< 50, 50 – 75, 76 – 100, > 100 mg/dl) considered in this decision; Q3 – creatinine (Cr) elevation (2 – 3 times; > 3 times; absolute level > 5 mg/dl regardless of change) considered important; Q4 – a rank order of parameters [BUN level, Cr change from baseline, oxygen saturation (O2 sat), potassium (K) level, and urine output]. (1 - most, and 5 - least influential). McNemar's and t-test was used for comparison.

Results: Surveys of 119/172 nephrologists who responded to all questions were analyzed. There were 87% males, 73% were in practice for > 5 years, and 70% practised in the U.S.

The proprtion of subjects likely to initiate early dialysis increased (76% to 94%) with predicted mortality ($p < 0.0001$). Proportion of subjects considering dialysis at a BUN level < 75 mg/dl increased from 17% to 40% across three cases ($p < 0.0001$). Proportion of subjects choosing absolute Cr to be influential went from 60% to 43% across the three cases ($p < 0.0001$). Mean ranking for O2 sat went down (more influential) and K level went up (less influential) as predicted mortality increased ($p < 0.0001$). Rank for BUN and Cr elevation were similar across cases.

Conclusions: More severily ill subjects are more likely to be initiated on early dialysis. Rank order analysis suggests that dialysis decision is influenced by "imminent" indications rather than elevation of Cr from baseline or BUN level.

Funding: Clinical Revenue Support

FR-PO1089

Recovery from Dialysis-Dependent Acute Tubular Necrosis Sumit Mohan, Edwin D. Huff, William M. McClellan, The FFBI Data Committee. *Fistula First Breakthrough Initiative.*

Background: Acute kidney injury is associated with an increased risk of end stage renal disease and a higher mortality, particularly in patients with underlying CKD. The need for continued renal replacement therapy (RRT) at the time of discharge from a hospitalization for an episode of acute renal failure (ARF) has increased over time. Acute Tubular Necrosis (ATN) remains the one of the most common causes of ARF among hospitalized patients and frequently requires RRT – and at times for prolonged periods. An estimated <1% of patients who experience ATN go on to develop ESRD, but the rate of recovery of renal function among patients who are dialysis dependent after an episode of ATN is unclear.

Methods: We reviewed ESRD registration data from the 2728 form in the United States for 2008 and 2009 and patient record updates signifying change in dialysis services at the patient's dialysis center due to renal recovery.

Results: We found that 3.48% of patients had a primary diagnosis of "Tubular Necrosis" (5836). Discontinuation of dialysis as a result of recovery of renal function over the subsequent 24 months was reported in 6.65% of all ESRD patients and 38% of patients with ATN ($p= 0.01$). Among all ESRD patients with recovery of renal function, 20% had a primary diagnosis of ATN at the initiation of dialysis. The median time to reported recovery of renal function for pts with dialysis dependent ATN was 41 days, compared to a median of 74 days for all pts with recovery of renal function.

Year	Number of new ESRD pts	% of patients with ATN as primary diagnosis	% of patients with recovery of renal function in ≤ 24 months	% of ATN patients with renal recovery in ≤ 24 months	Median duration to recovery of renal function
2008	101,359	3.36%	6.6%	37.6%	41 days
2009	104,040	3.59%	6.7%	38.2%	40 days

Conclusions: Recovery of renal function occurs in over a third of patients with dialysis dependent ATN – a significantly higher rate (see table) than the rest of the US ESRD program.

Funding: Other U.S. Government Support

FR-PO1090

Can Urinary Kidney Injury Biomarkers Affect Clinical Decision Making in the Emergency Department? Kai M. Schmidt-Ott,[1] Thomas L. Nickolas,[2] Eugenia Singer,[1] Catherine Forster,[2] Meghan E. Sise,[2] Abdallah Sassine Geara,[3] Philip Imus,[2] Friedrich C. Luft,[1] Jonathan M. Barasch.[2] [1]*Charite Berlin, Max Delbrueck Center, Germany;* [2]*Columbia University, NY;* [3]*Staten Island University Hospital, NY.*

Background: Conventional diagnostic strategies may inadequately detect patients at risk for poor clinical outcomes. Measuring urinary neutrophil gelatinase-associated lipocalin (uNGAL), a marker of kidney injury, may assist in identifying patients that require evaluation by a nephrologist.

Methods: Unselected patients were recruited from three emergency departments (n=1635) in the United States and Germany and serum creatinine (sCr) and uNGAL levels were measured. The composite outcome of the study was in-hospital hemodialysis initiation or mortality. Nephrology consults ordered within 24 hours of admission served as a surrogate of clinical decision making by physicians who were blinded to uNGAL measurements.

Results: uNGAL was an independent predictor of the composite outcome and improved net reclassification by 26.1% ($p=0.0001$). Patients who had sCr<1.4 mg/dl and uNGAL<104 ng/ml (sCr-/uNGAL-) (n=966) had low rates of clinical events (2.5%) and low rates of early nephrology consultation (2.6%), while patients who had sCr≥1.4 mg/dl and uNGAL≥104 ng/ml (sCr+/uNGAL+) (n=174) had high rates of clinical events (15.5%) and high rates of early nephrology consultation (50.9%). Patients who were sCr-/uNGAL+ (n=227) or sCr+/uNGAL- (n=236) were in an intermediate risk category (clinical event rates 5.3% and 5.1%, respectively). However, nephrology referral rates differed markedly between these categories: sCr-/uNGAL+ had an early referral rate of only 3.1%, while sCr+/uNGAL- patients had an early referral rate of 33.1% ($p<0.001$ by Chi-square test).

Conclusions: Stratification by sCr and uNGAL in the emergency department prospectively separated low risk (sCr-/uNGAL-), intermediate risk (sCr-/uNGAL+ or sCr+/uNGAL-) and high risk (sCr+/uNGAL+) patients. These risk categories were not adequately reflected in nephrology referral rates by physicians unaware of uNGAL levels, suggesting a marked potential for uNGAL to improve clinical decision-making.

Funding: NIDDK Support, Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1091

Renal Adverse Effects of Sunitinib Seon Ha Baek, Dong Ki Kim, Kook-Hwan Oh, Yon Su Kim, Jin Suk Han, Kwon Wook Joo. *Department of Internal Medicine, Seoul National University Hospital.*

Background: Sunitinib is an oral multitargeted small-molecule tyrosine kinase inhibitor mainly used in the treatment of metastatic renal cell carcinoma and its indication is being extended to other carcinomas. Although cases of mild proteinuria were frequently reported and high grade proteinuria and acute kidney injury have been recently reported, renal adverse effects of sunitinib have not been extensively reported. The aim of this study was to evaluate the incidence and risk factors of proteinuria and renal insufficiency (RI) associated with sunitinib.

Methods: We performed a retrospective medical record review of patients who had received sunitinib for more than 3 months. A new onset proteinuria was defined as ≥ 2+ and aggravation of preexisting proteinuria by more than 2 grades on dipstick test, or more than 2 fold increase on urine protein creatinine ratio. RI was defined as an increase ≥ 50 % or ≥ 0.5 mg/dL in serum creatinine (Cr) level.

Results: A total of 155 patients (mean age 58.7±12.6 yr) were enrolled with a mean baseline Cr of 1.24 mg/dl. Proteinuria developed in 15/111 (13.5%) patients and preexisting proteinuria was aggravated in 6/111 (5.4%) patients. Nephrotic range proteinuria was detected in 4 patients, and nephrotic syndrome was identified in only one patient. Following discontinuation of sunitinib, proteinuria was improved or disappeared in 12/17 (70%) patients but persistent in 5/17 (30%) patients. Risk factors for proteinuria were dyslipidemia (OR=8.38, 95% CI 1.76 to 39.98, $p=0.008$) and renal insufficiency (OR=4.07, 95% CI 1.20

to 13.83, p=0.025) at the start of sunitinib. RI was detected in 12/155(7.7%) patients and maximum Cr was 3.31 mg/dl. Risk factors of RI was old age (OR=1.08, 95% CI 1.01 to 1.16, p=0.023). RI was improved in 2 patients (16.6 %), but persisted in 10 patients (83.4 %) after the cessation of sunitinib.

Conclusions: There is a significant risk of developing renal adverse effects among patients receiving sunitinib. Although clinical significance of those adverse effects is still uncertain, proteinuria and renal dysfunction were common. Regular monitoring of renal adverse effects should be considered in patients who are on sunitinib.

FR-PO1092

Renal Tubular Cells Induce Early Pro-Inflammatory and Late Alternative Activation in Macrophages Sarah C. Huen, Lloyd G. Cantley. *Internal Medicine, Section of Nephrology, Yale University School of Medicine, New Haven, CT.*

Background: Macrophage infiltration into the kidney after ischemia reperfusion can contribute to both the initial injury as well as subsequent recovery. A classically activated inflammatory phenotype predominates early after reperfusion while an alternatively activated, anti-inflammatory phenotype predominates later during the repair phase. How paracrine signals from renal tubular cells influence these macrophage phenotypes is unknown.

Methods: Bone marrow-derived macrophages (BMM) were harvested from wild-type C57BL/6, MyD88-/-, Il-4ra-/-, and Stat6-/- mice. Mouse proximal tubular (MPT) cells were cultured in serum free media for 48 hours to generate MPT conditioned media (CM). BMM were cultured in the presence or absence of MPT CM and harvested for protein or mRNA analysis.

Results: The NF-kB protein p65 (RelA) is rapidly phosphorylated in wild-type BMM cultured in MPT CM, followed by increased expression of the classic pro-inflammatory markers Il-1b, Tnfa, Il-6, and Csf3 within 2 hours. By 12 hours after MPT CM incubation, the pro-inflammatory genes were downregulated, whereas alternative activation markers Arg1 and MR were highly upregulated. The initial pro-inflammatory response was prevented in BMM lacking MyD88 or following inhibition of NF-kB using the IKK2 inhibitor, SC-514. However, the late expression of Arg1 and MR was not prevented in BMM lacking the Il-4/Il-13 receptor Il-4ra or the downstream effector Stat6, suggesting that tubular cell-mediated alternative macrophage activation does not occur via the Il-4/Il-13 signaling pathway. Interestingly, Stat3 is activated in BMM at both early (30 minutes) and late (24 hours) time points after exposure to MPT CM, identifying this as a potential mediator of tubular cell-dependent alternative activation.

Conclusions: Paracrine signals from MPT cells induce sequential macrophage activation from a NF-kB dependent pro-inflammatory phenotype to an anti-inflammatory phenotype that is Il-4 and Stat6 independent. This sequential macrophage activation is similar to that seen in vivo after renal ischemia reperfusion injury, suggesting that tubular cells themselves can regulate the phenotype of infiltrating macrophages.

Funding: NIDDK Support

FR-PO1093

Renal Dendritic Cells Ameliorate Ischemia Reperfusion Injury Anju Yadav, Divya Anna Verghese, Barbara T. Murphy, Bernd Schroppel. *Nephrology and Transplantation Institute, Mount Sinai School of Medicine, New York, NY.*

Background: Renal DC represents an important population of immune cells within the kidney and found to be functionally important in nephrotoxic acute kidney injury (AKI). In this study we explored whether renal DCs are functionally important in ischemia reperfusion (IR) injury.

Methods: DTR-CD11cGFP mice were used with or without Diphtheria toxin injection prior IR injury (4 ng/g; i.p.). Depletion of renal DCs after DT injection was confirmed by flow cytometry analysis and IF. Bilateral renal pedicles were clamped for 30 minutes and kidneys were reperfused for 24 hrs. Renal function was assessed by serum creatinine and injury scored blindly by H&E. Single cell suspensions were made after renal tissue digestion with collagenase-D (2mg/ml) and flow cytometry analysis was done to phenotype renal immune cells.

Results: 30 minutes of IR caused significant renal histological and functional injury compared to sham controls assessed by serum creatinine and HP score. Depletion of CD11c before IR injury significantly worsened IR injury, evidenced by higher serum creatinine and more tubular damage than non-depleted mice (CD11c+).

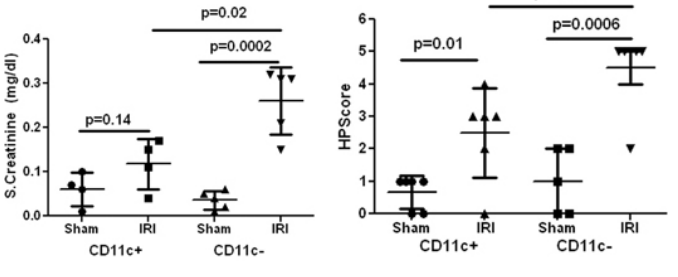


Fig. 1: DC depletion exacerbates IR injury. Serum creatinine (left) and HP score (right) of mice with or without CD11c depletion 24 hr after IR injury.

Renal DCs produced 2-fold more IL-10 by Western blot and 5-fold more mRNA transcripts after IR injury compared to sham controls. Total tissue IL-10 levels were similar in sham and DCs depleted renal immune cells. Furthermore we found that IL-10 gene expression was increased 2-fold when renal DCs were stimulated with the TLR2 ligand PGN, suggesting that IL10 production can be triggered by TLR activation after IR injury.

Conclusions: Renal DCs produce IL-10 after IR injury and afford significant protection for acute kidney injury.

PO1094

Regulatory T Cell Depletion during Established AKI Worsens Subsequent Brain Injury in Mice Manchang Liu,[1] Bing Wang,[1] Sylvain Dore,[2] Hamid Rabb.[1] *[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]University of Florida, Gainesville, FL.*

Background: Acute kidney injury (AKI) is well-established to lead to neurologic changes. Ischemic AKI in mice leads to brain cellular and soluble inflammation with significant an increase in keratinocyte-derived chemokine (KC) protein (J Am Soc Nephrol 2008 19:1360-70). Ischemic AKI enhances the susceptibility to the subsequent ischemic stroke (PO1748, ASN Renal Week 2009). In this study, we tested the mechanistic role of regulatory T cells (Treg) and KC receptor CXCR2 on ischemic stroke outcomes in AKI mice.

Methods: Young male C57BL/6 wild type mice (6-8 wk old) underwent either a sham operation or a 30 min of bilateral kidney warm ischemia following by reperfusion. On day 4 after kidney surgery, all mice underwent ischemic stroke by permanent (distal) middle cerebral artery occlusion (pMCAO) for 90 min and were followed up for 4 d. Mice were treated with antibody against Tregs (PC61) (500 µg per mouse, i.p.), antisera against CXCR2, or control antibody/sera at day 1 and at day 4 after AKI. Mice were sacrificed 8 days after kidney surgery for brain stroke infarct volume measurement.

Results: Depletion of circulating Treg was confirmed by flow cytometry analysis with a significant decrease in the percentage of CD4+CD25+Foxp3+ Treg of splenocytes (PC61 vs. IgG: 1 % vs. 14 %, p<0.001, n=10). AKI mice had higher brain infarct volumes after stroke compared with sham AKI stroke mice (AKI vs. Sham: 35.2% ± 3.4 vs. 23.9% ± 1.9, p=0.022, n=5). The degree of stroke infarct volume was associated with serum creatinine level (r=0.62, p=0.006, n=18). AKI mice treated with PC61 during established AKI had significant increase in stroke infarct volume when compared to AKI mice with IgG. (39.5% ± 1.6 vs. 32.9% ± 2.5, p=0.04, n=10 per group). No significant change was seen on stroke infarct volumes by blocking KC receptor CXCR2 (CXCR2 sera vs. Normal sera: 24.1% ± 5.1 vs. 20.9% ± 2.6, p=0.57, n=5-6).

Conclusions: These data demonstrate that ischemic AKI exacerbates subsequent stroke, and one underlying mechanism could be via regulatory T cells. Regulatory T cells have the potential, if harnessed appropriately, to decrease the high distant organ effects of AKI.

Funding: Private Foundation Support

FR-PO1095

Humoral Immune Response Is Enhanced by Renal Ischemia-Reperfusion Injury Richard C. Fuquay,[1] Brandon Renner,[1] Derek Strassheim,[1] Van Willis,[2] Kim McFann,[1] Joshua M. Thurman.[1] *[1]Nephrology, University of Colorado Denver Health Science Center, Aurora, CO; [2]Rheumatology, University of Colorado Denver Health Science Center, Aurora, CO.*

Background: Ischemia-reperfusion injury (IRI) is a model of acute kidney injury (AKI) that is characterized by robust renal inflammation and temporary renal failure of 48-72 hours. Renal transplant recipients that suffer delayed graft function (DGF) in the setting of prolonged ischemia time are felt to have an increased risk of long-term immunologic injury. We sought to investigate the effect of AKI on the humoral immune response to extra-renal antigens.

Methods: Mice have a reliable immunoglobulin response to nitrophenol keyhole limpet hemocyanin (NP-KLH), an innocuous T-cell dependent antigen.

Results: In mice immunized 24 hours before IRI vs. sham surgery there was no observed difference in NP-KLH specific antibody levels between the two groups up to 56 days after surgery. However, when mice were immunized 24 hours after IRI vs. sham surgery (the peak of renal failure in this model), the IRI-treated mice had increased levels of NP-KLH specific IgG1 (p=0.0376 by mixed model analysis) beginning at 35 days after surgery. The increased IgG1 response was not seen with unilateral ischemia, typified by local inflammation but not renal failure. Furthermore, the total IgG1 and IgM (not antigen-specific) were not different in IRI vs. sham mice; this effect is not due to polyclonal immunoglobulin upregulation. There was no difference between the two groups in the percentage of splenic lymphocytes expressing B220 (B cell marker) or CD 86 (marker of B-cell activation, also known as B7-2).

Conclusions: In summary, IRI-treated mice develop a greater antigen-specific IgG_1 response than sham-treated controls. This effect is not seen with unilateral IRI and is not due to global B cell activation or a polyclonal immunoglobulin increase. Experiments are ongoing with T-cell independent antigens and in mice with targeted deletion of innate immune factors. Ongoing experiments may provide a mechanistic explanation why DGF kidneys suffer long-term immunologic injury and reveal therapeutic interventions that could improve allograft survival.

Funding: NIDDK Support

FR-PO1096

Heat Shock Protein-70 (HSP70) Induced Renoprotective Effect Is Mediated by CD4+ CD25+ Foxp3+ Regulatory T Cells in Ischemia/Reperfusion (I/R) Induced Acute Kidney Injury Kichul Yoon, Won-Yong Cho, Sang-Kyung Jo, Sung Yoon Lim, Hyoung-Kyu Kim. *Internal Medicine, Anam Hospital, Korea University, Seoul, Korea.*

Background: Recent reports has demonstrated the immune modulatory effect of HSPs. HSP70 induced by heat preconditioning(HP) has been shown to decrease inflammation and injury in I/R induced AKI. CD4+ CD25+ Foxp3+ regulatory T cells(Tregs) has recently been recognized as an important player in decreasing kidney injury after I/R. The aim of this study was to test whether HP induced HSP70 exerts renoprotective effect through Tregs.

Methods: Thirty min bilateral I/R injury was done with or without heat preconditioning(42C for 15min) in mice. Quercetin for inhibition of HSP70, or PC61 for depleting Tregs, were administered and various molecular and flow cytometric analyses were performed.

Results: Splenocytes from HP mice demonstrated expansion of Tregs, and reduced proliferative response upon mitogenic stimuli. T cells from HP mice failed to reconstitute postischemic injury when adoptively transferred to T cell deficient mice in contrast to T cells from normal mice, suggesting that HP has immune modulatory function. While depleting Tregs before HP abolished the renoprotective effect, adoptive transfer of the cells back into Treg-depleted mice partially restored the beneficial effect of HP. Significantly increased Foxp3 gene expression as well as increased infiltration of Tregs into kidney were also observed in heat preconditioned ischemic kidneys. Immunohistochemistry and western blot demonstrated that HSP 70 was induced upon HP not only in kidney, liver or lung but also in immune cells in spleen. Inhibition of HSP70 by quercetin before HP suppressed the expansion of Tregs and this was associated with partial loss of beneficial effect of HP in I/R. Finally, transferring Tregs to quercetin-treated HP mice partially restored the beneficial effect of HP.

Conclusions: These results suggest that renoprotective effect of HSP70 might be partially mediated by their direct immune modulatory effect through Tregs. Further understanding of cytoprotective or immune modulatory mechanisms of various stress proteins might facilitate discovery of new targets or drug development in the field of AKI.

FR-PO1097

Inhibition of the c-fms Receptor Prevents Kidney Macrophage Accumulation and Is Renoprotective Following Ischemic Injury Meghan Clements, Michael Gershenovich, Christopher Chaber, Steven R. Ledbetter, Anna Zuk. *Cell Biology, Genzyme Corporation, Framingham, MA.*

Background: Colony stimulating factor-1 regulates proliferation, differentiation and survival of monocytes and macrophages by signaling through the c-fms macrophage receptor. Since the mononuclear phagocyte system mediates renal injury following ischemia-reperfusion (I/R), we hypothesized that inhibition of the c-fms receptor would be renoprotective.

Methods: A small molecule inhibitor of c-fms, was administered prior to and after bilateral renal ischemia.

Results: Vehicle-treated mice had significant increases in the number of CD11b+/F4/80+ macrophages in both the circulation and kidney 24 hrs post-reperfusion, correlating with peak levels of plasma creatinine and blood urea nitrogen (BUN). Treatment with a c-fms inhibitor significantly reduced CD11b+/F4/80+ macrophages in the kidney and blood, kidney levels of MCP-1 and plasma creatinine and BUN by approximately 50%. Thus, antagonism of c-fms prevents kidney macrophage accumulation and is renoprotective. However, macrophages also mediate kidney repair and fibrosis. By examining changes in monocyte/macrophage populations at various times post-reperfusion by CD11b and Ly6C expression, we show that CD11b+/Ly6CHigh cells appear 3 hrs post-reperfusion, which correlates with elevated tissue mRNA of pro-inflammatory IL-6 and CXCL1. This suggests that this population represents M1 macrophages. By 24 hrs post-reperfusion, the CD11b+/Ly6CHigh cells decrease and instead a CD11b+/Ly6CInt population dominates, returning to baseline by 5 wks post-reperfusion. A CD11b+/Ly6CLow population emerges 3 days post-reperfusion and is the only population elevated at 5 wks when interstitial fibrosis and inflammation are observed. Increased tissue mRNA of CD206 and arginase-1 suggest the Ly6CLow population represents M2 macrophages. Similarly, shifts in CD11b+/F4/80high/low populations are seen.

Conclusions: Collectively, these data define three distinct populations of monocytes/macrophages following renal I/R using the markers CD11b and Ly6C. Ongoing studies are investigating the role of these populations in kidney injury and repair and whether antagonism of c-fms could impact fibrosis.

Funding: Pharmaceutical Company Support

FR-PO1098

Mechanisms of Uric Acid-Mediated HMGB1 Translocation and Release from Endothelial Cells (EC) May Rabadi, Mei-Chuan Kuo, Tammer N. Ghaly, Seham Rabadi, Mia Weber, Michael S. Goligorsky, Brian B. Ratliff. *New York Medical College, Valhalla, NY.*

Background: Based on findings that after IRI HMGB1 is released by the kidneys into the circulation, we examined the cellular localization and release of HMGB1 in EC. Due to its early and robust release after IRI, uric acid was examined as a potential mediator of HMGB1 release from EC.

Methods: Treatment of EC with uric acid resulted in increased HMGB1 mRNA expression and protein translation, effects that were blocked by inhibition of TLR4 receptor.

Results: Uric acid treatment of EC lead to the translocation of HMGB1 from the nucleus to the cytosol and release from the cell into either the cell culture medium (in vitro) or the circulation (in vivo), as determined by western blot analysis and immunofluorescence. We next aimed at identifying the mechanism by which uric acid induces the release of HMGB1. Treatment of EC with uric acid and an inhibitor of intracellular calcium release (TMB-8) or an inhibitor of MEK/ERK pathway (U0126) resulted in the retention of HMGB1 in the nucleus, while diminishing cytoplasmic and extracellular levels of HMGB1. We also examined the role of HMGB1 acetylation in mediating its translocation. Treatment of EC with uric acid caused acetylation of HMGB1 leading to its translocation and release from EC, an effect blocked by pretreating cells with ethyl pyruvate. Once released, HMGB1 was observed to have a positive feedback mechanism that promoted further translocation and release of HMGB1 from endothelial cells. We also demonstrate that uric acid and released HMGB1 induce EC angiopoietin 2 mRNA expression and protein release while activating intracellular NF-kB (as measured by luciferase reporter assay) and the pro-inflammatory response.

Conclusions: Uric acid through the TLR4 receptor mediates the transcriptional and translational HMGB1 response in EC by a mechanism that involves the release of intracellular calcium and the MEK/ERK pathway and the acetylation of HMGB1, which resulted in its translocation and release from the cell. Angiopoietin 2 expression and NF-kB activity were increased after uric acid and HMGB1 treatment. Mobilization of acetylated HMGB1 was reduced with ethyl pyruvate treatment.

Funding: NIDDK Support

FR-PO1099

HMGB1 Release from Kidneys during Renal Ischemia-Reperfusion Injury May Rabadi, Tammer N. Ghaly, Seham Rabadi, Michael S. Goligorsky, Brian B. Ratliff. *Medicine, New York Medical College, Valhalla, NY.*

Background: Factors that initiate cellular damage and trigger the inflammatory response cascade and renal injury are incompletely understood after renal ischemia-reperfusion injury (IRI). HMGB1 is a DAMP molecule that binds to DNA, but upon signaling translocates from the nucleus to the cytoplasm and subsequently released from necrotic and/or damaged cells.

Methods: We observed that HMGB1 is released by the IRI kidneys into the venous circulation. Immunohistochemical analysis of kidneys at different times after IRI showed release of HMGB1 into the circulation within minutes and up to 1 hour after the initial insult.

Results: The degree of HMGB1 release by renal cells was dependent on the duration of ischemic insult with progressively increased release occurring when ischemic times increased from 25 min to 50 min. We next examined the effect released HMGB1 has in promoting the pro- and anti-inflammatory cascade. Circulating HMGB1 induced rapid systemic surge (within 1 hour) of cyto- and chemo-kines including TNF-α, eotaxin, G-CSF, IFN-γ, IL-10, IL-1α, IL-6. FACS analysis revealed circulating HMGB1 mobilizes endothelial progenitor cells (Flk+, CD34+, CD45-) from the bone marrow, indicating its potential regeneration promoting effects. To examine the therapeutic efficacy of blocking the release of HMGB1, we treated mice with ethyl pyruvate during IRI. Ethyl pyruvate has previously been shown to block the nuclear-cytoplasmic translocation of HMGB1 in monocytes/macrophages. Here we demonstrated that release of HMGB1 by IRI kidneys into the venous circulation was blocked by ethyl pyruvate pretreatment. Renal function after IRI was improved when HMGB1 release was blocked with ethyl pyruvate, as judged by the serum creatinine retention. In long-term studies, two months after the ischemic insult, renal function continued to show improvement as urine albumin:creatinine ratios were decreased in IRI mice treated with ethyl pyruvate, as compared to non-treated IRI mice.

Conclusions: Despite putative pro-regenerative signaling, HMGB1 exerts a robust systemic pro-inflammatory response in IRI and inhibition of its release results in the short- and long-term functional benefits.

Funding: NIDDK Support

FR-PO1100

Functional Consequences of Inhibiting Exocytosis of Weibel-Palade Bodies (WPB) in Acute Renal Ischemia (IRI) Kaoru Yasuda,[1,2] Peter Jose Hayek,[1] Brian B. Ratliff,[1] Jonathan Mares,[1] Silvia Bertuglia,[3] Paolo Mascagni,[4] Michael S. Goligorsky.[1] *[1]New York Medical College, Valhalla, NY; [2]Nagoya University Graduate School of Medicine, Japan; [3]University of Pisa Medical School, Italy; [4]Italfarmaco Research Centre, Milan, Italy.*

Background: Exocytosis of WPB represents a distinct response of endothelial cells to stressors and local release of WPB contents leads to systemic escalation of this response.

Methods: We (SB and PM) synthesized a glycine-(Nα-Et)lysine-proline-arginine (ITF1697) peptide and demonstrated that it inhibits IRI-induced exocytosis of WPB and protects microcirculation. Here, mice were implanted with Alzet osmotic pumps (10μg ITF1697/kg/min at volume of 1 μl/h) for the duration of 3 days, and subjected to bilateral renal IRI.

Results: *En face* staining of aortic endothelial cells showed that WPB were depleted after 40-180 min post-IRI and this was significantly blunted in aortic preparations obtained from mice treated with ITF1697. Ischemia resulted in a marked renal injury and elevation of serum creatinine in mice treated with a vehicle. In contrast, renal injury and elevation of creatinine level were significantly ameliorated in mice subjected to IRI and receiving ITF1697. ITF1697 prevented systemic response to IRI: a significant surge in the levels of eotaxin and IL-8 (KC) (both components of WPB), IL-1α and IL-1β, and RANTES

were all prevented or blunted by the administration of ITF1697, whereas the levels of an anti-inflammatory IL-10 and MIP-1α were upregulated in ITF1697-treated animals. WPB exocytosis contributed to IRI-associated mobilization of EPC and HSC and ITF1697 blunted stem/progenitor cell mobilization. One month after IRI, mice treated with ITF1697 showed a significantly more pronounced degree of scarring than non-treated animals.

Conclusions: 1) application of ITF1697 inhibits exocytosis of WPB; 2) the systemic inflammatory response of IRI is in part due to the exocytosis of WPB and its blockade blunts it; 3) ITF1697 improves short-term renal function after IRI, but not the long-term fibrotic complications.

Funding: NIDDK Support

FR-PO1101

Slit2 Prevents Renal Ischemia Reperfusion Injury Swasti Chaturvedi,[1] Amandeep Bajwa,[3] Liping Huang,[3] Grace Lam,[2] Yi-Wei Huang,[2] Guang-Ying Liu,[2] John Brumell,[2] Mark D. Okusa,[3] Lisa Robinson.[1] *[1]Nephrology, The Hospital for Sick Children, Toronto, Canada; [2]Research Institute, The Hospital for Sick Children, Toronto, Canada; [3]Medicine, University of Virginia Health System, Charlottesville, VA.*

Background: Acute kidney injury (AKI) affects approximately 5% of hospitalized patients and leads to significant morbidity and mortality. Inflammation marked by recruitment of circulating leukocytes, particularly neutrophils, to the injured kidney is a key component of AKI caused by ischemia-reperfusion injury (IRI). The neuronal guidance cue, Slit2 and its receptor roundabout (Robo) prevent axonal migration during the central nervous system development. We have shown that Slit2 inhibits neutrophils chemotaxis towards diverse chemoattractants. We further aimed to determine the effect of Slit2 on individual steps of neutrophil recruitment cascade and to explore its therapeutic role in renal IRI.

Methods: The effect of Slit2 on neutrophil capture and adhesion was studied using microfluidics. Neutrophil transendothelial migration was studied using transwell assays. To test whether Slit2 prevents IRI, we used a mouse model of bilateral renal pedicle clamping. To study the effect of Slit2 on innate immune responses to infection, hepatic bacterial colony counts were measured after inoculating mice with *L.monocytogenes*.

Results: Slit2 reduced both neutrophil capture and adhesion to TNF-α stimulated endothelium under shear stress conditions ($p < 0.05$). Further Slit2 reduced chemoattractant induced neutrophil transendothelial migration in transwell assays ($p < 0.05$). Pre-treatment of mice with Slit2 before inducing renal IRI prevented the rise in serum creatinine in a dose dependent manner ($p < 0.05$ at the highest dose of Slit2 tested). Slit2 also prevented neutrophil and macrophage infiltration into the post-ischemic kidney ($p < 0.05$). Additionally, renal tubular necrosis and injury were significantly reduced by Slit2. The hepatic L.monocytogenes bacterial load was not increased following Slit2 treatment.

Conclusions: These findings suggest that Slit2 could be used to prevent and treat the inflammatory cell recruitment in AKI, without increasing susceptibility to bacterial infection.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1102

The Effect p53 Inhibition in Ischemic Acute Kidney Injury Is Species Dependent and Is Determined by the Relative Contributions of Inflammation and Tubular Apoptosis Takashi Hato, Melissa D. Anderson, Timothy A. Sutton, Pierre C. Dagher. *Medicine/Nephrology, Indiana University School of Medicine, Indianapolis, IN.*

Background: Renal ischemia-reperfusion injury (IRI) is characterized by inflammation, tubular cell apoptosis and necrosis. We have previously shown that the p53 inhibitor pifithrin-α mitigates tubular cell apoptosis and protects renal function in a rat model of renal IRI despite the persistence of moderate inflammation.

Methods: To determine if the genetic absence of p53 also confers protection, we compared the outcomes of renal IRI in p53 knockout (KO) and wild-type (WT) mice.

Results: Surprisingly, KO mice have worse renal function after IRI compared to WT (mean creatinine 24 hrs post IRI: 0.96 mg/dl in KO vs. 0.39 mg/dl in WT, $p=0.01$). Histology in p53 KO mice after IRI reveals an increase in tubular necrosis, dilation, and cast formation compared to WT. Leukocyte infiltration is greater in KO mice compared to WT (mean F4/80+ cells 24 hrs post IRI: 788/section in KO vs. 441 in WT, $p=0.03$ and mean esterase+ cells 24 hrs post IRI: 237/section in KO vs. 96 in WT, $p=0.05$). The difference in leukocyte count is sustained one week after IRI. Unexpectedly, the frequency of tubular cell apoptosis is higher in p53 KO mice as compared to WT (TUNEL+ cells 24 hrs post IRI: 4.7 %/field in KO vs. 2.3 % in WT, $p=0.004$). Since these results conflict with the beneficial effects of pifithrin-α in the rat, we examined the effects of pifithrin-α on renal IRI in WT mice. The results were similar to those observed in p53 KO mice.

Conclusions: We conclude that the effects of p53 inhibition or its genetic absence on the outcome of renal IRI is species dependent and is determined primarily by the contribution of inflammation to IRI. In mice inflammation plays an important role in renal IRI and p53 provides an anti-inflammatory effect by reducing leukocyte survival and cytokine secretion, thus p53 inhibition worsens inflammation and renal injury. In rats, inflammation in renal IRI is less prominent and p53 inhibition provides an anti-apoptotic effect on renal tubular cells and improves function. Whether human renal IRI is better modeled by mice or rats remains to be determined.

Funding: NIDDK Support

FR-PO1103

Renal Tubular and Peritubular Capillary Injury in Hepatic Failure Induced Acute Kidney Injury in Rats Akira Shimizu,[1] Shinya Nagasaka,[1] Ayako Sato,[2] Honglan Piao,[2] Tetsuo Morioka,[2] Yukinari Masuda.[1] *[1]Analytic Human Pathology, Nippon Medical School, Tokyo, Japan; [2]Cellular Physiology, Institute of Nephrology, Niigata University School of Medicine, Niigata, Japan.*

Background: Acute kidney injury (AKI) is a common complication in the acute liver dysfunction. However, little is known about the mechanisms of AKI during the development of acute liver dysfunction. In this study, we characterize a rat model of AKI during the development of acute liver dysfunction following liver transplantation.

Methods: Acute hepatic failure was induced in rat by liver transplantation from DA (RT1a) to Lewis (RT1l) rats without immunosspression. Rats were dead around day 11 with severe acute liver dysfunction. We studied kidney samples at day 5, day 7, and day 9 to 11, focusing on the tubular and peritubular capillary (PTC) injury. In addition, hemodynamic events in PTCs in vivo were evaluated functionally and quantitatively by the use of a real-time confocal laser-scanning microscope (CLSM) system (Kidney Int 59: 252-259, 2001).

Results: During the progression of rejection in hepatic graft, acute liver dysfunction (T-Bil 7.9±1.8, $p<0.01$) and acute kidney injury (BUN 112.0±22.5; Cr 0.6±0.1, $p<0.05$) developed by day 11. During the development of AKI, renal tubular degeneration with bile pigment accumulation, mitochondrial degeneration, KIM-1 expression, and severe disruption of f-actin. TUNEL+ dead cells were noted in tubules and PTCs. In adittion, endothelial dysfunction in PTCs developed with decrease expression of eNOS, and marked reduced blood flow (540±162 vs 860±145 μm/sec in control, $p<0.05$). Interstitial edema occurred with inflammatory cell infiltration.

Conclusions: In conclusion, AKI developed in rats during the development of acute liver dysfunction and was characterized by renal tubular injury as well as endothelial dysfunction in PTCs with marked reduced blood flow.

Funding: Private Foundation Support

FR-PO1104

Role of Calcium/Calmodulin-Dependent Kinase Kinase 2 (CaMKK2) in Acute Kidney Injury Christina R. Kahl,[1] Thomas J. Ribar,[2] Gloria A. Preston,[1] Anthony R. Means.[2] *[1]UNC Kidney Center, University of North Carolina at Chapel Hill, NC; [2]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

Background: The multifunctional calcium/calmodulin-dependent kinases (CaMKs) are implicated in the inflammatory response of lymphocytes, dendritic cells and macrophages. Previously published studies have shown that treatment with a non-selective CaMK inhibitor ameliorates disease development and renal injury in a lupus mouse model. Since CaMKK2 regulates the activity of both CaMKI and CaMKIV and inflammation plays a key role in acute kidney injury (AKI), we hypothesized that the loss of CaMKK2 would be protective in mouse models of AKI.

Methods: Cohorts of *CaMKK2* null mice or wild type (WT) mice were injected with LPS (lipopolysaccharide) or folic acid to induce AKI based on established protocols. LPS causes a systemic inflammatory response and subsequent renal injury. Folic acid causes tubular injury and inflammation with AKI dramatically attenuated by neutrophil depletion in mice.

Results: *CaMKK2* null mice demonstrate normal renal function by BUN and creatinine, and kidneys demonstrate normal histology. Following LPS injection, CaMKK2 null mice are indeed protected from renal injury with lower BUN and creatinine values than WT mice (creatinine in mg/dL, 0.26 versus 0.50, $p<0.05$). Surprisingly, folic acid injection causes dramatic renal injury in CaMKK2 null mice similar to WT mice (creatinine 2.36 versus 1.60, not significant). The CaMKK2 null mice with AKI also had significantly higher kidney/body weight ratios than WT mice and extensive renal injury histologically.

Conclusions: Therefore, CaMKK2 is required for AKI following LPS treatment but it is not required for the development of folic acid induced renal injury, suggesting that it may regulate different inflammatory responses in AKI. These results are consistent with previously published studies demonstrating the role of CaMKs in macrophage and dendritic cell activation in response to LPS. The CaMKK cascade may represent a novel pathway for therapeutic modulation of acute kidney injury related to endotoxemia.

Funding: NIDDK Support

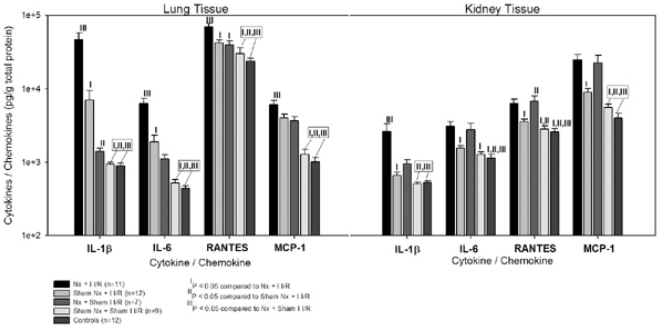
FR-PO1105

Acute Kidney Injury (AKI) in Rats with Pre-Existing Chronic Kidney Disease (CKD) Induces a Major Increase in Pro-Inflammatory Cytokines (IL-1β, IL-6) and Chemokines (RANTES, MCP-1) in Kidney and Lung Martin Skott,[1] Rikke Norregaard,[2] Tae-Hwan Kwon,[3] Jorgen Frokiaer,[2] Soren Nielsen.[1] *[1]Institute of Biomedicine, University of Aarhus, Aarhus, Denmark; [2]Institute of Clinical Medicine, University of Aarhus, Aarhus, Denmark; [3]Department of Biochemistry & Cell Biology, School of Medicine, University of Taegu, Korea.*

Background: AKI in patients with pre-existing CKD have increased co-morbidity and mortality. Although it is well established that AKI is associated with a major increase in pro-inflammatory cytokines and chemokines, it is unknown to which extent AKI in pre-existing CKD leads to changes in the expression of proinflammatory cytokines/chemokines. The aim of this study was to assess the changes in the expression of pro-inflammatory cytokines and chemokines in kidney and lung in response to AKI in rats with pre-existing CKD.

Methods: CKD was induced by 5/6 nephrectomy (5/6 Nx) for 6 weeks. AKI was induced by intestinal ischemia for 45 min followed by reperfusion for 90 min (II/R): 1) Nx+II/R; 2) Sham Nx+II/R; 3) Nx+Sham II/R; 4) Sham Nx+Sham II/R, 5) controls. Cytokines/chemokines were measured in homogenized whole kidney and lung preparations with LuminexTM 100.

Results: S-Cr increased significantly in response to II/R:from 66.2±7.3 to 88.9±8.3 in Nx rats, resp., and from 32.0±0.7 to 54.7±2.9 in Sham Nx rats. The levels of IL-1β, IL-6, RANTES, and MCP-1 in lung and kidney, were significantly higher in rats undergoing II/R compared to sham II/R. Importantly also in the 5/6 Nx rats II/R induced a significant increase in IL-1β, IL-6, RANTES, and MCP-1 expression in kidney and lung compared to sham 5/6 Nx. Morever the response was even more pronounced in the 5/6 Nx compared to the response in sham 5/6 Nx rats.



Conclusions: The results demonstrate a significant increase in pro-inflammatory cytokines and chemokines in response to AKI in rats with pre-existing CKD.

Funding: Private Foundation Support, Clinical Revenue Support

FR-PO1106

MCP-1 Is Involved in Potentiation of Lung Inflammation by Antecedent Ischemic Acute Kidney Injury Rajit K. Basu, Emily Donaworth, Prasad Devarajan, Hector R. Wong. *Divisions of Critical Care and Nephrology,*

Compartment	Sham-PBS	Sham-LPS	AKI-PBS	AKI-LPS	p value
Kidney	3.2 (2.5-3.9)	2.6 (2.2-3.9)	9.5 (5.2-13.2)	4.0 (2.6-8.2)	.112
Serum	59 (43-75)	69 (56-73)	108 (53-161)	139 (87-230)	< .001
Lung	1.1 (0.7-1.5)	9 (6-10)	1.4 (0.9-1.8)	11 (7-14)	.358
BALF	13.8 (7.2-25.8)	767 (707-1107)	12 (5.2-13)	5388 (4024-6662)	< .001

Figure 1. MCP-1 expression ubiquitously increases in ALI with antecedent AKI. MCP-1 expression, by Luminex xMAP, was elevated in all tissue compartments in AKI-LPS groups versus Sham-LPS groups. Results are medians with interquartile ranges shaded (ng/ml) (p values: Sham-LPS vs. AKI-LPS) (n=10-12/group)

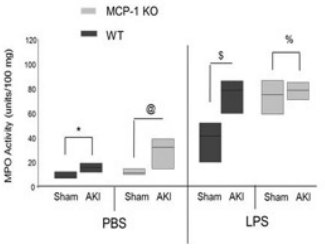


Figure 2. Elimination of MCP-1 abrogates AKI dependent lung priming. Lung myeloperoxidase activity for MCP-1 KO mice and wild type mice subjected to 2-hit model. MCP-1 KO mice receiving LPS did not show an augmented lung inflammation with antecedent AKI as opposed to their WT cohorts. n=6/group. (* p = .025, @ p = .016, $ p = .005, % p = not significant)

Taken together, our two-hit model demonstrates that ischemic AKI primes the lung for a heightened inflammatory response to subsequent injury and MCP-1 may be involved in this potentiation. The findings hold relevance for critically ill patients at risk of deleterious kidney-lung crosstalk.

Funding: Clinical Revenue Support

FR-PO1107

Natural IgM Anti-Leucocyte Autoantibodies(IgM-ALA) Block Immune Mediated Proinflammatory Cytokine Release through Inhibition of NF-kB Activation Sang Ju Lee,[1,2] Amandeep Bajwa,[1,2] Kailo H. Schlegel,[1,2] Liping Huang,[1,2] Mark D. Okusa,[1,2] Peter I. Lobo.[1,2] *[1]Department of Medicine, University of Virginia, Charlottesville, VA; [2]Center for Inflamation, Immunity and Regenerative Medicine, University of Virginia, Charlottesville, VA.*

Background: IgM-ALA reduce kidney ischemia-reperfusion injury (IRI) however, little is known about the mechanisms by which these antibodies attenuate IRI. IgM-ALA bind to receptors on leucocyte and endothelial cell membranes, tissues important in the pathogenesis of IRI. The purpose of the current studies was to examine the effect of IgM on cellular mechanisms that lead to attenuation of kidney IRI.

Methods: Wild type mice and IgM KO mice were subjected to kidney IRI with and without IgM pretreatment. At 24 hours we measured serum creatinine and evaluated kidney histology. In other studies, we cultured mouse glomerular endothelial cells (mGEC) and RAW264.7 macrophage cells using standard methods and tested the effect of IgM (25μg/ml) on these cells activated with LPS(0.1 to 1μg/ml) for 20 min to 24 hrs. We measured proinflammatory cytokine production by real time PCR and immunohistochemistry as well as phosphorylation and nuclear translocation of NF-kB by Western blot.

Results: LPS significantly increased CXCL1 production (p<0.001) by mGEC. IgM, but not IgM adsorbed with leukocytes to remove IgM-ALA, blocked CXCL1 production through inhibition of phosphorylation and nuclear translocation of NF-kB. IgM KO mice showed increased renal injury after IRI. IgM pretreatment, but not IgM depleted of IgM-ALA, significantly decreased sCr and tubular injury in wild type and IgM KO (p<0.01). Similar to in vitro data, IgM treated mice had significantly less labeling of MCP-1 and CXCL1 on kidney vascular endothelial cells co-expressing TLR4 and CD31 after IRI.

Conclusions: We show that IgM-ALA, in physiologic doses, inhibits proinflammatory cytokines production by preventing activation of NF-kB. These studies highlight the importance of naturally occurring IgM-ALA in regulating excess inflammation as occurs after ischemia reperfusion injury despite presence of competent regulatory T cells. IgM could pre-emptively be used to prevent renal IRI.

Funding: NIDDK Support

FR-PO1108

The Effect of mTOR-Inhibition on Cycling of NF-kappa B Activation in Kidney Ischemia-Reperfusion Injury in Mice Aleksandra V. Kezic,[1] Jan U. Becker,[2] Tung Yu Tsui,[3] Friedrich Thaiss.[4] *[1]Univ. Hospital, Dep. Int. Medicine, Belgrade, Serbia; [2]Univ. Hospital, Dep. Pathology, Hannover, Germany; [3]Univ. Hospital, Dep. Transplant Surgery, Hamburg, Germany; [4]Univ. Hospital, Dep. Int. Medicine, Hamburg, Germany.*

Background: Ischemia-reperfusion injury (IRI) remains an important initiator of acute renal transplant rejection and delayed allograft function. Multiple stimuli in IRI activate the NF-kappa B (NF-κB) pathway which plays a central role in the regulation of numerous downstream pathways. We therefore determined cycling of NF-κB activation after induction of IRI and the role mTOR-inhibition (Everolimus; eve) might play.

Methods: C57/BL6 mice were subjected to IRI by clamping both renal pedicles. Application of eve started one day before IRI induction in a dose of 1.5 mg/kg b.w. subcutaneously daily. Both eve treated and non-treated mice were sacrificed 30 min, 1h, 2h, 6h, 12h, 1d, 2d, 3d and 7d after IRI. Kidneys were examined by morphology and standard molecular biology techniques.

Results: After induction of IRI NF-κB was significantly increased at early (6h and 3d) and also later (7d) time points when compared with shame operated animals. NF-κB activation was paralleled by a biphasic increase in TNF-alpha and chemokine CCL2 and CCL5 expression. Eve treatment of mice significantly increased NF-κB activity and inhibited NF-κB cycling at all time points. The second peak of TNF-alpha expression was significantly increased while CCL2 and CCL5 expression was significantly reduced at all time points in eve treated animals. By morphology eve treatment significantly increased injury index at early (2d) (p<0.05), however significantly reduced at later (7d) time points (p<0.004) compared with non-treated mice. Interstitial infiltration of F4/80-positive cells after IRI was significantly reduced at later time points in eve treated animals (P<0.01).

Conclusions: NF-κB cycling could be demonstrated in kidneys after IRI during a 7 days observation period. The peaks of NF-κB activation might represent early epithelial and late immune activation pathways. The influence of mTOR inhibition during early epithelial and late immune mediated activation of NF-κB after IRI warrants further investigation.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1109

Activation of the Inflammasome in Cisplatin-Induced Acute Kidney Injury Dong Won Lee, Zhibin He, Quocan Nguyen, Ali Akcay, Alkesh Jani, Sarah Faubel, Charles L. Edelstein. *Kidney Disease and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: We have demonstrated increased caspase-1, IL-1α, -1β and IL-18 in cisplatin (Cis)-induced AKI. As caspase-1, IL-1α, -1β and IL-18 are activated in the inflammasome, the aim of the study was to further investigate the inflammasome in Cis-induced AKI. Inflammasomes are cytosolic complexes composed of NALPs, ASC and caspase-5. BID is a pro-apoptotic protein activated by caspase-1 in the inflammasome.

Results: Mice injected with Cis (25 mg/kg) developed AKI on day 3. On qPCR of whole kidney, NALP3 mRNA was increased on day 3 (p<0.05, 0.75 vs. 0.1 fold change). On immunoblot of whole kidney, there were a 2-fold increase in ASC (22 kDa) (p<0.05), a 3-fold increase in caspase-5 (47 kDa) (p<0.05) on days 2 and 3, and a 2-fold increase in cleaved BID (15 kDa) on day 3 (p<0.05). Caspase-5 activity was increased (p<0.05, 29.2 vs. 11.2 nmol/mg/min in Veh). In summary, the NALP3 inflammasome is activated in AKI as evidenced by increased caspase-1, NALP3, caspase-5, ASC and BID in whole kidneys. On immunoblots, NALP3 was present in the freshly isolated proximal tubules (PT), but not in endothelial cells or macrophages. Thus we further investigated the inflammasome in a model of PT treated with Cis 10 and 50 μM. Caspase-1 activity was increased (p<0.05, 2.3±0.3 and 3.2±0.5 vs. 0.9±0.2 nmol/mg/min in Veh). Active caspase-1 (10 kDa) was increased in Cis-treated PTs (p<0.05 vs. Veh). NALP3 was strongly expressed in PTs, but with no changes between groups. Parent BID (22 kDa), but not cleaved BID, was 2-fold increased in PTs (p<0.05). On ELISA, IL-1α activity was increased with Cis (p<0.05, 1.1 and 1.3 vs. 0.1 nmol/mg/min in Veh). IL-1β was increased in 50 μM Cis-treated PTs (p<0.05, 10.9 vs. 7.6 nmol/mg/min in Veh). In summary, in Cis-treated PTs, there is increased caspase-1, IL-1α and IL-1β.

Conclusions: In conclusion, components of the inflammsome are increased in both whole kidneys in vivo and PTs treated with Cis in vitro.

FR-PO1110

Hypoxic Response of Myeloid Cells in Renal Ischemia-Reperfusion Injury
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Background: After ischemia-reperfusion injury tubular cells undergo apoptosis and necrosis which triggers an inflammatory cell response. Infiltration of M1-type macrophages is detrimental in early phases, however alternatively activated M2-macrophages promote tissue repair. Hypoxia-inducible factors are critical for inflammatory macrophage responses and are differentially expressed in M1 and M2 macrophages. We therefore investigated whether HIF is critical for macrophage function and plasticity in ischemic kidney injury.

Methods: Floxed alleles of HIF-1α were deleted in myeloid cells by Cre-loxP recombination using LysM-Cre. Kidney ischemia-reperfusion injury was performed by clamping renal arteries for 25 minutes. Animals were analysed at 48 h and 72 h after reperfusion. Creatinine and urea levels were determined as renal function parameters. Gene expression analysis was done by real-time PCR and by immunohistochemistry.

Results: No renal phenotype is observed under control conditions in mice with deletion of HIF-1α in myeloid cells. Functional impairment 48h after renal ischemia reperfusion injury is not significantly different between wildtype and knockout mice. Creatinine levels in wildtype animals 72h after reperfusion are significantly better. Knockout animals do not exhibit this recovery of renal function. Macrophage infiltration is similar in both groups as assessed by F4/80 staining and MCP1 expression. However, TGFβ expression in injured kidneys is significantly lower in knockout animals.

Conclusions: Deletion of HIF-1α in myeloid cells results in a reduced capacity for functional recovery after renal ischemia-reperfusion injury. This is somewhat surprising since HIF-1α is implicated to be major isoform in M1-type macrophages. The observed phenotype does not seem to be the result of defects in motility or invasion of macrophages. Reduced expression of TGFβ in a HIF-dependent fashion indicates that this pathway plays a role in attenuating tissue damage. Thus our data add new and relevant insights into HIF-function in renal inflammatory cells.

Funding: Government Support - Non-U.S.

FR-PO1111

Regulation and Protective Role of Autophagy in Cisplatin Nephrotoxicity: zVAD-fmk Blocks Cisplatin-Induced Cleavage of ATG5, Beclin-1, and ATG12 but Impairs Autophagic Flux *In Vitro* and *In Vivo*
Alexandrea Holmes,[2] Cheng Yang,[1] Christian Herzog,[1] Gur P. Kaushal.[1,2] *[1]UAMS, Little Rock, AR; [2]CAVHS, Little Rock, AR.*

Background: Autophagy is a process of degradation of intracellular organelles and long-lived proteins via the lysosome and plays a survival role under stress conditions. We previously reported a cytoprotective role of autophagy in cisplatin-induced cytotoxicity and the current study has determined regulation and role of autophagy in cisplatin injury.

Methods: Autophagy was identified by immunostaining and by GFP-LC3-II formation *in vitro* and *in vivo*. Cleavage of ATG5, beclin-1, and ATG12 during the course of cisplatin injury to renal epithelial cells were determined by western blot. Overexpression of autophagy was done by transfection with mCherry-ATG5 and beclin-1 plasmids. Renal function and histology was determined by the established methods. Autophagic flux was determined by LC3-II and p62 accumulation and lysosomal inhibition.

Results: Cleavage of Atg proteins began as caspase activation was initiated suggesting autophagy proteins are a target of caspases. The pan-caspase inhibitor zVAD-fmk completely prevented cisplatin-induced cleavage of Atg proteins but promoted expression of LC3-II, p62, and blocked lysosomal cathepsins and calpains, suggesting blockage of lysosomal function and impairment in autophagic flux. ZVAD-fmk did not protect from cisplatin nephrotoxicity in vivo as evident from increased levels of BUN, creatinine, and impaired histology. Overexpression of ATG5 and beclin-1 prevented cisplatin-induced cell death signifying autophagy plays a survival role. Administration of the autophagy inhibitor chloroquine enhanced severity of renal dysfunction (BUN in mg/dL Con, 18.5; 3d CP, 75.5; 3d CP+Chlq, 128.5; 3d Chlq, 13.8; Creatinine in mg/dL Con, 0.24; 3d CP, 0.285; 3d CP+Chlq, 1.1; 3d Chlq, 0.155), and impaired histology and autophagic flux.

Conclusions: We demonstrate regulation of autophagy by caspase mediated cleavage of Atg proteins and protective role in cisplatin AKI. ZVAD-fmk did not protect cisplatin-induced AKI and impaired autophagic flux both *in vitro* and *in vivo*. These studies link autophagy as a potential therapeutic target for preventing cisplatin AKI.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1112

TGF-beta Signaling in the Proximal Tubule Modulates the Response to Acute Kidney Injury
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Background: Growth factors play an important role in the proximal tubule's response to acute kidney injury (AKI), but there are conflicting reports on the effects of transforming growth factor-beta (TGF-β) on the response to acute renal injury. Therefore, we investigated how TGF-β signaling in the proximal tubule affects kidney injury and repair after AKI.

Methods: Mice lacking the TGF-beta type II receptor (TβRII) specifically in the proximal tubules (γGT-Cre;Tgfbr2$^{flox/flox}$) were generated using the Cre/lox technique. AKI was induced by mercuric chloride ($HgCl_2$) at either a low or high dose, and morphology and renal function (BUN and creatinine) were assessed. Immunoblots of tissue lysates and immunohistochemistry were used to determine inflammation, proliferation, and apoptosis after injury. To better explain these *in vivo* results, we created proximal tubule epithelial cells (PTEC) with and without TβRII *in vitro*. Apoptosis was induced *in vitro* by both H_2O_2 and detachment (polyHEMA-coated plates) and cell adhesion, migration, and transepithelial resistance (TER) were measured.

Results: The conditional knockout mice were protected from AKI at lower doses of HgCl2. At early time points, there was no difference in inflammation or proliferation, but γGT-Cre;Tgfbr2flox/flox mice had less apoptosis. In contrast, at higher levels of HgCl2, injury was equivalent in both genotypes but the conditional knockout mice also had delayed recovery. Consistent with our in vivo data, the TβRII-/- cells in vitro were protected from apoptosis but had reduced adhesion and migration on collagen IV and fibronectin.

Conclusions: These results suggest that blocking TGF-β signaling in proximal tubules may protect against milder AKI by conferring resistance to epithelial apoptosis. However, in more profound injury, inhibiting TβRII may compromise recovery of the renal epithelium by interfering with cell/matrix interactions such as adhesion and migration. In conclusion, the effect of proximal tubular TGF-β signaling on AKI may vary depending upon the severity of injury.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-PO1113

Acute Reduction in Renal Calpain 10 Causes Mitochondrial and Renal Dysfunction
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Background: Calpain 10 is a calcium-dependent cysteine protease that is ubiquitously expressed. Calpain 10 is in kidney mitochondria and NDUFB8, NDUFV2 (complex I proteins), and ATP synthase β are mitochondrial calpain 10 substrates. Our laboratory showed that renal calpain 10 decreases with age in humans, rats, mice, and in diabetic rats. Furthermore, over-expression or knockdown of calpain 10 results in cell death. Therefore, we determined the effects of acute reduction of renal calpain 10 on mitochondrial and renal function.

Methods: One IV injection of 20 nmol of calpain 10 or scamble siRNA in the tail vein of 8 week old Fischer 344 rats. Rats were sacrificed at days 2, 5 and 7 for immunoblot analysis.

Results: One injection of calpain 10 siRNA decreased mitochondrial and cytosolic calpain 10 at all time points (days 2, 5, 7) compared to controls. This decrease in calpain 10 was kidney and calpain 10 specific. On day 7, kidney dysfunction was observed with a rise in serum creatinine from 0.8 mg/dL to 1.8 mg/dL. We hypothesized that loss of mitochondrial calpain 10 would cause calpain 10 substrates to accumulate and induce mitochondrial dysfunction. At all time points, ATP Synthase β and NDUFB8 protein levels in rats treated with calpain 10 siRNA increased 2.5-fold in the kidney cortex. In calpain 10 siRNA-treated animals, Mitofusin 2 was decreased at all time points, suggesting decreased mitochondrial fusion. Drp1, a mitochondrial fission protein that is associated with mitophagy and apoptosis, increased in the calpain 10 siRNA-treated animals on day 5. We analyzed markers of mitophagy, the process of removing damaged mitochondria, PINK1 and LC3-II. At days 2 and 5, both PINK1 and LC3-II increased, suggesting mitophagy is occurring. Cleaved caspase 3 was detected at days 5 and 7, providing evidence that calpain 10 is required for cell viability.

Conclusions: These results support our hypothesis that the loss of renal calpain 10 causes renal dysfunction sequentially through 1) the accumulation of mitochondrial calpain 10 substrates, 2) decreased mitochondria fusion and increased mitophagy, 3) increased mitochondrial fission, and 4) apoptosis.

Funding: Other NIH Support - NIH - Environmental Health Sciences Training Program in Environmental Stress Signaling (T32 ES012878), Veterans Administration Support

FR-PO1114

Role of Mitofusin 2 in the Renal Stress Response
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Background: Mitochondria are dynamic organelles that undergo constant remodeling, essential to their function as regulators of cell survival. Although mitofusin 2 (MFN2) is critical for mitochondrial morphology and function, its role in the renal stress response is unknown.

Methods: To delete MFN2 in vitro, primary cultures of proximal tubular cells from MFN2 floxed mice (MFN2f/f) mice were treated with Cre-expressing adenovirus or empty adenoviral vector (control). Mitochondrial morphology, outer mitochondrial membrane integrity, Bax activation (6A7 epitope exposure), oxygen consumption, and cell survival were evaluated under resting conditions and following ATP depletion. To conditionally knockout MFN2 (MFN2 cKO) in renal cells in vivo, MFN2f/f mice were crossed with those expressing Cre-recombinase under the control of the Pax2 promoter. Renal histology and organ function were assessed in MFN2 cKO pups and control littermates.

Results: MFN2-deficiency caused profound mitochondrial fragmentation but did not alter baseline oxygen consumption or survival of proximal tubule cells in culture at rest. In contrast, after ATP depletion, proximal tubular cells lacking MFN2 had more mitochondrial outer membrane injury and an 80% increase in apoptosis compared to MFN2-expressing control. Bax activation was comparable in cells that did or did not express MFN2, although MFN2 deficiency significantly increased mitochondrial Bax accumulation and enhanced release of both apoptosis inducing factor and cytochrome c, pro-apoptotic markers of

membrane injury. Conditional MFN2 knockout in vivo also caused severe mitochondrial fragmentation in renal epithelial cells but did not affect renal histology or organ function in newborn mice.

Conclusions: MFN2 is critical for renal epithelial cell survival after ATP depletion but is not required under resting conditions or during renal organogenesis. MFN2 deficiency decreases survival after stress by increasing Bax-mediated mitochondrial outer membrane injury and apoptosis, a primary cause of acute ischemic renal failure.

Funding: NIDDK Support

FR-PO1115

Tubule-Specific Ablation of Endogenous β-catenin Aggravates Acute Kidney Injury in Mice Dong Zhou,[1] Yingjian Li,[1] Peter Igarashi,[2] Youhua Liu.[1] *[1]Department of Pathology, University of Pittsburgh, PA; [2]Department of Medicine, University of Texas Southwestern Medical Center, Dallas, TX.*

Background: β-catenin is a unique intracellular protein that plays dual functions: as an integral component of the cell adherens junction complex, and as a principal signaling protein that mediates the canonical Wnt signaling. Inappropriate activation of β-catenin occurs predominantly in renal tubular epithelium, and is implicated in the pathogenesis of a variety of progressive chronic kidney disorders, such as diabetic and obstructive nephropathies. However, little is known about its function in normal physiologic state as well as after acute kidney injury (AKI). To explore this issue, we generated conditional knockout mice in which β-catenin gene is specifically ablated in renal tubules (designated as Ksp-β-cat-/-), particularly in the distal tubules and collecting duct epithelia by mating β-catenin floxed mice with Ksp-Cre transgenic mice. Ksp-β-cat-/- mice were phenotypically normal, without any appreciable defect in kidney morphology and function, suggesting that β-catenin is dispensable for renal tissue homeostasis. However, we found that tubule-specific ablation of endogenous β-catenin aggravated acute kidney injury induced by folic acid. Comparing with controls, Ksp-β-cat-/- mice displayed a higher serum creatinine level and more severe morphologic injury at 2 days after folic acid injection. Similarly, more apoptotic cells were detected in Ksp-β-cat-/- kidneys, which was accompanied by an increased Bax protein and decreased survivin mRNA expression in the kidneys. *In vitro*, activation of β-catenin via transfection of either Wnt1 or constitutively active β-catenin expression vectors protected human proximal tubular epithelial cells (HKC-8) from apoptosis induced by staurosporine. Consistently, activation of β-catenin also induced survivin and repressed Bax expression *in vitro*. These results suggest that endogenous β-catenin in renal tubules is pivotal for renal protection after acute kidney injury primarily through activating cell survival signaling.

Funding: NIDDK Support

FR-PO1116

Two Independent Pathways, BNIP3 and Sestrin2, Mediate Autophagy of Renal Tubular Cells in Acute Kidney Injury In Vitro and In Vivo Masayuki Ishihara,[1] Masayuki Bun,[2] Masayuki Hisa,[2] Kazu Hamada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Toru Kagawa,[1] Toshihiro Takao,[1] Yoshio Terada.[1] *[1]Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan; [2]Center for Innovative and Translational Medicine, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan.*

Background: Autophagy is one of the systems which protect life from many kinds of stresses. In the previous study we reported autophagy occurred in acute kidney injury (AKI) mice model. Autophagy is thought to play a protective role in renal tubules from stresses. However, little is known about signal transduction for autophagy in AKI. Bcl-2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3) is one of the target proteins of hypoxia inducible factor -1a (HIF-1a). Sestrin2 is one of the target proteins of p53. The aim of this study is to reveal the roles of BNIP3 and sestrin2 in autophagic pathway in AKI.

Methods: We used rat ischemia/reperfusion (I/R) AKI model in vivo and cultured renal tubular cells as an in vitro AKI model. The expression of BNIP3 and sestrin2 are up-regulated after I/R in proximal tubules in immunostaining and immunoblotting. BNIP3 mRNA and protein expressions were up-regulated in the hypoxia condition via HIF-1a dependently in vivo. Sestrin2 mRNA and protein expressions were up-regulated in the oxidative stress condition (H2O2) via p53 dependently. To examine BNIP3 and sestrin2 regulate autophagy or not, we established NRK cells which stably transfected with a fusion protein between green fluorescent protein and light chain 3 (LC3-GFP) as a marker of autophagy. Overexpression of BNIP3 and sestrin2 both induced autophagy in NRK-LC3-GFP cells and induced LC3 protein expression.

Results: The autophagosome induced by BNIP3 localized in mitochondria. Induction of autophagy by hypoxia was reduced by sestrin2 siRNA. Induction of autophagy by H2O2 was reduced by BNIP3 siRNA.

Conclusions: These observations disclose that autophagy in renal tubules in AKI is induced by at least two independent pathways, p53-sestrin2 pathway and HIF-1a-BNIP3 pathway. These two pathways may work differently according to the types of stresses to protect renal tubules in AKI.

FR-PO1117

Proximal Tubular Cells (PTCs) from AMP-Activated Protein Kinase (AMPK) Knock-Out (KO) Mice Are More Susceptible to Metabolic Stress Than Their Wild-Type WT Controls Wilfred Lieberthal,[1] Meiyi Tang,[1] Vimal Patel,[2] Jerrold S. Levine.[2] *[1]Medicine, Stony Brook Medical Center, Stony Brook, NY; [2]Medicine, University of Chicago, IL.*

Background: We have previously shown that inhibiting AMPK in PTCs from WT mice increases their susceptibility to apoptosis. There are two isoforms of the catalytic domain of AMPK, α1 and α2. In this study we examined the susceptibility to metabolic stress of AMPK KO mice that lack the α1 or α2 isoform of the catalytic domain.

Methods: Primary cultures of PTCs from WT and KO mice were incubated in dextose (6mM, 5mM or 4mM), without or with 2μM antimycin A (anti). Apoptosis, quantified by FACS analysis, was expressed as a % of the total number of cells. In PTCs that were not subjected to metabolic stress, gene expression was determined by real-time PCR and expressed in KO PTCs as a % of WT.

Results: Apoptosis was comparable in α1 KO and WT PTCs incubated in 6mM dextrose in the presence (5.4±2.1%) vs the absence of anti (10.0±3.2%). However, there was more apoptosis in α1 KO vs WT PTCs incubated in 5mM dextrose in the presence (18.2±3.2%) vs absence of anti (3.2±1.2%)($p<0.01$). In 4mM dextrose, the amount of apoptosis was even greater in α1 KO vs WT PTCs in the presence (33.2±.6%) vs absence (3.2±1.9%) of anti ($p<0.001$). In α2 KO and WT PTCs apopotosis was at control levels (~5%) in 6 and 5mM dextrose with and without anti. However, at 4mM dextrose, apoptosis was increased in α2 KO vs WT PTCs in the presence (21±2.1%) vs absence (3.2±1.3) of anti ($p<0.02$). We also found that the expression of genes encoding PPARγ coactivator-1α(PGC-1α) and cytochrome B was reduced to a comparable extent (by ~55%) in α1 and α2 KO mice, while the expression of cytochrome C was reduced to a greater extent in α1 vs α2 KO PTCs (by 42±7% vs 62±8% respectively)($p<0.02$).

Conclusions: Conclusions: i) AMPK KO mice are more susceptible than WT mice to apoptosis induced by metabolic stress; ii) α1 KO PTCs are more sensitive than α2 PTCs to the same metabolic stress; iii) Differences in the reduction in gene transcription between α1 and α2 KO mice may contribute to the differences in sensitivity of the α1 and α2 KO PTCs to metabolic stress.

Funding: Veterans Administration Support

FR-PO1118

Sexual Dimorphism of ER Stress-Induced Kidney Injury Hossam Mustafa, Adel Tarcsafalvi, Judit Megyesi, Rawad Hodeify, Peter M. Price. *Medicine, UAMS, Little Rock, AR.*

Background: Gender differences can affect the severity of AKI; ischemia-reperfusion AKI is more severe in males whereas cisplatin AKI is more severe in females. Sex hormones are thought to play a key role in these gender-related differences in susceptibility to kidney injury. In this study we observed differences in the severity of kidney injury between male and female mice (strain 129/Sv) in response to tunicamycin, an ER stress agent.

Methods: Tunicamycin was injected subcutaneously into mice, and kidney function, morphology, and proteins were assessed after 1, 3, and 5 days. In addition, some female mice were implanted with testosterone (5 mg/pellet, 21 day release) and some male mice with estrogen (β-estradiol 17-acetate, 5 mg/pellet, 21 day release).

Results: The ER-induced decline in kidney function was most severe five days after tunicamycin injection and was greater in male mice than in female mice. At this time, concentrations of BUN and creatinine in serum of male mice were 82.9 and 1.37 mg/100 ml, respectively, while in female mice were 18.5 and 0.4, which were not significantly different than levels in untreated controls. Similarly, protein markers of ER stress were also significantly more elevated in male mice. Specifically, levels of phosphorylated eukaryotic initiation factor-2α (phospho-eIF-2α), glucose-regulated protein (GRP-78) and CAAT/enhancer binding protein homologous protein (CHOP) were higher in tunicamycin-treated male mice. Testosterone administered to female mice by pellet implantation 2 weeks before tunicamycin resulted in a phenotype similar to male mice with a comparable decline in renal function and induction of ER stress markers. In contrast to testosterone, estrogen administered to male mice was not protective against tunicamycin-induced kidney injury.

Conclusions: We conclude that in response to ER stress, male mice are more vulnerable and have substantially greater acute kidney injury than female mice. The presence of testosterone, rather than estrogen-mediated protection, was responsible for this gender difference. ER stress contributes to AKI arising from several different insults and gender differences in the tolerance to this stress could play a significant role in induction of AKI.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1119

Peroxisomal Contribution to LPS Kidney Injury Radovan Vasko, Brian B. Ratliff, Ellen Nadel, Jun Chen, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

Background: Peroxisomes (PER) are ubiquitous organelles involved in metabolism of ROS through their generation and detoxification. The role of PER in inflammatory response of AKI remains obscure, although their activation seems to be protective. Our previous metabolomic study of ischemic AKI revealed accumulation of PER markers, suggestive of increased biogenesis of PER.

Methods: Here, we investigated the role of PER in LPS-mediated AKI. Sublethal dose of LPS in C57BL mice (wild-type, WT) did not lead to increased serum creatinine (SCr). However, in C57BL/6J-Lyst mice (model of defective lysosomal autophagy/pexophagy), LPS resulted in a significant increase in SCr and urinary albumin (24h; SCr 0.48+0.07 mg/dl, UACR 193+33 mg/g) compared to WT mice (SCr 0.23+0.03 mg/dl mg/dl, UACR 51+12 mg/g).

Results: Immunoblot analyses of kidney homogenates demonstrated significantly higher increases of PMP70 (a major PER membrane protein) and catalase (a marker for the PER matrix) in WT compared with C57BL/6J-Lyst mice after LPS treatment. Ex vivo, time course studies in HUVEC revealed a biphasic profile of PMP70 expression under LPS: an initial decline, followed by an elevation after 9h. PMP70 normalized after 24h and further increased compared to control cells without LPS stimulation. Expression profile of PMP70 under LPS was paralleled by catalase and p62, protein known to interact with ubiquitin and trigger autophagy. Activation of autophagy decreases the levels of p62. In our in vivo studies, PMP70 levels in autophagy-deficient C57BL/6J-Lys mice were decreased compared to WT, suggesting concomitantly impaired PER biogenesis by LPS. Peroxin14 (Pex14), a PER membrane docking protein that interacts with PER targeting sequence (PTS) containing proteins, showed down-regulation in LPS-treated HUVEC, suggesting impairment of PER assembly due to altered protein import. Fluorescence microscopy of HUVEC transfected with PTS-GFP demonstrated decreased colocalization of PTS with PER under LPS.

Conclusions: 1) LPS exerts a biphasic effect on PER: initial depletion followed by accumulation; and 2) both the impaired biogenesis and autophagic degradation of PER (pexophagy) seems to contribute to aggravation of AKI.

Funding: NIDDK Support

FR-PO1120

Roles of mTOR Pathway on Autophagy in the Proximal Tubular Epithelial Cells of Rat Kidneys

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Background: The mammalian target of rapamycin (mTOR) pathway plays important roles in several kidney diseases, including ischemia-reperfusion (IR) injury, chronic renal failure, diabetic nephropathy and polycystic kidney disease. Recently, we showed activation of the mTOR pathway in proximal tubular cells of rat kidneys after subtotally nephrectomy (Nx) (Nakagawa et al., Biochem Pharmacol, 79, 67-76, 2010; Nishihara et al., Am J Physiol Renal Physiol, 298, F923-F934, 2010). Although recent studies proposed that mTOR regulated autophagy through interaction with UNC51-like kinase 1 (Ulk1) in vitro, the regulatory mechanisms of autophagy via interaction between mTOR and Ulk1 in vivo were not clear. In this study, we aimed to clarify roles of the mTOR pathway on autophagy in the renal proximal tubules.

Methods: The amounts of phosphatidylethanolamine-conjugated form of microtubule associated protein 1 light chain 3 (LC3-II), a marker for activity of autophagy, in rat kidneys were examined after Nx, IR and cisplatin treatment.

Results: Immunofluorescent analysis showed that the positive signals for mTOR, Ulk1 and LC3 were detected in the proximal tubular epithelial cells of rat kidneys, and immunoprecipitation revealed the direct interaction between mTOR and Ulk1 in the rat kidneys. The levels of LC3-II in rat kidneys were significantly decreased to 16%, 27% and 52% compared to control rats after Nx, IR and cisplatin treatment, respectively. On the other hands, phosphorylated ribosomal protein S6 (a marker for activation of the mTOR pathway) was significantly increased to 283%, 138% and 152% compared to control rats after Nx, IR and cisplatin treatment, respectively. In addition, the levels of LC3-II in the rat kidneys after Nx, IR and cisplatin treatment were significantly increased to 517%, 147% and 185% as compared to vehicle treated rats by treatment with mTOR inhibitor everolimus.

Conclusions: Activation of the mTOR pathway in proximal tubular epithelial cells caused suppression of autophagy during kidney injury by direct interaction with Ulk1.

Funding: Government Support - Non-U.S.

FR-PO1121

Extracellular Signal-Related Kinase Inhibition Prevents Autophagy Induction and Sensitizes Proximal Tubular Cells to LPS-Induced Apoptosis

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Background: Sepsis-induced acute kidney injury (AKI) is a common and destructive complication encountered in hospitalized patients. Clinical studies have highlighted the negative association between sepsis-induced AKI and short-term and long-term renal function and mortality outcomes. As of yet, there are no approved therapies to treat or prevent sepsis-induced AKI. Autophagy is a complex intracellular degradation mechanism that endows cells with the ability to maintain homeostasis after exposure to stress-inducing stimuli, such as bacterial lipopolysaccharide. In these studies we investigated the autophagic response to bacterial lipopolysaccharide (LPS), the cytoprotective function of autophagy, and the signaling pathways required for autophagy induction.

Methods: Autophagy was evaluated by western blot for LC3II and quantification of fluorescent punctae in GFP-LC3 transfected proximal tubular epithelial cells (PTEC). MAPK inhibitor U0126 was used to determine the effect of Extracellular signal-related kinase (ERK1,2) on autophagy and cell survival in LPS exposed PTEC.

Results: Autophagy induction was demonstrated in LPS exposed PTEC by increased LC3II accumulation and a significantly higher quantity of GFP-LC3 punctae compared to control treated cells. Knockdown of Beclin-1, an essential part of the autophagy machinery, sensitized cells to LPS-induced apoptosis as evidenced by increased PARP-1 cleavage. Incubation of PTEC with the ERK-inhibitor U0126 inhibited the autophagic response to LPS as evidenced by decreased Beclin-1 induction and LC3II accumulation. Moreover, U0126 increased apoptosis in LPS-treated PTEC.

Conclusions: ERK-dependent autophagy induction is a counter-regulatory mechanism preventing apoptosis after LPS exposure and may therefore be an important protective mechanism against sepsis-induced AKI.

Funding: Other NIH Support - T32 DK007757-12(Leventhal); R01 DK078510(Ross)

FR-PO1122

Sensitivity of Diabetic Mice and High Glucose-Conditioned Renal Tubular Cells to Acute Injury Induced by Ischemia-Reperfusion and ATP Depletion

Jianping Peng, Zheng Dong. *Georgia Health Sciences University.*

Background: Acute kidney injury (AKI) leads to a worse outcome and prognosis in the patients with existing chronic kidney diseases including diabetic nephropathy. We hypothesize that kidney cells and tissues are more sensitive to injury under diabetic condition.

Methods: We tested this possibility by examining kidney cell and tissue injury in hyperglycemic and diabetic conditions.

Results: We first observed that significantly higher kidney injury and mortality were induced by renal ischemia-reperfusion in streptozotocin-induced diabetic mice. Consistently, renal ischemia induced more severe kidney injury in diabetic Akita mice than wild-type mice. In addition, ischemic kidney injury in these diabetic models showed a correlation with their blood glucose levels. To understand the mechanism of the injury sensitivity, we examined that the effect of high glucose on ATP-depletion induced apoptosis in cultured renal proximal tubular cells. The cells were cultured for two weeks in media containing 5.5 mM glucose, 30 mM glucose, or 30 mM mannitol, followed by ATP-depletion with anoxia or azide. ATP-depletion induced significantly higher apoptosis in high glucose-conditioned cells (60%) than the cells cultured with 5.5 mM glucose or 30 mM mannitol (15%). Consistently, caspase activity was significantly higher in high glucose-conditioned cells. During ATP-depletion, high glucose-conditioned cells also showed an earlier and higher Bax translocation and cytochrome c release.

Conclusions: Taken together, these results suggest that high glucose or hyperglycemia may sensitize renal tubular cells to mitochondrial or the intrinsic pathway of apoptosis, resulting in heightened kidney injury.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1123

Murine Double Minute-2 Links Inflammation and Tubular Healing in Acute Kidney Injury of Mice

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Background: The E3 ubiquitin ligase murine double minute (MDM)-2 promotes cancer cell survival and growth, by degrading p53, hence, MDM2 inhibition with nutlins emerges as novel cancer therapy. To test whether MDM2 also promotes regenerative cell growth we examined the effects of nutlin-3a during postischemic kidney injury (AKI).

Methods: We used bilateral or unilateral renal artery clamping (45 min.) in C57BL/6 wildtype and p53-deficient C57BL/6 mice. Mice were injected with nutlin-3a or vehicle on days -1, +1, +2 and +3. Renal pathological evaluation was performed at day 1 and 5 after IRI (immunohistochemistry, qPCR, western blot). For in vitro studies we stimulated p53-MDM2 double-deficient or p53 single-deficient mouse embryonic fibroblasts (MEFs) with LPS and analyzed them with ELISA, western blot and electrophoretic mobility shift assay.

Results: When nutlin-3a was injected 1 day before renal artery clamping it significantly reduced necrosis and apoptosis of tubular cells, neutrophil infiltration, and mRNA expression of IL-6, TNF-α, CXCL2, CCL2 at 1 day. This effect was identical in wildtype or p53-deficient mice documenting a p53-independent effect of MDM2. In-vitro experiments confirmed that MDM2 is required to induce the mRNA expression and secretion of NFκB-dependent cytokines upon Toll-like receptor stimulation by enhancing NFκB binding to its cytokine promoter binding sites. Although nutlin-3a protected from acute tubular necrosis, tubular damage was aggravated at day 5 in nutlin-treated mice as compared to controls. Nutlin treatment was associated with more TUNEL+ tubular cells but this effect was absent in p53-deficient mice which suggests that MDM2-mediated inhibition of p53 is required to allow tubular repair after AKI.

Conclusions: Together, MDM2's dual role in AKI encompasses NFκB binding to cytokine promoters, a p53-independent proinflammatory effect, as well as p53 inhibition for regenerative cell growth. We conclude that, MDM2 links inflammation and epithelial healing during AKI, two additional biological functions that need to be considered when inhibiting MDM2 for therapy.

FR-PO1124

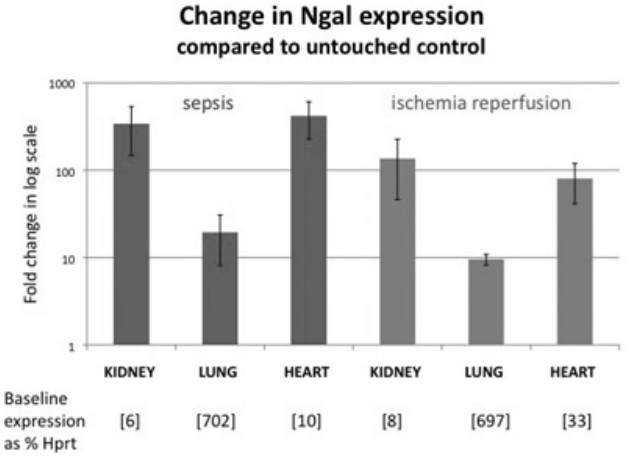
Acute Kidney Injury and Sepsis Independently Induce a Multi-Organ Stereotyped Inflammatory Response

Barbara Pedrycz, Catharine Compston, Valerie A. Luyckx, Thomas F. Mueller. *University of Alberta.*

Background: Acute kidney injury (AKI) and sepsis are independently associated with high mortality and frequently co-exist in critically ill patients who develop multi-organ dysfunction. Animal models have shown that AKI and sepsis are associated with distant organ inflammation. We compared pathways of inflammation and injury associated with AKI and sepsis in multiple organs, to determine whether local injury induces a stereotyped or situation-specific inflammatory response in distant organs.

Methods: 3 month old male C57BL/6 male mice underwent unilateral renal ischemia-reperfusion (IR) or cecal ligation and puncture (CLP) as models of AKI and sepsis respectively. Transcript levels of the acute phase response (APR) genes Osm, Osmr, Il6, Il6r, Lif, Lifr, Serpina3, and Ngal were measured at 24 hours by real-time RT-PCR in hearts, lungs and non-clamped kidneys of control (n=7), IR (n=3) and CLP (n=5) mice. Gene expression is represented as fold change over control and % HPRT.

Results: A similar qualitative inflammatory response was observed in distant organs after IR and CLP for all genes analyzed. Fold change of Ngal served as a general injury/leucocyte infiltration marker. Distant organ expression patterns were similar in CLP and IR mice (Figure 1).



Quantitatively, the degree of injury/inflammation was higher in CLP compared to IR mice. In hearts, kidneys and lungs respectively, Ngal expression increased by 417, 340 and 19 fold in CLP mice, compared to 33, 340 and 9 fold in IR mice. Baseline expression was consistently higher in the lung for all the APR genes, followed by heart and then kidney.

Conclusions: The injury-repair response is highly stereotyped across remote organs regardless of initiating event. Distant organ inflammation is therefore likely a significant mediator of multi-organ dysfunction contributing to the high morbidity and mortality in AKI and sepsis

Funding: Private Foundation Support, Clinical Revenue Support, Government Support - Non-U.S.

FR-PO1125

Endoplasmic Reticulum Stress Links Inflammatory and Fibrogenic Responses To Induce Kidney Fibrosis after Acute Injury Gang Li, Jianhua Huang, Yordanka Ivanova, Anna Zuk. *Genzyme Corporation, Framingham, MA.*

Background: Acute kidney injury (AKI)-induced aberrant repair has been implicated in the progression of chronic kidney disease (CKD), yet the mechanisms linking AKI to CKD are incompletely understood. Here, we examined the unfolded protein response (UPR) following AKI and hypothesized that activation of the maladaptive arm of UPR together with insufficient adaptive UPR induces inflammation and tubulo-interstitial fibrosis post AKI. In a mouse model of bilateral renal ischemia-reperfusion, the UPR is markedly induced post-reperfusion and correlates with renal dysfunction, tubular apoptosis, and inflammation. In contrast to the sustained induction of the adaptive UPR in young mice (8-10 wks), measured by stimulation of IRE1 and ATF6, aged mice (48-50wks) display only transient induction of these pathways. Additionally, in aged mice, there is substantially greater induction of CHOP and its downstream target GADD34, which associates with increased mortality and more severe renal injury. Importantly, CHOP induction is sustained, correlating with greater kidney fibrosis and inflammation 6wks post-reperfusion. To understand how epithelial cells contribute to fibrosis in a setting of AKI, an ATP depletion-repletion model of normal rat kidney epithelial cells was developed to recapitulate ischemia-reperfusion in vivo. Sustained CHOP induction together with transient induction of ATF6 is observed during repletion and is associated with induction of inflammatory (*Mcp-1, Il6,* and *Il8*) and fibrogenic genes (*Ctgf* and *Fgf*). Most importantly, induction of these genes is significantly inhibited when CHOP induction is blocked by siRNA, providing direct causal evidence of CHOP as a mediator of inflammatory/fibrogenic responses. In addition, a specific inhibitor of dsRNA-dependent protein kinase (PKR) substantially suppresses the induction of inflammatory/fibrogenic genes through inhibition of CHOP expression. These data provide new insight into how ER stress in general, and the CHOP branch in particular, contributes to kidney fibrosis and suggest that interventions targeting the CHOP branch of UPR may provide new opportunities to prevent kidney fibrosis.

Funding: Pharmaceutical Company Support

FR-PO1126

Suberoylanilide Hydroxamic Acid (SAHA) Suppresses the Progression of Renal Fibrosis in Aristolochic Acid Induced Renal Fibrosis Model in Mice Mineaki Kitamura,[1] Tomoya Nishino,[1] Kumiko Io,[1] Yoko Obata,[1] Yoshitaka Hishikawa,[2] Takehiko Koji,[2] Shigeru Kohno.[1] *[1]Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan; [2]Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.*

Background: The epigenetic modulation of genes is known to be a key mechanism for the control of gene expression and plays an important role in the progression of fibrosis. Among epigenetic mechanisms, histone acetylation is regulated by histone deacetylases (HDACs) and it leads to the transcriptional activation of genes. In the present study, we investigated the involvement of histone acetylation in the renal fibrosis induced by aristolochic acid (AA), and the effect of suberoylanilide hydroxamic acid (SAHA), which is a HDAC inhibitor on fibrotic lesions.

Methods: Male Balb/c mice were divided into three groups, AA, AA+SAHA, and control group. Renal fibrosis was induced by AA injection. SAHA or saline were injected subcutaneously for 3 weeks. The expression of type IV collagen, acetylated histone and HDAC2 were examined by immunohistochemistry. TUNEL staining was performed to evaluate apoptosis. Serum creatinine and urinary protein were analyzed to evaluate renal function.

Results: The expression of type IV collagen was significantly increased compared to control group and it was significantly attenuated by SAHA treatment. The number of histone acetylated cells was also increased in AA group, while there was no significant difference between AA and AA+SAHA groups. The number of HDAC2-positive and TUNEL-positive cells was significantly increased in AA group in comparison to control group. Of note, SAHA treatment decreased the number of them and inhibited the progression of renal dysfunction.

Conclusions: These results suggested that histone acetylation was involved in the progression of renal fibrosis and SAHA could attenuate renal fibrosis and renal function. The antifibrotic effect of SAHA might exert the suppression of apoptosis via HDAC inhibition. SAHA might be one of the candidates for a novel therapeutic agent in preventing renal fibrosis.

FR-PO1127

The HDAC-Dependent Suppression of Bmp-7 Transcription Countributes to the Dysregulation of the Innate Repair Mechanisms of the Kidney Following Prolonged Urinary Tract Obstruction Scott R. Manson, Paul F. Austin. *Surgery, Washington University, Saint Louis, MO.*

Background: While BMP-7 inhibits the pathogenesis of renal injury in a variety of disorders of the kidneys and urinary tract, little is known about the regulation of endogenous BMP-7 and its renal protective functions. This study examines the molecular regulation of BMP-7 in obstructive uropathies and the role which it plays in the repair of obstruction-induced renal injuries.

Methods: A reversible model of unilateral ureteral obstruction (UUO) was used to characterize the repair of the kidney following the correction of short-term obstructions that led to reversible renal injury and prolonged obstructions that led to irreversible renal injury. The role of BMP-7 was assessed by treatment with anti-BMP-7 or exogenous BMP-7.

Results: BMP-7 activity is required for the restoration of renal architecture and the resolution of fibrosis following obstruction-induced renal injury. However, prolonged UUO leads to the loss of BMP-7 expression and irreversible renal injury. Importantly, the restoration of BMP-7 activity through treatment with exogenous BMP-7 enhances kidney repair following prolonged UUO. In examining the mechanisms that lead to the loss of BMP-7, we found that UUO results in histone deacetylation in the Bmp-7 promoter and the subsequent repression of its transcriptional activity. Treatment with the histone deacetylase (HDAC) inhibitor trichostatin A during UUO stimulates the expression of BMP-7, the activation of its downstream signaling pathways, and the BMP-7-mediated suppression of TGF-β-dependent pro-fibrotic pathways. Finally, HDAC inhibition enhances kidney repair in obstructive uropathies that typically lead to irreversible injury.

Conclusions: BMP-7 plays a critical role in the repair of acute obstruction-induced renal injuries. However, prolonged obstruction leads to the dysregulation of kidney repair, in part, through the HDAC-dependent suppression of *Bmp-7* transcription. Importantly, HDAC inhibition restores BMP-7 expression and stimulates the innate repair mechanisms of the kidney during the treatment of obstructive uropathies.

Funding: Private Foundation Support

FR-PO1128

A Novel Method To Remove Iron from the Body by Urinary Excretion Andong Qiu,[1] Neal A. Paragas,[1] Roland Strong,[2] Jonathan M. Barasch.[1] *[1]Columbia; [2]Fred Hutchinson.*

Background: There are two types of iron overload disorders, hereditary (HH) and acquired hemochromatosis (AH). HH is caused by loss of function of genes that regulate iron metabolism, and AH is caused by blood transfusions and dietary overload. Since each RBC unit contains 250 mg of iron, rapid clearance of 25% of even a single unit delivers a bolus 10X the daily requirement for iron , resulting in biochemical evidence of toxicity. We sought novel methods to remove iron from the body with low-toxicity and high efficacy.

Methods: NGAL (Siderocalin, Lipocalin 2) is a carrier that can transport iron bound to a catecholate cofactor. The complex (25KDa) of NGAL:catecholate:Fe is filtered by the kidney, and then reabsorbed by the proximal tubule. Consequently, to safely remove iron

from the body (1) iron must be bound in a redox neutral fashion, (2) NGAL-catecholate-iron complex must not be reabsorbed by the proximal tubule, and (3) iron must be bound in a pH insensitive fashion, so that the complex does not dissociate in acidic urine.

Results: We have solved each problem by modifying a number of surface residues of NGAL-Siderocalin based on structural data. The mutant, called NGAL* was competent to bind the bacterial catecholate siderophore, enterochelin, producing a bright red coloration typical of the wild type complex. The complex NGAL*:enterochelin:iron was inactive in assays that measured the conversion of FeIII to FeII, an initiator of the Fenton reaction, and the complex was inactive in radical activation of fluorescein precursors. NGAL* failed to release iron even at pH4.0 consistent with prior studies of enterochelin stability. Finally, unlike the wild type NGAL-Siderocalin, the complex was found in the urine containing iron captured from the body.

Conclusions: Serum NGAL-Siderocalin (sNGAL) is cleared and metabolized by the kidney, while urine NGAL-Siderocalin (uNGAL) derives principally from cells in the distal nephron. However injection of NGAL* in the serum can cross into the urinary compartment by escaping reabsorption. Consequently, we suggest that NGAL* is a tool to treat iron overload syndromes typical of genetic diseases and multiple blood transfusions by safely clearing iron into the urine.

Funding: NIDDK Support

FR-PO1129

Apelin Ameliorates Renal Ischemia/Reperfusion Injury in Rats Mehmet Koc,[1] Zarife Ozdemir,[1] Naziye Ozkan,[2] Sule Cetinel,[2] Berrak Yegen.[1] *[1]Department of Physiology, Marmara University, Medical Faculty; [2]Department of Histology, Marmara University, Medical Faculty, Istanbul, Turkey.*

Background: Apelin is a peptide identified as the endogenous ligand of the human orphan G-protein-coupled receptor. It is highly expressed in the cardiovascular system and shows significant positive inotropic action and causes endothelium and nitric oxide-dependent vasodilatation. Recently, apelin was reported to protect myocardium against ischemia-reperfusion (I/R) injury by preventing the generation of free radicals. The potential protective effects of apelin in renal I/R injury were elucidated in this study.

Methods: Under ketamine anesthesia (100 mg/kg intraperitoneally, ip) male Sprague Dawley rats underwent right nephrectomy and I/R was induced by placing a microvascular clamp across left renal artery for 60 minutes (n=17), while the control group had sham operation with no clamp placement (n=8). Immediately after clamp placements, the rats were injected ip with either saline or apelin (50 μg/kg). Following a 24-h reperfusion period, the rats were decapitated to obtain serum and kidney tissue samples for the assessment of histopathological changes and the determination of malondialdehyde (MDA, an end product of lipid peroxidation), glutathione (GSH, a key antioxidant) levels and myeloperoxidase (MPO) activity, an index of tissue neutrophil infiltration.

Results: In saline-treated I/R group, serum creatinine and BUN levels were increased significantly as compared to sham-operated group, while apelin prevented I/R-induced elevations in creatinine and BUN ($p<0.05$). Renal MDA level in the saline-treated group was significantly increased ($p<0.05$), while apelin administration prevented I/R-induced increase in MDA level. Neither MPO activities nor GSH levels were significantly different among experimental groups. Histological analysis revealed severe renal injury in the saline-treated rats, while given scores in the apelin-treated I/R group were significantly reduced ($p<0.05$).

Conclusions: The present data demonstrate that apelin ameliorates renal I/R-injury by delimitating oxidative stress. Apelin merits further investigation for its supportive use in post-ischemic renal injury.

FR-PO1130

THR-184, Novel Peptide Agonist of BMP (Bone Morphogenetic Protein) Signaling, Is Efficacious in Preventing Ischemia-Induced AKI in Normal and CKD Rats Silvia B. Campos-Bilderback,[1] Ruben M. Sandoval,[1] Exing Wang,[1] Dattatreyamurty Bosukonda,[2] Peter C. Keck,[2] William Carlson,[2] Bruce A. Molitoris.[1] *[1]Medicine/Div of Nephrology, Indiana Univ. School of Medicine, Indianapolis, IN; [2]Thrasos Innovation Inc, Montreal, QC, Canada.*

Background: BMP 7 is known to reduce ischemic, nephrotoxic and obstructive acute kidney injury (AKI) in preclinical models. However, BMP is a potent bone and cartilage inducer in vivo, and these effects limit its therapeutic use in kidney diseases. To alleviate this side effect novel peptide agonists of the BMP pathway devoid of osteogenic activities have been developed. These peptides exert their beneficial effects in kidney by exerting anti-inflammatory and anti-apoptotic effects in renal proximal tubule cells.

Methods: THR-184 was studied in ischemia reperfusion studies in normal and chronic kidney disease (CKD) rats. THR-184 administered via intravenous infusion or bolus injection 30 min prior to or 30 min post injury to rats undergoing a 40-minute unilateral clamp, with contralateral nephrectomy.

Results: These studies resulted in a dose response with maximal reduction in serum creatinine (SCr) of greater than 50% untreated ischemic controls. IV infusion of THR-184 over 60 min starting thirty minutes post ischemic injury reduced SCr from 4.4 mg/dl ± 0.21 to 3.25 ± 0.17 ($P\leq0.01$) at 24 hours and 3.7 ± 0.15 to 2.5 ± 0.16 ($P\leq0.01$) at 48 hours. Ischemia reduced baseline GFR from 0.77 ± 0.02 ml/min/100g body wt to 0.01 ± 001 and 0.02± 0.01at 24 and 48 hr, respectively. THR-184 resulted in 6 and 5 fold increases in GFR at 24 and 48 hrs, respectively. At 5 days post ischemia THR-184 increased GFR to 0.22 +/-0.11 ml/ml/100gm body wt versus 0.09 +/-0.08 versus untreated ischemic rats ($P\leq0.01$). Finally, in rats with pre-existing ischemia-induced CKD and baseline SCr of 1.0 mg/dl, 30 minutes of clamp ischemia increased SCr to 4.6 mg/dl at 24 hrs and 3.1 at 48 hrs, while post injury treatment with THR-184 minimized the increase in SCr to 2.5 and 2.1 at 24 and 48 hrs, respectively.

Conclusions: These data indicate that THR-184, a peptide agonist of BMP signaling, is efficacious in preventing ischemia-induced AKI in normal and CKD rats.

Funding: Pharmaceutical Company Support

FR-PO1131

Novel Xanthine Oxidoreductase Inhibitor, Febuxostat, Protects Rat Kidney from Renal Ischemia-Reperfusion Injury Yoshitaka Isaka,[1] Hidetoshi Tsuda,[2] Noritaka Kawada,[1] Hirotsugu Iwatani,[1] Toshiki Moriyama,[1] Shiro Takahara,[2] Hiromi Rakugi.[1] *[1]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Renal ischemia-reperfusion (I/R) injury is unavoidable during kidney transplantations and leads to both early and long-term graft dysfunction. During I/R injury, the burst of reactive oxygen species (ROS) can trigger the inflammation and the tubular cell injury. Febuxostat is a novel selective inhibitor of xanthine oxidoreductase (XOR), approved for treating hyperuricemia. As XOR is a critical source of ROS, inhibition of XOR could be a therapeutic target for I/R injury. Therefore, we performed this study to test the therapeutic effect of febuxostat on renal I/R injury.

Methods: To test the protective effect of febuxostat, uninephrectomized Sprague-Dawley rats received saline (Veh) or febuxostat (Feb) at a dose of 10 mg/kg body weight orally 24 hour and 60 min prior to I/R injury. Renal I/R injury were induced by clamping the left renal artery for 45 min. At 24 hours after reperfusion, serum and kidney samples were harvested.

Results: Veh-treated I/R injured rats exhibited elevated serum creatinine (1.81±0.29 mg/dl) and BUN (111.2±7.5 mg/dl), which were significantly blunted in Feb-treated I/R injured rats (0.65±0.09 and 40.1±6.4 mg/dl, respectively). Histological analysis revealed that Feb-treated rats showed less tubular injury with reductions in CD68-positive macrophages infiltration and TUNEL positive tubular cells compared to Veh-treated rats.

Conclusions: In conclusion; novel xanthine oxidoreductase inhibitor, febuxostat, can protect kidney from renal I/R injury, and may contribute to preserve early and long-term kidney graft function.

FR-PO1132

Angiopoetin-1 and VE-Cadherin – EPC Antagonists in Acute Ischemic Renal Failure Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Background: Exogenously administered endothelial progenitor cells (EPCs) can protect the kidney from acute ischemic injury (iAKI). Over the last years, several EPC activators have been identified which increase the cells´ renoprotective capacity in iAKI (8-O-cAMP, melatonine). Angiopoietin-1 and -2 play critical roles in vascular homeostasis. Aim of the study was to analyze whether Angiopoietin-1 (Ang-1) modulates EPC-mediated renoprotection in iAKI.

Methods: EPCs were isolated from male C57BL/6N mice. After 5 days of culturing, cells were incubated with different substancies: Ang-1, Ang-1 + blocking peptide, Ang-1 + anti-VE-Cadherin, 8-O-cAMP+cRGD (±anti-VE-Cad.), respectively. After one hour of incubation, dye-labelled EPCs were systemically injected into recipient animals after bilateral renal ischemia of 40 minutes. Two days later, renal function and morphology were analyzed. In addition, the migratory cell activity was investigated in vitro.

Results: Mice were not prevented from acute renal failure if 0.5 × 106 untreated EPCs were injected. Animals injected with Ang-1 pretreated EPCs showed significantly worsening of postischemic renal function. These effects were completely reversible after combined cell incubation with Ang-1 and a specific blocking peptide. In order to analyze whether the effects of Ang-1 are mediated by its agonistic actions on VE-Cadherin, cells were incubated with Ang-1 and anti-VE-Cadherin. Renal function of cell injected mice declined further. Comparable effects were inducable by EPC pretreatment with 8-O-cAMP (+cRGD) and anti-VE-Cadherin (8-O-cAMP stimulates VE-Cadherin expression). In vitro analysis showed significantly reduced migratory EPCs activity after VE-Cadherin blockade.

Conclusions: Ang-1 decreases the renoprotective capacity of syngeneic murine EPCs in iAKI. These effects do not result from agonistic actions on VE-Cadherin. VE-Cadherin rather seems to antagonize Ang-1 in the setting of an EPC-based therapy of iAKI.

FR-PO1133

Intermedin Promotes Recovery from Acute Kidney Injury Induced by Renal Ischemia-Reperfusion Injury Rongshan Li,[1,2] Xi Qiao,[1,2] Li Zhao,[1,2] Haihong Zhao,[1,2] Yudong Chu,[1,2] Guozheng Feng.[1,2] *[1]Department of Nephrology, Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, China; [2]Shanxi Kidney Disease Institute, Taiyuan, Shanxi, China.*

Background: Acute kidney injury (AKI) induced by ischemia-reperfusion injury (IRI) is associated with high morbidity and mortality. The prognosis of renal IRI is determined by the extent of the injury and the process of the recovery. Our previous studies demonstrated that intermedin (IMD) protects against renal IRI by reducing injury, while its role in the recovery process is known yet.In this study, we investigate the effect of IMD on the recovery process after renal IRI and its mechanisms.

Methods: Eukaryotic expression vector encoding rat IMD gene or control empty plasmids was transfected into the kidney using an ultrasound-microbubble mediated system. The transfection rate was detected. Renal IRI model was induced by clamping left renal

artery for 45 min followed by reperfusion. One, 2, 3, 4, 7 and 14d of reperfusion, renal function, tubulointerstitial damage and PCNA positive cells were evaluated. The expression of angiogenesis-related proteins (HIF-1a, VEGF and Tie-2), nephrogenic proteins (Pax-2, ZO-1, Ncam, Wt-1 and Vimentin) as well as cell cycle related proteins (cyclin D1, cyclin E, CDK2 and CDK4) was measured by Western blot analysis or ELISA.

Results: IMD expression was significantly up-regulated in kidneys of rats in IMD transgene group than the control. IMD gene transfer significantly protected renal function and lessened morphological damage. The number of PCNA positive cell increased significantly 1d, 2d, 3d, 4d and 7d after IRI, and reached the peak at 7d. IMD gene transfer further increased the number of PCNA positive cell, which arrived the peak at 3d. The expression of HIF-1a, VEGF, Tie-2, Pax-2, ZO-1, Ncam, Wt-1 and Vimentin was significantly up-regulated 1, 2, 3 and 4d after IRI, and cyclin D1, cyclin E, CDK2 and CDK4 expression was markedly up-regulated 1, 2, 3, 4, 7 and 14d. In rats treated with IMD, we observed a 1- to 4-d earlier and more abundant reexpression of the above proteins than the control.

Conclusions: IMD can induce an earlier regeneration process after renal IRI, thus promotes recovery from renal IRI.

Funding: Government Support - Non-U.S.

FR-PO1134

DPP4 Inhibition Attenuates Ischemia-Induced Impaired Renal Function in a Unilateral Rat Model of Ischemia Reperfusion Lorenzo Glorie,[1] Anja Verhulst,[1] Veerle Matheeussen,[2] Ingrid De Meester,[2] Joanna Magielse,[3] Nina Hermans,[3] Patrick C. D'Haese,[1] Annelies De Beuf.[1] *[1]Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium; [2]Laboratory of Medical Biochemistry, University of Antwerp, Antwerp, Belgium; [3]Laboratory of Nutrition and Functional Food Science, University of Antwerp, Antwerp, Belgium.*

Background: Dipeptidyl peptidase 4 (DPP4) inhibitors increase the half-life of DPP4 substrates and improve glucose tolerance of diabetes patients. It has been shown that DPP4 inhibitors exhibit protective effects on ischemia reperfusion injury (IRI) of heart and lung. We studied the effect of vildagliptin (VG) on IRI-induced acute kidney injury in rats.

Methods: Male Wistars underwent 30' of left renal ischemia, followed by right nephrectomy. Saline, 1 or 10 mg/kg VG (VG1/VG10) was administered iv prior to ischemic or sham operation. Rats were sacrificed after 2, 12, 48 hours of reperfusion.

Results: DPP4 inhibition was confirmed by decreased activity in serum and kidney homogenates. VG resulted in a significant dose-dependent decrease of serum creatinine (1.31±0.32 and 0.70±0.19 mg/dl for VG1 and 10 resp. vs 1.91±0.28 mg/dl for controls at 12h; $p<0.01$). Tubular morphology (PAS-PCNA) revealed reduced necrosis of proximal tubules at 12h (62.1±18.0% in VG10 vs 77.5±22.0% in controls; $p<0.05$). VG had no effect on regeneration. VG resulted in a decreased apoptosis shown by the 2-fold decreased Bax/Bcl-2 mRNA expression and diminished presence of apoptotic bodies on TUNEL stained sections (414.1±231.7 in VG10 vs 1212.0±650.8 in controls; $p<0.05$). VG treatment also resulted in a early downregulation of several pro-inflammatory markers, including IL16 (3-fold), CXCL10 (2-fold), TNFα (2-fold) and upregulation of anti-inflammatory IL10 (1.5-fold). Furthermore, VG results in early downregulation of SOD3 and catalase mRNA at 2h, faster recovery at 12h and sign.reduction in expression of MDA at 48h (0.54±0.17 and 0.56±0.21 for VG1&10 resp. vs 1.00±0.35 µM in controls; $p<0.05$).

Conclusions: DPP4 inhibition results in functional and morphological protection of the kidney against IRI as reflected by changes of expression of relevant genes. The precise mechanism remains to be elucidated.

FR-PO1135

A New Class of Peptide Agonists Share Functional Properties of Bone Morphogenetic Protein To Protect Against Renal Ischemic Damage Dattatreyamurty Bosukonda,[1&3] Peter C. Keck,[1] Silvia B. Campos-Bilderback,[2] Ruben M. Sandoval,[2] Exing Wang,[2] Philippe Bey,[1] William Carlson,[1&3] Bruce A. Molitoris.[2] *[1]Thrasos Innovation, Inc., Montreal, QC, Canada; [2]Indiana University School of Medicine, Indianapolis, IN; [3]Mass General Hospital/ Harvard Medical School, Boston, MA.*

Background: Proximal tubule epithelial cells (PTEC) play a central role in the response of the kidney to insult by the production of chemokines and cytokines that signal inflammatory response. Bone morphogenetic protein-7(BMP-7),a member of the TGF-beta superfamily, has previously been shown to reduce inflammation and tissue damage in animal models of acute renal failure.

Methods: We have designed a new class of peptide agonists of molecular size,2 kDa.

Results: Like BMPs, the peptides bind both type I and type II BMP receptors to induce signal transduction (SMAD 1/5) in PTEC; unlike BMPs, they do not bind ALK6, a type I receptor that plays a predominant role in osteogenesis. Upon injury, PTEC express increased levels of cytokines and chemokines causing inflammation of PTEC which was prevented by the peptide agonists to protect PTEC. SPECT/CT Bio-imaging of rats treated with radioiodinated THR-184 demonstrated that the compound rapidly accumulated in kidneys and stomach, at levels 10 times that for the rest of the body. In kidney cortex, levels rose rapidly within the first 15 min [9.4% +/- 0.9% of injected doses, n=4] and remaining steady over 2 h after injection [private communication, Dr. Mary Rusckowski, U Mass Medical School, Worcester,MA]. In AKI animals after 45 min bilateral renal artery occlusion, and at 24 h after reperfusion, serum creatinine (Cr) increased 18 fold above basal values. The peptide agonist either by iv at 15 min before clamping or by oral at 2 h before and 1 h after clamping, strongly suppressed the serum Cr rise at 24 h after reperfusion, suggesting that the compound preserved kidney function.

Conclusions: These results suggest that the new class of peptide agonists while sharing the beneficial properties of BMP may provide novel therapeutic agents for kidney disorders involving inflammation and ischemic damage of PTEC.

FR-PO1136

Paricalcitol Prevents Cisplatin-Induced Renal Injury by Suppressing Apoptosis and Proliferation Jeong-Woo Park,[1] Soo Yeon Joo,[2] Eun Hui Bae,[1] Seong Kwon Ma,[1] Suhn Hee Kim,[3] Jongun Lee,[2] Soo Wan Kim.[1] *[1]Internal Medicine, Chonnam National University Medical School, Gwangju, Korea; [2]Physiology, Chonnam National University Medical School, Gwangju, Korea; [3]Physiology, Chonbuk National University Medical School, Jeonju, Korea.*

Background: Cisplatin (CDDP) is a widely used chemotherapeutic agent. However, approximately one-third of patients experience kidney injury with CDDP treatment. Paricalcitol is an active vitamin D analog that shows renoprotective action in various experimental nephropathy models. We investigated the efficacy of paricalcitol in preventing the progression of CDDP-induced kidney injury.

Methods: Male Sprague-Dawley rats were treated with vehicle (n=12), single CDDP alone (n=12, 6 mg/kg/day, *i.p.*), or single CDDP+paricalcitol (n=12, 0.2 µg/kg/day, *s.c.*) for 4 days. In another series of experiment, HK-2 cells treated with CDDP (50 µM), either with or without paricalcitol (0.2 ng/ml) were also examined.

Results: Paricalcitol counteracted the CDDP-induced decline in renal function. Paricalcitol also suppressed the expression of transforming growth factor-β1 (TGF-β1), Smad signaling, and the subsequent epithelial-to-mesenchymal process in CDDP-induced nephropathy. In HK-2 cells, paricalcitol suppressed the CDDP-induced increases in ERK1/2 and p38 phosphorylation and in fibronectin/connective tissue growth factor expression. Paricalcitol co-treatment also reduced CDDP-induced over-expression of p53, which coincided with a decrease in pro-apoptotic markers. It also augmented the up-regulated expression of p27kip1 and decreased the number of proliferating cell nuclear antigen-positive cells in CDDP-treated rat kidneys. Accordingly, paricalcitol co-treatment reduced the over-expression of cyclin-dependent kinase2/cyclin E induced by CDDP.

Conclusions: Our results suggest that paricalcitol can attenuate CDDP-induced renal injury by suppressing the induction of fibrotic, apoptotic and proliferative factors. Its underlying mechanisms may include the inhibition of TGF-β1, mitogen-activated protein kinase signaling pathways, p53-induced apoptosis, and the augmentation of $p27^{kip1}$ over-expression.

FR-PO1137

CCL19 and 21 Are Essential for Regulatory T Cell Function in Nephrotoxic Serum Nephritis Kathrin Eller, Alexander R. Rosenkranz. *Clinical Division of Nephrology, Medical University Graz, Graz, Austria.*

Background: CCL19 and CCL21 guide T lymphocytes via binding to CCR7 to the T cell areas in lymph nodes. Recently, we provided evidence that CCR7 knock-out (KO) mice display increased disease indices when subjected to nephrotoxic serum nephritis (NTS). Tregs of CCR7KO mice were found to migrate to the kidneys rather than to the lymph nodes and thereby lost their potential to efficiently suppress NTS.

Methods: To study the CCR7 counterparts CCL19 and CCL21 we used the plt-mouse model, which do not express CCL21 or CCL19 protein in secondary lymphoid organs but continue to express CCL21 at reduced levels in lymphatic endothelium.

Results: When plt-mice were subjected to NTS they displayed significantly increased disease activity as shown by increased albuminuria and histological changes. Even though significantly decreased numbers of CD4+ T cells were detected in the lymph nodes of plt-mice, subtyping revealed significantly increased numbers of activated CD4+CD69+ T cells and decreased numbers of CD4+CD25+FoxP3+ Tregs to infiltrate the lymph nodes of plt-mice after NTS induction. In line, Tbet and TNF-alpha mRNA expression were found to be increased in lymph nodes of plt-mice. Interestingly, significantly increased Tregs were found to infiltrate the kidney of plt-mice as compared to respective controls. When Tregs were transferred to plt-mice a delayed suppression of NTS was observed as compared to wild-type mice.

Conclusions: Together, CCL19 and CCL21 are significantly involved in the Treg homing in NTS. Tregs are only capable to sufficiently inhibit NTS when located to the lymph nodes. Since plt-mice still express CCL21 at reduced levels Treg transfer in plt-mice resulted only in a delayed suppression of NTS.

FR-PO1138

Th17/Treg Imbalance in Patients with Idiopathic Membranous Nephropathy Lili Liu, Yan Qin, Limeng Chen, Xue-Wang Li. *Division of Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: IMN was an autoimmune disease, which involve T and B lymphocyte dysfunction. Recently, regulatory T cells (Treg) cells and Th17 cells had been described as two distinct subsets of T helper cells. Th17/Treg balance may be important in the development/prevention of autoimmunity. The objective of this study was to evaluate whether the Th17/Treg balance was broken in IMN patients.

Methods: Forty-nine patients, diagnosed IMN by renal biopsy and excluded potential secondary factors, were enrolled in this study. Twenty-eight healthy volunteers served as healthy controls (HCs).The frequencies of peripheral Treg and Th17 subsets were evaluated

by flow cytometry. The peripheral relative mRNA expression of key transcription factors were determined by real-time RT-PCR. The concentrations of plasma cytokines were evaluated by ELISA. The infiltration of Treg cells and expression of IL-17 in renal tissue were determined by immunohistochemical staining.

Results: Compared with HCs, the frequency of peripheral Treg cells and plasma TGF-β1 level decreased ,while the frequencies of Th17 cells and plasma IL-23, IL-17 levels increased significantly in IMN patients.The key transcription factors of Treg and Th17(Foxp3 and RORγt), had similar alterations in HC and IMN patients. The Th17/Treg ratios increased along with increased proteinuria and decreased albumin levels in patients with IMN. IL-17 protein expression in the renal tissue of IMN patients increased significantly compared with that in control subjects. Infiltration of Treg cells was also detected in the renal tissue of IMN patients, while rare Treg cells had been seen in normal renal tissue. The infiltration of Treg cells in renal interstitium also related to a higher clinical remission in IMN patients.

Conclusions: Th17/Treg imbalance existed in IMN patients, suggesting a potential role of Th17/Treg imbalance in the pathogenesis of IMN.

FR-PO1139

Sexual Dimorphism of Foxp3+ T-Regulatory Cell Function Is HDAC6-Dependent Tatiana Akimova, Ulf H. Beier, Wayne W. Hancock. *Children's Hospital of Philadelphia & University of Pennsylvania.*

Background: Pre-menopausal women have 2-3 fold higher rates of autoimmune diseases compared to age matched males. While the basis for this difference is likely complex and multifactorial, little attention has been given to whether there are gender-based differences in Treg suppressive function, despite the fact that Foxp3 is an X-linked gene.

Methods: We analyzed Treg gene expression and suppressive function (SF) in normal 6-8 wk male and female littermate C57BL/6 mice, and in various KO strains.

Results: While male and female mice had comparable numbers of Tregs, male Tregs had greater SF. This disparity was also seen using male and female Tregs of various knockout mice, except in HDAC6-/- mice. HDAC6-/- C57BL/6 mice had the same number of Tregs as WT mice, but higher Foxp3 expression per cell (qPCR, Western blot) and a more mature Treg phenotype (assessed using CD44, CD62L, CD69 and CD103 markers). HDAC6-/- Tregs were also more suppressive than WT Tregs, but interestingly, female Tregs had better suppressive function than male Tregs, i.e. there was a gender-based reversal in HDAC6-/- Treg function. The SF mean ratio of adult Tregs was 1 (WT F) to 1.3 (WT M) to 2 (HDAC6 F) to 1.7 (HDAC6 M), and Tregs from HDAC6-/- females had more mature Treg phenotype (10-12% fewer naive Tregs). This difference was also seen upon treatment of Tregs from normal human donors (29 tests) with pan-HDAC or HDAC6-selective inhibitors; while male Tregs had 1.7±0.1 fold enhanced SF, females Tregs showed 2.2±0.2 fold increase (p=0.057). Binding of estrogen to the estrogen receptor-α induces HDAC6 and promotes proteasomal degradation of Smad2 and Smad3, and Tregs from HDAC6-/- mice showed increased phospho-Smad3. We are currently assessing the effects of HDAC6 targeting on Smad2/3-dependent events at the TGF-β-dependent Foxp3 enhancer.

Conclusions: Our studies show HDAC6, also an X-linked gene, is key to regulation of Foxp3 expression and function, and that its targeting can affect estrogen/estrogen receptor signaling. Use of HDAC6 selective inhibitors may provide a means to overcome the gender-based disparity in Treg function and be useful in the therapy of multiple autoimmune diseases, including those affecting the kidney.

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FR-PO1140

IKK2 Is Required for the Development of Regulatory T Cells Eveline Piella,[1] Anna Hermann,[1] Gunther Zahner,[1] Annette Erhardt,[2] Hans-Joachim Paust,[1] Jan-Eric Turner,[1] Ulf Panzer,[1] Joachim Velden,[3] Gisa Tiegs,[2] Rolf A. Stahl,[1] Friedrich Thaiss.[1] *[1]Univ. Hospital, III. Med. Department, Hamburg, Germany; [2]Univ. Hospital, Inst. Exp. Immunology, Hamburg, Germany; [3]Univ. Hospital, Inst. Pathology, Hamburg, Germany.*

Background: Regulatory T cells (Tregs) are characterized by their expression of the transcription factor Foxp3. In experimental glomerulonephritis (GN) Tregs seem to play a pivotal role, since their depletion leads to an aggravation of GN. Recent studies have shown that members of the NF-kappaB-family are involved in Foxp3 gene expression. Phosphorylation of the NF-kappaB inhibitor by the I-kappaB-kinase 2 (IKK2) is one of the crucial steps in NF-kappaB activation. In order to elucidate the potential role of NF-kappaB on the development of Tregs in vivo, mice with IKK2 deficient Foxp3^{+} cells were generated (Foxp3^{Cre}IKK2$^{fl/fl}$).

Methods: Foxp3^{Cre} (YFP) mice have been described by Dr. A.Y. Rudensky and IKK2$^{fl/lf}$ mice were kindly provided by M. Karin. Animals homozygous for Foxp3^{Cre} and IKK2$^{fl/fl}$ were sacrificed on days 3, 7 and >14 after birth. Cells from thymus, spleen and lung were isolated and the frequencies of Tregs analyzed by FACS. Periodic-acid-Schiff staining and immunohistochemistry were performed using standard techniques.

Results: Homozygous Foxp3CreIKK2fl/fl mice spontaneously develop a scurfy-like phenotype at day 12-27 after birth which manifests in a hunched posture and a skin disorder characterized by scaliness, swollen ears and a tail shielded by padded rings. Animals show enlargement of lymph nodes and a significant splenomegaly. Homozygous Foxp3CreIKK2fl/fl mice present a complete disruption of organ architecture at day >14 of the spleen. The kidneys did not show infiltrating inflammatory cells or failed tissue organization. The number of Tregs in the spleen and lung are significantly reduced but are not altered in the thymus compared to control animals.

Conclusions: Our data demonstrate that continuous IKK2 activity is essential to maintain thymic pre-programmed Foxp3 expression in mature Treg cells in the periphery. Thus, continued expression of IKK2 in mature Treg cells is indispensable to maintain the dominant tolerance which is mediated by these cells.

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FR-PO1141

Anti-CD45RB Not Only Augments homeostatic Proliferation but Uniquely Promotes Antigen-Specific Proliferation of Regulatory T Cells In Vivo Ying Wang, David M. Rothstein. *University of Pittsburgh, PA.*

Background: CD4+Foxp3+ Treg play an important role in transplant tolerance. By transferring CFSE-labeled Foxp3+ (Treg) or Foxp3- (Tconv) CD4 cells from congenic Foxp3-reporter mice into naïve WT recipients, we showed that Treg exhibit a higher constitutive rate of homeostatic proliferation (HP) than Tconv (~50% vs. <10% over 10d). Moreover, anti-CD45RB induces tolerance through a 2X increase in Treg which results by dramatically increasing Treg HP, even in the absence of exogenous antigen. While exogenous antigen is not required, Treg HP may still be antigen-driven. To address the signals involved, CFSE-labeled congenic CD4 cells were transferred into naïve wt mice and proliferation of Foxp3+ and Foxp3- cells assessed on d10. Treating mice with Cyclosporin A (CsA) greatly reduced both basal and anti-CD45RB-mediated HP of Treg. This inhibition by CsA was only partly restored by concomitant administration of rIL-2, suggesting that NFAT translocation was required. Moreover, when CFSE-labeled congenic CD4 cells were transferred into MHCII-/- mice, both basal and anti-CD45RB-mediated HP of Treg were markedly reduced. These studies suggest that antigen signaling is required for high rates of HP by Treg. OTII mice (TCR-transgene specific for Ova peptide) on a RAG-/- background, exhibit low but readily detectable Treg. Anti-CD45RB alone had no effect, whereas, immunization with Ova (20μg ip) expanded Treg % and number ~2X. Anti-CD45RB + Ova further increased Treg 3.3X vs. Ova alone, but had no effect on Tconv cell number. Next, OTII (non-RAG-/-) mice were used because they express sufficient Treg (3.6%) for cell transfer studies to assess proliferation. CFSE-stained CD4 cells from these OTII mice were adoptively transferred into congenic mice. Ova + anti-CD45RB specifically augmented proliferation of OTII Treg compared to Ova alone, while Tconv were unaffected. These results demonstrate that both basal and anti-CD45RB-mediated augmentation of Treg HP are antigen-dependent. Moreover, anti-CD45RB can specifically enhance the proliferative response of Treg to exogenous antigen in vivo – a new paradigm for expansion of Treg for therapeutic purposes.

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FR-PO1142

Regulatory T Cells Reverse Obesity-Linked insulin Resistance and Diabetic Nephropathy Kathrin Eller,[1] Alexander R. Rosenkranz,[1] Philipp Eller.[2] *[1]Clinical Division of Nephrology, Medical University Graz, Graz, Austria; [2]Clinical Division of Angiology, Medical University Graz, Graz, Austria.*

Background: FoxP3 expressing regulatory T cells (Tregs) are critical for maintenance of tolerance in rodents and men. Evidence is increasing that regulatory T cells improve insulin resistance in type 2 diabetes mellitus.

Methods: The study was designed to evaluate the role of Tregs in type 2 diabetes mellitus and end organ damage in the human and murine setting.

Results: We observed that fat mass, fasting blood glucose levels and TNF-α mRNA expression significantly correlated with FoxP3 transcripts in human visceral adipose tissue. To further evaluate the pathogenic role of Tregs in insulin resistance, we used the db/db mouse model, depleted of or transferred with Tregs, and followed the mice for 56 days. Treg-depletion using an anti-CD25 monoclonal antibody enhanced insulin resistance as shown by increased fasting blood glucose levels as well as an impaired insulin sensitivity. Moreover, Treg-depleted db/db mice developed increased signs of diabetic nephropathy, such as albuminuria and glomerular hyperfiltration. This was paralleled by a pro-inflammatory milieu in both, murine visceral adipose tissue and the kidney. Vice versa, adoptive transfer of CD4+FoxP+ Tregs significantly improved insulin sensitivity and diabetic nephropathy. Accordingly, there was increased mRNA expression of FoxP3 as well as less abundant pro-inflammatory CD8+CD69+ T cells in visceral adipose tissue and kidneys of Treg-treated animals.

Conclusions: In summary, our data suggest a potential therapeutic value of Tregs to improve insulin resistance and end organ damage in type 2 diabetes by limiting the pro-inflammatory milieu in VAT.

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FR-PO1143

Early IL-17 Production by gamma-delta T Cells in the Kidney Is Induced by IL-23 and Contributes to Tissue Injury in Murine Crescentic Glomerulonephritis Christian Krebs,[1] Jan-Eric Turner,[1] Hans-Joachim Paust,[1] Andre Pascal Tittel,[2] Sabrina Bianca Bennstein,[1] Oliver M. Steinmetz,[1] Catherine Meyer-Schwesinger,[1] Rolf A. Stahl,[1] Christian Kurts,[2] Ulf Panzer.[1] *[1]III. Medizinische Klinik, UK-Eppendorf, Hamburg, Germany; [2]Institut für Experimentelle Immunologie, Universitätsklinikum Bonn, Bonn, Germany.*

Background: The inflammatory cytokine IL-17A (IL-17) is thought to play a critical role in the pathogenesis of human and experimental crescentic glomerulonephritis. However, the cell types which contribute to renal IL-17 production in glomerulonephritis are not

well characterized. In addition, the mechanisms which induce IL-17 production by renal leukocytes remain to be elucidated.

Methods: To characterize IL-17-producing cells in renal inflammation, we performed a time course analysis (day 1 - 30) of IL17 production in a T cell-dependent model of crescentic glomerulonephritis in mice (nephrotoxic nephritis, NTN).

Results: Ten-colour flow cytometric analysis of intracellular IL-17 staining and surface makers identified gamma-delta T cells (gdTC) as an important source of early IL-17 production (day 3) in the kidney. From day 6 on classical CD4+ Th17 cells were the major source of IL-17. Interestingly, CD3+ T cells that lacked expression of conventional T cell markers (CD4, CD8, dTCR, NK1.1, CD1d-α-GalCer) contributed significantly to renal IL-17 production. Analysis of mice deficient for IL-23-receptor, IL-23p19, or IL-1β-receptor1 showed that early IL-17 production by gdTC in the kidney critically depends on IL-23, but not IL-1β. Upon depletion of dendritic cells in CD11c-DTR mice gdTC were significantly decreased in the kidney with marked reduction of IL-17 production. To address the function of IL-17 production by gdTC in NTN we reconstituted IL-17-deficient hosts with wild-type CD4+ T cells ± co-transfer of wild-type gdTC. The absence of IL-17-producing gdTC resulted in reduced neutrophil infiltration and ameliorated renal tissue damage.

Conclusions: In conclusion, our data identify three major cellular sources of IL-17 in the kidney and demonstrate a previously unrecognized important role of IL-17-producing gamma-delta T cells in the immunopathogenesis of glomerulonephritis.

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FR-PO1144

The Function of CD4 Lymphocytes in Experimental Autoimmune Glomerulonephritis Hans-Willi Mittrücker,[1] Julia Holzer,[1] Stefanie Hünemörder,[1] Hans-Joachim Paust,[2] Ulf Panzer,[2] Helmut Hopfer.[3] *[1]Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Nephrology, University Medical center Hamburg-Eppendorf, Hamburg, Germany; [3]Pathology, University Hospital, Basel, Switzerland.*

Background: Autoimmunity against the alpha3IV-NC1 domain of type IV collagen results in antiglomerular basement membrane (GBM) glomerulonephritis. Although auto-antibodies against alpha3IV-NC1 are central for the development of glomerulonephritis, there is increasing evidence for a contribution of alpha3IV-NC1-specific T-lymphocytes in disease. In this study, we will investigate the abundance and function of different T cell subsets at different phases of anti-GBM nephritis.

Methods: Experimental Autoimmune Glomerulonephritis (EAG) was induced in DBA/1 mice by repeated immunization with recombinant human alpha3IV-NC1 protein in complete and incomplete Freund's adjuvant.

Results: Immunized mice develop high serum titers of alpha3IV-NC1-specific antibodies. After 8 to 10 weeks, mice showed signs of progressive loss of kidney function, such as severe proteinuria and edema formation. Eventually, most mice died due to severe renal failure. Kidneys of immunized mice showed IgG deposition along the GBM and in the end stage of disease, a large fraction of glomeruli contained crescent-shaped inflammation, the hallmark of anti-GBM disease. At this time point, there was also massive tubulointerstitial damage with accumulation of T cells and macrophages. Immuno histology and FACS analyses of renal cells revealed massive accumulation of macrophages and T cells. T cells demonstrated a highly activated phenotype and upon polyclonal stimulation, a fraction of CD4 T cells produced IFNgamma and IL-17A.

Conclusions: In conclusion, our EAG model closely reproduces central features of human anti-GBM disease. Cytokine expression analyses point to a contribution of Th1 and Th17 cells in the progression of disease. Our current studies aim at clarifying the role of these different T cell subsets in anti-GBM disease.

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FR-PO1145

A Myeloperoxidase Peptide-Specific CD4+ Cell Clone Induces Injury in Murine Anti-Myeloperoxidase Glomerulonephritis Janet Chang, Joshua D. Ooi, Stephen R. Holdsworth, A. Richard Kitching. *Medicine, Monash University, Melbourne, Victoria, Australia.*

Background: Autoimmunity to myeloperoxidase (MPO) is important in vasculitis. Autoreactive CD4+ cells support MPO-ANCA production and may play a direct role in injury, but T cell epitopes are not known and a role for effector CD4+ cells is uncertain.

Methods: An immunodominant MPO T cell epitope was defined by responses to overlapping MPO 20aa peptides in C57BL/6 mice. T cell clones were generated against an MPO peptide, transferred into Rag1-/- mice, then disease triggered with either low dose anti-glomerular basement membrane antibodies (anti-GBM Ab) or anti-MPO Ab/LPS.

Results: Mice were immunized with the 5 strongest responding peptides (by IFN-γ ELISPOT). All peptides responded to themselves and to recombinant mouse MPO, but peptide (pep)52 responded most strongly to rmMPO and itself (proliferation, IFN-γ and IL-17A ELISPOT). Pep52-immunized mice made MPO-ANCA, but at lower titers than MPO immunized mice. MPO pep52 specific CD4+ clones were generated by immunizing C57BL/6 mice. An MPO-specific IFN-γ secreting CD4+ cell clone was transferred into Rag1-/- mice, that were then immunized with MPO pep52 (an OVA-specific clone was a control; these Rag1-/- recipients were OVA pep immunized). 7 days later, low dose anti-GBM Ab was injected. Mice receiving anti-MPO clones developed progressive glomerular disease, but mice receiving anti-OVA clones did not (glomeruli with necrosis: day 5 anti-OVA clone 4±1, anti-MPO clone 49±5; day 14 anti-OVA 7±2, anti-MPO 69±4%; both P<0.001) and albuminuria (alb:creat day 5 anti-OVA 15±2, anti-MPO 276±44; day 14 anti-OVA 19±2, anti-MPO 1080±307 μg/μmol; both P<0.001). In further experiments, the same protocol was followed but disease was triggered with 50μg/g MPO-ANCA iv and 0.5μg/g LPS ip. Only MPO clone recipients developed significant disease (day 14: glomeruli with necrosis, anti-MPO Ab + anti-OVA clone 6±1, anti-MPO clone 41±4%; urine alb:creat anti-OVA 28±5, anti-MPO 1163±124 μg/μmol, both P<0.0001).

Conclusions: These results define a pathogenic MPO T cell epitope and support a role for cell mediated effector responses, along with humoral responses, in the pathogenesis of MPO-ANCA associated glomerulonephritis.

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FR-PO1146

Profiles of CD4+ T Cells Reveal a Novel Subset and FoxP3 Splice Variants in ANCA Disease Meghan E. Free,[1] Maureen Su,[2] Donna O. Bunch,[3] J. Charles Jennette,[1] Ronald J. Falk.[3] *[1]Pathology and Laboratory Medicine, UNC Chapel Hill; [2]Pediatrics, UNC Chapel Hill; [3]UNC Kidney Center, UNC Chapel Hill, Chapel Hill, NC.*

Background: We hypothesized that the population dynamics of CD4+ T cells are altered in anti-neutrophil cytoplasmic autoantibody (ANCA) disease. We examined a novel T cell population (defined as CD127high CD25intermediate) and regulatory T cells (defined as CD127low CD25+ FoxP3+).

Methods: Analyses were performed using flow cytometry on lymphocytes stained with appropriate antibodies. Functional studies were completed using a standard suppression assay.

Results: The CD25int population was expanded in patients with ANCA disease (52.3% of CD4+ T cells) and SLE (52.2%) compared to healthy controls (31.2%) (p=0.008). Longitudinally, this CD25int population was stable regardless of disease status and medication. The majority of CD25int T cells expressed CD45RO indicating prior antigen stimulation and a subset of these produced IL-17A and expressed CCR6. Functionally, CD25int T cells from healthy controls moderately suppressed the proliferation of CD25low effector T cells and this suppressive ability was abrogated in ANCA disease. CD25int T cells continued to proliferate despite co-culture with regulatory T cells, demonstrating resistance to traditional suppression.

Regulatory T cells were elevated in active ANCA disease but return to normal levels at remission. These cells were unable to suppress effector cell proliferation which was associated with a FoxP3 splice variant missing exon 2.

Conclusions: In ANCA disease the majority of the CD25int population is 'antigen experienced' and may harbor autoreactive T cells. This expanded CD25int population can migrate into inflamed organs and secrete IL-17A, potentiating an inflammatory cascade with neutrophil influx. This CD25int population is resistant to suppression by regulatory T cells and lends insight into loss of peripheral tolerance in patients with ANCA disease. Lastly, with active disease, regulatory T cells harbor exon 2 deficient FoxP3 which may alter their suppressive function.

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FR-PO1147

Interleukin 1 Regulates Localised Proliferation and Activation of T-helper 17 (Th17) Cells in the Acutely Obstructed Kidney Jana Pindjakova, Shirley Hanley, Michelle M. Duffy, Rhodri Ceredig, Matthew D. Griffin. *Regenerative Medicine Institute (REMEDI), School of Medicine, National University of Ireland, Galway, Ireland.*

Background: Interleukin-1 (IL- 1) enhances pathogenic IL-17-producing T-helper (Th17) cells in autoimmunity but its effects on intra-renal Th17 cells during kidney disease are unknown. We have reported that IL-1, produced by mononuclear cells from acutely injured kidneys, enhances IL-17 production by *in vitro*-generated Th17 cells. We have now examined *in vivo* proliferation and activation of Th17 cells in the mouse unilateral ureteral obstruction (UUO) model with and without IL-1 receptor (IL-1R) blockade.

Methods: Groups of mice underwent 72hr UUO with continuous bromodeoxyuridine (BrdU) exposure via drinking water ± 100 mg/kg/day IL-1R antagonist (IL-1Ra, Anakinra). Cell suspensions of obstructed (Obstr) and control (Ctrl) kidneys were analyzed immediately or after 12hr anti-TCR-stimulation culture by multi-color flow cytometry for T-cell markers/chemokine receptors and for intracellular BrdU and IL-17. IL-17 production in kidney cell cultures was measured by ELISA. Relative quantification of IL-17 and IFNγ mRNAs in kidney cortex was performed by qRT-PCR.

Results: *In vivo* proliferation (%BrdU+) of CD4+ T-cells was higher in Obstr compared to Ctrl kidneys. In vehicle-treated mice, 3-6% of CD4+ T-cells from Obstr kidneys were IL-17+ following anti-TCR stimulation. IL-17+ cells were almost exclusively CCR6+. The %BrdU+ among CD4+CCR6+ cells of Obstr kidneys was higher than that of CD4+CCR6- cells (44% vs 25%), indicating greater in vivo proliferation. Compared to vehicle, IL-1Ra was associated with reduced BrdU+CD4+ T-cell numbers in Obstr kidneys, reduced proportion of IL-17+ cells among the BrdU+CD4+ T-cells and reduced IL-17 production by anti-TCR stimulated cells from Obstr. kidney. qRT-PCR indicated reduced mRNA for IL-17 (but not IFNγ) in Obstr kidney cortex of IL-1Ra- compared vehicle-treated animals.

Conclusions: Following UUO, intra-renal CCR6+ Th17 cells undergo high rates of proliferation and activation. IL-1 blockade reduces this accumulation of proliferative, IL-17-competent T-cells and may be of interest in Th17-mediated kidney diseases.

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FR-PO1148

Morphine Enhances HIV-1 Entry into Kidney Cells through a Novel Pathway Hersh Goel,[1] Mohammad Husain,[1] Joanna Mikulak,[2] Ashwani Malhotra,[1] Helena Schmidtmayerova,[1] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Istituto Clinico Humanitas, Rozzano (Milano), Italy.*

Background: Opiate addiction is considered to be a risk factor for the development of HIV-associated nephropathy (HIVAN). We hypothesized that opiates might be enhancing HIV entry into kidney cells.

Methods: Morphine pre-treated human tubular cells (HK2 and HRPTECs) were incubated with HIV-infected (HIV-LY) or control lymphocytes (LY) followed by evaluation of HIV infection of tubular cells by gag expression (real time PCR) and T cell apoptosis (FACS analysis). To determine the involved mechanism of T cell apoptosis, HK2s were pretreated with anti-PDL-1 antibody and then co-cultivated with HIV-LYs and LYs. Subsequently, HIV-LYs and LYs were evaluated for apoptosis and tubular cells for HIV expression. In addition, the effect of morphine on tubular cell PDL-1 expression was determined by FACS analysis. To determine the role of phagocytosis of the apoptosed HIV-LYs and opiate receptors, morphine-pretreated HK2s were co-cultivated with HIV-LYs in the presence of a caspase-3 inhibitor, naloxone (opiate receptor antagonist) or cytochalasin-B (an inhibitor of phagocytosis) followed by evaluation for tubular cell HIV expression.

Results: Morphine not only enhanced tubular cell PDL-1 expression but also promoted apoptosis of HIV-infected T cells; whereas, anti-PDL-1 antibody prevented morphine-induced T cell apoptosis. Morphine enhanced tubular cell HIV-1 expression; whereas, naloxone inhibited tubular cell HIV-1 expression. Since both caspase-3 inhibitor and cytochalasin B inhibited tubular cell HIV-1 expression, it would indicate that increased uptake of the apoptosed HIV-LY or its fragments had contributed to tubular cell HIV-1 expression.

Conclusions: Tubular cells not only facilitated apoptosis of HIV-1 infected T cells but also demonstrated capability of phagocytosing them. Morphine enhanced HIV-1 uptake by tubular cells by enhancing apoptosis of HIV-infected T cells. Since direct tubular cell HIV-1 entry did not induce productive infection, this would suggest that phagocytosed apoptosed T cells provided a suitable milieu for productive HIV-1 infection in tubular cells.

Funding: NIDDK Support

FR-PO1149

Adverse Host Factors Exacerbate Occult HIV-Associated Nephropathy Dileep Kumar,[1] Partab Rai,[1] Deepti D. Torri,[1] Rungwasee Rattanavich,[1] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal,[1] Iti Yadav.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Background: Animal studies indicate that single HIV gene expression is enough for the development of HIVAN. With the advent of highly active anti-retroviral therapy (HAART) the incidence of HIVAN has gone down and the patients with HIV infection are living almost a normal life. We hypothesized that HIV infection of kidney cells prior to the start of HAART might manifest in the form of overt HIVAN in the presence of adverse host factors during the latter time periods.

Methods: To test our hypothesis, Vpr mice (which display doxycycline [Doxy] specific podocyte Vpr expression) with 2, 3, and 4 angiotensinogen (Agt) copies (Vpr-Agt-2, Vpr-Agt-3, and Vpr-Agt-4) were administered Doxy for 3 weeks (to develop occult HIVAN; to develop overt HIVAN, 6 wks doxy is needed) followed by Doxy-free water during the next 3 weeks. Subsequently, renal biomarkers (urinary protein :creatinine ratio, blood pressure levels) were collected and kidneys were harvested for renal histology, immunohistochemical studies, protein, and RNA extraction.

Results: Vpr-Agt-2 did not develop proteinuria and blood pressure, and displayed minimal glomerular and tubular lesions, without any microcyst formation. Vpr-Agt-3 showed mild glomeular and tubular lesions and microcyst formation; whereas, Vpr-Agt-4 showed moderate proteinuria, hypertension, glomerular sclerosis, tubular dilatation, microcysts and expression of epithelial mesenchymal transition markers. Vpr-Agt-4 not only displayed enhanced renal tissue expression of Agt, renin, and ACE but also showed higher ($P<0.04$) renal tissue concentration of Ang II; there was no difference in plasma concentration of Ang II in Vpr-Agt-2 and Vpr-Agt-4 mice. However, renal tissues in Vpr-Agt-4 showed enhanced ($P<0.01$) expressions of transforming growth factro (TGF)-β, connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEGF) when compared with Vpr- Agt-2.

Conclusions: These findings indicate that adverse host factors such as the activation of the RAS, promotes the progression of occult HIVAN to apparent HIVAN.

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FR-PO1150

Aging Effects on Kidney Cellular and Soluble Inflammation during AKI Nada Alachkar, Yuhong Tao, Elizabeth M. Higbee, Hamid Rabb. *Nephrology, Johns Hopkins University, Baltimore, MD.*

Background: Elderly have more severe course of AKI with increased progression to CKD compared to the young. We hypothesized that changes in kidney inflammation in elderly could contribute to the severity of AKI.

Methods: We performed IRI surgery on young (Y) (5-7 weeks) and elderly (E) (1 year) C57BL/6 male mice using a well-established model. Briefly, microvascular clamp was placed on the left renal pedicle for 45 minutes and then removed. Mice were sacrificed 3 days (D) post-IRI. IRI and contralateral kidneys were harvested. We used Flow cytometry to quantify the percent of CD4+, CD8+, CD69+ and Foxp3+ Tregs in kidney mononuclear cells (KMNCs) population. We measured levels of 9 cytokines (IL-2, IL-6, IFN-γ, TNF-a, IL-1b, IL-10, MCP-1, IL-17 and TGF-β.

Results: Mice were sacrificed at baseline or 3D post-IRI (n=7-9/group). KMNCs count of Y and E mice 3D post IRI was similar (6.2x106±2 vs. 5.3x106 ±0.97, NS). At baseline, the percent of CD4+ was higher in E kidneys (18±1.4 vs. 13.5±1.9, P=0.04), also, the percent of activated cells, CD4+CD69+ was higher in E kidneys (53.5±7 vs. 25.5±5.3, P=0.007). Additionally, CD8+ and CD8+CD69+ were significantly higher in E kidneys at baseline (11.5±1.57 vs. 7.66±0.47, P=0.037) and (48.6±4.6 vs. 25.9±3.4, P=0.002). No difference was noted at baseline in Tregs percent between E and Y kidneys. There was no change in the percent of CD4+ or CD4+CD69+ at 3D post-IRI between E and Y kidneys or in CD8+ or CD8+CD69+. We found that CD8+ and CD8+CD69+ were higher 3D post-IRI in Y kidneys comparing to baseline (7.66±0.47 vs 13.98, P=0.08) and (25.5±5.3 vs 44.65±4.6, P=0.04) respectively; however, there was no difference in E kidney. Both Y and E kidneys showed significant rise in Tregs 3D post-IRI (18.7±5.0 vs. 6.5±1.2, P=0.025) and (14.0±2.4 vs 5.0±1.18, P=0.008). We found that IL-6 was significantly higher in the IRI E kidney compared to Y (13.2±0.85 vs. 10±0.5, P=0.04).

Conclusions: Elderly mouse kidneys at baseline have higher population of activated CD4 and CD8 cells. Furthermore, elderly kidneys have greater postischemic IL-6 production. Both cellular and soluble inflammatory changes in kidney could help explain the worse course of AKI in elderly.

FR-PO1151

Effect of N-acetyl Cysteine on Short- and Long-Term Cyclosporine-Induced Renal Injury in Mice ShangGuo Piao, Seokhui Kang, Sunwoo Lim, Byung Ha Chung, Chul Woo Yang. *Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, Seoul, Republic of Korea.*

Background: Previously, we reported that chronic CsA nephrotoxicity suppresses the expression of Klotho, and this is mediated by oxidative stress. This study was examined whether antioxidant, N-acetyl cysterine (NAC), protects the Klotho expression and kidney tissue damage against CsA induced nephrotoxicity.

Methods: Mice were divided 6 groups: vehicle group (VH, olive oil, 1 ml/kg/day), CsA group (30 mg/kg/day), NAC 150 group (150 mg/kg/day), NAC 300 group (300 mg/kg/day), CsA + NAC 150 group, and CsA + NAC 300 group, respectively. Under 0.01% sodium diet, mice received daily administrations of olive oil or CsA subcutaneously for 1 and 4 weeks. Mice also received drinking water with or without NAC. Degree of tubulointerstitial fibrosis (TIF) induced by CsA was expressed in TIF score. Oxidative stress was measured by urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Immunoblot analysis was performed for Klotho protein expression.

Results: Administration of NAC on CsA significantly decreased TIF level (NAC 150 group, 16 ± 4%; NAC 300 group, 17 ± 3% vs. 29 ± 1%, $p < 0.05$ vs. CsA group) compared to CsA group. Moreover, urinary 8-OHdG excretion was decreased in NAC treatment on CsA groups compared to CsA group (CsA + NAC 150 group, 282 ± 74 ng/day; CsA + NAC 300 group, 187 ± 49 ng/day vs. 409 ± 149 ng/day, $p < 0.05$ vs. CsA group). There was no significant different TIF level and urinary 8-OHdG excretion between two NAC treated groups on CsA. 1-week treated CsA plus NAC groups were similar to those of 4-week groups. Amount of Klotho protein in CsA treatment was significantly decreased at 1-week (56 ± 5% vs. 100 ± 8%, $p< 0.05$) and was further decreased at 4-week compared to VH group (13 ± 4% vs. 100 ± 18%, $p < 0.05$). NAC 150 treatment on CsA for 1 or 4 weeks was restored the Klotho protein expression (1-week, 79 ± 3% vs. 100 ± 5%; 4-week, 36 ± 3% vs. 100 ± 11%, $p < 0.05$ vs. CsA group).

Conclusions: NAC may have protective effect in CsA-induced renal injury associated with oxidative stress. Up-regulated Klotho expression may be relevant to improvement against acute and chronic CsA nephrotoxicity.

FR-PO1152

Activation of Hypoxia-Inducible Factor-1alpha Attenuate Inflammation, Fbrosis and Apoptosis in Cyclosporine Induced Nephropathy Sewon Oh,[1] Jeongmyung Ahn,[2] Ho Jun Chin,[1,3] Ki Young Na.[1,3] *[1]Seoul National University Bundang Hospital, Kyeong-Kido; [2]Maryknoll Hospital, Busan; [3]Seoul National University College of Medicine, Seoul.*

Background: Hypoxia-inducible factor (HIF) is a transcription factor that regulates cellular hypoxic responses. The activation of HIF has been proved to be effective in various kidney disease models. We investigated whether HIF activation could improve the renal injury in cyclosporine induced nephropathy, and determined its mechanism.

Methods: Renal tubular epithelial cells (HK-2 cells) were exposed to CsA at 10μM for 24hours and, cobalt chloride ($CoCl_2$) 150μM or dimethyloxalyglycine (DMOG) 1mM was added (CsA+$CoCl_2$, CsA+DMOG). Nuclear factor kappa B (NFκB) p65, phosphorylated NF-κB p65, vimentin and caspase-3 were determined by western blotting. Caspase-3 activity also measured using caspase-3 fluorometric assay. In animal experiments, male Sprague Dawley rats kept on a 0.05% low salt diet were treated with CsA for 28 days (15mg/kg/day, subcutaneous) and received a continuous infusion of $CoCl_2$ (10mg/kg/day) or DMOG (100mg/kg/day) during the whole experimental period. Immunohistochemistry (IHC) of ED-1 and tunnel staining were performed.

Results: CsA+CoCl2 or CsA+DMOG increased renal HIF-1α protein by western blot. Apoptotic cell death was decreased in CsA+CoCl2 both in vivo and in vitro (vs. CsA, $P<0.05$) and in CsA+DMOG, in vitro ($P<0.05$). In HK2 cell, the expression of caspase-3 was reduced in CsA+CoCl2 (CsA, 1.3±0.3; CsA+CoCl2, 0.4±0.1; $P<0.05$). Also, caspase-3 activity was decreased in both CsA+CoCl2 and CsA+DMOG ($P<0.001$). The amount of vimentin was decreased in CsA+CoCl2 (1.8±0.1 vs. 1.2±0.1, $P<0.05$). In addition, rats treated with CoCl2 or DMOG showed the improvements of arteriolopathy (CsA vs. CoCl2,

CsA vs. DMOG, P<0.05) and tubulointerstitial fibrosis (CsA vs. DMOG; P<0.05). The ratio of p NFκB p65 and NFκB p65 was decreased in CsA+CoCl2 (1.6±0.6 vs. 0.5±0.3, P<0.05) in HK2 cell. The number of infiltrating macrophages were reduced by CoCl2 and DMOG treatment (CsA vs. CoCl2, CsA vs. DMOG P<0.05).

Conclusions: Activation of HIF by $CoCl_2$ or DMOG attenuates renal injury by inhibition of inflammation, fibrosis and apoptosis.

FR-PO1153

Induction of Constitutively Active TGFβ Receptor 1 in Kidney Tubules Causes Epithelial Damage with Inflammatory Leukocyte Infiltration and a Switch in Resident Dendritic Cell Phenotype Madeleine E. Gentle, Shaolin Shi, Erwin P. Bottinger, Detlef O. Schlondorff. *Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Expression of constitutively active TGFβ Receptor 1 (TβR1) in tubular epithelia, using a doxycycline inducible system under the control of the Pax8 promoter, results in tubular cell apoptosis, progressive tubular damage and tubulo-interstitial fibrosis [Shi S et al., ASN2010, free communication].

Methods: To define the role of inflammation in this process we evaluated inflammatory infiltrates by immuno-fluorescence and flow cytometry.

Results: We demonstrate that induction of TβR1 activity in the tubules is associated with a progressive (3-7 days) and widespread tubulo-interstital leukocyte infiltration, including macrophages, T cells and dendritic cells (DC). T cell but not macrophage recruitment was inhibited by mitoTEMPO a scavenger of mitochondrial ROS. Overall DC number did not change following induction of tubular damage, but the predominant DC subtype switched from ~80% F4/80+ CD11c+ DC in the healthy kidney to ~ 60% F4/80- CD11c+ DC following induction of tubular TβR1 signaling.

Conclusions: Mitochondrial ROS is required for T cell recruitment but, surprisingly is dispensable for macrophage infiltration. DC play a key role in activating T cells and shaping subsequent adaptive immune responses. In view of the switch in DC phenotype observed upon disease induction it will be of interest to study the relative contributions of each DC population in disease progression in this model of progressive tubular damage.

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FR-PO1154

Human Proximal Tubule Epithelial Cells Modulate Autologous Dendritic Cell Function Andrew J. Kassianos, Xiangju Wang, Kerry (Kathrein) E. Roper, Helen G. Healy, Ray Wilkinson. *Conjoint Kidney Research Laboratory, Department of Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Australia.*

Background: Proximal tubule epithelial cells (PTEC) participate in the disease process in many human kidney diseases. We have recently demonstrated the capacity of activated PTEC to inhibit autologous immune responses (Wilkinson et al, NDT 2011, 26(5):1483-92). In order to further define this regulatory mechanism, we monitored for the first time activated PTEC interactions with autologous dendritic cells (DC), professional antigen-presenting cells that play a pivotal role in the induction and regulation of immune responses.

Methods: Primary PTEC and peripheral blood mononuclear cells were collected from patients undergoing nephrectomies. Purified $CD1c^+$ blood DC were cultured with autologous IFNγ-activated PTEC in the presence or absence of DC activator, polyinosinic:polycytidylic acid (PIC). DC responses were monitored by cytokine secretion, surface antigen expression and antigen-presenting ability.

Results: Unstimulated CD1c+ DC upregulated surface CD40 and CD86 and secreted higher levels of IL-6 and IL-8 in the presence of PTEC compared to DC alone, with minimal changes to CD80, CD83 and HLA-DR expression and no or low levels of IL-1β and IL-10 detected in any culture supernatants. PIC-stimulated CD1c+ DC expressed higher CD40 and CD86, produced elevated IL-6 and IL-1β and lower levels of IL-10 in the presence of PTEC than PIC-stimulated CD1c+ DC alone. Notably, PIC-stimulated CD1c+ DC displayed reduced CD83 levels in all donors and lower expression of HLA-DR in 4/5 donors when cultured with PTEC, with 3/4 of these donor DC inducing lower proliferation of allogeneic T cells in a mixed lymphocyte reaction (MLR) compared to PIC-stimulated CD1c+ DC alone. These results highlight a novel immuno-regulatory role for activated PTEC through inhibition of DC function.

Conclusions: Our data suggest that activated PTEC regulate human autologous immunity via complex interactions with DC. Further dissection of the mechanism of PTEC modulation of autologous immune responses may offer targets for therapeutic intervention in renal medicine.

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FR-PO1155

Suppression of Inflammatory Cytokines-Triggered NFκB Activation and iNOS Expression in Renal Tubular Epithelial Cells by Gap Junction Inhibitor Flufenamic Acid: A Critical Involvement of AMPK Yuan Chi, Qiaojing Yan, Ying Zhu, Masanori Kitamura, Jian Yao. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Background: Our previous studies demonstrated that dysfunction of gap junctions (GJs) prevents renal tubular cell injury in several pathological situations (*Br J Pharmacol 2010, 160:2055-68; Antioxid Redox Signal 2011, 14:2427-39*). Because the cytokines-elicited expression of iNOS and production of nitric oxide (NO) contribute to the renal tubular cell injury, we asked whether GJ inhibitors also interfere with the inflammatory responses.

Methods: NFκB activation was evaluated by using NRK/NFκB-SEAP reporter cells. NO was measured by the Griess reagent. Ca^{2+} was measured by using Fura-2 as an indicator. AMPK activation was evaluated by Western blot analysis of phosphorylation levels of AMPK at Thr-172. CAMKKβ was downregulated by siRNA.

Results: 1) Exposure of renal tubular cell line NRK-E52 to inflammatory mediators TNFα and IL-1β resulted in an NFκB-dependent expression of iNOS and production of NO. 2) Among several GJ inhibitors tested, flufenamic acid (FFA) strongly inhibited NFκB activation and suppressed the expression of iNOS and production of NO. In addition, it also inhibited MCP-1 expression. 3) This effect of FFA was mimicked by AMPK activator AICAR and abolished by CAMKKβ inhibitor STO-609, indicating a mediating role of CAMKKβ-AMPK pathway. 4) FFA induced an Ca2+-dependent activation of AMPKα. Indeed, FFA triggered a cyclosporine-preventable rise in intracellular Ca2+ and activation of CAMKKβ. Inhibition of CAMKKβ with specific inhibitor STO-609 or downregulation of CAMKKβ with specific siRNA completely abolished the FFA-induced activation of AMPK.

Conclusions: Taken together, we concluded that GJ inhibitor FFA potently suppressed the inflammatory cytokines-elicited activation of NFκB and expression of iNOS in renal tubular epithelial cells through activation of Ca^{2+}-CAMKKβ-AMPK pathway. FFA may be therapeutically employed for intervention of inflammatory tubular cell injury through suppression of inflammatory responses and disruption of gap junction-mediated propagation of cell death.

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FR-PO1156

Characterization and Localization of CD11c+F4/80+ Mononuclear Phagocytes in Healthy and Diseased Kidney Qi Cao, Junyu Lu, Changqi Wang, Vincent W.S. Lee, Ya Wang, Thian Kui Tan, Guoping Zheng, Yiping Wang, David C. Harris. *Centre for Transplant and Renal Research, Westmead Millennium Institute, Sydney, NSW, Australia.*

Background: Kidney tubulointerstitium contains resident macrophages (kMΦ) and dendritic cells (kDCs). Distinguishing between kMΦ and kDCs relies on specific cell-surface markers: CD11c the most reliable marker for DC and F4/80 for macrophages. However, a group of kidney cells has been found to express both dendritic and macrophage markers, but their function and distribution in kidney are unknown. In this study, function and localization of CD11c+F4/80+ cells, kMΦ and kDCs were examined in healthy and diseased kidney.

Methods: Adriamycin nephrosis (AN) was induced by 10 mg/kg adriamycin in BALB/c mice. Localization and function (phagocytosis and antigen presentation) of CD11c+F4/80+ cells, CD11c-F4/80+ kMΦ and CD11c+F4/80- kDCs were examined in normal and AN mice.

Results: CD11c+F4/80- kDCs comprised 0.6% of total kidney cells, CD11c+F4/80+ cells 2% and CD11c-F4/80+ kMΦ 1.6%. CD11c+F4/80- kDCs and CD11c+F4/80+ cells were mainly present in cortex of normal and AN kidney, while CD11c-F4/80+ kMΦ were present in cortex and medulla. CD11c+F4/80+ cells highly expressed macrophage markers including CD68, CD204 and CD206, but had lower expression of DC markers, including CD205 and CD103. Interestingly CD11c+F4/80+ cells from kidneys of mice with or without AN had a higher capability of phagocytosis and lower ability of antigen presentation than CD11c+F4/80- kDCs, indicating that CD11c+F4/80+ cells are more like macrophages.

Conclusions: CD11c+F4/80+ mononuclear phagocytes have high capability for phagocytosis and low for antigen presentation, indicating they should be defined as a subset of kidney macrophages. The function of these cells will be examined *in vivo* by depletion and reconstitution studies.

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FR-PO1157

Inflammasome Activation and Processing of IL-1β and IL-18 in Experimental Crescentic Glomerulonephritis in the Rat Simona Deplano,[1] Jennifer Smith,[1] Charles D. Pusey,[1] Robert J. Unwin,[2] Frederick W.K. Tam,[1] H. Terence Cook,[1] Jacques Behmoaras.[1] *[1]Renal Medicine, Imperial College London; [2]UCL Medical School, London, United Kingdom.*

Background: Inflammasomes are molecular platforms activated by cellular infection or stress, which trigger the release of active proinflammatory cytokines such as IL-1β and IL-18 through caspase-1 activation. The role of caspase-1 dependent inflammasome activation in crescentic glomerulonephritis (Crgn) is unknown.

Methods: We studied inflammasome activation in the macrophage-dependent model of nephrotoxic nephritis (NTN) in the Wistar-Kyoto (WKY) rat. Activation of Nlrp3 and AIM2 inflammasomes was studied in bone-marrow derived macrophages (BMDMs) isolated from WKY and NTN-resistant Lewis (LEW) rats. Ex-vivo investigation of the inflammasome activation was performed in cultured glomeruli isolated from WKY and LEW kidneys 4 days following NTN induction, a time point corresponding to maximal macrophage infiltration.

Results: We found that the majority of the Nlrp3-inflammasome genes are up-regulated in WKY BMDMs compared with LEW. Lipopolysaccharide (LPS) primed WKY BMDMs produced significantly higher levels of active IL-1β and IL-18 when stimulated with ATP compared with LEW. Similarly, the activation of the AIM2-inflammasome following LPS and poly(dA:dT) treatment resulted in increased IL-1β secretion in WKY BMDMs, suggesting that the increased IL-1β production is due to a dysregulated caspase-1 activity in the macrophages of this rat strain. This was indeed the case as we showed increased caspase-1 activity in WKY BMDMs when compared with LEW, and caspase-1 dependant

IL-1β and IL-18 production was significantly reduced following a specific caspase-1 inhibitor and after caspase-1 siRNA treatment. Importantly, we showed markedly increased active IL-18 and IL-1β production in WKY nephritic glomeruli, together with significantly increased caspase-1 activity suggesting that caspase-1 dependent inflammasome activation is driven by infiltrating macrophages in NTN.

Conclusions: This is the first report showing inflammasome activation in macrophage-dependant Crgn. These results reveal novel genetic factors controlling susceptibility to Crgn.

FR-PO1158

Proinflammatory Role of the Inflammasome Component Nlrp3, but Not Asc in Murine Immune Complex-Mediated Glomerulonephritis Kirstin Andersen, Nuru Eltrich, Volker Vielhauer. *Nephrologisches Zentrum, Ludwig-Maximilians-University, Munich, Germany.*

Background: Interleukin-1β (IL-1β) is an inflammatory mediator of immune complex-induced glomerulonephritis (GN). Caspase 1 activates IL-1β in an inflammasome-dependent intracellular process. Thus, we examined the functional role of the inflammasome components Nlrp3 and its adapter molecule Asc in autologous murine nephrotoxic serum nephritis (NTN).

Methods: NTN was induced in wild-type, Il1r1-, Nlrp3-, and Asc-deficient C57BL/6 mice after preimmunisation with rabbit IgG. At day 21 functional parameters, renal histology and renal leukocyte infiltrates were compared between the four groups and untreated wild-type controls. In addition, cellular and humoral immune responses against rabbit IgG were analysed.

Results: NTN was not inducible in Il1r1-deficient mice, confirming a crucial role of IL-1 signaling in this model. Nlrp3-deficient mice developed a less pronounced nephrotic syndrome compared to wild-type, including reduced albuminuria, less hypoproteinemia, and a tendency towards lower urea levels. Consistently, renal leukocyte infiltrates were significantly reduced in Nlrp3-/- mice. This correlated with a decrease in renal mRNA expression of inflammatory chemokines and cytokines. In contrast, NTN was not attenuated in Asc-/- mice.

Systemic immune responses were examined after restimulation of splenocytes with rabbit IgG. Nlrp3-/- splenocytes revealed a tendency towards lower INF-γ production and reduced numbers of activated CD69+ CD8+ T cells. Interestingly, the humoral immune response was increased in Nlrp3-/- mice, as indicated by higher autologous anti-rabbit IgG serum titers. Despite developing NTN, Asc-/- mice also demonstrated an attenuated T cell response, but similar anti-rabbit IgG levels compared to wild-type.

Conclusions: We identified Nlrp3 as an important pro-inflammatory mediator of immune complex GN. Surprisingly, we could demonstrate an inflammatory function of Nlrp3 independent of its adapter molecule Asc and the inflammasome, although deficiency of both molecules reduced cellular immune responses against the foreign antigen planted in the glomerulus. Thus, Nlrp3, but not Asc may be a new therapeutic target for immune complex GN.

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FR-PO1159

Accelerated Necrotizing Glomerulonephritis in Response to Nephrotoxic Serum in Mpv17-Deficient Mice Gabriella Casalena, Dmitrij Kollins, Ilse S. Daehn, Madeleine E. Gentle, Erwin P. Bottinger, Detlef O. Schlondorff. *Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Human MPV17 mutations cause hepatocerebral forms of mitochondrial DNA (mtDNA) depletion syndrome. Insertional deletion of Mpv17 in mouse was initially associated with glomerulosclerosis [Weiher H et al., Cell 1990]. Mpv17 is a mitochondrial protein with unknown function, but may be involved in control of mitochondrial DNA copy number and ROS production. MtDNA copy number was reduced in glomerular tufts of Mpv17-/- mice that manifested reduced lifespan and kidney failure [Viscomi C et al. Hum Mol Genet 2009].

Purpose: To determine whether Mpv17-deficiency alters lesions and manifestations of necrotizing glomerulonephritis (GN) typically associated with nephrotoxic serum nephritis model in mice.

Methods: Mpv17+/+ and Mpv17-/- mice (C57BL/6J inbred background) were injected with sheep nephrotoxic serum and sacrificed after one or seven days.

Results: Tissue oxidative stress assessed by 3-Nitrotyrosine IHC was increased at baseline in glomeruli of Mpv17-/- compared to Mpv17+/+ mice. Day 1 after NTS injection, PAS positive staining was apparently increased in Mpv17-/- but not in Mpv17+/+ mice, compared to controls. By day 7, glomerular lesions were present in all Mpv17-/- and increased compared to Mpv17+/+. Tubular casts were detectable only in Mpv17-/- mice. Serum creatinine was increased in Mpv17-/- compared with Mpv17+/+ (0.74 +/- 0.01 vs 0.48 +/- 0.09; $p<0.05$). ACR was not significantly different. Quantification of infiltrates by staining with anti-CD45 antibody showed no differences between Mpv17+/+ and Mpv17-/- at day 7 after NTS injection.

Conclusions: Loss of mitochondrial membrane protein Mpv17 was associated with increased baseline glomerular oxidative stress, and accelerated the onset and increased the severity of necrotizing GN induced by NTS

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FR-PO1160

Leukocyte Syk Activation in Antibody-Dependent Glomerular Injury Jessica Ryan, John Kanellis, David J. Nikolic-Paterson. *Department of Nephrology and Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.*

Background: Spleen tyrosine kinase (Syk) plays an important role in Fc-γ receptor signaling. Recent clinical trials using an Syk inhibitor have shown protection against joint damage in rheumatoid arthritis. However, the potential role of Syk in acute antibody-dependent glomerular disease has not been investigated. Therefore, we examined activation of Syk in human rapidly progressive glomerulonephritis by detecting phosphorylation of Tyr525/526 in the Syk activation loop.

Methods: Immunostaining for phosphorylated-Syk (p-Syk) was performed in renal biopsy sections in cohort of 68 patients, which included: MCD (3), TBMD (8), post-infectious glomerulonephritis (5), Class IV SLE (11), and ANCA vasculitis (9), IgAN (14), membranous nephropathy (7) and FGS (10).

Results: No p-Syk staining was seen in minimal change or thin membrane disease which lack antibody deposition and leukocytic infiltration. In contrast, all cases of post-infectious glomerulonephritis exhibited numerous p-Syk+ cells in glomeruli. Two-colour staining identified p-Syk+ cells as infiltrating neutrophils, and to a lesser extent, macrophages. Furthermore infiltrating leukocytes were also positive for phopspho-p38 and phospho-JNK, known downstream targets of Syk signalling, suggesting Syk-dependent leukocyte activation in post-infectious glomerulonephritis. Glomerular p-Syk+ cells were seen in 8/9 cases of ANCA vasculitis, 7/11 cases of Class IV SLE and 6/14 cases of IgAN. In these diseases, p-Syk staining was also localised to infiltrating neutrophils and possibly to some monocyte/macrophages. In addition, 1/7 cases of membranous and 2/10 cases of FGS exhibited glomerular p-Syk positive cells in areas of glomerulosclerosis. Furthermore, in some cases infiltrating p-Syk+ cells were also seen in areas of interstitial fibrosis and tubular atrophy.

Conclusions: Syk is activated in infiltrating leukocytes, predominantly neutrophils, in acute antibody-dependent glomerular disease. These findings support the therapeutic use of Syk inhibitors in rapidly progressive crescentic glomerulonephritis.

Funding: Other NIH Support - NHMRC Australia

FR-PO1161

Decreased Plasma RANTES Concentration in Children with Minimal Change Nephrotic Syndrome in Relapse Is Associated with Th2 Cytokine Profile Chang Yien Chan,[1] Wee Song Yeo,[1] Kar Hui Ng,[1] Subhra K. Biswas,[2] Hui Kim Yap.[1] *[1]Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [2]SIgN, A*STAR, Singapore.*

Background: Cytokine release secondary to viral infections may potentially trigger relapses in minimal change nephrotic syndrome (MCNS). This study aimed at investigating the cytokine profile in MCNS patients during relapse and remission, in order to enhance our understanding of disease pathogenesis.

Methods: Plasma cytokine profile was analyzed using multiplex suspension bead array system in 13 nephrotic children, aged 4 to 25 years with steroid-sensitive nephrotic syndrome in relapse and remission. Thirty-two age-matched healthy controls were included for comparison. Results were expressed as mean±SEM. Statistical analyses was done using a linear mixed model to compare the differences in cytokine levels between MCNS patients and age-matched controls. Wilcoxon signed rank test was used for paired analysis.

Results: As shown in the table below, of the 27 cytokines analyzed, plasma IL-5, IL-9, IL-10, and IL-13 were significantly increased and RANTES concentration was significantly decreased in MCNS relapse compared to age-matched controls ($p<0.05$). There was no significant difference in the concentrations of these cytokines between MCNS remission and controls. However, pairwise comparison showed that only RANTES concentration were significantly lower in patients in relapse compared to remission ($p=0.023$).

Cytokine	Controls (n=32)	MCNS Relapse (n=13)	MCNS Remission (n=13)
IL-5	3.78±0.38	5.09±0.71	5.89±0.72[1]
IL-9	12.7±1.23	18.6±4.14	22.9±4.07[1]
IL-10	1.25±0.24	2.00±0.54	3.77±0.58[2]
IL-13	4.27±0.40	5.42±0.47	6.54±0.64[1]
RANTES	390.6±51.6	231.6±25.6	172.5±14.6[2 3]

[1]$p<0.05$, comparing MCNS relapse with normal controls; [2]$p<0.01$, comparing MCNS relapse with normal controls; [3]$p<0.03$, comparing MCNS relapse with remission (paired)

Conclusions: In conclusion, the findings of increased IL-5, IL-9, IL-10 and IL-13 production during relapses in children with MCNS further support Th2 polarization in this disease. Decrease in RANTES production, a Th1 chemokine normally induced by viral infections, further confirms the immune dysregulation with Th2 polarization associated with relapses of MCNS.

Funding: Government Support - Non-U.S.

FR-PO1162

Effects of Everolimus on Proteinuria and Expression of Slit Diaphragm Proteins in Experimental Nephrotic Syndrome Rawi Ramadan,[1] Hoda Awad,[2] Zaid Abassi.[2] *[1]Nephrology, Rambam Medical Center, Haifa, Israel; [2]Physiology and Biophysics, Faculty of Medicine-Technion, Haifa, Israel.*

Background: Everolimus, a mTOR inhibitor, is used as a potent immunosuppressant in renal transplantation. Although various serious side effects of Everolimus have been described, including renal injury and proteinuria, other studies have demonstrated beneficial renal effects of the drug. Therefore, the aim of this study was to examine the effects of

different doses of Everolimus given as either early or late treatment on proteinuria and slit diaphragm proteins in adriamycin (ADR)-induced experimental nephrotic syndrome (NS).

Methods: Low or high dose of Everolimus (20 or 100 mg/L via drinking water) was administered to NS rats, beginning either 3 days prior to NS induction (early treatment) or 2 weeks after the induction of NS (late treatment). Daily and cumulative urinary protein excretion (UprV) were determined throughout the treatment period, which lasted 6 weeks. Moreover, the effects of Everolimus on GFR and key slit diaphragm proteins, namely nephrin and podocin, were assessed at the end of the study.

Results: While the low dose of Everolimus resulted in therapeutic plasma concentration of 4.9±0.6, the high dose yielded supra-pharmacological plasma level of 21.3±4.4 ng/ml. As expected, ADR administration induced gradual significant increase in daily and cumulative UprV, in association with glomerular injury as presented by decrease in nephrin and podocin abundance. Low dose of Everolimus as early, and to a lesser extent as late treatment, reduced UprV and increased plasma albumin levels. These beneficial effects of low dose Everolimus were associated with improvement in GFR and substantial preservation of glomerular podocin and nephrin immunoreactivities. In contrast, high dose of Everolimus aggravated renal dysfunction and did not preserve nephrin/podocin expression. However, protein excretion in NS rats treated with the high Everolimus dose was eventually reduced secondarily to its deleterious effects on GFR.

Conclusions: Our study indicates that Everolimus possesses antiproteinuric effect at a therapeutic dose, whereas at a high dose it aggravates pre-existing glomerular injury.

Funding: Pharmaceutical Company Support

FR-PO1163

Angiotensin Receptor Blocker Ameliorates Obesity-Induced Albuminuria and Inflammation and Modulates Adipose Macrophage Polarization

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Background: Activation of the angiotensin type 1 (AT1) receptor is implicated in the pathogenesis of both obesity and CKD. We investigated the effects of angiotensin receptor blocker (ARB) on obesity-induced albuminuria, adipose tissue macrophage polarization and inflammation.

Methods: WT mice (age 8-10 wks) were fed high-fat diet (HFD) for 24 weeks with or without ARB (HFD, n=5; or HFD+ARB, losartan 80 mg/L DW, n=6) and compared to mice fed normal chow (NC, n=5). Metabolic parameters, urine albumin/creatinine ratio (ACR) were assessed at intervals, and expression of adipose tissue M1 and M2 macrophage markers was assessed by qPCR. Data are expressed as mean±SE.

Results: HFD induced albuminuria, and ARB treatment significantly reduced this parameter (HFD 213.0±44.3, HFD+ARB 99.6±15.1 μg/mg, $p<0.05$). ARB treated mice on HFD also had significantly lower body weight (NC 28.5±0.7, HFD 56.0±3.3, HFD+ARB 41.5±4.0 g, $p<0.01$), body fat percentage (NC 11.6±1.6, HFD 33.7±1.5, HFD+ARB 17.5±2.5 %, $p<0.01$), blood glucose (HFD 191.7±10.3 vs HFD+ARB 140.5±8.4 mg/dl, $p<0.05$) and plasma insulin (HFD 6.8±0.6 vs HFD+ARB 3.0±0.8 ng/ml, $p<0.05$) vs HFD alone although food intake was comparable (HFD 2.6±0.4 vs HFD+ARB 2.6±0.2 g/day/mouse). ARB partially restored obesity-associated decrease in muscle mass (% of muscle mass/body weight: NC 73.1±0.9, HFD 59.5±1.2, HFD+ARB 72.3±2.3, $p<0.05$) and plasma adiponectin (NC 17.4±1.5, HFD 10.9±0.2, HFD+ARB 12.6±0.5 μg/ml, $p<0.05$). ARB abolished obesity-induced adipose tissue macrophage M1 markers (IL-1β mRNA/18srRNA: NC 1.0±0.2, HFD 1.5±0.1, HFD+ARB 1.0±0.2; MCP-1 mRNA/18srRNA: NC 0.6.±0.1, HFD 2.4±0.1, HFD+ARB 0.9±0.1, $p<0.01$ HFD+ARB vs HFD respectively) but further enhanced adipose tissue macrophage M2 markers (Ym-1 mRNA/18srRNA: NC 1.7±0.3, HFD 8.6±2.0, HFD+ARB 28.4±7.6, $p<0.05$ HFD+ARB vs HFD).

Conclusions: ARB protects against obesity and obesity-induced albuminuria, improves adipose metabolism and adipose tissue inflammation, linked to modulation of adipose tissue macrophage polarization. Our data suggest ARB is effective in treatment of obesity-related CKD.

FR-PO1164

Mechanisms of HO-1 Mediated Attenuation of Proteinuria: A Gene Profiling Study

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Background: Using a mouse model of proteinuria induced by administration of antibody (Ab) against the glomerular basement membrane (anti-GBM), and a model of proteinuria induced by administration of the glomerular epithelial cell (GEC) toxin, adriamycin, we assessed the effect of GEC-targeted expression of the cytoprotective enzyme heme oxygenase (HO)-1. A human (h) HO-1 cDNA sequence was targeted to glomerular epithelial cells (GEC) using a GEC-specific murine nephrin promoter. Specifically, we employed a 4.125-kb fragment of a mouse nephrin promoter downstream to which a FLAG-tagged hHO-1 cDNA sequence was inserted and subsequently generated transgenic mice from the FVB/N parental strain. GEC-specific targeting of hHO-1 was verified by immunalocalization of the FLAG-tagged transprotein. Administration of anti-GBM Ab or Adriamycin to transgenic (TG) mice with GEC-targeted hHO-1 induced comparable degrees of proteinuria. Protein excretion was attenuated in transgenic (Tg) mice with anti-GBM Ab or Adriamycin-induced proteinuria compared to wild type (WT) controls. To explore changes in expression of genes that could mediate this salutary effect, we performed gene expression profiling using microarray analysis of RNA isolated from the renal cortex of WT or TG mice with or without anti-GBM antibody induced proteinuria. Significant increases in expression were detected in nine MHC-class II genes, two IFN-γ inducible GTPases and three genes of the ubiquitin-proteasome system. The increase in MHC-class II and proteasome gene expression in TG mice with injury was validated by real time PCR or Western blot analysis.

Conclusions: The observations point to candidate mechanisms whereby GEC-targeted HO-1 over-expression attenuates proteinuria.

FR-PO1165

Hyperglycemia Promotes the Progression of HI-Associated Nephropathy

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Background: After the advent of highly active retroviral therapy patients with HIV infection are living almost a normal life. Thus, they are equally prone to develop diabetes mellitus and its associated complications. We hypothesized that development of hyhperglycemia in HIVAN (HIV-associated nephropathy) patients would exacerbate the progression of HIVAN. In the present study, we evaluated the effect of hyperglycemia on the progression renal lesions in a mouse model of HIVAN (Tg26).

Methods: Four weeks old Tg26 mice develop proteinuira and renal lesions. Four weeks old control (C) and Tg26 (HIV) mice (n=4) were either administered vehicle or streptozotocin (STZ, 150 mg/Kg, intraperitoneal, one dose). At the end of two weeks, mice were sacrificed. Blood and urine were collected and kidneys were harvested for renal histology, imunohistochemical, and immunoblotting studies. Renal tissues were probed for occurrence of oxidative stress and the activation of the renin-angiotensin system (RAS) by immunoblotting for angiotensinogen expression and measurement of Ang II production by ELISA.

Results: STZ-treated mice (C-STZ) developed higher proteinuria vs. control (C) mice (C, 0.3 ± 0.08 vs. C-STZ, 3.1 ± 1.0 g/gm creatinin; $P<0.01$); STZ-treated Tg26 mice (HIV-STZ) displayed greater proteinuria vs. vehicle-treated Tg26 mice (C-HIV) (HIV-STZ, 24.0 ± 0.8 vs. C-HIV, 15.7 ± 1.1 gm/gm creatinine; $P<0.01$). There was no difference in BUN levels between control and C-STZ; HIV-STZ displayed higher BUN levels when compared to C-HIV (C-HIV, 48 ± 2 vs. HIV-STZ, 59 ± 5 mg/dl; $P<0.05$). Although there was no difference in the severity of glomerular and tubular lesions between HIV-STZ and C-HIV but renal tissues of HIV-STZ displayed enhanced oxidative stress and the activation of the RAS.

Conclusions: Development of diabetes mellitus has potential to adversely effect the progression of HIVAN.

Funding: NIDDK Support

FR-PO1166

Urine Exosomes Maintain a Sterile Renal Tract by Inducing Bacterial Lysis

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Background: Exosomes are nanovesicles released by many cell types, and may play roles in cell-cell signaling, antigen presentation or apoptosis. Urine exosomes released by the kidney have no known function, are considered waste, and studies to date have focused on biomarker discovery. We asked if urine exosomes were functional.

Methods: Exosomes were isolated from second morning urine samples from healthy volunteers by differential centrifugation. Exosomal proteins were characterised by LC-MS/MS coupled with novel data-analysis and bioinformatic workflows, and the resulting protein catalogue interrogated for functional enrichment. Proteins with putative functional roles were confirmed by Western blot (WB) and electron microscopy (EM).

Growth of luciferase-expressing laboratory and clinical strains of E. coli and S. aureus was assessed by luminometry, and the morphological effects of exosomes on E. coli studied by EM.

Results: We identified a catalogue of proteins significantly enriched for those involved in innate immunity and bacterial killing ($p = 0.0004$), and confirmed their presence on exosomes by WB and immuno-EM. Intact exosomal protein fractions, but not the abundant urinary protein uromodulin, potently and dose-dependently inhibited the growth of laboratory and clinical isolates of E. coli and S. aureus. Incubation of uropathogenic E coli with urinary exosomes, but not with vehicle alone, resulted in bacterial lysis ($p < 0.0001$).

Conclusions: Urine exosomes are enriched for bacteriocidic and -static proteins, inhibit bacterial growth and induce lysis of uropathogenic bacteria. We propose a novel model of host defence whereby Trojan decoy exosomes are released from the kidney to maintain urinary tract sterility, by delivery to invading organisms of packaged defence proteins.

Funding: Private Foundation Support

FR-PO1167

HD5 Expression in the Human Kidney and Urinary Tract John D. Spencer, Andrew L. Schwaderer, David S. Hains. *Division of Nephrology, Research Institute at Nationwide Children's Hospital, Columbus, OH.*

Background: Urinary tract infections (UTI) are a common bacterial infection. Despite constant exposure to microbes, the urinary tract is usually sterile. Prior studies have indicated that the epithelial antimicrobial peptide (AMP), Human α-defensin 5 (HD5), contributes to the defense of the Gl and reproductive tracts. HD5's role in the kidney and urinary tract is unknown. This study was designed to characterize gene and protein expression of HD5 in the human kidney and urinary tract.

Methods: *Gene expression*: We isolated RNA from non-infected human kidney, pyelonephritic kidney, and bladder tissue and quantified *HBD5* using real-time PCR. *Protein expression*: HD5 expression was localized using immunohistostaining. To examine HD5 expression in the urine, we developed a sandwich ELISA and normalized urinary HD5 concentrations to mg of urine creatinine (UCr).

Results: *Gene expression:* Constitutive HD5 mRNA expression was detected in human kidney and bladder tissue. Absolute real-time PCR quantification revealed HD5 expression of 3583±532 HBD5/10ng total RNA in non-infected renal cortex, medulla, and pelvis. Expression did not vary by location within the kidney. With pyelonephritis, mean HD-5 expression increased to 6208±1056ng HD5/10ng total RNA ($p<0.04$). Protein expression: Immunohistochemistry localized HD5 to the urothelium of the bladder and ureter. In the kidney, expression was primarily in the collecting tubule and loop of Henle. With pyelonephritis, HD5 expression increased throughout the nephron and collecting duct. HD5 was not detected in non-infected human urine samples while mean HD5 expression increased to 27.07±4.67 μgHD5/mg UCr with UTIs ($p<0.01$).

Conclusions: Our results characterize the expression of HD5, a novel human urinary tract AMP. HD5 is expressed in the kidney, ureter, and bladder. HD5 expression and production increase and become detectable in the urine with infection making this molecule a potentially useful biomarker to augment our current urine dipsticks for rapid diagnosis of UTIs.

FR-PO1168

Ribonuclease 7: An Upregulated Antimicrobial Peptide during Urinary Tract Infections John D. Spencer, Andrew L. Schwaderer, David S. Hains. *Division of Nephrology, Research Institute at Nationwide Children's Hospital, Columbus, OH.*

Background: Although the urinary tract is constantly challenged by microbial invasion, it remains free from microbial colonization. Recent studies stress the importance of antimicrobial peptides (AMP) in preventing UTIs. Our lab has previously shown that Ribonuclease 7 (RNase7) is a potent AMP that contributes to urinary tract sterility. At baseline, RNase 7 is expressed in intercalated cells in the renal parenchyma and is secreted in the urine at levels sufficient to kill bacteria. This study was designed to characterize expression, production, and function of RNase7 in the urinary tract during infected states.

Methods: *Gene expression:* We isolated RNA from non-infected and pyelonephritic human kidney tissue and quantified *RNASE7* using real-time PCR. *Protein expression*: RNase7 expression was localized using immunofluorescence (IF). We developed a sandwich ELISA and normalized urinary RNase7 to mg of urine creatinine (UCr) in infected and sterile urine. *RNase7 function*: To evaluate the antimicrobial function of RNase7 on the microbial membrane of uropathogenic bacteria, we used atomic force microscopy (AFM).

Results: *Gene expression:* Absolute quantification of RNASE7 mRNA expression revealed that RNASE7 expression increased from 1028±149ng RNASE7/10ng total RNA in non-infected kidney tissue to 2927±590ng RNASE7/10ng RNA in kidneys with pyelonephritis ($p<0.04$). Protein expression: In infected kidney tissue, RNase7 expression was expanded to include the proximal nephron along with intercalated cells. Urinary RNase7 production increased with UTIs from a mean urinary RNase7 expression of 8.69±0.38μg/mg UCr to 12.53μg/mg UCr during infection ($p<0.02$). RNase7 function: Using AFM, we developed a proof-of-principle study to show that 2.5μM of RNase7 disrupts the microbial membrane of uropathogenic E.coli, Pseudomonas, and Enterococcus, causing cell death.

Conclusions: RNase7 is a potent AMP in the urinary tract. Our results indicate that RNase7 expression and production increases with infection. The functional relevance of increased RNase7 production was demonstrated by disruption of the microbial cell wall of common uropathogenic bacteria.

FR-PO1169

Lanthanum Carbonate Reduces Cumulative Oxalate Absorption and Prevents Nephrocalcinosis after Oxalate Loading in Rats Stef Robijn, Benjamin Arthur Vervaet, Patrick C. D'Haese, Anja Verhulst. *Laboratory of Pathophysiology, University of Antwerp, Belgium.*

Background: Hyperoxaluria is a risk factor for calcium oxalate (CaOx) nephrolithiasis/-calcinosis. Lanthanum carbonate (LC) is used as intestinal phosphate binder in dialysis patients to prevent hyperphosphatemia. As previously shown *in vitro* in our laboratory, lanthanum also has the ability to bind oxalate (Ox) in the pH range of the intestine. To evaluate *in vivo* intestinal Ox binding capacity of lanthanum, we investigated in rats whether LC is able to reduce Ox absorption and to prevent nephrocalcinosis (NC).

Methods: To investigate Ox absorption kinetics, 12 male Sprague-Dawley (SD) rats were divided into 2 groups: a group (n=6) receiving 1000 mg LC followed by 2 mmol Ox (2x molar La/Ox ratio) and a control group (n=6) receiving carboxymethylcellulose (VEH) followed by the same Ox dose by gavage. Serum Ox levels were measured at baseline, 30min and every hour up to 12h after gavage. Oxaluria was measured starting at 4h after Ox loading.

To evaluate the effect of LC on the development of NC, 26 male SD rats were divided into 2 groups: a group (n=13) receiving 1000 mg LC followed by 5 mmol Ox (0.8x molar La/Ox ratio) and a control group (n=13) receiving VEH followed by the same Ox dose by daily gavage for 7 consecutive days. After sacrifice, degree of NC was assessed on Von Kossa stained kidney sections and by renal Ca analysis as a measure of NC.

Results: Ox loading resulted in a biphasic pattern of transiently increased serum Ox levels in controls, which was almost completely abolished in LC treated animals. Lower serum Ox levels sustained in LC treated animals during the study period, resulting in a significantly reduced cumulative Ox absorption compared to controls. Furthermore, 6h after Ox loading oxaluria was blunted in LC treated animals, resulting in a significantly reduced CaOx crystalluria.

Administration of 1000 mg LC resulted in significantly lower median renal Ca content compared to controls: 0.14 (0.08–3.75) vs 1.12 (0.14–7.46) mg/g renal tissue.

Conclusions: LC significantly reduced cumulative Ox absorption and blunted oxaluria compared to controls. Furthermore the degree of NC was significantly lower in LC treated rats.

Funding: Government Support - Non-U.S.

FR-PO1170

Oxalobacter Formigenes Conditioned Medium Stimulates Oxalate Transport by Human Intestinal Cells Hatim A. Hassan, Donna L. Arvans, Ming Cheng, Mark W. Musch, Eugene B. Chang. *Medicine, University of Chicago.*

Background: The vast majority of kidney stones are composed of calcium oxalate, and minor changes in urinary oxalate affect stone risk. Intestinal oxalate secretion mediated by anion exchanger SLC26A6 plays a crucial role in preventing hyperoxaluria and related kidney stones. The probiotic bacterium Oxalobacter Formigenes (OF) plays a critical role in preventing recurrent calcium oxalate kidney stones. In addition to degrading intraluminal dietary oxalate, OF also interacts with colonocytes by inducing distal colonic oxalate secretion, leading to reduced urinary oxalate excretion. However, the mechanism(s) underlying OF-colon interaction remain(s) unknown. We therefore examined whether OF culture condition medium (CM) affects intestinal oxalate transport using human intestinal Caco2-BBE cells.

Methods: We measured apical [^{14}C]oxalate influx in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity.

Results: Preincubation of Caco2 cells with OF CM (1:50 dilution x 24 hours) significantly stimulated oxalate influx (by >106%) while OF growth medium (OM) has no effect, suggesting that soluble factors in the CM might be responsible for the observed stimulation by modulating the activity of the likely involved transporter(s) {SLC26A6, SLC26A2, and/or SLC26A3}. Importantly, CM from Lactobacillus Acidophilus has no effect on oxalate influx, indicating specificity. TER and incubation medium pH were not affected by the OM and CM. Heat-inactivation of the CM completely abolished the stimulatory effect, indicating that the secreted factor(s) is/are likely to be protein(s) or peptide(s). Pretreatment of the CM with pepsin destroyed the bioactivity of the CM, providing further evidence that the secreted factors are proteins or peptides. Selective ultrafiltration reveals that the secreted factors have a molecular mass between 10-30 kDa. Using real-time PCR, we observed in preliminary experiments that the CM led to a >4.0 fold increase in SLC26A3 mRNA, without affecting SLC26A2 or SLC26A6 mRNA expression.

Conclusions: We conclude that soluble factors from OF activate oxalate transport by Caco2 cells through mechanisms that likely include enhanced SLC26A3 mRNA expression.

Funding: NIDDK Support

FR-PO1171

Culture of Hepatocytes from Primary Hyperoxaluria Type I Liver Sweaty Koul,[1] Hari K. Koul.[1] [1]*Urology-Surgery, University of Colorado School of Medicine, Aurora, CO;* [2]*Nephrology, Mayo Clinic, Rochester, MN.*

Background: PH-I is an autosomal recessive disorder caused by loss of functional AGT enzyme in the liver, which results in excessive production of oxalate, causing recurring stones from childhood on and end stage renal failure. At present a combined liver and kidney transplants are needed for these patients,but quality of life as well as survival are poor. We evalauted fesibility of generating hepatocyte cell cultures from PH-I patients.

Methods: Liver tissue was obtained form surgical waste of PH-I patients . The liver sapmles were perfused with ice-cold Wisconsin solution. The samples were perfused with PBS supplemented with EGTA followed by Collagenase digenstion. The eluted cells were separated from undigested tissue, colloectd, spun down and separated on percol gradient to remove contaminantating debris. Cells were plated on collagen coated tissue culture dishes. Expression of various markers was visualized by immuno fluorescence microscopy. AGT was cloned from HEPG-2 cells and AGT was tagged with GFP to generate GFP-AGT construct. Replication deficient Adv-system eapressing GFP-AGT was generated as a tool to deliver AGT.

Results: We were able to grow hepatocytes from 4 out of 5 patient samples. The primary cultures of PH-1 hepatocytes stop growing after 4-5 passages. Initial pasage contains both hepatocytes as well as fibroblasts. After selecting the cells in epithelial select media, the cells express cyto-keratin but do not express vimentin, characteristics associated with absence of fibroblasts. Transfection of these cells with h-Tert in a reteroviral vector system resulted in a

cell line that we are able to grow to multiple passages. the cells retain cyto-keratin expresion and lack vimention, indicating maintenance of a phenotype. Adv-GFP-AGT resulted in sucessful transfection of all the cells in culture as visualized by GFP expression.

Conclusions: To the best of our knowledge this is the first report describing generation of hepatocytes from PH-1 patient liver and first direct demonstration of sucessful AGT-transfection of PH-1 hepatocytes in culture. These cells should provide a new tool to investigate cellular and alternate therapies against PH-I

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NIH-RO1 DK 54084; UROLITHIASIS: OXALATE RENAL CELL INTERACTIONS

FR-PO1172

Oxalate Absorption Is Independent of Cation Complex Formation Narae Ko,[1] Felix Knauf,[1] William G. Robertson,[2] Peter S. Aronson.[1] *[1]Nephrology, Yale University, New Haven, CT; [2]Physiology Dept, Royal Free and University College Medical School, London, United Kingdom.*

Background: Intestinal oxalate handling plays an important role in overall oxalate balance and risk for calcium-oxalate nephrolithiasis. We have previously shown that epithelial oxalate absorption is largely passive and paracellular through a low capacity, size-independent pathway for large solutes through the tight junction. A prediction of this model is that permeability to oxalate should be independent of its forming soluble complexes with cations.

Methods: Wild-type mouse duodenum was mounted in an Ussing chamber. Apparent permeability to [14C]-oxalate was measured simultaneously with that of [3H]-mannitol. Various oxalate species in the Ringer's buffer were calculated using the SUPERSAT program.

Results: In standard Ringer's buffer, the apparent permeability values for absorption of oxalate and mannitol were essentially identical. It was calculated that 0.79 of the 2.0 micromolar total oxalate in this solution was available as free oxalate, and the rest in soluble complexes predominantly with sodium, magnesium and calcium. The apparent permeability values for oxalate and mannitol were unchanged when the magnesium concentration was raised from 1.2 to 10 micromolar, which was calculated to decrease the free oxalate concentration from 0.79 to 0.33 micromolar as more oxalate was then complexed with magnesium. In contrast to the lack of effect of oxalate speciation on oxalate absorptive flux, the active component of oxalate secretion was inhibited by 80% when the free oxalate concentration was decreased.

Conclusions: We demonstrate that oxalate absorption in mouse intestine is independent of its forming soluble complexes with cations. In contrast, active oxalate secretion is highly dependent on the concentration of free oxalate.

Funding: NIDDK Support, Private Foundation Support

FR-PO1173

Urinary Tract Infection Increases the Risk of Calcium Oxalate Kidney Stone Formation Suchai Sritippayawan,[1] Somkiat Vasuvattakul,[1] Yasushi Nakagawa.[2] *[1]Renal Unit, Siriraj Hospital, Bangkok, Thailand; [2]Division of Biological Sciences, University of Chicago, IL.*

Background: Calcium oxalate(CaOx) can be complicated by urinary tract infection(UTI). There is no evidence about infection-induced CaOx stone formation. This study demonstrated that UTI can favor CaOx stone formation.

Methods: 73 kidney stones from patients with pyuria(urine white cell>5/HD) were analyzed by FTIR. CaOx monohydrate crystal aggregation assay was used to study the inhibitory activity of protein in fasting urine of 28 kidney stone formers(18CaOx, 10uric) and 85 nonstone formers. The assay was modified from the method described by Hess(Am JPhysiol1989;257(1Pt2):F99-106). The rate of aggregation was measured by a linear slope of the decreasing turbidity rate during particle sedimentation. Sample was 1μg of urine protein in <50μl volume. Negative control was buffer solution. Positive control was 1μg of nonstone former Tamm-Horsfall glycoprotein(THPc). Crystal aggregation was displayed as % of linear slope of sample per negative control. Urine protein having %aggregation greater than THPc(65%) was classified as bad inhibitor.

Results: There were 37CaOx, 27carbonate apatite, 6uric acid, 2struvite and 1ammonium urate stones. 85%of them were mixed stone. Stone formers trended to have %aggregation more than nonstone fomers(52±22%vs47±18%). The proportion of stone formers having bad inhibitor was significantly higher than nonstone formers(39%vs9%,X2p<0.01). Pyuria subjects had %aggregation significantly higher than nonpyuria subjects(55±19%,n=31vs46 ±19%,n=82,p=0.03). Among the nonstone formers, pyuria subjects had significantly more %aggregation than nonpyuria subjects(58±14%,n=14vs45±19%,n=71,p<0.01) and 28% of pyuria subjects had bad inhibitor comparing with 5.6% of nonpyuria subjects(X2p=0.02). In stone fomers, both pyuria and nonpyuria patients had the same %aggregation(52±24 %,n=17vs51±20%,n=11,p<0.01) but there was a trend of higher %aggregation in pyuria CaOx stone subjects than nonpyuria subjects(52±24%,n=11vs46±21%,n=17). Urine citrate/ creatinine was not different between pyuria and nonpyuria groups.

Conclusions: UTI can induced CaOx stone formation by enhancing COM crystal aggregation.

Funding: Government Support - Non-U.S.

FR-PO1174

Bladder Urolithiasis and Spontaneous Pyelonephritis Associated with Cutaneous Vesicostomy in a Murine Model of Functional Lower Urinary Tract Obstruction Brian Becknell,[1] Ashley R. Carpenter,[2] Andrew L. Schwaderer,[3] Kirk M. McHugh.[2] *[1]Department of Pediatrics, Nationwide Children's Hospital, Columbus, OH; [2]Center for Molecular and Human Genetics, Nationwide Children's Hospital Research Institute, Columbus, OH; [3]Division of Nephrology, Department of Pediatrics, Nationwide Children's Hospital, Columbus, OH.*

Background: *Megabladder (mgb-/-*) mice have impaired detrusor smooth muscle development *in utero*, and require cutaneous vesicostomy (CV) to prevent progression to ESRD and death. The long-term sequelae of CV in these mice have not been reported.

Methods: 50 *mgb-/-* males underwent CV at 3-7 weeks. Urine was subject to UA, microscopy, and culture. Moribund animals underwent necropsy, and kidneys and urinary tract were subject to histopathology using standard stains.

Results: CV relieved hydronephrosis in male mgb-/- mice, who otherwise progress to ESRD and die by 8 weeks. Males undergoing CV lived for 20 ± 10 weeks (range 8 - 32). 18% (9/50) mgb-/- males became moribund with palpable bladder masses after a variable observation period (15 ± 11 weeks, range 1 – 27). At necropsy, these animals had bladder stones, which in certain cases fully occupied the lumen, resulting in massive hydroureteronephrosis. No stones were observed in mgb-/- mice prior to CV. UA demonstrated pyuria and struvite crystals, as early as 7 days following CV. Stone analysis revealed 88% struvite. Urine aspirated from the renal pelvis of animals with bladder stones grew organisms associated with urease production and struvite stones, including Enterococcus, coagulase-negative Staphylococcus, and Pasteurella species. Kidney histopathology had features of acute and chronic pyelonephritis.

Conclusions: These findings parallel rare but significant complications observed in patients undergoing CV, and provide a unique murine model for struvite urolithiasis and spontaneous pyelonephritis.

Funding: NIDDK Support

FR-PO1175

Clinical Validation of a Novel Assay Used for Monitoring Treatment of Patients with Cystinuria Aditya Mattoo,[1] Frank Modersitzki,[1] Jacob H. Cohen,[4] Michael Grasso,[4] John R. Asplin,[2] David S. Goldfarb.[1,3] *[1]Nephrology, NYU Langone Medical Center, New York, NY; [2]Litholink, Chicago, IL; [3]Nephrology Section, New York Harbor VAMC, New York, NY; [4]Endourology, Lenox Hill Hospital, New York, NY.*

Background: Traditional cystine assays are unreliable in predicting response to therapy and clinical outcomes of cystinuria. A new assay, cystine capacity (CysCap), may be a superior method of correlating urinary parameters with dietary and pharmacological interventions and may predict disease recurrence.

Methods: We performed a single-center retrospective analysis of cystinuric patients to correlate urinary parameters with clinical outcomes. Patients with at least one CysCap analysis and adequate clinical follow-up, (≥ 1 year of documented clinical events), were included. Chart reviews were performed to obtain demographics, pharmacotherapy, lab values and clinical events, defined as urologic intervention, stone passage, renal colic without stone passage, and either new stone or stone growth seen on imaging.

Results: 37 patients met the inclusion criteria. They had a mean of 2.1 CysCap analyses and mean follow-up of 489 days. They experienced a mean of 3.1 clinical events with time to first clinical event at 258 days. Of the clinical events, 1.1 urologic interventions occurred with mean time to first intervention of 47 days. In a general linear model, CysCap was highly correlated with 24h urine cystine excretion, urine volume and urine pH (r2=0.71; P<0.001). Increasing excretion of urine urea nitrogen and urine sodium over 24h correlated with increasing 24h cystine excretion (r=0.499 and 0.480, respectively, p<0.01), but not with CysCap or cystine supersaturation. The CysCap values of those patients on and off cystine binding thiol drugs did not significantly differ between the two groups. CysCap, CysSS and 24hrCys excretion did not correlate with the number of clinical events or the time to first clinical event.

Conclusions: We conclude that the CysCap assay is internally valid as it correlates with 24h cystine excretion, urine volume and urine pH. However, in this retrospective analysis, we were unable to correlate CysCap with the number of clinical events or the time to first clinical event.

Funding: NIDDK Support, Other NIH Support - ORDR

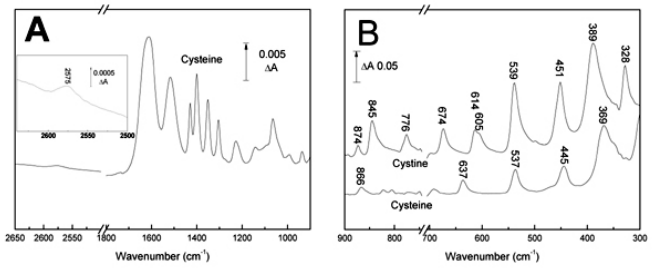
FR-PO1176

Infrared Vibrational Spectroscopy as a Diagnostic Tool for Cystinuria Robert J. Unwin,[1] Elspeth Anne Macdonald,[2] Amandine Marechal,[2] Peter R. Rich.[2] *[1]UCL Centre for Nephrology, University College London, London, United Kingdom; [2]Institute of Structural and Molecular Biology, University College London, London, United Kingdom.*

Background: Infrared (IR) vibrational spectroscopy can be used to analyze many chemicals and materials, and complex mixtures. Absorption bands arise from molecular vibrations and most molecules have characteristic IR spectra. There is growing interest in medical diagnostic uses of mid-IR 'molecular fingerprints' in the 1800-900 cm-1 range. Spectra can distinguish cells and tissues; deconvolution can provide quantitative analyses of fluid constituents. We assessed whether mid- or far-IR spectroscopy could provide a rapid and direct method for quantitative analysis of cystine in urine - normally 0.2 mM or less, but can be several mM in cystinuria.

Methods: In the mid-IR fingerprint region, cysteine and cystine in urine have similar absorbance spectra, overlapping with many other components, precluding their quantitation in this region. However, cysteine has an S-H absorption band at 2575 cm-1, and cystine has characteristic bands in the low frequency IR range at 845 cm-1 and 674 cm-1. Thus, cystine can be detected at 2575 cm-1 after chemical conversion to cysteine or, more directly, in dried samples from its far-IR absorption bands.

Results:



A. Mid-IR spectra of cysteine; 200 mM at pH 6.0 placed on a silicon ATR microprism; spectrum recorded with an MCT-B detector cooled to 77K and water contribution subtracted.

B. Far-IR spectra of solid cysteine and cystine in their protonated forms; samples in fine powder form placed on a diamond ATR prism equipped with KRS-5 optics; spectra recorded with a far-IR room temperature DTGS detector.

Conclusions: The practical quantitative limits of cysteine/cystine quantitation by these methods will be presented, together with quantitative analyses of IR spectra of urine samples from healthy volunteers and cystinuria patients.

FR-PO1177

Rapid Determination of Urinary 2,8-Dihydroxyadenine with Liquid Chromatography – Electrospray Tandem Mass Spectrometry Vidar O. Edvardsson,[1,2] Baldur Bragi Sigurdsson,[3] Margret Thorsteinsdottir,[2,3] Runolfur Palsson.[1,2] *[1]Landspitali - The National University Hospital of Iceland; [2]University of Iceland; [3]Arctic Mass, Reykjavik, Iceland.*

Background: Adenine phosphoribosyltransferase (APRT) deficiency is an autosomal recessive disorder of purine metabolism that leads to excessive urinary excretion of poorly soluble 2,8-dihydroxyadenine (2,8-DHA), causing radiolucent kidney stones and chronic kidney disease. Treatment with allopurinol reduces 2,8-DHA production and prevents stone formation and kidney injury. The purpose of this study was to develop a fast method for determination of urinary 2,8-DHA and other purines for therapeutic monitoring of allopurinol therapy.

Methods: Liquid chromatography - electrospray tandem mass spectrometry (LC-MS/MS) was designed for rapid quantification of 2,8-DHA and other purines, including 2-deoxyadenosine, adenine, adenosine, hypoxanthine, xanthine and oxypurinol through a series of experiments. Capillary and cone voltage, source and desolvation temperature, flow speed, gradient, gradient slope and salt concentration in the mobile phase were optimized by D-optimal design and related to LC-MS/MS responses, using partial least squares regression. To accurately quantify urinary 2,8-DHA, 6 aliquots of urine were collected immediately after the urine container had been inverted several times to suspend settled particles. The pH of the aliquots was adjusted to 10 with 2 μM NH_4OH which dissolved all precipitates before injection into the LC-MS/MS system.

Results: Quantitative analysis of 2,8-DHA and the other purine metabolites was achieved with 100% specificity in 6 minutes. The coefficient variation for different urine aliquots was <5% indicating a robust sampling method. Validation of the LC-MS/MS-based method, which included selectivity, limit of quantification, response function, intra- and inter-day precision and accuracy as well as recovery of all analytes, was well within pre-defined limits.

Conclusions: We have developed a rapid and reliable LC-MS/MS-based method for determination of urinary excretion of 2,8-DHA and other purine derivatives that will greatly facilitate therapeutic monitoring of allopurinol therapy in APRT deficient patients.

Funding: NIDDK Support, Other NIH Support - Office of Rare Diseases Research (ORDR).

FR-PO1178

Antiurolithiatic Activity of an Unani HerboMineral Formulation Sayeed Ahmad,[1, 2] Wasim Ahmad,[1] Mohammed Ahmed Khan,[1] Rabea Parveen,[1] Masood Shah Khan,[1] Ashwani Malhotra,[2] Mohammad Husain,[2] S.M. Arif Zaidi.[1] *[1]Hamdard University, New Delhi, India; [2]Division of Kidney Diseases and Hypertension, Feinstien Institute of Medical Research, North Shore LIJ University Hospital, Great Neck, NY.*

Background: Safoof-e-Pathar Phori (SPP) an Unani herbo-mineral formulation have been used since long in Unani System of Medicine for its anti-urolithiatic activity as a good non-invasive remedy. It is a powdered formulation containing *Didymocarpous pedicellata*; *Dolichous biflorus*; *Rheum emodi*; *Raphanus sativus*-processed salt; *Hordeum vulgare*- processed salt and Potassium nitrate. The anti-urolithiatic activity of herbo-mineral formulation (SPP) against induced calcium oxalate nephrolithiasis was carried out using ethylene glycol - ammonium chloride rat model.

Methods: The animals were divided in four groups control, toxic control and treatments (n=6). The drugs and toxicants were administered orally as per the protocol of rat model for 21 days. On 22nd day urine was collected and analyzed for of Ca^{++}, Mg^{++}, Na^{+}, K^{+} levels and crystalluria studies, whereas serum was used for blood urea nitrogen (BUN) and creatinine levels. The antioxidant markers of kidney tissues and histo-pathological examinations were also carried out.

Results: The SPP treatment (500 and 1000 mg/kg/day) significantly lowered the levels of BUN 49.35±2.30 at 500mg/kg and 23.74±2.34 at 1000mg/kg against toxicant 134.7±14.86 (P<0.001), while creatinine 0.27±0.01mg/dL at 500mg/kg and 0.20±0.01mg/dL at 1000mg/kg against toxicant 0.69±0.04mg/dL (P<0.001). The treatment with SPP showed significantly higher levels of Na+ (25.92±0.47mEq at 500mg/kg and 29.92±0.62mEq at 1000mg/kg against toxicant 10.13±0.85mEq), K+ (6.83±0.04mEq at 500mg/kg and 7.5±0.04mEq at 1000mg/kg against toxicant 4.4±0.03mEq) and Ca++ (5.95±0.02mg/dL at 500mg/kg and 6.28±0.16mg/dL at 1000mg/kg against toxicant 3.90±0.06mg/dL) (P<0.001).

Conclusions: The studies on crystalluria, histopathology and anti-oxidant markers supported the anti-urolithiatic potential of SPP in preventing calcium oxalate deposition without producing diuresis. Further, analysis for isolation of active component is under progress.

Funding: Government Support - Non-U.S.

FR-PO1179

Urinary Uric Acid Excretion in Pediatric Urolithiasis: Which "Cut-Off" Value To Use? Maria Goretti Penido,[1] Uri S. Alon.[2] *[1]Pediatric Nephrology Unit, Federal Univ. of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Bone and Mineral Disorders Clinic, Pediatric Nephrology, Children's Mercy Hospital (CMH), Univ. of MO, Kansas City, MO.*

Background: The "gold standard" for normal urinary uric acid (UA) excretion is <815 mg/24h/1.73m^2, but due to difficulties in 24h collection in pediatrics hyperuricosuria is often defined based on random urine UA/Cr ratio (normal <0.65), or UA/Cr factored for GFR [UA/Cr x serum Cr (SCr), normal <0.57, all values in mg/dl]. The latter is regarded as more accurate but requires blood work for SCr determination. However, the above "cut-off" values were established in healthy children; not in pediatric urolithiasis population. Hence the aim of this study was to examine their validity in such population.

Methods: Based on electronic records, we analysed all children diagnosed at CMH between Jan. 1999 and Dec. 2010 with radiologically documented primary urolithiasis who had diagnostic 24-h urine. Data extracted: age at diagnosis, BSA, 24-h urine volume, Cr, UA, and SCr. Tests were performed ≥1 m after stone expulsion.

Results: After excluding 6 with inappropriate collection based on urine creatinine (mg/kg/24h), there were 188 patients who had their urine tested for both UA expressed in mg/24h/1.73m2 and UA/Cr ratio. In 148 of them UA/Cr was also factored for GFR. Age at diagnosis was 11.9±3.7 (median 12.1). 9 patients had UA > 815 mg/1.73 m2/24h, 46 had UA/Cr ratio ≥ 0.65; 12 had UA/Cr x SCr ≥ 0.57. Compared to the "gold standard" the sensitivity of UA/Cr ratio was 64% and specificity 78%; when factored for GFR sensitivity was 30% and specificity 93%. The area under the ROC curve for UA/Cr ratio was 0.802 (95% CI 0.706-0.898) and that for the GFR corrected 0.766 (95% CI 0.661-0.871). The positive predictive value (PPV) of UA/Cr ratio was 15%; the negative predictive value (NPV) 97%; and after factoring for GFR PPV was 25% and NPV 95%.

Conclusions: The current "cut off" values for urine UA/Cr ratio and UA/Cr factored for GFR are good tools to rule out hyperuricosuria but not to positively diagnose it. Furthermore, in school-age children UA/Cr factored for GFR does not provide an advantage; thus it can be omitted and with it serum creatinine determination .

FR-PO1180

Demographic, Clinical, and Laboratory Characteristics of 137 Pediatric Nephrolithiasis Patients David J. Sas,[1] Amy E. Wahlquist.[2] *[1]Pediatrics, Medical University of South Carolina, Charleston, SC; [2]Biostatistics & Epidemiology, Medical University of South Carolina, Charleston, SC.*

Background: Evidence suggests that the incidence of kidney stone disease is increasing in children, yet there are few data documenting characteristics of pediatric stone formers. We sought to thoroughly describe various characteristics of our stone-forming pediatric population. A description of pediatric nephrolithiasis patients of this size and scope has not been performed in almost two decades.

Methods: We retrospectively reviewed the charts of pediatric patients with nephrolithiasis confirmed by imaging and collected data on over 120 demographic, clinical, laboratory, evaluation, management and follow-up variables on each patient.

Results: Data from 137 subjects was collected and analyzed. Forty-nine percent of our patients were female. Twenty-five percent of our stone formers lived in rural environments. The mean age of presentation with first stone tended to be earlier in males than females (8.2 vs. 9.9 years). Males were more likely to be obese than females (23.6% vs. 17.3%) and the rate of obesity was higher than the general pediatric population in both sexes. Ninety-eight percent of the 47 stones that were analyzed contained calcium. Forty percent of our stone formers had elevated calcium excretion. Thirty-six percent and 57% had elevated supersaturation of calcium oxalate and calcium phosphate respectively. Random calcium-to-creatinine ratio correlated with 24-hour calcium excretion 73% of the time. Patients with 1-3 recurrences tended to be older, but those with >3 recurrences had the youngest mean age of first presentation. Each stone former was, on average, exposed to just over two CT scans specifically to evaluate for nephrolithiasis.

Conclusions: Our data summarize many characteristics of pediatric stone formers and reveal intriguing results related to obesity, recurrence rates, and differences related to gender. Further investigation into potential contributors to the increasing incidence of pediatric kidney stone disease is warranted.

FR-PO1181

Vitamin D in Renal Stone Formers Nikhil Johri, Robert J. Unwin. *Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: Vitamin D (vitD) deficiency is common even among western population. There has been a resurgence of interest in the several health benefits of vitD, however concerns remain about vitD supplementation in those with history of kidney stones. We studied our cohort of idiopathic stone formers (ISF) to evaluate their vitD status and the impact of supplementation of 25-OH vitamin D (25D).

Methods: We retrospectively studied the prevalence of vitD deficiency in the ISF and compared metabolic parameters in 25D deficient (<30nmol/L) with those having normal levels (>75nmol/L) using an unpaired t-test. We also prospectively studied the impact of supplementing 25D in ISF. Total of 37 patients (21M, 16F) were prescribed tab colecalciferol 20,000 units once a week for 3 to 6 months along with advice about reducing meat intake, increasing proportions of fruit and vegetables and a list of oxalate-rich foods was provided. Bloods and 24 hr urine collections were done pre and post supplementation, and results compared using a paired t-test.

Results: 464 ISF were included, 67% males and 33% females, aged 15-81(mean 46.9 years). VitD deficiency was noted in 31%, while only 11.6% had normal vitD levels. The groups were no different in terms of age and renal function. Serum PTH levels were significantly higher in low vitD group (5.31±0.39 vs 3.30±0.19). Differences in urinary parameters influencing stone risk, such as U Ca/Cr, U Ox/Cr , U Cit/Cr , U UA/Cr were non significant. Studying vitD supplementation showed serum 25D levels were significantly higher (19.43±5.72 vs 52.76±26.62) and PTH levels (5.91±3.28 vs 4.61±1.95) significantly lower after supplementation. No significant differences were seen in serum calcium, phosphate and creatinine. 24hr U UA/Cr ratio was significantly lower (0.28±0.07 vs 0.25±0.06) and U Phos/Cr ratio significantly higher (2.04±0.46 vs 2.26±0.59) post supplementation. No significant difference was seen in U Ca/Cr, U Ox/Cr and U Cit/Cr pre and post supplementation.

Conclusions: VitD deficiency is common in our ISF cohort. Higher serum 25D levels were not associated with higher urinary calcium, oxalate, citrate and urate excretions. Supplementing 25D when coupled with dietary advice on stone risk does not result in an adverse change in urine composition.

FR-PO1182

Effects of the Calcium Sensing Receptor Promoter Region Polymorphisms in Kidney Stone Disease Andrea Aloia,[1] Annalisa Terranegra,[1, 2] Giuseppe Vezzoli,[2] Teresa Arcidiacono,[2] Elena Dogliotti,[1] Alessandra Mingione,[1] Francesco Rainone,[2] Laura Soldati.[1] *[1]Department of Medicine, Surgery, Dentistry, Università Studi Milano, Milan, Italy; [2]Unit of Nephrology, Dialysis, Hypertension, San Raffaele Hospital, Milan, Italy.*

Background: The calcium-sensing receptor (CaSR) is a candidate gene of calcium nephrolithiasis (CN). Previously, we found an association between the CaSR gene region including P1 and P2 promoters and CN. Particularly, the rs7652589 and rs1501899 SNPs, localized up and downstream of promoters, were strongly associated to CN, both idiopathic and primary hyperparathyroidism CN.

The aims of this study were to test the association of CaSR gene promoter SNPs with CN and their effects on CaSR expression in renal tissue.

Methods: SNPs genotyping was performed by Taqman genotyping assays of rs7648041, rs7648044, rs6776158 and rs1048213, located into P1 and P2, in 165 idiopathic calcium stone formers and 213 controls matched for age and gender. CaSR mRNA level was evaluated in 109 normal kidney medulla tissues by Real-time PCR and genotyped for the rs7652589, rs1501899 and rs6776158 SNPs.

Results: A fine mapping of the CaSR gene promoter region has been performed in controls and stone formers for the SNPs placed in P1 and P2. The data showed that SNP rs6776158, localized in P1, resulted strongly associated with the disease. The rs6776158 minor allele showed a higher frequency in stone formers than in controls (37.8% vs 26.4%, p=0.005). CaSR mRNA of kidney medulla tissues was related to the kidney stones associated SNPs. A decreased CaSR mRNA was found in homozygous subjects for the minor allele of rs6776158 and rs1501899 vs heterozygous and homozygous for the common allele (rs6776158: n=97, 2.75±0.22 vs n=12, 1.69±0.33, p=0.016; rs1501899: n=97, 2.75±0.22 vs n=12, 1.69±0.33, p=0.016). No variation was found with rs7652589. A decreased mRNA was also observed in subjects carrying haplotype combining the variant alleles at rs6776158 and rs1501899 SNPs (2.70±0.21 vs 1.28±0.32 with p=0.0062).

Conclusions: These findings confirmed the association of CN with CaSR promoter region. Moreover, the minor alleles of P1 SNPs were associated to a reduced expression of kidney CaSR mRNA.

Funding: Government Support - Non-U.S.

FR-PO1183

Protective Effect of Thiazides on Bone Mass in Hypercalciuric Nephrolithiasis Armando Luis Negri, Francisco Rodolfo Spivacow, Elisa Elena Del Valle. *Nephrology, Instituto de Investigaciones Metabólicas, Buenos Aires, Argentina.*

Background: A very important but under addressed area in hypercalciuric nephrolithiasis is the accompanying bone disease. Decreased bone mineral density (BMD) and defects in bone remodeling has been described in these patients. Although thiazide diuretics are useful in preventing stone recurrence, there are still controversies about their efficacy in preventing bone loss.

Methods: We retrospectively analyzed the long term effects of treatment with thiazides on 48 patients (40 women and 8 men) with hypercalciuric nephrolithiasis studied between 2003 and 2010. Their mean age was 45.1 ± 12 years, BMI: 24.2 ±4.2 and normal renal function. Bone densitometry was performed in all patients at the lumbar spine (LS) and femoral neck (FN) at baseline and at the end of follow-up. Urinary Calcium was measured in 24 hour urine collections and bone remodeling was assessed using total and bone ALP, and β crosslaps, The lowest possible dose of a combination of hydrochlorothiazide/ amiloride was used to control calciuria, (range: 12.5 to 50 mg /d and 1.25 to 5 mg, respectively). We also evaluated the appearance of adverse effects to thiazides.

Results: The mean follow-up was 71.3 ± 48.0 months. 24h urinary calcium decreased from 306.5 ± 76.4 to 195.7 ± 58.4 mg/24h (p <0.001), and Ca / kg BW decreased from 5.0 ± 1.1 to 3.24 ± 0.98 (p <0.001). BMD at LS (basal: 1,047 ± 0.22; end follow-up: 1072 ± 0.14 g/cm2;p: NS) and at the FN (basal: 0.855 ± 0.120; end follow-up 0.864 ± 0.130 g/cm2; p: NS) remained stable; The percentage increase of BMD was 2.38 % at the LS and 1.4% at the FN. Bone remodeling markers did not change significantly during follow-up. Mild hypokalemia was observed in 39.4%of the patients, hyperglycemia was seen in 7 patients (2 in the diabetic range), hypotension in 4, palpitations, cramps and headaches in 2 patients in each case. Triglycerides, uric acid and plasma sodium showed no changes.

Conclusions: Low dose thiazide/amiloride combination produced significant long-term reduction in urinary calcium with preservation of bone mass. There were mild adverse effects that did not cause suspension of medication.

FR-PO1184

Race Differences in Patients Receiving Urologic Interventions for Nephrolithiasis in a Large Urban Community Hospital Albert M. Osei, Shweta Punj. *Medicine, Division of Nephrology, John H. Stroger Jr. Hospital of Cook County, Chicago, IL.*

Background: The prevalence of nephrolithiasis has increased and caucasians are more likely than African Americans and Hispanics to have renal stones. However, the race distribution of patients requiring interventions to remove urinary tract stones is unknown.

Methods: We reviewed the electronic and manual surgical logs of all procedures done by our genitourinary service over a two year period. Patients who had any urinary tract stone removal procedures were identified and the full operative reports were retreived and reviewed for the location of the stone, method of removal and the age at the time of the procedure . The demographic characteristics of the patients were also obtained from the hospital electronic records. The racial breakdown of all the patients utilizing the hospital services for that periord was also obtained.

Results: Two hundred and eighty four patients comprising 158 men and 126 women underwent surgical interventions to treat urinary tract stones during that period. The racial distribution were 35% Hispanic, 29 %White, 23% black and 13% for other races. During that same period, the racial distribution of patients using the hospital services were 30%, 20%, 45% and 5% for Hispanics, Whites, Blacks and other races respectively. The mean ages were 43, 46 and 49 years for Hispanics, Whites and Blacks respectively. The male to female ratio was 1.2:1 in all three groups. Thirty one patients had bladder stones and of these 42% percent were black. Nine (43%) of the 21 cases of staghorn calculi were among Hispanics. Extracorporeal short wave lithotripsy(ESWL) at 43% was the commonest procedure but 38%, 45% and 42% of Hispanics, Whites and Blacks respectively underwent the procedure

Conclusions: Hispanics were disproportionately over respresented among patients needing interventions for stone removal in this single center review. This suggests the renal stone burden may be higher among Hispanics than reported or they form stones which tend to require surgical removal. Blacks tend to required surgical removal of bladder stones more than Hispanics or Whites.

FR-PO1185

Regulation of Renal Calcium Reabsorption by Serum Calcium in Hypercalciuric and Control Subjects Kristin J. Bergsland, Elaine M. Worcester. *Nephrology Section, University of Chicago, IL.*

Background: Calcium (Ca) stone formers with idiopathic hypercalciuria (IH) have reduced renal Ca reabsorption, but the mechanism is not understood.

Methods: In the General Clinical Research Center, we studied 29 IH (17 male) and 17 (7 male) control (C) subjects. 27 of 29 IH formed Ca stones. We collected 15 urine and 20 blood samples over a 15 hour day, both fasting and with 3 meals of known composition.

Results: Fractional excretion of Ca (FECa) of all subjects was higher fed than fasting, but FECa of IH exceeded C both fasting and fed (Table). Serum Ca (SCa) and parathyroid hormone (PTH) did not differ significantly between IH and C either fasting or fed. SCa rose and PTH fell with feeding in IH but did not change in C.

	FECa (%)		SCa (mmol/L)		PTH (pg/ml)	
Group	Fast	Fed	Fast	Fed	Fast	Fed
C	1.6 ± 0.3	2.9 ± 0.3[b]	2.33 ± 0.02	2.37 ± 0.02	35 ± 3	28 ± 3
IH	2.7 ± 0.2[a]	5.3 ± 0.2[a,b]	2.31 ± 0.02	2.38 ± 0.02[c]	39 ± 2	29 ± 2[c]

Mean ± SEM; a, differs from C, p<0.01; b, differs from fasting, p<0.01; c, differs from fasting, p<0.05

In a general linear model (GLM) along with subject and food status, SCa varied with FECa (F=7.4, p<0.01), but PTH did not. SCa is part of FECa which presents potential for artifact, so we decomposed FECa into its two terms: urine Ca excretion (UCa) and filtered load of Ca (FLCa, using ultrafilterable Ca). FLCa did not differ between IH and C either fasting or fed. By GLM, UCa varied with FLCa and subject type; SCa had an independent effect on UCa during feeding (F=5.1, p=0.03). The association of SCa with UCa in IH is more striking when SCa is stratified into 'high' or 'low' SCa, defined as above or below the mean

by subject type within food period (Table). High SCa IH had increased UCa compared to low SCa IH, which was significant during feeding (0.52 ± 0.01 vs 0.46 ± 0.01 mmol/hr, p<0.001) and not attributable to FLCa. UCa did not differ by SCa group in C either fasting or fed. In IH, PTH was significantly lower in the high SCa group both fasting and fed (p<0.001), but PTH did not differ in C by SCa group.

Conclusions: SCa appears to modulate the effect of food intake on Ca excretion and PTH level in IH but not C, which implicates altered Ca signaling in the abnormal Ca metabolism of IH.

Funding: NIDDK Support

FR-PO1186

Does FGF23 Play a Role in Pediatric Idiopathic Hypercalciuria? Maria Goretti Penido,[1] Marcelo S. Tavares,[1] Uri S. Alon.[2] *[1]Pediatric Nephrology Unit, Federal Univ. of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Bone and Mineral Disorders Clinic, Pediatric Nephrology, Children's Mercy Hospital, Univ. of MO, Kansas City, MO.*

Background: Various mechanisms were proposed as pathophysiology of idiopathic hypercalciuria (IH). Based on recent findings and suggestion by Worcester & Coe (2008), the aim of this study was to explore a potential role for FGF23 in pediatric IH.

Methods: We studied 29 controls (19M) and 58 children with IH (35M); of whom 24 before treatment (untreated) and 34 after 6 months treatment either with K-citrate alone (20) or combined with thiazides (14). Plasma FGF23 was assessed using C-terminal ELISA (Immunotopics, San Clemente, CA). We also measured serum PTH, 25OH Vit D, P, Ca, creatinine; and 24h urine calcium (UCa), phosphate and creatinine.

Results: No differences in age were noted between controls (15.4±8.3) and patients (16.0±5.0), nor between untreated (16.8±5.4) and treated (15.5±4.7), and no difference in gender distribution. The incidence of lithiasis in the untreated (75%) and treated patients (76%) was the same. Plasma FGF23 in controls was 85.5±34.8 compared with all patients 69.6±52.0 RU/mL (p=0.0019). However, FGF23 in untreated patients 77.6±36.9, was not different from controls, whereas in treated patients it was 64.0±60.4, significantly lower than in controls (p<0.0001) and untreated children (p=0.02). In all IH patients combined there was a tendency of correlation between FGF23 and UCa (r= 0.22; p=0.09). There were no differences between the untreated and treated IH children regarding serum creatinine, Ca, 25D, PTH, urine creatinine or phosphate. Treated patients had significantly lower UCa 2.5±0.7 vs. 5.6±1.2 mg/kg in untreated (p<0.0001); higher TP/GFR 4.0±0.6 vs. 3.4±0.7 (p<0.001) and serum P 4.4±0.5 vs. 4.0±0.6 mg/dl (p=0.007).

Conclusions: Treatment of IH patients resulted in significantly lower UCa excretion rate, lower plasma FGF23 and elevated TP/GFR and serum P, without significant changes in serum PTH. We conclude that the reversal of hypercalciuria may directly or indirectly affect phosphate metabolism, perhaps via calcium retention in bone or changes in 1,25 $(OH)_2$ Vit D metabolism. Further studies on this topic are needed.

FR-PO1187

Abnormal Arterial Stiffness and Bone Density in Calcium Renal Stone Formers Antonia Fabris,[1] Antonio Lupo,[1] Francesco Fantin,[2] Pietro Manuel Ferraro,[3] Chiara Caletti,[1] Gabriele Comellato,[2] Mauro Zamboni,[2] Giovanni Gambaro.[3] *[1]Nephrology, University of Verona; [2]Geriatrics, University of Verona; [3]Nephrology, Catholic University, Rome, Italy.*

Background: Kidney stone formers (SF) are at increased risk for myocardial infarction for still unclear reasons. Reduced bone mass is a frequent finding in calcium SF. An inverse relationship between bone density and abnormal arterial stiffness partly related to vessel calcifications has been reported. Abnormal arterial stiffness is a strong predictor of CV mortality.

To elucidate the causes of the increased CV risk in SF we investigated whether they have abnormal arterial stiffness.

Methods: Recurrent calcium SF (23) and 19 age and sex matched controls underwent DEXA to determine bone mineralization, and pulse waive velocity (PWV) by Complior, and augmentation index (AI) by pulse pen. BMI, triglycerides, cholesterol, blood pressure were not different between groups. All had normal renal function. None had diabetes, or hyperparathyroidism or were actual and past smokers. Females were not in menopause. Main characteristics of the 2 groups are shown in the table.

	Stone formers	Controls	P-value
Augmentation Index	12.3±8.2	5.7±11.8	0.042
PWV charotid-radial	10.0±2.0	9.2±2.0	0.202
PWV charotid-femoral	10.6±2.6	9.1±2.8	0.075
T-score LS*	-1.45 (0.80)	-0.10 (0.90)	0.025
T-score femoral neck*	-1.60 (0.70)	0.00 (0.85)	<0.001
T-score hip*	-1.30 (0.40)	0.25 (0.90)	<0.001

Mean ± SD or * median (interquartile range)

Results: A trend to higher values of PWV charotid-femoral in SF was shown; the AI was significantly higher in this group suggesting an increased stiffness of small arteries and a preclinical atherosclerosis condition.

Multivariate analysis, adjusted for age, gender and BMI, disclosed an inverse relationship with femoral neck mineralization (T-score, p= 0.014) and hip mineralization (T-score, p= 0.015).

Conclusions: A bone-vessel liaison which leads to atherosclerosis has been discovered in a number of conditions. Present data extend to calcium nephrolithiasis this paradigma although the mechanisms are not immediately obvious. Certainly, classical CV risk factors were scantly represented in these SF. Our findings may explain the increased CV morbility and mortality of renal SF.

Funding: Clinical Revenue Support

FR-PO1188

Nephrocalcinosis, Renal Failure and Bone Disease in Patients with Dent Disease and Medullary Sponge Kidney: Comparison with Patients with Reccurrent Calcium Nephrolithiasis Antonia Fabris,[1] Antonio Lupo,[1] Luisa Maria Bertizzolo,[2] Franca Anglani,[3] Giovanni Gambaro,[2] Angela D'angelo.[3] *[1]Nephrology, University of Verona; [2]Catholic University of Rome; [3]University of Padua.*

Background: Medullary Sponge Kidney (MSK) is a developmental and functional disorder with Nephrocalcinosis (NC) and Lithiasis (LT); familial occurrence and the discovery of rare GDNF variants suggest a role of genetic factors.Dent Disease (DD) is a X-linked proximal defects, caused by CLCN5^{+} or OCRL1 mutations,which may present with NC and/or CN. RCN is a common disease affecting adults;earlier occurrence may suggest a hereditary cause.

Methods: RCN, MSK and DD groups were divided into subgroups on the base of specific characteristics; the prevalence of 8 cardinal clinical signs tailored to DD clinical pattern were analysed.

Characteristics in pts with RCN, MSK and DD.

Subgroups	RCN +m.a.	RCN -m.a.	MSK Bilt.	MSK Mono.	CLC5+	CLCN5/ OCRL1	Fam.CLCN5
Pts (males)	53(23)	38(14)	36(17)	21(10)	40(100)	17(100)	7(100)
Age	28±11	30±8	32±11	37±7	14±9	17±16	27±16
HyperUCa%	66	0	78	28	97	64	57
HypoPPi%	19	18	21	38	33	44	34
NC%	0	0	100	100	90	29	42
LT%	100	100	100	100	27	29	42
LMWP+UPi%	47	0	41	14	30	52	71
BD%	35	7	52	4	42	17	0
RF%	0	0	0	0	12	35	0

Results: NC, present by definition in all pts with MSK, was not detected in RCN and was significantly (p<,01) more frequent in CLCN5+ than in CLCN5- DD pts. RF, not present in our MSK and RCN pts, was always associated with NC in CLCN5+ DD pts, but only sporadically in CLCN5-DD pts. No pt. among DD familial cases and MSK pts have RF, despite having NC. Bone disease (BD) in each group is similar (31-36%) but higher than in the general population. It correlates to neither age nor gender, but significantly only to renal hypercalciuria (p<,05) thus confirming the secondary nature of BD complication.

Conclusions: Pathogenetic mechanisms other than nephrocalcinosis trigger RF in DD pts. The lack of BD and RF in DD familial case pts indicates that disease phenotype is mitigated and that it might be resulting from the presence of modifier genes. In 1/3 of MSK pts the presence of a Fanconi-like syndrome also suggest proximal tubular damage.

FR-PO1189

Factors Predicting Medical and Nutritional Therapy Adherence for Reccurent Nephrolithiasis Roy A. Jhagroo,[1] Kristina L. Penniston.[2] *[1]Medicine, UW Madison, Madison, WI; [2]Urology, UW Madison, Madison, WI.*

Background: Nephrolithiasis has a 10% lifetime prevalence in the general population as well as carries a 2.1 Billion dollar economic burden per year. While medications as well as nutritional recommendations have been found to prevent kidney stones, factors affecting compliance with these prevention methods remain poorly understood.

Methods: We used targeted patient surveys to access patient compliance with various modalities of treatment. 155 surveys were returned by mail or at time of the next clinic visit. Nutritional advice if given was evaluated for it's clarity of explanation, comprehension as well as implementation. Medical therapy was also assessed for it's clatrity of explanation, comprehsion and adherence.

Results: A patient described understanding of the rationale for the nutritional therapy recommended it was associated with compliance with the diet (p < 0.001)).

Supporting this, it was also noted that if patients felt that the explanation of the dietary recommendations was satisfactory, they were compliant with diet recommendations(p < 0.0001).

In our survey women reported lower compliance with pharmacologic therapy than men.(p < 0.0003) Specifically for Hydrochlorothiazide women reported lower compliance (p = 0.05)).

A tendency was also noted regarding the poor compliance of women with regard to potassium citrate which was not found in men. (p = 0.09)

In addition, a tendency for women to feel that the rationale behind the medications were not well explained to them was noted(0.058).

Conclusions: Compliance with medications as well as nutrition seems to be influenced by gender as well as provider provided education.

Identification of characteristics affecting adherence provides an opportunity for us to maximize the effect of our therapys. It seems that explaination of the rational behind the use of medications and diet modification is vital for compliance.

These results are also suggestive that women should be given more attention to ensure compliance and possibly would benefit from Nutritional consultation routinely rather than as needed.

FR-PO1190

Etiology of Pediatric Primary Urolithiasis: 12 Year Experience in a Mid-Western Children's Hospital Maria Goretti Penido,[1] Tarak Srivastava,[2] Uri S. Alon.[2] *[1]Pediatric Nephrology Unit, Federal Univ. of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Bone and Mineral Disorders Clinic, Pediatric Nephrology, Children's Mercy Hospital, Univ. of MO, Kansas City, MO.*

Background: Urolithiasis has become more prevalent due to changes in habits and increasing affluence, and possible ecologic changes. The aim of this study was to evaluate the current etiology of pediatric primary stone disease.

Methods: Using electronic data, we identified all children seen at CMH in Kansas City between Jan. 1999 and Dec. 2010 with radiologically documented urolithiasis who had diagnostic 24-h urine analysis. 56 patients with secondary causes (e.g. CF, Crohn's, primary hyperpara, etc.) were exluded. Data extracted included: age at diagnosis, gender, weight, BMI, imaging thechnique used; 24-h urine volume, creatinine (Cr), Ca, uric acid, citrate, oxalate, cystine; serum Cr, electrolytes and minerals; and costs.

Results: After omitting 11 whose urine Cr (mg/kg/24h) showed inappropriate collection, there were 222 patients (48% male), all with normal serum Cr, electrolytes and minerals. Annual rate of primary urolithiasis tripled from 9.7±6.4 to 27.3±8.0 (p= 0.002); 73% were diagnosed by ultrasound and the rest by CT. Age at diagnosis was 11.8±3.8 and BMI 20.5±5.7: 15% were overweight. 147 patients (63.0%) had urine flow < 1.0 ml/kg/24h; in 54 (24.3%) as only abnormailty. Hypercalciuria was observed in 47% of patients, hypocitraturia in 10%, and 54% had high Ca/citrate ratio (≥0.33). Mild idiopathic absorptive hyperoxaluria was found in 3 patients and hyperuricosuria in 11 (all 14 had at least 1 additional abnormality). 1 had cystinuria.

Conclusions: We conclude that "oliguria" and hypercalciuria continue to be the most common abnormalities, followed by hypocitraturia. The significant increase in stone incidence could not be attributed to increased utilization of CT *per se*. Incidence of obesity in the urolithiasis population was not higher than in the general pediatric population. Hyperoxaluria and cystinuria are rare; to identify 1 patient they require screening of 74 at ≥$3500 in the former, and 222 patients at ≥ $42,000 in the latter entity; hence both may not be indicated in 1st analysis.

FR-PO1191

Metabolic and Medical Risk Factors in Pediatric Patients with Urolithiasis Sermin Saadeh, Brett C. Ferguson, Rossana G. Baracco Maggi, Gaurav Kapur, Amrish Jain, Tej K. Mattoo, Rudolph P. Valentini. *Pediatric Nephrology, Children's Hospital of MI, Detroit, MI.*

Background: Urolithiasis is an uncommon medical condition in pediatrics. However, it can be associated with morbidity and recurrence. Compared to adults, a higher proportion of pediatric stone patients have predisposing conditions for recurrence (metabolic disorders, structural abnormalities, infections) often combined with dietary and environmental factors. **Aim:** Evaluate risk factors for pediatric kidney stone formation and recurrence.

Methods: Retrospective chart review of patients evaluated for urolithiasis in the pediatric nephrology clinic from 2005 to 2010.

Results: Charts of 41 patients were reviewed. Median age was 10.3 years (0.6-17.5 yrs), male to female ratio was 1.6:1. Most patients were Caucasian (24/41). The most common presenting symptoms were abdominal colic (68%), gross hematuria (27%), UTI (24%) and dysuria (24%). The imaging modality leading to diagnosis was CT scan in 46%, ultrasound in 34% and x-ray in 15%.

Metabolic work-up in the form of 24-hr urine collection and/or stone analysis was done. Results of 24-hr urine collection on 34 patients are described in table 1. A metabolic abnormality was found in 88% of patients. Hypercalciuria was the most common metabolic abnormality, followed by hypocitraturia. Stone analysis in 13 patients revealed calcium oxalate stones in 7/13 (54%), followed by calcium phosphate stones in 4/13(31%), cystine stone in 1/13(7.6%), and ammonium urate stone in 1/13 (7.6%). 34% of patients had predisposing conditions (neurogenic bladder, loop diuretics, bladder augmentation, ketogenic diet), and 44% had a positive family history for stones.

Results of 24-hr urine collection in 34 patients

24-hr urine collection results	n (%)
Hypercalciuria	9 (26)
Hypocitraturia	7 (20.5)
Hypercalciuria and hypocitraturia	7 (20.5)
Cystinuria	5 (15)
hyperoxaluria	1 (3)
Hypercalciuria and hypomagnesiuria	1 (3)
Non-diagnostic	4 (12)

Conclusions: Hypercalciuria and hypocitraturia are the most common metabolic disorders in our pediatric population with urolithiasis. Patients with urolithiasis should be evaluated for the presence of predisposing conditions to help reduce the risk of recurrence.

FR-PO1192

Urine Levels of Inter-α-Trypsin Inhibitor Proteins Differ According to Age and Stone Forming Status Ran Pang,[1,2] Michael P. Linnes,[1] Amy E. Krambeck,[3] Samuel Edeh,[1] Andrew D. Rule,[1] John C. Lieske.[1] *[1]Department of Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Department of Urology, Guang An Men Hospital, China Academy of Chinese Medical Science, Beijing, China; [3]Department of Urology, Mayo Clinic, Rochester, MN.*

Background: The urine of many individuals is supersaturated favoring the formation of calcium oxalate and calcium phosphate crystals. However, proteins are thought to inhibit nucleation, growth, and aggregation of these crystals. Many protein crystallization inhibitors have been identified, including the inter-α-trypsin inhibitor (IαI) family, which have also been implicated during pathologic calcification in cartilage and vessels. In the current study we investigated urinary levels of IαI proteins in an incident stone forming cohort with matched controls.

Methods: Urine samples were collected from 37 first time stone formers (SF) and controls (C), matched by gender and age. IαI family proteins including heavy chain1 (HC1), heavy chain2 (HC2), and heavy chain3 (HC3) were detected by Western blot and quantitated using Image J. SF and C were divided into four groups based on age: younger (25-50 yrs) SF (n=19), older (51-65 yrs) SF (n=18), younger C (n=20), and older C (n=17).

Results: HC1 was higher in younger SF (1.20±0.70) than in older SF, (0.60±0.32), ($p<0.001$), as were HC2 (1.23±1.24 vs.0.63±0.31, $p<0.01$) and HC3 (1.21±0.34 vs.0.92±0.34, $p<0.05$). Conversely, urinary IαI heavy chains were similar in the younger and older C groups, which also matched the older SF patients.

Conclusions: These results suggest that urinary IαI protein levels differ by age and stone forming status. Although age was not found to affect IαI trimer levels in the controls, significantly higher amounts of the heavy chains were found in the younger SF population. Deposition of IαI heavy chains is thought to protect against pathologic calcification in soft tissues, including cartilage. Therefore, increased levels of heavy chains in the urine of younger SF could represent a feed back protective mechanism against further stone formation. This response seems to be diminished in the urine of older SF, in whom the pathogenesis of kidney stones may differ.

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FR-PO1193

Does Dietary Education Improve 24 Hour Urine Stone Risk Profiles in Children? Katherine E. Twombley,[1] Nicol Corbin Bush,[2] Candace F. Granberg.[2] *[1]Pediatrics, UT Southwestern Medical Center, Dallas, TX; [2]Urology, UT Southwestern Medical Center, Dallas, TX.*

Background: Dietary education (DE) is a widely practiced therapy for pediatric stone formers. However, few studies show the effects of dietary intervention on 24hr urine stone risk profiles in children. We analyzed 24hr urines before and after DE among our pediatric stone formers to evaluate if urinary stone risk profiles improved.

Methods: We performed a retrospective review of 24hr urine samples in stone forming children. Inclusion criteria included patient age <18yrs, radiographically-confirmed stones, DE handout and preDE and postDE 24hr urines. DE handout advised increased water, decreased sodium(Na) and oxalate(Ox), and moderate calcium(Ca) intake. Statistical analyses of 24hr urine parameters were performed with paired t-test.

Results: Among 24 patients with an average age of 10.8yrs (3-18), 11M:13F, improvement in urine volume was the only significant change, increasing from 33 to 45ml/kg/day (p=0.03) or 1.1 to 1.6 liters/day (p=0.002). Urine Na (3.5 vs. 3.7mEq/kg/day), Ca (3.5 vs. 4.4mg/kg/day), and Ox (0.66 vs. 0.70mg/kg/day) did not change despite DE. Likewise, there was no improvement in the supersaturations for calcium oxalate (1.7 vs. 1.4, p=0.2), brushite (1.2 vs. 1.3, p=0.6) and sodium urate (2.9 vs. 2.7, p=0.3).

Conclusions: Among pediatric stone formers, urine output improved with DE, however urine supersaturation indices, Na, Ox and Ca did not. Since 24 hour urine is the gold standard measurement for dietary Na intake, our results suggest poor patient compliance with low sodium diet (despite written DE) may contribute to persistent hypercalcuria in some patients.

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FR-PO1194

Transglutaminase 2 Accelerates Vascular Calcification in CKD Neal X. Chen,[1] Kalisha O'Neill,[1] Kraiwiporn Kiattisunthorn,[2] Xianming Chen,[1] Sharon M. Moe.[1,3] *[1]Indiana University School of Medicine; [2]Faculty of Siriraj Medical School, Mahidol University, Bangkok, Thailand; [3]Roudebush VA Medical Center, Indianapolis, IN.*

Background: Transglutaminase 2 (TG2) is a calcium dependent enzyme that is a co-receptor with ß1 integrins and fibronectin on the cell membrane, connecting the cytoskeleton and the extracellular matrix (ECM). TG2 can cross link nearly all ECM proteins through transamidation or deamidation, increasing osteoblast differentiation and arterial remodeling. Furthermore, vascular smooth muscle cells (VSMC) from TG2 null mice cannot calcify in response to hyperphosphatemia. We therefore hypothesized that increased TG2 activity leads to accelerated vascular calcification in CKD.

Methods: In the current study, we used thoracic aortas and VSMC form the Cy rat, a model of CKD-MBD compared to normal rats to examine the role of TG2 in vascular calcification using real time PCR, immunostaining, Western blot and biochemical assays.

Results: Histological evaluation revealed that TG2 expression is increased in area adjacent to calcification in arteries from CKD rats, whereas there is minimal TG2 expression in arteries from NL rats. VSMC isolated from CKD rats had a 50% increase in TG2 expression compared to NL rats. We also isolated matrix vesicles (MV) from Rat VSMC incubated with control or calcification media and found high levels of TG2 are present in calcified MV whereas no detectable TG2 was found in control MV. To further confirm the role of TG2 in vascular calcification, VSMC from normal or CKD rats were incubated with calcification media in the presence of various concentrations of the TG2 inhibitor cystamine. The results demonstrated that there is significantly greater calcification and alkaline phosphatase (ALP) activity in VSMC from CKD rats compared to that in VSMC from normal rats (10.9 3.5 vs. 3.3 0.5 mol/mg protein, calcification; 11.4 1.6 vs. 6.7 1.4 U/g, ALP). Inhibition of TG2 activity by cystamine dose dependently decreased calcification and ALP activity in VSMC from CKD rats but had no effect on that in NL VSMC.

Conclusions: These data demonstrate a role of TG2 in the pathogenesis of vascular calcification in CKD, likely through its effects on the ECM.

Funding: Veterans Administration Support

FR-PO1195

Magnesium Reduces Vascular Calcification In Vitro Addy Rosa Montes de Oca Gonzalez,[1] Fatima Guerrero,[1] Juan R. Muñoz-Castañeda,[2] Julio Manuel Martínez Moreno,[2] Juan Antonio Madueño Domenech,[2] Carmen Herencia,[2] Yolanda Almaden Peña,[2] Escolastico Aguilera-Tejero,[1] Mirjam Peter,[3] Jutta Passlick-Deetjen,[4] Sonja Steppan,[3] Mariano Rodriguez.[2] *[1]Depto. Medicina y Cirugia Animal, University of Cordoba, Spain; [2]Hospital Reina Sofia, RedinRen, IMIBIC, Spain; [3]Fresenius Medical Care Deutschland GmbH, Bad Homburg, Germany; [4]University of Duesseldorf, Germany.*

Background: Vascular calcification (VC) in uremic patients is associated with high serum phosphorus (P). P binders containing magnesium (Mg) are successfully used for control of hyperphosphataemia. An association of increased serum Mg with reduced VC has been described. Thus, the question to be addressed is whether Mg may exert a direct effect on VC.

Methods: Rat aortic rings (AO) were incubated in vitro for 7 days with a P concentration of 2.8mM and 1.8mM calcium (Ca) with increasing concentrations of Mg (0.6, 1.4, 2.6mM).

Results: Ca content in AO increased from (mean±SE) 0.21± 0.04 in controls without P added, to 4.8±0.6 (mcg/mg protein) with 2.8mM P/0.6mM Mg. Increasing the Mg concentration to 1.4 and 2.6mM significantly ($p<0.01$) reduced the AO Ca content to 1.3±0.7 and 0.25±0.08 respectively, the later value was not different from control. Compared to control values, the decrease in VC induced by Mg was associated with decreased expression of the osteogenic markers Cbfa1 and osterix. Similar results were obtained using human vascular smooth muscle cells (VSMC). Even with concentration of P as high as 3.3mM the change in Mg from 0.6 to 1.4mM reduced Ca content from 11.9±1.9 to 2.1±0.2; a higher concentration of Mg did not produce a further decrease in VC. To test the ability of Mg to reverse VC, VSMCs were incubated for 5 days with 3.3mM P. The Ca content was 8.8±0.6 mcg/mg which increased to 12.1±2.0 after 9 days. The addition of 1.4mM Mg at day 5 produced a markedly decreased Ca content at day 9 (2.5± 0.3; $p<0.01$); this value was not different from control without P added.

Conclusions: An increase in Mg to the upper limits of normal, not only prevented, but also reversed VC in vitro. Thus, P lowering with Mg containing P binders may help to reduce VC through a decrease in P and a direct beneficial effect of Mg.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1196

Stanniocalcin-1 (STC1) Is Upregulated in Uremic Rats and Induces VSMC Calcification Magdalena Gonzalez, Rodrigo Andaur, Solange C. Valdés, Peter W. Murphy, Luis F. Michea. *Facultad de Medicina, Universidad de Chile, Santiago, Chile.*

Background: Calcification of arterial vascular smooth muscle cells (VSMC) is frequent in end-stage renal disease and correlates with hyperphosphatemia. VSMC calcification may involve the osteochondrogenic trans-differentiation. STC1 is an autocrine/paracrine homodimeric factor that promotes osteochondrogenic differentiation in developing bone of mammals. We hypothesized that STC1 is an inducer of VSMC calcification that increases in arterial calcification, observed in chronic renal failure (CRF).

Methods: Male SD rats were nephrectomized (5/6 kidney ablation) and separated into the following groups: standard diet, high phosphate diet (HP), vitamin D plus HP and sham operated rats under standard or HP. Physiological parameters were determined, after 5 weeks animals were sacrificed to obtain blood, urine and aorta samples.

Results: We observed hypertension and low creatinine clearence in all NPX groups ($n=5, P<0,05$). HP diet caused increased [Phosphate]plasma in all NPX groups ($n=6, P<0,05$). Plasma STC1 increased in NPX+HP rats, NPX+VD rats and NPX+HP+VD rats (2.0±0.6, $P<0,05$). To test the direct effect of STC1 on VSMC mineralization, we used A7r5 cells (Aortic, ATCC CRL-1444) incubated in the presence of HP (2,5mM) or VD (100nM), with or without STC1 (10nM) up to 12 days. High CaCl2 (2mM)+HP medium was the positive control of mineralizartion. We observed that STC1+HP induced a time-dependent increase in mineralization (measured Alizarin-red staining, 11.4± 2.9 fold of control levels), even higher that HP+CaCl2 medium (7.9± 2.1, $n=6, P<0,05$).

Osteochondrogenic differentiation was evaluated by measuring the mRNA abundance of the Sry-box containing gene-9 (Sox-9) and Core Binding Factor Alpha-1 (Cbfa-1). STC1 induced a dramatic increase of both transcripts (>2000 fold; $n=3-4, P<0.01$). Addition of HP induced a further increase of osteochodrogenic factors mRNAs ($n=3-4; P<0,01$).

Conclusions: Our data demonstrate increased plasma STC1 levels in uremic rats subjected to pro-calcifying conditions and the induction/potentiation of VSMC calcification by STC1. Supported by FONDECYT 1090223, Fondecyt-FONDAP 15010006.

Funding: Government Support - Non-U.S.

FR-PO1197

Elevated Extracellular Phosphate Levels Down-Regulate Both Akt and AMP-Dependent Kinase in Endothelial Cells Yutaka Taketani, Tomoyo Kitamura, Hironori Yamamoto, Eiji Takeda. *Department of Clinical Nutrition, University of Tokushima, Japan.*

Background: Hyperphosphatemia is an independent risk factor for cardiovascular diseases (CVD) in general population as well as chronic kidney disease (CKD) patients. In addition, phosphate toxity has been emerged from cardiovascular disease to various aging-related diseases such as diabetes mellitus, based on the studies of klotho-deficient mice with premature aging-like phenotype. We hypothesized that hyperphosphatemia can regulate multiple kinases relating on various metabolic processes.

Methods: Most of kinases can be regulated by phosphorylation or dephosphorylation, therefore we investigated the changes of phosphorylation status in response to elevation of extracellular phosphate in human aortic endothelial cells (HAECs) by phosphoproteomic analysis with anti-phospho-signal transduction molecules antibody array.

Results: We found that incubation of HAECs with high phosphate medium (3 mM) decreased phosphorylation of both AMPK at threonine 172 and Akt at serine 473, but control phosphate medium (0.9 mM) did not change them. In addition, high phosphate medium also decreased phosphorylations of the downstream effector proteins of AMPK and Akt, such as endothelial nitric oxide synthase (eNOS), p70S6K and acetyl-CoA carboxylase (ACC).

Conclusions: Akt is a key molecule of insulin signaling pathway. AMPK is known as energy sensor that can regulate glucose and lipid metabolism in response to intracellular AMP/ATP ratio. Therefore, elevated extracellular phosphate levels down-regulated Akt and AMPK, suggesting that hyperphosphatemia may cause abnormal glucose and lipid metabolism found in the klotho-deficient mice.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1198

Role of Local and Systemic Pyrophosphate Metabolism in Vascular Calcification Koba A. Lomashvili, Faten Hasounah, W. Charles O'Neill. *Renal Division, Emory University, Atlanta, GA.*

Background: Pyrophosphate (PPi) is an important endogenous inhibitor of vascular calcification but it is not known whether inhibition is dependent on local (vascular smooth muscle) or systemic PPi. PPi is synthesized by ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) and both humans and mice lacking ENPP1 develop vascular calcification. We transplanted aortas between Enpp1-/- and Enpp+/+ mice to determine the relative role of local and systemic PPi in inhibiting vascular calcification.

Methods: Sections of abdominal aorta (5-8 mm) were transplanted via end-to-end anastomosis between 2-4 month old Enpp1-/- mice and wild-type littermates (Enpp1+/+). Transplants from Enpp1+/+ mice into Enpp1+/+ mice were also were performed as a control. Animals were fed a 1.5% phosphorus diet for 45 days before sacrifice. Calcium was measured in transplanted abdominal aorta grafts (excluding suture lines), recipient abdominal aorta (adjacent to the allograft), and recipient thoracic aortas by the cresopthalein method after drying and extraction with 1 M HCl.

Results: Aortic calcium contents (nmol/mg dry weight) are shown in the table below. Plasma calcium and phosphorus were 1.3 ± 0.05mM and 2.06 ±0.18mM in Enpp1+/+ recipients and 1.24±0.07mM and 1.99±0.19mM in Enpp1-/- recipients. Transplantation into Enpp1+/+ mice substantially reduced but did not eliminate calcification of Enpp1-/- aortas. Enpp1+/+ aortas calcified when transplanted into Enpp1-/- mice but much less than native Enpp1-/- aortas.

Aortic calcium contents (nmol/mg dry weight)

Donor	Recipient	n	Donor aorta	Abdominal aorta	Thoracic aorta
Enpp1-/-	Enpp1+/+	8	32.7 ± 7.2	12.6 ± 3.6	9.7 ±2.9
Enpp1+/+	ENPP1-/-	7	49.3 ± 15.0	475 ±138*	529 ± 132
Enpp1+/+	Enpp1+/+	8	14.4 ± 4.8	10.1 ± 1.2	8.8 ± 0.9

Conclusions: Inhibition of vascular calcification is dependent on both locally produced and systemic pyrophosphate.

Funding: NIDDK Support

FR-PO1199

A Label-free Serum Test Measuring Overall Calcification Inhibition Andreas Pasch,[1,2] Stefan Farese,[1,2] Jurgen Floege,[3] Willi Jahnen-Dechent.[1] *[1]Biomedical Engineering, Biointerface Laboratory, RWTH Aachen University, Aachen, Germany; [2]Department of Nephrology and Hypertension, University Hospital Bern, Inselspital, Bern, Switzerland; [3]Department of Nephrology, RWTH University of Aachen, Aachen, Germany.*

Background: Accelerated vascular and soft tissue calcification is a major problem in patients with chronic kidney disease (CKD). As serum is supersaturated with regard to calcium and phosphate, inhibitors of calcification critically determine pathological calcification. Therefore, an assay measuring the overall calcification inhibitory capacity in blood would be helpful to make informed therapy decisions.

Methods: We developed a label-free assay to quantify calcification-inhibitory properties contained in serum. The assay measures the formation of protein-mineral aggregates in real time.

Results: Using this assay, we demonstrate that in the presence of high amounts of calcium and phosphate, primary calciprotein particles (CPPs) are formed in serum. Primary CPPs are spherical colloidal particles of 50-100 nm diameter. Subsequently, these primary CPPs undergo spontaneous transition to spindle shaped secondary CPPs. Primary CPPs are mainly comprised of fetuin-A and albumin, as demonstrated by protein gel and Western blot analyses. The size of the resulting secondary CPPs is regulated mainly by two serum-inherent proteins: fetuin-A and albumin, with albumin synergistically substituting low fetuin-A concentrations. We furthermore demonstrate that the transition step is delayed in the presence of magnesium, and accelerated in the presence of phosphate.

Conclusions: We have developed a novel test to assess the overall calcification inhibitory capacity of serum. This test may have an important role in the identification and specific treatment of calcification-prone CKD patients.

Funding: Government Support - Non-U.S.

FR-PO1200

Assessment of the Frequency of Pulmonary Calcification and Its Influence on Respiratory Function in Patients with Chronic Kidney Disease Joanna Matuszkiewicz-Rowinska, Magdalena Dylewska, Jerzy Przedlacki. *Nephrology, Dialysis & Internal Diseases, Medical University of Warsaw, Warsaw, Poland.*

Background: The extraosseus calcification accompanying CKD appears, among other, in cardiovascular system and lungs. Disturbed calcium-phosphate (Ca-P) metabolism and alkaline pH are taken as the most important risk factors of pulmonary calcification (PC). The aim of study was to assess the PC frequency and its effect on respiratory function among pts with 3-5D stage of CKD.

Methods: Thirty seven pts with CKD (23 males and 14 females, aged 53.0±8.5 years, 13 in stage 3-4 and 24 in stage 5 (treated with hemodialysis) were included into the study. The exclusion criteria were: obturative, restrictive and interstitial lung diseases, smoking, tuberculosis or anti-mycobacterium therapy actual or in past, neoplasms, heart failure and overhydration. The protocol of the study included: laboratory tests (Ca, P, CaxP product, alkaline phosphatase, PTH), lung tests (spirometry, pletysmography and diffusing capacity of the lung for carbon monoxide (DLCO), high resolution computed tomography (HRCT) of lungs and scintigraphy with the use of 99mTc-MDP.

Results: Only mild PC were found recognized by HRCT alone: in 13/24 pts with 5 CKD and in 1/13 pts with 3-4 CKD ($p<0.01$). Bone scintigraphy did not reveal any calcification. No disturbances of lung tests were found. The mean duration of hemodialysis was longer among the pts with PC (9.9±8.2 years) vs without PC (2.6±6.9 years) ($p<0.01$). No statistical differences in age, gender, duration of CKD, severity of Ca-P disorders and its therapy were found among pts with and without PC. Clinical symptoms: chest pain and dyspnea were observed more frequently in pts with PC (10/13 and 9/13 respectively) than without PC (2/11 and 3/11 respectively) ($p<0.004$ and $p<0.04$ respectively). No difference in frequency of chronic, nonproductive cough was observed among both groups.

Conclusions: We conclude that despite a relatively high frequency of PC, the severity of PC was mild and they were not associated with restrictive disturbances nor impaired DLCO in functional lung tests. HRCT is an useful tool in detection of PC in CKD patients.

FR-PO1201

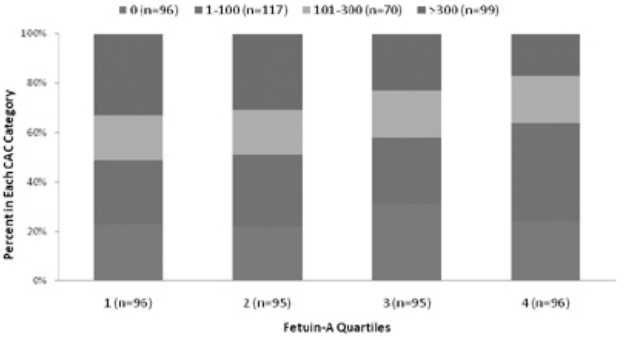
The Associations of Fetuin-A with Subclinical Cardiovascular Disease in Community-Dwelling Persons: The Rancho Bernardo Study Joachim H. Ix,[1] Elizabeth Barrett-Connor,[2] Christina Wassel,[2] Kevin M. Cummins,[2] Jaclyn Bergstrom,[2] Lori B. Daniels,[2] Gail A. Laughlin.[2] *[1]Medicine, UCSD, San Diego, CA; [2]Family and Preventive Medicine, UCSD, San Diego, CA.*

Background: Fetuin-A is a hepatic secretory protein that inhibits arterial calcium deposition in vitro. Lower fetuin-A levels are associated with arterial calcification and death in ESRD populations. The association of fetuin-A with subclinical cardiovascular disease (CVD) in other settings is unknown. In 1,375 community-living individuals without prevalent clinical CVD, we sought to determine the association of fetuin-A with subclinical CVD.

Methods: Plasma fetuin-A concentrations measured by ELISA. Peripheral arterial disease (PAD) was defined by ankle brachial index (ABI) < 0.90, coronary artery calcification (CAC) was defined by CT, and cIMT > 75th% by ultrasound. Logistic regression, ordinal regression, and linear regression evaluated adjusted associations of fetuin-A with each subclinical measure, respectively.

Results: Mean age was 70 ± 11 years, 64% were female, and mean eGFR was 68±16 ml/min/1.73m2. Fetuin-A levels were inversely associated with CAC severity.

Distribution of coronary artery calcification categories by fetuin-A quartiles among 382 community-living individuals without clinically apparent cardiovascular disease: The Rancho Bernardo Study



In ordinal logistic regression using CAC categories (0, 1-100, 101-300, >; 300), each SD higher fetuin-A was associated with a 29% lower odds of CAC severity (proportional odds ratio 0.71; 95% CI 0.58, 0.88; p=0.001) in models adjusted for demographics, lifestyle factors, traditional CVD risk factors and kidney function. In contrast, no association of fetuin-A was observed with PAD or high common or internal cIMT in adjusted or unadjusted models.

Conclusions: Lower fetuin-A levels are associated with greater CAC severity independent of CKD and other covariates, but not PAD or cIMT. If confirmed, fetuin-A may mark calcium deposition within the vasculature, but not atherosclerosis per se.

Funding: Other NIH Support - NHLBI, Veterans Administration Support

FR-PO1202

Vitamin E Reduces Calcification in Vascular Smooth Muscle Cells Exposed to High Phosphate Addy Rosa Montes de Oca Gonzalez,[1] Juan Antonio Madueño Domenech,[2] Fatima Guerrero,[1] Juan R. Muñoz-Castañeda,[2] Yolanda Almaden Peña,[2] Ignacio Lopez,[1] Mariano Rodriguez,[2] Escolastico Aguilera-Tejero.[1] *[1]Medicina y Cirugia Animal, Universidad de Cordoba, Spain; [2]Instituto Maimonides de Investigacion Biomedica de Cordoba (IMIBIC), Spain.*

Background: Hyperphosphatemia and oxidative stress are consistent findings in patients with chronic kidney disease. Vascular smooth muscle cells (VSMC) exposed to high phosphate (P) undergo phenotypic transition to osteogenic cells. Recent evidence suggests that reactive oxygen species can also induce calcification in VSMC. In addition, high P levels may result in oxidative stress. The present study investigates whether high P induces oxidative stress in VSMC and the effect of a natural antioxidant (vitamin E) in preventing phosphate-induced calcification.

Methods: Human VSMC were cultured for 9 days in normal P (Control) or high P (3.3mM) with or without vitamin E (10 µM). Lipid Peroxidation –LPO- (spectrophotometry), Advanced Glycation End Products –AGEs- (ELISA), Alkaline Phosphatase –ALP- activity (p-nytrophenyl phosphate method), Cbfa1 mRNA (RT-PCR), and Calcium deposition (spectrophotometry of acid extract) were measured.

Results: High P increased LPO and AGEs, enhanced ALP activity and gain of expression of Cbfa1, and induced calcification of VSMC. The addition of vitamin E to the high P media reduced LPO and AGEs. Furthermore, vitamin E prevented the increase in ALP activity and Cbfa1 expression and attenuated calcification of VSMC.

Treatment	LPO ([MDA] uM/ mg protein)	AGEs (µg/mg protein)	ALP activity/mg protein	mRNA Cbfa1/β-actin	Ca (µg/mg prot)
Control	0.94±0.10	18.64±1.07	57.74±4.89	1.00±0.01	0.42±0.08
P 3.3mM	1.81±0.26 (a)	28.48±3.87(a)	89.12±5.57 (a)	2.87±0.37 (a)	2.72±0.32 (a)
P 3.3mM + Vit.E 10 µM	0.83±0.36 (b)	12.36±1.92 (b)	48.39±3.36 (b)	0.70±0.08 (b)	1.18±0.31(a,b)

Mean±SE; a: P<0.05 vs Control; b: P<0.05 vs P 3.3 mM

Conclusions: In conclusion, oxidative stress seems to play an important role in phosphate-induced VSMC calcification and antioxidants may have a role in preventing vascular calcification.

Funding: Government Support - Non-U.S.

FR-PO1203

Vascular Calcification in Kidney Transplantation – A Comparison to Predialysis Chronic Kidney Disease Sinee Disthabanchong, Kotcharat Vipattawat. *Division of Nephrology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Phayathai, Bangkok, Thailand.*

Background: Vascular calcification (VC) is prevalent in patients with reduced kidney function as a result of mineral metabolism derangement and uremic environment. The increased VC burden has been shown to predict cardiovascular events and mortality. Evidence suggested VC decreased after kidney transplantation (KT) compared to patients who remained on dialysis. The magnitude of VC in KT recipients compared to predialysis CKD patients whose kidney function are equivalent has never been established.

Methods: 91 chronic stable KT recipients who were at least 1 year post surgery and 102 predialysis CKD patients were included in the study. VC was analyzed by plain radiographs, lateral lumbar spine x-ray for abdominal aorta and pelvic x-ray was iliac and femoral arteries, and scored according to the methods described previously. Important factors associated with VC were also determined.

Results: Higher percentages of CKD patients were diabetic. The median KT duration was 7.5 years (range 1-17 yrs). The average GFR of KT and CKD patients were 45.4 and 41.4 mL/min/1.73 m2 respectively. Over 50% of CKD and KT patients had VC, mostly in the abdominal aorta. Substantial increase in pelvic arterial calcification was observed in KT compared to CKD. Total VC score was also significantly higher in KT. In patients without DM, heightened VC scores in abdominal aorta, pelvic arteries and combined sites were observed in KT. Increased pelvic arterial calcification was demonstrated especially in a subgroup with GFR < 45 mL/min/1.73 m2. Age and DM were associated with VC in both groups, whereas dialysis vintage emerged as an independent factor associated with VC in KT. Multivariate analysis of the entire population demonstrated being a KT recipient was the strongest factor associated with VC.

Conclusions: In conclusion, increased VC in chronic stable KT recipients compared to predialysis CKD patients was observed that was likely the result of past dialysis experience. While KT was able to restore renal function, it could not fully reverse VC.

Funding: Government Support - Non-U.S.

FR-PO1204

Serum Phosphorus Is Associated with Coronary Artery Calcification and Obstruction in Patients with Preserved Renal Function Ana L.E. Cancela,[1] Raul D. Santos Filho,[2] Silvia M. Titan,[1] Luiz Antonio Machado Cesar,[2] Carlos Eduardo Rochitte,[2] Eulogio Martinez,[2] Luciene M. dos Reis,[1] Fabiana G. Graciolli,[1] Vanda Jorgetti,[1] Rosa M. Moyses.[1] *[1]Nephrology, Universidade de São Paulo, São Paulo, Brazil; [2]Heart Institute (InCor) Universidade de São Paulo, São Paulo, Brazil.*

Background: Serum phosphorus has been associated with mortality and cardiovascular events in the CKD and general populations. In vitro studies suggest that excessive phosphorus induces vascular calcification and endothelial dysfunction. Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone and has been correlated to atherosclerosis in the community.

Methods: This cross-sectional study included 290 patients (167 males) with suspected coronary artery disease (CAD) and a MDRD creatinine clearance > 60ml/min/1,73m^2 undergoing elective coronary angiography. Coronary obstruction was quantified using the Friesinger score (FS). Coronary artery calcification was assessed by MSCT.

Results: Serum phosphorus was higher in patients with an Agatston score (AS) > 10HU when compared to the group with a AS ≤ 10 HU (3.63±0.55 vs 3.49±0.52mg/dL, p=0.019). In the univariate and multivariate analysis, each mg/dL of elevation in the serum phosphorus implied a higher risk of presenting an AS > 10 HU [Odds Ratio (OR) =1.92, CI 1.56-3.19; p=0.01]. Patients were divided using the median Friesinger score (4 points) as the cutoff value. Serum phosphorus was higher (3.6±0.5 vs. 3.5±0.6 mg/dl, p=0.04) and intact FGF23 was lower (median 40.3 IQR 24.1-62.2 pg/mL vs. 45.7 IQR 31.7-76.1 pg/mL, p=0.01) in the FS > 4 group. In the multivariate logistic regression analysis, a rise of 1 mg/dL of serum phosphorus carried a 74% increase in the risk of having a FS higher than 4 (OR 1.74, CI 1.06-2.88; p=0.03) and FGF23 was a negative predictor of FS both in the univariate (OR 0.32, CI 0.14-0.71; p=0.005) and multivariate analyses (OR 0.26, CI 0.11-0.63; p=0.002). Serum calcium and parathormone were not associated with CAD..

Conclusions: In patients with suspected coronary artery disease and preserved renal function, phosphorus was predictive of both coronary artery calcification and obstruction. There was a negative association between FGF23 and coronary obstruction.

Funding: Government Support - Non-U.S.

FR-PO1205

Phosphorus (P) Management Trends of Hemodialysis Patients in a Large Dialysis Organization Jamie Heise,[1] Kamyar Kalantar-Zadeh,[2] John Brian Copley,[1] Moshe Fridman,[3] Rajnish Mehrotra.[2] *[1]Medical Affairs, Shire Pharmaceuticals, Chesterbrook, PA; [2]Nephrology, Harbor- UCLA Medical Center, Torrance, CA; [3]Statistics, AMF Consulting, Los Angeles, CA.*

Background: Increased serum P is a robust predictor of all-cause and cardiovascular mortality in dialysis patients. Though P control in the last decade has improved overall, performance varies widely among facilities.

Methods: 104,471 patients treated at a large dialysis provider were selected for analysis. Facilities with at least 50 patients (N=940) were aggregated and ranked by the proportion of patients with serum P < 5.5 mg/dl for the calendar month. The top and bottom 5% of facilities were compared for the achievement of targets for other laboratory measures (calcium, Ca; parathyroid hormone, PTH, and albumin), P-binder and vitamin D use, geographic region, and use of a central pharmacy.

Results: 76% of hemodialysis patients achieved serum P < 5.5 mg/dl. The top 5% of facilities (n=47) treated 4011 patients and the bottom 5% of facilities (n=56) treated 4081 patients. P control was achieved in 91% of patients of top-performing facilities compared to 60% of the bottom-ranked facilities. While there was no difference in the proportion of patients achieving serum albumin target (87% vs. 88%; p=0.40), top-performing facilities were more likely to achieve targets for serum Ca (< 9.5 mg/dl; 99% vs., 97%, p < 0.0001) and PTH (150-600 pg/ml; 74% vs. 70%, p=0.007). Furthermore, top-performing facilities were more likely to use non-calcium P-binders (sevelamer, 69% vs. 61%, p=0.02; lanthanum, 15% vs. 12%, p=0.04). There was no significant difference in the use of either vitamin D (84% vs. 81%; p=0.09) or cinacalcet (29% vs. 28%; p=0.58), or use of a central pharmacy between the top and bottom 5% of facilities.

Conclusions: Despite a high overall rate of P control, differences exist at the unit level in proportion of patients with serum P < 5.5 mg/dl across dialysis facilities. Use of non calcium binders may provide better P, Ca, and PTH control. Identifying practice patterns that allow a larger proportion of patients to achieve P control have the potential of further improving outcomes.

Funding: Pharmaceutical Company Support

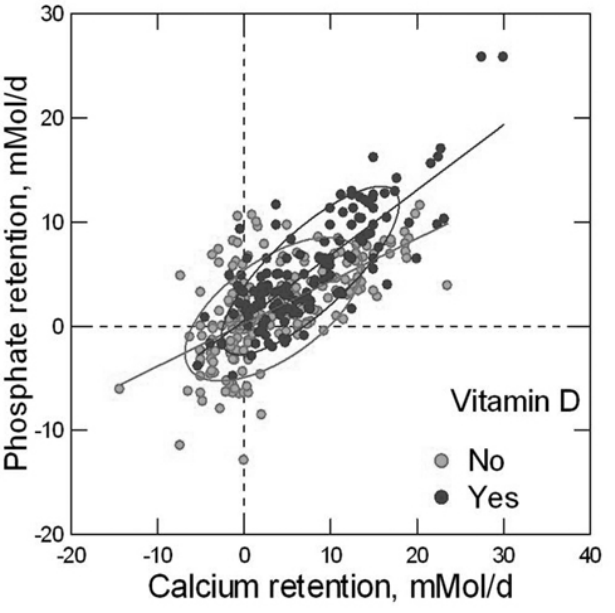
FR-PO1206

Calcium Retention and Vitamin D Drive Phosphate Retention in Chronic Kidney Disease Anna L. Zisman, Elaine M. Worcester. *Department of Medicine, University of Chicago Pritzker School of Medicine, Chicago, IL.*

Background: Phosphate retention is thought to entrain a sequence of abnormalities that stimulate PTH and FGF23 secretions and suppress activated vitamin D production and secretion. Calcium based phosphate binders are used to offset this, though recently concerns have arisen that this may promote cardiovascular disease in CKD patients.

Methods: We have gathered 233 formal calcium and phosphate balance periods in 30 adult patients with CKD from the literature to test whether intake of calcium or vitamin D promotes phosphate retention.

Results: Patients ranged in age from 19 to 72 (43.1 ± 13.4 yrs) and suffered from either CKD Stage 3 (n=2), 4 (n=5), or 5 (predialysis, n=23). Dietary calcium intake ranged from 96 mg to 9278 mg (1309 ± 1295 mg), and phosphate intake ranged from 191 mg to 1800 mg (786 ± 279 mg) per day. Calcium retention was directly proportional to calcium intake, and increased with vitamin D intake. Phosphate retention was associated with phosphate intake only in the setting of vitamin D supplementation (p<0.001). Vitamin D supplementation was tested in 88 balance periods (32 activated vitamin D, 56 nutritional vitamin D). When calcium retention is not positive, phosphate retention centers around a median and mean of 0 (figure). When calcium retention is >0, virtually all phosphate retention points are >0, and retention varies with that of calcium (p<0.0001). When vitamin D is added, phosphate retention is increased for any degree of calcium retention (1.29 ± 0.29 vs. 6.53 ± 1.11, p<0.0001).



Conclusions: In CKD, phosphate retention varies directly with calcium retention, more so with vitamin D supplementation. As calcium retention is a key driver of phosphate retention in CKD, and is dependent on calcium intake, caution should be exercised in calcium loading in patients with CKD.

Funding: NIDDK Support

FR-PO1207

Cinacalcet May Prevent Serum Fetuin A Reduction in Dialysis Patients Monika Staszkow,[1] Ewa Wojtaszek,[1] Zbigniew Bartoszewicz,[2] Stanislaw Niemczyk,[1] Jerzy Przedlacki,[1] Joanna Matuszkiewicz-Rowinska.[1] *[1]Nephrology, Dialysis & Internal Diseases, Medical University of Warsaw, Warsaw, Poland; [2]Department of Internal Diseases and Endocrinology, Medical University of Warsaw, Warsaw, Poland.*

Background: Cinacalcet, a novel calcimimetic, targeting the calcium-sensing receptor (CaSR) markedly reduces parathyroids activity. The CaSR has also been found in the arterial wall, and may play a role in the prevention of vascular calcification.

The aim of the study was to examine the impact of cinacalcet on serum fetuin A (sFA), osteoprotegerin (sOPG), and vascular calcification measured by coronary artery calcification score (CAC) in dialysis pts.

Methods: We enrolled 35 pts, aged 53±10years, on dialysis for 55±63 months) with serum iPTH ≥300 pg/ml; 19 of them received cinacalcet (58±32 mg/d) for 52 weeks, 16 pts remained on standard therapy. CAC scores were obtained by multi-detector computed tomography before and after the study.

Results: There was a significant reduction in sFA in the control group (from 31.7 ± 10.6 to 24.8 ± 7.3 ng/ml, p = 0.013), while in cinacalcet group sFA gradually although insignificantly increased (from 31.9±18.4 to 36.4 ± 14.5 ng/ml). This has resulted in a significant difference between the groups at the end of the study (p = 0.034). In both groups a significant increase in sOPG was seen (from 8.3±4.4 to 14.4±4.3 pmol/l, p<0.0001 and from 11.9±10.7 to 20±9.3 pmol/l, p<0.0001, respectively). The mean CAC score increased slightly (18%) in the control group (from 1081±2076 to 1272±1887 jA; NS) and remained unchanged in cinacalcet group. Both proteins correlated strongly with CAC: sFA negatively (r= -0.41, p=0.01) and sOPG positively (r=0.52, p=0.026) indicating an important and opposite their role in vascular calcification. There was also a positive correlation between time on dialysis and sOPG (r=0.47, p=0.006), as well as CAC (r=0.56, p=0.0009).

Conclusions: These data demonstrate that FA and OPG play an important role in vascular calcification in dialysis pts, the first as an inhibitor and the second as a risk factor. Calcimimetic may prevent sFA reduction in this population. Prevention of vascular calcification is probable however the larger randomized studies are needed.

FR-PO1208

Predominant Osteogenic Characteristics in FGF-23 Secreting Oncogenic Osteomalacic Tumors Yuki Nagata,[1] Yasuo Imanishi,[1] Jun Hashimoto,[2] Keisuke Kobayashi,[1] Akimitsu Miyauchi,[3] Hiroshi Kaji,[4] Koka Motoyama,[1] Katsuhito Mori,[1] Masaaki Inaba.[1] *[1]Department of Metabolism, Endocrinology & Molecular Medicine, Osaka City University Graduate School of Medicine; [2]Osaka Minami Medical Center; [3]Miyauchi Medical Center; [4]Kinki University Faculty of Medicine, Japan.*

Background: Oncogenic osteomalacia (OOM), or tumor induced osteomalacia (TIO), is a rare disease characterized by renal phosphate wasting and hypophosphatemic osteomalacia due to the secretion of fibroblast growth factor 23 (FGF-23) from causative mesenchymal tumors. OOM tumors express phosphate-metabolism related factors such as FGF23, DMP1, MEPE and FRP-4, which are reported to be physiologically expressed in osteogenic lineages. To determine whether OOM tumors have osteogenic characteristics, the expressions of osteoblast/osteocyte specific genes in OOM tumors were investigated at the transcriptional and translational levels.

Methods: Seventeen causative OOM tumors and 6 histopathologic classification-matched non-OOM tumors were analyzed by quantitative real-time RT-PCR and immunohistochemistry. Fluorescent immunohistochemistry was also applied to investigate co-localization of the gene expressions in OOM tumors.

Results: Sixteen genes were significantly elevated in OOM tumors compared to non-OOM tumors in 30 genes for osteoblast/osteocyte or mesenchyme specific genes. In these 16 genes, OOM tumors exhibited positive staining in both phosphate-metabolism related factors (FGF23, DMP1, MEPE) and other osteoblast/osteocyte specific genes (Runx2, osterix, osteocalcin, sclerostin). Fluorescent immunohistochemistry revealed that localizations of FGF23, DMP1 and osterix expressions were well merged in some OOM tumors, however, the predominant localizations of FGF23 was different from those of DMP1 in other OOM tumors. These tumors exhibited co-localizations of FGF23 and osterix, and those of DMP1 and osteocalcin.

Conclusions: OOM tumors have osteogenic characteristics and express phosphate-metabolism related factors. Co-localization of some phosphate-metabolism related factors and osteoblast/osteocyte specific genes suggests osteogenic characteristics contribute to express phosphate-metabolism related factors.

FR-PO1209

Plasma Concentrations of Klotho Protein in Patients with Chronic Renal Failure Toshiyuki Nakao, Hideaki Iwasawa, Ami Hayashi, Yoshitaka Miyaoka, Yoshie Kanazawa. *Department of Nephrology, Tokyo Medical University, Tokyo, Japan.*

Background: Klotho (Kl) is known as an anti-aging protein predominantly expressed in the kidney, parathyroid gland and choroid plexus of the brain. It is a co-receptor specific to fibroblast growth factor 23 (FGF-23) and regulates metabolism of minerals such as calcium and phosphate. The extracellular domain of Kl is secreted into extracellular fluid, but little is known about the plasma levels of Kl and their contributing factors in patients with chronic renal failure (CRF).

Methods: We measured plasma Kl by sandwich ELISA. The studied subjects were 110 CRF patients, which included 69 non-dialyzed (ND), 25 hemodialyzed (HD) and 16 peritoneal dialyzed (PD) patients. We simultaneously measured the serum levels of FGF-23, intact PTH, creatinine (Cr), calcium (Ca), phosphate (P) and albumin (Alb).

Results: The mean plasma Kl concentrations were 551.0±177.8 in the ND , 400.3±140.7 in the HD and 577.3±243.6 pg/ml in the PD patients, and it was significantly lower in the HD than in the ND and PD patients. There were significant differences in serum FGF-23 levels among the ND, HD and PD patients (p<0.05), and the serum FGF-23 levels were highest in the PD patients and lowest in the ND patients. In the ND patients, plasma Kl showed significantly negative correlations with Cr (r =-0.244, p=0.043), P (r=-0.273, p=0.023) and Alb (r=-0.266, p=0.027), while serum FGF-23 showed significantly positive correlations with Cr (r=0.498, p=0.001), P (r=0.398, p=0.010) and i PTH (r=0.397, p=0.010). In the HD patients, plasma Kl significantly positively correlated with Cr (r=0.474. p=0.017), while serum FGF-23 significantly positively correlated with Ca (r=0.543, p=0.009) and P (r=0.478, p=0.024) In the PD patients, plasma Kl significantly positively correlated with Ca (r=0.569, p=0.021), but serum FGF-23 did not. Serum FGF-23 negatively correlated with Alb in both the HD (r=-0.464, p=0.030) and the PD (r= -0.530, p=0.035) patients, but plasma Kl did not. Plasma Kl did not correlate with either serum FGF-23 or i PTH among any groups.

Conclusions: In conclusion, the contributing factors to plasma Kl concentration are different from those to serum FGF-23 level in patients with CRF.

FR-PO1210

Effects of Frequent Hemodialysis on Measures of Chronic Kidney Disease Mineral and Bone Disorder (CKD-MBD): The Frequent Hemodialysis Network Daily and Nocturnal Trials John T. Daugirdas,[1] Glenn M. Chertow,[2] Andreas Pierratos,[3] Juan Carlos Ayus,[4] Tom H. Greene,[5] Brent W. Miller,[6] Sam H. James,[7] Gerald Schulman,[8] Alan S. Kliger,[9] Cynthia A. Kendrick,[10] The FHN Trial Group.[11] *[1]U Illinois Chicago; [2]Stanford U; [3]U Toronto; [4]Renal Consultants of Houston; [5]U Utah; [6]Washington U St. Louis; [7]U California San Francisco; [8]Vanderbilt U; [9]Yale U; [10]Cleveland Clinic; [11]NIDDK, NIH.*

Background: Phosphorus is believed to be better controlled with more frequent hemodialysis.

Methods: In the Frequent Hemodialysis Network (FHN) Daily and Nocturnal Trials, we examined the effects (month 12 vs. baseline) of treatment assignment to 6/week dialysis on predialysis serum phosphorus (P) and on prescribed dose of phosphorus binder expressed as an equivalent phosphorus binder dose (EPBD, g/day) where phosphorus-binding equivalence was calculated on a weight basis relative to calcium carbonate.

Results: In the Daily Trial (n=245), where the prescribed 6/week session length was 1.5-2.75 hours, assignment to frequent hemodialysis was associated with a 0.55 mg/dL decrease in mean serum P relative to the conventional hemodialysis arm (95% CI -0.86 to -0.23) while EPBD was reduced by 1.60 g/day (95% CI -2.73 to -0.48). In the Nocturnal Trial (n=87), where the prescribed 6/week session length was 6-8 hours, assignment to frequent dialysis was associated with a 1.23 mg/dL decrease in mean serum P vs. control (95% CI -1.77 to -0.68), and with an EPBD reduction of 3.78 g/day (95% CI -4.96 to -2.60). Only 20% of subjects (3/15) adherent to 6/week nocturnal treatment (weekly time > 35 hrs) required phosphorus binders, and 60% (9/15) of these subjects required addition of phosphorus to the dialysate. The length of the preceding interdialytic interval was related to higher predialysis serum P, averaging +0.47±0.18 (SE) and +0.64±0.17 mg/dL per 48 h in the Daily and Nocturnal Trials, respectively.

Conclusions: Our results confirm findings in prior observational and nonrandomized studies of improved control of hyperphosphatemia with 6/week dialysis. The improvement was greater when prescribed 6/week treatments were of longer duration.

Funding: NIDDK Support

FR-PO1211

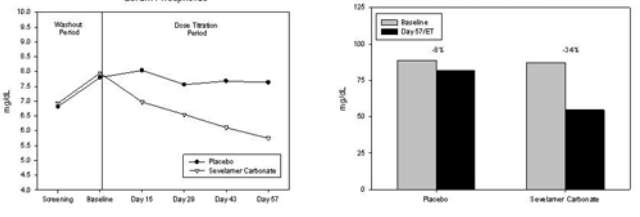
A Randomized, Double-Blind, Placebo-Controlled, Dose-Titration Study of Sevelamer Carbonate in Hemodialysis Patients Nan Chen. *Shanghai Ruijin Hospital, Shanghai, China.*

Background: While clinical practice guidelines advocate that hyperphosphatemic CKD patients should be treated with phosphate binders, there is little information on how these patients respond when not treated and how the pattern of hyperphosphatemia changes over time.

Methods: This randomized, double-blind, dose-titration study compared placebo and sevelamer carbonate in HD patients. Patients on calcium-based binders prior to the study were randomized to either placebo (n=70) or sevelamer carbonate (n=135) for an 8-wk treatment period. Starting dose was 800 mg TID with meals for both groups. If serum phosphorus was >5.5 mg/dL, blinded study drug was increased by 800 mg per meal.

Results: The maximum prescribed daily dose was 8.8 ± 1.6 g/day for placebo and 7.1 ± 2.5 g/day for sevelamer carbonate.

Mean serum phosphorus decreased significantly with sevelamer carbonate (p<0.0001) but remained persistently elevated with placebo throughout the study. The sevelamer carbonate group achieved better phosphorus control compared to pre-study calcium-based binder. There were also statistically significant mean decreases from baseline in serum total and LDL-cholesterol with sevelamer carbonate (p<0.0001), but not placebo. The magnitude of LDL-cholesterol reduction found in the current study is similar to the reduction found in SHARP.



Sevelamer carbonate was well-tolerated. Overall, the AEs experienced by the patients in the sevelamer carbonate and placebo groups were similar and consistent with the patients' underlying renal disease.

Conclusions: This study demonstrates that hyperphosphatemia develops quickly following the cessation of phosphate binders and remains persistently elevated when not treated. This study also demonstrates that sevelamer carbonate is an effective phosphate binder that also decreases LDL-cholesterol which is important given the increased cardiovascular risk profile of many dialysis patients.

Funding: Pharmaceutical Company Support

FR-PO1212

Comparison between PA21, a New Iron-Based Non-Calcium Phosphate Binder and Lanthanum and Sevelamer Carbonate in Uremic Rats

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Background: In a previous study, we demonstrated that PA21, a new calcium-free, iron based phosphate binder effectively controlled hyperphosphatemia and iPTH levels, and was superior to calcium carbonate in preventing the development of vascular calcifications in rats with chronic renal failure (CRF). This ongoing study expands on our previous findings and compares the efficacy of PA21 with lanthanum (La) and sevelamer carbonate (Se) on hyperphosphatemia, and secondary hyperparathyroidism.

Methods: CRF was induced in rats using 0.75% adenine-enriched high phosphorus 1.3 % diet for 4 weeks. Then, rats were randomized to receive the same % of active ingredient of each binder in the diet without adenine for another 4 week period. The concentration (%) of each binder was chosen to deliver the same amount of active pharmaceutical ingredient to each rat: PA21 5%, La 2%, Se 1.5%. N=6/group.

Results: At randomization, no difference was observed for serum calcium (Ca), phosphorus (P), or creatinine (creat) concentration between the 4 groups. Food intake was comparable in all groups during the study. At sacrifice, the following results were found:

	Body weight g	Creatinine µmol/l	P mmol/l	Ca mmol/l	iPTH pg/ml	U. P/creat
CRF placebo	311±8.6	157±14	4.2±0.6	2.4±0.5	3704±868	21±4.6
CRF PA21	300±6.3	136±14	2.1±0.1 ***	2.5±0.03 *	1277±411**	4.6±1.0**
CRF La	306±5.8	144±21	2.5±0.1 ***	2.5±0.5 *	1109±355**	10±1.1***
CRF Se	305±5.7	130±8	2.4±0.1 ***	2.6±0.01 *	1000±212**	7.3±1.1*[/sup]

***p<0.001, **p<0.01, *p<0.05 vs CRF placebo

Conclusions: These experimental data show that the iron based, calcium-free phosphate binder PA21, is at least as effective as La and Se in controlling P and iPTH in rats with CRF.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1213

Serum Phosphorous (Phos) as a Predictor of Macrovascular Events and Mortality within a Large Ethnically Diverse Population with and without CKD

John J. Sim,[1] Antoine C. Abcar,[1] Ning Smith,[2] Joanie Chung,[2] Dean A. Kujubu,[1] Simran K. Bhandari,[1] Scott A. Rasgon,[1] Kamyar Kalantar-Zadeh.[3] *[1]Nephrology & Hypertension, KPSC LAMC, Los Angeles, CA; [2]Research & Evaluation, KPSC, Pasadena, CA; [3]Nephrology, Harbor UCLA, Torrance, CA.*

Background: We sought to determine whether higher serum Phos was a risk for ischemic heart disease (IHD), congestive heart failure (CHF), cerebrovascular events (CVE), and mortality outcomes.

Methods: Retrospective cohort study 1/1/1999 -12/31/2009 persons ≥18yrs with Phos ≥ 1 and min 1 yr continuous followup. Subjects were categorized into population based quartiles using time dependent average Phos. The association btwn Phos quartiles and the primary outcome (composite of new hospitalization diagnoses of IHD, CHF, and CVD, and mortality) was examined using Kaplan Meier analysis and multivariate Cox Proportional Hazard model adjusting for age, gender, hypertension, diabetes, estimated glomerular filtration rate (eGFR), and charlson comorbidity index.

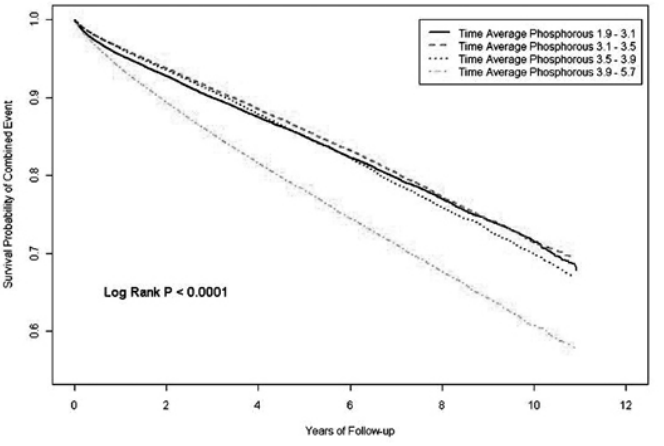
Results: Total 209,865 persons included in cohort with mean age 54 yrs, 61% females, and 41% whites. Median followup was 3.1yrs. 13% had eGFR<60ml/min. Time dependent average Phos were categorized into 4 quartiles (mg/dl); 1.9-3.0, 3.1-3.4, 3.5-3.8, and 3.9-5.7. Compared to lowest Phos quartile, adjusted HR were increased with higher Phos.

Adjusted HR for Outcomes

Phos Quartile	Composite (95%CI)	IHD	CHF	CVE	Mortality
1.9-3.0	-	-	-	-	-
3.1-3.4	0.92 (0.89-0.95)	0.94	1.07	1.20	0.90
3.5-3.8	0.98 (0.95-1.02	1.02	1.19*	1.06	0.94
3.9-5.7	1.46 (1.41-1.50)	1.29*	1.78*	1.23*	1.16*

* p<0.05

Linearity was demonstrated for combined outcomes with HR 1.25 for 0.5 mg/dl increase in Phos. Survival curves demonstrated lower event free rates with higher Phos.



Conclusions: Higher serum Phos demonstrated greater risk for IHD, CHF, CVE, and mortality outcomes within a large population primarily without CKD.

Funding: Pharmaceutical Company Support

FR-PO1214

Serum Calcium Phosphate, and PTH Levels: A Comparison of American and Japanese CKD Patients

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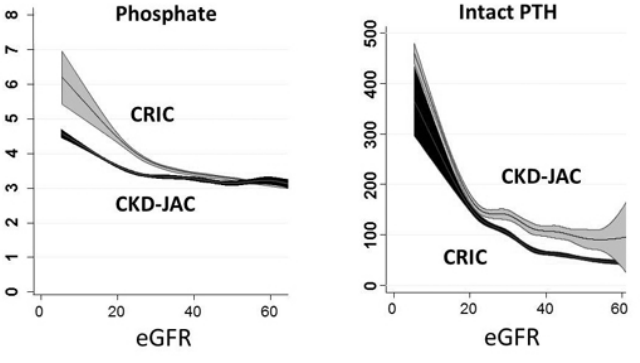
Background: The DOPPS revealed that American hemodialysis patients have two times higher PTH levels compared to their Japanese counterparts despite comparable levels of serum calcium (Ca) and phosphate (P). There are no comparative data in predialysis patients with CKD.

Methods: We compared baseline levels of serum Ca, P, PTH, urinary phosphate (a proxy for net absorbed phosphate), urinary calcium, and use of phosphate binders and vitamin D preparations among participants of the CRIC (Chronic Renal Insufficiency Cohort) and CKD-JAC (Japanese Cohort) studies. Since PTH was measured by different assays, we calibrated the PTH levels using a range of samples from a set of 108 patients with CKD not enrolled in either study.

Results: CRIC and JAC enrolled 3939 and 2977 patients, respectively. Serum P and urinary P/creatinine (P/Cr) (0.62 vs.0.43) were significantly higher in CRIC participants regardless of eGFR and despite their significantly greater likelihood of receiving phosphate binders (7.7 vs.4.4%). However, PTH levels were significantly higher in JAC even after extensive adjustment. Serum Ca levels were also significantly higher in CRIC, while Urinary Ca/Cr were comparable.



Conclusions: Higher phosphorus intake reflected by urine P/Cr possibly could explain the higher serum P levels in American patients. Higher PTH in the Japanese might be explained by the absence of vitamin D-fortified food in Japan. Future cross-national comparisons of serum vitamin D and FGF23 levels, and dietary history will help define these differences.

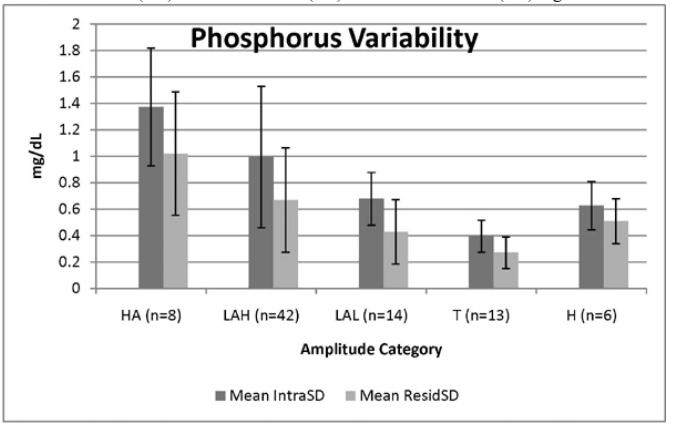
FR-PO1215

Methods Quantifying Variations in Serum Phosphorus Concentrations in Hemodialysis (HD) Patients Katie E. Cardone,[1,2] Alissa Lynn Phillips,[1] Rachid Daoui,[3] Christopher D. Hoy,[3] Shari A. Meola,[3] George R. Bailie.[1,2] *[1]Albany College of Pharmacy and Health Sciences, Albany, NY; [2]Albany Nephrology Pharmacy Group (ANephRx), Albany, NY; [3]Hortense and Louis Rubin Dialysis Center, Clifton Park, NY.*

Background: Medication & diet are used to manage CKD-MBD. Fluctuations may lead to frequent regimen changes and contribute to poor adherence. However, limited data exist regarding [phosphorus (P)] variability in HD pts. Methods to quantify variations in serum hemoglobin have been defined, but have not been used to assess [P] variability. The purposes of this study were to 1)determine extent & magnitude of [P] fluctuation in HD patients using various methods, 2)define typical ranges of fluctuation for each method.

Methods: Retrospective chart review of [P] was conducted at 3 HD centers. All adult HD patients who received treatment in the first quarter of 2009 (between 1/1/2009 and 3/31/2009), who had >2 [P] lab value were included. Patients were censored at death, transfer, hospitalization or HD discontinuation. [P] fluctuation was determined using 3 methods of variability: intrapatient standard deviation (intraSD), residual SD (residSD) and amplitude of variation (high amplitude (HA), low amplitude high (LAH), low amplitude low (LAL), high values only (H), low only (L), or target range only (T). Patients were divided by amplitude classification. Mean(SD) intraSD and residSD were determined for each amplitude group.

Results: Eighty-three patients met study criteria. Half of all patients had LAH fluctuation. Mean(SD) intraSD was 0.86(0.5) & residSD was 0.59(0.4) mg/dL.



Conclusions: [P] variability is significant among HD patients, and may be measured in various ways. It is unclear which method of variability best correlates with clinical outcomes, and warrants further study.

FR-PO1216

Effect of Lanthanum Carbonate Compared with Calcium Carbonate on CAPD Patients in Korea: A Randomized Prospective Study Yong Kyu Lee,[1] Sug Kyun Shin,[1] Ho Yung Lee.[2] *[1]Nephrology, NHIC Ilsan Hospital, Goyang, Geongido, Korea; [2]Nephrology, Severance Hospital, College of Medicine, Seoul, Korea.*

Background: Hyperphosphatemia can result in hyperparathyroidism, metabolic bone disease, cardiovascular calcification, and mortality. But, controlling hyperphosphatemia with calcium-based phosphate binders can lead to hypercalcemia, hypoparathyroidism, arterial calcification, low bone turnover.

Methods: This study is a randomized prospective study designed to compare effect of lanthanum carbonate with calcium carbonate in controlling serum calcium, phosphate, iPTH levels on CAPD patients. Patients who were on CAPD for at least 6 months were screened for 4 weeks. Within those patients, patients whose serum phosphate were over 5.6mg/dL at 0 week were randomly assigned to either lanthanum carbonate or calcium carbonate.Serum calcium, phosphate, iPTH level and other biochemical parameters were examined every 4 weeks for 24 weeks.

Results: A total of 44 patients were enrolled in this study. Out of 44 patients, 11 patients were dropped out due to adverse effect of each drug. After 24 weeks of treatment, both lanthanum carbonate and calcium carbonate reduced serum phosphate level significantly, from 6.59 ± 0.76 to 4.61 ± 0.63 mg/dL and from 6.55 ± 0.54 to 4.91 ± 0.75 mg/dL, respectively. Calcium X phosphate product was reduced in both groups, from 59.66 ± 9.00 to 42.55 ± 8.63 mg2/dL2 and from 60.01 ± 7.18 to 46.21 ± 6.38 mg2/dL2, respectively. Serum iPTH level in Lanthanum Carbonate group was not significantly different, but in calcium carbonate group, the level decreased significantly, from 221.96 ± 223.94 to 151.17 ± 176.09 pg/dL. Serum calcium level was not elevated significantly in both groups.

Conclusions: Lanthanum carbonate is as effective as calcium carbonate in reducing serum phosphate level, and serum iPTH level tends to be steadier in lanthanum carbonate group compared to calcium carbonate group in CAPD patients as in HD patients. Though it was not significantly different, lanthanum carbonate tends not to elevate calcium level in CAPD patients compared to calcium carbonate. But high incidence of G-I adverse effect in lanthanum carbonate group needs to be further evaluated.

FR-PO1217

Role of Sodium-Dependent Phosphate (Pi) Transporter (Npt2b) on Salivary Pi Secretion Hiroko Segawa,[1] Tomo Mukai,[1] Saori Ohnishi,[1] Shohei Sasaki,[1] Akiko Ohi,[1] Shoji Kuwahara,[1] Shinsuke Kido,[1] Sawako Tatsumi,[1] Yasuko Ishikawa,[2] Otoya Ueda,[3] Naoshi Horiba,[3] Kou-Ichi Jishage,[3] Naoshi Fukushima,[3] Ken-Ichi Miyamoto.[1] *[1]Department of Molecular Nutrition, Institution of Health Bioscience, University of Tokushima Graduate School, Tokushima, Japan; [2]Department of Medical Pharmacology, Institution of Health Bioscience, University of Tokushima Graduate School, Tokushima, Japan; [3]Pharmaceutical Research Department 1, Chugai Pharmaceutical Co., Ltd., Gotenba, Shizuoka, Japan; [4]Genome Antibody Product Research Department, Chugai Research Institute for Medical Science Inc., Gotenba, Shizuoka, Japan.*

Background: Hyperphosphatemia is recognized as a contributor to vascular calcification in patients with chronic kidney disease (CKD) and hemodialysis (HD) patients and is independently associated with cardiac mortality. Dietary phosphate (Pi) restriction, and the Pi binders are important therapy for dialysis patients with hyperphosphatemia. Recently, Savica et al reported the salivary secretion of Pi to be an important determinant of hyperphosphatemia in patients with CKD and in those with ERSD under chronic dialysis (JASN 20:639,2009, J Renal Nutrition 21, 39, 2011). In the present study, we investigated the role of type IIb sodium-dependent Pi transporter (Npt2b) on salivary Pi excretion in mice.

Methods: Pilocarpine was injected intravenously into wild-type mice (Wt mice, C57BL6/J) and Npt2b-null mice (Npt2b+/+, and Npt2b+/-). During the 5 min after administration, saliva was collected by pipette.

Results: In Wt mice fed a high Pi diet, the levels of plasma and salivary Pi are significantly higher than those in Wt mice fed a low Pi diet. The expression of Npt2b protein was detected at the apical side of duct cells in the salivary glands, suggesting that ductal cells appears to be able to reabsorb Pi, thereby modifying the Pi concentration in the final saliva. The levels of Npt2b protein were decreased about 50% in the salivary glands of Npt2b+/- mice. The salivary Pi concentrations were significantly increased in Npt2b+/- mice compared with those in Npt2b+/+ mice.

Conclusions: These data suggest that Npt2b is involved in Pi secretion by salivary glands.

Funding: Government Support - Non-U.S.

FR-PO1218

FGF23 Is Expressed in Coronary Arteries of Patients Undergoing Heart Transplantation Natalie A. van Venrooij,[1] R.C. Pereira,[1] Yin Tintut,[2] Linda Demer,[2] Michael C. Fishbein,[3] Katherine Wesseling-Perry,[1] Isidro B. Salusky.[1] *[1]Pediatric Nephrology, UCLA; [2]Cardiology, UCLA; [3]Pathology, UCLA, Los Angeles, CA.*

Background: Elevated levels of FGF23 have been associated with left ventricular hypertrophy, vascular calcification, and mortality in patients with chronic kidney disease (CKD). Vascular calcification in patients with advanced CKD is an active process, with vascular smooth muscle cells (VSMC) acquiring osteogenic properties; however, the stage of kidney disease at which this transformation occurs and the contribution of FGF23 to this process are undefined.

Methods: To evaluate the relationship between renal function, vascular calcification, and vascular FGF23 expression, immunohistochemistry for FGF23, DMP1, and osteopontin and Von Kossa staining was performed in coronary arteries from 27 patients who underwent cardiac transplantation between February 2008 and 2010. 24 hr CrCl, HgA1c, and HSCRP values were obtained pre-transplantation.

Results: 53% of subjects had positive staining for FGF23. Table 1 describes the characteristics of patients with FGF23-positive and FGF23-negative staining. * indicates $p<0.05$.

Variable	FGF-23 positive (n=14)	FGF-23 negative (n=13)
Age (years)	65.4 ± 1.6*	53.8 ± 3.2
Gender (Male)	87%	92%
Ethnicity		
White	62%	50%
Black		25%
Hispanic	8%	8
Other	31%	17%
Diabetics	29%	33%
Prior smokers	62%	50%
Hypertensive history	50%	50%
S-Calcium (mg/dL)	9.0 ± 0.2	8.9 ± 0.2
S-Phosphorus (mg/dL)	3.6 ± 0.2	3.9 ± 0.4
Alkaline Phosphatase (IU/L)	83 (73, 102)	83 (62, 102)
Creatinine Clearance (ml/min/1.73m^2)		
CKD (%)		
none	45 (34, 97)≠	89 (57, 105)
Stage 2	36%	46%
Stage 3	0%	23%
Stage 4	57%*	31%
	7%*	0%
CRP (mg/dL)	8.0 (2.7, 35.5)*	32.0 (18.7, 88.6)
HgA1C (%)	6.4 ± 0.2	6.1 ± 0.1

Patients with positive FGF23 staining were older, had lower CRP levels, and were more likely to have a CrCl<60 ml/min/1.73m² than those with no vascular FGF23 expression. 36% of patients with positive FGF23 staining had normal renal function. FGF23 colocalized with DMP1 and FGF23 expression correlated directly with vascular calcification score and DMP1 expression.

Conclusions: Osteogenic transformation of VSMC occurs in individuals with very early and no CKD and progresses with worsening kidney function; whether FGF23 plays a role in the development of cardiovascular calcification or is solely a marker of the disease process remains to be determined.

FR-PO1219

Intact Fibroblast Growth Factor 23 Levels Predict Cardiovascular Death and Events before Dialysis Inception but Not after the Start of Dialysis
Chikako Nakano,[1] Takayuki Hamano,[1] Naohiko Fujii,[2] Isao Matsui,[1] Kodo Tomida,[1] Kazunori Inoue,[1] Akihiro Shimomura,[1] Yoshitsugu Obi,[1] Noriyuki Okada,[3] Yoshiharu Tsubakihara,[3] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Internal Medicine, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Osaka, Japan; [3]Nephrology and Hypertension, Osaka General Medical Center, Osaka, Japan.*

Background: High fibroblast growth factor23 (FGF23) levels were reported to be risk factors for mortality in incident and maintenance hemodialysis patients. The association of FGF23 levels with cardiovascular diseases (CVD) among predialysis chronic kidney disease (CKD) patients remains unclear.

Methods: In this prospective cohort study, we enrolled 738 CKD outpatients of 2 nephrology units (mean estimated glomerular filtration rate (eGFR), 35mL/min/1.73m²). The endpoint was composite of any CVD event with hospitalization and cardiovascular death.

Results: For a median duration of 4.4 years, 86 patients developed the outcomes including 62 events before dialysis initiation. Cox proportional hazards analyses revealed that high FGF23 levels predicted the outcomes preceding dialysis therapy (hazard ratio [HR] per SD of lnFGF23, 1.93; 95% confidence interval [CI], 1.17-3.21). If we did not censor the cases at dialysis inception and followed those after that, FGF23 did not predict the outcomes (HR, 1.26; 95%CI, 0.85-1.87). Interactions between FGF23 and prior CVD or diabetes mellitus (DM) were not significant. Multiple imputation of missing values did not change the results. Adding FGF23 to the base model of age, sex, DM, prior CVD, pulse pressure, and eGFR led to a net reclassification improvement of 6.87% (P=0.04) due to better identification of the patients not developing CVD (specificity gain).

Conclusions: Intact FGF23 levels among predialysis CKD patients predicted CVD events and death before dialysis inception, but did not predict the outcomes during the entire follow-up including the period after the start of dialysis.

FR-PO1220

Klotho Plasma Levels in CKD Patients: Relation to Renal Function, FGF-23 and Parathyroid Hormone Levels
Sarah Seiler, Franziska Flügge, Esther Herath, Anja Weihrauch, Danilo Fliser, Gunnar H. Heine. *Internal Medicine IV, Saarland University Hospital, Homburg, Saar, Germany.*

Background: The single-pass transmembrane protein Klotho acts as co-receptor for the phosphaturic hormone FGF-23. It has been hypothesized that soluble Klotho, which results from cleavage of the extracellular domain of Klotho by a membrane-standing protease, might exert systemic effects on calcium phosphate metabolism. CKD patients have diminished renal expression of the Klotho gene, and lower renal excretion of the Klotho protein. However, data on plasma levels of circulating Klotho in CKD patients are not available.

Methods: We studied 312 CKD stage 2-4 patients in our ongoing CARE FOR HOMe study. We measured plasma levels of intact parathyroid hormone (iPTH), Klotho and FGF-23, and assessed urinary fractional phosphate excretion (FePi). We estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease study equation 4.

Results: Our patients had a mean eGFR of 44.2±15.7 ml/min/1.73 m², and were 65±12 years of age. Median FGF-23 level was 99.0 rU/ml (IQR 60.3-158.6 rU/ml), median Klotho level 537.9 pg/ml (IQR 450.1-665.7 pg/ml). With progressive impairment of renal function, FGF-23 and iPTH levels, and FePi increased (all p<0.001), while Klotho plasma levels did not (p=0.37). FGF-23 and iPTH levels were correlated with serum calcium and phosphate as well as with FePi, while Klotho was not correlated with any of these parameters:

Table 1

	Klotho (pg/ml)	FGF-23 (rU/ml)	iPTH (pg/ml)
Klotho (pg/ml)	-	r=-0.06; p=0.22	r=0.06; p=0.25
FGF-23 (rU/ml)	r=-0.06; p=0.22	-	r=0.17; p=0.002
iPTH (pg/ml)	r=0.06; p=0.25	r=0.17; p=0.002	-
Calcium (mmol/l)	r=0.01; p=0.88	r=-0.01; p=0.89	r=-0.20; p<0.001
Phosphate (mg/dl)	r=-0.07; p=0.21	r=0.18; p=0.001	r=0.17; p=0.002
FePi (%)	r=0.03; p=0.63	r=0.18; p=0.001	r=-0.20; p<0.001

Conclusions: In our cohort of CKD stage 2-4 patients we confirmed a gradual increase of FGF-23 and iPTH levels with declining renal function. In contrast, Klotho plasma levels were neither associated with renal function, nor with parameters of calcium phosphate metabolism. In the long-term follow-up of our study we will assess the predictive power of Klotho plasma levels for future cardiovascular or renal events in CKD patients.

FR-PO1221

A Randomized Cross-Over Trial Evaluating FGF23 and PTH in the Treatment of CKD Stage 3b
Inger Hjørdis Bleskestad,[1] Anders Hartmann,[2] Harald Bergrem,[1] Lasse G. Goransson.[1] *[1]Stavanger University Hospital, Norway; [2]Oslo University Hospital, Norway.*

Background: FGF23, a phosphaturic hormone is secreted from bone and the level increases as renal function declines. The level of FGF23 is associated with increased mortality in haemodialysis-patients. The use of active vitamin D and phosphate binders as recommended in international guidelines, may affect the level of FGF23 and thereby clinical outcome. We investigated the effects of a phosphate binder and active vitamin D on the serum levels of FGF23 and PTH in patients with CKD stage 3b (30-45 ml/min/1.73m²).

Methods: Seven women and 14 men where included, mean age 65.6 ± 12.2 years. They were randomized in a 1:1 ratio to receive one of two treatment sequences. Group 1: alphacalcidol 0.25 µg once daily for two weeks followed by sevelamer carbonate 800 mg TID with meals for two weeks after a two-week washout period. Group 2: vice versa. Nineteen patients completed the study. The 25(OH) vitamin D level at baseline was 97.6 ± 25.0 mmol/l.

Results: There were no significant period or "carry-over" effects for FGF23 or PTH. There were no significant treatment effects on FGF23 or PTH (p=0.604 and p=0.243 respectively). The period-difference for FGF23 was however unexpectedly positive for both groups.

In group 1 the FGF23 level was higher after treatment with alphacalcidol compared to sevelamer carbonate (mean 105.8 ± 41.6 vs. 79.1 ± 36.5 pg/ml, p=0.047 (CI: 0.4-52.9), for PTH lower (median: 26.5, range: 14.6-55.2 vs. median 36.1, range 13.4-106.9 pg/ml, p=0.011 (CI: 3.5-13.8). In group 2 the FGF23 level increased after treatment with sevelamer carbonate and throughout the washout period non-significantly.

Conclusions: In this cross-over trial with alphacalcidol and sevelamer carbonate in patients with CKD stage 3b, the response on the FGF23 level seems to depend on whether alphacalcidol or sevelamer carbonate is initiated as the first line of therapy. Initiating therapy with sevelamer carbonate increases FGF23 levels while this response is mitigated in the group of patients given alphacalcidol followed by sevelamer carbonate. This observation needs to be confirmed in larger studies.

FR-PO1222

KAI-4169, a Novel Peptide Agonist of the Calcium Sensing Receptor, Suppresses Parathyroid Hormone, Parathyroid Gland Hyperplasia, and Ectopic Calcification in a Rodent Model of Chronic Renal Dysfunction
Sarah Walter, James Tomlinson, Amos Baruch, Shawn Alexander, Jin Dong, Kevin Q. Yin, Dirk B. Mendel, Derek Maclean, Felix D. Karim, Randolph M. Johnson. *Research, KAI Pharmaceuticals, South San Francisco, CA.*

Background: Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) is a frequent and serious complication of CKD that is linked to bone abnormalities and increased risk for cardiovascular disease. KAI-4169 is a novel peptide agonist of the calcium sensing receptor (CaSR). The efficacy of KAI-4169 was evaluated in the 5/6 nephrectomy model, a preclinical rat model of chronic renal dysfunction.

Methods: Three separate studies were done using this model. In the first study, animals were randomly assigned to receive daily doses of placebo, KAI-4169 (1 mg/kg) by IV bolus or cinacalcet (10 mg/kg by oral gavage) for 4 weeks. In the second two studies, KAI-4169 was administered for ~6 weeks as a thrice weekly subcutaneous bolus (3 mg/kg) Control animals were either treated with vehicle or left un-treated.

Results: In the first study, parathyroid hormone (PTH) was the primary endpoint examined. 48 hours after the last dose, animals treated with KAI-4169 had significantly reduced levels of PTH compared with placebo-treated rats. In contrast, PTH had returned to baseline or to above baseline values for the cinacalcet-treated rats by 16 hours after the last dose. Control rats in the second and third studies developed elevated PTH (~1500 pg/mL), parathyroid gland hyperplasia (as measured by gland weight and BrdU staining), elevated serum creatinine and significant vascular and soft tissue calcification. Repeat-dose administration of KAI-4169 reduced PTH levels, attenuated parathyroid gland hyperplasia, significantly reduced aortic and renal calcification and was associated with a significant reduction in serum creatinine compared with baseline values.

Conclusions: KAI-4169 is a novel peptide agonist of the human CaSR that reduces PTH levels and improves markers of CKD-MBD. KAI-4169 is currently in clinical development as an IV treatment for CKD-MBD/SHPT in hemodialysis patients.

Funding: Pharmaceutical Company Support

FR-PO1223

The Impact of Parathyroidectomy on Circulating FGF23 Levels and Renal Phosphate Handling
Liesbeth Viaene, Bjorn K.I. Meijers, Kathleen Claes, Pieter Evenepoel. *Nephrology, University Hospital, Leuven, Belgium.*

Background: The full relationship between fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) remains incompletely understood, with regard to the reciprocal regulation of their transcription and secretion as well as with regard to their phosphaturic actions. Recent experimental evidence suggests PTH directly affects FGF23 secretion.

Methods: We performed a prospective observational study in 24 renal transplant recipients (14 male; age 51±12y) with persistent hyperparathyroidism and hypercalcemia referred for parathyroidectomy (PTX). Parameters of mineral metabolism (including fasting calcitriol, and biointact FGF23 and PTH) and renal phosphate handling (fasting fractional phosphorus excretion, FE_{phos}) were assessed immediately before PTX and at discharge (day 11±8). Twenty healthy volunteers (8 male, age 34±10 years) served as controls.

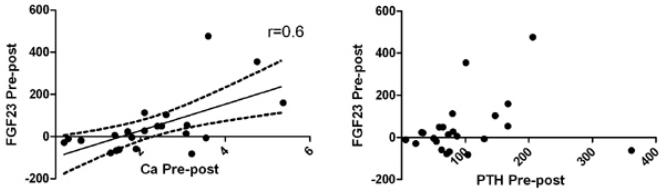
Results: PTX was successful in all patients.

Evolution of parameters of mineral metabolism before and after PTX

	Pre-PTX	Post-PTX	Controls	p ANOVA
eGFR	45*	41*	94	<0.0001
Calcium	10.9*	8.7$	9.2	<0.0001
Phosphate	2.1*	3.3$	3.5	<0.0001
PTH (ng/L)	99*	10$	17	<0.0001
FGF23 (ng/L)	90*	71*	27	<0.0001
Fe Phosphate	44*	22*$	12	<0.0001

Median values are shown; *p<0.05 vs controls; $ p<0.05 vs post-PTX

Overall, FGF23 levels did not decline after PTX. Delta calcium (p= 0.01), but not delta PTH (p =0.3) correlated with delta FGF23 (figure).



The FE_{phos} significantly decreased after PTX, but remained significantly higher as compared to controls (12±7%; p=0.01) despite similar phosphorus concentrations (3.5±0.3 mg/dL).

Conclusions: Our data suggest that calcium rather than PTH directly affects the efflux of FGF23 from bone. PTH and FGF23 act synergistically to increase phosphaturia.

FR-PO1224

Phosphate Restriction Extends the Life of Uremic Rats with Extensive Vascular Calcification Eduardo Slatopolsky, Duk H. Lee, Jane L. Finch. *Medicine/Renal Division, Washington University School of Medicine, St. Louis, MO.*

Background: Numerous studies have demonstrated the role of hyperphosphatemia in the pathogenesis of secondary hyperparathyroidism, cardio-vascular disease and the progression of renal failure. The purpose of this study was to determine if, in rats with chronic kidney disease (CKD) and severe vascular calcification, a significant reduction in phosphate intake could prolong the life of these animals.

Methods: CKD was induced by 5/6th nephrectomy. A group of normal rats served as control (NC). All rats were fed a high-phosphate diet containing 1.4% phosphorus (P) and 0.8% calcium (Ca). After 3 months some rats from both groups were sacrificed. The remaining uremic rats were divided into the following 3 groups: uremic rats +1.4% P diet (UHP), uremic + 1.4% P diet + sevelamer (4%) (UHP+S) and uremic + a very low P diet, 0.1%, (ULP). These rats were sacrificed after 10 weeks.

Results: After the first 3 months, the serum P in uremic rats increased from 5.9 ± 0.34, in normal rats, to 10.3 ± 0.76 mg/dl. The Ca x P increased from 56.7 in normal rats to 94.3 mg2/dl2. Aortic calcium content was also increased (NC: 0.5 ± 0.06 vs. uremic: 46.2 ± 14.2 mg/mg dry wt). Uremic rats also exhibited positive aortic staining for von Kossa, RUNX2 and osteopontin. After the study was continued for an additional 10 weeks, the serum P in the UHP group was 14.8 ± 5.14 mg/dl vs. UHP+S rats (9.8% ± 2.87), ULP rats (3.7 ± 0.02) and NC rats (3.9 ± 0.03). Mortality in the UHP group was 78%. Mortality was reduced to 30% by treatment with sevelamer (UHP+S)and further reduced by the 0.1 % P diet to just 8% (ULP). Positive staining for aortic von Kossa, RUNX2 and osteopontin was increased in UHP rats. Phosphorus restriction inhibited this.

Conclusions: These studies clearly demonstrate that a significant reduction in mortality in uremic rats with severe vascular calcification can be achieved by intensive control of P restriction.

Funding: Other NIH Support - WUCKDR O'Brien Center Grant(P30DK079333), Pharmaceutical Company Support

FR-PO1225

Changes in Fibroblast Growth Factor 23 during Treatment with Vitamin D Analogs. Comparison of Alfacalcidol and Paricalcitol in CKD5D Patients, with Secondary Hyperparathyroidism, in a Randomised Controlled Trial Ditte Hansen,[1] Knud Rasmussen,[1] Lars Rasmussen,[2] Susanne Møller Pedersen,[2] Lisbet Brandi.[1] *[1]Medical Department, Roskilde Hospital, Denmark; [2]Clinical Biochemistry and Pharmacology, Odense University Hospital, Denmark.*

Background: Fibroblast growth factor 23 increases renal phosphate excretion and decreases levels of circulating 1,25 dihydroxyvitamin D. In patients with chronic kidney disease, fibroblast growth factor 23 levels are markedly elevated by unknown mechanisms. Treatment with vitamin D analogs may influence on the level of fibroblast growth factor 23 in hemodialysis patients. Furthermore, the level of fibroblast growth factor 23 may predict the response to treatment of secondary hyperparathyroidism

Methods: In a Danish multicenter study, intravenous alfacalcidol and paricalcitol were compared in hemodialysis patients with secondary hyperparathyroidism in a randomised 2 x 16 week cross-over study, with 6 weeks wash-out period preceding treatment and between treatment periods. In 57 of the enrolled patients, blood samples were frozen before and after each treatment period, and available for measurement of fibroblast growth factor 23.

Results: Alfacalcidol and paricalcitol increased fibroblast growth factor equally (period 1: 223% versus 314%; P=0.384 and period 2: 174% versus 227%; P=0.510) and the levels returned to pre-treatment levels during the six week wash out period. Independent predictors of rise in fibroblast growth factor 23 were baseline levels of fibroblast growth factor (P<0.01), changes in ionised calcium (P<0.01) and phosphate (P<0.01) and cumulative dose of vitamin D analogues (P=0.024). Pre-treatment levels of fibroblast growth factor 23 were independently associated with the level of parathyroid hormone after 16 weeks of treatment with vitamin D analogs (P=0.016).

Conclusions: Alfacalcidol and paricalcitol increase levels of fibroblast growth factor 23 significantly in hemodialysis patients. However, the impact of such increase is not known. Baseline FGF23 levels predicts PTH response to treatment

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1226

Parathyroid Hormone (PTH) Levels and Mortality among Hemodialysis (HD) Patients Not Receiving Treatment for Secondary Hyperparathyroidism (SHPT): Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Francesca Tentori,[1] Mary K. Guidinger,[1] William G. Goodman,[2] Ronald L. Pisoni,[1] Yun Li,[1,3] Ryan D. Kilpatrick,[2] Juergen Bommer,[4] Masafumi Fukagawa,[5] Brian Bieber,[1] Bruce M. Robinson.[1,3] *[1]Arbor Research Collaborative for Health; [2]Amgen, Inc.; [3]U of Michigan; [4]Dialysezentrum Heidelberg; [5]Tokai University School of Medicine.*

Background: Very high PTH levels have been associated with mortality in HD patients. Agents prescribed for SHPT, namely vitamin D analogs and calcimimetics, may have impacted those results. To add insight into whether PTH levels per se are associated with mortality we tested this association among DOPPS participants not receiving treatment for SHPT.

Methods: Of 17,476 DOPPS participants from 12 countries in 1996-2008, 5,728 (30.2%) were categorized as "untreated", i.e. had no evidence of vitamin D (oral or IV) or cinacalcet prescription for 12 months following study entry. Using the most recent PTH value at the end of the 12-month period, the association between PTH levels and mortality over study follow-up was evaluated in Cox models with different levels of adjustment.

Results: Compared to those receiving SHPT therapy, untreated patients had shorter duration of HD and lower serum PTH (mean 217.0 ± 314.8 vs. 306.9 ± 384.0 pg/ml for treated group). Among untreated patients, age was inversely associated with PTH. The association between PTH and mortality in untreated patients is shown.

			Model A	Model B	Model C	Model D
			Unadjusted	Adjusted for demographics + co morbidities*	Model B + serum albumin, Kt/V and hemoglobin	Model C + serum calcium and phosphorus
PTH (pg/mL)	% of patients	Mean age, years	HR (CI)	HR (CI)	HR (CI)	HR (CI)
<50	19.0	63.4	1.30 (1.11, 1.53)***	1.27 (1.07, 1.52)***	1.24 (1.04, 1.49)**	1.29 (1.08, 1.55)***
50-149	35.9	64.3	1.20 (1.04, 1.39)**	1.10 (0.94, 1.28)	1.09 (0.94, 1.27)	1.11 (0.96, 1.30)
150-299	25.5	62.9	1	1	1	1
300-499	10.7	61.0	0.99 (0.79, 1.24)	1.15 (0.92, 1.44)	1.18 (0.94, 1.48)	1.16 (0.93, 1.46)
≥500	8.9	56.4	0.99 (0.78, 1.24)	1.35 (1.08, 1.69)***	1.36 (1.07, 1.73)**	1.33 (1.05, 1.67)**

*Age, sex, black race, years on dialysis, body mass index, and 13 summary co morbid conditions. Models were stratified by phase & country and Accounted for facility level clustering. **P≤0.05. ***P≤0.01.

Conclusions: In an international cohort of HD patients without evidence of SHPT treatment for 12 months, PTH levels < 50 and ≥500 pg/ml were associated with elevated mortality (p<0.05), with the possibility of lowest mortality risk in the KDOQI target range of 150-300 pg/ml. Our results indicate an opportunity for improvement in clinical practice, since a high % of untreated patients had PTH levels above the lowest risk range.

FR-PO1227

The Association of Dietary Phosphorus Intake with Serum Fibroblast Growth Factor-23 and Vitamin D Metabolites in Chronic Kidney Disease: The Seattle Kidney Study Kalani T. Yamamoto, Alina Kostina, Bryan R. Kestenbaum. *Kidney Research Institute, University of Washington, Seattle, WA.*

Background: Phosphorus is excreted through the kidneys by phosphaturic hormones fibroblast growth factor-23 (FGF-23) and parathyroid hormone (PTH) and impairs activation of vitamin D. The impact of dietary phosphorus on mineral metabolism hormones in the setting of chronic kidney disease (CKD) is unclear. We tested the hypothesis that dietary phosphorus, measured using prospective food diaries, would be associated with key mineral metabolism biomarkers among CKD patients.

Methods: We studied 60 patients from the Seattle Kidney Study, a prospective study of CKD, who were not taking activated vitamin D or phosphorus binders and agreed to complete food diaries. We assessed mean dietary phosphorus intake using 5-day

prospective food records and 24-hour recall, with data entered into the Nutrition Data System for Research software system. We measured serum intact FGF-23 and PTH using immunoassays and vitamin D metabolites using mass-spectroscopy.

Results: Mean 5-day phosphorus intake by food diary was 1231 ±355 mg/day in men and 1033 ±327 mg/day in women. Correlation of 5-day prospective dietary phosphorus with 24-hour dietary recall phosphorus was low (r2 = 0.10). Dietary phosphorus, assessed by either method, was not associated with any of the measured mineral metabolism biomarkers.

Association of dietary phosphorus intake with FGF23, PTH, and vitamin D metabolites

		Per 200 mg increase in dietary phosphorus (95% CI)*	
Mineral	N	Diary	FFQ
1,25-vitamin D†	59	-1.65 (-5.48, 2.19)	1.00 (-0.62, 2.62)
Vitamin D24,25†	59	0.31 (-0.09, 0.72)	-0.15 (-0.32, 0.02)
PTH	59	-12.5 (-28.9, 4.01)	-0.59 (-8.02, 6.85)
FGF-23	59	3.24 (-7.9, 14.4)	-1.24 (-6.16, 3.69)

*Models adjusted for age, race, gender, BMI, eGFR , and total kcal. †Additionally adjusted for 25-OH vitamin D

Conclusions: Despite large variation in dietary phosphorus intake across individuals with CKD, dietary phosphorus was not associated with FGF-23, PTH, or vitamin D metabolites.

Funding: Other NIH Support - NIH NIDDK T32 "Research Training in Renal Disease"

FR-PO1228

Comparison among Different Dialysate Calcium Concentrations in Bicarbonato Hemodialysis Carlo Basile, Pasquale Libutti, Francesco Casucci, Piero Lisi, Carlo Lomonte. *Division of Nephrology, Miulli General Hospital, Acquaviva delle Fonti, Italy.*

Background: A correct net calcium (Ca) mass balance during hemodialysis (HD) is crucial in the treatment of renal osteodystrophy.

Methods: Twenty-two stable anuric uremic patients underwent three 4h-bicarbonate HD sessions, each with a different dialysate total Ca (tCa) concentration (1.25, 1.375 and 1.50 mmol/l). Hourly measurements of plasma water ionized Ca (pw iCa), of inlet and outlet dialysate iCa and plasma parathyroid hormone (PTH) concentrations were effected. iCa and tCa mass balances (iCaMBs and tCaMBs) were measured from the dialysate side (Genius batch dialysis system, FMC, Germany).

Results: Mean hourly pw iCa concentrations were statistically significantly higher with a dialysate tCa concentration of 1.50 mmol/l. Mean tCaMBs were positive (diffusion gradient from the dialysate to the patient), being more and more higher by increasing dialysate tCa concentrations (+ 75 ± 122 mg, + 182 ± 125 mg, + 293 ± 228 mg, respectively) ($P < 0.0009$). Only 6 out of the 66 tCaMBs were negative (exclusively with 1.25). Mean tCaMBs were less positive than mean iCaMBs for each of the dialysate tCa concentration studied, even though the mean difference between tCaMBs and iCaMBs (9.8%) did not reach the level of statistical significance. The scatter plot of tCaMBs during every HD session (y axis) vs. the area under the curve of the hourly inlet dialyzer diffusion concentration gradients (dialysate iCa concentration - pw iCa concentration) was: $y = 4.667x - 140.24$ ($R2 - 0.155$, $P < 0.001$). It means that when the measured diffusion concentration gradient is 0, the tCaMB is - 140.2 mg, which represents about 3 l of ultrafiltrate. Mean Δ values of plasma PTH of each treatment (i.e. the mean differences between post- and pre-HD levels) were statistically significantly different among the three treatments ($P < 0.021$), being positive with a dialysate tCa concentration of 1.25 mmol/l and negative with both a dialysate tCa concentration of 1.375 and 1.50 mmol/l.

Conclusions: A dialysate tCa concentration of 1.375 mmol/l should be preferred being able to keep the patient in a mild positive tCaMB, to avoid HD hypercalcemia and not to stimulate PTH secretion.

Funding: Clinical Revenue Support

FR-PO1229

The Interaction of Secondary Hyperparathyroidism(SHPT) and Fibroblast Growth Factor 23(FGF23) Changying Xing, Rui Wang, Huijuan Mao. *Dept. of Nephrology, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu Province, China.*

Background: To investigate the FGF23 before and after parathyroidectomy (PTX) with forearm autotransplantation in patients with advanced SHPT and the cause of resistance of SHPT to FGF23 in hemodialysis patients.

Methods: 1. Serum iFGF23 was detected from 21 patients with advanced SHPT patients before PTX day 3. To further observe immediate changes of FGF23 after PTX, serum iFGF23 was detected before and in 20 minutes, third, fifth, seventh day after PTX. Furthermore, the 25(OH) D3, iPTH, calcium, phosphorus, calcium and phosphorus product changes were observed before and after PTX. 2. The specimens of parathyroid tissue were taken from 8 patients with PTX and were divided into two types: diffuse type (D- type) and nodular type (N-type). The expression of Klotho and FGFR1 were detected by Western blot. 3. Parathyroid cells gotten from patients with SHPT were randomly divided into four groups in vitro as follows: blank-control group, FGF23 stimulation: FGF23 (0.1μg/ml) stimulation group, FGFR3 antibody + FGF23 stimulation group: According to the recommended concentration of FGFR3,0.5μg/ml and1.0μg/ml FGFR3 and FGF23 (0.1μg/ml) co-stimulated for 0h, 2h, 6h, 12h, 24h, 36h.

Results: 1.FGF23 was significantly elevated in SHPT patients. In 21 PTX patients, postoperative FGF23 were significantly decreased compared with preoperative levels, and this was followed by a reduction in iPTH levels. Calcium levels, phosphorus levels, and calcium-phosphorus product levels were significantly decreased after PTX, and this was followed by a reduction in plasma FGF-23 levels in time-course study. 2.Nodular type of parathyroid tissue exhibited lower Klotho and FGFR1 expression than diffuse type of parathyroid tissue. 3.The administration of recombinant FGF23 0.1μg/ml and FGFR3 antibody 1.0μg/ml together, the level of PTH was decreased at 2h.

Conclusions: Parathyroid glands regulate circulating FGF23 levels in SHPT. Nodular type of parathyroid tissue exhibited lower Klotho and FGFR1 suggestes the depressed expression of the Klotho–FGFR1 complex in hyperplastic glands. FGF23 and FGFR3 antibody together, downregulate the level of PTH at 2h.

FR-PO1230

Maxacalcitol Ameliorates Tubulointerstitial Fibrosis in the Obstructed Kidney by Abrogating Smad3-Dependent TGF-β1 Production, without Affecting the Renal Renin Expression Kazunori Inoue,[1] Isao Matsui,[1] Takayuki Hamano,[2] Akihiro Shimomura,[1] Chikako Nakano,[1] Atsushi Takahashi,[1] Yoshiyasu Ueda,[1] Yoshitsugu Takabatake,[1] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, PA.*

Background: Active vitamin D (aVD) is one of the candidates protecting the kidney from tubulointerstitial fibrosis(TIF) because aVD negatively regulates renin in renal diseases with high local renin. However, the therapeutic effects of aVD under low renin condition are not well-established.

Methods: Six-week-old male Sprague-Dawley rats were randomly divided into four groups; sham + vehicle (S+V), sham + maxacalcitol (22-oxacalcitriol (OCT))(S+O), unilateral ureteral obstructed (UUO) + vehicle (U+V), or UUO + OCT (U+O). Vehicle or OCT at a dose of 0.5 μg/kg BW was administrated subcutaneously twice a day. All animals were sacrificed 3 days after UUO because renal renin was temporary down-regulated in UUO kidney on day 3.

Results: Fibrotic parameters (such as tubular injury index, interstitial volume index, and collagen I positive area) and mRNA expression levels of collagen I, collagen III, and fibronectin 1 were elevated in group U+V in comparison with two sham groups. OCT significantly ameliorated these fibrotic changes. Because mRNA and protein levels of renal renin in Group U+O were comparable to those of group U+V, the amelioration of TIF by OCT seemed not to be mediated through suppression of renal renin. Neither renal angiotensin II levels nor plasma renin activities were affected by OCT. OCT significantly suppressed TGF-β1/Smad pathway both in the obstructed kidney and in cultured NRK52E cells. We found that TGF-β1 up-regulates mRNA of TGF-β1 itself in NRK52E cells. Because a selective inhibitor of TGF-β1 type I receptor or a Smad3 inhibitor abrogated the TGF-β1-induced TGF-β1 production, this vicious circle seemed to be Smad3-dependnent. OCT suppressed this vicious circle.

Conclusions: Our findings provide a new rationale for aVD therapy in fibrotic kidney diseases even without renin up-regulation.

FR-PO1231

Combinational Usage of Vitamin D Status and the Earliest Marker Fibroblast Growth Factor 23 Improves Risk Stratification for Renal Outcome Chikako Nakano,[1] Takayuki Hamano,[1] Naohiko Fujii,[2] Isao Matsui,[1] Kodo Tomida,[1] Kazunori Inoue,[1] Akihiro Shimomura,[1] Yoshitsugu Obi,[1] Noriyuki Okada,[3] Yoshiharu Tsubakihara,[3] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Internal Medicine, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan; [3]Nephrology and Hypertension, Osaka General Medical Center, Osaka, Japan.*

Background: High serum phosphate, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels and vitamin D deficiency, when studied separately, were found to predict the progression of chronic kidney disease (CKD). However, not all mineral bone disease (MBD)-related factors have been measured simultaneously.

Methods: This prospective cohort consisted of 738 predialysis outpatients (mean estimated glomerular filtration rate (eGFR), 35 mL/min/1.73m^2) in 2 nephrology units. The endpoint was doubling of serum creatinine or dialysis initiation.

Results: At baseline, the increase in intact FGF23 levels with eGFR decline was earlier than changes in the other MBD-related factors. For a median duration of 4.4 years, 213 patients reached the endpoint. In Cox proportional hazards model, high FGF23 and low 25-hydroxyvitamin D (25D) levels predicted CKD progression (interaction P=0.11), while 1,25-dihydroxyvitamin D, PTH, phosphate levels, or active vitamin D therapy did not. Adding FGF23 and 25D to the base model of age, sex, diabetes, proteinuria, eGFR, prior cardiovascular disease, systolic blood pressure, hemoglobin, and albumin led to a net reclassification improvement of 9.05% (P=0.001). Dividing patients into 4 groups by the median of 25D and FGF23, adjusted hazard ratios for the outcome of High FGF23-Low 25D, High FGF23-High 25D, and Low FGF23-Low 25D groups were 2.52 (95%CI 1.13-5.62), 1.96 (0.88-4.36), and 1.47 (0.63-3.43), respectively, compared to Low FGF23-High 25D group (P for trend=0.01). Treating death as a competing risk did not change the results.

Conclusions: Combinational use of two markers improved risk stratification.

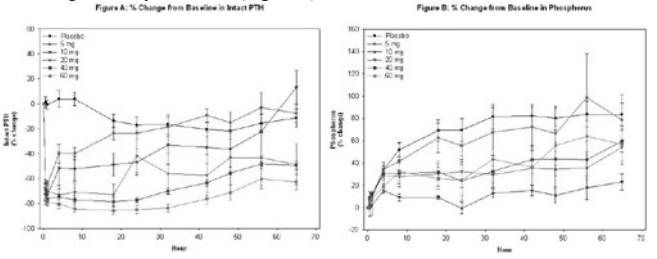
FR-PO1232

The Effect of KAI-4169, a Novel Treatment for Chronic Kidney Disease-Mineral and Bone Disorder, on Serum Phosphorus Kinetics Post-Hemodialysis Kevin J. Martin,[1] Gregory Bell,[2] Saling Huang,[2] Karen Pickthorn,[2] Marwan O. Kaskas,[3] Marializa Bernardo,[4] Peter F. Mount,[5] David A. Power,[5] Geoffrey A. Block.[6] *[1]Saint Louis University; [2]KAI Pharmaceuticals; [3]Northwest Louisiana Nephrology; [4]Southwest Houston Research; [5]Austin Hospital; [6]Denver Nephrology.*

Background: KAI 4169 is a novel, long-acting, peptide agonist of the calcium sensing receptor under development for the treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) in ESRD.

Methods: Twenty-eight subjects on hemodialysis were given a single dose of KAI 4169 or placebo. The 5, 10 and 20 mg cohorts were studied in a 2-period cross-over design while the 40 and 60 mg cohorts were randomized to KAI-4169 or placebo with 8 subjects per cohort. Immediately following hemodialysis, subjects were admitted to a Phase 1 unit and observed for 3 days. Baseline laboratory testing was performed 2 hours post hemodialysis.

Results: Following injection of KAI 4169 post dialysis, there is a rapid 60-80% decrease in the levels of intact PTH followed by a dose dependent return towards baseline over the following ~70 hours (Figure A). Phosphorus values which were decreased by dialysis, rose rapidly over the first 8 hours to a plateau and then increased more slowly during the remaining interdialytic interval (Figure B).



Interestingly, the rate of return to the plateau level of phosphorus was markedly modified by KAI-4169. The 5 mg dose had minimal effect but higher doses markedly decreased the rise of serum phosphorus towards pre-dialysis values.

Conclusions: These observations suggest that the marked reduction in PTH sustained over 72 hours appears to alter serum phosphorus kinetics post dialysis such that phosphorus efflux, presumably from bone, is markedly attenuated. This would suggest that phosphorus efflux from bone is a significant contributor to the generation and maintenance of hyperphosphatemia in patients with secondary hyperparathyroidism on hemodialysis.

Funding: Pharmaceutical Company Support

FR-PO1233

Vitamin D Deficiency in Acute Kidney Injury David E. Leaf,[1] Myles S. Wolf,[2] Leonard Stern.[1] *[1]Department of Medicine, Division of Nephrology, Columbia University Medical Center, New York, NY; [2]Division of Nephrology, University of Miami, FL.*

Background: Chronic kidney disease and end-stage renal disease are associated with high rates of vitamin D deficiency. In contrast, little is known about vitamin D homeostasis in acute kidney injury (AKI).

Methods: We recruited 28 participants with AKI and 30 controls from the medical intensive care unit and general hospital wards of Columbia University Medical Center. The following serum values were measured at baseline and repeated 5 days later: 25-hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), 24R,25-dihydroxyvitamin D_3 ($24R,25(OH)_2D_3$), Vitamin D Binding Protein (VDBP), Fibroblast Growth Factor 23 (FGF-23), and Parathyroid Hormone (PTH).

Results: Compared with controls, participants with AKI had lower baseline levels of 25(OH)D, 1,25(OH)2D3, 24R,25(OH)2D3), and VDBP, and higher levels of FGF-23 and PTH. Among participants with AKI, there was a significant inverse correlation between FGF-23 and 25(OH)D (p=0.018), however, no significant correlations were found between FGF-23 and 1,25(OH)2D3, nor between FGF-23 and 24R,25(OH)2D3.

Baseline serum values in control vs. AKI, median (IQR)

	Control	AKI	p-value
25(OH)D (ng/ml)	14 (8-21)	8 (5-15)	0.049
1,25(OH)2D3 (pg/ml)	25 (15-35)	15 (10-22)	0.013
24R,25(OH)2D3 (ng/ml)	1.5 (0.6-2.6)	0.9 (0.3-1.2)	0.009
VDBP (mg/dl)	29 (25-36)	23 (14-31)	0.009
FGF-23 (RU/ml)	263 (87-577)	1546 (253-2988)	0.003
PTH (pg/ml)	40 (30-80)	74 (50-148)	0.013
Ca (mg/dl)	9.6 (9.3-9.8)	9.1 (9.0-9.4)	0.001
PO4 (mg/dl)	3.4 (2.9-4.0)	4.7 (3.6-5.8)	0.001

Conclusions: AKI is associated with vitamin D deficiency and elevation of FGF-23. While elevated FGF-23 may contribute to vitamin D deficiency in AKI, the mechanism does not appear to be mediated by CYP24 and enhanced catabolism of 25(OH)D, given our findings of a decreased $24R,25(OH)_2D_3$. The mechanism may be related to alterations of vitamin D distribution or to diminished substrate delivery, as the VDBP levels were also decreased. The significance of these preliminary findings is unclear and will require future investigation. Vitamin D deficiency in AKI may have important physiologic consequences such as impaired host immunity, and may be a marker of poor outcomes.

Funding: Private Foundation Support

FR-PO1234

No Change in Methylation Pattern of Parathyroid *VDR* and *CaR* Genes in Secondary Hyperparathyroidism in the Rat Jacob Hofman-Bang,[1] Eva Graversen,[1] Ewa Lewin.[1, 2] *[1]Department of Nephrology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [2]Department of Nephrology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark.*

Background: Secondary hyperparathyroidism (sHPT) due to uremia is characterized by decreased expression of the vitamin D receptor (*VDR*) and the calcium sensing receptor (*CaR*) in the parathyroids. Previous results from our lab showed a resistance towards normalization of the gene expression of *VDR* and *CaR* after an isogenic kidney transplantation in rats. In parathyroid tissue cultures CaR expression is also significantly reduced. Methylation of cytosine nucleotides in CpG islands - CpG being the DNA di-nucleotide - is often detected during epigenetic down-regulation of gene expression.

The possibility of a change in the parathyroid promoter methylation was therefore examined in uremic rats on a high phosphate diet for 8 weeks (n=9) as compared to sham operated rats. Furthermore, the methylation pattern was examined in 24 h parathyroid tissue cultures(n=7).

Methods: Utilizing melting temperature profiling of the PCR products after bisulfite treatment of genomic DNA from rat parathyroids, we assessed the methylation pattern of gene promoter regions harbouring CpG islands in the *CaR* and *VDR* genes. Gene expression was assessed by real time PCR.

Results: The parathyroids of uremic rats and the parathyroid cultures all showed the same low methylation pattern, as seen in sham rats. Verifying the low expression of CaR in vitro, we also observed a low expression of VDR, PTH and Klotho at 24 hours.

Conclusions: In the rat model of sHPT due to uremia and in tissue cultures of normal parathyroid glands no aberrant methylation pattern was detected in the promoter regions of the parathyroid *CaR* and *VDR* genes. As such, a change in the methylation of the promoters in the parathyroids could not explain the decreased expression of *CaR* and *VDR* genes in experimental uremia. Furthermore, the low *CaR* and *VDR* gene expressions *in vitro* after 24 h could not be attributed to promoter methylation.

Funding: Government Support - Non-U.S.

FR-PO1235

The Fasting Fractional Excretion of Phosphate Is Correlated with FGF23 Levels in CKD Patients Not on Dialysis Patricia Quadros Branco,[1] Ricardo Vizinho,[1] Teresa Adragao,[1] Andre L. Weigert,[1] Maria Augusta Cabrita Silva Gaspar,[1] Rita Birne,[1] Cristina Jorge,[1] Artur P. Mendes,[1] Jorge Dickson,[1] Joao Faro-Viana,[2] Jose Matias Barata.[1] *[1]Nephrology Department, HSC, Carnaxide, Lisboa, Portugal; [2]Pathology Department, CHLO.*

Background: In CKD patients (pts) increase of FGF23 levels precedes PTH and phosphate (P) increase; 24h-phosphaturia is reduced in advanced CKD stages and 24h-fractional excretion of P (FEP) is not consistently correlated with FGF23. This study aimed to analyze, in a group of CKD not on dialysis pts the correlation of fasting FEP and FGF23 levels.

Methods: We studied 86 CKD (74% male; 21% diabetic; age 65±13 years; CKD stages 3: 48%, 4: 43%, 5: 9%). FGF23 (C-Terminal, Immutopics) was evaluated by ELISA. FEP (%) calculated using formula (UPO4 x PCr):(PPO4 x UCr) x 100**.** Blood and urine samples were taken simultaneously in a fasting steady state, after discarding overnight urine. Glomerular filtration rate (eGFR) was estimated by the MDRD4 equation.

Results: P>4.5 mg/dL was found in 17% pts, iPTH>normal in 85%, FEP>20% in 88% and FGF23>100 rU/mL in 87%. 43% pts had 25-OH-vitD deficiency (<15 ng/ml) and 42% insufficiency (15-30 ng/ml). In univariable analysis, logFGF23 was inversely correlated with eGFR (p<0.001) and albumin (p<0.022) and directly correlated with P levels (p<0.001), iPTH (p=0.001) and with FEP (p=0.001); FEP was inversely correlated with eGFR (p<0.001) and directly with PTH (p<0.001). Patients with P>4.5 mg/dL showed higher FGF23 levels (p<0.001) and FEP values (p=0.010).

In multivariable analysis, adjusting for age, eGFR and PTH levels, logFGF23 was directly correlated with P levels (p<0.001) and FEP (p=0.008) and inversely with albumin levels (p=0.01); FEP was inversely correlated with eGFR (p<0.001) and P levels (p=0.001) and directly with logFGF23 (p=0.008).

Conclusions: In summary, in this group of CKD pts, P levels were increased only in 17% of pts; while FEP>20% was detected in 88% of pts. Fasting FEP was inversely associated with eGFR and positively associated with FGF23 levels. FEP evaluated by this simple method may be a useful tool in the assessment of the altered mineral metabolism of CKD pts.

Funding: Private Foundation Support

FR-PO1236

Safety and Efficacy of Cholecalciferol (D3) Treatment in Hemodialysis Patients: Effects on Bone and Mineral Metabolism Maria Krassilnikova, Peter S. Heeger, Brian D. Radbill, Anita Mehrotra. *Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: 25OH vitamin D (25OHD) deficiency is common in hemodialysis patients, and uncontrolled studies show that repletion may be associated with a decline in PTH. To determine the safety and efficacy of D3 repletion and its effect on bone and mineral metabolism, we carried out a randomized controlled study of D3 repletion in a cohort of dialysis outpatients.

Methods: We screened 97 patients for 25OHD deficiency. 79 patients with 25OHD levels <25ng/mL were randomized to treatment with D3 (50,000 IU/week to goal >35 followed by 10,000 IU/week) or control (no therapy) in a 2:1 ratio. After 6 weeks, 3, and 6 months, patients were assessed for any side effects, medications and monthly parameters of bone and mineral metabolism were reviewed, and 25OHD levels were measured.

Results: In the D3 treatment arm, baseline median 25OHD increased from 13.5ng/mL to 40.5 at 6 weeks (n=51/p<0.001), 43.4 at 3 months (n=38/p<0.001) and 38.5 at 6 months (n=20/p<0.001). Baseline median serum Ca was 9.3mg/dL and remained unchanged at 6 weeks (9.2/p=0.785), 3 months (9.4/p=0.984), and 6 months (9.4/p=0.852). Median PTH was 360pg/mL at baseline, 381 at 6 weeks (p=0.212), 372 at 3 months (p=0.757), and 292 at 6 months (p=0.97).

At our dialysis center, therapy with activated Vit D is dose-adjusted to serum PTH level. Intriguingly, normalization of 25OHD with oral D3 resulted in a significant decrease in dose of activated Vit D from 4mcg 3x/week at baseline to 2mcg 3x/week at 6 months (n = 20/p=0.026).

In contrast, in the control arm (n=28), we did not observe changes in median 25OHD (13.1ng/mL at baseline vs. 10.4 at 6 months, n=13, p=0.944). There were no changes in Ca (9.1mg/dL at baseline vs. 8.8 at 6 months, p=0.158) or PTH (293pg/mL at baseline vs. 329 at 6 months, p=0.158). Moreover, activated Vit D requirements were unchanged.

Conclusions: The early results of this randomized trial indicate that D3 repletion is safe and effective in hemodialysis patients and suggest that repletion lowers dose of activated Vit D required to maintain optimal bone mineralization. Because oral D3 is inexpensive, these findings could have important economic implications.

Funding: Private Foundation Support

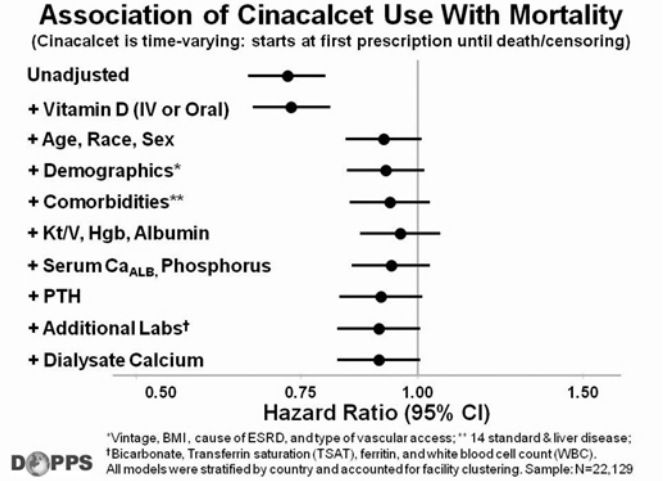
FR-PO1237

Cinacalcet Therapy & Mortality among Hemodialysis (HD) Patients: International Results from the Dialysis Outcomes & Practice Patterns Study (DOPPS) Francesca Tentori,[1] Diane Steffick,[1] Ronald L. Pisoni,[1] Brenda W. Gillespie,[1,2] Peter G. Kerr,[3] Stefan H. Jacobson,[4] Takashi Akiba,[5] Bruce M. Robinson.[1,2] *[1]Arbor Research Collab for Health, Ann Arbor, MI; [2]Univ of Michigan, Ann Arbor; [3]Monash Medical Centre & Univ, Australia; [4]Danderyd Hospital, Stockholm, Sweden; [5]Tokyo Women's Medical Univ, Japan.*

Background: Elevated parathyroid hormone (PTH) is associated with poor outcomes in dialysis patients. By acting on parathyroid calcium-sensing receptors, calcimimetics (cinacalcet) may provide better PTH control than vitamin D (VitD) alone. Among VitD-treated patients from a US dialysis provider, those also using cinacalcet had lower mortality. We studied cinacalcet use & mortality in a HD cohort from 12 countries with wide variation in clinical practices.

Methods: 22,129 patients in DOPPS3 & 4 (2005-2010) were analyzed; 4,534 (20%) used cinacalcet. Cox models were used to predict survival with a time-varying cinacalcet indicator set at first reported prescription.

Results: Cinacalcet use rose over time from 8% (2005) to 19% (2010) & varied across facilities in each country (e.g. 0 to 90% in US). Patients on cinacalcet were younger (Δmean=5.8 yrs), had been on dialysis longer & had fewer comorbidities than those not on cinacalcet. At study entry, VitD (IV/oral) use was 71% for cinacalcet patients & 58% for non-cinacalcet patients. Mortality risk was lower for cinacalcet users in unadjusted models (HR=0.73, p<.001); this association was explained mostly by adjustments for demographics (HR=0.92, p=.08) & slightly by other factors (HR=0.91, p=.08).



Conclusions: Cinacalcet use varies widely between facilities, likely due to differences in policies & provider preferences. In this international HD cohort, mortality risk is slightly lower with cinacalcet use. Whether this finding is due entirely to patient health status or to a drug effect requires additional investigation.

Funding: Pharmaceutical Company Support

FR-PO1238

Characterization of KAI-4169, a Novel Peptide for the Treatment of Chronic Kidney Disease – Mineral and Bone Disorder, in a Phase I Study in Healthy Males Kevin J. Martin,[1] Gregory Bell,[2] Karen Pickthorn,[2] Saling Huang,[2] Peter Hodsman,[3] Munro Peacock.[4] *[1]Saint Louis University School of Medicine; [2]KAI Pharmaceuticals; [3]Nucleus Network; [4]Indiana University.*

Background: KAI-4169 is a novel peptide agonist of the CaSR that is being developed as a therapy for patients with chronic kidney disease-mineral and bone disorder (CKD-MBD). This 'first-in-man' study was conducted to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of KAI-4169 in healthy males.

Methods: KAI-4169, administered as an IV bolus, was evaluated in a single-center, randomized, double-blind, placebo-controlled, single-dose, dose escalation Phase 1 study. Eight subjects were enrolled in each of 4 cohorts (0.5, 2, 5, 10 mg) and were randomized 6:2 to KAI-4169 or placebo. Serum for routine chemistries and PTH were obtained prior to dosing and every 2-6 hours following dosing for 48 hours. Plasma for KAI-4169 analysis were collected at regular intervals following dosing. Urine was collected 24 hours prior to dosing and for 48 hours following dosing.

Results: The plasma terminal half-life of KAI-4169 was ~20 hours and KAI-4169 exposure was dose-dependent. KAI-4169 resulted in dose-dependent reductions in iPTH with a mean percent change from baseline at 30 minutes folowing dosing of -3.5, -21.7, -55.4, -69.0 and -72.6% for placebo, 0.5, 2, 5 and 10 mg groups, respectively. Dose-dependent reductions in serum calcium were observed with a mean maximal reduction of ~16% at the highest dose. There was no significant change in the urine Ca/Cr ratio but tubular reabsorption of Ca tended to decrease with dose in the period immediately following dosing. Mean serum phosphorus (Pi) changes were similar to placebo except in the 10 mg cohort where it increased; however, apparent dose-dependent reductions in the Pi/Cr ratio and dose-dependent increases in tubular reabsorption of Pi were observed.

Conclusions: KAI-4169, a novel peptide agonist of the CaSR, was well tolerated at single doses up to 10 mg in healthy men and resulted in sustained dose-dependent reductions in PTH and calcium. KAI-4169 may represent a novel therapeutic approach for the treatment of CKD-MBD.

Funding: Pharmaceutical Company Support

FR-PO1239

Evidence for Increased Catabolism of 25(OH)D in CKD Hala Alshayeb,[1] Barry M. Wall,[2] Arif Showkat,[1] Geeta G. Gyamlani,[2] Valentin David,[1] Bing Dai,[1] Leigh Darryl Quarles.[1] *[1]Nephrology, UTHSC, Memphis, TN; [2]Nephrology, VAMC, Memphis, TN.*

Background: Low circulating 25(OH)D, a marker of vitamin D deficiency, is prevalent in CKD. 1,25$(OH)_2$D is synthesized from 25(OH)D by CYP 27b1 and both D metabolites are catabolized via Cyp24-mediated hydroxylation. Complex derangements in vitamin D metabolism occur in CKD, including PTH stimulation of Cyp27b1 and FGF23 and active vitamin D analogue stimulation of Cyp24. The contribution of Cyp24-mediated catabolism to the lower serum 25(OH)D in CKD is not clear.

Methods: We examined serum FGF23 and 25(OH)D levels and kidney Cyp24 mRNA expression in a nutritionally replete Col4a3$^{-/-}$ mouse CKD model. In addition, we assessed the ability of cholecalciferol po 10,000 IU/week for 8 weeks to increase serum 25OHD level in CKD (n=14) and non-CKD (n=14) patients with 25(OH)D levels ≤ 20 ng/ml. We assessed the effects of concomitant treatment with activate vitamin D analogues on cholecalciferol-induced changes in serum 25(OH)D in patients with ESRD (n=14).

Results: The Col4a3-/- mice exhibited reductions in renal function that were associated with progressive increments in serum FGF23 levels and elevations of Cyp24 message expression in the kidney. Serum FGF23 concentrations were negatively correlated with serum 25(OH)D levels in Col4a3-/- mice (Pearson r=-0.65, p<0.00001). CKD and non-CKD patients had similar baseline 25(OH)D and 1,25(OH)2D concentrations, but the CKD group had significantly higher baseline PTH levels(167±117 vs 60± 27). The change in 25OHD and 1,25(OH)2D levels were lower in CKD vs non-CKD (12±9 vs 19±8, p=0.05) and (4±24 vs 16±32, p =0.1). Serum iPTH levels decreased significantly after treatment in CKD (-42±68, p=0.04) but not in non-CKD (-10±25, p=0.16). In the ESRD group co-treatment with doxercalciferol resulted in significantly lower cholelcalciferal induced increments in 25(OH)D (11±6) compared to the non-doxercalciferol treated group (22±12) (p=0.04).

Conclusions: Experimental CKD in mice have FGF23-associated reductions in 25(OH) D and patients with impaired renal function are resistant to cholecalciferol treatment possibly due to FGF23 and/or doxercalciferol-mediated Cyp24 catabolism of 25(OH)D.

Funding: NIDDK Support

FR-PO1240

Vitamin D Deficiency in Fabry Disease Christiane Drechsler,[1] Stefan Pilz,[3] Christoph Wanner.[1] *[1]Dept of Medicine 1, Div of Nephrology, University of Wuerzburg, Germany; [2]Dept of Endocrinology, University of Graz, Austria.*

Background: Patients with Fabry disease frequently develop left ventricular (LV) hypertrophy and renal fibrosis. Due to heat intolerance and inability to sweat, patients tend to avoid sunlight exposure. We hypothesized that subsequent vitamin D deficiency may contribute to hypertrophic cardiomyopathy (HCM). This study investigated the vitamin D status and its association with LV mass, HCM and adverse clinical symptoms in patients with Fabry disease.

Methods: 25-hydroxyvitamin D (25[OH]D) was measured in 111 patients with genetically proven Fabry disease. LV mass and HCM were assessed by echocardiography and magnetic resonance imaging. In cross-sectional analyses, associations with adverse

clinical outcomes were determined by linear and binary logistic regression analyses, respectively, and adjusted for age and sex.

Results: Patients had a mean age of 40±13 years (42% male), and mean 25(OH)D of 23.5±11.4 ng/ml. Those with severe vitamin D deficiency (25[OH]D ≤ 10ng/ml) had an adjusted 10fold higher risk of HCM compared to those with sufficient 25(OH)D levels >30ng/ml (p=0.005). The mean LV mass was meaningfully different with 170±75 g in severely deficient, 154±60 g in deficient and 128±58 g in Vitamin D sufficient patients, respectively (p=0.013). With the severity of vitamin D deficiency, the median levels of proteinuria increased, as did the prevalences of depression, edema, cornea verticillata and the need for medical pain therapy.

Conclusions: Vitamin D deficiency was strongly associated with HCM and higher LV mass in patients with Fabry disease as well as adverse clinical symptoms. Whether vitamin D supplementation improves complications of Fabry disease, requires randomized controlled trials.

FR-PO1241

Regulators of Bone Mineral Disorder and Vascular Function in Patients with Chronic Kidney Disease Doris T. Chan,[1,2] Ashley B. Irish,[3] Gursharan K. Dogra.[1] *[1]Renal Unit, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia; [2]School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, Australia; [3]Department of Nephrology, Royal Perth Hospital, Perth, Western Australia, Australia.*

Background: In light of the known association between hyperphosphatemia and mortality, regulators of bone mineral disorder (BMD) such as fibroblast growth factor-23 (FGF-23), 25-hydroxyvitamin D (25OHD) and fetuin-A may contribute to excess cardiovascular risk in patients with chronic kidney disease (CKD) by mechanisms which involve alteration in vascular function.

Methods: In this cross-sectional study of patients with stage 3-5 CKD (n=125) and 40 healthy controls (HC), regulators and markers of BMD [FGF-23, fetuin-A, 25OHD, calcium, phosphate (PO4), intact parathyroid hormone (iPTH)] and measures of vascular function [endothelium-dependent flow mediated dilatation (FMD) and systemic arterial compliance (SAC)] were evaluated.

Results: Compared with HC, CKD subjects had lower 25OHD, higher PO4, fetuin-A and FGF-23 levels (all p<0.05) but had no difference in serum calcium levels. CKD subjects with lower FMD (median cut-off <4.19%) had higher FGF 23 (P=0.05) and fetuin (p=0.02) levels while those with impaired large artery compliance (median cut-off <14.58ml/mmHgx10) had lower 25OHD level (p=0.05) but with no difference in serum calcium, PO4 and iPTH. In a stepwise multiple regression analysis, higher FGF-23 was independently associated with reduced FMD (beta=-0.28, p=0.001) while low 25OHD was independently associated with impaired large artery compliance (beta=0.19, p=0.01).

Conclusions: Increased serum FGF-23 level in CKD is independently associated with endothelial dysfunction, whereas low 25OH was independently associated with impaired large artery compliance. These data support a link between regulators of BMD and vascular dysfunction in CKD.

Funding: Pharmaceutical Company Support, Private Foundation Support

FR-PO1242

Comparison of Alfacalcidol and Paricalcitol for Treatment of Secondary Hyperparathyroidism in Hemodialysis Patients: A Randomised Cross-Over Study Ditte Hansen,[1] Knud Rasmussen,[1] Henning B. Danielsen,[8] Helmut Meyer-Hofmann,[2] Egidijus Bacevicius,[2] Jens K. Madsen,[9] Birgitte Godskesen Tougaard,[9] Peter Marckmann,[4] Jorgen Nielsen,[6] Svend Kreiner,[7] Peter Thye-Rønn,[5] Thomas G. Lauridsen,[3] Lisbet Brandi.[1] *[1]Roskilde University Hospital; [2]Aalborg University Hospital; [3]Region Hospital Holstebro; [4]Odense University Hospital; [5]Esbjerg Hospital of Southwest Denmark; [6]Holbaek Hospital; [7]Department of Biostatistics, University of Copenhagen; [8]Viborg Hospital; [9]Århus Universityhospital Skejby, Denmark.*

Background: Alfacalcidol and Paricalcitol are vitamin D analogs used for secondary hyperparathyroidism in CKD patients. Their ability to suppress secondary hyperparathyroidism, without elevating phosphate and calcium outside the accepted range, have never been compared in a randomised clinical trial before.

Methods: In a Danish multicenter randomised 2 x 16 weeks cross-over study, intravenous alfacalcidol and paricalcitol were compared in chronic hemodialysis patients with secondary hyperparathyroidism. Doses were increased every second week until parathyroid hormone were sufficiently suppressed or phosphate or ionised calcium were increased above accepted range.

Results: A total of 86 hemodialysis patients were randomised. Due to the presence of a period effect only data from the first treatment period (n=80) were available for statistical tests. The proportion of patients reaching a 30% decrease in parathyroid hormone during the last four weeks of the treatment period were similar in the two groups (alfacalcidol 82% and paricalcitol 93% (P=0.180)). There was no difference in the incidence of hypercalcemia or hyperphosphatemia between the treatment groups. A significant interaction effect between baseline parathyroid hormone and treatment was found (P=0.012), suggesting that alfacalcidol suppressed parathyroid hormone irrespective of baseline level, whereas suppression by paricalcitol depended on baseline level.

Conclusions: We found no over-all difference between alfacalcidol and paricalcitol with respect to suppression of secondary hyperparathyroidism and induction of hypercalcemia and hyperphosphatemia in hemodialysis patients.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1243

Lack of Signalling through Vitamin D Receptor Leads to Stress-Induced Premature Senescence in Vascular Smooth Muscle Cells Petya Valcheva, Noelia Torremade, Milica Bozic, Elvira Fernandez, Jose M. Valdivielso. *Experimental Nephrology, IRBLLEIDA, Lleida, Spain.*

Background: Vitamin D has been associated with cardiovascular health in a number of epidemiological studies. In vascular smooth muscle cells (VSMC), vitamin D regulates proliferation and calcification. In the artery wall, angiotensin II (Ang II) is a potent mediator of oxidative stress and provokes premature senescence in VSMC. Ang II levels are controlled by renin expression, which promoter is regulated by vitamin D. Thus, a defect of vitamin D signalling in the artery wall could lead to an increase in Ang II levels and its adverse effects.

Methods: Proliferation of VSMC of VDRKO and WT mice was tested in vitro and in vivo. The expression of the cell cycle regulators involved in the G1/S transition checkpoint were analyzed. The local production of Ang II and of Ang II-induced free radicals, and its involvement in the phenotype of VDRKO cells was tested.

Results: Proliferation rates of VSMC from VDR null mice (VDRKO) are lower than wild type (WT) both, in vitro and in vivo, together with an increased expression of p19Arf, p27Kip1, p21Cip1 and p57Kip2, and lower levels phosphorylated pRb and cyclins D and E. Furthermore, VDRKO cells showed an increase in the expression of cathepsin D and Ang II type 1 (AT1) receptor, together with an increased production of Ang II. Also, superoxide anion production was higher in the mutant cells, and was inhibited with both losartan and DPI. Thus, VDRKO cells showed premature senescence together with an increase in free radicals produced by NADPH oxidase.

Conclusions: Absence of VDR signalling in VSMC leads to premature senescence due to the increased local production of AngII, and an increase in free radicals, which suggests a possible role of the vitamin D system in vascular diseases associated with hyperproliferation of VSMC.

Funding: Government Support - Non-U.S.

FR-PO1244

Effects of Cinacalcet Treatment on Serum Soluble Klotho Levels in Hemodialysis Patients with Secondary Hyperparathyroidism Hirotaka Komaba, Hisae Tanaka, Masahiro Koizumi, Hiroo Takahashi, Kaichiro Sawada, Takatoshi Kakuta, Masafumi Fukagawa. *Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan.*

Background: Klotho is a transmembrane protein and acts as a cofactor for fibroblast growth factor 23 (FGF23). In patients with end-stage renal disease, parathyroid expression of Klotho and FGF receptor 1 is decreased, which may contribute to the pathogenesis of secondary hyperparathyroidism (SHPT) by causing parathyroid resistance to FGF23 to inhibit parathyroid hormone (PTH) secretion. Klotho also exists as a soluble circulating protein, which is generated by cleavage of the extracellular domain of the molecule. However, the role of soluble Klotho in patients with SHPT is largely unknown.

Methods: This was a *post hoc* analysis of data from a 52-week, multicenter, open-label, single-arm trial that examined the effect of cinacalcet on SHPT in patients undergoing hemodialysis. Soluble Klotho and full-length FGF23 levels were measured at baseline and study weeks 12, 24, and 52, using serum samples of 51 patients who participated and completed the study.

Results: At baseline, median soluble Klotho was 398 pg/ml (interquartile range, 268 to 588 pg/ml), which was slightly lower than the reported values in healthy subjects. After 12 weeks of cinacalcet treatment, soluble Klotho decreased significantly but only slightly (median, 378 pg/ml; interquartile range, 266 to 568 pg/ml), and returned to the baseline values thereafter. There were no significant associations between changes in soluble Klotho and changes in any other parameters of mineral metabolism, including serum calcium, phosphorus, intact PTH, and FGF23.

Conclusions: Despite significant alterations in mineral and bone metabolism during treatment with cinacalcet, there were only slight and transient reductions in soluble Klotho levels. Further studies are needed to determine the role of soluble Klotho in altered mineral metabolism in patients with SHPT.

Funding: Private Foundation Support

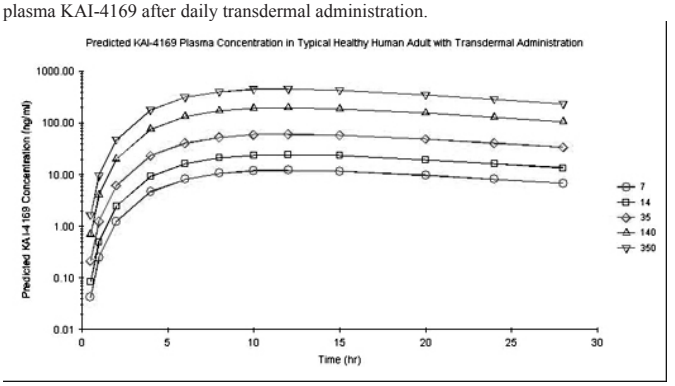
FR-PO1245

PK/PD Modeling of Transdermal Delivery of a Novel Peptide, KAI-4169, for the Treatment of Chronic Kidney Disease-Mineral and Bone Disorder Karen Pickthorn,[1] Jun Shen,[2] Andrew M. Vick,[2] Sarah Walter,[1] Saling Huang,[1] Gregory Bell,[1] Randolph M. Johnson.[1] *[1]KAI Pharmaceuticals, South San Francisco, CA; [2]Seventh Wave, Chesterfield, MO.*

Background: KAI-4169, a novel peptide agonist of the calcium sensing receptor that inhibits PTH secretion, is in development for the treatment of CKD-MBD. Microporation of the stratum corneum is a painless method of enabling transdermal delivery of peptide compounds. Systemic delivery of KAI-4169 using transdermal microporation patch technologies was evaluated in animals and was used with clinical Phase 1 PK and PTH data obtained following IV KAI-4169 to healthy volunteers (HV) and hemodialysis patients (HD) to develop a quantitative PK/PD model for daily dosing with a KAI-4169 patch.

Methods: KAI-4169 disposition parameters were directly extracted from Phase 1 data. From preclinical data, bioavailability was estimated and a transit absorption model constructed to estimate the number of compartments and mean transit time for drug to

pass through these compartments to reach the central circulation. Using these parameters, a 1-compartment distribution with transit absorption model was constructed to simulate plasma KAI-4169 after daily transdermal administration.



Phase 1 PK/PD data were then leveraged to simulate PTH response with KAI-4169 patch administration in Stage 4 CKD patients.

Results: The novel peptide KAI-4169 can be delivered systemically by patch technologies. In HV and HD subjects, KAI-4169 demonstrated low PK variability and a predictable exposure-response relationship. Simulations performed with the integrated PK/PD model indicate that daily dosing with KAI-4169 patch will result in dose-dependent sustained exposure to KAI-4169 with corresponding reductions in PTH.

Conclusions: Daily transdermal patch administration of KAI-4169 is predicted to provide sustained PTH control in Stage 4 patients with CKD-MBD.

Funding: Pharmaceutical Company Support

FR-PO1246

Calcidiol Is Less Effective Than Calcitriol in Regulating VDR Target Gene Expression in Human Coronary Artery Smooth Muscle Cells J. Ruth Wu-Wong,[1] Myles S. Wolf,[2] Masaki Nakane.[1] *[1]Renal Research, VidaGene, Chicago, IL; [2]Miller School of Medicine, University of Miami, FL.*

Background: Vitamin D receptor (VDR) activation by agonists such as calcitriol, paricalcitol and doxercalciferol is associated with cardiovascular benefits in chronic kidney disease patients, but whether VDR's hormone, calcitriol, and prehormone, calcidiol, exhibit similar effects requires more studies.

Methods: Primary culture of human coronary artery smooth muscle cells (HCASMCs) were treated with the VDR agonist calcitriol or the prehormone calcidiol in the presence of normal (0.9 mM) or elevated (2.06 mM) phosphate (Pi). The expression of VDR target genes were determined by real-time PCR and proteins detected by Western blotting. The expression and activity of CYP27B1 (the enzyme responsible for converting calcidiol to calcitriol) was also measured.

Results: Treating HCASMCs with 2.06 mM Pi for 24 -48 hr significantly elevated the VDR mRNA (233%) and protein levels (181%). Calcitriol and calcidiol induced CYP24A1 expression with EC50 values at 70 and 662 nM, respectively; the effects of both compounds were not affected by high Pi. Calcitriol and calcidiol stimulated the expression of thrombomodulin with EC50 at 61 and 156 nM at 0.9 mM Pi, respectively. When the Pi concentration was increased to 2.06 mM, the EC50 of calcitriol on stimulating thrombomodulin was at 31 nM, but the EC50 of calcidiol was increased to 780 nM. At either 0.9 or 2.06 mM Pi, calcitriol suppressed the expression of thrombospondin-1 with IC50 at 9-12 nM, while calcidiol had no significant effect. Mechanistic analysis demonstrated that HCASMCs expressed the CYP27B1 mRNA and protein, which was not significantly affected by elevated Pi. HCASMCs converted calcidiol to calcitriol at a low rate (about 20% in 24 h).

Conclusions: HCASMCs express CYP27B1 and are capable of converting calcidiol to calcitriol, but the conversion rate is low, which may explain why the prehormone calcidiol is less effective than calcitriol in regulating VDR target gene expression.

FR-PO1247

Relationship between Parathyroid Hormone(PTH)and Serum Phosphorus (P) Levels before and during Treatment with Cinacalcet among Hemodialysis (HD) Patients with Secondary Hyperparathyroidism (SHPT) Kerry Cooper, Yumi Kubo, Holly Tomlin, William G. Goodman. *Amgen Inc.*

Background: Efforts to control hyperphosphatemia in patients on HD focus mainly on reducing gastrointestinal P absorption with dietary P restriction and P binders. Much less attention is given to SHPT as a cause of hyperphosphatemia despite the known effect of PTH to mobilize both P and calcium from bone.

Methods: We thus evaluated the relationship between PTH and P levels before and during 26 weeks of treatment with Sensipar®/Mimpara® (cinacalcet) using pooled data from Phase III studies. All subjects had a PTH>300pg/mL and calcium≥8.4mg/L at study entry. Per criteria, all subjects were on constant doses of vitamin D sterols and P binders at baseline and mean doses did not change during follow-up. The severity of SHPT at baseline was categorized by PTH levels: 300-500pg/mL; 500-800pg/mL; and >800pg/mL. Among cinacalcet-treated subjects (N=656), the median (Q1,Q3) of all PTH and P levels between study weeks 12 and 26 were compared with median PTH and P values at baseline.

Results: The median serum P at baseline was incrementally higher by category of disease severity, being highest in those with PTH>800 pg/mL. Serum P decreased during treatment with cinacalcet regardless of baseline disease severity. Both the absolute change and percentage decrease in serum P were incrementally greater with increasing disease severity. In fact, the largest reductions in serum P were seen in cinacalcet-treated subjects with baseline PTH>800 pg/mL. For all cinacalcet-treated subjects, the reductions in PTH and serum P were positively associated.

	PTH (pg/mL) 300-500 (N=242)	500-800 (N=228)	>800 (N=186)
Baseline P (mg/dL), median (Q1, Q3)	5.82 (4.81, 6.73)	5.88 (4.89, 7.10)	6.55 (5.45, 7.67)
P after Cinacalcet Therapy [median, (Q1, Q3)]			
Mean P During Therapy (mg/dL)	5.21 (4.30, 6.31)	5.28 (4.35, 6.28)	5.64 (4.74, 6.77)
Absolute Change from Baseline (mg/dL)	-0.48 (-1.29, 0.26)	-0.62 (-1.58, 0.41)	-0.86 (-1.73, 0.10)
Percent Change from Baseline (%)	-7.95 (-21.94, 5.28)	-10.11 (-26.27, 6.81)	-12.46 (-26.51, 2.22)
PTH after Cinacalcet Therapy [median (Q1, Q3)]			
Mean PTH During Therapy (pg/mL)	191.10 (135.00, 298.49)	281.10 (1 72.29, 426.00)	703.10 (384.12, 1134.00)
Absolute Change from Baseline (pg/mL)	-197.56 (-263.09, -74.81)	-345.31 (-453.30, -201.95)	-464 (-733.80, -105.60)
Percent Change from Baseline (%)	-51.07 (-66.45,20.85)	-54.99 (-72.46, -30.87)	-39.60 (-65.02, -8.62)

Conclusions: Hyperphosphatemia worsens in subjects with advanced SHPT and serum P levels decrease when PTH levels are lowered during treatment with cinacalcet. Inadequately controlled SHPT contributes substantially to the hyperphosphatemia that characterizes patients undergoing HD.

Funding: Pharmaceutical Company Support

FR-PO1248

25-Hydroxyvitamin D3 Levels Are Predictors of Morbidity and Mortality in Hemodialysis Patients Patrícia Matias,[1,2,3] Cristina Jorge,[1,2,3] Carina Ferreira,[1,2,3] Marília Borges,[4] Inês Aires,[1,2,3] Tiago Amaral,[1,2,3] Marco Mendes,[1,2,3] Célia Gil,[1,2,3] José Cortez,[4] Aníbal Ferreira.[1,2,3] *[1]Dialysis Clinic, Hemodial, Vila Franca de Xira, Portugal; [2]Dialysis Clinic, Dialverca, Alverca, Portugal; [3]NIDAN, Lisboa, Portugal; [4]Laboratório Dr. Fernando Teixeira, Lisboa, Portugal.*

Background: Vitamin D deficiency has been associated with the development of cardiovascular (CV) disease and mortality in the general population.

The aim of this prospective study was to evaluate the relationship between 25-hydroxyvitamin D3 [25(OH)D3] and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] levels and mortality in chronic hemodialysis (HD) patients.

Methods: With this purpose, we measured [25(OH)D3] and [1,25(OH)2D3] serum levels twice (end of Winter and of Summer) and correlated them with hospitalizations and overall and CV mortality for the following 48-month period. All patients were submitted to oral cholecalciferol supplementation according to baseline calcidiol levels during the studied period.

We studied 223 HD patients with mean age (± SD) of 62.7±15.3 years, 48% female, 27% diabetics, with mean HD time of 42.9±39.3 months. Univariate and multivariate analysis were performed and a $p<0.05$ was considered significant.

Results: During the study, 44% of the patients were hospitalized at least once and 29% of the patients died (mainly from CV causes). [25(OH)D3] levels were significantly lower in the patients that died from all causes (16.6±8.2 vs. 23.7±13.1 ng/mL, $p< 0.001$) and in patients that died from CV causes (16.4±8.7 vs. 22.6±12.6 ng/mL, p=0.006). [25(OH)D3] levels were also lower in patients hospitalized during the study (18.5±9.3 vs. 23.9±11.7 ng/mL, p=0.001). [1,25(OH)2D3] levels were similar in all groups.

In multivariate analysis, lower levels of [25(OH)D3] were predictors of hospitalization (p=0.01), death from all causes (p=0.001) and death from CV causes (p=0.02). Patients with [25(OH)D3] deficiency (< 15 ng/mL) had a significantly lower survival at the end of the 48-month studied period ($p<0.001$).

Conclusions: In conclusion, [25(OH)D3] serum levels seem to be a good marker of morbidity (according to hospitalizations) and mortality (overall and CV) in HD patients.

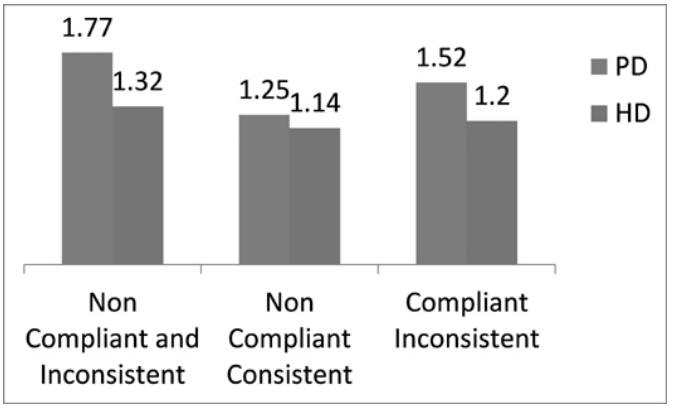
FR-PO1249

Serum Phosphorous Variability Is Associated with Elevated Mortality Risk in Hemodialysis and Peritoneal Dialysis Patients Stefanos Zenios,[1,3] Hector J. Rodriguez,[2] Constantia Petrou.[3] *[1]Graduate School of Business, Stanford University; [2]DaVita Inc; [3]Culmini Inc.*

Background: To determine the association between serum phosphorous levels and variability, and mortality in ESRD patients.

Methods: Data on all ESRD patients from a major dialysis chain from 1/1/2006 to 12/1/2009 were divided into six-month intervals generating a total of 119,191 patients and 852,239 six-moth windows. For each patient-interval we obtained treatment modality (PD vs HD), average and standard deviation of serum phosphorous levels, age, gender and race, comorbidity indicators, Charlson index, ICD-9-CM codes, medical tests, dialysis adequacy, pre and post dialysis weight and blood pressure, medication doses and compliance. Patients were classified: compliant when average serum phosphorous was below the K/DOQI guidelines standard of 5.5 mg/dl, non-compliant otherwise, consistent when standard deviation of serum phosphorous was below the all-patient average of the standard deviations, inconsistent otherwise. Time dependent proportional hazards regression for time to death was performed seperately for HD and for PD.

Results: 57% of patients were compliant, 56% were consistent, 40% were compliant and consistent. After adjusting for comorbidities, other lab tests, and medication dosing and compliance, higher serum phosphorous levels and variability were associated with higher mortality in both HD and PD patients. The Figure presents relative mortality risk (compliant/consistent is the baseline) - all values were significantly higher than 1 (p-value < 0.001)



Conclusions: Serum phosphorous variability was found to be an independent predictor of mortality in both PD and HD patients. Optimal medical management should focus on managing both serum phosphorous levels and their variability. Unmeasured variables may account for patients with higher and more variable phosphorous levels having higher mortality.

Funding: Pharmaceutical Company Support

FR-PO1250

Vitamin D Catabolism in Chronic Kidney Disease Cortney R. Bosworth, Gregory Levin, Cassianne Robinson-Cohen, Bryan R. Kestenbaum, Jonathan Himmelfarb, Bessie A. Young, Ian H. de Boer. *University of Washington, Seattle, WA.*

Background: The current paradigm of decreased 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] activation in chronic kidney disease (CKD) is incomplete because it overlooks the role of vitamin D catabolism. 24,25-dihydroxyvitamin D [24,25$(OH)_2$D], the first product in the metabolism of 25-hydroxyvitamin D [25(OH)D] by CYP24A1, is a biomarker of vitamin D catabolism. We developed a novel assay to quantify serum 24,25$(OH)_2$D and studied its determinants, functional significance, and health outcomes in CKD.

Methods: We performed a case-cohort study in the Nephrology clinic-based Seattle Kidney Study. We randomly sampled 277 of 531 participants for inclusion in descriptive analyses and added 34 cases for mortality analyses (total N=90 cases of death). Vitamin D metabolites were measured from frozen baseline serum using HPLC-tandem mass spectrometry. The Barlow method was used for assigning case-cohort sampling weights in survival analyses.

Results: At baseline, participants had a mean eGFR of 46ml/min/1.73m2 and a mean age of 61 years. 83% were men and 69% were white. 24,25(OH)2D concentration declined with kidney function. For eGFR ≥60, 45-59, 30-44, 15-29, and <15 ml/min/1.73m2, mean serum 24,25(OH)2D concentrations were 3.6, 3.2, 2.6, 2.6, and 1.7ng/ml, respectively (p trend <0.001). Black race, diabetes, and acidosis were also associated with lower 24,25(OH)2D concentration. 24,25(OH)2D concentration was more strongly correlated with parathyroid hormone (PTH) concentration (r=-.37, p<0.001) than 25(OH)D (r=-.24) or 1,25(OH)2D (r=-.16). 24,25(OH)2D concentration below the cohort median (2.4ng/mL) was associated with an estimated 87% greater risk of death (95% CI 3%, 240%), p=0.04, after adjustment for age, sex, race, diabetes, coronary artery disease, eGFR, albuminuria, bicarbonate, and 25(OH)D. This association was not attenuated by further adjustment for 1,25(OH)2D, PTH, or fibroblast growth factor-23.

Conclusions: CKD is a state of stagnant vitamin D metabolism characterized by both decreased 1,25$(OH)_2$D production and decreased vitamin D catabolism. Low 24,25$(OH)_2$D, a novel marker of vitamin D catabolism, is associated with increased risk of death.

Funding: Private Foundation Support

FR-PO1251

The Vitamin D Analog Eldecalcitol (ED-71) Is a Potent Inducer of Intestinal Phosphate Absorption and Sodium-Phosphate Cotransporter IIb Alex J. Brown, Cynthia S. Ritter. *Renal Division, Washington University School of Medicine, St. Louis, MO.*

Background: The vitamin D analog eldecalcitol (ED-71) has been developed for treatment of osteoporosis. We have previously reported that this analog has greater potency than calcitriol, the natural vitamin D hormone, on intestinal calcium and phosphate (Pi) absorption. In the present study, we further investigated the actions of eldecalcitol on Pi absorption and induction of the sodium/phosphate cotransporters.

Methods: Vitamin D-deficient rats were treated orally with 0, 20, 50 or 150 pmol of eldecalcitol or calcitriol q.o.d. for 12 days. Duodenal Pi absorption was measured by the duodenal loop method. Phosphate transporter mRNAs were quantified by RT-qPCR. In a second experiment, normal rats were gavaged with vehicle, eldecalcitol (50 pmol) or calctriol (50 or 1000 pmol) at 72 and again at 24 h prior to sacrifice, and phophate transporters expression was determined in the duodenum, jejunum, ileum, kidney and lung.

Results: Duodenal Pi absorption was maximally increased by 8-fold with the 20 pmol dose of eldecalcitol, while none of the doses of calcitriol were effective. This action of eldecalcitol was attributable to a dramatic 24-fold induction of NaPi-IIb (SLC34A2) mRNA in the duodenum. Other duodenal sodium-phosphate cotransporters, Pit-1 (SLC20A1) and Pit-2 (SLC20A2) were not increased. In normal rats, eldecalcitol increased NaPi-IIb 8- and 3-fold in the duodenum and jejunum, respectively, but had no effect in ileum or lung, despite the presence of the VDR and induction of 24-hydroxylase mRNA. Calcitriol had no effects in any of the tissues. Renal NaPi-IIa and NaPi-IIc were not regulated by either vitamin D compound under these conditions.

Conclusions: These studies indicated that the vitamin D analog eldecalcitol is a unique potent stimulator of intestinal Pi absorption via induction of NaPi-IIb. The mechanisms responsible are under investigation.

Funding: Pharmaceutical Company Support

FR-PO1252

Vitamin D Suppresses High Glucose-Induced GLUT1 Expression in Mesangial Cells by Targeting NF-κB Dilip K. Deb, Youli Wang, Yunzi Chen, Yan Chun Li. *Department of Medicine, University of Chicago, IL.*

Background: Glucose transporter 1 (GLUT1) is a facilitative glucose transporter that is up-regulated by high glucose (HG). In diabetes GLUT1 overexpression in mesangial cells stimulates glucose uptake and the synthesis of extracellular matrix, leading to glomerular injury. Glomerular GLUT1 overexpression in transgenic mice causes glomerulosclerosis even in non-diabetic conditions. Our previous studies showed that vitamin D reduces glomerulosclerosis in diabetic nephropathy.

Methods: We used mesangial cell cultures to explore the molecular mechanism underlying vitamin D inhibition of glomerulosclerosis

Results: Exposure of primary mesangial cells to HG media (30 mM) markedly stimulated GLUT1 expression at mRNA and protein levels, and the stimulation was blocked by 1,25-dihydroxyvitamin D (1,25-VD). Consistently 1,25-VD also inhibited HG-induced glucose influx in mesangial cells. The HG-stimulation was blocked by NF-κB inhibitor BAY 11-7082, suggesting the involvement of NF-κB in HG induction of GLUT1. A putative cis-κB site was identified in the GLUT1 enhancer-2 at + 17282 by in silico analysis. EMSA showed that this κB site could be bound by p65. ChIP assays with anti-p65 antibodies confirmed the interaction of p65 with this κB site in mesangial cells. HG increased p65 binding to the κB site, which was blocked by 1,25-VD. Luciferase reporter assays using pEn-GLUT1-Luc plasmid showed that HG induced luciferase activity in the wild-type plasmid, but not in a mutant plasmid in which this κB site was mutated. The HG-induced luciferase activity was inhibited by 1,25-VD treatment.

Conclusions: Taken together these data suggest that 1,25-VD suppresses HG-induced GLUT1 expression by targeting the κB-mediated pathway. Suppression of GLUT1 may contribute to the reno-protective activity of vitamin D against glomerulosclerosis in diabetes.

FR-PO1253

Serum 25-Hydroxyvitamin D Levels and Vascular Calcification in Predialysis and Dialysis Patients with Chronic Kidney Disease Dong Ho Yang,[1] So-Young Lee,[1] Yoon Hee Lee,[2] Hoon Jung.[3] *[1]Internal Medicine, Bundang CHA General Hospital, CHA University, Seongnam, Geonggi-do, Republic of Korea; [2]Pathology, Gangnam CHA Hospital, CHA University, Seoul, Republic of Korea; [3]Internal Medicine, Seoul Bukbu Geriatic Hospital, Seoul, Republic of Korea.*

Background: The role of vitamin D in the process of vascular calcification is controversial in patients with chronic kidney disease. We investigated whether serum 25-hydroxyvitamin D associates with vascular calcification in predialysis and dialysis patients.

Methods: We included 209 patients. Vascular calcification was evaluated by examining plain X-rays of pelvis and hands as previously described. The augmentation index (AIx) was assessed with a commercially available device (VP-2000, Colin Corporation).

Results: We found a high prevalence of vitamin D deficiency in our population (77.0%). Vascular calcification was present in 36.4% of all patients. The presence of vascular calcifications was significantly associated with lower 25(OH)D levels in predialysis, dialysis and all patients. Multivariate analysis showed that 25(OH)D levels were inversely associated with simple vascular calcification score ≥ 1 (OR; -0.037, 95% CI; 0.86 - 0.99, P = 0.037). Lower 25(OH)D levels were associated with higher AIx in predialysis and all patients, but this inverse relationship was abolished in multivariate analysis.

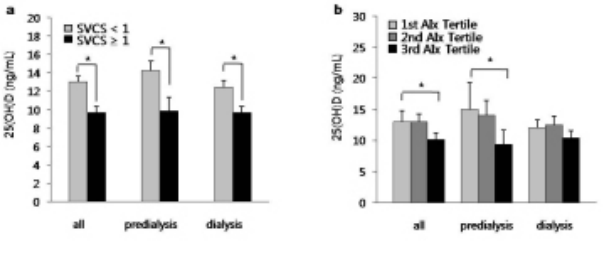


Figure. 1. (A) Mean 25(OH)D levels according to SVCS. (B) Mean 25(OH)D levels by tertiles of the augmentation index. *P < 0.05

Conclusions: We showed an independent relationship between low serum 25(OH)D levels and vascular calcification in both predialysis and dialysis patients.

FR-PO1254

Long-Term Effect of Cinacalcet Hydrochloride Treatment on the Parathyroid Gland Volume in Patients with Advanced Secondary Hyperparathyroidism

Shunsuke Yamada,[1] Masatomo Taniguchi,[1] Masanori Tokumoto,[3] Kazuhiko Tsuruya,[2] Satoru Fujimi.[4] *[1]Deparatment of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [3]Division of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan; [4]Department of Nephrology, Fukuoka Renal Clinic, Fukuoka, Japan.*

Background: Cinacalcet hydrochloride has been shown to lower serum PTH and to improve achievement rate of the guidelines for secondary hyperparathyroidism (SHPT). However, long-term effect of cinacalcet hydrochloride treatment with intravenous vitamin D therapy on the parathyroid gland (PTG) volume has not been fully elucidated.

Methods: The present study comprised of 60 hemodialysis patients from 10 dialysis centers in Japan, who received more than two years of cinacalcet treatment for advanced SHPT, resistant to intravenous vitamin D therapy (intact PTH; 568 ± 268 pg/mL). The serial changes in the biochemical parameters and maximum PTG volume were determined. We also investigated the factors that determined "marked PTG reduction", and the achievement rate of the guideline recommended by the Japanese Society of Dialysis Therapy (JSDT); 8.4 ≤ calcium ≤ 10.0 mg/dL, 3.5 ≤ phosphorus (P) ≤ 6.0 mg/dL, 60 ≤ intact PTH ≤ 180 pg/mL. "Marked PTG reduction" was defined as more than 30% reduction in maximum PTG volume after two years of treatment, and was used as the endpoint in the following analysis.

Results: Cinacalcet treatment with a mean dose of 47.5 mg/day for two years achieved approximately 25% reduction in PTG volume overall. A total of 33 patients showed "marked PTG reduction". The achievement rate of the JSDT guideline increased from 5% to 48%. Multivariate logistic regression analysis revealed that lower serum P level, less number of detected PTG, and PTG volume < 500 mm3 at baseline were significantly associated with "marked PTG reduction".

Conclusions: Cinacalcet treatment with intravenous vitamin D therapy for two years effectively reduced maximum PTG volume even in patients with advanced 2HPT, leading to the increased achievement rate of the JSDT guideline.

Funding: Private Foundation Support

FR-PO1255

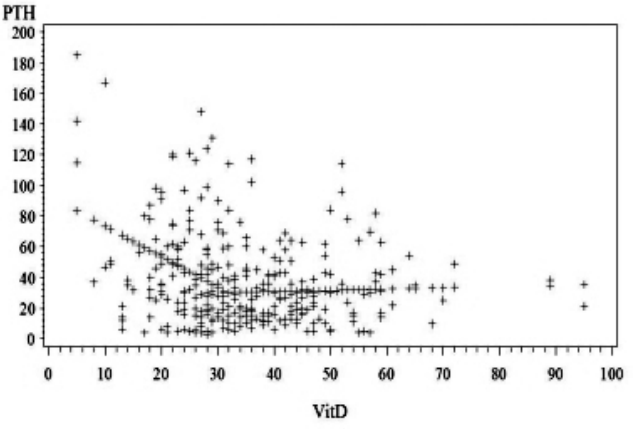
Serum 25-Vitamin D Level in Children: Do We Need To Change the Reference Range Based on 2011 Institute of Medicine (IOM) Report?

Tarak Srivastava, Uttam Garg, Melanie Ruiz, Hongying Dai, Uri S. Alon. *Bone and Mineral Disorders Clinic, Children's Mercy Hospital, Univ. of Missouri, Kansas City, MO.*

Background: The 2011 IOM Report recommends serum 25-Vitamin D (S-VitD) of ≥20 ng/ml as normal value. The traditional reference range (RR) of S-VitD for children in our clinical lab has been ≥30 ng/ml. Characteristically, in subjects with normal kidney function the onset of Vit D insufficiency is indicated by a rise in serum PTH (S-PTH). Considering the new IOM report, the aim of this study was to re-examine our RR for S-VitD based on S-PTH.

Methods: The database of clinical lab of our tertiary children's hospital from Jul 09 to Jan 11 was analyzed for S-VitD, S-PTH and serum creatinine (S-Cr). Data were included only of the child's initial sample and if all measurements done on same blood sample. S-VitD was measured by Tandem Mass Spectrometry on AB4000 QTrap, S-PTH by immunoassay (Immulite™) and S-Cr on Vitros autoanalyzer. To exclude patients with kidney failure, primary hyperpara and pseudohypopara we included only children with S-Cr ≤0.6 mg/dL and S-PTH ≤200 pg/ml.

Results: There were 304 samples with S-Cr ≤0.6 mg/dL and S-PTH ≤200 pg/ml. Using S-VitD <30, 39% were insufficent/ deficient compared to 10% for S-VitD <20 ng/ml. The inflection point by Gaussian-Newton method for S-VitD (Figure) was at 31.7 ng/ml (95%CI 26.4-37.0). S-PTH ≥75 pg/ml was recorded in 23 children (19.3%) with S-VitD <30 ng/ml compared with only 12 (6.5%) with S-VitD ≥30 ng/ml (χ2-test p=0.0006). OR for S-PTH ≥75 for S-VitD ≥30 was 0.29 (95%CI 0.14-0.61). Similar analysis in 553 children with S-Cr ≤1.0 mg/dL yielded identical results with inflection at 30.1 ng/ml (95%CI 24.1-36.1).



Conclusions: Based on both the inflection point for S-VitD and incidence of elevated S-PTH, and in contrast to the IOM recommendation, we suggest to maintain in children a cut-off value of ≥30 ng/ml.

FR-PO1256

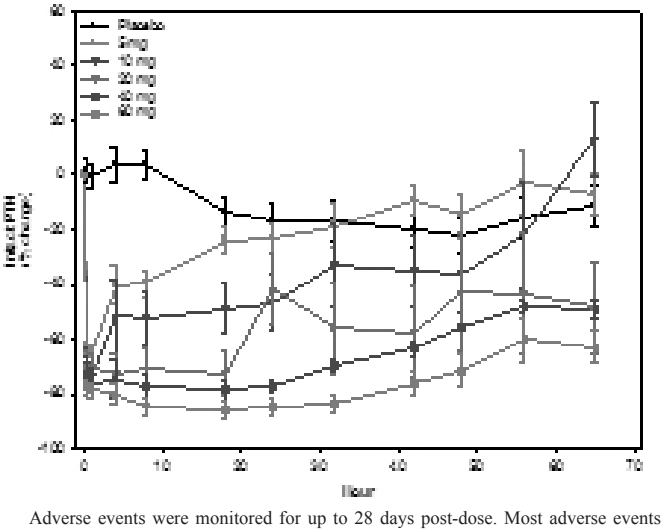
Characterization of KAI-4169, a Novel Peptide for the Treatment of Chronic Kidney Disease – Mineral and Bone Disorder, in a Single-Dose Study in Hemodialysis Subjects

Kevin J. Martin,[1] Gregory Bell,[2] Karen Pickthorn,[2] Saling Huang,[2] Marwan O. Kaskas,[3] Marializa Bernardo,[4] Peter F. Mount,[5] David A. Power.[5] *[1]Saint Louis University; [2]KAI Pharmaceuticals; [3]Northwest Louisiana Nephrology; [4]Southwest Houston Research; [5]Austin Hospital.*

Background: KAI-4169, a novel peptide agonist of the calcium sensing receptor (CaSR), is being evaluated in hemodialysis subjects as a treatment for CKD-MBD. This randomized, double-blind, placebo-controlled, single-dose, dose-escalation study was designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of KAI-4169 administered as an IV bolus.

Methods: Twenty-eight subjects were enrolled in one of five cohorts (5, 10, 20, 40, 60mg). Cohorts 1-3 were treated in a 2-period crossover design with 4 subjects/cohort. In Cohorts 4 and 5, 8 subjects were randomized 1:1 to KAI-4169 or placebo. Major inclusion criteria included hemodialysis for at least 3 months, serum iPTH >300 pg/mL, serum cCa ≥ 9.0 mg/dL and the dose of vitamin D or analogs had to be stable for at least 3 weeks prior to screening. Subjects were admitted to a clinical research unit following hemodialysis and were observed for 3 days prior to discharge for hemodialysis.

Results: Treatment with KAI-4169 resulted in rapid suppression of iPTH with mean maximal reduction from baseline of 64, 73, 75, 84 and 86 % at the 5, 10, 20, 40 and 60 mg dose levels, respectively.



Adverse events were monitored for up to 28 days post-dose. Most adverse events were mild or moderate and resolved without sequelae. No drug-related GI adverse events were reported.

Conclusions: KAI-4169, a novel peptide agonist of the CaSR, administered as an IV bolus resulted in sustained, dose-dependent reductions in serum PTH and was well tolerated at single doses up to 60 mg. KAI-4169 represents a novel therapeutic approach for the treatment of CKD-MBD.

Funding: Pharmaceutical Company Support

FR-PO1257

Are Current KDOQI/JSDT Guidelines Sufficient for Preventing the Progression of Secondary Hyperparathyroidism? Sato Megumi,[1] Masanor Jotoku,[2] Yuzuru Sato,[2] Ryota Ikee,[3] Masataka Tsunoda,[4] Naomi Sasaki,[5] Nobuo Hashimoto.[5] *[1]Department of Nephrology, Sato Junkankikanaika, Matsuyama, Ehime, Japan; [2]Department of Internal Medicine, Sato Junkankikanaika, Matsuyama, Ehime, Japan; [3]Department of Nephrology and Dialysis, H.N.Medic Kitahiroshima, Kitahiroshima, Hokkaido, Japan; [4]Department of Nephrology and Dialysis, H.N.Medic Spporo-Higashi, Sapporo, Hokkaido, Japan; [5]Department of Nephrology and Dialysis, H.N.Medic, Sapporo, Hokkaido, Japan.*

Background: There are several guidelines for secondary hyperparathyroidism(2°HPT) in the world, and each guideline has each standard values for Ca, P and PTH. In this study we compared the incidence of refractory 2°HPT between the dialysis patients who had been categorized by the current guidelines from the Kidney Disease Outcomes Quality Initiative(KDOQI) and Japanese Society for Dialysis Therapy (JSDT).

Methods: 180 chronic hemodialysis patients were enrolled into the study protcol. The patients were divided into the next 3 groups based on the averaged cCa and P. Group A: cCa and P met KDOQI guideline (n=107), Group B: gap area between JSDT and KDOQI (n=47), Group C: the rest patients (n=26). Refractory 2°HPT was identified as the administration of Cinacalcet hydrochloride(CH) or receiving of parathyroidectomy (PTx). The incident rates of refractory 2°HPT in each group were evaluated after 3-year-followup period.

Results: The mean values in cCa, P, i-PTH were shouwn in table 1. The incident rate in each group was significantly different from each other; 1/107 (0.9%) in Group A, 12/47 (25.5%) in Group B and 14/26 (53.9%) in Group C(Table 1).

Parameters of each group

	Group A(n=107)	Group B(n=47)	Group C(n=26)
cCa(mg/dL)	9.1±0.3	9.6±0.3	9.9±0.8
P(mg/dL)	4.9±0.4	5.4±0.5	6.1±1.1
i-PTH(pg/mL)	174.4±62.2	168.4±102.1	299.3±240.9
PTx. or CH	1/107	12/47	14/26
rate of incidence	0.9%	25.5%	53.9%

Conclusions: The JSDT guideline is not sufficient to prevent the onset of refractory 2°HPT because the incidence in Group B was significantly higher than that in Group A. In conclusion, ideal standard values in cCa and P should be stricter than the current values in both JSDT and KDOQI guidelines for preventing refractory 2°HPT.

FR-PO1258

Proteinuria Modifies Response to Cholecalciferol in CKD Amay Parikh,[1] Herbert S. Chase,[2] Linda Vernocchi,[1] Leonard Stern.[1] *[1]Columbia University, New York, NY; [2]Biomedical Informatics, Columbia University, New York, NY.*

Background: We previously showed that the response to cholecalciferol replacement therapy in CKD, assessed by the 25-OH Vitamin D (25VitD) level, was associated with eGFR: Non-responders displayed a worsening eGFR over time compared to Responders. The mechanism of the observation was unknown. The aim of this study was to test whether the effect on eGFR by the cholecalciferol response was modified by proteinuria.

Methods: 309 pts (Stages 2-5) were identified from 2001-2010 at the CKD Clinic at Columbia University. Demographics, creatinine, proteinuria (24 hr urine protein or protein/creatinine ratio), and VitD were extracted from the Columbia CKD Program database. Pts received cholecalciferol 10,000 IU capsules weekly as initial therapy. We repleted VitD to a target level of 40-60 ng/ml. When levels above 40 were not achieved, doses were titrated up to a maximum of 50,000 IU weekly. Pts reaching a level of 40 ng/ml were designated Responders. Pts not reaching a level of 40 ng/ml were designated Non-responders. The MDRD eGFR was calculated.

Results: 147/309 pts had proteinuria data and 93/147 pts with a mean follow up of 1.5 years had a concurrent creatinine. The initial mean 25VitD level was lower in the NON-RESPONDERS (19 vs. 23 ng/ml, p=0.02). The initial mean proteinuria in NON-RESPONDER (1.47 g/d) was higher than RESPONDER (0.89 g/d) (p<0.004). During 50 months of follow-up, proteinuria levels remained lower in the RESPONDERS (p=0.04). No difference was observed in microalbuminuria between the two groups. With similar initial eGFR (NON-RESPONDERS 27 vs. RESPONDERS 30 ml/min/1.72m^2, p=0.09) and elevated proteinuria, the eGFR declined faster in the NON-RESPONDERS. Regression modeling showed that proteinuria modifies the effect of cholecalciferol response on the change in eGFR (p=0.02).

Conclusions: Response to treatment with VitD is associated with lower initial levels of proteinuria, better preservation of eGFR, and a reduction in proteinuria over time. The mechanism of the observation is unknown but may be related to alterations of VitD metabolism or binding protein characteristics in CKD. Future research is planned to address these issues.

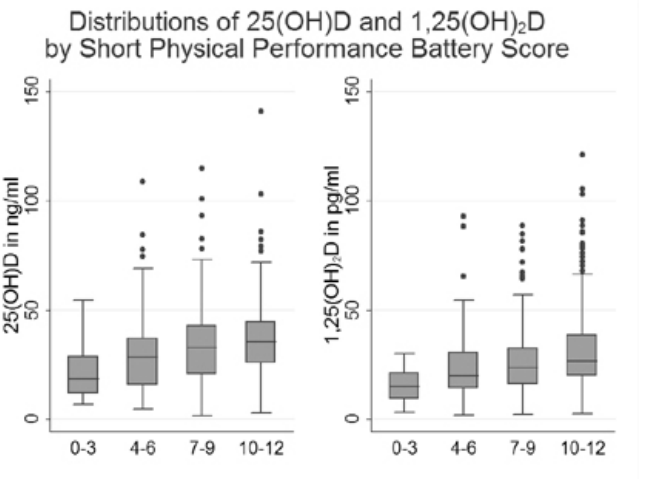
FR-PO1259

Vitamin D Levels and Physical Performance in Chronic Kidney Disease: A CRIC Study Peter P. Reese,[1] Mat Davis,[1] Harold I. Feldman,[1] Anne Cappola,[1] Mary B. Leonard.[2] *[1]University of Pennsylvania, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: Risk factors for poor physical function in CKD have not been established. CKD is associated with low vitamin D levels, and vitamin D deficiency may impair muscle strength.

Methods: Physical performance was assessed using the Short Physical Performance Battery (SPPB) in 1056 Chronic Renal Insufficiency Cohort (CRIC) participants, median (interquartile range) age 64 (57-70) years, eGFR 45 (34-55) ml/min/1.73m^2; 54% male; 58% white, and 35% black race. The SPPB score (0-12) summarizes tests of gait speed, timed chair-raises and standing balance, and is a validated predictor of death and disability that was developed among elderly persons. Serum 25(OH)D and 1,25$(OH)_2$D concentrations were quantified by HPLC tandem mass-spectrometry and I^{125} RIA, respectively.

Results: Median SPPB score was 9 (IQR 7-10). 42% of black and 11% of whites were 25(OH)D deficient [<20 ng/ml]. Median 1,25(OH)2D was 24 pg/ml. In univariate analyses, 25(OH)D and 1,25(OH)2D were positively associated with SBBP scores.



In multivariable linear regression, lower SPPB was significantly associated with older age, female sex, black race, greater BMI, cardiovascular morbidities, lower eGFR, 25(OH) D deficiency, and lower 1,25$(OH)_2$D levels.

Linear Regression Model of SPPB Score

Predictor	Beta	p
Age, vs <50 yr		
50-59	-0.41	0.07
60-69	-0.67	0.001
≥70	-1.53	<0.001
Female	-0.44	<0.001
Black Race	-0.56	0.001
Congestive heart failure	-0.49	0.03
Stroke	-0.19	0.001
Diabetes	-0.23	0.09
Anemia	-0.30	0.03
BMI, vs <25 kg/m²		
25-29	-0.10	0.57
30-34	-0.50	0.009
≥35	-0.98	<0.001
Income, vs <$20,000		
20,000-49,999	0.61	0.004
50,000-99,999	0.86	<0.001
≥100,000	1.33	<0.001
eGFR, vs <15 ml/min/1.73m²		
15-29	0.96	<0.01
30-59	1.04	0.001
≥60	1.22	0.001
25(OH)D >20 ng/mL	0.38	0.02
Log 1,25$(OH)_2$D	0.32	0.008

Conclusions: eGFR, and levels of 25(OH)D and 1,25$(OH)_2$D were independently associated with selected physical performance measures.

Funding: NIDDK Support

FR-PO1260

Vitamin D Deficiency (VDD) Is Associated with Increased Frequency of Vascular Access Dysfunction (VAD) in Chronic Hemodialysis Patients (HDPs) Reuben Valentin,[1] Jose A. Velez,[1] Khaldoun Soudan,[1] Christine L. Gear,[1] Jonathan A. Gelfond,[1] Wajeh Y. Qunibi.[1] *[1]Division of Nephrology, University of Texas Health Science Center at San Antonio, TX; [2]Epidemiology and Biostatistics, University of Texas Health Science Center at San Antonio, TX.*

Background: VDD is prevalent worldwide and is associated with obesity, diabetes, hypertension and other cardiovascular risks. Vitamin D receptors are present in vascular smooth muscle and endothelial cells. Vascular access complications in HDPs are a major source of morbidity and increased health costs. In this study we sought to examine the relationship between 25OHD levels and dialysis access interventions for thrombosis, failure to mature, pseudoaneurysm, steal syndrome, stenosis within 1 year of initial 25OHD level.

Methods: We reviewed medical records of 256 HDPs who had 25OHD level and arteriovenous fistula or arteriovenous graft. We performed univariate Poisson regression with the number of access interventions regressed onto pre-specified demographic and laboratory variables and then included significant variables as covariates in a multiple logistic regression of the odds of having one or more intervention.

Results: Mean age was 55±12.5 years. 48% were female. Mean 25OHD level was 19.1±11 ng/ml. Serum calcium, 25OH, hemoglobin, platelets, albumin were significantly associated with access interventions (p<0.05). The per-unit change in intervention risk associated with 25OHD level remained significant (p=0.0246; 95%CI [0.94, 1.00]) after adjusting for these variables.

Multiple Logistic Regression of Risk of Access Intervention

Variable	Log Odds	Std_Err	OR	95% CI	P-Value
Calcium	-0.136	0.17	0.87	(0.62, 1.22)	0.4276
25OHD	-0.033	0.01	0.97	(0.94, 1)	0.0246
Hemoglobin	0.011	0.09	1.01	(0.85, 1.2)	0.9005
Platelet	2.632	1.76	13.9	(0.44, 441.98)	0.1359
Albumin	-0.531	0.38	0.59	(0.28, 1.25)	0.1659

The adjusted OR of the risk of access intervention for patients with 25OHD deficiency (<15 ng/mL) was 1.98 (p=0.0142: 95%CI [1.15, 3.41]) compared to those with 25OH ≥15 ng/mL.

Conclusions: VDD is associated with the development of VAD. Treatment of VDD for the purpose of preventing VAD must await randomized, controlled studies.

FR-PO1261

Parathyroid Hormone, Calcium, and Phosphorus Metabolism in Children Undergoing Chronic Peritoneal Dialysis Yoon Jung Lee, Joo Hoon Lee, Young Seo Park. *Pediatrics, University of Ulsan College of Medicine, Seoul, Korea.*

Background: Chronic kidney disease-mineral and bone disorder (CKD-MBD) remains a significant problem in children on dialysis. Parathyroid hormone (PTH), calcium and phosphorus metabolism is a key component of CKD-MBD and PTH is at the center of the mineral imbalance, consequent skeletal disease and growth failure. However, the optimal PTH target in CKD patients is a matter of long-standing controversy.

Methods: We assessed the status of CKD-MBD in children on peritoneal dialysis for more than 1 year at Asan Medical Center between May 2001 and July 2010 and compared biochemical profile with recommendations of KDOQI guidelines. We also analyzed the relationship between PTH and growth.

Results: Twenty-five boys and 16 girls with median age of 16 years (3-22 years) were included. The median age at initiation of peritoneal dialysis was 10 years (0-17 years) and mean±SD duration of peritoneal dialysis was 39.9±22.0 months. Mean±SD values of serum PTH, Ca, P and CaXP during the period of peritoneal dialysis were 466.2±539.5 pg/mL, 9.7±1.1 mg/dL, 6.1±1.8mg/dL and 58.9±17.0 mg2/dL2,, respectively. More than 50 percent of patients had mean values of Ca, P, CaXP and PTH above the range recommended in the KDOQI guideline. Six patients (14.6%) showed clinical symptoms and/or radiological signs of CKD-MBD including rickets, bone pain, bone deformities and tissue calcifications. These patients exhibited more severe secondary hyperparathyroidism than those without bone disease (830.9±364.8 vs 378.0±261.0 pg/mL, P<0.005). Mean±SD HtSDS at initiation of peritoneal dialysis was -1.60±1.34. Mean ΔHtSDS was not different between patient with mean PTH >;300 pg/mL and <300 pg/mL (-1.04±1.66 vs -1.31±1.36, P=0.415) and annual ΔHtSDS was not correlated with time-averaged PTH levels regardless of growth hormone therapy.

Conclusions: Biochemical profile was outside the guideline targets in more than 50% percent of patients despite effort to control Ca, P and PTH. Signs and symptoms of CKD-MBD were observed in 15 percent of patients whose PTH level was significantly higher. There were no relationships between growth and time-averaged PTH concentrations regardless of growth hormone therapy.

FR-PO1262

Cinacalcet Treatment Decreases Parathyroid Gland Volume in Hemodialysis Patients with Secondary Hyperparathyroidism – A Long-Term Follow-Up Study Mitsuru Ichii,[1] Eiji Ishimura,[1] Senji Okuno,[2] Naoki Tsuboniwa,[2] Shinya Nakatani,[1] Kyouko Norimine,[2] Kenjirou Yamakawa,[2] Shigeichi Shoji,[2] Tomoyuki Yamakawa,[2] Yoshiki Nishizawa,[1] Masaaki Inaba.[1] *[1]Metabolism, Endcrinology, and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Shirasagi Hospital, Osaka, Japan.*

Background: We and others have recently reported that cinacalcet treatment significantly decreases parathyroid gland volume (PTV) in hemodialysis patients with secondary hyperparathyroidism (SHPT) in 6 months (Ichii, et al. Nephron Clin Pract;115:c195, 2010) and in 12 months (Komaba, et al. Clin J Amer Soc Nephrol 5:2305, 2010). However, further long-term effect of cinacalcet on PTV is unknown. In the present study, we examined changes in PTV after cinacalcet treatment for longer term.

Methods: Cinacalcet was administered in patients with SHPT in whom active vitamin D and phosphate binders could not sufficiently reduce PTH levels. Dosage of cinalcalcet was adjusted according to the Japanese guideline for the treatment of SHPT (Ther Apher Dial 12:514, 2008). PTV was measured four times by ultrasonography before (- 12.3±3.8 months), at the start of ,at 7.0±2.9 months and at18.6±2.8 months after cianalcet treatment in 49 patients with SHPT (61±9 years, hemodialysis duration 166±85 months).

Results: Intact-PTH was significantly increased from 500±279 pg/ml 12 months before, to 607±288 pg/ml at the start of cinacalcet treatment (p<0.01). It was decreased to 244±132pg/ml at 7.0 months (p<0.0001), and to 202 ± 116pg/ml at 18.6 months (p<0.0001). PTV was significantly increased from 681±638 mm3 12 months before, to 994±754 mm3 at the start (p<0.0001). PTV was significantly decreased to 737±553 mm3 (p<0.001) at 7.0 months, and was continuously decreased to 643±552 mm3 (p<0.001) at 18.6 months. Although, out of 49 patients, there were 12 patients in whom PTV was increased during the period, intact-PTH in these patients was significantly decreased (p<0.0001).

Conclusions: In the present study, cinacalcet treatment was demonstrated to decrease PTV, along with decreasing intact PTH, for longer periods. This study suggests that cinacalcet treatment may postpone parathyroidectomy and/or reduce its cases.

FR-PO1263

Serum 25-Hydroxyvitamin D Concentration and Risk of Clinical Disease Events in a Community-Based Population of Older Adults Gregory Levin,[1] Cassianne Robinson-Cohen,[1] Bryan R. Kestenbaum,[1,2] Ian H. de Boer.[1,2] *[1]University of Washington; [2]Kidney Research Institute.*

Background: Circulating concentrations of 25-hydroxyvitamin D [25(OH)D] are used to define vitamin D deficiency. While not optimal, threshold 25(OH)D concentrations for populations with chronic kidney disease have largely been extrapolated from the general population. Current clinical 25(OH)D targets are based on associations with markers of bone and mineral metabolism. These targets may not reflect optimal levels for other chronic diseases that are potentially affected by vitamin D and do not account for seasonal 25(OH) D variation. The goal of this study was to evaluate the relationship of serum 25(OH)D concentration with the incidence of major disease outcomes that are pathophysiologically relevant to vitamin D.

Methods: We studied 1,662 Caucasian older adults from the community-based Cardiovascular Health Study. We measured baseline serum 25(OH)D concentration using a high performance liquid chromatography-tandem mass spectrometry assay that conforms to National Institute of Standards and Technology reference standards. We defined the primary outcome measure as the time to a composite outcome of incident hip fracture, myocardial infarction, cancer, or death from any cause.

Results: Over 11 years median follow-up, the composite outcome occurred in 1,041 participants (63%). The association of 25(OH)D < 20 ng/ml with the composite clinical outcome varied by season (p=0.03). Serum 25(OH)D concentration below the lowest season-specific 22nd percentile (< 14 ng/mL in winter, < 18 ng/mL in spring, < 22 ng/mL in summer, and < 19 ng/mL in fall) was associated with an 18% greater adjusted risk of the composite outcome (95% confidence interval 1%, 38%). Compared with a static 25(OH) D threshold of 20 ng/mL, season-specific thresholds reclassified 8% of participants and improved risk prediction (net reclassification index 2.8%, p=0.047).

Conclusions: These findings suggest that threshold concentrations of 25(OH)D associated with increased risk of relevant clinical health events center near 20 ng/mL. In addition, season-specific targets for 25(OH)D concentration may be more appropriate than static targets when evaluating health risk.

FR-PO1264

VS-110: A Novel Vitamin D Receptor Modulator with Cardiovascular Protective Effects in 5/6 Nephrectomized Uremic Rats J. Ruth Wu-Wong, Megumi Kawai, Yung-Wu Chen, Masaki Nakane. *Renal Research, Vidasym, Chicago, IL.*

Background: Vitamin D receptor modulators (VDRMs) such as calcitriol, paricalcitol and doxercalciferol are commonly used to manage hyperparathyroidism secondary to chronic kidney disease (CKD). A majority of CKD patients die from cardiovascular complications. Clinical observations demonstrate that VDRM therapy may provide cardiovascular and survival benefit for CKD patients. However, current on-market VDRMs have a narrow therapeutic index (TI) at 1-4-fold (estimated from the hypercalcemic toxicity and PTH suppressing efficacy). Hypercalcemia remains a serious concern, which leads to the need for frequent drug dose titration and serum calcium monitoring. Significant clinical benefit can be derived from a VDRM with expanded TI and cardiovascular protective effects.

Methods: The 5/6 nephrectomized (NX) male Sprague-Dawley rats at Week 6 after the surgery exhibited established uremia, elevated parathyroid hormone (PTH), endothelial dysfunction and left ventricular hypertrophy.

Results: Treatment of 5/6 NX rats by VS-110, a novel VDRM, at 0.01 - 1.0 μg/kg (oral gavage, once daily, for two weeks) suppressed serum PTH effectively without raising serum calcium, demonstrating a >50-fold TI. Similar results were obtained when VS-110 was given to 5/6 NX rats by i.p., 3x/week for two weeks. When the 5/6 NX uremic rats were treated with VS-110 (0.01 - 1 μg/kg) for two weeks, VS-110 improved endothelium-dependent aortic relaxation, reduced left ventricular (LV) fibrosis and attenuated LV hypertrophy in a dose-dependent manner without affecting serum calcium. Real-Time PCR showed that VS-110 induced CYP24A1 and CD14 expression in HL-60 cells with EC50 values at 6.8 and 0.4 nM, respectively. VS-110 induced HL-60 differentiation with an EC50 value at 1.7 nM (vs. calcitriol at 13.9 nM) and inhibited the proliferation of primary human keratinocytes with an IC50 value at 1.4 nM (vs. calcitriol at 10 nM).

Conclusions: These studies demonstrate that VS-110 is a novel VDRM with greatly expanded TI and an overall therapeutic product profile that supports clinical development for expanded use in pre-dialysis CKD patients to realize the cardiovascular protective effects of VDR activation.

Funding: Pharmaceutical Company Support

FR-PO1265

Rise in Uncontrolled Secondary Hyperparathyroidism (SHPT) among Blacks after Implementation of the US Prospective Payment System (PPS): Results from the DOPPS Practice Monitor (DPM) Francesca Tentori,[1] Douglas S. Fuller,[1] Ronald L. Pisoni,[1] Justin M. Albert,[1] Marc Turenne,[1] Bruce M. Robinson.[1,2] *[1]Arbor Res Collab for Hlth, Ann Arbor, MI; [2]Univ of MI, Ann Arbor, MI.*

Background: Increased financial constraints may lead to lower utilization of intravenous (IV) vitamin D analogs under the new bundled prospective payment system (PPS), leading to poorer control of secondary hyperparathyroidism (SHPT). Because Black patients require higher vitamin D doses (Wolf JASN 2008) on average, they may be particularly susceptible to this change.

Methods: The DOPPS Practice Monitor (DPM) provides timely, public reporting of trends in dialysis care as the new PPS and QIP are implemented (www.dopps.org/dpm). The DPM follows a nationally representative sample of ~140 US dialysis units with ≥20 chronic hemodialysis patients. We studied trends in parathyroid hormone (PTH) values and SHPT therapies from July 2010 to February 2011.

Results: The % of patients with PTH measured over 3 months did not change (93-96%). The median PTH value rose among Blacks from 296 (interquartile range [IR]: 214-469) to 379 pg/ml (IR: 236-606), and among non-Blacks from 244 (IR: 173-354) to 283 pg/ml (IR: 192-435). The prevalence of severe uncontrolled SHPT (defined as PTH >600 pg/ml) rose sharply among Blacks (from 16 to 26%) and slightly among non-Blacks (from 9 to 11%). Preliminarily, these changes do not appear to be due to decreased overall use of SHPT treatments (% prescribed IV vitamin D rose slightly in both race groups, cinacalcet use rose slightly in blacks) or to changes in serum calcium or phosphorus.

Distribution of PTH values by race group in US-DOPPS

	Month	N	Mean (pg/ml)	Median (pg/ml)	< 150 pg/ml	150-300 pg/ml	301-600 pg/ml	> 600 pg/ml
Black	10-Jul	685	420	296	12%	39%	33%	16%
	10-Aug	915	419	299	10%	40%	35%	15%
	10-Sep	880	412	301	11%	39%	35%	15%
	10-Oct	881	437	321	10%	37%	35%	19%
	10-Nov	910	469	338	11%	30%	37%	23%
	10-Dec	970	493	374	9%	29%	37%	25%
	11-Jan	908	498	375	9%	28%	39%	24%
	11-Feb	866	484	379	10%	29%	35%	26%
Non-black	10-Jul	1,652	326	244	18%	48%	25%	9%
	10-Aug	2,096	316	238	19%	48%	23%	9%
	10-Sep	2,003	305	236	18%	50%	24%	8%
	10-Oct	1,956	314	245	20%	46%	26%	9%
	10-Nov	2,097	325	256	18%	44%	28%	10%
	10-Dec	2,069	338	268	17%	41%	32%	10%
	11-Jan	1,879	358	281	16%	38%	33%	13%
	11-Feb	1,816	371	283	14%	40%	35%	11%

Conclusions: Our results indicate a notable increase in PTH levels overall, and in severe uncontrolled SHPT (>600 pg/ml) among Blacks, over the early PPS transition period. Additional evaluation of the causes of this trend and its potential consequences are warranted.

Funding: Pharmaceutical Company Support

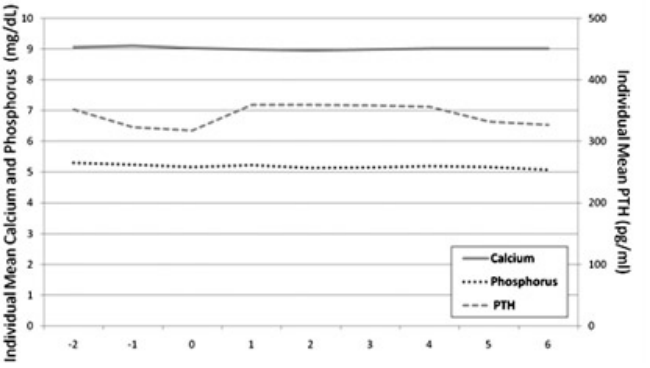
FR-PO1266

Effects of Switching from Intravenous Paricalcitol to Doxercalciferol on Dialysis Patient Bone and Mineral Outcomes T. Christopher Bond, Steven M. Wilson, Mahesh Krishnan, Tracy Jack Mayne. *DaVita Clinical Research, Minneapolis, MN.*

Background: The relative effectiveness of various forms of intravenous vitamin D among hemodialysis (HD) patients is still in question. We assessed outcomes before and after a planned switch of dialysis patients from paricalcitol to doxercalciferol.

Methods: This single-arm, prospective study measured pre/post levels of phosphorus, corrected calcium and parathyroid hormone (PTH) to assess non-inferiority of clinical outcomes in dialysis facilities that switched all of their patients from paricalcitol to doxercalciferol. Patient (n=828) lab values for the 2 months before the switch were compared to the 6 months following the switch at the patient level using mixed models. Similar facility (n=7) comparisons compared the 6 months before the switch to the 6 months following the switch. Pre-set criteria for inferiority were a rise in phosphorus or calcium ≥ 0.5 mg/dL or a rise of PTH of ≥ 100 pg/mL.

Results: The individual-level analysis showed no meaningful differences in mean laboratory values before and after the switch (Figure). All confidence intervals for repeated measures mixed model analyses excluded the pre-set criteria, indicating non-inferiority of doxercalciferol versus paricalcitol. Patient-time within range for all 3 laboratory measures was constant or increased across the time frame of the study. The facility-level analysis of laboratory values showed results similar to those seen in the individual-level analysis. There was no change in hospitalizations or hospitalized days per patient-year.



Conclusions: All individual-level and facility-level assessments support the conclusion that doxercalciferol is non-inferior to paricalcitol on bone and mineral outcomes in HD patients. The small increase in PTH coincides with the increase in the KDIGO upper limit from 300 to 600 pg/mL.

Funding: Pharmaceutical Company Support

FR-PO1267

Cinacalcet Hydrochloride Induces Apoptosis of Parathyroid Cells in Humans and In Vitro: Histological and Cytological Analyses Ryoko Tatsumi, Takatoshi Kakuta, Kaichiro Sawada, Genta Kanai, Hirotaka Komaba, Masafumi Fukagawa. *Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan.*

Background: Secondary hyperparathyroidism develops during the long course of chronic kidney disease. Recently, cinacalcet hydrochloride has been available as one of the treatment options for secondary hyperparathyroidism. There have been a number of reports on the effects of cinacalcet on biochemical parameters of secondary hyperparathyroidism. In this study, we examined histological and cytological changes of parathyroid glands removed from patients with severe secondary hyperparathyroidism, particularly focusing on apoptosis in parathyroid cells.

Methods: The study subjects were 16 dialysis patients who underwent parathyroidectomy for severe secondary hyperparathyroidism in our hospital from April 1, 2007 to March 31, 2010, in whom 8 patients had been treated with cinacalcet hydrochloride and the others had not. We compared the number of TUNEL-positive cells between cinacalcet group and non-cinacalcet group. We also examined the effects of cinacalcet on parathyroid cell death in vitro cell culture with the TUNEL staining, using parathyroid cells from patients with severe secondary hyperparathyroidism.

Results: The number of TUNEL-positive cells in cinacalcet group was significantly higher than that of non-cinacalcet group (12.7 ± 4.3 versus 3.2 ± 2.4 per 1000 cells, P <0.001). In vitro examination also showed significant increases of apoptotic cells with the addition of 10 μM cinacalcet hydrochloride into the culture medium, further supporting the apoptotic effect of cinacalcet on parathyroid gland cells.

Conclusions: These results suggest that treatment with cinacalcet induces apoptosis in hyperplastic parathyroid cells in patients with secondary hyperparathyroidism.

FR-PO1268

Differential Effects of Active Vitamin D Compounds on Secondary Hyperparathyroidism and Vascular Calcification W. Charles O'Neill,[1] Xiaonan H. Wang,[1] Hartmut H. Malluche.[2] *[1]Emory University; [2]University of Kentucky; [3]Amgen, Inc.*

Background: Active vitamin D compounds suppress secondary hyperparathyroidism but can also produce vascular calcification. Whether PTH can be suppressed at doses that do not augment vascular calcification is unclear. Since phosphate also affects PTH secretion and vascular calcification, we reasoned that the differential effects on PTH and vascular calcification depend on phosphate intake.

Methods: Renal failure was produced in rats by feeding adenine, and calcitriol or paricalcitol was given 3 times per week at varying doses. After 28 days, aortic calcium was measured by the cresopthalein method after extraction in HCl. PTH was measured by immunoassay using an antibody against rat PTH 1-34.

Results: Plasma calcitriol, measured by radioimmunoassay 48 hours after dosing, was 6.3 ± 1.8, 7.5 ± 1.0, 24.6 ± 4.1, and 79.6 ± 6.1 pg/ml at 0, 10, 40, and 100 ng/kg calcitriol, indicating that the higher doses resulted in persistent elevations. With 1.06% dietary phosphorus, suppression of PTH (348 ± 66 vs. 719 ± 85 pg/ml, p<0.001) required 100 ng/kg calcitriol, while 40 ng/kg increased aortic calcium (3695 ± 733 vs. 1077 ± 865 nmol/mg, p<0.005; normal: <25). Similarly, with 0.73 % phosphorus intake, 40 ng/kg calcitriol increased aortic calcium (5091 ± 1055 vs. 367 ± 217 nmol/mg, p<0.005) without suppression of PTH (304 ± 138 vs. 276 ± 55 pg/ml). Paricalcitol increased aortic calcification at 160 ng/kg (2623 ± 562 vs. 1586 ± 388 nmol/mg, p=0.007) while 320 ng/kg was required to suppress PTH (197 ± 77 vs. 636 ± 56 pg/ml, p <0.001). However, with a 0.4% phosphorus diet (normal rat chow), PTH was suppressed at 40 ng/kg calcitriol (29 ± 16 vs. 570 ± 109 pg/ml, p=0.002) without significantly increasing aortic calcification (558 ± 551 vs. 88 ±

47 nmol/mg). Aortic calcium content correlated weakly with plasma phosphate (r=0.28, p <0.001) but not with PTH or calcium. A significant increase in plasma calcium occurred only with paricalcitol.

Conclusions: Suppression of secondary hyperparathyroidism by calcitriol without promoting vascular calcification is dependent on phosphate intake. There was no advantage of paricalcitol over calcitriol.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-PO1269

Chronic Exposure to Laminar Shear Stress Induces an Anti-Coagulant, Anti-Inflammatory Phenotype in Glomerular Endothelial Cells and Results in Communication with Podocytes Sadie Slater, Gavin Iain Welsh, Moin Saleem, Peter W. Mathieson, Simon C. Satchell. *Academic Renal Unit, University of Bristol, Bristol, United Kingdom.*

Background: The importance of podocyte to glomerular endothelial cell (GEnC) communication in the glomerulus is widely recognised. In systemic circulations chronic laminar shear stress (LSS) plays a crucial role in determining EnC behavior and regulating EnC communication with smooth muscle cells. Here we investigate signaling pathways activated by LSS in GEnC and the effect on co-cultured podocytes.

Methods: Conditionally immortalised human GEnC and podocytes were utilised. GEnC were cultured under static conditions, or on an orbital shaker set to generate 10dynes/cm2 LSS, for 24, 48, 72 or 96h. Western blotting examined changes in protein expression. A nitrate assay measured nitric oxide (NO) production. Changes in GEnC barrier properties in response to LSS were measured using an ECIS (Electric Cell Impedance Sensing) system. Changes in podocyte protein expression and barrier properties in response to GEnC LSS were investigated using a GEnC-podocyte co-culture model, or by placing conditioned media from static or LSS GEnC on podocytes.

Results: LSS in GEnC increased expression of KLF2 ($p<0.0001$), thrombomodulin ($p<0.003$), eNOS ($p<0.003$), and NO ($p<0.005$). These changes are associated with ERK5 phosphorylation and were prevented by addition of a MAPK kinase inhibitor, OU126. ECIS demonstrated GEnC monolayer resistance decreased in response to LSS. Podocytes exposed to spermine, a nitric oxide donor, showed decreased monolayer resistance and increased phosphorylation of a key protein in actin regulation, VASP. Similarly, GEnC LSS media decreased podocyte monolayer resistance and increased VASP phosphorylation, compared to static media.

Conclusions: We have demonstrated chronic LSS in GEnC results in an anti-inflammatory, anti-coagulant phenotype, mediated via the MEK5 pathway, leads to an increase in NO. Mediators (possibly NO) produced by GEnC during LSS decrease podocyte barrier properties and increase VASP phosphorylation. This is the first data to directly demonstrate GEnC to podocyte communication and suggests an important role in glomerular homeostasis.

FR-PO1270

Interferon Beta Modulates Glomerular Endothelial Cell(GEnC) Barrier Properties through Activation of the Small GTPase Rap1 Georgina Cope,[1] Candida Tasman,[3] Peter W. Mathieson,[1] David O. Bates,[2] Gavin Iain Welsh,[1] Simon C. Satchell.[1] *[1]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [2]Microvascular Research Laboratories, University of Bristol, Bristol, United Kingdom; [3]Dementia Research Group, University of Bristol, Bristol, United Kingdom.*

Background: We have previously shown that IFNβ protects rats from albuminuria in experimental glomerulonephritis. In-vitro exposure of glomerular endothelial cell (GEnC) monolayers to 1000 units/ml of IFNβ reduced their permeability, suggesting that IFNβ may act directly on cells of the glomerular filtration barrier to maintain its permselectivity. Others have shown that endothelial barrier properties are enhanced by activation of the small GTPase Rap1. We therefore tested the hypothesis that IFNβ acts via Rap1.

Methods: Pull down assays were used to detect activated Rap1 post IFNβ. An ECIS (electric cell-substrate impedance sensor) system was used to monitor GEnC monolayer resistance in the presence of IFNβ with or without GGTI-298 (Rap1 inhibitor)or siRNA to Rap1a. Immunoprecipitation and immunofluorescence were used to determine the effects of IFNβ on Rap1 association with actin and Rap1-GTP-interacting adaptor molecule (RIAM) and its subcellular location.

Results: IFNβ induced Rap1 activation at a level comparable to 8'me cAMP, an activator of Rap1a. Co-incubation with GGTI-298 significantly reduced the tightening of the GEnC barrier provoked via IFNβ. To determine the isoform of Rap1 responsible we used targeted siRNA to Rap1a. This resulted in Rap1a knockdown and attenuated the increase in resistance caused by IFNβ for 6 hours, while GGTI-298 mediated a sustained inhibition. Activation of Rap1 in GEnC via IFNβ induced its association with actin and RIAM. This interaction was reduced by co-incubation of GGTI-298 and IFNβ. IFNβ induction of the Rap1a: actin association did not cause cytoskeletal rearrangement or changes in cell shape suggesting it is not a cytoarchitectural regulator.

Conclusions: IFNβ mediated tightening of GEnC monolayers is partially dependent on Rap1 activation. Modulation of this pathway in glomerular cells may have therapeutic potential in proteinuric renal disease.

FR-PO1271

α-Actinins Suppress Podocyte Contractility Fangfang He,[1] Hui Chen,[1] Philip A. Bondzie,[1] Julie A. Tomolonis,[1] Daniel J. Becker,[2] Martin R. Pollak,[3] Joel M. Henderson.[1] *[1]Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA; [2]Division of Nephrology, Brigham and Women's Hospital, Boston, MA; [3]Division of Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: Mutations in the gene encoding α-actinin-4, an actin-filament crosslinking protein, cause an autosomal dominant form of focal segmental glomerulosclerosis. α-actinins may modulate actomyosin contractility through actin binding and crosslinking, or through other mechanisms. Changes in podocyte actomyosin contractility associated with altered α-actinin function may compromise glomerular structural integrity, thereby leading to glomerular damage. The aim of this study is to define the role of α-actinins in modulation of podocyte contractility.

Methods: α-actinin-1 and α-actinin-4, two isoforms significantly expressed in cultured murine podocytes, were independently ablated in these cells using lentiviral RNA interference. Ablated and control cells were plated on glass and polyacrylamide elastic substrates. Immunostaining was performed to characterize α-actinin and filamentous actin distribution, and traction force microscopy was used to measure cell traction.

Results: Highly efficient ablation of both α-actinin-1 and α-actinin-4 was achieved at the protein level using the lentiviral system. Both α-actinin-1 and α-actinin-4 ablation were associated with disorganization of actin stress fibers. α-actinin-1 and α-actinin-4 ablation increased cell traction by 68% and 35%, respectively, relative to controls.

Conclusions: Both α-actinin-1 and α-actinin-4 participate in cytoskeletal organization and traction modulation in murine podocytes. Our results indicate that α-actinins tend to suppress podocyte contractility. These findings suggest that altered α-actinin function (e.g., gain of function mutations) could cause glomerular damage by altering podocyte contractility.

Funding: NIDDK Support

FR-PO1272

Endocytic Proteins in Podocytes Affect the Stability of Foot Processes in Kidney Glomeruli Keita Soda, Xuefei Tian, Rena Zheng, Shuta Ishibe. *Internal Medicine, Section of Nephrology, Yale University, School of Medicine, New Haven, CT.*

Background: Membrane trafficking linking actin regulation plays a pivotal role in maintaining cell homeostasis. However, membrane dynamics in podocytes remain unclear. We show that endocytic proteins, Dynamins (Dyn1 and Dyn2) and Synaptojanin 1(SJ1) affect the stability of glomerular filtration barrier in the kidney.

Methods: Podocyte specific double knockouts of Dyn1 and Dyn2 were generated using the Cre-LoxP system (Dyn dKO). Kidney sections were analyzed with H and E, PAS, trichrome staining and electron microscopy (EM). Albuminuria and urine creatinine were measured by ELISA. Endocytosis assays were performed on primary wild type (WT) and mutant podocytes.

Results: Dyn dKO mice were born at the expected Mendelian frequency. However, these mice failed to gain weight by 4 weeks (1.3±0.1 vs. 1.4 ±0.1, 10.5±0.4 vs. 8.5±0.3, 21.8±1.8 vs. 13.3±0.8 (wild vs. Dyn dKO;p1, 4 and 8weeks)(g)), developed severe albuminuria normalized to urine creatinin(16.3±0.9 vs. 716.7± 37.0, 15.4±0.8 vs. 1246.7±43(wild vs. Dyn dKO;4 and 8 weeks) (μg/mg)), and creatinine measurements demonstrated progressive renal failure (0.35±0.06 vs. 0.78±0.09, 0.34±0.06 vs. 1.3±0.04, 0.32±0.05 vs. 1.47±0.06 (wild vs. Dyn dKO;4,8 and 10 weeks)(mg/dL)) with the majority of Dyn dKO mice dying by 10 weeks. Histological examination resulted in lesions characteristic of focal segemental glomerulosclerosis that progressed to global sclerosis and interstitial fibrosis, while EM demonstrated severe foot process effacement. To further explore the role of the endocytic pathway, Dyn binding partner SJ1 was examined. SJ1 null mice had severe albuminuria at birth (25±5 vs 890±67 (wild vs. SJ1 KO)(μg/mg)) and foot process effacement. Compared to wild type podocytes, Dyn dKO or SJ1 null podocytes had robust Arp2/3 accumulation at clathrin coated pits as well as endocytic defects characterized by significantly decreased Nephrin uptake compared to wild type (Dyn dKO; <4%, SJ1 KO; <70%, $p<0.05$ respectively).

Conclusions: These findings provide insight into Dynamins and Synaptojanin 1 playing a fundamental role regulating endocytosis and actin dynamics within the glomerular filtration barrier.

FR-PO1273

Calprotectin, an Endogenous Toll-Like Receptor 4 Ligand (TLR4), Is Critical for Induction of Glomerulonephritis Ruth J. Pepper,[1] Phoebe E.H. Sharp,[2] Gurjeet Bhangal,[2] Hsu-Han Wang,[1] Ruth M. Tarzi,[2] Charles D. Pusey,[2] H. Terence Cook,[2] Alan D. Salama.[1] *[1]Centre for Nephrology, UCL; [2]Imperial College London.*

Background: Calprotectin an endogenous TLR4 agonist, expressed in neutrophils, monocytes and infiltrating macrophages. We investigate disease induction using the murine nephrotoxic nephritis (NTN) model in calprotectin deficient mice, as well as potential amplification of human monocyte calprotectin expression by MPO-ANCA and PR3-ANCA, investigation of serum levels and cell surface expression in patients.

Methods: Accelerated NTN experiments performed on wild-type (WT) and calprotectin deficient mice (Cal-/-), with day 7 sacrifice. Renal histology was scored for thrombosis and macrophage infiltration, and serum measurement of urea and creatinine. In human

studies, healthy control monocytes and patients in remission were stimulated with ANCA, and calprotectin expression determined by flow cytometry (FACS). Serum levels measured by ELISA. Calprotectin surface expression of neutrophil and monocytes in patients and controls determined by FACS.

Results: Cal-/- mice were protected from glomerulonephritis with significantly lower serum urea than WT ($p<0.05$), less thrombosis and proteinuria and significantly less macrophage and T cell glomerular infiltration ($p<0.001$, $p<0.005$). In human studies, monocytes isolated from remission vasculitis patients significantly upregulate calprotectin expression following MPO-ANCA stimulation ($p<0.01$), control IgG or PR3-ANCA did not induce a significant increase. AAV patients (non-renal involvement) who subsequently relapse have higher serum calprotectin at 1 and 6 months after treatment compared to non-relapsers. Patients demonstrate significantly higher calprotectin surface expression in neutrophils and monocytes compared to controls ($p<0.05$).

Conclusions: Mice deficient in calprotectin are protected from renal injury, demonstrating calprotectin has a critical role in the initiation of glomerulonephritis. We demonstrate significantly higher serum levels in those who subsequently relapse. MPO-ANCA can promote further upregulation of monocyte calprotectin levels. Patients have increased surface expression of calprotectin on neutrophils and monocytes.

FR-PO1274

Substrate Elasticity and Geometry Affect Podocyte Cell Mechanics Eric Schordan,[1] Sandra Schordan,[1] Rudolf Merkel,[2] Bernd Hoffmann,[2] Nicole Endlich,[1] Karlhans Endlich.[1] *[1]Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany; [2]Institute of Complex Systems (ICS-7), Forschungszentrum Juelich GmbH, Juelich, Germany.*

Background: Podocyte foot processes cover the outer aspect of the capillaries in the renal glomerulus. They are important determinants of the permeability of the glomerular filtration barrier. In addition, podocytes stabilize the capillary wall with a contractile actin cytoskeleton counteracting the high hydrostatic capillary pressure. Chronic kidney disease is predominantly caused by damage and failure of podocytes, e.g. by increased capillary pressure.

Methods: In the present study we investigate the cytoskeleton and cell mechanics of podocytes in response to different elasticities and geometries of the substrate.

Results: We find that soft substrates (8 to 56 kPa mimicking basement membrane elasticity) as compared to stiff substrates (490 kPa or rigid plastic) modify cell morphology by inducing cell orientation, actin cytoskeleton reorganization and formation of longer processes. Directed cell migration in a wounding assay is impaired on soft substrates, whereas cell spreading remains unaffected. The geometry of non-planar soft substrates, mimicking the curvature of the capillary wall, is sensed by podocytes and leads to morphological changes. Traction forces of podocytes, measured on soft substrates with embedded displacement markers, increase with increasing substrate stiffness. Recently, we have shown that stress fibers are elongated at focal adhesions and move centripetally over time. Here we show that elongation rate correlates with traction force.

Conclusions: Our data demonstrate that elasticity and geometry affect podocyte cell mechanics. Moreover, there is a coupling between substrate elasticity, traction force and stress fiber formation.

Funding: Government Support - Non-U.S.

FR-PO1275

Nephrin Regulates Lamellipodia Formation by Assembling a Protein Complex That Includes Ship2, Filamin and Lamellipodin Puneet Garg, Leslie Cook, Madhusudan M. Venkatareddy, Rakesh Verma. *Internal Medicine/ Renal, University of Michigan, Ann Arbor, MI.*

Background: Actin dynamics has emerged at the forefront of podocyte biology. Identification of human disease with mutations in actin associated proteins presenting with proteinuria and chronic kidney disease supports the importance of actin dynamics in podocytes. Numerous mouse knock out models of actin associated proteins further demonstrates its importance in not only development but maintenance and repair of the podocytes. Podocyte intercellular junction receptor Nephrin plays a vital role in regulating actin dynamics.

Methods: To identify the proteins that interact with Nephrin we generated a library of His-tagged SH2 domains of SH3-SH3 domain containing proteins.

Results: Here we report recruitment and regulation by Nephrin of a protein complex that includes ship2 (SH2 domain containing 5'inositol phosphatase), Filamin (FLN) and lamellipodin (LPD). These proteins are important in regulation of actin dynamics, lamellipodia formation and focal adhesion dynamics. Nephrin activation resulted in phosphorylation of actin crosslinking protein FLN in a p21 activated kinase dependent manner. FLN exists in a complex with Ship2 in the cytoplasm. This leads to ship2 enrichment at the sites of actin polymerization. Ship2 dephosphorylates PIP3 to generate PI (3,4) P2. This further leads to recruitment of scaffold protein LPD which binds specifically to PI (3,4)P2 via its PH domain. Nephrin activation in cell culture results in formation of lamellipodia, a process that requires specialized actin dynamics at the leading edge along with focal adhesion turnover. Using the previously described CD16-Nephrin clustering system, Nephrin ligation resulted in abnormal morphology of actin tails in human podocytes where ship2, FLN and LPD was knocked down. We also observed decreased efficiency of lamellipodia formation and cell migration in these knock down cells.

Conclusions: We propose that Nephrin-Neph1 complex not only initiates actin polymerization but is also able to regulate and assemble a protein complex that is necessary to regulate the architecture of the generated actin filament as well as focal adhesion dynamics.

Funding: NIDDK Support

FR-PO1276

Indomethacin Blocks the Fluid Flow Shear Stress (FFSS)-Induced Increase in Glomerular Albumin Permeability (P_{alb}) *In Vitro* Tarak Srivastava,[1] Ellen T. McCarthy,[2] Ram Sharma,[3] Mukut Sharma.[3] *[1]Nephrology, CMH, UMKC, Kansas City, MO; [2]Nephrology, KUMC, Kansas City, KS; [3]Nephrology, KC VA Med Ctr, Kansas City, MO.*

Background: FFSS, the force tangential to the podocyte surface that results from flow of ultrafiltrate, increases with SNGFR. Increased SNGFR occurs in congenital or acquired solitary kidneys and in early diabetic nephropathy, conditions that result in microalbuminuria and CKD. We have shown that FFSS: alters the actin cytoskeleton in podocyte monolayers and increases COX-2 expression and prostaglandin E_2 (PGE_2) levels. We have also shown that PGE_2, increases albumin permeability (P_{alb}) in isolated glomeruli and that increased P_{alb} precedes proteinuria in models of diabetes and hypertension. We hypothesized that increased FFSS alters filtration barrier through COX2 metabolites.

Methods: Glomeruli from Sprague-Dawley rats (200-225 g) were subjected to 0.3 dynes/cm^2 FFSS for 30, 60 or 120 mins followed by a recovery for 120 mins at 37°C. Indomethacin (2.5μM) was included in the medium in some experiments. Changes in glomerular P_{alb} were determined using an *in vitro* assay. Untreated baseline and time matched glomeruli were used as controls. Murine podocytes were subjected to FFSS for 120 minutes and analyzed for changes in gene expression using Affymetrix GeneChipMouse Exon 1.0 ST Array.

Results: Results show that FFSS increased Palb at 30, 60 and 120 minutes. The effect of FFSS on Palb persisted after 120 minutes of recovery. Partial results presented in the Table below show that indomethacin significantly blocked the effect of FFSS on Palb

Group	P_{alb}±SEM	*P* value
Control	0.02±0.04	
FFSS [120 min]	0.77±0.06	0.001*vs.* Control
FFSS [120 min] + Recovery [120 min]	0.62±0.06	0.001*vs.* Control
Indomethacin+FFSS+Recovery	0.16±0.11	0.001 *vs.* FFSS

n=20 glomeruli/group

Initial analysis of the gene array results has shown a >3 fold upregulation of COX-2 expression in FFSS-treated compared to control podocytes (data not shown here).

Conclusions: These results suggest that flow-induced increase in FFSS increases P_{alb} through PGE_2, an arachidonic acid metabolite of the COX pathway. We postulate that changes in the glomerular filtration barrier secondary to FFSS may explain the microalbuminuria in CKD.

Funding: NIDDK Support, Private Foundation Support

FR-PO1277

The Podocyte Slit-Diaphragm Molecule MAGI-1 Is Required for Nephrin Localization and Function Justin Vadaparampil, Lewis Kaufman. *Nephrology, Mount Sinai School of Medicine, New York, NY.*

Background: In podocytes, MAGUK with inverted domain structure-1 (MAGI-1) is specifically expressed at the slit-diaphragm and functions as a linker protein that directly interacts with several other critical proteins including nephrin, α-actininin-4, and synaptopodin. Previously, the function of MAGI-1 and its roles in podocyte dynamics in proteinuric diseases were entirely unknown.

Methods: To study the mechanisms of MAGI-1 function in greater detail, we generated stable MAG-I deficient podocytes using targeted shRNA lentiviral infections of an established human cell line.

Results: MAGI-1 deficient podocytes have significant abnormalities in cellular morphology characterized by a simplified and overall smaller cell size, less intricate cytoskeletal architecture, and less complex projections and llamelopodia. Podocytes lacking MAGI-1 also divide at a dramatically lower rate and adhere abnormally to many components of the glomerular basement membrane including collagen type IV and laminin.

To begin to investigate MAGI-1 function, we examined the impact of MAGI-1 deficiency on the localization of its known interacting partners. In MAGI-1 deficient podocytes transfected with a FLAG-nephrin expression construct, membrane expression of nephrin was almost completely lost with significant accumulation in the cytoplasm. These results were confirmed when analyzing endogenous nephrin in fully differentiated podocytes. Ongoing work is focused on dissecting involved mechanisms.

Conclusions: Our data suggests that MAGI-1 deficiency has a signficant impact on podocyte architecture in culture. These effects may be mediated by the loss of nephrin surface expression in the setting of MAGI-1 deficiency. We speculate that loss of MAGI-1 results in destabilization of the protein complex associated with nephrin's cytoplasmic domain resulting in increased nephrin endocytosis.

Funding: NIDDK Support

FR-PO1278

Sumoylation Determines Localization and Stability of Nephrin at the Plasma Membrane Irini Schaefer, Erik Himmelseher, Hermann G. Haller, Mario Schiffer. *Nephrology, Medical School Hannover, Hannover, Germany.*

Background: The podocyte slit diaphragm is a delicate extracellular protein structure that has to withstand bloodpressure associated changes in glomerular perfusion pressure and thus requires constant renewal. A disordered endocytosis of nephrin could cause a misplaced localization and could therefore lead to destabilization of the slit diaphragm. Sumo (small ubiquitin-like modifier) is a ubiquitin-like protein with 20% identity to ubiquitin. In vertebrates the Sumo-family has at least four members: Sumo-1, -2, -3 and a recently reported member Sumo-4. Sumoylation of proteins on lysine residues can block

ubiquitination of the same site thus lead to stabilization. The aim of our studies was to analyze the role of Sumoylation in regulating localization and stability of nephrin.

Methods: First we analyzed sumoylation of nephrin by in vitro Sumoylation. For mapping the potential sites that can be modified by Sumoylation we used Sumosp 2.0. We found one predicted Sumoylation sequence at the cytoplasmic tail of nephrin that is conserved in mouse and human. To explore if this predicted site is modified by Sumoylation we created nephrin-mutants in which lysine was converted to arginine. To show that Sumoylation may regulate stability of nephrin we examined the steady-state level by treatment with cycloheximide. Furthermore we performed endocytosis-assays, subcellular fractionization and confocal microscopy.

Results: We could show that nephrin is sumoylated by Sumo-1, -2 and -3. By immunoprecipitation and in vitro Sumoylation we could verify that the lysine-mutant can not be sumoylated. Cycloheximide-experiments showed that the lysine-mutant has a lower steady-state level compared to WT-nephrin. Furthermore endocytosis of the lysine-mutant is enhanced and subcellular fractionization and confocal microscopy showed that the lysine mutant accumulated at the perinuclear region and cytosol.

Conclusions: These data suggest that Sumoylation determines localization and enhances stability of nephrin. Next to glycosylation and phosphorylation, Sumoylation is a posttranslational modification that may play a role in the tight regulation of nephrin trafficking and turnover at the slit diaphragm.

Funding: Government Support - Non-U.S.

FR-PO1279

Role of Guanine Nucleotide Exchange Factor-H1 in Complement-Mediated RhoA Activation in Glomerular Epithelial Cells Flaviana Mouawad, Lamine Aoudjit, Ruihua Jiang, Tomoko Takano. *Medicine, McGill University, Montreal, QC, Canada.*

Background: In the rat model of membranous nephropathy (passive Heymann nephritis), complement C5-9 causes visceral glomerular epithelial cell (GEC) injury. We reported previously that complement activates the small GTPase, RhoA, in GEC *in vitro* and *in vivo*. The present study addresses the role of guanine nucleotide exchange factor (GEF)-H1, an activator of RhoA, in complement-mediated RhoA activation in GEC.

Methods: Rat GEC and immortalized mouse podocytes were used. Complement stimulation was done by serially exposing cells to antibody and normal human serum (NS) or de-complemented serum (control). Affinity precipitation by RhoG17A, which binds to active GEF, followed by immunoblotting was used to quantify GEF-H1 activity. RhoA activity in live cells was visualized by fluorescence resonance energy transfer. Passive Heymann nephritis was induced by an intravenous injection of anti-Fx1A antiserum in male Sprague-Dawley rats.

Results: GEF-H1 protein was expressed in mouse podocytes, rat GEC, and rat glomeruli. In vitro, NS, but not control serum, activated GEF-H1 activity, starting at 15 min, which continued to increase up to 40 min. Complement-mediated GEF-H1 activation was absent when C8-deficient serum was used but was restored when C8 was added back, indicating that it is dependent on C5b-9. RhoA activity was also increased by complement in a time course similar to GEF-H1. RhoA activation was most distinct in the sub-membrane area of cellular protrusions and was followed by retraction of the protruded area. Complement-mediated GEF-H1 activation was reduced by the inhibitors of the Extracellular-signal Regulated Kinase (ERK) pathway (U0126 and PD98057). In vivo, GEF-H1 and RhoA were activated in the glomeruli from rats with passive Heymann nephritis at 14 days after disease induction.

Conclusions: Complement C5b-9 activated GEF-H1 in GEC in a time course similar to RhoA. GEF-H1 activation is likely to contribute to complement-mediated RhoA activation in GEC, which may lead to morphological changes of GEC and proteinuria in a rat model of membranous nephropathy.

Funding: Government Support - Non-U.S.

FR-PO1280

The Novel Podocyte Protein 4.1O Interacts with Slit Diaphragm Proteins Nephrin, and Glepp1, and Ameliorates Nephrin Endocytosis Eva Koenigshausen,[1] Sinja Ohlsson,[1] Marcus G. Pezzolesi,[2] Magdalena Woznowski,[1] Ivo Quack,[1] Sebastian Alexander Potthoff,[1] Andrzej S. Krolewski,[2] Lars C. Rump,[1] Lorenz Sellin.[1] *[1]Nephrology, Heinrich Heine University, Duesseldorf, Germany; [2]Section on Genetics and Epidemiology, Joslin Diabetes Center - Harvard Medical School, Boston, MA.*

Background: Microalbuminuria is as an early marker for diabetic nephropathy. A GWAS for diabetic nephropathy revealed FRMD3 as a candidate gene in type 1 diabetics. FRMD3 encodes for the protein 4.1O. 4.1 proteins serve as adaptors between plasma membrane proteins and the actin cytoskeleton. The 4.1O orthologue in zebrafish is required for prevention of proteinuria. Nephrin is endocytosed upon binding to the adaptor protein β-arrestin2. The expression and molecular function of 4.1O in human podocytes is unknown so far.

Methods: Human podocytes were differentiated over 14 days. RNA and protein were isolated and followed by RT-PCR or western blot respectively for 4.1 family members. Cells expressed 4.1O and nephrin or GLEPP1 or truncations of the indicated proteins. After cell lysis co-immunoprecipitation was performed. Using monoclonal and polyclonal antisera immunofluorescence for 4.1O was done.

Results: 4.1 family members 4.1O, 4.1G, 4.1B and 4.1N are expressed in human podocytes. 4.1O shows a punctated cytoplasmic distribution with staining of the plasma membrane. 4.1O interacts with nephrin and GLEPP1 but not with Neph1. The interaction domain of 4.1O within the nephrin C-terminus. 4.1O binds to nephrin within its very c-terminal domain. In addition protein 4.1O forms homodimers. 4.1O reduces the interaction of nephrin with β-arrestin2 and .

Conclusions: FRMD3 (4.1O) has been shown to be a candidate gene for diabetic nephropathy in type 1 diabetics. In zebrafish, its deletion leads to proteinuria. 4.1O is a novel human podocyte protein that interacts with nephrin and GLEPP1. Binding of 4.1O to nephrin prevents β-arrestin2 binding to nephrin. It is therefore conceivable that 4.1O is a relevant adaptor to slit diaphragm proteins and the actin cytoskeleton. Furthermore it is postulated that 4.1O plays an important role in the protection for progression of proteinuric kidney disease by inhibition of nephrin endocytosis.

FR-PO1281

RhoA Regulates the Expression of Fibronectin Via Nuclear Factor of Activated-T Cells in Podocytes Lei Zhu, Ruihua Jiang, Lamine Aoudjit, Tomoko Takano. *Medicine, McGill University, Montreal, QC, Canada.*

Background: RhoA is a small GTPase which regulates the actin cytoskeleton. We reported previously that high level expression of active RhoA in podocytes in mice induces glomerulosclerosis, accompanied by fibronectin upregulation. The aim of this study was to investigate the mechanism by which RhoA upregulates fibronectin expression in podocytes. Role of nuclear factor of activated-T cells (NFAT) was investigated.

Methods: Differentiated mouse podocytes or cultured rat podocytes were used for studies with cultured cells. Double luciferase assay was used to study the activation of the fibronectin promoter and the NFAT pathway. $[Ca^{2+}]i$ was determined using Fluo-4. RhoA activity was monitored by fluorescence resonance energy transfer in live cells. Nuclear extracts from glomeruli were used to study NFAT activation *in vivo* by electromobility shift assay (EMSA).

Results: Constitutively active (CA)-RhoA activated the fibronectin promoter, which was inhibited by BAPTA (Ca2+ chelater), W7 (calmodulin inhibitor), FK506 (calcineurin inhibitor), and VIVIT (NFAT inhibitor). Angiotensin II, which is known to activate RhoA in podocytes, also transactivated the fibronectin promoter, which was inhibited by the same set of inhibitors. CA-RhoA activated the NFAT-responsive promoter and CA-NFAT in turn activated the fibronectin promoter, which was further augmented by an activator of protein kinase C, phorbol 12-myristate 13-acetate. RhoA-mediated NFAT activation was inhibited by BAPTA and W7. Calpeptin, an activator of RhoA, increased [Ca2+]i in a time course similar to RhoA activation. Induction of CA-RhoA expression also increased [Ca2+]i significantly. NFAT activation, studied by EMSA, was detected in glomerular nuclear extracts from rats with puromycin aminonucleoside nephrosis and from mice which express constitutively active RhoA in podocytes.

Conclusions: RhoA activates the $[Ca^{2+}]i$ -NFAT pathway in podocytes. RhoA-induced fibronectin upregulation is, at least in part, mediated by gene transactivation via NFAT. Thus, the RhoA-NFAT pathway may contribute to the development of certain forms of glomerulosclerosis.

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FR-PO1282

Albumin Exposure Induces an Inflammatory Response and Reduces VEGF Expression in Cultured Podocytes Kayo Okamura,[1] Patrick Daniel Dummer,[2] Jeffrey B. Kopp,[2] Judith Blaine.[1] *[1]Medicine, University of Colorado Denver, Aurora, CO; [2]National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD.*

Background: Albuminuria exposure induces an injury response in cultured proximal tubular cells, but the effects of albumin on cultured podocytes have not been as well investigated. *In vivo*, podocytes express multi-ligand receptors that facilitate albumin uptake, including low-density lipoprotein-related protein 2 or megalin and neonatal Fc receptor.

Methods: We studied human urine-derived podocyte-like cells, transformed with human telomerase reverse transcriptase and temperature-sensitive SV40 T antigen (Sakairi, Am J Physiol 2010). Podocytes were cultured under non-permissive conditions (37°C) at which they express differentiation markers including WT1, synaptopodin, and nestin. Podocytes were exposed to medium supplemented with 10% heat-inactivated fetal bovine serum (final albumin concentration ~ 2 mg/ml), supplemented with either 5 mg/ml recombinant human serum albumin or dextran of a similar molecular mass as an oncotic control. Cytokine mRNA levels were assessed by quantitative RT-PCR over an 8 hour time course and cytokine medium levels were assessed over a 48 hour time course. Results are expressed as fold-change compared to oncotic control.

Results: Albumin exposure increased expression of interleukin-1beta (IL-1b) expression (peak mRNA increase ~ 8 fold at 3 hours)), increased tumor necrosis factor alpha (TNFa) expression (peak mRNA increase ~3 fold at 3 hours, peak protein increase ~15 fold at 6 hours), increased interleukin-6 expression (peak mRNA increase ~ 7 fold and peak protein increase ~8 fold at 48 hours). In addition, vascular endothelial growth factor (VEGF) mRNA expression was reduced by ~3 fold at 6 hours compared to dextran treated controls.

Conclusions: These results suggest that exposure of cultured podocytes to albumin concentrations similar to those present in nephrotic urine induce an inflammatory factor cascade, with increased expression of IL-1b and TNFa followed by IL-6, and reduce VEGF mRNA expression. Together these may contribute to dysfunction of podocytes and glomerular endothelial cells in nephrotic syndrome.

Funding: NIDDK Support

FR-PO1283

Podocyte Failure Caused by Hypertrophic Stress in Rats Expressing a Podocyte-Specific Dominant Negative 4E-BP1 Transgene of the mTOR Pathway. Acceleration of Proteinuria, FSGS and Progression to ESKD by Conditions Causing Glomerular Growth, and Prevention by Calorie Restriction

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Background: Progressive podocyte depletion drives progressive glomerulosclerosis. Podocyte depletion could result from reduction in podocyte number, size and/or function.

Methods: To test the hypothesis that reduced capacity of podocytes to respond to hypertrophic stress would lead to glomerulosclerosis we developed Fischer 344 rats expressing a human AA-4E-BP1 dominant negative transgene driven by the podocyte-specific podocin promoter. 4E-BP1 (in the mTOR pathway) controls CAP-dependent translation and plays a role in determining cell size.

Results: Tg rat podocytes uniformly expressed the transgene, were born with the expected ratios without increased proteinuria and normal kidney histology. At 100g homozygous (but not heterozygous) rats had reduced podocyte and glomerulular tuft volume. Both hetero and homozygous tg rats developed proteinuria, FSGS and reached ESKD by 12 months. Accelerated glomerular hypertrophy was induced by uninephrectomy (NX). Wt rats with NX develop minor proteinuria and few FSGS lesions by 14 weeks. In contrast NX of heterozygous tg rats caused proteinuria after 3 weeks, FSGS by 8 weeks and ESKD by 14 weeks. NX of homozygous tg rats caused rapid development of high level proteinuria, glomerulosclerosis and ESKD by 9 weeks. Proteinuria occurred in direct proportion to body weight gain. Morphometry showed development of a mismatch between glomerular size and total podocyte volume in hetero and homozygous rats. Both proteinuria and FSGS were completely prevented by calorie restriction.

Conclusions: Reduced capacity of podocytes to respond to hypertrophic stress in response to growth signaling through the mTOR pathway can trigger proteinuria, FSGS and progression to ESKD. Calorie restriction could be a useful adjunctive therapy to slow and/or prevent progression to ESKD.

Funding: NIDDK Support

FR-PO1284

Motor Protein Myo1c Is a Critical Component of the Glomerular Filtration System

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Background: Podocyte cells along with their specialized junctions "slit diaphragm" form the key components of glomerular filtration assembly. These structures are critical for the glomerular filtration function including the selective passage of low molecular weight waste products and the retention of blood plasma proteins. Our recent study identifies Myo1c as a novel Nephrin and Neph1 interacting motor protein that transports these proteins from cytoplasm to podocyte cell membrane.

Methods: This study investigates the role of Myo1c in maintaining the glomerular filtration function using zebrafish as a model system.

Results: Knockdown of Myo1c gene in zebrafish using antisense morpholino resulted in abnormal developmental phenotype.

In addition, the knockdown also induced pericardial edema and whole body edema at 72hpf (arrows) which suggests impairment of pronephric osmoregulatory function. Histological analysis of mutant fish revealed dilated nephric tubules consistent with loss of slit diaphragm proteins Nephrin and Neph1 in zebrafish. Further investigation to determine changes in the podocyte foot processes using electron microscopy and loss of filtration barrier using transgenic fish is under progress.

Conclusions: In conclusion, Myo1c is a component of Nephrin and Neph1 signaling pathway and plays a role in maintaining glomerular function.

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FR-PO1285

Septin-7, a Novel Interaction Partner of CD2AP, Negatively Regulates Glucose Uptake

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Background: Podocytes are insulin sensitive and take up glucose in response to insulin using glucose transporters GLUT4 and GLUT1. Glucose uptake depends on nephrin, which interacts with VAMP2 residing on GLUT4 storage vesicles (GSVs) and facilitates the fusion of the vesicles with the plasma membrane. CD2AP, an adaptor protein essential for the glomerular filtration barrier, interacts with nephrin and a number of proteins involved in various signaling and vesicular trafficking pathways.

Methods: Here we describe the identification of the small GTPase septin-7 as a novel interaction partner of CD2AP by pull-down assay and mass spectrometry and show by 2-deoxy-D-glucose uptake assay that septin-7 regulates glucose uptake.

Results: Septin-7 partially co-localizes with CD2AP in rat glomeruli and pull-down assay on rat glomerular lysate indicates that their interaction is mediated by the 3rd SH3 domain of CD2AP and the C-terminus of septin-7. Further, co-immunoprecipitation assays show that both CD2AP and nephrin interact with septin-7. Septins are filament-forming proteins and also septin-7 is expressed in cultured human podocytes in a filamentous pattern that depends on the intact actin cytoskeleton. In addition to regulating cytokinesis, mammalian septins function in exocytosis. We found that septin-7 interacts with regulators of vesicular trafficking both on GSVs and plasma membrane suggesting that septin-7 could regulate the exocytic transport of GSVs. In line with this, knockdown of septin-7 in HIRc cells, rat fibroblasts that stably express human insulin receptor, increased the glucose uptake activity of the cells. Further, the interaction of VAMP2 and ectopically expressed nephrin increased in septin-7 depleted HIRc cells.

Conclusions: The data indicate that septin-7 may form a filamentous barrier and thus hinder vesicle trafficking, and consequently, depletion of septin-7 facilitates the fusion of the vesicles with the plasma membrane. Involvement of nephrin suggests that septin-7 may participate in the regulation of glucose transport also in podocytes.

Funding: Private Foundation Support

FR-PO1286

Ang II Induces Nephrin Dephosporylation and Podocyte Injury through Caveolin 1-Dependent Mechanism Zhilong Ren,[1] Wei Liang,[1] Cheng Chen,[1] Pravin C. Singhal,[2] Guohua Ding.[1] *[1]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Medicine, North Shore-Long Island Jewish Health System, Manhasset, NY.*

Background: It has been suggested that nephrin contributes to the mechanism of Ang II- induced podocyte injury. Caveolin-1 has been demonstrated to play a crucial role in signaling transduction. In the present study, we evaluated the effect of AngIIon nephrin phosphorylation in podocytes and whether caveolin-1 was involved in this process.

Methods: Cultured podocytes were exposed to Ang II(10^{-6}mol/L) and pretreated with or without losartan(10^{-5}mol/L) for variable time periods. Nephrin and caveolin-1 expression and their phosphorylation were evaluated by Western-blotting and immunofluorescence. Caveolar membrane fractions were isolated by sucrose density gradient centrifugation, then the distribution and interactions between ATI, nephrin, Csk and caveolin-1 were analyzed using Western-blotting and co-immunoprecipitation. Podocyte apoptosis was evaluated by cell nucleus staining with Hochest-33342.

Results: Caveolin-1 was expressed in podocytes and had a low level of phosphorylation in control condition. After Ang II stimulation, caveolin-1 phosphorylation was increased, but its total protein expression had no significant change. Nephrin and caveolin-1 were found to be co-localized in caveolae fractions. AT1 and Csk moved in caveolae fractions and had a interaction with caveolin-1 after the stimulation of AngIIfor 3-hour. Transfection of caveolin-1 plasmid(pEGFPC3-cav-1) significantly increased AngII-induced nephrin dephosphorylation and podocyte apoptosis. Furthermore, knockdown caveolin-1 protein using siRNA inhibited nephrin dephosphorylation and ameliorated Ang II-induced podocyte apoptosis.

Conclusions: In conclusion, these data indicate that AngIIinduces nephrin dephosphorylation and podocyte injury through a caveolin-1-dependent mechanism.

Funding: Government Support - Non-U.S.

FR-PO1287

Role of Scribble for Podocyte Function and Cell Polarity Eugen Widmeier,[1] Bjorn Hartleben,[1] Nicola Wanner,[1] Sung Tae Kim,[2] Jeffrey H. Miner,[2] Dontscho Kerjaschki,[3] Gerd Walz,[1] Tobias B. Huber.[1] *[1]Renal Division, University Hospital Freiburg, Germany; [2]Nephrology Department, Washington University St. Louis; [3]Pathology, Medical University Vienna, Austria.*

Background: The kidney filter represents a unique assembly of podocyte epithelial cells that tightly enwrap the glomerular capillaries with their foot processes and the interposed slit diaphragm. So far, very little is known about the guidance cues and polarity signals required to regulate proper development and maintenance of the glomerular filtration barrier. We recently demonstrated that the evolutionarily conserved apical Par3-Par6-aPKC complex, a fundamental regulator of cell polarity proteins interact with Neph1 and Nephrin at the slit diaphragm of the glomerular filtration barrier and that podocyte-specific deletion of aPKCiota in mice results in severe proteinuria, nephrotic syndrome, progressive glomerulosclerosis and death at 3-4 weeks after birth. The cell polarity protein Scribble, a large, multidomain scaffold protein, is known to be essential for 3-dimensional tissue morphogenesis. Here we report, that Scribble translocates from the lateral side of polygonal shaped immature podocytes to the basal cell membrane during podocyte differentiation. Immunogold electron microscopy reveals membrane associated localisation of Scribble predominantly basal of the slit diaphragm and partially at the slit diaphragm. To further study the role of Scribble for podocyte differentiation Scribble flox/flox mice were generated by introducing lox-p-sites into the Scribble introns 1 and 8 and these mice were crossed to Cre deleter mice and Podocin-Cre mice. Constitutive Scribble knockout animals die during embryonic development around E14. Kidney culture experiments of constitutive Scribble knockout mice reveal unaffected foot process development and slit diaphragm formation. Podocyte specific Scribble knockout mice develop normally and show no histological, ultrastructural or clinical abnormalities for up to 12 months of age. Ongoing research evaluates the role of Scribble under glomerular pathophysiological conditions.

Funding: Government Support - Non-U.S.

FR-PO1288

Functional and Spatial Analysis of *C. elegans* SYG-1 and SYG-2, Orthologs of the Neph/Nephrin Cell Adhesion Module Directing Selective Synaptogenesis Elke Neumann-Haefelin,[1] Nicola Wanner,[1, 2] Gerd Walz,[1, 3] Tobias B. Huber.[1, 2, 3] *[1]Renal Division, University Hospital, Freiburg, Germany; [2]Spemann Graduate School of Biology and Medicine, Albert-Ludwigs-Universität, Freiburg, Germany; [3]Centre for Biological Signalling Studies (bioss), Albert-Ludwigs-Universität, Freiburg, Germany.*

Background: The assembly of specific synaptic connections represents a prime example of cellular recognition. Members of the immunoglobulin superfamily are ancient proteins in both mammals and invertebrates, where they constitute a trans-synaptic adhesion system. The correct connectivity patterns of the IgSF proteins nephrin and Neph1 are crucial for the assembly of functional neuronal circuits and the formation of the kidney slit diaphragm, a synapse-like structure forming the filtration barrier.

Methods: Here, we utilize the nematode *C. elegans* model for studying the requirements of synaptic specificity mediated by nephrin-Neph proteins.

Results: In C. elegans, the nephrin/Neph1 orthologs SYG-2 and SYG-1 form intercellular contacts strictly in trans between epithelial guidepost cells and neurons specifying the localization of synapses. We demonstrate a functional conservation between mammalian nephrin and SYG-2. Expression of nephrin effectively compensated loss of syg-2 function in C. elegans and restored defective synaptic connectivity further establishing the C. elegans system as a valuable model for slit diaphragm proteins. Next, we investigated the effect of SYG-1 and SYG-2 trans homodimerization respectively. Strikingly, synapse assembly could be induced by homophilic SYG-1 but not SYG-2 binding indicating a critical role of SYG-1 intracellular signalling for morphogenetic events and pointing towards the dynamic and stochastic nature of extra- and intracellular nephrin-Neph interactions to generate reproducible patterns of synaptic connectivity.

Conclusions: In summary, our findings corroborate that *C. elegans* is a useful tool for investigating fundamental nephrin/Neph protein functions. Furthermore, we present novel insights into the mechanisms of SYG-1 and SYG-2 homotypic adhesion properties and intracellular functions.

Funding: Government Support - Non-U.S.

FR-PO1289

Effect of Amyloidogenic Immunoglobulin Light Chains on the Contractile Cytoskeleton of Human Podocytes In Vitro Laura Econimo,[1] Julie A. Tomolonis,[1] Laura M. Dember,[2] David C. Seldin,[2] Lawreen H. Connors,[2] Joel M. Henderson.[1] *[1]Pathology and Laboratory Medicine, Boston University Medical Center, Boston, MA; [2]Amyloid Treatment & Research Center, Boston University Medical Center, Boston, MA.*

Background: Podocyte injury and proteinuria are hallmarks of renal involvement in light-chain (AL) amyloidosis, but the mechanisms underlying these effects are not well established. Recent work suggests that amyloidogenic light chains (LC) may have direct toxic effects on cells. Given the importance of the cytoskeleton to podocyte barrier function, we hypothesized that amyloidogenic LC may elicit a toxic effect on podocytes, characterized by derangement of cytoskeletal structure and function. In this study, we investigated the effect of amyloidogenic LC on human podocyte cytoskeletal structure and contractile function in vitro.

Methods: Human LC were isolated from urine of patients with: renal amyloidosis and severe proteinuria; amyloidosis without renal involvement; and multiple myeloma. Human podocytes were plated on glass and polyacrylamide elastic substrates, and exposed to soluble LC, control protein (recombinant transthyretin, rTTR) or vehicle for 24 hr. After protein exposure, immunostaining was performed to characterize filamentous actin distribution and soluble protein endocytosis, and traction force microscopy was used to measure cell traction.

Results: All of the proteins to which podocytes were exposed (LC or TTR) were confirmed to be endocytosed by the cells. Cells exposed to amyloidogenic LC appeared small and exhibited cytoskeletal disorganization, as compared to cells receiving other treatments. Cells exposed to amyloidogenic LC showed a significant decrease (up to 62%) in traction force magnitude compared to vehicle control; cells exposed to other LC or rTTR exhibited less marked decreases (20 to 31%) in traction force relative to control.

Conclusions: The results suggest that amyloidogenic LC have a direct toxic effect on podocytes, manifest as contractile cytoskeleton dysfunction.

Funding: NIDDK Support

FR-PO1290

Complement Modulates the Ubiquitin-Proteasome System and Endoplasmic Reticulum-Associated Degradation in Glomerular Epithelial Cells Thomas M. Kitzler, Joan Papillon, Julie Guillemette, Simon S. Wing, Andrey V. Cybulsky. *Department of Medicine, McGill University, Montreal, QC, Canada.*

Background: In experimental membranous nephropathy, complement C5b-9 induces sublethal glomerular epithelial cell (GEC) injury and proteinuria. C5b-9 also activates mechanisms that restrict injury or facilitate recovery. The actions of C5b-9 are mediated by pathways, including mitogen-activated protein kinases and protein kinase C (PKC). Recently, we showed that in GECs, C5b-9 augmented the function of the ubiquitin-proteasome system (UPS); moreover, proteasomal inhibition exacerbated complement-mediated cytotoxicity. This study addresses mechanisms by which complement modulates the UPS.

Methods: We monitored UPS function by transfection of a UPS reporter, GFP^u (CL1 degron fused with green fluorescent protein). By analogy, CD3δ-yellow fluorescent protein (YFP) was employed as a reporter of endoplasmic reticulum-associated degradation (ERAD). Ubiquitin mRNA was quantified by RTq-PCR.

Results: In GECs, sublytic C5b-9 decreased GFPu due to proteasomal degradation. This reduction in GFPu was attenuated by the c-jun N-terminal kinase (JNK)-directed inhibitor, SP203580, or by expression of a dominant-negative JNK mutant, whereas inhibitors of the extracellular signal-regulated kinase and p38 pathways were ineffective. Complement-induced GFPu degradation was also blocked by the PKC-directed inhibitor, GF109203X, or by depletion of PKC (prolonged pretreatment with phorbol myristate acetate). Complement increased the level of CD3δ-YFP in GECs. The overall ubiquitination of proteins was enhanced in complement-treated GECs and in glomeruli of rats with experimental membranous nephropathy, although ubiquitin mRNA was unchanged in GECs.

Conclusions: In GECs, complement increased ubiquitination of proteins, consistent with increased protein misfolding, and augmented overall UPS function, which was at least in part dependent on JNK and PKC. In parallel, ERAD was impaired, perhaps

due to an overabundance of misfolded proteins in the ER, and/or reduced capacity for retrotranslocation to the proteasome. Therapeutic modulation of UPS function may be a novel strategy to limit complement-mediated GEC injury.

FR-PO1291

Cell Surface Heparan Sulfate Glycosaminoglycans Regulate Podocyte Cell-Cell Recognition Kevin J. McCarthy, Deborah J. McCarthy. *Pathology, LSU Health Sciences Center, Shreveport, LA.*

Background: Studies from our laboratory showed that heparan sulfate glycosaminoglycans (HS) on cell surface proteoglycan (PG) core proteins serve as podocyte adhesion co-receptors. HS-null podocytes have decreased ability to attach, spread, and migrate compared to control cells. In this present report, we investigated if cell surface HS mediates cell-cell interactions in podocytes.

Methods: Immortalized Ext1 $^{fl/fl}$ podocytes were used for the study, deletion of the floxed Ext1 allele was done via adenoviral delivery of a Cre recombinase construct (HS-null podocytes). Control podocytes were infected with the same adenovirus construct but lacking the Cre recombinase cassette (HS+ podocytes). Differentiated, non-replicating HS+ and HS- podocytes were labeled in vitro using cell permeant fluorescent dyes CMFDA or CMAC for identification during co-culture experiments. For co-culture experiments, equal numbers of differentially labeled HS+ and HS- were admixed and added to microwell slides. Fluorescent images were digitized at 0, 4, 12, 24, 48, hours and 1 week post-seeding. Short term (<24hrs) interactions were also explored by time lapse microscopy. Controls for cell monolayer development consisted of seeding equivalent numbers of HS+ and HS- cells in separate wells.

Results: At T=0, fluorescent imaging of mixed cultures showed that both HS+ and HS- podocytes were dispersed in a random suspension. At T=2 hours, podocytes from both groups had attached and spread to the substrate in a random pattern. Between 6-12 hrs, HS+ podocytes had begun to aggregate into clusters, whereas the HS- podocytes remained oriented into cords. At longer intervals, (24hrs-48hrs), the HS+ cells remained as clusters, the HS-cells consistently excluded-at no time were heterotypic aggregates (HS+: HS-) seen. Exogenous addition of heparin had no effect on sorting.

Conclusions: The data show that cell surface HS mediates cell-cell interactions in a homophilic manner and that heterophilic interactions, i.e. HS+:HS- aggregates do not occur. Thus, besides playing a role in cell-matrix interactions in podocytes, cell surface HS also plays a previously uncharacterized role in mediating cell-cell interactions in podocytes.

Funding: NIDDK Support

FR-PO1292

Regulation of Inflammatory Response in Renal Glomeruli Depends on the State of Podocytes Sahithi Josna Panchagnula,[1] Simon C. Satchell,[2] Moin Saleem,[2] Lorraine Harper,[1] Julie M. Williams,[4] Helen M. McGettrick,[1] Samantha Tull,[1] Edward Rainger,[1] Caroline O.S. Savage.[3] *[1]University of Birmingham, Birmingham, United Kingdom; [2]University of Bristol, Bristol, United Kingdom; [3]Glasko Smith Kline, Stevanage, United Kingdom; [4]Wellcome Trust Clinical Research Facility, Birmingham, United Kingdom.*

Background: We have hypothesized that podocytes modulate inflammatory responses cooperatively with glomerular endothelial cells (GEnC) in both health and disease.

Methods: Using an *in vitro* static co-culture system, the effects of podocytes on GEnCs to recruit neutrophils was studied in the absence and presence of ANCA. The effect was also studied by injuring the podocytes with puromycin aminonucleoside (PAN).

Results: Co-culture of GEnC and podocytes stimulated with higher concentrations of TNF-α (100U/ml), demonstrated significant reduction in neutrophil recruitment by up to 50% when compared to GEnC mono-cultures. Neutrophil recruitment in co-cultures vs monocultures was 18.83 ± 4.8% and 31.48 ± 5.5%, n=4, using primary cells and 22.3 ± 1.9% and 45.16 ± 4.5%, n=31, using immortalised cell lines. IL-6 was found to be key mediator involved in the inhibition of neutrophil recruitment. The neutrophil interactions in co-cultures were dependent on CXCR2 and ENA-78. The ability of ANCA to enhance neutrophil recruitment by GEnC mono-cultures with TNF-α (2U/ml), was mitigated in co-cultures and the effect was reversed with increasing TNF-α. Co-cultures did not show inhibition in neutrophil recruitment following podocyte injury by PAN.

Conclusions: Healthy podocytes modulate GEnC responses to cytokine stimuli via IL-6. Modulation can occur with ANCA activation of neutrophils to a certain level but is lost with increased endothelial activation or when podocytes are injured. Collectively, we demonstrate importance of podocytes in modulating endothelial responses to injury.

FR-PO1293

Transforming Growth Factor (TGF)-β1 Induced DNA Methylation of Wilms' Tumor Suppressor Gene (WT1) Promoter in Human Podocytes Hiroko Hamatani, Keiju Hiromura, Toru Sakairi, Satoshi Takahashi, Hidekazu Ikeuchi, Akito Maeshima, Yoshihisa Nojima. *Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

Background: WT1, firstly identified as a tumor suppressor gene, is essential for normal podocyte function. Previous reports have shown that WT1 promoter is often methylated in cancers, leading to transcriptional silencing. Recently, it was reported that TGF-β1 downregulates WT1 expression in podocytes. We investigated the possibility that the reduction of WT1 expression is caused by its promoter methylation.

Methods: A conditional immortalized human podocyte cell line was treated with 3 ng/ml of TGF-β1. A human renal tubular epithelial cell line (HK-2), which does not express WT1, was used as the positive control for the methylated WT-1 promoter. The degree of DNA methylation was determined by quantitative methylation-specific PCR (Q-MSP) and bisulfate sequence.

Results: We first determined the effect of TGF-β1 on WT1 expression. Both WT1 mRNA and protein expression were reduced by the treatment of TGF-β1. We next examined the degree of methylation of WT1 promoter by Q-MSP. The level of DNA methylation was low in untreated podocytes (2.9±4.5%). In contrast, increased methylation was detected in podocytes treated with TGF-β1 for 11 days (14.3%±10.6%). Untreated HK2 showed hypermethylation (99.1±0.96%). Bisulfite sequencing also revealed some methylation of CpG sites at WT1 promoter in TGF-β1-treated podocytes, whereas almost no methylation was observed in untreated podocytes. Finally, pretreatment with demethylation agent, 5-aza-2'-deoxycytidine (5-aza-dC), partially prevented the reduction of WT1 mRNA (37.1±11.3% vs 65.9±26.2% of untreated cells, TGF-β1 alone vs TGF-β1 + 5-aza-dC, determined by real time PCR).

Conclusions: Our data showed that TGF-β1 induces DNA methylation of WT1 promoter in human cultured podocytes. The reduction of WT1 expression by TGF-β1 is considered to be partly caused by the DNA methylation of WT1 promoter.

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FR-PO1294

Insulin Stimulates the Production of VEGF-A in the Podocyte Dependent on the Insulin Receptor Lorna J. Hale, Peter W. Mathieson, Moin Saleem, Gavin Iain Welsh, Richard Coward. *Academic Renal Unit, Bristol University, Bristol, United Kingdom.*

Background: VEGF-A is a pro-survival factor abundantly produced by the podocyte, that is critical for glomerular function. Diabetic nephropathy is the leading cause of end stage renal failure in the developed world; however in this condition the role VEGF-A is unclear. Using transgenic mice with podocyte specific deletion of the insulin receptor (IR) (podIRKO) we have recently shown that insulin sensitivity of the podocyte is critical for normal glomerular function (Welsh et al Cell Metabolism 2010). One of the features we observed was increased podocyte apoptosis over time. We therefore set out to investigate if podocyte VEGF-A is controlled by insulin.

Methods: We used conditionally immortalised human podocytes in vitro and murine in vivo models to study the role insulin in the production of VEGF-A. We knocked out the podocyte specific IR by 75% in human cells using ShsiRNA and by at least 90% in mice using cre-lox technology.

Results: In vitro - Insulin rapidly increased the level of mRNA production in human podocytes within 30 minutes of insulin stimulation (increase of 115%. * $p<0.05$. $N\geq5$ experiments) and this was detectable at the protein level (30% increased VEGF-A protein production within 24 hours [$p<0.01$ n=9 experiments]). This was reduced in podocytes with IR knockdown by 30% ($p<0.001$ n=7).

In vivo- VEGF-A was studied both pre and post the development of albuminuria (which occurs at 5 weeks in the podIRKO model). At 4 weeks the production of VEGF-A was diminished by 40% by measuring quantitative PCR of a single cell podocyte suspension and this continued and was reflected by decreased in-situ VEGF-A hybridisation signal after albuminuria has developed in podIRKO animals, when measured at 8 weeks.

Conclusions: VEGF-A is insulin inducible in the podocyte. Our results suggest that when podocyte insulin resistance is present then VEGF-A production is reduced. This occurs prior to the development of albuminuria. Reduced insulin stimulated VEGF-A production could explain the development of podocyte induced apoptosis observed in our transgenic models and also that commonly observed in human diabetic nephropathy.

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FR-PO1295

Podocyte Membrane Traffic Modulates HIV Accumulation Via DC-SIGN Dileep Kumar,[1] Joanna Mikulak,[2] Partab Rai,[1] Rungwasee Rattanavich,[1] Mohammad Husain,[1] Ashwani Malhotra,[1] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Immunology, Istituto Clinico Humanitas, Rozzano (Milano), Italy.*

Background: HIV infection of podocytes has been considered to play a critical role in development of HIV-associated nephropathy. Podocytes display a dysregulated growth and proliferative phenotype. We recently reported that DC-SIGN facilitates HIV-1 entry into conditionally immortalized human podocytes (CIHPs) (Am J Physiol 299:F664-F673, 2010). In the present study, we examined the role of DC-SIGN in membrane traffic-modulated podocyte viral accumulation.

Methods: CIHPs were pulsed with primary R5 and X4 strains for two hours and then thoroughly washed. Subsequenlty, CIHPs were assayed for viral accumulation by RT-PCR at different time periods. To determine the role of DC-SIGN for HIV-1 sequestration in CIHPs, control, anti-DC-SIGN-antibody treated CIHPs and siRNA/DC-SIGN transfected CIHPs were pulsed with HIV. To study the effect of membrane traffic, CIHPs were pretreated with either buffer, cytochalasin-B (an inhibitor of actin filament), colchicine (an inhibitor of microbules), Brefeldin A (an inhibitor of Golgi-ER pathway) and then pulsed with HIV. Cells treated under these conditions were evaluated for HIV accumulation and DC-SIGN expression.

Results: CIHPs rapidly internalized R5 and X4 HIV-1 primary strains via endocytosis. Both anti-DC-SIGN antibody and siRNA/DC-SIGN attenuated HIV accumulation in CIHPs. Pretreatment of CIHPs with both colchicine and cytochalasin B increased podocyte HIV-1 accumulation and thus, showed the role of actin and microtubules in the podocyte viral trafficking. Since Brefeldin A also enhanced podocyte HIV-1 accumulation, it seemed that

Golgi-ER pathway might be linked to podocyte viral sorting. Interestingly, CIHPs treated with colchicine, cytochalasin B, and Brefedin A showed a two-fold increase in DC-SIGN expression. Thus, it appears that in addition to the blockade of the membrane traffic and enhanced expression of DC-SIGN has contributed to HIV entry into CIHPs.

Conclusions: These findings indicated that alteration of membrane traffic promoted podocyte HIV-1 accumulation by enhancing podocyte expression of DC-SIGN.

Funding: NIDDK Support

FR-PO1296

aPKC lambda/iota and zeta Are Required for Podocyte Process Development

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Background: The kidney filter represents a unique assembly of podocyte epithelial cells that tightly enwrap the glomerular capillaries with their foot processes and the interposed slit diaphragm. Previously, we identified the Par3, Par6, aPKC polarity complex as novel interactors of Nephrin and Neph1 at the slit diaphragm. Podocytes express both isoforms of aPKC, lambda/iota and zeta. Genetic deletion of aPKC lambda/iota in podocytes results in severe proteinuria, nephrotic syndrome, progressive glomerulosclerosis and death at 3-4 weeks after birth. Interestingly, while podocyte-specific aPKC lambda/iota KO mice develop such a severe glomerular phenotype they still born with a podocyte footprocess network. We speculated that the aPKC zeta isoform might act as functional backup compensating for the loss of aPKC lambda/iota during glomerular development. Here we report that podocyte-specific double knockout of both aPKC isoforms lambda/iota and zeta results in a dramatic phenotype of podocytes that fail to form correct primary and secondary processes. This severe phenotype leads to a glomerular developmental arrest, mesangiolysis, incomplete capillary network and perinatal death.

These results suggest that aPKC signaling is the essential step in formation of the podocyte process network identifying a fundamental signaling program for the regulation of the complex three-dimensional podocyte network.

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FR-PO1297

The Reno-Protective Effect of Ethyl Pyruvate in Streptozotocin-Induced Diabetic Nephropathy Model

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Background: Pyruvate is an endogenous anti-oxidant and anti-inflammatory substance. The present study was implemented to investigate the protective effect of pyruvate against the development and progression of diabetic nephropathy in an *in-vitro* and *in-vivo* model.

Methods: Diabetic condition was induced by injecting streptozotocin(STZ, 65mg/kg) intraperitoneally from the S-D rats. Those that developed diabetes after 48 h were treated with ethyl pyruvate (40mg/kg) intraperitoneally every other day. Rat mesangial cells cultured primarily from the S-D rat were treated in high glucose (HG, 50mM) or normal glucose (NG, 5 mM) conditions.

Results: HG increased mRNA and protein expression levels of MCP-1, TGF-b1, laminin, fibronectin and type IV collagen in a time dependent manner. And NADPH oxidase-dependent reactive oxygen species (ROS) generation was increased in HG-stimulated cultured mesangial cells. Ethyl pyruvate (EP) decreased NADPH-dependent ROS generation and reduced mRNA and protein levels of MCP-1, TGF-b1, laminin, fibronectin and type IV collagen in a dose dependent manner. Diabetic rats without pyruvate treatment and nondiabetic rats were used for control. Pyruvate-treated diabetic rats exhibited decreased albuminuria, and NADPH-dependent ROS generation. Immunohistoche-mistry showed reduced laminin, type IV collagen and fibronectin deposition in the glomeruli compared with nontreated diabetic rat. Parallel changes were shown in the tissue mRNA and protein expression level of MCP-1, TGF-b1, laminin, fibronectin and type IV collagen in the kidney.

Conclusions: These findings suggest that pyruvate protects against kidney injury via NADPH oxidase inhibition.

Funding: Private Foundation Support

FR-PO1298

Distribution of the Hindiii Restriction Fragment Length Polymorphism and CR1 Expression on Blood Leukocytes of Patients with Primary Glomerulonephritides and Lupus Nephritis

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Background: CR1 is a membrane receptor for C3b and C4b expressed on blood cells. Being involved in the processing and clearance of immune complexes (IC) and regulation of B-cell function, it protects from the development of IC diseases. CR1 expression on erythrocytes was supposed to be linked to high (H) and low (L) expression alleles identified by HINDIII restriction fragment length polymorphism (RFLP). Intriguingly, there are no studies that would have examined the influence of this polymorphism on the level of CR1 expression on blood leukocytes (BL) in patients with primary and secondary glomerulonephritides (GN).

Methods: The surface expression of CD35 was determined by flow cytometry analysis of whole blood samples from 65 patients with primary GN (PGN), 31 with lupus nephritis (LN) and 44 healthy controls (C), with back gating on monocytes (M, CD14), neutrophils (N, CD15) and B-lymphocytes (CD19). DNA was isolated from BL, amplified using primers specific for intron 27 of CR1 gene and HINDIII RLFP analysis was performed thereafter.

Results: Among 140 examined individuals, the HH allele was found in 65%, HL in 29.9% and LL in 5.71% of them. Unexpectedly, the highest expression of CR1 on all cell types was associated with the LL allele. In this respect, significant differences were found between individuals having the LL vs. HH alleles ($p<0.001$) and LL vs. HL alleles ($p<0.05$). The comparison of subjects having the HL and HH alleles revealed that those with the HL allele displayed significantly higher CR1 expression on B-cells ($p<0.01$) and N ($p<0.05$), but this could not be demonstrated on M. The prevalence of the HH allele was the highest in the examined groups (PGN, 60%; LN, 64.5%; C, 72.7%), followed by HL (30.8%, 32.3% and 25%) and LL allele (9.2%, 3.2% and 2.3%, respectively).

Conclusions: We found a genetic link between the HINDIII RLFP and CR1 expression on BL in Polish population. However, no impact of this polymorphism on the development of IC diseases could be confirmed.

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FR-PO1299

Toward the Practical Use of Nephrin Peptides as a TRPC6 Inhibitor

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Background: There are lines of evidence that overactivation of TRPC6, is involved in the podocyte pathophysiology (Winn M, Science. 2005) (Reiser J, Nat. Genet. 2005) (Moller C C, JASN. 2007). However, regulatory mechanism of TRPC6 remains unclear. We reported phosphorylation of TRPC6 Y284 is necessary for the binding with PLC-γ1, which promotes the membrane expression. Nephrin also binds to pY284 and interferes the phospho-dependent activation of TRPC6 by disrupting the PLC-γ1-TRPC6 interaction. (Kanda S, MoBC. 2011). Here we analyzed the phosphorylation status of Y284 in glomeruli, and examined whether Nephrin peptide has an inhibitory effect on TRPC6 activity.

Methods: We raised a phospho-specific anti-TRPC6 antibody against pY284. We introduced four kinds of Nephrin peptides, each of which consist of 12 amino acids, into the cytoplasm of cultured cells by a peptide delivery system and assessed the TRPC6 activity by biotinylation assay and calcium assay using Fura-2 loaded cells.

Results: In isolated rat glomeruli, Y284 phosphorylation was observed weakly, and augmented by a phosphatase inhibitor. We identified Nephrin cytoplasmic region (1216-1227) is necessary for its binding with phospho-TRPC6. Two different Nephrin peptides around this region inhibited phospho-dependent TRPC6 membrane trafficking. Toward an in vivo application of Nephrin peptides, we conjugated a motif of 11 consecutive arginines to the most effective Nephrin peptide and delivered it into cultured podocytes. The peptides were delivered into almost all the cells. This Nephrin peptide reduced the membrane expression of phospho-TRPC6 to 57.1±2.8 % and the phospho-dependent Ca uptake to 79.9±6.7 % as compared to a control peptide.

Conclusions: Nephrin peptides inhibits phospho-dependent TRPC6 activation, and have a potential as a therapeutic reagent with appropriate delivery in vivo.

FR-PO1300

Nephrin Missense Mutations Alter Cellular Trafficking and Induce Endoplasmic Reticulum Stress

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Background: Nephrin, a key component of the filtration slit diaphragm, undergoes post-translational modifications in the endoplasmic reticulum (ER). Mutations in nephrin lead to proteinuria. We examined the effects of missense mutations in nephrin on protein folding in the ER, cellular trafficking, and induction of the unfolded protein response (UPR).

Methods: Wild type (WT) nephrin, and the I171N, G270C, S366R, S724C and R743C mutant cDNAs were expressed in 293T cells or glomerular epithelial cells (GECs) by transient transfection. Association of nephrin with the ER chaperone, calnexin, was studied by co-immunoprecipitation. Activation of the UPR was assessed by monitoring expression of the ER chaperone, Grp94, phosphorylation of eukaryotic translation initiation factor-2α subunit (eIF2α), and induction of C/EBP homologous protein-10 (CHOP), as well as activating transcription factor-6 (ATF6)-luciferase reporter activity.

Results: All nephrin mutants showed increased association with calnexin, compared with WT nephrin. The I171N and G270C mutants increased expression of Grp94 in 293T cells, and stimulated ATF6-luciferase activity in both 293T cells and GECs. Nephrin S366R and S724C tended to induce the UPR, but changes in Grp94 and ATF6-luciferase activity were less consistent. The R743C mutant did not enhance Grp94 expression, nor ATF6-luciferase activity. All nephrin mutants did not increase eIF2α phosphorylation, nor CHOP expression. Immunofluorescence microscopy showed WT nephrin at the plasma membrane, while the I171N and S366R mutants were perinuclear, colocalized with calnexin. Morever, the two nephrin mutants induced aggregation of the ER chaperone, calreticulin, compared with WT. Treatment of cells with castanospermine (which reduces the interaction of nephrin with calnexin) resulted in a portion of nephrin I171N and S366R appearing at the plasma membrane.

Conclusions: Certain nephrin mutants show impaired folding in the ER, and activate the ATF6 branch of the UPR. Induction of ER chaperones may represent a cytoprotective response, allowing cells to withstand proteotoxic injury. Blocking the interaction of nephrin with calnexin results in a partial rescue of certain nephrin mutants to the plasma membrane.

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FR-PO1301

Cyclin-Dependent Kinase Cdk5 Regulates TRPC6 Function at the Slit Diaphragm Henning Hagmann,[1] Bernhard Schermer,[1] Jeffrey W. Pippin,[2] Thomas Benzing,[1] Stuart J. Shankland,[2] Paul T. Brinkkoetter.[1] *[1]Department of Nephrology, University of Cologne, Germany; [2]Department of Nephrology, University of Washington, Seattle, WA.*

Background: The slit diaphragm (SD) bridges the 30-40nm wide intercellular gap in between neighbouring podocyte foot processes. It not only displays a structural component of the glomerular filter but it is also a site of highly active signalling to ascertain podocyte function and survival. Among the proteins involved are the Nephrin-Neph signalling complex as well as the non-selective cation channel TRPC6. Hyperactive TRPC6 channel activity has been shown to cause podocyte demise and glomerular disease (FSGS) [1].

Results: Here, we provide evidence that the atypical cyclin dependent kinase Cdk5 is a key regulator of TRPC6 activity in podocytes. Cdk5 and its activator p35 interacted and co-localized with SD-proteins in detergent resistant membrane fractions in vitro and phosphorylated the non-selective cation channel TRPC6 directly. In contrast, no effect was observed for Cdk5-Cyclin I, another activator present in podocytes. We further investigated the physiological impact of phosphorylation on TRPC6 protein stability and membrane localization. Phosphorylation of TRPC6 led to enhanced translo-cation of the channel protein to the plasma membrane and increased TRPC6-channel activity, as assessed in whole cell voltage clamp experiments in Xenopus oocytes and fluorometric Ca2+-measurements in cultured podocytes.

Conclusions: Our data suggests that Cdk5, a kinase involved in synaptogenesis and synaptic plasticity of neurons, plays a crucial role at the slit diaphragm. It acts directly on slit diaphragm proteins and regulates TRPC6 signalling by direct phosphorylation. This new regulatory role of Cdk5 at the podocytes intercellular junction makes it tempting to speculate that the podocyte slit diaphragm and the neuronal synapse, indeed, share great similarities and raises an array of questions concerning slit-diaphragm turnover and remodelling.

1. Winn, M.P., et al., A mutation in the TRPC6 cation channel causes familial focal seg-mental Glomerulosclerosis. Science, 2005. 308(5729): p.1801-4.

Funding: Government Support - Non-U.S.

FR-PO1302

Protein Kinase G Negatively Regulates Angiotensin II-Induced Transient Receptor Potential Cation Channel-6 Activity in Cultured Podocytes Gentzon Hall, Peter J. Lavin, Rasheed A. Gbadegesin, Guanghong Wu, Alison Byrd, Alison Homstad, Michelle P. Winn. *Medicine/Nephrology, Duke University Medical Center, Durham, NC.*

Background: The transient receptor potential cation channel, type 6 (TRPC6) is a non-selective, Ca^{2+}-permeable cation channel that causes hereditary FSGS. Despite mounting evidence for the role of TRPC6 in various glomerular pathologies, the development of selective TRPC6 inhibitors has been complicated by the high degree of sequence homology between TRPC family members. Recent insights into the functional regulation of TRPC6 have revealed protein kinase G (PKG) as a key negative modulator of TRPC6 activity through phosphorylation of 2 highly conserved residues, threonine 69 (T69; T70 in humans) and serine 321 (S321; S322 in humans). In an effort to characterize the role of PKG-mediated inhibition of TRPC6 activity in podocytes, we evaluated TRPC6 phosphorylation at threonine 69 (T69) in mouse podocytes.

Methods: Using a combination of immunoblotting, siRNA-mediated gene knockdown, adenoviral gene expression, and scratch wound healing assays in conditionally immortalized and primary murine podocyte cultures, we examined the role of PKG-mediated TRPC6 phosphorylation.

Results: In unstimulated podocytes, TRPC6 is heavily phosphorylated at T69. This basal phosphorylation is attenuated 4 fold with siRNA-mediated gene knockdown of PKG and 2 fold with Angiotensin II (Ang II) stimulation. Pretreatment of podocytes with the PKG signaling agonists SNAP and 8-br-cGMP and the soluble guanylate cyclase agonists YC-1 and Bay 41-2272, completely attenuated Ang II-induced T69 dephosphorylation. Additionally, pharmacologic agonism of PKG pathway signaling attenuated TRPC6-dependent podocyte migration by 30%-50%. Similarly, in Ang II-stimulated podocytes overexpressing a phosphomimetic mutant of human TRPC6 (TRPC6 T70E/S322E), podocyte migration was significantly reduced by 30%.

Conclusions: Taken together, these data provide evidence for the role of PKG in the modulation of TRPC6 activity in podocytes and highlight the potential therapeutic value of NO/cGMP/PKG agonism in the treatment of TRPC6-associated podocytopathies.

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FR-PO1303

PKCε Is the Upstream Regulator of Dynamin in Podocytes Beina Teng,[1] Sanja Sever,[2] Changkyu Gu,[2] Hermann G. Haller,[1] Mario Schiffer.[1] *[1]Division of Nephrology, Department of Medicine, Medical School Hannover, Hannover, Germany; [2]Nephrology Division, Department of Medicine, Massachusetts General Hospital, Charlestown, MA.*

Background: We have previously demonstrated that the novel PKC isoform (PKCε) participated in signaling pathways controlling actin filament dynamics. PKCε deficiency in podocytes resulted in defective actin arrangement including cell adhesion, migration, spreading and motility, which led to a progressive reduction of podocytes and a spontaneous glomerulosclerosis phenotype in PKCε-/- mice in vivo.

Methods: Wildtype or mutant PKCε (dominant negative), wildtype or mutant dynamin (deletion or dominant negative) were re-expressed in PKCε-/- podocytes via adenoviral infection to further confirm the role of PKCε in podocyte deregulation.

Results: Re-expression of wildtype PKCε but not the mutant resulted in increased formation of stress fibres, focal adhesion complexes, lamellipodia and filopodia in PKCε-/- podocytes. Loss of PKCε in podocytes dramatically reduced the activities of Rho family small GTPases that are key upstream regulators of actin-cytoskeleton rearrangement, which could be reversed by re-expression of wildtype PKCε.

Dynamin, a large GTPase known to regulate either actin or actin-binding proteins to modulate the actin reorganization, was detected to be a binding partner of PKCε in vitro. The expression level of dynamin was not significantly changed in PKCε-/- podocytes compared to wildtype podocytes. Overexpression of constitutively active dynamin(dynE/K) construct via adenoviral infection in PKCε deficient podocytes but not the dominant negative dynamin (dynK/A, dynK/E) or deletion mutant dynamin (dynΔPRD) significantly enhanced the formation of stress fibers, focal adhesion complexes and lamellipodia. Overexpression of dynamin also alters the signaling of Rho family small GTPases especially the Rac1 activity and its localization. All the disordered actin-cytoskeletal phenotypes due to PKCε deficiency could be partically rescued by overexpression of constitutively active dynamin.

Conclusions: PKCε might be an upstream regulator of dynamin in the signaling processes that modulate the dynamics of actin cytoskeleton in podocytes.

FR-PO1304

CLIC5A Enhances Ezrin Phosphorylation and Association with the Actin Cytoskeleton Abass Almomany, Laiji Li, Barbara J. Ballermann. *Medicine, University of Alberta, Edmonton, AB, Canada.*

Background: CLIC5A is a glomerulus-predominant protein that co-localizes with podocalyxin and Ezrin in podocyte foot processes. Ezrin couples transmembrane proteins like podocalxin to cortical actin when it is phosphorylated on T567(P-Ezrin). In CLIC5A deficient mice, podocyte P-Ezrin and total Ezrin are reduced, podocyte structure is abnormal, and adriamycin-induced injury is more severe than in wild-type mice. Here, we explored whether Ezrin phosphorylation is regulated by CLIC5A.

Methods: Wild-type CLIC5A was cloned into pCDNA3.1. Cos-7 cells, lacking endogenous CLIC5A, were transfected with CLIC5A or vector. Total cell lysates, and soluble and cytoskeletal fractions were prepared using Triton X-100 lysis buffer.

Results: Immunoblots of total cell lysates showed that total Ezrin abundance was similar in CLIC5A and vector transfected cells. However, a much greater proportion of Ezrin was phosphophorylated at T567 in the CLIC5A-transfected, compared to vector transfected cells. Furthermore, in CLIC5A-transfected cells there was a dramatic, 8-10 fold greater association of Ezrin with the cytoskeletal fraction than in vector-transfected cells, and most of this Ezrin was phosphorylated. The PKC inhibitor staurosporine (100 nM 30 min) strongly inhibited Ezrin phosphorylation and its association with the cytoskeletal fraction, while the RhoA kinase inhibitor Y-27632 (15 μM 30 min) had no effect on Ezrin phosphorylation. Akt activation was similar in CLIC5A- and vector-transfected cells. The phosphatase inhibitor calyculin A increased P-Ezrin abundance in the total cell lysates and Ezrin association with the cytoskeletal fraction. The rate of Ezrin de-phosphorylation, quantified by determining P-Ezrin abundance as a function of time after addition of staurosporine, did not differ in CLIC5A- and vector-transfected cells.

Conclusions: Hence, CLIC5A promotes Ezrin phosphorylation and its association with the cytoskeletal fraction in transfected Cos-7 cells. Enhanced Ezrin phosphorylation is likely PKC- (not RhoA or AKT) mediated, and is not due to inhibition of phosphatase activity. We propose that in podocytes, CLIC5A may maintain the association of Ezrin with actin by regulating Ezrin phosphorylation.

Funding: Government Support - Non-U.S.

FR-PO1305

Glomerular Endothelial Cells Express Glutamate Receptors: Implication for Glomerular Filter Permeability Min Li,[1] Silvia Armelloni,[1] Laura Giardino,[1] Alessandro Corbelli,[1] Masami Ikehata,[1] Deborah Mattinzoli,[1] Piergiorgio Messa,[2] Maria Pia Rastaldi.[1] *[1]Renal Research Laboratory, Fondazione IRCCS Policlinico & Fondazione D'Amico, Milan, Italy; [2]Nephrology and Dialysis, Fondazione IRCCS Policlinico, Milan, Italy.*

Background: We have previously shown that podocytes express glutamate receptors (NMDR and Grm1), and that changes of glomerular glutamate signaling cause podocyte damage and proteinuria. Aim of this study was to start uncovering molecular mechanisms linking dysregulated glutamate signaling to proteinuria.

Methods: In-vitro filter permeability was measured by a novel 3D co-culture system with podocytes and endothelial cells grown on the opposite sides of a membrane. BSA passage from the endothelial to the podocyte supernatant was assessed by spectrometry.

Immunostainings (IF, immunoEM), western blot (WB) and in-cell ELISA served to evaluate NMDR and Grm1 expression and MAPKinase activation.

Results: IF, WB and immunoEM showed that glomerular endothelial cells express NMDAR and Grm1, as it occurs in brain capillaries.

In the 3D co-culture system, BSA permeability was increased not only by podocyte damage but also by incubation of endothelial cells with a neurotoxic dose of glutamate (1 mM).

Application to podocytes of alpha-latrotoxin (a-LTX), a neurotoxin causing bulk glutamate release from synaptic vesicles, induced as well a marked increase in BSA permeability, that was prevented by endothelial cell incubation with the NMDAR antagonist MK-801, but not with the Grm1 antagonist CPCCOEt.

In-cell ELISA demonstrated that glutamate exposure (either direct on endothelial cells, or indirect by a-LTX applied to podocytes) causes endothelial activation of the MAPKinase pathway. The effect is exclusively depending on endothelial NMDAR activation because it is abrogated by endothelial pre-incubation with MK-801, but not with CPCCOEt.

Conclusions: Our data provide a first molecular link between podocyte glutamatergic signaling and increased filter permeability. From our experiments we can hypothesize that leakage of albumin is caused by excessive entry of calcium through the NMDAR into endothelial cells and leads to endothelial dysfunction through activation of the MAPKinase pathway.

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FR-PO1306

A Novel Function of the MYPT Family Member TIMAP in Endothelial Cells (EC)

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Background: TIMAP is a prenylated EC-predominant protein phosphatase 1 (PP1c) regulatory subunit in the myosin phosphatase (MYPT) family, first identified by us in glomerular EC. MYPTs control PP1c activity toward myosin light chains (MLC). Whether TIMAP shares this MYPT function is unknown. Here we explored whether TIMAP regulates phosphorylation of MLC2 (P-MLC2)in EC.

Methods: Two point mutations were introduced in the PP1c binding site of TIMAP, producing TIMAP(PP1c-). Immunoprecipitates of EC-expressed wild-type TIMAP (TIMAP(WT)), but not TIMAP(PP1c-) contained PP1c protein and phosphatase activity. We reasoned that TIMAP(PP1c-) might inhibit de-phosphorylation of P-MLC2 by the TIMAP/PP1c holoenzyme in a dominant negative fashion and therefore increase P-MLC2 abundance.

Results: Endogenous TIMAP and MLC2 co-immunoprecipitated from EC lysates, and in migrating EC, stably expressed TIMAP(PP1c-) exquisitely co-localized with MLC2 at the tip of trailing projections. Contrary to expections, expression of TIMAP(PP1c-) in EC was associated with markedly reduced P-MLC2 in cellular projections, compared to EC transfected with vector or TIMAP(WT) (n=3). By western blot, the proportion of MLC2 phosphorylated on T18/S19 was also lower in EC expressing TIMAP(PP1c-) compared to TIMAP(WT). Upon Rho kinase inhibition with Y27632, P-MLC2 was rapidly dephosphorylated, but the dephosphorylation rate in EC expressing TIMAP(WT) and TIMAP(PP1c-) was identical (n=3). Expression of TIMAP(PP1c-), but not TIMAP(WT) induced formation of long projections in EC, similar to those observed when myosin activity was inhibited. Finally, free MLC2 could be phosphorylated on T18/S19 by myosin light chain kinase (MLCK) in vitro. Also, MLC2 bound directly to TIMAP in vitro. However, the MLCK-mediated phosphorylation of MLC2 bound to immobilized TIMAP(WT) was almost completely blocked, even when PP1c was absent.

Conclusions: The findings that TIMAP interacts with MLC2, that TIMAP devoid of PP1c inhibits MLC2 phosphorylation in EC and in vitro, and mimics myosin inhibitors functionally in EC, lead us to propose the novel mechanism that the T18/S19 phosphorylation site of MLC2 becomes inaccessible to kinases when MLC2 is bound to TIMAP.

Funding: Government Support - Non-U.S.

FR-PO1307

The Impact of Interplay between c-mip and WT1 Activity in the Pathophysiology of Podocyte Diseases

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Background: C-maf inducing protein, c-mip, was identified in our lab as a key component in the molecular pathogenesis of acquired nephrotic syndromes (NS). The Wilm's tumor suppressor gene *WT1* is expressed constitutively in podocytes and is essential for the functional integrity of glomerular filtration barrier. Mutations of *WT1* gene are associated with NS but the mechanisms of proteinuria are unclear. Because WT1 may act as transactivator or transrepressor, it is not known whether the podocyte dysfunction mainly results from downregulation or overexpression of target genes or both. We report here a functional antagonism between c-mip and WT1 may play a critical role in the pathophysiology of podocyte diseases.

Methods: All adult patients analyzed in this study suffered from idiopathic NS. IHC was performed for WT1 in human biopsy and mouse kidney sections. Chromatin immunoprecipitation, EMSA, WT1 decoy oligonucleotide transfection and luciferase activity assays were performed in M15 and podocyte cell lines. Ubiquitin E3 ligase activity detection kit was used to evaluate the E3 activity of c-mip. siRNA was performed to knockdown c-mip in vivo.

Results: Under physiological conditions, WT1 binds to c-mip promoter via WT1 responsive elements and inhibits its transcriptional induction in podocytes. Conversely, the abundance of c-mip in podocytes was strongly increased in patients presenting with Denys-Drash syndrome. We showed that the induction of c-mip in mouse model for Frasier syndrome precedes the development of the massive albuminuria. In acquired idiopathic NS, c-mip was upregulated in podocytes, while WT1 protein level was reduced. We demonstrated that c-mip interferes with the constitutive transactivation of WT1 mediated by NF-kB. Moreover, we showed that c-mip interacts with WT1 and exhibits an E3 ligase activity, which targets WT1 to proteasome degradation. Intravenous injection of c-mip-siRNA specifically prevented the repression of WT1 in LPS-induced proteinuria in mice.

Conclusions: The negative cross-talk between WT1 and c-mip is required for glomerular integrity and might be a critical determinant in the pathophysiology of podocyte diseases.

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FR-PO1308

EphB4 Forward Signaling Maintains Podocyte Homeostasis during Thy1.1 Nephritis

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Background: Glomerular diseases starting with mesangiolysis have a high potential for recovery, the molecular mechanisms remain however to be defined. Eph receptor tyrosine kinases and their ligands (ephrins) play a pivotal role in the homeostasis of many adult organs and are also widely expressed in the kidney. The aim of our study was thus to assess their role in the glomerular recovery from Thy1.1 nephritis, a rat model of reversible mesangioproliferative glomerulonephritis (GN).

Methods: Western blotting and immunohistochemical methods were used to follow the expression and activation status of Eph/ephrins in healthy and nephritic glomeruli. Next, NPV-BHG712, a novel inhibitor of EphB4 phosphorylation, was applied to control or nephritic rats.

Results: EphB4 and ephrinBs were expressed in healthy glomeruli and upregulated during Thy1.1 nephritis. EphB4 was mostly expressed at the apical membranes of podocytes, whereas ephrinBs were located at the foot processes. Importantly, EphB4 was strongly phosphorylated around day 9 of nephritis. NPV-BHG712 induced no glomerular changes in controls. Nephritic animals treated with vehicle showed neither morphological evidence of podocyte injury nor loss of podocytes. In contrast, NPV-BHG712 application to nephritic rats induced glomerular microaneurysms, podocyte damage and loss. Prolonged NPV-BHG712 treatment resulted in increased albuminuria and substantially disregulated mesangial recovery. Additionally, NPV-BHG712 inhibited capillary repair by intussusceptive angiogenesis, suggesting a previously unrecognized role of podocytes in regulating intussusceptive vessel splitting.

Conclusions: In aggregate, our results identify EphB4 signalling as a novel pathway allowing podocytes to adapt to transient capillary collapse and thus survive.

Funding: Government Support - Non-U.S.

FR-PO1309

Divergent Mechanisms of Proteinuria and Fibrogenesis in a Mouse Model of Adriamycin Nephrosis

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Background: Transforming growth factor (TGF-β) is an important mediator of renal fibrogenesis. We initially showed that PI3K and TGF-β contribute to collagen I expression in vitro. Extending these findings, we presented at ASN 2010 a modified mouse model of adriamycin (ADR) nephropathy manifesting proteinuria and FSGS-like histology. Here, we evaluated the role of the p110γ isoform of PI3K in this model.

Methods: A single intravenous injection of ADR to 8 week-old 129Sv/j mice induced glomerulopathy. Kidney tissue and cultured mouse podocytes were evaluated by using standard immunohistochemistry and qPCR.

Results: In-vivo administration of soluble TGF-β receptor II protected against glomerular disease, but did not change ADR-induced proteinuria or tubular injury. Akt phosphorylation was detected by immunostaining of ADR-treated mouse kidneys, suggesting PI3K activation. mRNA encoding p110γ, but not other PI3K isoforms, was selectively up-regulated in ADR-kidneys. Though p110γ is highly enriched in leukocytes, we found that cultured podocytes express p110γ and that p110γ staining colocalizes with nephrin in ADR-kidney glomeruli. In-vivo blockade of p110γ by AS605240 prevented both proteinuria and glomerulosclerosis in ADR-treated mice. In cultured podocytes, AS605240 prevented ADR-induced podocyte damage, protecting against cytoskeletal disorganization and apoptosis. These data suggest that p110γ mediates podocyte injury at the initiation of the disease process. In ADR-kidneys, AS605240 treatment prevented TGF-β expression. In cultured human kidney epithelial cells, AS605240 did not affect collagen induction by TGF-β, whereas pan-PI3K inhibition by LY294002 blocked collagen induction. These data suggest that PI3K p110γ contributes to disease initiation and podocytes injury, whereas other PI3K isoforms play a role in subsequent, TGF-β-mediated collagen expression.

Conclusions: We suggest that PI3K p110γ mediates podocyte injury, resulting in proteinuria, through cell signaling that is not directly dependent on TGF-β/Smad3 pathway activation. Conversely, TGF-β/Smad3 signaling is not involved in proteinuria, but instead has a significant role fibrogenesis.

Funding: NIDDK Support

FR-PO1310

Hypoxia-Inducible Factor 1α Expression Is Upregulated and Accerelates Fibrosis in Adriamycin-Induced Murine Glomerulonephropathy Tomoko Hayashida, Xiaoyan Liang, Susan C. Hubchak, H. William Schnaper. *Pediatrics, Northwestern University, Chicago, IL.*

Background: Hypoxia-inducible factor (HIF)-1α has been associated wtih ischemic renal injury, but its fibrogenic role in normoxia is less characterized. We recently reported that transforming growth factor (TGF)-β upregulates HIF-1α expression in normoxic cultured human kidney cells. Transcriptional inhibition of HIF-1α blocked TGF-β1-induced type I collagen expression (Basu, JASN 2011). Here, we tested a possible role for HIF-1α in a mouse model of kidney fibrosis.

Methods: Glomerulonephropathy was induced in male 129x1/Svj mice by a single injection of adriamycin (ADR, 15 mg/kg, iv). Some mice were transferred to a hypoxia chamber (10% oxygen) the day after the ADR administration. On day 10, mice were sacrificed 1 hr after Hypoxyprobe administration (60 mg/kg, iv), which accumulates under hypoxia ($pO2 < 10$ mmHg) and is detectable by immunochemistry post mortem. Kidneys were harvested to evaluate histology and mRNA expression by real-time PCR.

Results: As described, ADR-treated mice in normoxia develop proteinuria and TGF-β-mediated histopathological changes consistent with FSGS, associated with increased ECM mRNA expression starting at day 7 and plateaus by day 14. (Finer and Hayashida, ASN2010). Hypoxyprobe adducts were minimally detected in control mouse kidney cortex, whereas they strongly decorated proximal tubules of the ADR-treated mouse kidneys. HIF-1α mRNA expression, determined in whole kidney lysates, was increased 4.3x by ADR compared to control. Glomeruli of the ADR-treated mice showed mesangial expansion and fibrosis but no Hypoxyprobe staining, suggesting a non-hypoxic mechanism for glomerular changes. The kidneys of ADR mice housed in hypoxia showed greater histopathological changes. COL1A2 mRNA expression in whole kidney lysates was not affected in control mice in hypoxia, whereas hypoxia exacerbated COL1A2 mRNA expression in ADR-treated mice (1.89x vs. 5.47x, respectively, compared to normoxic control). Hypoxia did not increase whole-kidney HIF-1α expression greater than that seen with ADR alone.

Conclusions: These results suggest a synergestic role for HIF-1α in both hypoxic and normoxic kidney fibrosis.

Funding: NIDDK Support

FR-PO1311

Renoprotective Effect and Mechanism of Bortezomib in Adriamycin-Induced Nephropathy Rats Qiao Zhou, Ying Lu, Fang Zhong, Xu Hao, Cong Li, Shanmai Guo, Weiming Wang, Nan Chen. *Nephrology, Ruijin hospital, Shanghai, China.*

Background: Many studies indicate that the proteasome inhibitor could exert potent anti-fibrosis/inflammatory effects . Bortezomib demonstrates a potent antitumor activity against several human cancers and has been clinically used in patients with refractory multiple myeloma.

Methods: Adriamycin nephropathy was induced in Sprague-Dawley rat by a intravenous injection of adriamycin. 4 weeks after injection, bortezomib was given intraperitoneally (30μg/kg or 60μg/kg, twice in week) for 4 weeks. At the end of study, the biochemical indicators were measured and the pathological changes of the renal tissue were evaluated by light microscope. Transmission electron microscopy was used to observe the ultrastructure change of rat kidney. Immunohistochemistry and mRNA expression was applied to observe the expression levels of FSP-1, α-SMA, ColI, Col III, TGF-β, Smad2, Smad3 and the macrophage infiltration in rat kidney.

Results: During the course of nephrotic syndrome, serum creatinine(Scr) and blood urea nitrogen (BUN) were significantly elevated ($p<0.05$). Histological examinations of kidney tissue demonstrated evident tubulo-interstitial inflammation and fibrosis ($p<0.05$). Compared with untreated adriamycin nephrotic group, bortezomib treatment could significantly reduce the level of urine protein, the Scr and BUN, which was accompanied by the attenuation of the macrophage infiltration, pathological changes and ultrastructure change in rat kidney. Immunohistochemistry results showed that the expression of FSP-1, α-SMA, ColI, Col III, TGF-β and Smad3 significantly increased in adriamycin-induced nephritic group($p<0.05$), but no significant difference in Smad2 ($p>0.05$) was found. Compared with the untreated adriamycin-induced nephrotic group, bortezomib treatment could significantly decreased the expression of FSP-1, α-SMA, ColI, Col III, TGF-β and Smad3 ($p<0.05$), but Smad2 was increased ($p<0.05$).

Conclusions: The results demonstrated that bortezomib could significantly ameliorate nephrotic syndrome in adriamycin-induced nephropathy rats and progression of renal fibrosis through a TGF-β/Smad-dependent pathway.

FR-PO1312

Podocyte Damage in Fabry Disease Max C. Liebau,[1,2] Fabian Braun,[1] Claudia Larissa Weitbrecht,[1] Roman-Ulrich Mueller,[1,3] Moin Saleem,[4] Bernhard Schermer,[1,3] Thomas Benzing,[1,3] Markus Cybulla,[5] Christine E. Kurschat.[1,3] *[1]Department of Nephrology, University Hospital Cologne, Cologne, Germany; [2]Department of Pediatrics, University Hospital Cologne, Cologne, Germany; [3]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Germany; [4]Children's Renal Unit, Bristol Royal Hospital for Children, Bristol, United Kingdom; [5]Department of Medicine IV, University Hospital Freiburg, Freiburg, Germany.*

Background: Fabry disease is an X-linked lysosomal storage disorder resulting from an inborn deficiency of lysosomal α-galactosidase A (α-gal A). This deficiency leads to accumulation of neutral glycosphingolipids, mostly globotriaosylceramide (Gb3), in various tissues including kidney, heart, vessels and brain. End-stage renal disease is very common in hemizygous males and limits life expectancy in these patients but renal involvement leading to proteinuria, hematuria, and renal insufficiency is not well understood. Histological studies suggest that the accumulation of Gb3 in podocytes plays an important role in the pathogenesis of glomerular damage. However, the pathophysiological role of Gb3 in podocytes is difficult to study due to the lack of an appropriate animal or cell culture model.

Methods: We have established a human cell culture model of podocyte damage in Fabry disease by using RNA interference technology in combination with lentiviral gene transfer. An established human podocyte cell line was transduced with various short hairpin RNA (shRNA) constructs against human α-gal A. Reduction of α-gal A mRNA levels and α-gal A activity were confirmed by qPCR and by a photometric assay.

Results: Lipid chromotography revealed Gb3 accumulation in α-gal A knockdown cells. We observed a decrease of AKT phosphorylation as well as reduced levels of phospho-mTOR in α-gal A knockdown cells, associated with an increase in autophagy.

Conclusions: Our data suggest that podocyte disease in Fabry patients is linked to a dysregulation of autophagy.

This novel cell line will serve as a promising tool for further studies on the pathophysiological mechanisms responsible for glomerular dysfunction in Fabry disease.

Funding: Government Support - Non-U.S.

FR-PO1313

Inverted Formin 2 Is Essential for Maintenance of Podocyte Morphology and Signaling Hua Sun, Martin R. Pollak, Johannes S. Schlondorff. *Nephrology, Beth-Israel Deaconess Medical Center, Boston, MA.*

Background: Podocytes are terminally differentiated cells whose morphology and function depend on the fine regulation of actin skeleton dynamics. Mutations in inverted formin 2 (INF2), an actin regulating protein, can cause familial focal segmental glomerulosclerosis (FSGS). INF2 serves as a potential regulator of actin dynamics through its dual polymerization/depolymerization activity and by interference with diaphanous-related formin (mDia) mediated actin polymerization. Here we investigated the role of INF2 in the maintenance of actin-based morphology and signaling in podocytes to gain insight into the mechanism of this form of disease.

Methods: In cultured human podocytes, slit diaphragm (SD) signaling was initiated by nephrin clustering and phosphorylation. The distribution pattern of SD proteins and the nephrin signalsome was compared with/without INF2 knockdown by siRNA sequences targeting INF2. The translocation of SD proteins and signalsome components were observed by immunofluorescent stain.

Results: In cultured podocytes, nephrin and podocin localized along the ruffles of the cells, at the tip of actin filaments. The phosphorylated nephrin recruited adaptor protein NCK/actin serving protein mDia to the signalsome, and induced local actin tail formation. With INF2 knockdown, the de novo expressed nephrin or podocin was stuck in the cytoplasmic endosomes rather than being transported to the ruffles of the cells. The recruitment of mDia and the formation of actin tails were disrupted.

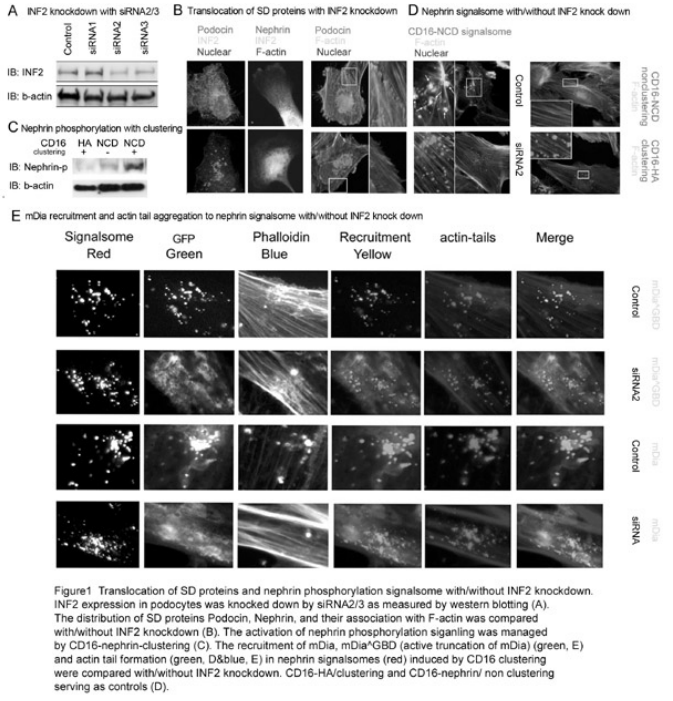

Figure1 Translocation of SD proteins and nephrin phosphorylation signalsome with/without INF2 knockdown. INF2 expression in podocytes was knocked down by siRNA2/3 as measured by western blotting (A). The distribution of SD proteins Podocin, Nephrin, and their association with F-actin was compared with/without INF2 knockdown (B). The activation of nephrin phosphorylation siganling was managed by CD16-nephrin-clustering (C). The recruitment of mDia, mDia^GBD (active truncation of mDia) (green, E) and actin tail formation (green, D&blue, E) in nephrin signalsomes (red) induced by CD16 clustering were compared with/without INF2 knockdown. CD16-HA/clustering and CD16-nephrin/ non clustering serving as controls (D).

Conclusions: INF2 is an essential protein in maintaining the actin cytoskeleton-dependent morphology of podocyte, distribution of SD proteins, and the response of actin-regulating signal transduction initiated by nephrin phosphorylation. The decomposition of foot process integrity may be associated with the deficiency of INF2 in the SD complex-cytoskeleton pathway.

FR-PO1314

Role of Calcium-Indepent Phospholipase A_2-γ in Complement-Mediated Glomerular Epithelial Cell Injury Hanan Elimam, Tomoko Takano, Joan Papillon, Andrey V. Cybulsky. *Nephrology, McGill University, Montreal, QC, Canada.*

Background: In experimental membranous nephropathy, complement C5b-9-induces glomerular epithelial cell (GEC) injury and proteinuria. The effects of C5b-9 are mediated via signaling pathways, including calcium-independent phospholipase A_2γ (iPLA$_2$γ), protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs), that is extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. The iPLA$_2$γ pathway is cytoprotective. The present study addresses the mechanisms of iPLA$_2$γ activation.

Methods: Cultured GEC were stably transfected with iPLA$_2$γ cDNA. GEC were incubated with antibody and normal serum (to assemble complement C5b-9) or heat-inactivated serum (control). To study MAPKs in iPLA$_2$γ activation, COS-1 cells were transfected with iPLA$_2$γ and cyclooxygenase-1 (COX-1). PLA$_2$γ activity was monitored by quantifying prostaglandin E_2 (PGE_2) production. Mutations in two putative ERK phosphorylation sites in iPLA$_2$γ (S168, S271A) were created by PCR-based mutagenesis.

Results: Complement-mediated production of PGE2 was amplified in GEC that overexpress iPLA2γ, compared with control cells, and the effect of iPLA2was blocked by the iPLA2γ inhibitor, bromoenol lactone (BEL). In GEC that overexpress iPLA2γ, complement-mediated PGE2 production was reduced significantly by depletion of PKC, as well as by chemical inhibitors of MAPK/ERK kinase-1 (MEK1) and p38, but not JNK. PKC activation did not, however, stimulate PGE2. In COS-1 cells that overexpress iPLA2γ and COX-1, PGE2 production and ERK activation were induced by expression of constitutively active MEK1 or by stimulation with epidermal growth factor (EGF)+ionomycin, and the effect of EGF+ionomycin was inhibited by BEL. Activation of S168A, S271A and S168A/S271A iPLA2γ mutants was comparable to the wild type enzyme.

Conclusions: Complement-mediated activation of iPLA$_2$γ is mediated via PKC and ERK. PKC is necessary, but is insufficient for iPLA$_2$γ activation. ERK is necessary and sufficient, but appears not to act directly on iPLA$_2$γ. Defining the mechanisms by which complement contributes to GEC injury and proteinuria will provide opportunities for development of novel therapeutic approaches.

Funding: Government Support - Non-U.S.

FR-PO1315

Serum Albumin-Induced Podocyte Cox-2 Expression Is Inhibited by Glucocorticoids and MAPK Inhibitors Shipra Agrawal,[1] Adam J. Guess,[1] Ruma Pengal,[1] Rainer Benndorf,[1,2] William E. Smoyer.[1,2] *[1]Center for Clinical and Translational Research, Research Institute at Nationwide Children's Hospital, Columbus, OH; [2]Department of Pediatrics, Ohio State University, Columbus, OH.*

Background: Proteinuria is a hallmark of glomerular disease, the third leading cause of ESRD in the US. Proteinuria is also a known risk factor for progressive glomerular disease, and results in increased podocyte exposure to serum albumin (SA). Additionally, oxidized forms of SA have been found in patients with glomerular disease. Based on this, we analyzed the ability of SA and oxidized SA to induce Cox-2 in podocytes, and whether glucocorticoids (GC), thiazolidinediones (TZDs) or selected MAPK inhibitors could block this induction.

Methods: Cultured murine podocytes were treated with SA and oxidized SA at physiological concentrations and cell viability and Cox-2 expression were quantified. In addition, since GC, TZDs and MAPK inhibitors have all been reported to reduce both proteinuria and direct podocyte injury, we also analyzed their ability to regulate SA-induced Cox-2 expression.

Results: SA increased Cox-2 mRNA and protein expression in podocytes in a dose-dependent manner. Moreover, SA-induced Cox-2 expression was reduced by inhibitors of ERK1/2, p38 MAPK, and MK2, but not SAPK/JNK, although all of these were activated by SA exposure. In addition, GC, but not TZDs, inhibited SA-induced Cox-2 expression. Notably, treatment with oxidized SA led to a greater induction of Cox-2 protein than un-treated SA. Lastly, increasing doses of SA led to progressive reductions in podocyte viability.

Conclusions: Physiologic concentrations of SA induce podocyte Cox-2 expression, which can be inhibited by both GC and selected MAP inhibitors. Since GC and MAPK inhibitors are now known to directly protect podocytes against injury, our results suggest that Cox-2 may be a molecular mediator of SA-induced podocyte injury.

Funding: NIDDK Support

FR-PO1316

15-Deoxy-$D^{12,14}$-Prostaglandin J_2 Inhibits the Expression of Chemokines by Blocking NF-κB Nuclear Translocation Via PPARg-Independent Mechanism in Lipopolysaccharide-Stimulated Renal Tubular Epithelial Cells Ying Lu, Qiao Zhou, Fang Zhong, Xu Hao, Cong Li, Weiming Wang, Nan Chen. *Nephrology, Ruijin Hospital, Shanghai, China.*

Background: 15d-PGJ_2 is a high-affinity ligand for peroxisome proliferator-activated receptorg (PPARg), and has been suggested to exert anti-inflammatory effects in vivo. The aim of the study was to investigate the effect and mechanism of 15d-PGJ_2 on the expression of chemokines in Lipopolysaccharide–stimulated renal tubular epithelial cells.

Methods: Confluent renal tubular epithelial cells (HK-2 cells) were separated into four groups: control group, LPS-stimulated group, 15d-PGJ_2 alone group and 15d-PGJ_2-pre-treated LPS group. Chemokines including interleukin-8 (IL-8) and chemoattractant protein-1 (MCP-1) were determined by Real-time PCR and ELISA. The location of nuclear factor-κB (NF-κB) was detected by immunofluorescence analysis. The phosphorylated IκBα (p-IκBα) in cytoplasm and NF-κB in nucleus were analyzed by western blot. To evaluate the inhibitory effects of 15d-PGJ_2 were PPARg-dependent or independent, PPARg was knocked down by RNA interference (RNAi) .

Results: The results showed that, compared with the control group, IL-8 and MCP-1 were significantly increased at both transcription and post-transcription level in LPS-simulated group. Accordingly, p-IκBα in cytoplasm as well as NF-κB in nucleus was significantly increased in LPS-stimulated HK-2 cells compared with the control group. Pre-treatment HK-2 cells with 15d-PGJ2 abolished LPS-induced IL-8 and MCP-1 overproduction. Knockdown of PPARg by RNAi in HK-2 cells shows that 15d-PGJ2 also inhibited LPS-induced IL-8 and MCP-1 overexpression in these cells. And nuclear translocation of p65 and phosphorylation of IκBα induced by LPS were restored by 15d-PGJ2 in both HK-2 cells and PPARg-knockdown HK-2 cells.

Conclusions: In the study, we demonstrated that 15d-PGJ_2 could inhibit the expression of chemokines by blocking NF-κB translocation into nucleus via a PPARg-independent mechanism in LPS–stimulated renal tubular epithelial cells.

FR-PO1317

Regulation of Fatty Acid Oxidation Profoundly Affects Palmitic Acid Induced Podocyte Cell Death Kapil Dev Kampe,[1] Jonas Sieber,[1] Peter H. Mundel,[2] Andreas Werner Jehle.[1,3] *[1]Department of Biomedicine, Molecular Nephrology, University Hospital, Basel, Switzerland; [2]Division of Nephrology, Massachusetts General Hospital, Boston; [3]Department of Internal Medicine, Kantonsspital Bruderholz, University of Basel, Basel, Switzerland.*

Background: Diabetes mellitus type 2 is associated with altered lipid metabolism leading to elevated levels of free fatty acids (FFAs). Recently, we have reported the antagonistic effects of palmitic acid versus monounsaturated FFAs (MUFAs) on podocyte survival (Am J Physiol Renal Physiol. 2010 Oct;299(4):F821-9). The objectives of this study were to elucidate whether inhibition or stimulation of fatty acid oxidation (FAO) may affect palmitic acid induced podocyte cell death.

Methods: Conditionally immortalized murine podocytes were differentiated for at least 11 days and palmitic acid or oleic acid were complexed to BSA. Apoptosis and necrosis

were quantified by annexin V and propidium iodide labeling. We used etomoxir, a carnitine-palmitoyltransferase 1 (CPT1) inhibitor, to reduce FAO. Contrariwise, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) was used to stimulate FAO. Levels of pAMPK, pACC, AMPK and ACC were measured by immunoblotting. We used tritiated palmitic acid and measured tritiated water to assess oxidation of palmitic acid.

Results: Etomoxir (10-200μM) dose-dependently increases palmitic acid induced cell death up to 200%. Contrariwise, we show that AICAR significantly decreases palmitic acid induced apoptosis and necrosis by 49.5±1.5%, (p=0.004), and 55.7±4.0% (p=0.01) respectively. AICAR upregulated the phosphorylation of both AMPK and ACC. Functionally, AICAR significantly increases oxidation of palmitic acid by 124.0±4.9% (p<0.001) as assessed by the metabolism of tritiated palmitic acid. Similarly, oleic acid increases oxidation of palmitic acid by 51.3±9.6% (p=0.001).

Conclusions: Our results unveil that the regulation of FAO via the AMPK-ACC-CPT1 pathway profoundly affects palmitic acid induced podocyte cell death. Although oleic acid significantly increases FAO of palmitic acid this effect can only partially explain the protective effect of MUFAs for palmitic acid induced podocyte cell death.

FR-PO1318

DNase I Induces Other Endonucleases, DNA Damage and Apoptosis Pathways in Kidney Tubular Epithelial Cells by Its DNA Cleavage Activity Tariq Fahmi,[1] Xiaoying Wang,[1] Eugene Apostolov,[1] Alexei G. Basnakian.[1,2] *[1]University of Arkansas for Medical Sciences, Little Rock, AR; [2]Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: Every mammalian cell has cytotoxic endonucleases which degrade host DNA prior and after cell death. The endonucleases have similar mode of action and often simultaneously induced to cleave DNA after cell injury. The mechanism for the simultaneous induction of endonucleases is unknown. Induction of DNase I, the most active kidney endonuclease, has been observed in kidney ischemia-reperfusion (IR), and its inactivation was protective against toxic or ischemic kidney injuries suggesting that DNase I is responsible for kidney cell death. We hypothesized that DNase I may be a universal regulator of other endonucleases, as well as some apoptosis and DNA damage pathways.

Methods: To test this, rat kidney tubular epithelial NRK52E cells were transfected with rat DNase I gene or its inactive mutant in pECFP expression vector for 6, 12 and 24 hrs, while control cells were transfected with "empty" pECFP. DNase I-CFP expression was monitored by fluorescence. RNA was analyzed for all known cytotoxic endonucleases and ~200 markers of DNA damage and apoptosis pathways using real-time RT-PCR.

Results: The measurements showed certain endonucleases, DNA damage and apoptosis pathways were induced by active DNase I, including: (1) several cytotoxic endonucleases except DNase II, with the maximal effect for DNase gamma and DNase X; (2) beta-actin, the only known endogenous inhibitor of DNase I; (3) Fas, previously described being transcriptionally regulated by DNase I, and its inhibitor FAIM; (4) caspases 3, 7, 8 and 12; (5) DNA polymerase beta (indicative of single-stranded DNA breaks); (6) p53 and Tp53bp2 (likely a result of DNA damage); and (7) Apaf 1 (likely a result of p53 activation). Immunohistochemical analysis of rat kidneys subjected to IR injury showed that DNase I and some of these markers are induced at 16 hrs after ischemia.

Conclusions: These results suggest for the first time that DNase I activity may induce other endonucleases and several key regulators of DNA damage and apoptosis pathways during kidney injury.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1319

Ethanol Causes Oxidative Stress, Alters Actin Cytoskeleton and Decreases Expression of Cytochrome P450 Isoforms in Murine Podocytes Ellen T. McCarthy,[1] Jianping Zhou,[2] Ryan G. Eckert,[1] Shuhua Wang,[1] Tarak Srivastava,[3] Ram Sharma,[2] Mukut Sharma.[2] *[1]University of Kansas Medical Center, Kansas City, KS; [2]Kansas City VA Medical Center, Kansas City, MO; [3]Children's Mercy Hospital, Kansas City, MO.*

Background: The contribution of ethanol (EtOH) to progression of chronic kidney disease is controversial, although EtOH is associated with hypertension. Effects of EtOH on podocytes are unknown. We have shown that EtOH alters podocyte expression of cytochrome P450 (CYP450) isoforms involved in 20-hydroxyeicosatetraenoic acid (20-HETE) synthesis and that 20-HETE is protective in several models. We hypothesized that EtOH causes oxidative stress and alters podocyte structure and that 20-HETE provides protection from injury.

Methods: Immortalized podocytes were incubated with EtOH (1 to 20 μL/mL) for 1 to 24 hr. These concentrations correspond to blood alcohol levels seen in humans. We examined podocyte superoxide generation using the fluorescent probe hydroethidine (HE), visualized podocyte actin cytoskeleton using confocal microscopy and measured expression of CYP4a12a and CYP4a12b using quantitative RT-PCR. 20-HETE (100 nM) was included in the incubation medium in some groups.

Results: Incubation with EtOH increased superoxide levels, indicated by increased HE fluorescence. Furthermore, EtOH caused disruption of actin filaments. These effects were dose- and time-dependent. Inclusion of 20-HETE abrogated the increase in superoxide and prevented actin cytoskeletal disruption. EtOH (20 μl/ml) decreased expression of CYP4a12a and CYP4a12b at 24 hr (P<0.02 and P<0.001 vs control, respectively) while lower concentrations increased the expression of these isoforms.

Conclusions: EtOH in meaningful concentrations increased oxidative stress and altered actin cytoskeleton in podocytes, effects reversed by 20-HETE. We posit that excessive EtOH consumption may alter glomerular structure and function by mechanisms that include increased oxidative stress and decreased synthesis of protective eicosanoids. Such changes may exacerbate underlying glomerular pathological conditions and contribute to progressive renal injury. In contrast, moderate EtOH intake may provide protection by increasing 20-HETE.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1320

Role of Podocytes´ Collagen Receptors in Renal Fibrosis of COL4A3 Knockout Mice Diana Rubel, Rainer Girgert, Gerhard A. Mueller, Oliver Gross. *Dep. of Nephrology and Rheumatology, University Medicine Goettingen, Goettingen, Germany.*

Background: Podocytes sense the composition of glomerular basement membrane by collagen receptors such as DDR1 (discoidin domain receptor 1) and ITGA2 (Integrin α2). Therefore, collagen receptors might play an important role in maintenance normal composition of the glomerular basement membrane and in type IV collagen diseases such as Alport syndrome. In the present study, we compared renal signaling of DDR1/ITGA2 and COL4A3 (Alport) knockout mice with wildtype controls.

Methods: Phosphorylation of Akt/PKB and STAT1 was analyzed by Western Blotting. Quantification of protein bands was carried out by ImageJ and the ratio of phosphorylated to unphosphorylated protein was calculated. Matrix accumulation and fibrosis was analyzed by immuno-histochemistry.

Results: Kidney lysates of wildtype mice showed an age-dependent (50d, 100d, 150d) increase of phosphorylated Akt/PKB. Even the lowest level of activated Akt in 50 d wildtype mice was higher than in COL4A3 knockouts of any age. Mice without ITGA2- and DDR1-expression exhibited the same expression profile, but have a higher level of phosphorylated Akt (paralleled by less matrix accumulation) compared to COL4A3 knockout mice. Phosphorylated STAT1 also increases with age in wildtype mice. In contrast to Akt/PKB, activation of STAT1 is at its maximum at 4.5 weeks of age and slightly decreases until week 9 in COL4A3 knockout mice. The fraction of phosphorylated STAT1 in Alport mice is lower than in wildtype mice.

Conclusions: Akt1 is involved in cellular survival pathways. Therefore, decreased activation of STAT1 by the DDR1 pathway in mutant mice indicates a role of DDR1 in podocyte injury during renal fibrosis. A lower activation of ITGA2 pathway in COL4A3 knockout mice results in decreased levels of phosphorylated STAT1 causing less cdc42 activity and decreased actin polymerization. Further studies will focus on the role of actin polymerization in the various genotypes, downstream signaling pathways and the cross-talk of ITGA2/DDR1-signaling in podocytes.

FR-PO1321

Expression of NIPP1 in AGE-BSA Treated Podocytes Marita Liebisch, Gunter B. Wolf. *Internal Medicine III, University of Jena, Jena, Germany.*

Background: Patients with diabetes mellitus have increased level of advanced glycation end products (AGEs) accumulating in different tissues including the kidney. The formation of AGEs causes in molecular dysfunctions of molecules and is finally followed by organ damage leading to podocyte damage, a typical feature of diabetic nephropathy.

The nuclear inhibitor of PP1 (NIPP1) inhibits the phosphatase activity of PP1, which is involved in the regulation of numerous cellular processes such as intracellular transport or metabolism. Moreover, NIPP1 is important in spliceosome assembly and pre-mRNA splicing. By performing differential display analysis we have recently found out that NIPP1 gene expression was differentially regulated in Co-BSA and AGE-BSA treated differentiated podocytes. The aim of this study was to address the NIPP1 expression in podocytes.

Methods: The NIPP1 mRNA expression in cultured differentiated podocytes was analyzed by Real Time PCR, the protein concentration in corresponding samples by Western Blot analyses and immunofluorescence staining. In addition, it was analyzed whether the reduced NIPP1 expression correlates with changes in cell cycle or cell proliferation.

Results: Real Time PCR and Western Blot analyses exhibited a significantly reduced NIPP1 expression in AGE-BSA treated podocytes compared with Co-BSA treated cells. Immunofluorescence showed a NIPP1 translocation from the cytoplasma to the nucleus compared to the control cells. NIPP1 siRNA transfection of podocytes reduced NIPP1 expression in podocytes and induced p27Kip1 a cell-cycle inhibitor, previously shown to be induced by AGE-Bsa in podocytes. Moreover, Podocytes revealed a lesser proliferation rate when NIPP1 expression is reduced either by AGE-BSA or NIPP1 siRNA.

Conclusions: AGE-BSA treated podocytes demonstrated a reduced NIPP1 expression compared to control cells. This decreased NIPP1 expression may explain several pathophysiological alterations of podocytes in diabetic nephropathy such as hypertrophy, cell-cycle arrest, and apoptosis.

Funding: Government Support - Non-U.S.

FR-PO1322

Role of Connective Tissue Growth Factor in High Glucose-Induced Epithelial-Mesenchymal Transition of Podocytes Hou-Yong Dai, Rining Tang, Min Zheng, Jie Ni, Linli Lv, Kun Ling Ma, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: Our previous studies demonstrated that connective tissue growth factor (CTGF) plays an important role in the early kidney hypertrophy and fibrosis of diabetic nephropathy, while the potential mechanism is still unclear. This study was to investigate the role of CTGF in high glucose-induced epithelial–mesenchymal transition in podocytes.

Methods: The differentiated podocytes cultured under different conditions for 24 hours at 37°C were divided into four groups as follows: Control (5 mM GS), 5 mM

GS supplemented with 50 ng/ml CTGF, high glucose (30 mM GS) and high glucose supplemented with CTGF(50 ng/ml). The morphology of cultured podocytes was performed by phase contrast microscopy. To study the relative marker of epithelial–mesenchymal transition (EMT), the mRNA and protein expression were analyzed by real-time RT-PCR and western blotting, respectively. In addition, the effect of inhibition CTGF with anti-CTGF antibody on high glucose-induced EMT of podocytes was investigated.

Results: High glucose induced phenotypic transition of podocytes from an arborized morphology to a cobblestone morphology. After addition of CTGF to high glucose, podocytes developed a feature of spindle. In high glucose conditions, podocytes underwent EMT, demonstrating that the epithelial marker of nephrin was decreased, in contrast, the mesenchymal marker of desmin was increased. Of note, exogenous CTGF in synergy with high glucose promoted EMT, moreover, high glucose-induced EMT of podocytes was prevented by CTGF inhibition with anti-CTGF antibody.

Conclusions: This study demonstrated that CTGF was involved in high glucose-induced EMT of podocytes, which suggested inhibition of CTGF might be a potential target for prevention of diabetic nephropathy.

Funding: Government Support - Non-U.S.

FR-PO1323

Connective Tissue Growth Factor Binds to Epidermal Growth Factor Receptor To Modulate Renal Inflammation Sandra Rayego-Mateos,[1] Raquel Rodrigues-Diez,[1] Raúl R. Rodrigues Díez,[1] Carolina Lavoz,[1] Janos Pato,[2] Gyorgy Keri,[2] Alberto Ortiz,[3] Jesus Egido,[3] Marta Ruiz-Ortega.[1] *[1]Nephrology, Universidad Autónoma, Madrid, Spain; [2]Vichem Chemie Ltd., Budapest, Hungary; [3]ISCFJD-UAM, Madrid, Spain.*

Background: Connective tissue growth factor (CTGF) has suggested as a risk marker for chronic kidney diseases. CTGF inhibition ameliorates experimental renal damage. Some authors hypothesize that CTGF is an antifibrotic therapeutic target, but is important first to understand its renal effects and molecular effectors. In vivo, CTGF induces renal recruitment of inflammatory cells via upregulation of cytokines and chemokines. There is no described a known specific CTGF receptor. Our aim was to investigate whether CTGF interact with epidermal growth factor receptor (EGFR) in the kidney, evaluating the signalling pathways and cellular responses.

Methods: Systemic administration of CTGF (2.5ng/g BW) to mice was done, a group was also treated with the EGFR kinase inhibitor Erlotinib (40mg/Kg). In vitro studies were done in tubular epithelial cells.

Results: In vivo, CTGF caused EGFR phosphorylation in the kidney, mainly located in tubuloepithelial cells. Treatment with Erlotinib inhibited CTGF-induced renal changes observed at 24 hours, including ERK activation (a downstream EGFR signalling), up-regulation of proinflammatory factors and interstitial inflammatory cell infiltration. In cultured tubular epithelial cells, CTGF increased EGFR phosphorylation. This process was not due to EGFR transactivation, as shown by the lack of effect of matrix metalloproteases inhibitors (GM6001 and TAPI-2). Direct binding was demonstrated by cross-linking and immunoprecipitation studies, that leads to CTGF-EGFR heterodimers formation. EGFR activation is regulated by integrins, as shown by different approaches: RGD peptides, neutralizing antibody and RNA silencer of αV and β3 integrin. Moreover, erlotinib inhibited CTGF-induced proinflammatory mediators and ERK activation in tubular cells.

Conclusions: Our results suggest that CTGF directly binds to EGFR and activates its signalling pathway, leading to modulation of downstream mechanisms, such as ERK activation, and cellular responses, including renal inflammation.

Funding: Government Support - Non-U.S.

FR-PO1324

In Vivo Manipulation of Glutathione S-Transferase A4 Prevents 4-hydroxynonenal-Induced Renal Tubular Cell Damage Anlin Liang, Yun Wang, Lauren Elizabeth Woodard, Matthew H. Wilson, Jie Du, William E. Mitch, Jizhong Cheng. *Nephrology Division, Baylor College of Medicine, Houston, TX.*

Background: Lipid peroxidation yields toxic products that can damage cell membranes, including aldehyde 4-hydroxynonenal (4-HNE). Since glutathione S-transferase, GSTA4, can eliminate 4-HNE, we studied a mouse model of unilateral ureteral obstruction (UUO) to determine if changing GSTA4 expression influences UUUO-induced fibrosis.

Methods: Hydrodynamic injection or an inducible, piggyBac transposon gene delivery system were used to study if GSTA4 protects against tubule cell damage and fibrosis in mice with UUO or after release of UUO (RUUO). WT and GSTA4 KO mice were studied.

Results: At 3 days of UUO, 4-HNE and its protein adducts increased while GSTA4 decreased. UUO in GSTA4 KO mice increased the interstitial expression of fibroblast markers (SMA-α, FSP-1 and TGF-β) vs results in WT mice. Tubular cell damage was also more severe vs results in WT mice. To raise GSTA4 activity, we hydrodynamically injected GSTA4 into the renal vein. This reduced 4-HNE levels and the interstitial fibrosis studied by UUO. To determine if long-term activation of GSTA4 would restore the tubule architectures damaged by UUO, we studied mice bearing an inducible GSTA4 expression accomplished using a PiggyBac transponson system. In these mice GSTA4 was expressed in response to doxycyline. After 3 days of UUO, we released UUO (i.e., RUUO) and 3 weeks later, we found GSTA4 was overexpressed. This decreased SMA-α and collagen I staining in the interstitium.

Om GSTA4 KO mice, UUO increased autophagy in tubule cells. In NRK52E cells, GSTA4 overexpression blocked 4-HNE-induced expression of LC3-II, consistent with reduced autophagy. GSTA4 expression also inhibited 4-HNE-mediated tubule cell damage with downregulation of Occludin and E-cadherin. The mechanism involved an UUO-induced increase in the Snail transcription factor plus GSK-3β phosphorylation. These responses were blocked by GSTA4 overexpression.

Conclusions: Thus, the UUO-induced fibrosis and tubule cell damage involves 4-HNE and is blocked by GSTA4.

Funding: NIDDK Support, Private Foundation Support

FR-PO1325

Cdc42-Interacting-Protein-4 Promotes the Translocation of β-catenin to Nucleus in TGF-β1- Induced Renal Tubular Epithelial-to-Mesenchymal Transdifferentiation Chuou Xu, Qiaodan Zhou, Rui Zeng, Lily Liu, Gang Xu. *Division of Nephrology, Department of Internal Medicine, Tongji Hospital Medical College, HUST, Wuhan, Hubei, China.*

Background: Epithelial-to-mesenchymal transdifferentiation (EMT) in kidney is the transition of tubular epithelial cells into myofibroblasts, it is considered as one of the most important events underlying chronic renal diseases. During the process of EMT, epithelial cells lose the expression of E-cadherin. Snail family members are E-cadherin transcriptional repressors which have been implicated in promoting EMT. Evidence demonstrates that translocation and accumulation of β-catenin in the nucleus lead to enhanced binding to members of the T cell factor (TCF) family and lymphoid enhancer factor (LEF) family, in turn, β-catenin/TCF/LEF complexes activate target genes such as Snail family. Thus, it is essential to investigate what effects translocation of β-catenin to nucleus. Cdc42-interacting protein 4 (CIP4), a Cdc42 effector protein involved in cytoskeletal organization and actin polymerization, is suggested to associated with β-catenin and paly a role in EMT. We investigated this potential role of CIP4 in TGF-β1-induced renal tubular EMT.

Methods: Expression,interaction and colocalization of proteins were detedced by western blotting,immunoprecipitation and confocal iamging respectively.

Results: Expression of CIP4 was upregulated in rat proximal tubule cell line (NRK52E) stimulated by TGF-β1 accompanied by reduced expression of E-cadherin and increased expression of mesenchymal marker α-SMA. The interaction between CIP4 and β-catenin was detected by immunoprecipitation, CIP4 colocalized with β-catenin in cell membrane/cell nucleus before/after TGF-β1 stimulation by confocal images. In normal condition, overexpression of CIP4 promoted translocation of β-catenin to nucleus accompanied by the reduced expression of E-cadherin. Furthermore, to knockdown CIP4 by using siRNA, we found that β-catenin translocated to nucleus was decreased, expression of E-cadherin was reversed.

Conclusions: In conclusion, CIP4 promotes translocation and accumulation of β-catenin to nucleus in TGF-β1-induced renal tubular EMT ,which is accompanied by reduced expression of E-cadherin.

FR-PO1326

Mechanisms of Proteinuria Induced by Rho A GTPases Liming Wang,[1] Matthew Jay Ellis,[1] David Howell,[2] Robert F. Spurney.[1] *[1]Department of Medicine, Duke University and Durham VA Medical Centers, Durham, NC; [2]Department of Pathology, Duke University and Durham VA Medical Centers, Durham, NC.*

Background: Podocytes play a pivotal role in maintaining the integrity of the glomerular filtration barrier. A growing literature suggests that this function is regulated by small GTPases belonging to the Rho GTPase family.

Methods: A constitutively active Rho A (V14Rho) or a dominant negative Rho A (N19Rho) were introduced into cultured podocytes using protein transduction by tagging the proteins with the TAT human immunodeficiency virus (HIV) protein sequence [V14Rho(+) or N19Rho(+)]. Cell impermeable proteins lacking the TAT sequence were used as controls [V14Rho(-) or N19Rho(-)]. To investigate the role of Rho A in podocyte biology in vivo, we created transgenic (TG) mice that expressed either V14Rho or N19Rho specifically in podocytes using a doxycycline inducible strategy.

Results: V14Rho(+) enhanced both stress fiber formation and podocyte apoptosis, and the apoptotic effect was blocked by the Rho kinase inhibitor Y27632. In contrast, N19Rho(+) had no significant effect on podocyte apoptosis but decreased stress fiber formation and promoted the formation of monomeric actin (G actin). In TG mice, induction of either V14Rho or N19Rho caused a significant increase in albuminuria and foot process effacement. The mechanisms of these adverse effects, however, appeared to be different. Induction of V14Rho caused a reduction in expression of nephrin at both the mRNA and protein levels without affecting expression of the actin-associated, cytoskeletal protein synaptopodin. In contrast, induction of N19Rho had no effect on nephrin mRNA or protein levels but decreased synaptopodin expression.

Conclusions: These data suggest that basal Rho A activity has beneficial effects in podocytes, perhaps by stabilizing the glomerular architecture. In contrast, high levels of Rho A activity also promote podocyte injury by mechanisms that appear to be different from the mechanisms that alter glomerular filtration barrier function following Rho A inhibition.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1327

Complete Differentiation of Rab3A-KO Podocytes Induced by GABAA-R Agonists Silvia Armelloni, Min Li, Laura Giardino, Alessandro Corbelli, Masami Ikehata, Deborah Mattinzoli, Piergiorgio Messa, Maria Pia Rastaldi. *Renal Research Laboratory, Fondazione IRCCS Policlinico & Fondazione D'Amico, Milan, Italy.*

Background: We have previously shown that Rab3A-KO mice have spontaneous macroalbuminuria with podocyte cytoskeletal changes and decreased expression of specific molecules. Aim of this study was the identification of intracellular pathways possibly linking Rab3A absence to podocyte damage.

Methods: RealTime-RTPCR-arrays were used to quantify differential gene expression. Cell proliferation was evaluated by FACS analysis of BrdU incorporation and by "FUCCI assay", based on cell transfection of Cdt1 and geminin, fused to fluorescent markers. Activation of the MAPKinase pathway, and expression of GABA Receptors and cytoskeletal molecules were investigated by Western Blot and immunostaining.

Results: Rab3A-KO podocytes showed increased mRNA expression of Ntrk2, Fgf2, Tro, NpY, and NpYr1 and a decreased expression of Nrg1 than WT cells. All overexpressed molecules are involved in proliferation and maturation of neuronal precursors and act by activating the MAPKinase pathway. Nrg1 instead regulates neuronal differentiation and synapse development.

FACS analysis showed increased BrdU incorporation in KO podocytes and the FUCCI method detected more KO than WT cells in the G2 phase of the cell cycle.

KO cells also displayed higher p-MAPK/MAPK ratio than WT podocytes.

Further, mRNA and protein of GABAA-Receptors (GABAA-R) were higher in KO than WT podocytes. Incubation of KO-cells with GABAA-R antagonists induced further p-MAPK increase, while incubation with a GABAA-R agonist, not with a GABAB-R agonist, caused p-MAPK reduction, increased the expression of the cytoskeletal molecules Arg and synaptopodin, and improved alpha-actinin-4 distribution.

Conclusions: Our results show that Rab3A null podocytes are less differentiated and, as it occurs in neuronal cells, a series of early genes induce proliferation through activation of the ERK/MAPK pathway.

Our data also show the potential involvement of GABAA-Receptors in these processes; ionotropic GABAA-R activity decreases MAPK activity and can be modulated by specific agonists, which improve cell differentiation by cytoskeletal regulation.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1328

Gα12 Activation Leads to Upregulation of IL-8 (CXCL-8) Ilene Boucher,[1,2,3] Wanfeng Yu,[2,3] Tianqing Kong,[1,3] Bradley M. Denker.[1,2,3] *[1]Medicine, Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Medicine, Renal DIvision, Beth Israel Deaconess Hospital, Boston, MA; [3]Harvard University Medical School, Boston, MA.*

Background: Injury activates numerous signaling pathways, several of which can stimulate GPCRs. Angiotensin II, LPA, and thrombin receptors couple to Gα12 (Gα12/Gα13 is one of four Gα families) and are activated with injury. Gα12 can be activated by reactive oxygen species (ROS) and regulates cell junctions, migration, proliferation and apoptosis. Preliminary in vivo studies suggest an important role for Gα12 in the renal response to ischemia/reperfusion injury (IRI), but the mechanisms are unknown. Inflammation mediated by secreted cytokines such as IL-6, IL-8 are important components of the injury response, but a role for Gα12 regulating inflammation with injury has not been identified.

Methods: To determine if Gα12 regulates inflammatory cytokines, previously characterized tet-off inducible (-dox) MDCK cells expressing constitutively active Gα12 (Q229L; QLα12) were analyzed in triplicate using Affymetrix microarrays and compared with non-induced cells (+dox). Cell supernatants were analyzed in triplicate for IL-8 secretion by ELISA.

Results: Relative message levels for the IL-6 and IL-8 showed no significant change (defined as 2-fold with $p<0.05$), but IL-8 was significantly upregulated (34 fold). We confirmed increased IL-8 secretion by ELISA. There was 1020± 155 pg/mL of baseline IL-8 secreted in the controls (+dox) and with QLα12 expression (-dox) the amount increased 5-fold to 5820±167pg/mL.

Conclusions: Preliminary studies with IRI in Gα12-/- mice reveal a less severe injury phenotype and studies are in progress to quantify the inflammatory response and determine if the putative IL-8 orthologue (CXCL-15) is inhibited with injury. Taken together, we hypothesize that Gα12-stimulated cytokines may be a novel therapeutic target to prevent inflammation in acute kidney injury.

Funding: NIDDK Support, Other NIH Support - NIH GMS

FR-PO1329

The Role of Rho Kinase in the Regulation of K-Cadherin Expression in Tubule Epithelial Cells Nileshkumar Shah,[1] Iain Macphee,[2] Mysore Keshavmurthy Phanish,[1] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]St George's University of London, London, United Kingdom.*

Background: Regulation of cadherin expression if of critical importance for maintaining cell contact and epithelial integrity but it can also regulate various signaling cascades important in determining cell proliferation and phenotype. In the kidney TGFβ1-mediated reduction in E-cadherin (Cdh1) is associated with epithelial mesenchymal transition. However, our group demonstrated that unlike rodent models K-cadherin (Cdh6) is expressed in human proximal tubule, not Cdh1, and that diabetic nephropathy is associated with its loss. Hence, we have investigates the regulation of Cdh6 by TGFβ1 in human proximal tubule epithelial cells (PTEC).

Methods: Human primary PTEC were grown in supplemented media; 0.25% serum: experiments were carried out at passage 3. Cadherin expression was monitored by Western Blotting and qPCR. Cells were grown to 75% confluence and treated with TGFβ1 1.25-5.0 ng/ml for 1-48 h.

Results: Previously our group and others have demonstrated that TGFβ1 causes a rapid and sustained reduction in Cdh1 expression at concentrations as low as 1 ng/ml. In primary human PTEC TGFβ1 only caused a significant reduction in Cdh6 mRNA at 5 ng/ml; this reduction was rapid but transient returning to control levels by 24h. TGFβ1-induced reduction in Cdh6 protein also required 5ng/ml but was only statistically significant at 48h. We subsequently investigated the signaling cascades underlying this reduction in protein. Previously we have published that complete abrogation of TGFβ1-induced reduction in Cdh1 required knock-down of Smads2 & 3; however the k/d of Smad2, Smad3 or both, by RNAi did not change the affect of TGFβ1 on Cdh6. We also investigated the affect of inhibiting a range of kinases, including Alk5, Erk1/2, p38, Jnk, PI3-kinase and Rho kinase (Rock). Only inhibition of the TGFβ type1 receptor, Alk5 and Rock limited the effect of TGFβ on Cdh6 expression.

Conclusions: We conclude that the regulation of Cdh6 (K-cadherin), the native cadherin of the human proximal tubule, is more resistant to TGFβ1 than Cdh1 (E-cadherin) and occurs through distinct mechanisms.

FR-PO1330

Endothelin(ET)-1, Via ET_AR, Activates β-Arrestin-Mediated Signaling Pathways and Promotes Podocyte Migration: Implications for Proliferative Lesions in Chronic Kidney Disease Simona Buelli,[1] Elena Gagliardini,[1] Laura Rosano,[2] Anna Pezzotta,[1] Anna Bagnato,[2] Giuseppe Remuzzi,[1,3] Ariela Benigni.[1] *[1]Mario Negri Institute, Bergamo, Italy; [2]Regina Elena National Cancer Institute, Roma, Italy; [3]Ospedali Riuniti, Bergamo, Italy.*

Background: Podocytes acquire a migratory phenotype and form bridges named synechiae between the glomerular tuft and the Bowman's capsule, contributing to hyperplastic lesion formation in crescentic glomerulonephritis and podocytopathies (JASN 20:2593,2009). The stimulus enabling podocyte migration is unknown. Previous evidence showed a role of ET-1 in podocyte cytoskeletal rearrangement in vitro (Am J Pathol 169:1965,2006) and podocyte loss in experimental diabetes (Am J Physiol Renal 297:F1448,2009). Cancer cell invasiveness is driven by ET-1-mediated recruitment of β-arrestin -a signal transducer and adapter protein of G protein coupled receptors- to ET_AR resulting in β-catenin signaling activation, essential for motility (PNAS 106:2806,2009). Here we study if this mechanism operates in proliferative kidney disorders, and whether ET-1 triggers podocyte migration via β-arrestin-mediated signaling.

Methods: Migration of differentiated mouse podocytes and β-arrestin signaling pathway were assessed by wound healing assay and immunoprecipitation/immunoblotting.

Results: ET-1 (100nM) promotes podocyte migration via ETAR as selective antagonist BQ123 significantly prevents cell motility. ET-1 upregulates podocyte constitutive expression of β-arrestin mRNA and protein and induces the formation of ETAR/β-arrestin/Src signaling complex. By inducing Src activation, ET-1 promotes EGFR transactivation and Akt phosphorylation associated with active β-catenin accumulated in the cytoplasm, events preceding transcription of pro-migratory genes. Mice with adriamycin-induced nephropathy unexpectedly exhibit glomerular synechiae and few crescents at 4wk. Podocytes forming synechiae express high β-arrestin-1 levels.

Conclusions: These results indicate that, as in cancer cells, ET-1 promotes podocyte migration and activates ET_AR/β-arrestin/β-catenin pathways providing new clues for therapies based on ET receptor antagonists in podocytopathies associated with crescentic lesions.

Funding: Private Foundation Support

FR-PO1331

Characterization of a β_2 Adrenoceptor Pharmacophore That Predicts Mitochondrial Biogenesis Lauren P. Wills, Richard Trager, Christopher C. Lindsey, Yuri K. Peterson, Craig Cano Beeson, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: The renal tubular epithelia have capacity for repair and regeneration after insults, which can be improved by compounds that promote mitochondrial biogenesis.

Methods: To examine the impact of the β-adrenergic signaling pathway on mitochondrial biogenesis, primary renal proximal tubule cells (RPTC) and adult feline cardiomyocytes (AFC) were exposed for 24 hr to multiple β-adrenoceptor (β-AR) agonists: isoproterenol (non-selective β-AR agonist), BRL 37344 (selective β_3-AR agonist), and formoterol (selective β_2-AR agonist). The Seahorse Biosciences analyzer was used to quantify FCCP-uncoupled oxygen consumption rate (OCR), a marker of maximal electron transport chain activity and mitochondrial biogenesis.

Results: Isoproterenol and BRL 37244 did not alter mitochondrial respiration at any of the concentrations examined (10-1000 nM). Formoterol exposure (30 nM) resulted in increased FCCP-uncoupled OCR and mitochondrial DNA (mtDNA) copy number. The effect of formoterol on OCR in RPTC was inhibited by the β2-AR antagonist propanolol. To examine the effects in vivo, C57BL/6 mice were exposed to 100 μg/kg of formoterol for 24 or 72 hr. Formoterol exposure increased kidney and heart mtDNA copy number, induced the mitochondrial regulator PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-α), and induced multiple genes coding for electron transport chain

proteins (ATP6, ATP synthase β, COX1 and ND6). Pharmacophore evaluation of formoterol identified two chemically similar compounds from the Sigma Library of Pharmacologically Active Compounds (tomoxetine and nisoxetine), and two from the ChemBridge DIVERSet that caused significant increases in mitochondrial respiratory capacity.

Conclusions: These data have been utilized to develop a discrete pharmacophore model capable of predicting novel compounds with mitochondrial biogenic properties. This project was funded through grant number: GM084147.

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FR-PO1332

A Role for PI3-kinase in Regulating the EMT-Antagonist Protein, SARA

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Background: TGF-β1 promotes renal fibrosis, in part by promoting epithelial to mesenchymal transition (EMT). We have previously shown that the Smad anchor for receptor activation (SARA) helps maintain an epithelial cell phenotype, and that reduction of SARA by TGF-β1 or other means stimulates events associated with EMT. Investigation of signaling pathways that might regulate SARA expression demonstrated a role for PI3-kinase signaling, as evidenced by the fact that, like TGF-β1, the PI3-kinase inhibitor LY294002, caused a reduction in SARA expression and a concomitant increase in the expression of the EMT marker αSMA. However, our data suggested that the mechanism by which PI3-kinase inhibition depletes SARA differs from the mechanism that results from prolonged TGF-β1 treatment.

Methods: Standard protein, transfection, qPCR, and immunocytochemical techniques were employed.

Results: To confirm the effects of chemical PI3-kinase inhibition, we established an HKC proximal tubule cell line that stably expresses an shRNA for the p85α-regulatory subunit of PI3-kinase. The activity of Akt, a downstream target of PI3-kinase, is inhibited by LY294002, but was enhanced in p85α knock-down cells. However, knock-down cells still have depleted SARA expression and increased αSMA expression. These data suggest that maintenance of SARA expression likely is mediated by PI3-kinase but independent of pAkt. It is known that SARA associates with early endosomal subcellular compartments. While, LY294002 treatment did not interfere with SARA localization, it did cause alterations in endosome size and number. Potassium depletion, used to block internalization to the early endosome, inhibited the LY294002-induced SARA depletion. Further, co-immunoprecipitation studies demonstrated a SARA-p85α interaction that was reduced by LY294002 under control conditions, but was enhanced under conditions of inhibited internalization.

Conclusions: Our data therefore suggest that SARA is protected from degradation via its interaction with PI3-kinase at the cell membrane, but that the separation of this complex upon internalization to altered endosomal compartments leads to reduced SARA expression.

Funding: NIDDK Support

FR-PO1333

PI3K, but Not Smad3, Is Required for Normoxic Induction of HIF-1αexpression by TGF-β

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Background: The transforming growth factor (TGF)-β pathway, acting through the Smad signaling proteins, has been shown to interact with hypoxia-inducible factor (HIF)-1α in models of hypoxic kidney injury. Previously, we showed that TGF-β1 increases HIF-1α transcriptional activity and protein expression in a human proximal tubule cell (HKC) line under normoxia (21% O_2) in a receptor-dependent manner. Blockade of the Smad3 signaling pathway with the dominant-negative Smad3A construct reduced normoxic TGF-β1-stimulated HIF-1α transcriptional activity. Interestingly, a dominant-negative HIF-1α construct reduced normoxic basal and TGF-β1-stimulated Smad3 transcriptional activity, including the activation of the COL1A2 gene promoter. However it was not clear from these studies if Smad3 was required for normoxic induction of HIF-1α expression by TGF-β1 or if phosphoinositide-3 kinase (PI3K), a mediator of Smad3-independent TGF-β effects, might play a role.

Methods: Smad3 wild type (wt) and null mouse embryo fibroblasts (MEF) were stimulated with 1 ng/ml TGF-β1 for 6 hrs in 21% O_2-5%CO_2 before harvest for Western analysis. In select experiments, Smad3 null MEF were pretreated with 20 μM of the PI3K inhibitor LY294002 (LY) before stimulation with 1 ng/ml TGF-β1 and Western analysis.

Results: Smad3-wt MEF treated with TGF-β1 exhibited a 6.8 ± 3.3-fold increase in HIF-1α protein expression over vehicle treated cells. Although levels were reduced, HIF-1α was still expressed in Smad3-null MEF and TGF-β1 stimulated a 1.9 ± 0.6-fold increase. In Smad3-null MEF, LY reduced normoxic basal HIF-1α protein levels and abrogated TGF-β1-stimulated HIF-1α expression. Thus, TGF-β-stimulated HIF-1α expression was enhanced by, but not dependent upon, Smad3.

Conclusions: Together, our data suggest that PI3K mediates normoxic TGF-β-stimulated HIF-1α expression in a Smad3-independent manner, and raise the possibility that PI3K/HIF-1α and TGF-β/Smad3 participate in parallel pathways to promote fibrogenesis in normoxia or hypoxia.

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FR-PO1334

Mammalian Diaphanous 1 (mDia1) Plays a Central Role in RAGE Induced Redox Signaling

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Background: RAGE (Receptor for Advanced Glycation end Products) is a multiligand receptor of the immunoglobulin superfamily involved in fundamental disease processes characterized by vascular pathology. Vascular stress leads to up-regulation of RAGE ligands in the vessel wall, thereby facilitating their engagement and activation of RAGE to induce pro-inflammatory and tissue-damaging responses. We previously reported that the cytoplasmic domain of RAGE interacts with mDia-1, a member of the formin family involved in actin and microtubule reorganization.

Methods: In the present study, we tested the role of mDia1 in RAGE induced oxidative stress generation and redox signaling pathways. We established primary cultures of murine aortic smooth muscle cells (SMCs) from wild-type, RAGE null and mDia-1 null mice. Recombinant S100B was used as a prototypic RAGE ligand.

Results: We found that: 1/ RAGE activation leads to the translocation of cSRC kinase to the membrane, followed by Rac1 and Nox1 activation. Nox4 was not affected. 2/ RAGE induced superoxide production and consequent phosphorylation of GSK3β were required for WT SMCs migration. 3/ Presence of mDia1 was critical for RAGE induced cSRC translocation at the membrane, Rac1 and Nox1 activation, AKT/GSK3 β phosphorylation and VSMC migration. Finally, in vivo, in a mouse model of guidewire induced femoral artery denudation, we found that mice devoid of mDia-1 had less neointimal expansion. Furthermore, NADPH oxidase activity, and phosphorylation of AKT/GSK3beta were lower in arteries of animals devoid of mDia1.

Conclusions: We conclude that mDia1 integrates oxidative and signal transduction pathways triggered at least in part by RAGE ligands, therefore acting as a regulator of pathological neointimal expansion.

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FR-PO1335

A Novel Role of Tuberin in the Regulation of Cell Matrix Proteins in Proximal Tubular Cells

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Background: Matrix protein accumulation is major pathological features of diabetic nephropathy that eventuate in renal failure. PI3-K/Akt pathway phosphorylates tuberin on specific residues result in its inactivation. Tuberin inactivation leads to activate mTOR pathway, which increases protein translation and cell growth. Hyperglycemia increases cell matrix proteins but the pathogenic mechanisms are not fully understood.

Methods: Inactivation of tuberin and activation of downstream signal of mTOR, collagen IV and fibronectin expression were measured in kidney cortical tissue of control and type 1 diabetic animals and in primary proximal tubular cells (PPT) incubated with normal or high glucose. In addition, promoter transcription activity of fibronectin was measured in PPT cells treated with high glucose (HG) using luciferase assay.

Results: Our data show that inactivation of tuberin resulting activation of the mTOR pathway enhances matrix proteins accumulation in PPT cells exposed to HG and in kidney cortex of rats with type1 diabetes. We find also that high glucose enhances phosphorylation/inactivation of tuberin and results in increased phosphorylation of S6 Kinase, a major downstream target of mTOR. This is associated with increased fibronectin and collagen IV protein expression in cultured PPT cells. Our data show that the increase in fibronectin and collagen IV protein expression in tuberin-null cells is reversed upon the introduction of tuberin cDNA. In addition, our data show that blockade of mTOR with rapamycin prevents HG-induced increase in fibronectin promoter transcriptional activity in PPT cells.

Conclusions: In conclusions, we show for the first time a novel role of tuberin in the regulation of cell matrix proteins in proximal tubular cells. Our data provide an evidence that diabetes enhances tuberin phosphorylation/inactivation and results in an increase cell matrix proteins accumulation in kidney cortex.

FR-PO1336

Curcumin Blocks Ang II Induced TNFα Secretion by Inhibiting Phosphorylation of TNFα Converting Enzyme (TACE)

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Background: Angiotensin II (Ang II) by increasing TNFα secretion, can secondarily induce inflammation and aggravate renal failure. We showed that curcumin (CUR) ameliorates renal failure in rats by blocking TNFα secretion. However, the mechanism by which TNF secretion relates to Ang II/CUR is poorly elucidated. Phosphorylation of TNFαconverting enzyme(TACE)facilitates release of the active form of TNFα. Various signaling pathways including the MEK/ERK pathway mediate the phosphorylation of TACE. We theorized that Ang II, by activating the ERK pathway, can induce TACE

phosphorylation and CUR, which is known to block ERK phosphorylation, can prevent TACE activation and reduce TNFα secretion.

Methods: Thioglycolate elicited peritoneal macrophages (MO) from Sprague Dawley rats were plated for cell culture. The MO were treated with Ang II in the presence and absence of 10 μM CUR and/or 10 μM of ERK inhibitor PD 98059 (PD) for 24 hours; control MO were exposed to ethanol. TNFα was measured by ELISA. MO lysates were made with phosphatase and protease inhibitors (n= 4-6).

Results: Ang II treated MO had a 65% increase in TNFα secretion (p[let]0.01), CUR and PD blunted it by 40±3.9% ($p<0.01$) and 17±4.2% ($p<0.05$) respectively. Western blot analysis of macrophage lysates showed that Ang II increased ERK phosphorylation by 3 fold, which was effectively reduced by PD ($p<0.01$) and CUR ($p<0.05$). TACE was immunoprecipitated from MO lysate and immunoblotted with phosphoserine and phosphothreonine antibodies. Compared to controls total phosphorylation of TACE following Ang II treatment was increased by 41±5.1% ($p<0.01$) and only by 20±6.5 % ($p<0.01$) and 34 + 5.0 % ($p<0.05$) respectively, with CUR and PD pre-treatment [CUR vs PD $p \leq 0.05$].

Conclusions: Ang II increases TNFα secretion by phosphorylating TACE, a process effectively blocked by CUR. Although CUR was less effective than PD in blocking ERK phosphorylation it was more effective in blocking TNFα secretion and TACE phosphorylation. The superiority of CUR to PD may be a function of Ang II activating other phosphorylation pathways such as PKC, inhibitable by CUR but not by the MEK inhibitor PD.

FR-PO1337

High Glucose Induced Classically Activated Macrophage Facilitates Process of Epithelial Mesenchymal Tansition in Renal Proximal Tubular Epithelial Cells

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Background: It is the status of macrophage activation rather than infiltrated number in kidney tissue plays the key role in determining the outcome of kidney diseases. There are two types of macrophage activation including classical (M1) and alternative (M2) types. M1 induces inflammation and tissue injury while M2 may provide anti-inflammatory effect and promote tissue repair. Our previous study had demonstrated that macrophage-renal tubular cell binding could drive TGF-β1 expression by tubular cells. This study was to investigate the effect of high glucose (HG) on macrophage activation type and consequent effect on epithelial mesenchymal tansition (EMT) in renal proximal tubular epithelial cells (PTEC).

Methods: U937 and HK-2 cells were used in all experiments. High glucose (30mM) was used to stimulate U937 and M1 marker iNOS, TNF-α and IL-12 were detected by ELISA. Stimulated U937 were co-cultured with HK2 cells followed by measurement of EMT marker α-SMA,fibronectin and E-cadherin by western immunoblotting.

Results: Treatment of U937 cells with HG significantly increased the expression of iNOS mRNA ($P<0.05$) and the activity of iNOS ($P<0.05$), with the maximal iNOS activity achieved at hour 9 ($P<0.01$). Incubation of 30mM of HG with U937 cells for 24 hours increased expression of TNF-α ($P<0.01$) and IL-12 ($P<0.01$), with the peak level up to 15 times higher than control. In addition, co-culture of HG treated U937 with HK-2 cells for 24 hours decreased expression of E-cadherin mRNA ($P<0.05$) and protein ($P<0.05$), whereas α-SMA and fibronectin were significantly increased ($P<0.05$). The mRNA expression of α-SMA and fibronectin in HK2 cells were also increased after incubation with HG treated U937 ($P<0.05$). This change was similar with the positive control with IFNγ+LPS treatment to U937, which represents M1 activation, while it was not observed in the osmotic control (treatment with mannitol).

Conclusions: High glucose shifts macrophage differentiation into classically activated (M1) macrophage, which could facilitate the process of EMT in HK2 cells.

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FR-PO1338

Erbin Inhibits TGF-β1-Induced Epithelial-to-Mesenchymal Transition in Renal Tubular Epithelial Cells through an ERK Dependent Pathway

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Background: Epithelial-to-mesenchymal transition (EMT) plays a crucial role in the progression of renal interstitial fibrosis, which ultimately leads to end-stage renal failure. Erbin, a member of LAP family, is recently reported to inhibit Smads and ERK pathway which are two important intracellular signaling involved in TGF-β1-induced EMT.However, the role of Erbin in the regulation of EMT and the underlying mechanisms remain to be fully understood. To that end, we aimed to evaluate the expression of Erbin in renal interstitial fibrosis (RIF) and the potential role of Erbin in tubular EMT stimulated by TGF-β1.

Methods: Western blotting anaysis for Erbin, E-cadherin and α-SMA; RT-PCR for Erbin; Immunohistochemistry for Erbin expression in Rat renal tissue;

Masson staining for rat renal tissue;

Immunofluorescence test for E-cadherin and α-SMA.

Results: In this study we demonstrated that the expression of Erbin was up-regulated in the tubular epithelia of 5/6-nephrectomized rats. We also showed here that TGF-β1 induced Erbin expression both in mRNA and protein level in NRK52E cells during their acquisition of an EMT phenotype. Importantly, elevated expression of Erbin inhibited ERK signaling and partial reversed EMT stimulated by TGF-β1. In the mean time, reducing Erbin expression by specific siRNA enhanced ERK phosphorylation with no effect on smads signaling, promoted the E-cadherin suppression and induced α-SMA in response to TGF-β1, which could be rescued if cells were treated with the inhibitor of MEK1/2 U0126. However, in the absence of TGF-β1, Erbin failed to affect ERK activation and EMT process.

Conclusions: These results demonstrate that Erbin is a negative feedback molecule induced by TGF-β1 and inhibits TGF-β1-induced EMT via ERK signaling pathway.

FR-PO1339

Differential SMAD3 Linker Region (LR) Phosphorylation Is Specific for Different Cell Tissue-Types and Phenotypes

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Background: Regulation of TGF-β/Smad3-mediated fibrogenesis involves complex interaction among canonical and non-canonical signaling pathways. Here, we report the specificity of phosphorylation at several sites (Thr179, Ser204, Ser208, and Ser213) within the Smad3 LR. Previously we showed that ERK is required for collagen reporter activity; and that intact phosphoacceptor sites at Ser204 or Ser208 are required for collagen promoter activation by Smad3, whereas the remaining Thr179 and Ser213 may have opposing effects.

Methods: To further investigate a role for ERK in LR phosphorylation we pre-treated serum-starved murine embryonic fibroblasts (MEFs) with the MEK inhibitor, PD98059 (PD), prior to TGF-β1 stimulation (2ng/ml, 1h). Smad3 LR phosphorylation at Thr179, Ser204, Ser208, and Ser213 was assessed by western blotting. LR phosphorylation was also examined in human renal tubule epithelial cells (HKC), human mesangial cells and mouse podocytes.

Results: Phosphorylation at Thr179, Ser204, Ser208, and Ser213 of the Smad3LR by TGF-β1 was confirmed in wild type MEFs. In HKC, both Thr179 and Ser208 were phosphorylated while in mesangial cells, Ser208 and Ser213 were phosphorylated, none of which were affected by PD. In podocytes, phosphorylation at Thr179, Ser208 and Ser213 were detected (effect of PD not tested). In embryonic fibroblasts, PD inhibited Ser204 phosphorylation but did not affect the remaining sites (Thr179, Ser208 or Ser213). This is consistent with the notion that Ser204 is a primary ERK target and the remaining sites (Thr179, Ser208 or Ser213) are phosphorylated by other mediators.

Conclusions: The ability of TGF-β1 to stimulate, and of PD to inhibit, Ser204 phosphorylation in embryonic fibroblasts but not in HKC is consistent with previous findings that show PD to inhibit TGF-β1-stimulated collagen reporter activity in embryonic fibroblasts and mesangial cells but not in HKC. Further investigation of the role of the Smad3 LR in renal cell function may offer new approaches to selectively modifying TGF-β1-stimulated responses in progressive kidney disease.

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FR-PO1340

Connective Tissue Growth Factor (CTGF) Antagonizes BMP Signalling in Renal Cells with Pathogenic Consequences for Cellular Behaviour and Function

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Background: Initiation and progression of Diabetic Nephropathy (DN) is characterized by alterations in TGF β superfamily signalling, while there is increasing evidence for regulation of BMP signalling activity by extracellular BMP antagonists including CTGF. It has recently been shown that CTGF inhibits BMP-7 activity in experimental DN and in cultured renal cells however the relationship between BMP-2/4 signalling and CTGF has not been investigated in renal cells.

Methods: Biotinylated BMP-4 allowed measuring of changes in receptor binding.We used structural comparison modeling to predict changes in the binding energy between the wild-type CTGF VWC substituted for the equivalent domain of CV-2 by in silico mutagenesis.

Results: Analysis of complex structures of the TGF β1 superfamily member BMP-2 and Crossveinless-2 (CV-2) is informative: The VWC domain of CTGF shares significant homology with SD-1 of CV-2 and is structurally highly conserved.Mutagenesis studies identified key amino acids within the VWC domain that mediate the antagonism of BMP-2. We have shown in mesangial cells that CTGF antagonizes BMP-2/4 induced phospho smad 1/5/8. This effect was reversed by pre treatment of cells with anti-domain 2, a humanized monoclonal antibody directed against the VWC domain of CTGF. CTGF also inhibited BMP-4 receptor binding and BMP-4 induced alkaline phosphatase. CTGF mRNA and protein levels are significantly increased in the STZ-type 1 diabetic mice, concurrent with decreased levels of phosphorylated smad 1/5/8. Injection of wildtype Zebrafish Danio Rerio with in vitro transcribed CTGF mRNA also induces an anti-BMP phenotype.

Conclusions: Although previously undescribed inhibition of BMP-2/4 activity by CTGF may play a crucial role in the progression of DN with anti-CTGF therapies proving promising.

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FR-PO1341

HIF1 Modulates Chromatin Conformational Change by Recruiting KDM3A and Enhances Regulation of Glucose Transporter 3 in Endothelial Cells
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Background: Hypoxia plays a crucial role in both acute and chronic kidney injury. Hypoxia inducible factor 1 (HIF1) is a master regulator of the gene expressions especially to organize glycolysis, cell proliferation and cell survival under hypoxia. Recently HIF1 was reported to regulate subsets of histone demethylases, which modify the chromatin structures. However, its role in epigenetic regulation remains unknown. We aimed at clarifying a new epigenetic mechanism via HIF1, which serve as the first-line defense against hypoxic milieu in various organs including the kidney.

Methods: We performed chromatin immunoprecipitation with deep sequencing (ChIP-Seq) to identify the genome-wide binding sites of HIF1 and clarified the histone modifications in human umbilical venous endothelial cells. We also examined chromatin conformation capture assay to identify the chromatin structural change under hypoxia.

Results: We identified that HIF1 binds to the distal regions from the transcriptional starting sites and plays an important role as an enhancer of glucose transporter 3 (SLC2A3). The reporter assay confirmed that the distal regions of SLC2A3 up-regulate the luc activity under hypoxia and overexpression of HIF1. We clarified the distal region enhances SLC2A3 expression by changing chromatin conformational structure via HIF1. Furthermore KDM3A (JMJD1a; jumonji-domain containing 1a) is a member of histone demethylases and up-regulated by HIF1 under hypoxia. We demonstrated that KDM3A is recruited to the enhancer regions of SLC2A3 by HIF1 and demethylates dimethyl-H3K9 to up-regulate the expression under hypoxia, while KDM3A is not recruited to those enhancer regions when HIF1 is knocked down. These results demonstrate that HIF1 is essential to change the chromatin conformation and recruit KDM3A under hypoxia.

Conclusions: Our findings provide new insights into the regulatory mechanism of HIF1 and epigenetic modifications of KDM3A in HUVEC under hypoxia.

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FR-PO1342

Interferon Gamma Promotes Starvation-Induced Autophagy by Activating the GCN2-ATF4 Pathway
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Background: The aim of this study is to characterize the mechanisms and the consequences of IFNγ-induced autophagy in human epithelial cells.

Methods: Human Renal Cortical Cells (HRCCs) were exposed to 10 ng/ml IFNγ for 24 to 72 hours. Mechanisms and consequences of autophagy were evaluated by western blotting, real time PCR, immunofluorescence and small interfering RNA. An immunohistological study was performed from biopsies of human renal allograft affected by acute rejection and BK virus nephropathy to evaluate autophagy in these situations.

Results: IFNγ increased autophagic flux in HRCCs. Autophagy inhibition by RNA interference directed toward Beclin1 significantly reduced cell viability, suggesting that autophagy promotes cell survival during IFNγ-induced stress.

Indoleamine Dioxygenase (IDO) was strongly upregulated in HRCCs during IFNγ exposure and tryptophan was metabolized in the culture medium. Tryptophan supplementation reduced autophagic flux, suggesting that IFNγ-induced autophagy is a consequence of IDO-mediated tryptophan depletion.

GCN2, an eIF2α kinase activated during amino acid depletion, is phosphorylated following IFNγ treatment. GCN2 activates the eIF2α-ATF4-CHOP pathway leading to autophagy. IFNγ-induced autophagy is significantly reduced by RNA interference directed toward GCN2, suggesting that IFNγ-induced autophagy depends on an intact GCN2 pathway.

Finally, we demonstrate that the tubular expression of the autophagic marker LC3a is strongly upregulated during acute cellular rejection and BK virus nephropathy, conditions which are associated with IFNγ expression, in human renal allograft biopsies, compared to controls.

Conclusions: We report that IFNγ induces autophagy in human tubular cells and promotes cell survival. IDO-induced tryptophan depletion promotes autophagy. IFNγ-induced autophagy is dependant on an intact GCN2-eIF2α pathway leading to the upregulation of the autophagy inducers ATF4 and CHOP. Our results also suggest that autophagy is triggered during acute cellular rejection and BK virus nephropathy in human.

Funding: no

FR-PO1343

A Circadian Oscillator Kid-1, Whose Transcription Is Directly Regulated by c-Myc, Participates in the Transcriptional Mechanism of Per2 through Intervening in the Network of c-Myc and Tif1β
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Background: Discerning the role of the intrinsic kidney clock has important implications for cell cycle of renal tubular epithelial cells and renal function. We reported that a Zinc-finger transcriptional repressor Kid-1 is controlled by the kidney clock, but the mechanism of rhythmic Kid-1 mRNA expression remained to be determined.

Methods: There is no canonical E-box element in the promoter of Kid-1 gene. To determine whether Kid-1 is directly regulated by CLOCK, we knocked down CLOCK in NIH3T3 cells by siRNA. Predictably, Kid-1 mRNA expression level unchanged. Intriguingly, when the expression level of c-Myc was knocked down by siRNA, the level of Kid-1 mRNA was upregulated. c-Myc is a critical repressor for circadian Cyclin D1 transcription through its initiator element. Therefore, we searched Kid-1 gene for analogous sequences.

Results: We found a putative initiator element ACATTTC in Kid-1 promoter region, whose sequence is conserved among mice, rats and human. Luciferase assay and Chromatin immunoprecipitation (ChIP) assay in NIH3T3 cells confirmed that c-Myc negatively regulated Kid-1 transcription through its initiator element. Western blot analysis confirmed that transient overexpression of Kid-1 in NRK52E cells resulted in downregulation of c-Myc and silencing of Kid-1 by siRNA in NRK52E cells resulted in upregulation of c-Myc. Moreover, when Kid-1 was knocked down, the expression levels of Per2 mRNA and its protein product markedly decreased. ChIP assay confirmed that the more amount of lysine9 trimethyl histone H3 interacted with Per2 promoter region when Kid-1 was knocked down. Moreover, the association of c-Myc with Tif1β which is a transcriptional repressor appeared in Per2 promoter region when Kid-1 was knocked down.

Conclusions: These results suggest that Kid-1, whose transcription is negatively regulated by c-Myc, participates in the transcriptional mechanism of *Per2* through intervening in the network of c-Myc and Tif1β.

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FR-PO1344

An HIF-1α-Independent Neoangiogenic Pathway Regulated by the Unfolded Protein Response during Renal Ischemia
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Background: Ischemic injuries stress kidney tissue and challenge cell viability. Adaptive responses to ischemia are mostly mediated by the activation of the transcriptional factor HIF-1α. Emerging evidence suggest that other adaptive pathways promoting neoangiogenesis may be implicated.

Ischemia promotes glucose deprivation, ATP deprivation, hypoxia that triggers Endoplasmic Reticulum (ER) Stress followed by the adaptive response termed Unfolded Protein Response (UPR). The aim of this study was to test whether the UPR would promote neoangiogenesis independently of HIF-1α pathway during ischemic stress.

Methods: qPCR, immunoblots and ELISA were performed on human kidney tubular cell lines (HK2) exposed to glucose deprivation and/or hypoxia to evaluate the expression of the UPR markers BiP, CHOP and ATF4 and the expression of neoangiogenic inducers Vascular Endothelial Growth Factor (VEGF) basic Fibroblast Growth Factor (bFGF), Angiopoietin 1, Angiogenin and Platelet Derived Growth Factor. ARN interference directed against the three transducers of the UPR, ATF6, PERK and IRE1 was performed to test which UPR axis is involved in mediating VEGF and bFGF expression.

Results: Glucose deprivation and hypoxia significantly increased the expression of VEGF, bFGF both at the mRNA and protein levels. Glucose deprivation did not alter HIF-1α protein or mRNA levels suggesting that this pathway is not involved, whereas HIF-1α is upregulated during hypoxia.

Glucose deprivation, not hypoxia, increases the expression level of the UPR markers BiP, CHOP, ATF4, and the spliced form of XBP1 mRNA, suggesting that glucose deprivation, not hypoxia, promotes ER stress and triggers the UPR.

RNA interference directed against PERK decreased VEGF and bFGF secretion whereas RNA interference against Ire1α and ATF6 do not decrease these markers.

Conclusions: This work demonstrates that ischemia increases the expression of neoangiogenesis inducers VEGF and bFGF independently of the HIF-1α pathway in human kidney tubular cells and that the Perk arm of the UPR could play a role in neoangiogenesis during kidney ischemia.

FR-PO1345

Hypercritical Role of mRNA Regulation for Hypoxia Inducible Factor 1 alpha (HIF-1alpha) Expression Ralf Mrowka,[1] Anja Bondke Persson,[2] Andreas Steege,[3] Andreas Patzak,[2] Pontus Persson,[2] Michael Fähling.[2] *[1]KIM III, Experimental Nephrology, Universitätsklinikum Jena, FSU, Jena, Germany; [2]Physiology, Charité - Universitätsmedizin Berlin, Berlin, Germany; [3]Nephrology, Universität Regensburg, Regensburg, Germany.*

Background: Genes are regulated at multiple levels such as transcriptional as well post-transcriptional level. The expression of hypoxia-inducible factor 1 (HIF-1) is critical for many processes such as erythropoesis, vascular growth, anaerobic metabolism, and iron transport mechanisms. Beside the oxygen-dependent hydroxylation of the regulative HIF-1alpha subunit, there is increasing evidence for non-oxygen dependent pathways affecting HIF-1 regulation. In this study we addressed the question as to whether the HIF-1alpha mRNA is important for the regulation of the transcription factor complex and whether there are trans-acting factors that might act as regulators for HIF-1 activity.

Methods: We quantify the impact of transcriptional as well as post-transcriptional gene regulation for the factor HIF-1alpha. Further, we present a computational and experimental approach to identify RNA-binding proteins (RNA-BPs) that modulate mRNA stability of selected genes. For this purpose, we used gene expression data of a large set of microarray experiments available from the Stanford microarray database. We also applied modelling techniques to gain insight into the signalling dynamics of HIF-1.

Results: Based on large-scale expression data, we show that HIF-1alpha mRNA turnover is crucial for the activity of HIF-1 and expression of its downstream targets. We identify a hypercritical requirement in the HIF-1alpha mRNA level which highly impacts the activity of HIF-1 as a transcription factor. Further, we show, both computationally and experimentally, that the gene expression levels of a number of RNA-binding proteins is directly correlated with that of HIF-1 target genes.

Conclusions: Our integrated modelling and experimental approach highlights the importance of mRNA regulation especially under conditions that affect HIF-1alpha protein turnover. We identify a number of RNA binding proteins that modulate HIF-1alpha expression level and subsequently HIF-1 function.

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FR-PO1346

Regulation of HuR Expression in Renal Ischemia Suman Govindaraju, Beth S. Lee. *Physiology and Cell Biology, Ohio State University Medical Center, Columbus, OH.*

Background: HuR is a ubiquitously expressed RNA-binding protein that is increasingly shown to be a master regulator of genes associated with growth,cell division and stress response.We have demonstrated that renal HuR is specifically upregulated in proximal tubule (PT) cells following an ischemic event, where it protects these cells from subsequent apoptosis. The purpose of this study is to evaluate the transcriptional control of HuR in PT cells.

Methods: Cultured proximal tubule (PT) cell lines were grown under normal conditions or ATP depletion to mimic ischemic stress.Levels of HuR mRNA transcripts were determined by competitive RT-PCR and ribonuclease protection assays.Levels of HuR protein were determined by Western blotting.Gel mobility shift assays were used to detect binding of transcription factors to the 5' UTR of the HuR gene.

Results: We demonstrate that HuR mRNA is expressed in two forms with alternate 5' untranslated regions that are differentially expressed during normal growth and stress. We are exploring why PT cells express these forms under different cellular conditions, as insights into regulation of HuR will elucidate mechanisms controlling expression of genes promoting growth and cell survival.We are currently characterizing the mechanisms that regulate transcription of the alternate transcript, which is efficiently transcribed during normal growth, but down-regulated following cellular stress. Studies so far reveal that transcription factors Sp1 and NF-κB play a critical role in regulating this form of HuR mRNA. Further, we found that NF-κB stimulation of HuR expression results in its participation in a positive feedback loop that promotes expression of Akt, a protein kinase well-established as a mediator of cell survival.

Conclusions: HuR mRNA exists in two alternate forms which differ in the length of their 5'UTR and are differentially expressed during normal growth and cell stress. Elucidating these mechanisms will help us understand its protective role in ischemia-reperfusion injury, ischemic pre-conditioning and other models of renal cellular stress.

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FR-PO1347

5-Hydroxytryptamine-Class 2 Receptor-Mediated Mitochondrial Biogenesis as a Potential Therapeutic Strategy for Treatment of Acute Kidney Injury Jennifer L. Blakely, Sara M. Garrett, Craig Cano Beeson, Rick G. Schnellmann. *Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, Medical University of South Carolina, Charleston, SC.*

Background: Renal proximal tubule cells (RPTC) exposed to acute oxidant injury exhibit mitochondrial dysfunction; recovery of cellular function in these cells is temporally related to recovery of mitochondrial function, indicating that the mitochondria are a potential novel target for the treatment of AKI. Previous work in our laboratory demonstrated that DOI, a 5-hydroxytryptamine 2 receptor ($5HT_2$) pan-agonist, stimulates mitochondrial biogenesis in vitro by increasing the expression of peroxisome-proliferator-receptor-γ-coactivator-1α (PGC-1α), the "master regulator" of mitochondrial biogenesis. Additionally, it was demonstrated that DOI accelerates the recovery of mitochondrial function after exposure of RPTC to acute oxidant injury, suggesting that 5-HT receptor agonists may represent a novel therapeutic strategy for the treatment of mitochondrial and cell injury.

Methods: The goal of these studies was to further explore the role of $5HT_2$ receptors as targets for induction of mitochondrial biogenesis using the Seahorse Biosciences analyzer as a respirometric screen in primary cultures of rabbit RPTC. Concentration-response experiments were conducted using six $5HT_2$ receptor agonists and six $5HT_2$ receptor antagonists.

Results: Four compounds increased maximal FCCP-uncoupled respiration, a test for mitochondrial biogenesis. CP-809101, a highly selective 5HT2c agonist, yielded a maximal biogenic response at 100 nM whereas three 5HT2A antagonists, SB-242084, ketanserin and MDL-100907, yielded a maximal biogenic response at 100 nM, 100 nM and 1 nM, respectively.

Conclusions: Based on these results, we postulate that $5HT_{2c}$ agonism is responsible for the mitochondrial biogenic effect of DOI on cells exposed to oxidant injury. Furthermore, we suggest that $5HT_{2A}$ receptor antagonism signals mitochondrial biogenesis through a novel pathway.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1348

FoxO1 Is the Dominant Mediator of the Skeletal Muscle Atrophy Due to CKD and FoxO1 Can Be Inhibited *In Vivo* by miR-486 Jing Xu,[1] Zhaoyong Hu,[2] Yanlan Dong,[2] William E. Mitch.[2] *[1]Department of Nephrology, Changhai Hospital, Shanghai, China; [2]Nephrology Division, Baylor College of Medicine, Houston, TX.*

Background: The loss of muscle protein induced by chronic kidney disease (CKD) occurs via the ubiquitin-proteasome system (UPS). A pathway that activates the UPS in muscle includes an upregulation of the expression of atrophy-related E3 ligases, Atrogin-1 and MuRF-1. It is generally accepted that forkhead transcription factors (FoxOs) can stimulate expression these E3 ligases, it is not known which isoform (Foxo1, Foxo3 or FoxO4) activates muscle proteolysis *in vivo*. Identifying which FoxO is required could lead to therapeutic strategies.

Methods: We studied mice with muscle-specific deletion of FoxO1(MFKO) and examined how CKD (subtotal nephrectomy) affects muscle protein degradation. We also investigated if blocking FoxO1 expression by a micro RNA strategy changes muscle wasting.

Results: MFKO did not alter the expression of FoxO3a or FoxO4 in muscle and CKD did not cause significant muscle atrophy in MFKO mice. The expression of Atrogin-1/MuRF-1 in muscle and the rate of muscle protein degradation were blocked by ~70% ($p<0.01$). Since miR-486 can damp FoxO1 translation, we examined if miR-486 influences protein metabolism in muscle cells. After transfecting a miR-486 mimic into primary cultures of mouse myotubes, the FoxO1 protein level decreased and its phosphorylation increased. Control myotubes treated with dexamethasone (Dex) had increased Atrogin-1/MuRF-1 expression and protein degradation and these responses were largely blocked by expression of miR-486. In mice, we electroporated the miR-486 mimic into the mixed fiber, tibialis anterior (TA) muscles of CKD or Dex-treated mice. Muscle mass (the ratio of TA muscle weight to tibia length) in CKD mice significantly improved after electroporation of the miR-486 mimic and there was significant depression of FoxO1 protein and Atrogin-1/MurF-1 expression.

Conclusions: Our results demonstrate that FoxO1 is a dominant mediator of the CKD-induced activation of Atrogin-1/MuRF-1, and their contribution to muscle protein degradation. Manipulation of miR-486 could potentially blunt catabolic responses in muscle.

Funding: NIDDK Support

FR-PO1349

FGF23 Is Independently Associated with Vascular Calcification but Not Bone Mineral Density in Patients at Various CKD Stages Sophie Liabeuf,[1,2] Lucie Desjardins,[1,2] Cédric Renard,[4] Aurélie Lenglet,[1,2] Horst-Dieter Lemke,[5] Gabriel Choukroun,[2,3] Tilman B. Drueke,[2] Ziad Massy.[1,2,3] *[1]Pharmacology Department - Clinical Research Center, Amiens University Hospital, Amiens, France; [2]INSERM ERI 12, University of Picardie Jules Verne, Amiens, France; [3]Nephrology Department, Amiens University Hospital, Amiens, France; [4]Radiology Department, Amiens University Hospital, Amiens, France; [5]EXcorLab GmbH, Obemburg, Germany.*

Background: The hormone fibroblast growth factor 23 (FGF23) is involved in mineral homeostasis but may also have a role in vascular calcification and bone mineralization. Previous studies related to FGF23 and vascular and bone outcomes have been restricted to dialysis patients. The aim of the present study was to establish whether or not plasma FGF23 levels are associated with aortic and coronary calcification, arterial stiffness and bone mineral density in patients with early as well as late stages of CKD.

Methods: One hundred and fifty-three patients with CKD stages 2-5D were included in a cross-sectional study. In addition to routine biochemistry and intact FGF23 determinations, aortic and coronary calcification and stiffness and bone mineral density (BMD) were assessed by multislice spiral computed tomography and automated pulse wave velocity (PWV).

Results: Plasma intact FGF23 levels were elevated in CKD patients; the elevation preceded that of serum phosphate in early-stage CKD. Patients with elevated FGF23 levels had higher aortic and coronary calcification scores than patients with lower FGF23

levels. Multivariate linear regression analysis indicated that only age ($p<0.001$) and FGF23 ($p=0.008$) were independently associated with aortic calcification score. Plasma FGF23 was neither associated with PWV nor with BMD.

Conclusions: Our data suggest that plasma FGF23 is an independent biomarker of vascular calcification in patients with various CKD stages including early stages. The association between vascular calcification and FGF23 levels appears to be independent of BMD. It remains to be seen whether this association is independent of bone turnover and bone mass.

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FR-PO1350

microRNA-29 Is a Regulator of TGFβ-Dependent Fibrogenesis Phillip Kantharidis, Bo Wang, Rosemarie Carew, Chris Tikellis, Merlin C. Thomas, Mark E. Cooper. *JDRF Danielle Alberti Memorial Centre for Diabetes Complications, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia.*

Background: Glomerular and interstitial fibrosis is a common pathogenic pathway for progressive kidney disease. The renal accumulation of extracellular matrix (ECM) is primarily driven by increased levels of pro-fibrotic mediators, including TGF-β1. Recent data have suggested an important role for specific microRNAs in enhancing fibrogenic signalling and sustaining pro-fibrotic phenotypes. In particular, the 3'UTR of most collagens contains target sites for miR-29 mediated translational repression. This study examines the potential functions of the microRNA-29 in TGF-dependent fibrogenesis.

Methods: The ectopic expression of pre-miR-29a/b/c or pre-miR-Control (miR-C) was induced in rat proximal tubular cells, primary messangial cells and immortalised human podocytes using Oligofectamine. Cells were then exposed to TGF-β1 (1-10ng/mL) for 3-10 days and the expression of collagen assessed using RT-PCR and immunoblotting.

Results: Treatment of renal cells with TGF-β1 reduced the expression of the miR-29a/b/c and increased fibrogenesis. By contrast, ectopic expression of miR-29 repressed the expression of collagen I and IV, at an mRNA and protein level, and attenuated TGF-dependent fibrogenesis in both renal cell lines. Luciferase-reporter constructs incorporating the 3'UTR of collagen I and IV demonstrated that TGF-β1 increased luciferase activity (collagen expression) and that miR-29 was able to prevent this increase.

Conclusions: miR-29 potentially plays an important role in TGF-β1-mediated collagen synthesis in renal cells. These miRNAs potentially represent a new target for the development of anti-fibrotic therapies for the treatment of chronic kidney disease.

Funding: Government Support - Non-U.S.

FR-PO1351

Increased Zinc-α2-glycoprotein White Adipose Tissue Synthesis in Chronic Kidney Disease: Nutritional and Metabolic Impacts Caroline Pelletier,[1 2 3] Laetitia Koppe,[1 2 3 4] Emilie M. Kalbacher,[1 2 3 4] Denis Fouque,[2 4] Christophe O. Soulage.[1 3] *[1]CarMeN u1060, INSERM, France; [2]Univ Lyon 1, France; [3]INSA, Lyon, France; [4]HCL, Lyon, France.*

Background: Chronic kidney disease (CKD) is frequently associated with protein energy wasting which has been recognized as a predictive factor of mortality. Zinc-α2-glycoprotein (ZAG) has been proposed as a new adipokine involved in body weight control through its lipid mobilizing activity.We hypothesized that the uremic environment in CKD may interact with the adipose tissue, resulting in an over-production of ZAG and therefore contributing to metabolic disturbances observed in CKD patients.

Methods: ZAG level was quantified in mouse 3T3-L1 adipocytes after incubation in culture medium containing either urea (30mM), plasma from healthy volunteers, CKD or haemodialysis (HD) patients (20%,v/v). ZAG was also measured in white adipose tissue (WAT) from 5/6 nephrectomized or controls rats. Plasmas from 8 healthy volunteers, 8 CKD and 8 HD patients and subcutaneous adipose tissue (SAT) biopsies from 5 CKD patients and 9 non uremic individuals were collected. ZAG protein content was quantified in plasma, white adipose tissue or 3T3-L1 adipose cells by Western blotting.

Results: Uremic plasma but not urea or control sera, induced a significant increase of ZAG protein content (+224%,$p<0.001$) in 3T3-L1 adipocyte associated with an increase in basal lipolysis (+153%,$p<0,005$). 5/6 nephrectomized rats exhibited a significant decrease in WAT accretion (-44%,$p=0,006$) and a higher content of ZAG in WAT (+598%,$p<0,02$). ZAG protein level in WAT was negatively correlated with adipose tissue mass ($p=0,006$). Human plasma concentration of ZAG was increased in CKD patients as compared with healthy volunteers (+279%,$p=0,001$). Human SAT from CKD patients showed a higher content of ZAG protein level (+234%,$p=0,042$).

Conclusions: Undefined circulating factors in CKD, but not urea itself, increase ZAG production in adipocytes. These results suggest that the increase of ZAG serum levels reported in CKD patients could be due to an overproduction of ZAG by adipose tissue. The increase in ZAG could be a major contributor of fat mass loss and PEW in CKD patients.

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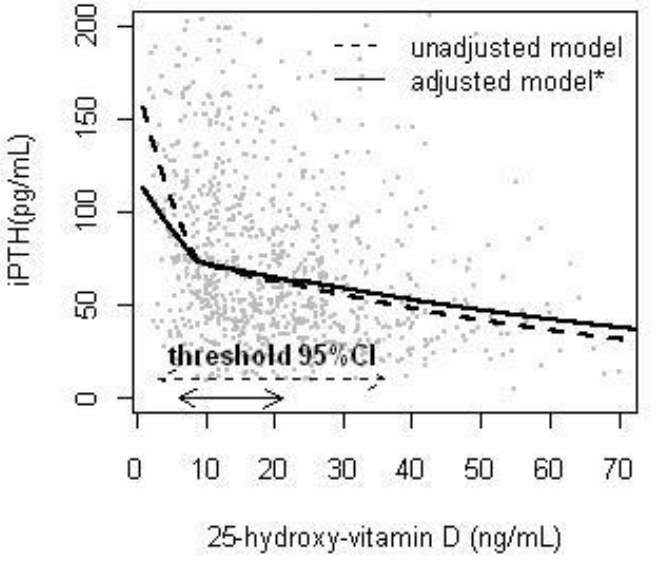
FR-PO1352

The Relationship between Vitamin D and Parathyroid Hormone in Chronic Kidney Disease: Is There a Threshold ? Marie Metzger,[1] Pascal Houillier,[2] Martin Flamant,[3] Jean-Philippe Haymann,[4] Marc Froissart,[2] Benedicte Stengel,[1] Pablo A. Urena.[5] *[1]Inserm U1018, Villejuif, France; [2]Paris Descartes University, Paris, France; [3]Bichat Hospital, Paris, France; [4]Tenon Hospital, Paris, France; [5]Clinique du Landy, St Ouen, France.*

Background: Vitamin D is essential for the regulation of parathyroid hormone (PTH) synthesis. According to the last KDIGO guidelines, serum 25(OH)D concentration might be measured in CKD patients and vitamin D deficiency corrected using treatment strategies recommended for the general population. However, there is no consensus on what define the adequate 25(OH)D values in CKD patients regarding to the level of PTH.

Methods: We used data from the NephroTest cohort including 916 adult patients with ND-CKD stages 1 to 5 and free from vitamin D supplementation to assess the relationship between PTH and 25(OH)D, and to test the existence of a threshold.

Results: Median values (IQR): measured GFR by 51Cr-EDTA: 38(28-52) mL/min/1.73m²; 25(OH)D: 17(11-27) ng/mL; PTH: 64(40-107) pg/mL. Mean ionized calcium (sd): 1.21 (0.07) mmol/L. PTH values were better predicted by a linear piecewise regression model of log(PTH) on 25(OH)D with a threshold of 9 ng/mL than by a linear regression model ($p=0.01$). Adjusting for ionized calcium and measured GFR significantly improved model predictions ($r^2=0.38$ vs 0.10 for the model without adjustment) and reduced the 95%CI around the 9 ng/mL threshold = [6-21 ng/mL]. Estimated slopes were significantly negative before and after the threshold.



*estimated for mGFR of 30-45 ml/min/1.73m² and mean ionized calcium

Conclusions: As in general population, the relation between serum PTH and 25(OH)D is non linear. The rise in serum PTH concentration markedly accelerates when serum 25(OH)D levels fall below 9 ng/mL with an upper 95%CI around 20 ng/mL. This result suggests maintaining serum 25(OH)D concentration above 20 ng/mL to improve the management of secondary hyperparathyroidism in CKD patients.

Funding: Government Support - Non-U.S.

FR-PO1353

Regulation of Hematopoeitic Growth Factor-Inducible Neurokinin, a Tubular Injury and Repair Marker, by Glucose in Mouse Distal Convoluted Tubule Cells and Patients with Diabetic Nephropathy Ying Wang, Anuja P. Shah, Lili Tong, Janine A. La Page, Sharon G. Adler. *Internal Medicine, LABiomedical Research Institute at Harbor-UCLA, Torrance, CA.*

Background: Microalbuminuria (MACR) is less sensitive/specific for diabetic nephropathy (DN) than previously thought, but is used to indicate disease activity/treatment response. Implicit is that therapy predominantly (but not exclusively) targets the glomerulus, but progression correlates better with tubulointerstitial change. We reported urine HGFIN/creatinine (uHGFIN/cr) as a sensitive/specific CKD biomarker in animals/patients, including some with DN (Patel-Chamberlin et a: KI 79:1138, 2011). De novo co-expression of HGFIN with LC3 II in injured distal nephron cortical tubule cells indicated expression in tubules undergoing autophagy. Others reported that HGFIN (aka Gpnmb) mediates autophagy/repair in injured tubules (Li et al: FASEBJ 24:4767, 2010). The regulation of HGFIN expression in diabetes has not been studied.

Methods: We: 1) Measured HGFIN mRNA/protein in mDCT cells cultured with varying glucose (Glu) concentrations; and 2) Assessed uHGFIN/cr values in DN patients randomized to placebo or minocycline (posited anti-apoptotic).

Results: 1) HGFIN/18s mRNA increased linearly in mDCT cells incubated with Glu at 10, 20* and 25* mM (*p<0.05 vs 5.5 mM), but trended down at 30 mM. HGFIN protein increased linearly at 10* and 20* mM Glu (*p<0.05 vs 5.5 mM) but trended down at 25 and 30 mM. 2) In a subset of clinical trial patients, with the randomization blind intact, MACR and uHGFIN/cr decrements were strongly associated; MACR stability/worsening was associated with higher uHGFIN/cr excretion.

Conclusions: 1) HGFIN mRNA/protein levels in mDCT cells are regulated at least in part by medium Glu content. Moderately high Glu increased, but extremely high Glu attenuated, HGFIN expression. 2) uHGFIN/cr correlated with treatment responses in DN. Taken together, these data suggest that HGFIN, which influences autophagy by regulating phagosomal-lysosomal trafficking, is regulated at least in part by Glu exposure, reflects tubular injury in vivo, is modifiable in DN, and may be a target for novel DN therapies and a measure of therapeutic success or failure.

Funding: Private Foundation Support

FR-PO1354

Osteoprotegerin Is Associated with Inflammation, Atherosclerosis and Mortality in CKD Patients Stage 3-5

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Background: Osteoprotegerin (OPG) regulates bone mass by inhibiting osteoclast differentiation and activation, and also plays a role in vascular calcification. Recent research has implicated OPG in atherogenesis, but epidemiological confirmation of this is sparse. In this study we evaluated the relationships between OPG levels and inflammatory and oxidative stress (OS) markers, atherosclerosis and mortality in CKD patients stage 3-5.

Methods: 162 patients (median age 61 years, 62% males; 50 hemodialysis (HD), 57 peritoneal dialysis (PD) and 50 CKD stages 3-4) were studied. All survivors completed 36 months of follow-up. Clinical characteristics were documented, and markers of mineral metabolism (including fibroblast growth factor-23; FGF-23), inflammation (high-sensitivity C-reactive protein; hsCRP, and interleukin-6; IL-6) and OS (8-OH-deoxyguanosine; 8-OHdG) as well as intima-media thickness (IMT; expressed as mean IMT) in common carotid arteries through B-mode ultrasonography were measured at baseline.

Results: After 36-months follow-up, 38 patients (23 PD, 17 HD and 3 CKD stage 3-4) had died. The survival rate by Kaplan–Meier analysis was significantly different according to OPG levels ($\chi 2 = 14.33$; P = 0.002). Increased OPG levels were positively associated with IL-6 (r= 0.38, p<0.001), FGF-23 (r= 0.26, p<0.001), hsCRP (r= 0.0.24, p=0.003), and 8-OHdG (r= 0.44, p<0.0001). In addition, OPG was positively associated with troponin I (r= 0.54, p<0.001) and IMT (r= 0.39, p<0.0001). Moreover, in Cox analysis, only OPG (HR=1.07(95% confidence interval (95% CI) 1.02-1.13) and hsCRP (HR= 1.02 (95% CI 1.01-1.04) were independently associated with increased risk of death.

Conclusions: Although the role of OPG in the vascular biology is poorly understood, these results suggest that elevated levels of serum OPG are associated with atherosclerosis and all-cause mortality in CKD patients stage 3-5.

Funding: Pharmaceutical Company Support

FR-PO1355

Protein-Energy Wasting Abolishes the Association between Fat Mass and Bone Mineral Density in End-Stage Renal Disease Patients

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Background: Low bone mineral density (BMD) is common in end-stage renal disease (ESRD) patients and leads to worse outcome. Many studies have demonstrated a positive association between fat mass and BMD. However, it is not known if protein-energy wasting (PEW) affects this association in ESRD.

Methods: 332 ESRD patients from an ongoing prospective cohort study including incident patients who were close to beginning dialysis replacement therapy were included. Total BMD was measured by dual energy X-ray absorptiometry (DXA) and expressed as T-score, indicating the number of standard deviations from the mean scores for 30-year old normal men and women separately. Fat mass (total fat mass, truncal fat mass and non-truncal fat mass) distribution was measured by DXA also. Subjective global assessment (SGA) was used as a surrogate of PEW. Spearman rank correlation analysis was used to determine the association between T-score and selected parameters.

Results: 100 patients had signs of PEW (SGA≥2), and these patients had lower BMD t-score compared to the non-wasting group (-1.09±1.18 vs -0.26± 1.31, p<0.0001,). BMD t-score positively correlated with truncal fat mass (r=0.2775, p<0.0001), non-truncal fat mass (r=0.2381, p<0.0001) and total fat mass (r=0.3017, p<0.0001) in non-wasted ESRD patients. However, in wasted patients these associations did not attain statistical significance (truncal fat mass, r=0.1604, ns; non-truncal fat mass, r=0.1538, ns; total fat mass, r=0.1564, ns).

Conclusions: Whereas BMD t-score positively correlated with truncal fat mass, non-truncal fat mass and total fat mass in non-wasted ESRD patients, no such associations were found in wasted ESRD patients. Our findings suggest that presence of PEW abolishes the normal and expected association between fat mass and BMD.

Funding: Pharmaceutical Company Support

FR-PO1356

Proteinuria in Injection Drug Users Associated with Vitamin D Deficiency

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Background: Proteinuria is common in injection drug users and HIV-infected persons. Vitamin D deficiency may contribute to proteinuria.

Methods: To study whether vitamin D deficiency is associated with higher risk of proteinuria (urine protein/creatinine ≥200 mg/g on 2 occasions) in this patient population at risk for kidney disease, we conducted a cross-sectional study in 277 HIV-infected and 647 HIV-uninfected individuals in the AIDS Linked to the Intravenous Experience Cohort.

Results: Most participants (73%) had 25-OH vitamin D levels<20 ng/mL. Deficient persons were likely to be black and less likely to be HIV or hepatitis C-infected versus vitamin D-replete persons (Table 1).

Table 1. Participant characteristics by vitamin D status

Characteristic	≥20 ng/mL	<20 ng/mL	P-value
Mean age, y (SD)	48.5 (8.9)	49.1 (7.6)	0.35
Male, %	67	65	0.59
Black, %	86	93	0.001
Injection drug use within 6 months,%	37	36	0.80
HIV-infected,%	37	28	0.008
Median CD4+ count, cells/mm^3 (IQR)	332 (189-584)	269 (154-449)	0.05
Median HIV RNA, copies/mL (IQR)	65.5 (<40-21800)	1570 (<40-218000)	0.06
AIDS history,%	6	4	0.20
ART within 6 months, %	62	50	0.06
Hepatitis C positive,%	91	83	0.003
Diabetic, %	11	10	0.70
Hypertensive,%	47	53	0.06
Mean proteinuria, mg/g (SD)	236 (382)	286 (751)	0.33
Median eGFR,mL/min|1.73m^2 (IQR)	98.2 (80.4-114.7)	102.5 (85.5-114.6)	0.20

Abbreviations: SD, standard deviation; IQR, interquartile range; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate

In univariate analysis, vitamin D was not associated with proteinuria (OR≡1.36 per log-1ng/mL lower, p≡ 0.40). After adjustment for age, race, HIV and hepatitis C serostatus, diabetes, hypertension, and CKD, lower vitamin D levels were associated with over a 2-fold higher risk of proteinuria (OR≡2.27 per log-1ng/mL lower, p≡0.04).

Conclusions: Vitamin D deficiency is prevalent in injection drug users, though fewer HIV-infected persons have low vitamin D levels. After accounting for CKD and CKD risk factors, lower vitamin D levels are associated with a higher risk of proteinuria. Studies are needed to determine predictors of vitamin D deficiency and whether vitamin D repletion ameliorates proteinuria in this patient population.

Funding: NIDDK Support, Other NIH Support - NIDA

FR-PO1357

Plasma 25-Vitamin D, 1,25-Vitamin D, Parathyroid Hormone (iPTH), and Fibroblast Growth Factor 23 (FGF23) Levels Do Not Predict Cognitive Function in Patients with Advanced Chronic Kidney Disease (CKD)

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Background: Little is known about the relationship of abnormalities of mineral metabolism with cognitive impairment in CKD patients.

Methods: We investigate the longitudinal association between 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 levels and cognitive function in 605 patients with advanced CKD not requiring dialysis (n=247) and ESRD (n=358), aged, 67± 12 years who participated in a randomized clinical trial evaluating the effects of folic acid and B vitamins on death in subjects with advanced CKD. Cognitive function was measured with the telephone interview for cognitive status – modified, a brief telephone cognitive assessment similar to the mini-mental state exam. We used linear regression analyses to examine the association between abnormalities of mineral metabolism levels with impairment of cognitive function.

Results: Initial cognitive function was impaired in approximately 20% of patients regardless of treatment assignment (vitamin or placebo) or kidney disease status (advanced CKD or ESRD). Increasing log 1,25D levels were protective of cognitive impairment (β= 1.96±0.87; p=0.02) and increasing log iPTH ((β= -1.40±0.57; p=0.04) and log FGF23 levels (β= -1.04±0.29; p=0.004) were associated with worsening cognitive status in unadjusted analyses. In multivariate analyses including all predictor variables, age, gender, race, smoking, years of education, body mass index, treatment assignment, hypertension, diabetes and serum calcium and phosphate levels the associations between log 1,25-vitamin D, log iPTH, and log FGF23 were no longer significant (p>0.05 for each). Increasing log 25-vitamin D level was not associated with cognitive function in unadjusted and adjusted analyses.

Conclusions: In this study of patients with advanced CKD, plasma levels of 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 were not independently associated with cognitive outcomes in patients with advanced CKD.

Funding: NIDDK Support, Veterans Administration Support, Pharmaceutical Company Support

FR-PO1358

Mineral Metabolism and Physical Function in Patients Undergoing Hemodialysis Ranjani N. Moorthi,[1] Sharon M. Moe,[1] Joline L.T. Chen,[2] Carmen Castaneda-Sceppa.[4] *[1]Indiana University School of Medicine, Indianapolis, IN; [2]Boston University School of Medicine, Boston, MA; [3]Tufts Medical Center, Boston, MA; [4]Northeastern University, Boston, MA.*

Background: Chronic kidney disease patients have a phenotype of premature aging, inclusive of poor physical function. Declining eGFR is related to poor physical performance using both objective and subjective measures. Abnormalities of parathyroid hormone (PTH), Phosphorus (Pi), Calcium (Ca) as well as 25-hydroxy Vitamin D (25OHD) deficiency are widespread in dialysis patients and may play a role in poor physical / muscle function in these patients. In this analysis, we explore this potential relationship.

Methods: We examined the cross-sectional relationship of 25(OH)D, PTH, Pi, Ca and physical performance in the baseline population of a published RCT of low intensity exercise in 44 hemodialysis patients, using stored serum samples. Outcomes of interest were the Short Physical Performance Battery (SPPB), which includes measures of strength, endurance and balance and measures of lean body mass by Dual-energy X-ray absorpiometry (DXA). Leisure time physical activity was assessed by self report using the Physical Activity Scale for the Elderly (PASE).

Results: Serum samples were analyzed in 41 patients with median dialysis vintage of 2 years. 25OHD levels were less than 10 ng/ml in 38% of patients. A 10 pg/ml increase in PTH was associated with a 0.05 kg decrease in whole body lean mass (p=0.02) after adjusting for age, gender, randomization group, 25OHD, Ca and Pi. No relationship was noted between 25OHD, Ca or Pi and lean muscle mass. Also, no baseline mineral metabolism parameters were associated with SPPB or PASE scores.

Conclusions: We found a relationship between high PTH and low muscle mass by DXA. High PTH increases intracellular Ca in muscle, and therefore likely plays a role in muscle metabolism. Loss of muscle mass can be debilitating for CKD patients. We found no associations between 25OHD levels, Ca or Pi and muscle mass/ function or on measures of self report. This analysis provides further grounds for conducting interventional studies of agents to lower PTH in CKD patients to prevent loss of muscle mass.

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FR-PO1359

Cholecalciferol Supplementation Does Not Affect Insulin Sensitivity in Non-Diabetic Patients with Moderate Impairment of Renal Function Hamid R. Dezfoolian, Ellen Christoffersen, Maria Svensson. *Kidney Diseases, Sahlgrenska University, Gothenburg, Sweden.*

Background: Reduced insulin sensitivity is a well-known phenomenon in end-stage renal disease (ESRD), but is also present at other manifestations of kidney diseases such as micro albuminuria, nephrotic syndrome and mild-moderate reduced renal function. Alterations in vitamin D metabolism have been suggested to influence the insulin resistance.

The aim of this study was to evaluate the potentially effect of 10 weeks high dosage vitamin D3 (cholecalciferol) supplementation in non-diabetic patients with moderate chronic kidney disease (CKD).

Methods: 24 patients with non-diabetic CKD stages 3-4 (Glomerular filtration rate (GFR) measured using Iohexol® clearance 15-60 ml/min/1.73m^2), low serum 25-OH-vitamin D levels (< 75 nmol/L) and elevated fasting serum insulin levels (>10 mU/L) were included in a randomized, placebo-controlled, two-way cross-over study to receive daily either 3200 IU (80 µg) vitamin D3 (cholecalciferol; TillVal D®) or placebo in 10 weeks. Insulin sensitivity was assessed at the end of each treatment period as M-value, i.e. glucose infusion rate divided by lean body mass (estimated with bio impedance) during the assumed steady state (60-120 min) using a hyperinsulinemic (40 mU/m^2/min) euglycemic (5.6 mmol/L) clamp.

Results: 19 (79%) patients (M/F 13/6, age 65.8±13.7 years, BMI 28.8±4.7 kg/m2, GFR 35±10.7 ml/min/1.73m2 , serum 25-vitamin D 51±15 nmol/L and fasting insulin 15±7 mU/L; mean±sd) completed both treatment and placebo periods. No significant difference in insulin sensitivity (mean M-values) was found between cholecalciferol supplementation and placebo (8.0±3.5 vs. 8.1±3.3 mg/kg lean body mass/min, p=0.89) despite significantly difference in mean serum 25-OH-vitamin D between the placebo and vitamin D3 periods (52±14 vs. 85±17 nmol/L, p <0001).

Conclusions: Preliminary analyses reveal that supplementation with high dosage vitamin D3 (cholecalciferol) does not alter insulin sensitivity (M-value) in patients with non-diabetic moderate impaired kidney function (CKD 3-4).

FR-PO1360

Hypomagnesemia and Glomerular Hyperfiltration in Diabetes Mellitus Type 2 P.C. T. Pham,[1] P.M. T. Pham,[2] P.T. T. Pham.[3] *[1]Nephrology, UCLA-OVMC, Sylmar, CA; [2]Medicine, GLA VA Medical Center, Sepulveda, CA; [3]Kidney and Pancreas Transplant, UCLA, Los Angeles, CA.*

Background: Glomerular hyperfiltration (GlomHyper) and hypomagnesemia are commonly observed in diabetes mellitus type 2 (DM2). We examine the relationship between DM2 GlomHyper and hypomagnesemia.

Methods: DM2 patients without known kidney disease evaluated at UCLA-OVMC during January-March 2001 were included. Data retrieved include serum creatinine, hemoglobin, hemoglobin A1C (HbA1C), routine electrolytes, lipid profiles, urinalyses, history of hypertension, and pharmacy profiles. Estimations of the presenting glomerular filtration rate (eGFR) were determined by the CKD-epi formula. Multivariate analyses were performed to determine if any clinical factors were associated with GlomHyper, defined as eGFR greater than 120 mL/min/1.73 m^2.

Results: There were 550 patients (54% females); mean age 57.5±11.0 years; eGFR 95.7±14.8 mL/min/1.73 m2. Twenty-nine patients had GlomHyper. GlomHyper had significant negative correlations with age, hypertension, serum calcium, and the use of aspirin, RAS inhibitors, and diuretics, and a positive correlation with HbA1C. Although the correlation between serum magnesium and GlomHyper did not reach statistical significance, it had a significant negative correlation with eGFR. Analysis of the interaction between magnesium and calcium levels (calcium x magnesium) revealed a more significant correlation with GlomHyper than either calcium or magnesium level alone, Pearson coefficients: -0.13 (p=0.008),-0.11 (p=0.03), and -0.08 (p=0.07), respectively. Another multivariate analysis revealed a significant correlation with lower magnesium levels and GlomHyper in the stratum with calcium levels below median, but not in the higher calcium stratum (coefficient: -0.27, p=0.0001).

Conclusions: The interaction factor (magnesium x calcium) revealed the strongest correlation with GlomHyper compared to either factor alone. We speculate that the DM2 GlomHyper induces urinary loss of both cations, but while hypocalcemia directly exacerbates GlomHyper *via* hypocalcemia-associated afferent arteriolar vasodilation, hypomagnesemia exerts an indirect effect *via* hypoparathryoidism-induced hypocalcemia.

FR-PO1361

Fibroblast Growth Factor 23 and Chronic Kidney Disease Progression Madhumathi Rao,[1] Joachim H. Ix,[2] Priyanka Jain,[1] Temitope Ojo,[1] Andrew G. Bostom,[3] Vaidyanathapura S. Balakrishnan.[1] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Nephrology, University of California- San Diego, CA; [3]Renal Diseases, Rhode Island Hospital, Providence, RI.*

Background: Recent reports have suggested that fibroblast growth factor 23 (FGF-23) may be a risk factor for CKD progression. We assessed the relationship between circulating levels of intact FGF-23 and other mineral metabolism related factors (parathyroid hormone (PTH), serum calcium (Ca) and phosphate (P)) and their relationship to progression to end stage renal disease (ESRD).

Methods: We followed 196 clinically stable adult CKD (eGFR>15 ml/min/1.73m2) patients prospectively for a median of 35 months. Plasma levels of intact FGF-23 were measured by ELISA (Immutopics, San Clemente, CA).

Results: FGF-23 levels were above reference ranges in 6% with median (P25-P75) of 17 (11-29) pg/mL. The mean±SD age of the study population was 61±15 years, 57% were male, 19% African American and 32% diabetic; 19% of subjects were CKD KDOQI stage 1 and 2, 52% stage 3 and 29% stage 4. Hyperphosphatemia (P>4.5 mg/dL) was present in 9% and elevated PTH (PTH>65 pg/mL) in 58%. FGF-23 and PTH levels correlated inversely with eGFR and were positively correlated with each other (p<0.001). PTH levels were inversely related to serum Ca (r=-0.24, p=0.002) and FGF-23 levels to serum P levels (r=0.17, p=0.02). ESRD occurred in 35 participants, who had significantly lower eGFR and higher PTH and FGF-23 levels at baseline (all p<0.001). In a multivariate Cox model, the strongest predictors of CKD progression were eGFR, level of proteinuria and PTH at baseline (HR , 95% CI for doubling: 1.8, 1.2-1.8). FGF-23 levels were not related to outcome (HR 1.1, 95%CI: 0.7-1.8).

Conclusions: FGF-23 levels are inversely correlated with eGFR and contribute to abnormal mineral metabolism in CKD, but do not predict CKD progression. PTH may have a role in predicting CKD progression apart from its role as a risk factor for cardiovascular disease.

Funding: NIDDK Support

FR-PO1362

Differences between Hospitals in Attainment of Parathyroid Hormone Treatment Targets in Chronic Kidney Disease Do Not Reflect Differences in Quality of Care Mieke J. Peeters,[1,2] Arjan D. Van Zuilen,[3] Jan A.J.G. van den Brand,[1] Peter J. Blankestijn,[3] Marc A.G.J. Ten Dam,[2] Jack F. Wetzels.[1] *[1]Radboud University Nijmegen Medical Center, Nijmegen; [2]Canisius Wilhelmina Hospital, Nijmegen; [3]University Medical Center Utrecht, Netherlands.*

Background: Transparency in quality of care (QoC) is stimulated and hospitals are compared and judged on the basis of their performance on specific treatment targets. In patients with chronic kidney disease (CKD), QoC differed significantly between hospitals. [NDT 2010;25:3647-3654] This was not explained by available patient characteristics. In this analysis we explored additional parameters to explain differences between centers in attainment of PTH treatment targets.

Methods: Using baseline data of the MASTERPLAN study, we selected one of the worst (center A) and one of the best (center B) performing hospitals. Differences between the two treatment centers were analyzed from the year prior to start of the MASTERPLAN study until the baseline evaluation and determinants of PTH were assessed.

Results: 101 patients from center A (median PTH 9.9 pmol/L, 63 patients above target) and 100 patients from center B (median PTH 6.5 pmol/L, 32 patients above target), were included. In multivariate analysis kidney transplant status, MDRD-4, and treatment center were independent predictors of PTH. However, when MDRD-6 (which accounts for serum urea and albumin) was used instead of MDRD-4, the center effect was reduced. Moreover, after calibration of the serum creatinine assays treatment center no longer influenced PTH. Analysis of clinical practice did not reveal differences in PTH management between the centers. Notably, hyperparathyroidism resulted in a change in therapy in less than 25% of patients.

Conclusions: Results of hospital performance comparisons should be interpreted with great care. We show that differences in PTH control between center A and B are not explained by differences in treatment, but depend on incomparable patient populations and laboratory techniques. We propose the use of the MDRD-6 formula to estimate GFR in analyses in which kidney function plays a central role.

Funding: Pharmaceutical Company Support, Private Foundation Support

FR-PO1363

An Analysis of the Presence of Pathogens in Hemodialysis Subjects Using Ibis Technology: Results from the IMPACT Study Comparing Paricalcitol and Cinacalcet for the Treatment of Secondary Hyperparathyroidism (SHPT) Markus Ketteler,[1] Kevin J. Martin,[2] Mario Cozzolino,[3] David J. Goldsmith,[4] Amit Sharma,[5] Michael Amdahl,[6] Samina Khan.[6] *[1]Klinikum Coburg; [2]Saint Louis U; [3]Paolo Hosp; [4]Guy's Hosp; [5]Boise Kidney and Hypertension Inst; [6]Abbott.*

Background: Hemodialysis patients have an increased risk of acquiring infections due to immunocompromised state and repeated venipuncture. In a significant number of dialysis patients, the nature of infection remains obscure, leading to suboptimal treatment. Timely and accurate diagnosis of pathogens causing clinical or subclinical infections is essential to optimize care in this patient population.

Methods: IMPACT was a randomized 28 wk, phase 4, international, open-label study of subjects undergoing hemodialysis receiving IV (IV Stratum) or oral paricalcitol (Oral Stratum) with supplementary cinacalcet for hypercalcemia, or cinacalcet with low-dose vitamin D. Ibis, a technology combining PCR and electrospray ionization mass spectrometry, can detect a broad range of pathogens. Baseline and final samples were tested using Ibis. Ibis is currently an investigational research tool; the clinical implications and interpretations of these results are not established.

Results: In IV Stratum there were 62 paricalcitol subjects and 64 cinacalcet subjects (60% male, mean age:61±12 years, mean duration of dialysis:4.1±4.0 years). In Oral Stratum there were 72 paricalcitol subjects and 70 cinacalcet subjects (65% male, mean age:65±13 years, mean duration of dialysis:3.9±3.2 years). Ibis results are shown below.

	IV Stratum		Oral Stratum	
	Paricalcitol N=62	Cinacalcet N=64	Paricalcitol N=72	Cinacalcet N=70
Bacterial or Fungal infections, n				
Baseline	0	2	9	7
Final	6	6	8	4
Viral infections, n				
Baseline	1	1	3	5
Final	1	0	4	2

Conclusions: Overall, a variety of bacterial, fungal, and viral species were detected using Ibis. This is the first report of Ibis results in hemodialysis subjects. Once widely available, Ibis technology may help aid in the timely identification of pathogens leading to optimal treatment of infections.

Funding: Pharmaceutical Company Support

FR-PO1364

Circulating Klotho Is Decreased in Pediatric Hemodialysis Patients Daniel Ranch,[1] Mazen Y. Arar,[1] Farzana Perwad.[2] *[1]Dept. of Pediatrics, University of Texas Health Science Center, San Antonio, TX; [2]Dept. of Pediatrics, University of California, San Francisco, CA.*

Background: Klotho is both a membrane-bound and circulating protein produced by the kidney. Membrane-bound klotho is a critical cofactor for the hormone fibroblast growth factor-23 to regulate phosphorus and vitamin D homeostasis. Circulating klotho protein has been detected in blood and urine in humans; however its functions are unknown. In mice, klotho deficiency induces vascular calcifications, osteopenia, and premature aging, a phenotype similar to chronic kidney disease (CKD). Wild-type mice with CKD have decreased plasma and urinary klotho compared to controls. Over-expression of klotho in these mice decreases soft tissue calcification, preserves renal function and delays progression of CKD. Recent studies in adult CKD patients showed a progressive decrease in urinary klotho with worsening kidney function, but whether serum klotho levels are affected by CKD is unknown in both adult and pediatric patients. In this study we hypothesized that circulating klotho is decreased in pediatric patients with CKD.

Methods: Twelve pediatric patients on chronic hemodialysis (4 males and 8 females, 2-17 yrs of age) and 9 healthy controls (1 male and 8 females, 3-12 yrs of age) were recruited.

Results: Serum klotho levels ranged from 1263 to 5265 pg/ml in healthy controls, and from 378 to 5389 pg/ml in hemodialysis patients. Mean serum klotho levels were lower in hemodialysis patients compared to controls (1542 ± 1569 vs 2741 ± 1355 pg/ml, $p<0.05$). In hemodialysis patients, serum klotho levels inversely correlated with age ($r=0.74$, $p<0.01$) and directly with serum calcium concentrations ($r=0.62$, $p<0.05$). No significant correlation was found between serum klotho and serum phosphorus ($r=0.33$), calcium-phosphate product ($r=0.23$), serum intact parathyroid hormone ($r=0.2$), serum 25(OH)D ($r=0.12$), or serum 1,25(OH)2D ($r=0.26$). No correlation between serum klotho levels and age or serum calcium was found in the control group.

Conclusions: These results prove our hypothesis that circulating klotho is decreased in pediatric CKD patients. Further studies are needed to determine the role of klotho in CKD pathophysiology.

FR-PO1365

Obesity-Related Glomerulopathy: Mast Cells Infiltration and Tubulointerstitial Lesion Xufang Wang, Ming-Chao Zhang, Chun-Xia Zheng, Honglang Xie, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China.*

Background: Tubular atrophy and interstitial fibrosis are the most valuable predictors of renal function in obesity related glomerulopathy (ORG). However, the studies of ORG have been mainly focused on glomerular injury, and we know little about the mechanism of tubulointerstitial injury. Mast cells have been implicated in tubular injury in multiple kidney diseases, including diabetic nephropathy. To investigate if mast cells are also involved in tubulointerstitial injury of ORG, we examined mast cell infiltration and its correlation with tubular injury and renal function.

Methods: 39 patients with biopsy-proven ORG and 10 non-obese donors as control were included in this study. Tryptase and CD68 were used for detecting mast cells and macrophages by immunohistochemistry.

Results: Mast cells were detected in fibrotic interstitial areas mostly, but not within glomeruli. The density of mast cells was significantly increased in ORG compared with the control group, and it was well correlated with body mass index (BMI) ($r=0.364$, $P=0.023$), systolic blood pressure ($r=0.459$, $P=0.003$), serum creatinine ($r=0.637$, $P<0.001$), tubular atrophy ($r=0.47$, $P=0.003$) and interstitial fibrosis ($r=0.669$, $P<0.001$), in contrast, it was negatively correlated with eGFR ($r=-0.559$, $P<0.001$). Macrophages accumulated focally in the tubular interstitium with other infiltrated cells, and their density was positively correlated with the number of interstitial infiltrating cells ($r=0.476$, $P=0.002$). No correlation was found between the numbers of mast cells and macrophages. Multivariate regression modeling showed that the number of mast cells was the critical factor for eGFR ($R2=0.44$, $P=0.036$).

Conclusions: Our data show that patients with ORG have an increase in the number of interstitial mast cells, which was correlated positively with BMI, blood pressure, serum creatinine, and tubular injury, but negatively with eGFR, suggesting a role for mast cells in tubulo interstitial lesion. Further functional studies are required to determine the role for mast cells in tubular injury of ORG.

FR-PO1366

Fibroblast Growth Factor-23 Predicts Renal Failure Progression in Children with Chronic Kidney Disease Elke Wuehl,[1,2] Gianluigi Ardissino,[1] Tomasz F. Urasinski,[1] Salim Caliskan,[1] Franz S. Schaefer.[1,2] *[1]ESCAPE Trial Group; [2]Pediatric Nephrology, Center for Pediatrics and Adolescent Medicine, University of Heidelberg, Heidelberg, Germany.*

Background: Fibroblast growth factor-23 (FGF-23), a phosphaturic hormone involved in calcium phosphate homeostasis, appears to predict renal disease progression in adults with non-diabetic CKD (Fliser, JASN 2007).

Methods: To determine renal survival according to FGF-23 serum levels in children with CKD stage II-IV, 232 children participating in the ESCAPE trial (age 11.5±4 yrs, GFR 45±18 ml/min/1.73m²; underlying renal disease: hypo/dysplasia (N=164), glomerulopathies (N=28), hereditary or other (N=40)) were analyzed. All patients received fixed dose ACE inhibition and were followed prospectively by 2-monthly examinations for up to 5 years. The study endpoint was defined by eGFR loss >50% from baseline, GFR <10ml or start of renal replacement therapy. FGF-23 levels were determined at baseline (C-terminal human FGF-23 ELISA (Immutopics, San Clemente, CA, USA)).

Results: Serum FGF-23 levels (mean 52±81 RU/ml) were correlated with estimated GFR ($r=-0.47$, $p<0.0001$), the annualized change of eGFR at baseline (-0.21, $p=0.002$), the urinary protein-creatinine ratio (0.24, $p=0.0007$) and serum phosphate levels (0.20, $p=0.004$). The risk of attaining the renal endpoint was 59.5% in patients with serum FGF-23 in the upper distribution quartile, as compared to 26.9% in patients with lower levels ($p<0.0001$). The relative risk of attaining the endpoint was increased by FGF-23 independently of age, eGFR, proteinuria, serum phosphate and the underlying renal diagnosis. Each increase of FGF-23 by 50 RU/ml was associated with an increase in the relative risk by 19% (Hazard ratio 1.19, CI 1.035-1.367, $p=0.01$).

Conclusions: Serum FGF-23 is an independent predictor of progressive renal failure in children with CKD undergoing ACE inhibition. Further studies are needed to evaluate the interplay between FGF-23, parathyroid hormone, Vitamin D, calcium and phosphate levels on renal disease progression and cardiovascular morbidity and mortality.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1367

Changes in Serum Uric Acid Have Reciprocal Effect on eGFR Change: A 10-Year Follow-Up Study of Community-Based Screening in Okinawa, Japan Kunitoshi Iseki. *Dialysis Unit, University Hospital of the Ryukyus, Nishihara, Okinawa, Japan.*

Background: Hyperuricemia is common among hypertension and metabolic syndrome therefore it could be a cause and result of these comorbid conditions. Few studies, however, have examined the relationship between the presence/absence of hyperuricemia and the changes in eGFR among the large cohort of general population.

Methods: We examined the participants who visited twice at 1993 and 2003 screening in Okinawa, Japan, and have data serum creatinine and uric acid. Total number was 16,796. Serum creatinine was measured by Jaffe method in 1993 and the enzymatic method in 2003 Serum creatinine was measured by the enzymatic method (converted to the value of measured by enzymatic method) and the estimated GFR (eGFR, ml/min/1.73m²) was

calculated by the Japanese Society of Nephrology (Matsuo S et al. Am J Kidney Dis 2009). Hyperuricemia (H) was defined as serum uric acid 7.0mg/dl and over in both sexes, and others were Normouricemia (N). Based on the absence or presence of H in 1993 and 2003 screening, we categorized into 4 groups as: Group 1 to 4 of N/N, H/N, N/H, and H/H. UA, serum uric acid (mg/dl). Trends were analyzed by analysis of variance.

Results: In all categories, ΔUA was significantly associated withΔeGFR, suggesting that increase (decrease) in serum uric acid was associated with decrease (increase) in eGFR. Subjects with persistent hyperuricemia (H/H) and developed hyperuricemia (N/H) showed higher decline in eGFR, whereas those with persistent normouricemia (N/N) and returned to normouricemia (H/N) showed slower decline in eGFR.

Relation between change in eGFR & UA

	Group 1	Group 2	Group 3	Group 4
Number	12794	1007	1453	1376
UA, '93/'03	4.9/5.1	7.9/6.1	6.1/7.8	8.0/8.2
eGFR, '93/'03	81.3/71.8	74.0/70.0	78.9/64.4	76.6/66.7
eGFRin '93	90 and over	60-89	45-59	<45
Number	4551	9970	2126	146
ΔeGFR/ΔUA	-3.0**	-3.0**	-2.6**	-1.5#

**P<0.0001, #P=0.020 by ANOVA

ΔeGFR/ΔUA* were calculated after adjusting for age, sex, and baseline eGFR.

Conclusions: Results suggest that maintaining normal range of serum uric acid is important to maintain eGFR.

Funding: Government Support - Non-U.S.

FR-PO1368

CKD Stage Modifies the Association of Urine Microalbumin-Creatinine Ratio (UACR) with Loss of Kidney Function in a Large Cohort of US Veterans

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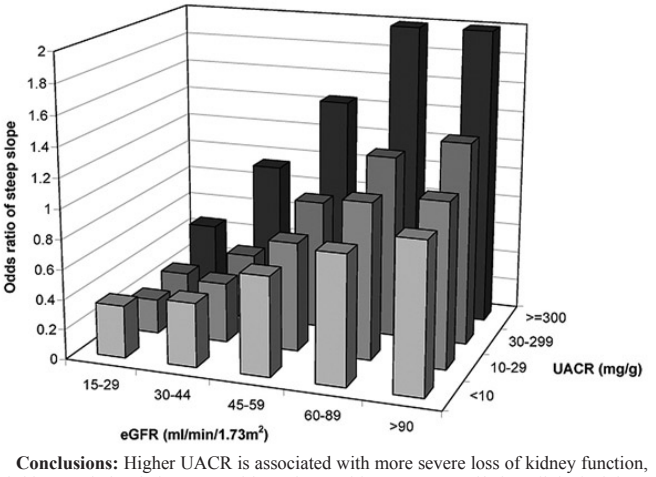
Background: The association of albuminuria with the progression of CKD in patients with various stages of CKD is not well characterized.

Methods: We examined the association of UACR with the slopes or eGFR in a nationally representative cohort of 275,479 US veterans with at least 3 eGFR values recorded after 2005. Slopes were calculated using a median (interquartile range) of 8 (5-13) eGFR values over up to 5 years of follow-up. Associations of UACR with slopes overall and stratified by baseline CKD stage were examined in linear and logistic regression models. Models were adjusted for sociodemographics, comorbidities, blood pressure and laboratory variables.

Results: The median (interquartile range) of eGFR slopes was -1.25 ml/min/1.73m2/year (-3.85, 0.92). A 1-unit increment in UACR on natural-log scale was associated with -0.37 ml/min/1.73m2/year steeper slopes (95%CI: -0.38, -0.36; p<0.001) in unadjusted analyses and with -0.25 ml/min/1.73m2/year steeper slopes (-0.26, -0.23; p<0.001) after multivariable adjustments. A linear association of UACR with steeper slopes (defined as slopes <-4 ml/min/1.73m2/year) was seen in patients with eGFR ≥45 ml/min/1.73m2. In patients with eGFR 30-44 only UACR >300 was associated with steeper slopes, and in patients with eGFR 15-29 the association appeared U-shaped, with UACR <10 mg/g also showing an association with steeper slopes (Figure).



Conclusions: Higher UACR is associated with more severe loss of kidney function, and this association is incremental in patients with eGFR >45 ml/min. Clinical trials to examine the effect of lowering UACR to improve renal outcomes in early CKD stages are indicated.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1369

Aristolactam-DNA Adducts in the Renal Cortex: Biomarkers of Internal (Environmental) Exposure to Aristolochic Acid

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Background: Endemic (Balkan) nephropathy (EN) is a chronic tubulointerstitial disease that is highly associated with urothelial cell carcinomas (UUC) of the upper urinary tract. Both diseases have been linked to exposure to aristolochic acid (AA), a nephrotoxin and carcinogen produced by Aristolochia plants that grow in wheat fields in endemic sites and contaminate flour used to prepare bread. AA reacts with DNA to form aristolactam-DNA (AL-DNA) adducts that lead to A:T transversions in the tumor suppressor gene TP53. Due to inefficient repair, adducts persist for years in the renal cortex and thus serve as an exposure biomarker. Here, we present evidence linking AA exposure to UUC in endemic regions of Bosnia, Croatia and Serbia that harbor EN.

Methods: DNA was extracted from renal cortex and tumors obtained from 67 patients from endemic sites who underwent nephroureterectomy for UUC. Ten subjects from nonendemic sites served as controls. Renal cortical AL-DNA adducts were quantified by a ^{32}P-postlabelling assay. TP53 mutations in UUC were identified by chip-sequencing technology.

Results: Most endemic subjects had observed Aristolochia plants in their wheat fields in the past, and were therefore likely to have ingested AA-contaminated bread. We detected renal cortical AL-DNA adducts in 70% of the endemic cohort, and the chemical identity of these lesions was verified by mass spectroscopy. A:T mutations in TP53 were present in 25% of the endemic cases, and AL-DNA adducts were also found in 94% in these cases, emphasizing the close association of these two biomarkers. In contrast, neither AL-DNA adducts nor TP53 mutations were detected in nonendemic subjects.

Conclusions: Aristolochic acid is the primary causative agent of urothelial carcinomas of the renal pelvis and ureter in EN subjects.

Funding: Other NIH Support - NIEHS PO1ES004068 and RO1ES019564, Government Support - Non-U.S.

FR-PO1370

Sustained-Release Tablets of Orally-Active Prostacyclin Analogue, Beraprost Sodium, for Patients with Non-Diabetic Chronic Renal Failure

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Background: Increasing evidence points to the protective effects of prostacyclin on kidney in pathophysiological conditions. Several nonclinical studies have suggested beraprost sodium (BPS), an orally active prostacyclin analogue, prevents progression of chronic renal failure (CRF) by maintaining renal blood flow and attenuating tubulointerstitial hypoxia.

Methods: This study was designed as a randomized double-blind placebo-controlled comparative study in CRF patients [serum creatinine (SCr) 1.5 to 4.5 mg/dL (male), 1.3 to 4.0 mg/dL (female)] to evaluate the effect of sustained-release tablets of BPS (TRK-100STP) on the progression of non-diabetic CRF in patients. The patients were treated with TRK-100STP twice daily at 120 μg/day (n=36), 240 μg/day (n=41) or placebo (n=35) for 28 weeks after a 22-week run-in period in which the patients were treated with the placebo.

Results: The primary endpoint (difference in 1/SCr slope between the run-in period and treatment period for the 240 μg group) showed no statistically significant difference, however, a significant change was observed in the 120 μg group. Sub-population analysis of patients with a SCr of 2.0 mg/dL or higher showed a significant amelioration of the 1/SCr slope in the 240 μg group, and improvement of the eGFR in both active groups. The main ADRs observed in the 112 patients were headache and hot flush, which were expected from BPS's vasodilative effects, in addition to diarrhea and vomiting. However, none of the ADRs was clinically significant.

Conclusions: The results indicate that TRK-100STP has a beneficial effect on the progression of non-diabetic CRF, especially in patients with SCr of 2.0 mg/dL or higher. An international P-IIb/III study using a renal composite endpoint has already started in Japan, China, Hong Kong, South Korea, Taiwan, Malaysia, Thailand and Philippines.

Funding: Pharmaceutical Company Support

FR-PO1371

Risk Factors for Renal Disease Progression after Orthotopic Liver Transplantation

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Background: Chronic kidney disease (CKD) is an important long-term complication in orthotopic liver transplantation (OLTx) patients. Few studies have assessed risk factors for CKD progression in post-Tx patients in the era of the Model for End-Stage Liver Disease (MELD).

Methods: Using medical record and UNOS registry data from May 1995 to April 2009, this non-concurrent prospective cohort study followed 419 primary OLTx adults at a tertiary hospital for up to 2 years. Pre-Tx and 3-, 6-, 12-, and 24-month post-Tx glomerular filtration rates were estimated (eGFR, using the CKD-Epi formula), along with other baseline clinical parameters. The pre-MELD era was defined as time until February 2002.

Results: The mean age was 51 years, 67% of the cohort was male, and 39% received OLTx in the pre-MELD era. The median pre-transplant and 12-month post-Tx eGFRs were 82 and 63 ml/min/1.73m2, respectively, representing a 20% decline in eGFR. After 24 months, the median eGFR was 59. Over 12 months, eGFR declined >33% in 31% of participants; and for those followed for 24 months, 42% declined >33%. Among those with pre-Tx eGFR ≥90, 60-89, and 30-59 ml/min/1.73m2, 70%, 52%, and 9% declined to a lower eGFR category over 12 months, respectively. Decline in eGFR primarily occurred in the first three months post-Tx. Progression to a lower eGFR category was associated with age>55 (versus age<45 years; adjusted odds ratio, AOR=8.0, p<0.001), and marginally associated with hemodialysis in the post-Tx period (AOR=4.8, p=0.08), and diabetes (AOR=1.9, p=0.09). No association was present with gender, race, hepatorenal syndrome, hypertension, or hepatitis C virus. After 24 months, eGFR decline >33% was lower in the MELD era compared to the pre-MELD era (AOR=0.5, p=0.03).

Conclusions: Renal function declined substantially after OLTx, and was associated with older age, post-Tx hemodialysis requirement, diabetes, pre-MELD era, and higher pre-Tx eGFR.

FR-PO1372

Fibroblast Growth Factor 23 Is a Risk Factor for Rapid Progression of Chronic Kidney Disease in Elderly Patients Eiichiro Kanda,[1] Sei Sasaki.[2] *[1]Tokyo Kyosai Hospital, Tokyo, Japan; [2]Tokyo Medical and Dental University, Tokyo, Japan.*

Background: The level of fibroblast growth factor 23 (FGF23) is increased at later stages of chronic kidney disease (CKD). FGF23 as a risk factor for the progression of CKD in elderly CKD patients has not been fully established.

Methods: 105 elderly CKD patients who had never used calcium or vitamin D supplements were enrolled in this study in Tokyo, Japan. We compared estimated glomerular filtration rate (eGFR) at the start of the study with that two months later and evaluated whether the decrease in eGFR was more than 5%. Urinary protein, serum calcium, phosphate, 1,25$(OH)_2$ vitamin D (1,25-VD), intact parathyroid hormone (iPTH), and FGF23 levels were measured at the start of the study. Factors were assessed using multivariate logistic model and regression analysis adjusted for age and gender.

Results: The following results were obtained: average age (SD), 73.2 (7.7) years; eGFR, 45.7 (24.1) ml/min; median urinary protein, 201.1 mg/day (IQR 61.9-700.6); median FGF23, 49.0 pg/ml (IQR 34.0-71.0); female, 32.4%. A decreased eGFR was observed in 22.9% of the patients. The percentages of patients with high FGF23 levels (>71.0 pg/ml) were as follows: Stage 3, 50%; Stage 4, 50%; Stage 5,100%. Univariate analysis showed that FGF23 level correlated with eGFR, urinary protein, serum calcium, phosphate, 1,25-VD, and iPTH levels (P<0.0001). Multivariate analysis showed that FGF23 level was associated negatively with eGFR (P<0.0001) and positively with urinary protein level (P=0.005).

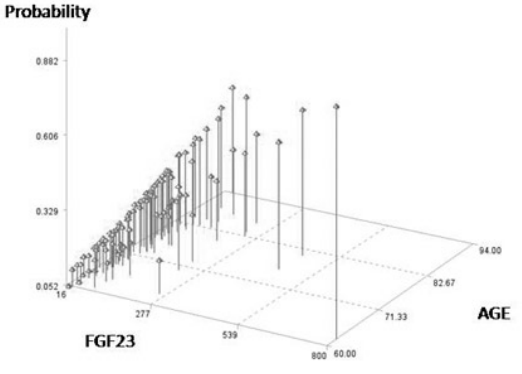


Figure Probability of the decrease in GFR increases according to FGF23 levels and age.

High FGF23 levels were associated with the decrease in GFR adjusted odds ratio, 4.35 (95% confidence interval 1.54-12.23).

Conclusions: In CKD patients, our findings suggest suggested that FGF23 level is associated with the progression of CKD. To detect and prevent the progression of CKD, FGF23 level should be monitored from an early CKD stage.

FR-PO1373

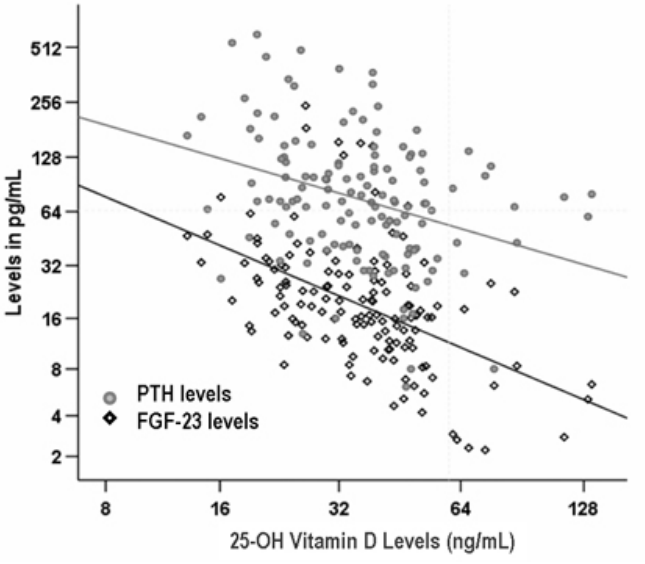
Vitamin D Deficiency in Chronic Kidney Disease and Its Role in Disease Progression Priyanka Jain,[1] Temitope Ojo,[1] Carmen Castaneda-Sceppa,[2] Vaidyanathapura S. Balakrishnan,[1] Madhumathi Rao.[1] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Health Sciences, Northeastern University, Boston, MA.*

Background: 25-OH Vitamin D (VitD) deficiency is common in chronic kidney disease (CKD) and is associated with increased morbidity and mortality. We examined risk factors for VitD deficiency and its association with mineral metabolism parameters and CKD progression.

Methods: 196 clinically stable adult CKD (eGFR >15 ml/min/1.73m2) patients were followed for a median of 35 months. VitD levels were measured by liquid chromatography and tandem mass spectroscopy. Intact parathyroid hormone (PTH) in serum was measured by non-competitive chemiluminescent immunoassay and intact fibroblast growth factor 23 (FGF-23) in plasma by ELISA (Immutopics, San Clemente, CA).

Results: The mean±SD age of the study population was 61±15 years; 57% were male, 19% African American and 32% diabetic; 19% of subjects were CKD KDOQI stage 1 and 2, 52% stage 3 and 29% stage 4; 34% were VitD insufficient and 25% VitD deficient. VitD levels did not vary by age, gender, race or severity of CKD, but showed a significant inverse correlation with PTH and FGF-23 levels (r, p-value: -0.24, 0.002; -0.22, 0.002). A 10 ng/mL increase in VitD levels was associated with 0.9 (95% CI 0.8-1.0) pg/mL lower PTH levels (p=0.007) and 0.9 (95% CI 0.8-1.0) pg/mL lower FGF-23 levels (p=0.004). Low VitD levels appeared to be associated with a greater risk of CKD progression (HR, 95%CI per 10 ng/mL increase: 0.5, 0.4-0.8; p=0.002) on univariate analysis but not after adjustment for baseline kidney function and PTH levels.

Conclusions: In summary, low VitD levels are common in patients with CKD and appear to have an impact on regulatory factors involved in mineral metabolism. The implications for longer term outcomes need further study.



Funding: NIDDK Support

FR-PO1374

Comparison between the Steroid Pulse Mono-Therapy and the Combination Therapy of Steroid Pulse and Tonsillectomy for IgA Nephropathy Ayami Ochi, Takahito Moriyama, Kayu Nakayama, Kosaku Nitta. *Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Steroid pulse therapy reported by Pozzi C *et al.*has been recognized as long-term effective therapy for IgA nephropathy (IgAN). In this decade, the combination of tonsillectomy and steroid pulse therapy has been widely performed in Japan. However there is no report to compare those two therapies.

Methods: In this prospective cohort analysis, we compared clinical findings at renal biopsy, histological findings according to the Oxford classification, and the ratio of complete remission rate (RR) of urinary protein excretion (U-Prot) and urinary red blood cells (U-RBC) at one year after treatment between 26 newly diagnosed IgAN patients received tonsillectomy and steroid pulse therapy (TaSP group), and 15 newly diagnosed IgAN patients received steroid pulse mono-therapy (SP-group).We defined clinical remission as U-Prot< 0.5 g/gCr and U-RBC <5 counts/HF.

Results: Clinical and histological characteristics at baseline between both groups did not differ (TaSP vs. SP group; mean eGFR: 68.2 vs. 68.4 ml/min, mean U-Prot: 0.63 vs. 0.51 g/day, and mean U-RBC 25 vs. 20 counts/HF). RR of U-Prot analyzed by the Kaplan-Meier method did not differ between both groups (76.9 vs. 60.0 %). However RR of U-RBC was significantly higher in TaSP group than in SP group (80.0 vs. 33.0 %, Log rank test; P=0.0053). Moreover, RR of both U-Prot and U-RBC was significantly higher in TaSP group than in SP group (65.0 % vs. 13.3 %, Log-rank test; P=0.0029). The cox regression analysis showed that combination therapy was associated with clinical remission (HR:12.07, 95%CI: 2.80-90.91, P= 0.0003).

Conclusions: Combination therapy of tonsillectomy and steroid pulse therapy showed higher clinical remission rate of urinary findings at one year after treatment in comparison to the steroid pulse mono-therapy in patients with IgAN. Long term observation for renal survival should be analyzed in the future.

FR-PO1375

von Willebrand Factor Synthesis and Circulating Half Life and Osteoprotegerin Levels Are Progressively Elevated in Stage 3-5 CKD Patients Cynthia M. Pruss,[1] Spencer Barr,[1] Julie Grabell,[2] Angie Tuttle,[2] Michael A. Adams,[1] Jocelyn S. Garland,[2] Paula James,[2] Rachel M. Holden.[2] *[1]Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; [2]Medicine, Queen's University, Kingston, ON, Canada.*

Background: Von Willebrand Factor (VWF) and osteoprotegerin (OPG) are co-secreted by the endothelium and remain associated in the circulation. VWF and OPG are biomarkers for endothelial dysfunction and have been linked to cardiovascular disease but have not been evaluated longitudinally in individuals with CKD.

Methods: VWF antigen (VWF:Ag), VWF propeptide (VWFpp), OPG, and IL-6 levels were measured at baseline and at 4 years in a cohort of individuals with stage 3-5 CKD (N=54, mean age 62 years, 44% female) and were compared to age matched controls (N=38, 67.2 years, 58% female). The VWFpp to VWF:Ag ratio (VWFpp/VWF:Ag) was used to evaluate VWF circulating time, with ratios <1 representing increased VWF half life.

Results: At baseline, VWF:Ag, OPG and IL-6 were significantly higher than age-matched controls. Within the CKD cohort, there were significant increases in VWF:Ag, VWF:pp, VWFpp/Ag, and OPG between baseline and 4 years. The VWFpp/VWF:Ag ratio was significantly lower in CKD patients indicating an increase in VWF circulating half life. OPG levels correlated positively with the VWF:Ag ($p<0.001$), VWFpp ($p<0.001$), and negatively with GFR ($p<0.05$) when all CKD measurements were pooled.

Average Values for CKD Patients and Normal Controls

	CKD, Initial	CKD, 4 years	Normal Controls
VWF:Ag (U/ml)	1.67 ± 0.65	1.93 ± 0.72	1.32 ± 0.47
VWFpp (U/ml)	1.30 ± 0.43	1.75 ± 0.46	1.38 ± 0.29
VWFpp/VWF:Ag	0.83 ± 0.22	0.98 ± 0.30	1.17 ± 0.18
OPG (ng/ml)	2.91 ± 1.59	7.89 ± 4.23	1.13 ± 1.54
IL-6 (pg/ml)	7.38 ± 13.1	8.77 ± 17.41	2.42 ± 3.33
GFR (ml/min)	27.9 ± 10.6	23.0 ± 10.0	(-)

Values presented are average ± SD.

Conclusions: The role of endothelial dysfunction is emerging as a key contributor to cardiovascular disease. This study demonstrates that there is a significant increase in VWF synthesis in the earlier stages of CKD that is progressive over time. The relationship between endothelial dysfunction, VWF synthesis and OPG requires further study.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1376

Genetic Variant of the Renin-Angiotensin-Aldosterone System (RAAS) and Renal Survival in Japanease Patients with Chronic Kidney Desease (CKD) Yasukazu Makino, Tadashi Konoshita. *Third Department of Internal Medicine, Fukui University School of Medicine, Eiheiji, Fukui, Japan.*

Background: Chronic kidney disease (CKD) is a public health problem, and inhibiting progression of CKD patients is a major task for the nephrology.The renin-angiotensin-aldosteron system (RAAS) may play pivotal role in the progression of CKD. And genetic polymorphism of RAAS has been reported to be associated with the development of some type of renal disease. However there has been few investigation of RAAS genetic variation and CKD.progression in a large population-based study.

Methods: We enrolled 1055 CKD patients who consulted nephrologist in our hospital between January, 1995, and December, 2010. All patients were Japanease. 486 (48.2%) reached end stage renal disease (ERSD) at an average age of 55.1±22.3 years. We estimeted the association between cumultive renal survival and five polymorphism of RAAS. The investigated genetic polymorphism are renin enhancer region (REN) C-5312T, angiotenogen (AGT) M235T, angiotensin converting enzyme ACE insertion/deletion, angiotensinIItypeIrecptor (ATR1) A1166C, and aldosterone synthase CYP 11B2 C-344T. For statistical analysis of the time course to ESRD,a cumulative survival analysis using the Kaplan-Meiyer method with log-rank test.

Results: Cumulative renal survival in CKD was significantly less in those with TT genotype in (REN C-5312T) [log-rank,P=0.0071 X2=7.380].There was no association between cumulative survival and M235T, ACEI/D,A1166C,and C-344T polymorphism.

Conclusions: REN C-5314T polymorphism may play a role CKD progression. And this polymorphism affect prognosis in CKD patient.

FR-PO1377

Circulating Bone Morphogenetic Protein-1 (BMP7) and Transforming Growth Factor-β1 (TGFβ1) as Potential Biomarkers for Renal Endpoint in Patients with Type 2 Diabetes Mellitus Muh Geot Wong,[1] Min Jun,[2] Usha Panchapakesan,[1] Mark Woodward,[2] Xinming Chen,[1] John P. Chalmers,[2] Vlado Perkovic,[2] Carol A. Pollock.[1] *[1]Kolling Institute of Medical Research, University of Sydney, NSW, Australia; [2]George Institute for Global Health, University of Sydney, Camperdown, NSW, Australia.*

Background: Albuminuria and reduced estimated glomerular filtration rate (eGFR) are known predictors of decline in kidney function for patients with diabetes mellitus. There is an increasing need for additional biomarkers which can predict renal outcome in the earlier stages of diabetes. Aims: To assess the baseline circulating value of TGFβ1 and BMP7 in patients with type 2 diabetes mellitus, and to establish the relationship of these markers with the risk of progressive diabetic nephropathy.

Methods: Serum samples from 124 participants of the ADVANCE Collaborative Group study, were studied. Cases were defined as those who developed a renal endpoint; ie. doubling of serum creatinine to at least 200umol/l, the need for renal replacement therapy, or death due to renal disease. Using propensity score methodology, controls were matched for age, sex, race, baseline estimated glomerular filtration rate (eGFR) (<60 vs. >60), urinary albumin:creatinine ratio (UACR), baseline blood pressure, baseline HbA1c, known macrovascular disease, history of retinopathy and treatment allocation. Enzyme linked immunosorbent assays were used to analyse total and active circulating TGFβ1 and BMP7 at baseline.

Results: Individuals with type 2 diabetes who developed renal endpoints (n=52) had a significantly higher circulating total TGFβ1, 12684.7 pg/ml vs. 7501.6 pg/ml, ($p<0.01$) and lower circulating BMP7 levels 6.89 pg/ml vs. 19.33 pg/ml ($p<0.0001$), compared to controls (n=72) respectively. Adjusted sensitivity analyses revealed TGFβ1 is a positive predictor (OR=1.9, 95% CI 1.30-2.30, p= 0.0002) and BMP7 is a negative predictor (OR=0.37, 95% CI 0.25-0.62 $p<0.0001$) for renal endpoints, independent of other conventional risk factors such as UACR and eGFR and each other.

Conclusions: Circulating BMP7 and TGFβ1 have prognostic potential in predicting poor renal outcomes in type 2 diabetes mellitus.

FR-PO1378

Aging and Progression of Chronic Kidney Disease Rachel Bregman, Renata De Souza Mendes, Carla C.S. Lemos, Frances Silva, Maria Ines Barreto Silva. *Nephrology, State University of Rio de Janeiro, Rio de Janeiro, Brazil.*

Background: Aging is a reality in the modern world and is pointed as a risk factor for Chronic Kidney Disease (CKD). The aim of this study was to analyze the progression of CKD in elderly.

Methods: We evaluated 236 patients (group1) with 60-74 years (y) and 72 patients with 75 years or more (group 2). Mean age (y) (±SD) was 68±4 and 80±4; time on treatment (y) was 3.8±3 and 3.6±3 respectively. Group 1 presented as baseline diseases hypertension (H) in 37% and diabetes mellitus (DM) in 41%; and group 2 H:53% and DM:30%. Patients were under treatment with a multidisciplinary team. Glomerular filtration rate was estimated (eGFR) by MDRD.

Results: At the time of referral MDRD (ml/min) was 32±12 (group1) and 36±13 (group 2). The rate of progression of CKD was -1.2±5 ml/min/y and -1.2±4 ml/min/y respectively. Proteinuria (mg/g creatinine) expressed as median (interquatile interval) was: all patients 281(125-961); group1: 288 (127-1088), group 2: 273 (95-890). Laboratory data showed all the parameters within the normal range. SBP was higher than 130mmHg in 22% and 46% in group1 and group 2 respectively. Analysing patients together SBP correlated ($p=0.033$) with the decrease of eGFR and proteinuria ($p=0.006$).

Population profile

	glucose mg/dl	SBP mmHg	DBP mmHg	albumin g/dL	cholesterol mg/dl	hemoglobin g/dl
60-74 years	108±24	136±17	79±7	4.3±0.3	191±38	12.3±1.8
75 years<	110±32	134±14	78±8	4.3±0.3	188±41	12.2±1.4

SBP:systolic blood pressure, DBP:diastolic

Conclusions: DM was less prevalent in older patients. High values of SBP suggest a decrease of arterial stiffness, and the correlation of high SBP with the decrease of eGFR and proteinuria, suggest endothelial dysfunction. The rate of decline of eGFR was below 4ml/min/y in 81% of the patients. We suggest that elderly respond well to CKD treatment implemented by a multidisciplinary team. Early referral (stage 3/4) was probably another point corroborating to retard progression of CKD. Therefore, we postulate that old people are likely to have a good prognosis of CKD, implicating in quality of life since the need of renal substitutive therapy is postponed.

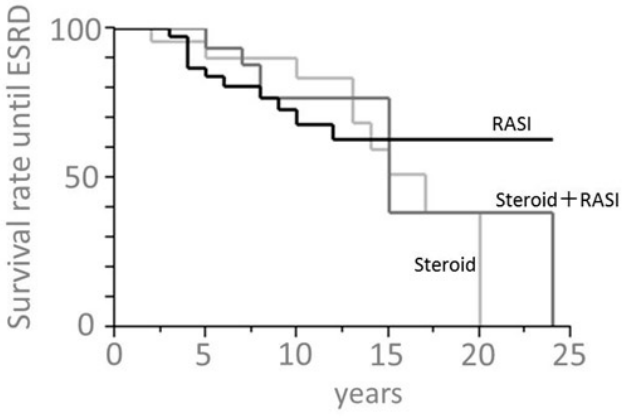
FR-PO1379

The Long-Term Beneficial Effect of Inhibitors of Renin-Angiotensin Aldosterone System (RASI) for Advanced IgA Nephropathy with Impaired Renal Function Takahito Moriyama, Ayami Ochi, Kayu Nakayama, Kosaku Nitta. *Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

Background: The adaptation for steroid therapy and the effect of RASI for advanced IgA nephropathy (IgAN) patients with impaired renal function is still controversial.

Methods: In this retrospective cohort analysis, we divided 91 IgAN patients with an estimated glomerular filtration rate (eGFR) of less than 60 ml/min and proteinuria greater than 0.5 g/day and lower than 3.5 g/day into three groups: the RASI group (RASI: n=39), the steroid group (STEROID: n=22), and combination of RASI and steroid therapy group (COMBI: n=30). We analyzed the clinical and histological background, renal survival rate until progression to end stage renal disease, and the risk factors for progression.

Results: The clinical and histological backgrounds were not significantly different among the three groups (eGFR (ml/min) and proteinuria (g/day): RASI; 46.4 and 1.37, STEROID; 48.5 and 1.52, and COMBI; 46.2 and 1.58). Until 2 years after treatment, proteinuria and U-RBC decreased from baseline in the three groups ($P<0.001$), and the decline of reduction of proteinuria was highest in COMBI (RASI 21.6%, STEROID 23.7%, and COMBI 53.0% decrease from baseline U-Prot). However, the renal survival rate until ESRD was not significantly different among the three groups.



Proteinuria at 2 years after treatment was an independent risk factor for progression by Cox multivariate analysis (HR 1.38, 95%CI 1.06-1.79, P=0.0173), however treatment of steroid and combination therapy was not.

Conclusions: The beneficial effect of RASI on renal survival of advanced IgAN with impaired renal function is equal to that with steroids and combination of steroid and RASI. RASI may be a sufficient therapy for advanced IgAN with impaired renal function and the adaptation of steroid therapy should be considered carefully.

FR-PO1380

The Relationship of Urine Proteinuria to Outpatient "AKI" and Renal Progression Steven J. Rosansky,[1,2] James W. Hardin,[2] Frankie Richards,[1] Kathlyn Sue Haddock,[1] Ann M. O'Hare,[4] William F. Clark.[3] *[1]Dorn Research Institute, WJBD VA Hospital, Columbia, SC; [2]Dept of Biostatistics, School of Public Health University of SC, Columbia, SC; [3]London Health Sciences Center, University of S Ontario, London, ON, Canada; [4]Nephrology, VA Seattle, Seattle, WA.*

Background: Outpatient "AKI" and change of renal function per year as measured by MDRD e GFR may be important parameters in the management of CKD patients. Proteinuria has been reported to be associated with a higher frequency of inpatient ICD coded AKI. The current study examines the relationship of urine proteinuria, renal function decline and outpatient AKI

Methods: All outpatient creatinine and urinalysis data from through December 31, 2008, were obtained for patients with an initial serum creatinine of ≥ 1.3 mg/dl during 1989-2003. Lab data was utilized from 3776 patients who had at least one urinalysis and ≥3 creatinine values over ≥3 years. Each subject's creatinine values (same methodology throuhout study)were separated into 90-day consecutive windows. "AKI "was defined by sequential outpatient serum creatinine values where a second creatinine was ≥ 50 percent higher than a prior serum creatinine in a 90-day window. The change in outpatient 4 variable MDRD e GFR (ml/min/1.73m^2/year) was calculated using the average of the first and last creatinine values and was examined according to three urinalysis categories, using all urinalysis data per patient, no proteinuria; < 2plus proteinuria; and ≥ 2plus proteinuria. Growth curve analysis was utilized to examine the relationship of all covariates to renal function change.

Results: Patients had an average of 23 creatinine and 9.4 urinalysis measures during an average follow-up of 9.6 years. AKI occurred in 10.4% of the overall population,9.1 percent of whites and 14.3 percent of blacks(p<.001).

	Proteinuria		
	None	< 2+	≥ 2+
Urinalysis Group (n)	1995	1357	424
AKI Episodes (% of population)	5.4	13	26
Decline in MDRD eGFR (ml/min/1.73^2/yr)	0.71	1.37	2.18

for all results, p<.001

Conclusions: In conclusion, outpatient "AKI" occured more frequently in blacks than whites.Higher urinalysis protein levels were associated with more AKI episodes and faster decline in e GFR..

FR-PO1381

Determinants of Arterial Stiffness in Patients with Chronic Kidney Disease Stage 3 Natasha J. McIntyre,[1] Richard J. Fluck,[1] Chris W. McIntyre,[1,2] Maarten W. Taal.[1] *[1]Department of Renal Medicine, Royal Derby Hospital, Derby, Derbyshire, United Kingdom; [2]Department of Vascular Medicine, University of Nottingham, United Kingdom.*

Background: Early stage CKD is associated with increased cardiovascular (CV) risk but the underlying mechanisms remain uncertain. Arterial stiffness (AS) is associated with increased CV risk in advanced stages of CKD, but it is unknown whether AS is relevant to CV disease in early CKD. We therefore investigated AS in subjects with CKD stage 3 in a primary care setting.

Methods: 1741 patients with eGFR 59-30ml/min/1.73m^2; mean age 73±9 yrs, were recruited from Primary Care Practices for the Renal Risk In Derby (R^{2}ID) Study. A detailed medical history and clinical assessment was obtained as well urine and serum biochemistry testing. Carotid to femoral pulse wave velocity (PWV) was measured, as a marker of AS, using a Vicorder™ device (Skidmore Medical Ltd,UK).

Results: Univariate analysis revealed significant correlations between PWV and previously identified risk factors for CV disease such as age (r=0.443), systolic BP (r=0.330), Body Mass Index (r=-0.134), Log urinary Protein to Creatinine ratio(r=0.128), Waist to Hip ratio (r=0.122), Diastolic BP(r=0.083), eGFR (r=-0.067), Log High sensitivity CRP(r=0.066) and HDL Cholesterol (r=-0.058). PWV was significantly higher in males (9.6m/sec vs.10.3m/sec), diabetics (9.8m/sec vs.10.3m/sec), and those with a history of CV events (9.8m/s vs.10.3 m/sec). Multivariable liner regression analysis identified independent determinants of higher PWV (Table; R2=0.29)

Independent determinants of higher PWV

	β	p value
Age	0.407	<0.0001
Systolic BP	0.184	<0.0001
Body Mass Index	-0.120	<0.0001
Diabetes Mellitus	0.110	<0.0001
Diastolic BP	0.097	<0.0001
eGFR	0.060	0.008
HS CRP	0.049	0.028
Female Gender	-0.048	0.039
HDL Cholesterol	-0.049	0.041
Albumin to creatinine ratio	0.045	0.044

Conclusions: Age was the dominant determinant of AS in this cohort of elderly patients with CKD stage 3. Nevertheless, reduced eGFR, albuminuria and several factors associated with CKD (including hypertension, inflammation, and dyslipidaemia) were all also identified as independent determinants. Long term follow-up will investigate the importance of AS as an independent risk factor for CV events in this cohort.

Funding: Other NIH Support - Kidney Research UK and The British Renal Society

FR-PO1382

Neutrophil/Lymphocyte Ratio Independently Predicts Cardiovascular Events in Patients with Moderate To Severe Chronic Kidney Disease Yalcin Solak,[1] Mahmut Ilker Yilmaz,[2] Alper Sonmez,[3] Mutlu Saglam,[4] Erdinc Cakir,[5] Hilmi Umut Unal,[2] Kayser Caglar,[2] Murat Karaman,[6] Seyid Ahmet Ay,[6] Mujdat Yenicesu.[2] *[1]Nephrology, Selcuk Uni., Konya, Turkey; [2]Nephrology, GATA, Ankara, Turkey; [3]Endocrinology, GATA, Ankara, Turkey; [4]Radiology, GATA, Ankara, Turkey; [5]Biochemistry, GATA, Ankara, Turkey; [6]Internal Medicine, GATA, Ankara, Turkey.*

Background: Cardiovascular (CV) diseases are leading cause of death in patients with chronic kidney disease(CKD).Neutrophil/Lymphocyte ratio(NLR) has been shown independently predict mortality and poor outcomes in patients with myocardial infarction, heart failure and advanced malignancy.We aimed to evaluate value of NLR in predicting fatal and nonfatal CV events in patients with stage 3-5 CKD.

Methods: Stage 3-5 CKD patients were followed-up for time-to-event analysis until occurrence of fatal or nonfatal CV events.Endothelium-dependent vaso-dilatation (FMD) and endothelium-independent vasodilatation(NMD) compared with NLR, CRP and routine laboratory data at baseline. Associations of NLR and endothelial dysfunction and other laboratory parameters were determined.Prevalence of fatal and nonfatal events according to NLR was calculated.Estimate survival time for each category are calculated.

Results: 225 patients included in the study (70 patients with stage 3,74 stage 4 and 81 stage 5 CKD).There was an inverse association between NLR and eGFR.Notably, while total WBC and neutrophil counts did not show significant difference across CKD stages, lymphocyte counts significantly decreased from stage 3 to stage 5 CKD. Multivariate analysis showed that associates of FMD only included NLR, diabetes, NMD, serum albumin and eGFR. During a mean follow-up period of 39 (2-42) months, 14 CV deaths, 52 non-fatal cardiovascular events were registered. Univariate and multivariate COX analyses showed that NLR was a significant independent predictor of fatal and nonfatal CV events (hazard ratio 1.58, p=0.001). When entire cohort divided by median NLR (2.81), 63 out of composit CV events occurred in patients with NLR above 2.81. Survival time was significantly longer in patients with lower NLR compared with patients with higher NLR.

Funding: Other NIH Support - GATA

FR-PO1383

Relationships between Physical Activity and Nutrition with Kidney Function in CKD Patients Robert G. Fassett,[1,3] Iain Robertson,[2] Madeline J. Ball,[2] Dominic P. Geraghty,[2] Jeff S. Coombes.[3] *[1]Renal Medicine, University of Queensland, Brisbane, Queensland, Australia; [2]Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; [3]Human Movement Studies, University of Queensland, Brisbane, Queensland, Australia.*

Background: The Lipid lowering and Onset of Renal Disease (LORD) trial was a three-year randomised, double-blind, placebo-controlled trial investigating the effects of atorvastatin on kidney function in CKD patients. The study design included measures of physical activity and nutrition every nine months. The aim of this sub-study was to investigate the relationships between physical activity and nutrition with kidney function.

Methods: 132 patients with serum creatinine levels >120µmol/l, not taking lipid-lowering therapy and at all levels of proteinuria and serum cholesterol were enrolled. For this sub-study data was available for 120 patients and they were followed for a mean of 2.9 years. Physical activity and nutrition were assessed every nine months using the Active Australia questionnaire and 4-day diet diaries analysed with Foodworks software respectively. eGFR was calculated using the MDRD formula. The association (Odds Ratio) between a number of predictors and eGFR were estimated using repeated measures ordinal

logistic regression. An OR >1.00 indicates a positive association, and an OR <1.00 indicates a negative association. General linear modelling was used to determine relationships between physical activity and nutrition with the change in eGFR.

Results: eGFR was positively associated with phosphate intake (OR 2.3, 95% CI 1.5-3.4, P<0.001), and negatively with folate (OR 0.6, 95% CI 0.4-1.0, P<0.03). They were no significant associations between physical activity and kidney function. Weekly physical activity was not associated with changes in kidney function and there were no group differences (placebo = slope 0.19 (SD 3.51), atorvastatin = 0.41 (2.73)). There was a significant group difference (P=0.009) between time in high intensity physical activity and eGFR (placebo = -0.24 (2.52), atorvastatin = 0.72 (2.07)).

Conclusions: Atorvastatin improves kidney function in patients with chronic kidney disease undertaking high intensity physical activity.

FR-PO1384

Malnutrition-Inflammation Score Is Associated with Handgrip Strength in Non-Dialysis-Dependent Chronic Kidney Disease Patients Fernanda C. Amparo,[1, 2] Antonio C. Cordeiro,[1, 3] Juan J. Carrero,[3] Lilian Cuppari,[2] Bengt Lindholm,[3] Celso Amodeo,[1] Amanda G.M.R. Sousa,[1] Maria A. Kamimura.[2] *[1]Dante Pazzanese Institute of Cardiology, São Paulo, Brazil; [2]Nutrition Program, Federal University of São Paulo, São Paulo, Brazil; [3]Baxter Novum and Renal Medicine, Karolinska Institute, Stockholm, Sweden.*

Background: Handgrip strength (HGS), a marker of muscle function, predicts mortality in earlier stages of chronic kidney disease (CKD). Protein-energy malnutrition and inflammation are coexisting deleterious conditions commonly shared by CKD patients (pts) that affect muscle function. We investigated whether the Malnutrition-Inflammation Score (MIS), developed for dialysis pts, is associated with HGS in non-dialysis-dependent (NDD) CKD pts.

Methods: We cross-sectionally evaluated 166 pts with NDD-CKD stages 2-5 (59 [51-67] years; 63% men). MIS was calculated as previously described, excluding the count for dialysis vintage. HGS was assessed in the dominant arm. Anthropometric parameters, laboratory data and bioelectrical impedance analysis were recorded.

Results: HGS correlated positively with lean body mass index [LBMI] (r=0.40; P<0.01), body cell mass [BCM] (r=0.63; P<0.01) and 24h urinary creatinine clearance [24hCrCl] (r=0.42; P<0.01), and negatively with age (r = -0.18; P=0.02) and MIS (r=-0.42; P<0.01). Pts were divided into two groups according to the sex-specific HGS median, (low [n=80] and high [n=86] HGS). Those with lower HGS were older (63[55 – 69] vs. 57 [50 – 64] years; P < 0.01), had lower LBMI (19.8 ± 3.3 vs. 21.0 ± 3.3 kg/m2; P=0.02), lower BCM (23.5 ± 6.4 vs. 28.1 ± 6.3 kg; P<0.01), lower 24hCrCl (15.3 [10.3 – 25.6] vs. 24.4 [12.1 – 36.4] ml/min/1.73m2; P<0.01), and higher MIS (8 [5-10] vs. 5 [3-7]; P<0.01). Linear regression analyses showed an association between MIS and HGS in both, crude and adjusted models (Table).

Model	Covariates	Coefficients	95% CI	P	R^2
1	MIS	-1.36	-1.82 to -0.90	<0.01	0.17
2	1+ age, gender	-1.15	-1.54 to -0.76	<0.01	0.43
3	2+ diabetes, 24hCrCl	-0.92	-1.31 to -0.53	<0.01	0.48
4	3+ BCM, C-reactive protein	-0.55	-0.99 to -0.11	0.02	0.53

Conclusions: The MIS score was associated with HGS in our population, indicating that MIS may be used to predict muscle function in NDD-CKD pts.

Funding: Government Support - Non-U.S.

FR-PO1385

Recent Status of Vitamins B1, B2, and C in Japanese Patients with End-Stage Renal Disease Toshikazu Wada, Hideaki Iwasawa, Ami Hayashi, Yoshitaka Miyaoka, Toshiyuki Nakao. *Nephrology, Tokyo Medical University, Tokyo, Japan.*

Background: Water-soluble vitamin deficiencies have been recognized in end-stage renal disease (ESRD) patients. Recently, the numbers of ESRD elderly patients in Japan have been increasing. We investigated the status of vitamins B1 (VB1), B2 (VB2), and C (VC) in Japanese ESRD patients not receiving renal replacement therapy (RRT) and those receiving maintenance hemodialysis (HD), and determined the kinetics of these vitamins in HD patients. We also investigated the status of these vitamins in ESRD elderly patients.

Methods: Plasma VB1, VB2, and VC levels were obtained in 46 non-RRT ESRD patients (age: 63.0 ± 14.0 years). In 82 HD patients (66.2 ± 12.5 years), the plasma vitamin levels were obtained before and after a single HD session and at the start of the next HD session. Dialysis clearance of these vitamins was assessed.

Results: The proportions of patients whose plasma VB1, VB2, and VC levels were under the normal range were as follows - non-RRT patients: 13.0%, 13.0%, 41.3%; HD patients: 12.2%, 12.2%, 51.2%; those above the normal range were as follows - non-RRT patients: 15.2%, 30.4%, 19.6%; HD patients: 11.0%, 24.4%, 19.5%, respectively. The patients who were prescribed vitamins showed high plasma vitamin levels. The plasma VC level significantly decreased after a single HD session (P < 0.05), and significantly increased at the start of the next HD session (P < 0.05); the plasma VB1 and VB2 levels did not significantly change in patients without vitamin supplements. The mean dialysis clearance of VC was significantly higher than those of VB1 and VB2 (both P < 0.05). In the elderly patients without vitamin supplements, non-RRT patients had significantly lower plasma VB1 levels than non-elderly patients (P = 0.006), and HD patients had significantly lower plasma VC levels than non-elderly patients (P = 0.001).

Conclusions: Low plasma VB1, VB2, and VC levels were commonly found in ESRD patients, especially in elderly patients. Control of the balance between vitamin intake and loss based on individual requirements is essential for ESRD patients.

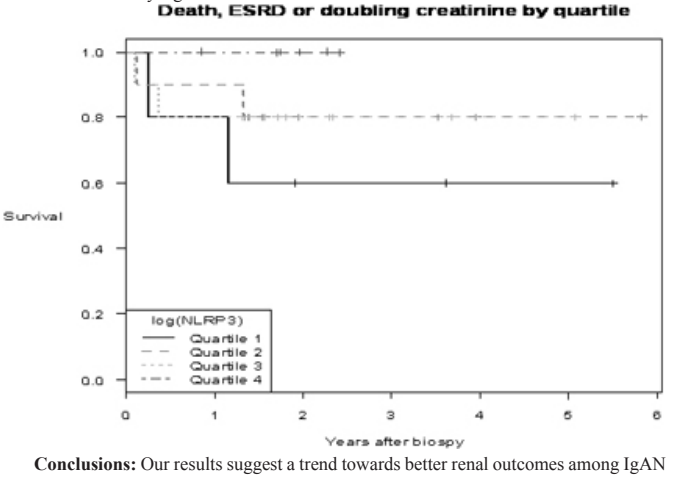
FR-PO1386

Association of NLRP3 Gene Expression with Human IgA Nephropathy Progression Xiangyu (Wendy) Wang, Justin Chun, Andrea Soo, Sofia B. Ahmed, Kiril Trpkov, Brenda Hemmelgarn, Daniel A. Muruve. *University of Calgary, Canada.*

Background: IgA nephropathy (IgAN) is characterized by variable clinical features and outcomes. The identification of reliable disease biomarkers will help define prognosis and therapeutic outcomes. Inflammation mediated by NOD-like receptors may be involved in the pathogenesis of IgAN. The increased expression of NLRP3 mRNA has been demonstrated in IgAN. However the association of NLRP3 gene expression with IgAN progression is an unexplored area.

Methods: Biopsies of 39 patients with IgAN from September 2003 to December 2007 were randomly chosen for analysis of NLRP3 mRNA expression by quantitative real-time PCR. Patients with advanced kidney disease(creatinine, Cr>300umol/L; n=3) and missing Cr (n=5) were excluded. A total of 31 cases of IgAN were analyzed. NLRP3 gene expression was analyzed as a logarithmic variable due to its non-normal distribution, and categorized into quartiles. The outcome was kidney failure, defined as a composite of doubling of serum Cr, ESRD or death. Subjects were followed from the time of their kidney biopsy to March 31, 2009 for the outcome of kidney failure.

Results: The mean age of the 31 subjects was 47 years, of whom 58% were male. The composite renal outcome was reached in 6 (19%) of the patients. The association between log NLRPs quartiles and survival is shown in Figure 1. There was an inverse and graded association between NLRP3 gene expression and kidney failure -- subjects with the highest NLRP3 gene expression were least likely to develop kidney failure, although the results were not statistically significant.



Conclusions: Our results suggest a trend towards better renal outcomes among IgAN subjeicts with higher NLRP3 mRNA expression. The presence of NLRP3 in IgAN may indicate reversible or treatment responsive inflammation although larger studies are required to confirm these findings.

FR-PO1387

Insulin Resistance and Type 2 Diabetes Patients in Difference Stage of Nephropathy Bancha Satirapoj, Ouppatham Supasyndh, Rattanawan Dispan, Prajej Ruangkanchanasetr, Panbubpa Choovichian. *Internal Medicine, Phramongkutklao Hospital, Bangkok, Thailand.*

Background: Diabetic nephropathy (DN) is one of the most serious complications of diabetes mellitus due to its high morbidity and mortality. In type 2 diabetic patients resistance to the action of insulin has been documented, but only a few studies have evaluated the risk of diabetic subjects with insulin resistance for developing DN. In this study we assessed insulin resistance in different stage of DN.

Methods: This is a cross-sectional study of 230 patients with type 2 diabetes. Patients were divided into three groups according to levels of urinary albumin-to-creatinine ratio (ACR) and urine protein: normoalbuminuria (ACR < 30 mg/g), incipient nephropathy (ACR 30-299 mg/g), and overt nephropathy (ACR ≥ 300 mg/g and/or persistent proteinuria). Insulin resistance was evaluated by homeostasis model assessment (HOMA-IR).

Results: There was a trend toward a higher HOMA-IR in overt and incipient nephropathy, but failed to reach statistic significance. Compared with the first quartile of HOMA-IR, the fourth quartile of HOMA-IR was associated with a significantly higher risk of overt nephropathy: crude odds ratio (OR) 4.14; 95% CI 1.69-10.11 and adjusted OR 5.79; 95% CI 1.72-19.52. In contrast, HOMA-IR quartiles were not significantly associated with the development of incipient nephropathy. Known duration of diabetes (adjusted OR 1.09; 95% CI 1.05-1.14), history of hypertension (adjusted OR 2.83; 95% CI 1.24-6.47), diabetic retinopathy (adjusted OR 6.48; 95% CI 3.01-13.96), and urinary albumin-to-creatinine ratio (adjusted OR 1.29; 95% CI 1.14-1.47), were also associated with development of diabetic nephropathy.

Conclusions: In addition to known risk factors of DN, high insulin resistance was independently associated with development of overt nephropathy in type 2 diabetes, but not incipient nephropathy.

Funding: Government Support - Non-U.S.

FR-PO1388

Left Ventricular Hypertrophy, Endothelial Dysfunction & Inflammation in CKD Dimitrios J. Poulikakos, Ana Sofia Rocha, Nihil Chitalia, Juan Carlos Kaski, Debasish Banerjee. *Renal and Cardiology Units, St Georges Hospital, London, United Kingdom.*

Background: Left ventricular hypertrophy [LVH] is common in CKD patients; however the mechanism is not clear. Inflammation and associated endothelial dysfunction [ED] are present in CKD and predict adverse cardiovascular outcomes. The relationships of inflammation, ED and LVH in different stages of CKD; predialysis, dialysis and post kidney transplantation [KT] are unclear.

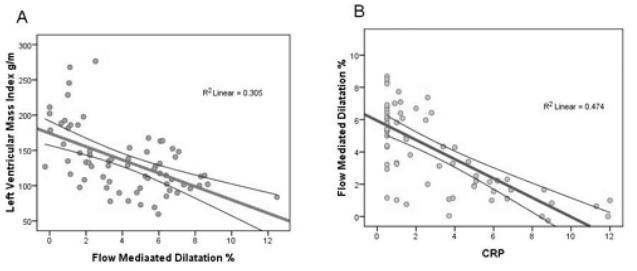
This study examined the relationship of inflammation, ED and LV mass in predialysis, dialysis and KT patients.

Methods: This study included 39 patients with different stages of CKD. Patients with heart failure, recent MI, infection and cancer were excluded. ED was measured using the brachial artery flow mediated dilatation [FMD]. Echocardiogram was used to estimate LV mass. LV mass index (LVMI) was calculated using Penn formula and indexed by height. Inflammation was measured using high sensitivity CRP.

Results: The clinical characteristics in 17 pre-dialysis, 9 haemodialysis and 13 KT patients were: age 56±12 [mean±SD] yrs, 33% female, 20% diabetes, 10% smokers, BMI 26±4 kg/m2, SBP 139±19mmHg, DBP 81±11mmHg, cholesterol 4.3±1.3mmol/L and Hb 12.7±1.6 g/L.

LVMI was high 157±45g/m and FMD was 2.6±2.1%. The hsCRP was 4.5±3.4mg/L. With increasing CRP the FMD decreased (r=-0.70,p<0.001) and with decreasing FMD, LVMI increased (r=-0.55, p<0.001), see figure 1. Increasing LVMI was associated with increasing CRP (r=0.51, p<0.001). In multivariate analysis the relationship between LVMI and FMD remained significant after adjusting for age, gender, diabetes, and hypertension (Adj. beta =-0.507, p=0.01).

Figure 1 Relationship of LV hypertrophy, endothelial dysfunction and inflammation



LVMI increases with worsening endothelial function in A; and FMD worsens with increasing CRP in B

Conclusions: The study demonstrates an association of inflammation, endothelial dysfunction and LV mass in all CKD patients.. This suggests Inflammation may have a role in the development of LV hypertrophy due to endothelial dysfunction.

FR-PO1389

A Multicenter Assessment of Depressive Symptoms in Patients Undergoing Hemodialysis: Women over 60 Years Should Receive the Attention and Monitoring Carmen B. Tzanno-Martins,[3] Fernanda Ribeiro Nisihara,[2] Geison Stein Meirelles Ramos,[1] João Paulo L.B. Martins,[1] Elzo R. Junior.[1] *[1]Home Dyalisis Center, São Paulo, Brazil; [2]Renal Class, São Paulo, Brazil; [3]Cine Integrado de Nefrologia, Guarulhos, Brazil.*

Background: Recent studies report that 10% to 25% of the general population has depressive symptoms. These symptoms are more frequent in women (15 to 20%) and elderly individuals (2 to 14%). These values can reach 30% in elderly nursing home residents.

Depressive symptoms can arise from psychosocial problems, or associated medical conditions such as cancer, chronic pain and chronic degenerative diseases, including Chronic Kidney Disease.

Recently, it has been shown that the prevalence of depression in patients with chronic kidney disease undergoing hemodialysis varies from 5% to 22%, with frequent occurrence of depressive symptoms with the onset of treatment.

Objective: To assess the prevalence of depressive symptoms using the Beck Inventory (screening test for diagnosis of depression) in chronic hemodialysis patients in 3 centers in the state of Sao Paulo, Brazil (n = 567) and correlate with demographics.

Methods: The Beck Depression Inventory was administered to a random sample of 70% of patients in three clinics (n = 397/567). We use statistical tests as chi-square with Fischer's post-test.

Results:

Final results of the Beck Depression Inventory applied to female patients

	under 60 years	over 60 years	Total
No depressive symptoms	80 (74.1%)	36 (54.5%)	116 (66.7%)
Depressive symptoms	28 (25.9%)	30 (45.5%)	58 (33.3%)
Total	108 (100.0%)	66 (100.0%)	174 (100.0%)

We conclude that the percentage of patients on chronic hemodialysis with depressive symptoms is higher than the general population (28.71%).

Conclusions: The data suggest that female patients had higher frequency of depressive symptoms compared to males regardless of age (25.9% X 21.7% under 60 years old, and 45.5% vs. 30.6% over 60 years old) (p <0.05). However, we found a greater number of female patients with depressive symptoms over the age of 60 years (25.9 X 45.5%) (p <0.05). Our data suggest that these patients should be accompanied with measures of psychological support during treatment.

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FR-PO1390

Incidental Imaging Is Enough To Screen for Acquired Cystic Kidney Disease Laura J. Maursetter, Hemender Singh Vats, Micah R. Chan. *Department of Nephrology, University of Wisconsin-Madison, Madison, WI.*

Background: Acquired cystic kidney disease (ACKD) is a known complication of End Stage Renal Disease (ESRD). The pathophysiology is unknown but uremia seems to be a common factor. There is the potential for these cysts to turn malignant adding increased mortality to the ESRD population. The rate of ACKD increases with time on hemodialysis (incidence 50% at 3 years and 90% at 5-8 years) and it has been suggested that screening patients after they have received hemodialysis for three years is an ideal time to avoid complications and reduce mortality. The prevalence of renal cell carcinoma in ESRD patients has been debated (1.6 to 4%) but has been consistently shown to be higher than the general population.

Currently no official screening guidelines have been established to assess transplant or ESRD patients for ACKD. Given the complexity of ESRD patients and the comorbidities that are associated with this disease, we predict sufficient renal imaging may be incidentally done through work up of other medical issues to make additional screening unnecessary.

Methods: 80 ESRD patients from Wisconsin Dialysis Incorporated, located in Madison, Wisconsin, were randomly identified as having been on dialysis for more than 3 years. The electronic medical record of each patient was reviewed for the time period after the patient had reached 3 or more years on dialysis. All imaging studies were reviewed for any ultrasound, CT scan or MRI images that could have incidentally found suspicious renal cysts. Any images that did not report findings of the renal system were disregarded.

Results: There was a total of 670.2 dialysis years reported in the 80 ESRD patient charts that were reviewed. Evaluation of each electronic medical record determined that 57.5% of ESRD patients have imaging performed randomly that could incidentally diagnose renal disease. None of the indications listed for the imaging tests was for ACKD screening.

Conclusions: Although the prevalence of renal cell carcinoma is much greater in the ESRD population, when taking into account the incidental screening, cost of the screening test and rate of disease occurrence, it is not cost effective to implement a screening system.

FR-PO1391

Responsiveness to Erythropoiesis Stimulating Agents (ESA-R) Predicts ESRD in Non Dialysis CKD Patients Roberto Minutolo,[1] Bruno Cianciaruso,[2] Vincenzo Bellizzi,[3] Giuseppe Conte,[1] Luca De Nicola.[1] *[1]Nephrology, Second Univ. of Naples; [2]Nephrology, Univ. Federico II, Naples; [3]Nephrology, Univ. Hospital, Salerno, Italy.*

Background: RCTs on complete normalization of anemia in CKD show that lower ESA-R predicts poor cardiovascular (CV) outcome. It is unknown whether this association persists in clinical practice where the target of partial anemia correction is pursued.

Methods: We verified the prognostic role of ESA-R in 194 consecutive CKD patients, regularly seen in outpatient nephrology clinics, that started ESA in the 2002-06 period. Exclusion criteria were causes of anemia other than CKD or recent transfusion. ESA-R was calculated as $(Hb_1\text{-}Hb_0)$/time/ESA dose (g/dL/mo per 1000 U/wk of ESA). Renal death (ESRD or death) was recorded from the 1st control after starting ESA to Apr 2011.

Results: Age was 64±16 y, 48% were males, 34% had diabetes and 32% CV disease. At baseline, BMI was 27±5 kg/m2, BP 140±21/77±11 mmHg, phosphate [/A]4.3±0.9 mg/dL, TSAT 23±9%, ferritin 158±139 ng/mL, CRP 0.8±0.9 mg/dL. These variables were similar across tertiles of ESA-R while, from lower to higher tertile of ESA-R, GFR increased (22±12, 22±14, 28±13 mL/min/1.73m2 , P=0.01) and proteinuria decreased (1.1 [0.3-2.2], 0.6 [0.2-1.8], 0.5 [0.1-1.3] g/d P=0.04). First control occurred after 1.4±0.5 mo.

	ESA-R<0.08 (n=65)	ESA-R 0.08-0.24 (n=64)	ESA-R>0.24 (n=65)	P
Basal Hb (g/dL)	10.1±0.7	9.9±0.8	9.7±0.9	0.006
Hb 1st control (g/dL)	10.2±0.8	11.0±0.9	11.8±1.2	0.0001
First ESA dose (IU/wk)	4738±2041	5141±2525	4397±1937	0.16
During initial 6 months				
Months with Hb<11	4.0 [2.2-5.5]	1.5 [0.8-3.8]	0.8 [0.5-1.4]	0.0001
Mean ESA dose (IU/wk)	4761±2207	4719±1844	3482±1582	0.0001

During follow-up (median 2.9 yrs), 99 patients reached ESRD and 35 died. At Cox analysis (adjusted for age, gender, BMI, diabetes, CV disease, BP, GFR, proteinuria, Hb, CRP, phosphate), lowest tertile of ESA-R predicted renal death (HR 2.61, 95%CI 1.46-4.64) and ESRD (2.46, 1.27-4.78).

Conclusions: ESA-R predicts renal prognosis in CKD patients followed in nephrology clinics, where ESRD is the predominant outcome and ESA is commonly used at low dose. A potential role for persistent hypoxia in patients with more advanced renal damage is suggested.

FR-PO1392

AKB-6548, a New Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor Increases Hemoglobin While Decreasing Ferritin in a 28-Day, Phase 2a Dose Escalation Study in Stage 3 and 4 Chronic Kidney Disease Patients with Anemia Charlotte Hartman,[1] Mark T. Smith,[2] Cindy Flinn,[1] Isaiah Shalwitz,[1] Kevin G. Peters,[1] Robert Shalwitz,[1] Volker H. Haase.[3] *[1]Akebia Therapeutics, Inc., Cincinnati, OH; [2]Kidney Care Associates, LLC, Augusta, GA; [3]Vanderbilt University Medical Center, Nashville, TN.*

Background: Current treatment of anemia associated with chronic kidney disease (CKD) with erythropoiesis-stimulating agents (ESAs) can lead to supraphysiological levels of circulating erythropoietin (EPO) that persist for days, a profile that may be associated with increased cardiovascular side effects. Therefore, a drug that provides moderate increases and reestablishes the diurnal variation in EPO may be a better treatment for patients with CKD. AKB-6548, a new short-acting hypoxia-inducible factor prolyl hydroxylase inhibitor, was selected to induce controlled daily rises in EPO that closely simulate physiologic responses to changes in altitude.

Methods: In a Phase 2a dose escalation study, 10 CKD patients received AKB-6548 once daily for 28 days. Dosing began at 400mg in CKD Stage 3 patients and 300mg in CKD Stage 4 patients. It increased by 100mg for each week that absolute reticulocyte count (ARC) did not increase by 18,000 above the baseline (BL) average.

Results: Dosing was generally well tolerated. Results, including both Stage 3 and 4 CKD patients, demonstrated that hemoglobin rose from 9.91g/dL at BL to 10.54g/dL by Day 29. Ferritin decreased from 334.10ng/mL at BL to 271.70ng/mL by Day 29. We conclude that AKB-6548 is well-tolerated and increases hemoglobin while decreasing ferritin in a dose-dependent manner in patients with Stage 3 or 4 CKD.

Conclusions: The consistent rise in hemoglobin and the concurrent fall in ferritin over the course of the study suggest an efficacious daily dose of AKB-6548 begins between 300 and 400mg.

	Baseline	Day 29	Paired t-test
Hemoglobin (g/dL)	9.91±0.63	10.54±0.89	p=0.0019
Ferritin (ng/mL)	334.10±209.82	271.70±181.31	p=0.0016

Funding: Pharmaceutical Company Support

FR-PO1393

Hemoglobin Variability in Chronic Kidney Disease and Type 2 Diabetes in TREAT Julie Lin,[1] Marc A. Pfeffer,[1] Ajay K. Singh,[1] Nairne William Scott-Douglas,[2] Chao-Yin Chen,[3] Jerome A. Rossert,[3] Peter Ivanovich,[4] Giuseppe Remuzzi, Mark E. Cooper. *[1]Medicine, Brigham and Women's Hospital, Boston, MA; [2]Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; [3]Amgen, Thousand Oaks, CA; [4]Nephrology, Northwestern University, Chicago, IL.*

Background: Sparse data are available on the natural history of hemoglobin (Hb) change in contemporary patients with CKD, anemia, and type 2 DM.

Methods: TREAT placebo arm patients (n=2,026) with type 2 DM, eGFR 20-60 ml/min/1.73 m^2, and Hb≤11 g/dL received "rescue" ESA therapy with darbopoetin at 0.45 mcg/kg/month (1/4 of active therapy start dose) only when Hb<9 g/dL.

Results: During mean f/u of 2.4 yrs, 1,113 (55%) did not have a Hb <9 g/dL nor received ESA. 913 (45%) had at least one Hb<9 g/dL and received "rescue", 321 (16%) received a single ESA dose while 334 (16%) got >4 monthly doses. Median Hb in non-"rescue" group was 10.7 g/dL at baseline (BL), 11.1 g/dL at 12 mos (n=829), and 11.2 g/dL at 24 mos (n=537). In the "rescue" group, median Hb was 10.0 g/dL at BL, 10.0 g/dL at 12 mos (n=684), and 10.1 g/dL at 24 mos (n=422). Besides lower baseline Hb (p<0.001), "rescue" group had lower eGFR (30 vs. 35 ml/min/1.73 m2), higher proteinuria (0.8 vs. 0.3 g/g Cr), ferritin (162 vs. 118 ug/L) and TSAT (23% vs. 22%) (all p≤0.001). Previous ESA use (13% vs 8%, p<0.001) and blood tx (11% vs 8%, p=0.02) were more frequent in the "rescue" group. During the trial, the rescue group was more likely to receive IV iron (25% v. 17%, p<0.001) and blood tx (35% v. 16%, p<0.001).

Conclusions: In the TREAT placebo arm, Hb remained stable with no or minimal ESA over 2.4 years follow-up, demonstrating that most patients with moderate anemia, eGFR 20-60 ml/min/1.73 m^2, and type 2 DM have stable Hb without implementing chronic ESA therapy.

Funding: Pharmaceutical Company Support

FR-PO1394

Comparison of High Dose Ferric Carboxymaltose to Oral or IV Iron in Subjects with Iron Deficiency Anemia (IDA) Not Suitable for Oral Iron Lynda A. Szczech,[1] David B. Bregman,[2] David Morris,[3] Angelia Butcher,[2] Todd Koch,[2] Lawrence Tim Goodnough,[4] Jane E. Onken.[1] *[1]Duke Clinical Research Inst, NC; [2]Luitpold Pharmaceuticals, PA; [3]Webbwrites, NC; [4]Stanford Univ, CA.*

Background: Ferric carboxymaltose (FCM) is a non-dextran IV iron that permits larger infusions compared to other IV irons marketed in the US. This trial randomized patients with IDA to FCM or other intravenous iron products to evaluate effect on hemoglobin as well as hemodynamics and other safety parameters.

Methods: Patients eligible for this randomized controlled trial had IDA due to any etiology (menorrhagia, GI, or CKD) with hemoglobin (Hb) ≤ 11.0 g/dL at screening. Subjects in Cohort I underwent a 14 day (run-in) course of oral iron. Subjects with an increase in Hb ≥ 1 g/dL were not randomized. All other subjects were randomized to FCM (2 injections of 750 mg @ 100 mg/minute on Days 0 and 7) or oral iron for 14 more days. The primary efficacy endpoint was the change in Hb from baseline to highest value between baseline and Day 35. Subjects with a history of severe intolerance to oral, considered too anemic for oral, or were intolerant of oral iron during the run-in were assigned to Cohort II and randomized to FCM as above or another IV iron (IV standard of care [IVSOC]). IVSOC was iron sucrose for 90% of subjects. For both cohorts, safety was assessed for 120 days, including an adjudicated composite endpoint (death, myocardial infarction, stroke, unstable angina, congestive heart failure, arrhythmia, hypertension, or hypotension).

Results: In Cohort I, the change in Hb (g/dL) from baseline to highest value between baseline and Day 35 was 1.57 for FCM (N=244) vs. 0.80 for oral iron (N=251)(p = 0.001). In Cohort II, the change in Hb (g/dL) was 2.90 for FCM (N=245) vs. 2.16 for IVSOC (N=237)(p = 0.001). FCM was well tolerated. Results were consistent regardless of etiology or Hb status at baseline. There was no significant difference between FCM and comparator with respect to the composite safety endpoint

Conclusions: In this head to head trial, FCM was safe and effective for the treatment of IDA in subjects unsuitable for oral iron regardless of etiology or Hb status at baseline.

Funding: Pharmaceutical Company Support

FR-PO1395

Red Blood Cell Survival in Patients on Maintenance Dialysis Frederiek E. Vos, John B.W. Schollum, Carolyn V. Coulter, Terence C. Doyle, Stephen Duffull, Robert J. Walker. *Medicine, University of Otago, Dunedin, New Zealand.*

Background: Shortening of red blood cell (RBC) survival contributes to the anemia of chronic kidney disease. The toxic uremic environment accounts for the reduced RBC life span. The impact of mechanical damage caused by hemodialysis to the shortened life span remains unclear. Reductions up to 70% in RBC survival have been reported in uremic patients. To date, no accurate, well-controlled RBC survival data exists in dialysis patients on different dialysis modalities and under erythropoiesis stimulating agent (ESA) therapy. Aim of this study was to determine RBC survival in hemodialysis (HD) and peritoneal dialysis (PD) patients compared to healthy subjects.

Methods: In this observational study, 14 HD patients and 5 PD patients were recruited. In addition, 14 healthy subjects matched according to age and gender to the HD patients were included to assess the normal range of RBC survival. All dialysis patients were either on ESA therapy or received regular iron supplementations. RBC survival was determined by radioactive chromium labeling and included correction for potential losses due to elution and vesiculation.

Results: Over 85% of dialysis patients were anemic (Hb 12.0 g/dL ± 1.1), hemoglobin concentrations were not significantly different among HD and PD subjects. The median RBC survival was significantly reduced by 20% in hemodialysis patients compared to healthy subjects (58.1 (54.6 – 71.2) vs 72.9 (63.4 – 87.8) days, p < 0.05). No difference was shown among the PD and HD group (55.3 (49.0 – 60.2) vs (58.1 (54.6 – 71.2) days, p = ns).

Conclusions: Despite current ESA therapy, reduced RBC survival contributes to CKD-related anemia, but the reduction is less than previously reported. Mechanical damage related to HD does not appear to contribute to the reduced RBC life span.

Funding: Government Support - Non-U.S.

FR-PO1396

Serum Hepcidin Levels Predict the Progression of Renal Anemia in Patients with Non-Dialysis Chronic Kidney Disease Kakuya Niihata,[1] Naohisa Tomosugi,[2] Takuya Uehata,[3] Tatsuya Shoji,[1] Yusuke Sakaguchi,[1] Akira Suzuki,[1] Terumasa Hayashi,[1] Noriyuki Okada,[4] Yoshiharu Tsubakihara.[1] *[1]Department of Kidney disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [2]Medical Research Institute, Kanazawa Medical University, Kahoku, Japan; [3]Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Japan; [4]Department of Clinical Laboratory, Osaka General Medical Center, Osaka, Japan.*

Background: We reported that hemoglobin (Hb) levels in patients with non-dialysis chronic kidney disease (CKD) were correlated with serum hepcidin (Hep-25) in the cross-sectional study. In this study, we examined whether Hep-25 levels predict the progression of renal anemia in patients with non-dialysis CKD.

Methods: Study design: Prospective observational cohort study. Materials: 355 ambulatory patients with CKD (stage1-5) in Osaka General Medical Center between

February 1, 2007, and June 30, 2007. Exclusion criteria: Those who had received renal replacement therapy, or those who had received erythropoiesis-stimulating agent (ESA) therapy within 6 months before the baseline evaluation. Outcome: Starting ESA therapy by the study end date, i.e., December 31, 2010. Predictor: Hep-25 levels. Statistics: Restricted cubic spline curve analysis. Hazard ratios were obtained using Cox proportional hazard models adjusted for age, sex, estimated glomerular filtration rate (eGFR) and Hb. We divided the patients by the median of ferritin levels and did the same analysis for each group.

Results: The mean age (standard deviation [SD]) was 61.8 (14.9) years;56.6% of the patients were men. The mean (SD) eGFR and Hb were 47.5 (25.0) mL/min/1.73m2 and 12.7 (1.8) g/dL.The total number of events was 80. The Cox proportional regression analysis showed that Hep-25 was a significant predictor of progression of renal anemia (p=0.04, Linearity p=0.09), In high ferritin group, Hep-25 was also a significant predictor (p=0.01, Linearity p=0.02) , whereas not in low ferritin group (p=0.09, Linearity p=0.04).

Conclusions: The Hep-25 levels significantly predict the progression of anemia in patients with non-dialysis CKD. Relationship between Hep-25 and progression of anemia varies according to ferritin levels.

FR-PO1397

Procalcitonin vs. C-reactive Protein for Predicting Infection in Chronic Kidney Disease Patients Ji Hyeon Park, Seung Tae Han, Jaeyoung Yoon, Hye Ryoun Jang, Jung Eun Lee, Wooseong Huh, Dae Joong Kim, Yoon-Goo Kim, Ha Young Oh. *Department of Medicine, Samsung Medical Center, Seoul, Korea.*

Background: Recently, procalcitonin (PCT) is used widely as a surrogate marker for predicting infection in chronic kidney disease (CKD) patients because nonspecific elevation of C-reactive protein (CRP) caused from chronic inflammation was reported in these patients. However, it is uncertain whether PCT is more accurate or cost effective for detecting infection compared to CRP in CKD patients. We investigated the clinical usefulness of PCT and CRP in patients with CKD stage 3-5.

Methods: Two hundred patients with CKD were included. In the group I, all 159 patients had culture-proven infection. Patients had CKD stage 3, 4, or 5 (n=30, 30, and 99). The 98 CKD stage 5 patients in group I were divided into three subgroups: no dialysis (n=31), hemodialysis (HD) (n=34) and peritoneal dialysis (PD) (n=34). A total of 41 patients (group II) were CKD stage 5 without infection. Group II were divided into two subgroups: HD (n=27) and PD (n=14). Simultaneously measured serum PCT and CRP levels were analyzed in each group.

Results: Both serum PCT and CRP levels were significantly higher in infection group compared to noninfection group.

Procalcitonin(PCT) and C-reactive protein(CRP) levels in each group

	Infection+		Infection-	
	CRP(mg/dl)	PCT(ng/ml)	CRP(mg/dl)	PCT(ng/ml)
CKD 5 with HD	9.80 (22.47)*	4.50 (42.01)†	1.29 (2.35)	0.48 (0.80)
CKD 5 with PD	3.30 (9.78)*	1.32 (10.74)*	0.50 (1.41)	0.41 (0.46)

Data represent median values (interquartile range).*P<0.001 versus infection-, †P=0.01 versus infection-.

Serum PCT and CRP showed distinct positive correlation in group I (r= 0.568, p<0.01) and weak positive correlation in grop II (r=0.357, p=0.012). PCT showed a sensitivity of 95.1% and a specificity of 19.5% predicting infection, whereas the sensitivity and specificity of CRP were 96.7% and 29.3%, respectively.

Conclusions: Our study demonstrates that measuring CRP is still accurate and cost effective for predicting infection in CKD patients. PCT may not be superior to CRP in regard to both diagnosis and cost benefit in CKD patients.

FR-PO1398

C.E.R.A. Once-Monthly (QM) Maintains Stable Hemoglobin (Hb) Levels in High-Risk (HR) Patients (pts) with Chronic Kidney Disease (CKD) Not on Dialysis: An Analysis of 4 European Trials Jean-Philippe Ryckelynck,[1] Bruno Cianciaruso,[2] Peter F. Barany,[3] Carlo A. Gaillard.[4] *[1]CHU Clemenceau, Caen, France; [2]University Federico II, Naples, Italy; [3]Clintec, Karolinska Institutet, Stockholm, Sweden; [4]University Medical Center, Amsterdam, Netherlands.*

Background: To explore Hb stability with C.E.R.A. QM in different types of HR pts with CKD not on dialysis converted from shorter-acting erythropoiesis-stimulating agents (ESAs).

Methods: Adult CKD pts not on dialysis were converted to C.E.R.A. QM to achieve target Hb values of 10.0-12.0 or 10.5-12.5 g/dL. 4 HR subgroups were defined based on epoetin or darbepoetin alfa dose (≥8000 IU or 40 μg weekly, respectively) before switching, median Hb fluctuations (≥0.31 g/dL) during screening, pre-existing cardiovascular (CV) risk factors (diabetes or cardiac), or median baseline NT-proBNP (≥670 pg/mL). Hb parameters were evaluated over 8 weeks after a 16-week titration period.

Results: Pts (n=245), median age 70 (range 24-93) years, were classified into subgroups as shown.

Subgroup criteria	Pts (n)
ESA dose	
High	74
Low	171
Hb fluctuations*	
≥0.31 g/dL	122
<0.31 g/dL	122
Pre-existing CV risk factors	
No	98
Yes	147 (diabetes 101, cardiac 89)
Baseline NT-proBNP**	
≥670 pg/mL	63
<670 pg/mL	71

*Data missing for 1 pt; **unavailable for 1 trial

Mean Hb in the HR subgroups was: 11.5 g/dL (high dose), 11.5 g/dL (Hb fluctuations ≥0.31 g/dL), 11.6 g/dL (CV risk factors), 11.4 g/dL (NT-ProBNP ≥670 pg/mL). The corresponding Hb fluctuations were: 0.41, 0.46, 0.45, and 0.47 g/dL. Hb stability (mean Hb within target or change from baseline ≤1 g/dL) was maintained by 82%, 75%, 73%, and 84% of pts, respectively. There were no significant differences in any of these parameters between the HR and low-risk groups. HR pts required higher C.E.R.A. doses. Cardiac and vascular adverse events (AEs) were more frequent in HR pts; differences in the frequency of serious AEs were non-significant.

Conclusions: C.E.R.A. QM maintains Hb stability across different types of HR pts not on dialysis.

Funding: Pharmaceutical Company Support

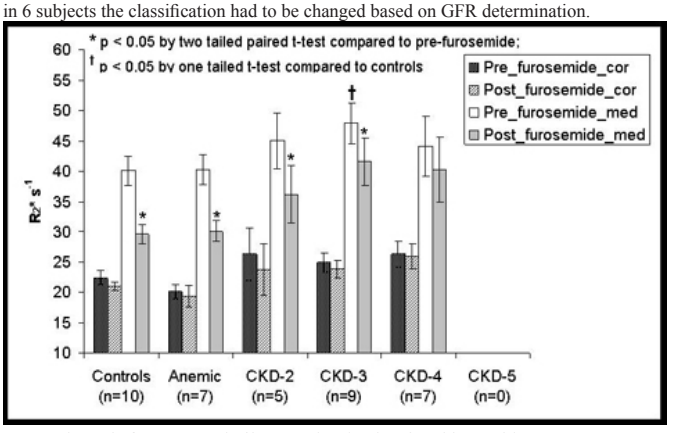
FR-PO1399

Blood Oxygenation Level Dependent MRI in Chronic Kidney Disease: A Preliminary Cross-Sectional Study Pottumarthi V. Prasad,[1] Muhammad Haque,[1] Luping Li,[1] Ujala Bokhary,[1] Rajiv Agarwal,[2] Stuart M. Sprague.[3] *[1]Radiology, NorthShore University HealthSystem, Evanson, IL; [2]Medicine, Indiana University, Indianapolis, IN; [3]Medicine, NorthShore University HealthSystem, Evanston, IL.*

Background: There is growing evidence for increased renal hypoxia in CKD and to support BOLD MRI as a useful tool for monitoring intra-renal oxygenation status. Here, a study was performed to compare BOLD MRI measurements in subjects at different stages of CKD.

Methods: A total of 38 subjects participated to-date: healthy control (n=10), anemic (n=7), CKD stage 2 (n=5), 3 (n=8), 4 (n=6), and 5 (n=3) based on eGFR. All subjects came to the study after an overnight fast and BOLD MRI studies were performed using a 3.0 T scanner and mGRE sequence before and after administration of 20 mg of furosemide (i.v.). R2* was used as BOLD parameter and is a reflection of the level of hypoxia. On a separate day, GFR was measured by iothalamate for accurate staging.

Results: Even though GFR by iothalamate and eGFR values were correlated (R2=0.7), in 6 subjects the classification had to be changed based on GFR determination.



As shown in figure, a trend of increased R2* values in subjects with CKD was apparent. Medullary R2* decreased significantly post-furosemide in all the groups (except CKD-4) and there was progressive reduction in magnitude of response in subjects with CKD.

Conclusions: Our preliminary experience with BOLD MRI measurements in patients with CKD suggest increasing levels of hypoxia both in cortex and medulla and lower medullary response to furosemide. This may suggest that increased hypoxia is probably related to reduced oxygen supply consistent with present understanding of the pathophysiology. Future studies should ask subjects to not take ACEi or ARBs prior to the BOLD MRI (Manotham K, ASN 2006 PO188) to observe potentially higher level of hypoxia.

Funding: NIDDK Support

FR-PO1400

Patients with CKD Have Reduced Levels of Circulating MicroRNA 145 and 155 Kraiwiporn Kiattisunthorn,[1] Neal X. Chen,[2] Kalisha O'Neill,[2] Xianming Chen,[2] Sharon M. Moe.[2,3] *[1]Medicine, Faculty of Siriraj Medical School, Mahidol University, Bangkok, Thailand; [2]Medicine, Indiana University School of Medicine, Indianapolis, IN; [3]Roudebush Veterans Affairs Medical Center, Indianapolis, IN.*

Background: Atherosclerotic cardiovascular disease is highly prevalent across the spectrum of CKD, especially in dialysis patients. MicroRNAs (miRNAs) can modulate cellular proliferation, differentiation and apoptosis and are shown to be associated with cancer and cardiovascular disease. A recent study has demonstrated that circulating hsa-miR-145 and hsa-miR-155 expression decreased in non CKD patients with cardiovascular disease (CAD). The objective of the current study is to determine the miRNA profile in normal and CKD patients with or without CAD.

Methods: miRNAs were isolated from 5 different pooled serum collections from healthy volunteers and 5 different pooled samples from hemodialysis patients by QIAzol Lysis Reagent and miRNeasy Mini Kit (Qiagen). Quantitative real-time PCR was performed to determine the expression of miR-145 and miR-155 using specific primers and normalized by U6 SnRNA.

Results: The results demonstrated that compared to healthy controls, miR-155 was down-regulated by 68% in hemodialysis patients. We also collected serum from CKD patients and found there is 23% decrease for miR-145 and 49% decreased for miR-155 ($p=0.04$) in the serum of stage 4 CKD patients with CAD compared to those of sex and age-matched stage 4 CKD patients without CAD. There was no difference in age, diabetes, albumin, calcium or phosphorus, but PTH was higher in those with CAD.

Conclusions: Circulating miR-155 levels were significantly lower in dialysis patients than that in healthy controls. Furthermore, CKD patients with CAD had decreased miR-145 and miR-155 compared to those without CAD. These results suggest that miR-145 and miR-155 may be novel biomarkers of CKD and CAD. Further studies are needed to validate these results.

Funding: Veterans Administration Support, Government Support - Non-U.S.

FR-PO1401

Urinary Excretion of Hepcidin 20, 22, and 25 Is Dependent on Iron Storage but Not on Each Serum Level Takahiro Kuragano, Hiroshi Nonoguchi, Takeshi Nakanishi. *Division of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

Background: We have already demonstrated that serum hepcidin-25 is dependent on iron storage but not inflammatory cytokines in hemodialysis patients without apparent inflammation. Recently, enhanced urinary hepcidin 25 excretion in patients with acute kidney injury has been suspected as an index of the tubular damage. To clarify the kinetic of hepcidin isoform in predialysis patients and the renal handling of hepcidin, we evaluated serum and urinary hepcidin isoform levels in patients with various stages of chronic kidney disease (CKD).

Methods: We measured serum and urinary levels of hepcidin 20, 22, and 25 in 46 predialysis CKD patients and 10 healthy controls by LC-MS/MS methods. Serum levels of iron, TIBC, transferrin, ferritin, creatinine (Cr), urea nitrogen (UN), IL-6 and TNF-α levels were measured. Urinary levels of β2-microglobulin (MG), N-acetyl-β-D-glucosaminidase (NAG), Liver type fatty acid binding protein (L-FABP), and albumin were also measured. Urinary level of each hepcidin isoform was normalized by Cr.

Results: There were no significant differences in serum hepcidin isoform or urinary levels between control and CKD patients. Serum levels of hepcidin 20, 22, and 25 were significantly correlated each other. However, there was no significant correlation between serum and urinary levels of each hepcidin isoform. Serum hepcidin 20 ($p<0.01$,$R=0.67$), 22 ($p<0.01$, $R=0.60$), and 25 ($p<0.01$, $R=0.62$) levels were significantly correlated with serum ferritin levels, but not with serum levels of creatinine, UN, IL-6, and TNF-α. Urinary hepcidin 20 ($p<0.01$, $R=0.67$), 22 ($p<0.01$, $R=0.60$) and 25 ($p<0.01$, $R=0.62$) levels were also significantly correlated with serum levels of ferritin, but not with urinary β2MG, NAG, FABP and albumin.

Conclusions: In CKD patients, there was no significant correlation between serum and urinary hepcidin isoform levels. Furthermore, each hepcidin isoform in serum as well as urine was faithfully correlated with iron storage but not with renal function, tubular damage, or inflammatory conditions. Further clarification regarding renal handling of hepcidin needs to be determined.

FR-PO1402

The Effect of Dietary Magnesium on Vascular Calcification and Erectile Dysfunction in a Rat Model of Chronic Kidney Disease Alexis Jozefacki,[1] Maria Tina Maio,[1] Kristin M. McCabe,[1] Navid Shobeiri,[1] Mason Curtis,[1] Spencer Barr,[1] Rachel M. Holden,[1,2] Michael A. Adams.[1] *[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; [2]Department of Medicine, Queen's University, Kingston, ON, Canada.*

Background: Erectile dysfunction (ED) is highly prevalent in patients with chronic kidney disease (CKD). We have previously shown that the pudendal artery (PA) is critical to the vasculogenic erectile response and that this artery is prone to vascular calcification (VC). Magnesium (Mg) has been shown to inhibit VC in vitro. We determined the effect of high dietary Mg on VC in the PA and on the associated erectile response in a rodent model of CKD.

Methods: Male Sprague-Dawley rats (375-400g) received a 0.25% adenine diet containing either control Mg (0.05%; n=8) or high Mg (0.2%; n=8) for 5 weeks. The o-cresolphthalein complexone method was used to assess the Ca content in the PA. Erectile response (average # of erections per 30 minute period) was assessed at weeks 1 and 5 using an apomorphine bioassay.

Results: The mean (SD) creatinine was 378(85) uM. Animals receiving the high Mg CKD diet had significantly higher ($p<.05$) levels of serum Mg compared to the control Mg diet. The Ca content in the PA was significantly lower in the high Mg group (23.75±14.6 nmoles Ca/mg tissue; $p<0.05$) than in the control Mg group (107.4±95.8 nmoles Ca/mg tissue). The Mg:Ca ratio in the PA decreased as Ca content in the PA increased and a plateau was attained as calcification progressed. Erectile responses were normal (>2.5 erections/30 mins) at week 1. At 5 weeks, the control Mg group had a significant decline in erectile response compared to both week 1 ($p<0.05$) and the high Mg group ($p<0.05$). The inverse correlation between PA Ca content and erectile response ($r=-0.7$) was close to attaining significance ($p=0.06$).

Conclusions: These results demonstrate that high dietary Mg inhibits VC in the PA and attenuates the decline in erectile responses observed in this rodent CKD model that may be due, in part, to the reduction in Ca content. Further studies are necessary to determine the mechanism by which Mg inhibits PA calcification and attenuates the progression of ED.

FR-PO1403

Uremic Toxins May Hange the Pharmacokinetics of Erythromycin in Healthy Volunteers, Chronic Kidney Disease and End-Stage Renal Disease Lynda A. Frassetto, Sam H. James, Hong Sun, Maribel Reyes, Leslie Benet. *UC San Francisco.*

Background: Elimination of non-renally excreted drugs is altered in chronic kidney diease (CKD); non-renal (hepatic) clearance (CLh) increases for some drugs and decreases for others. We hypothesize that drugs that require transporters for uptake or excretion may be affected by circulating uremic toxins thereby changing pharmacokinetic (PK) parameters. We studied the PK of E in healthy controls (HC), CKD and ESRD patients on hemodialysis (HD). We previously demonstrated that the uremic toxin carboxy methylpropylfuranpropanoic acid (CMPF) inhibits the transport of both E and its metabolite N-demethyl E and indoxyl sulfate (IS) inhibits the metabolism to the metabolite. CMPF and IS correlated significantly and inversely with CLh of E; CMPF $r= -0.46$, IS $r= -0.44$, both $p<0.05$.

Methods: We studied 12 CKD, 12 ESRD, and 12 HC subjects. eGFR was calculated using the MDRD equation. HD patients were studied on non-dialysis days. Each subject took 1 oral dose of E (250mg for HC and ESRD, 125mg for CKD; all data was dose adjusted). PK blood samples were drawn at 0, 0.5,1, 1.5, 2, 4, 6, 8, 10, 12, 14, and 24 hours (h). Urine samples were collected at 4 h intervals for 24 h. Plasma and urine concentration for E and N-demethyl E were determined by LC-MS/MS. Values expressed as mean ± SD.

Results: eGFR (mL/min/1.73m2): HC 86±11; CKD 28±7; ESRD ~0.

table1

	E			N-demethyl E		
	HC	CKD	ESRD	HC	CKD	ESRD
Cmax, ng/mL	473±297	568±370	764±529	38±26	35±21	*#65±51
T1/2, h	3.7±2.5	*7.8±5.3	*#5.5±2.7	3.6±1.9	4.8±2.2	*5.6±3.6
AUC0-∞, ng/mL*h	1173±789	*2230±1696	*2309±1805	120±71	192±136	*298±241
CLr, L/h/kg	0.04±0.01	*0.01±0.01	—	—	—	—
V/F, L/kg	22±12	*30±27	18±12	—	—	—
CL/F, L/h/kg	5.59±5.32	3.28±3.45	3.18±2.99	—	—	—

Apparent volume of distribution, V/F; * $p<0.05$ vs HC; # $p<0.05$ vs CKD

The metabolite/parent AUC was significantly greater in the ESRD than the CKD group (0.13±0.08 vs. 0.08±0.03, $p=0.04$).

Conclusions: Oral CL (CL/F) for the CKD and ESRD patients is the same suggesting the effect of uremic toxins on hepatic elimination is similar in both CKD and ESRD. In only the CKD patients the uremic toxins have a further effect increasing V/F, prolonging T1/2 and decreasing production of the metabolite.

Funding: Clinical Revenue Support

FR-PO1404

Comparative Analysis of CKD-EPI, MDRD, and AASK-MDRD Estimations of Glomerular Filtration Rate in a HIV-Infected Population Tahira P. Alves,[1] Pingsheng Wu,[2] Talat Alp Ikizler,[3] Timothy Sterling,[4] Samuel Stinnette,[4] Peter Rebeiro,[4] Suvro Ghosh,[1] Todd Hulgan.[4] *[1]Medicine, Nephrology, University of Texas Health Science Center and Audie L. Murphy VA Hospital, San Antonio, TX; [2]Medicine and Biostatistics, Vanderbilt University Medical Center, Nashville, TN; [3]Medicine, Nephrology, Vanderbilt University Medical Center, Nashville, TN; [4]Medicine, Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN.*

Background: Chronic kidney disease (CKD) is common among HIV-infected persons (HIV+) and very few studies have compared creatinine-based estimates of glomerular filtration rates (eGFR) in HIV+ persons.

Methods: We compared the MDRD, CKD-EPI, and AASK-MDRD (sub analysis of HIV+ blacks) equations in calculating eGFR level and the subsequent categorization into CKD stages 1-5. Wilcox signed rank test, Intra-class correlation coefficient (ICC), and Bland-Altman plots were used to compare the agreement of CKD-EPI vs. MDRD vs. AASK-MDRD (subanalysis of HIV+ blacks).

Results: Among the 2468 individuals included in this cross-sectional observational cohort study, 21% were female and 33% were black. Median CD4 count 327 cells/mm^3, VL 8,055 copies/mL, BMI 25kg/m^2, and albumin measured 4.3g/dL. CKD-EPI calculations

of eGFR resulted in a higher eGFR compared with the MDRD equation (median: 103 vs. 98, P<.01). The ICC of the two equations was 0.56 (95% confidence interval [CI]: 0.53,0.59). The ICCs of the three equations were: 0.96 (95%CI 0.95,0.96) between CKD-EPI and AASK-MDRD; 0.76 (95%CI: 0.73,0.78) between AASK-MDRD and MDRD; and 0.72 (95%CI: 0.68,0.75) between CKD-EPI and MDRD. Among HIV+ blacks, the AASK-MDRD equation resulted, on average, in the highest eGFR value (median: 109.3), with CKD-EPI the second (median: 107.1), and the MDRD with the lowest value (median: 104.8).

Conclusions: In conclusion, the results of all three equations closely agreed, but the CKD-EPI generated a higher eGFR than the MDRD. The AASK-MDRD equation generated the highest eGFR among blacks. Further studies are needed to determine the predictive value of these creatinine based eGFR on the risk of CKD progression, ESRD and mortality in the HIV+ population.

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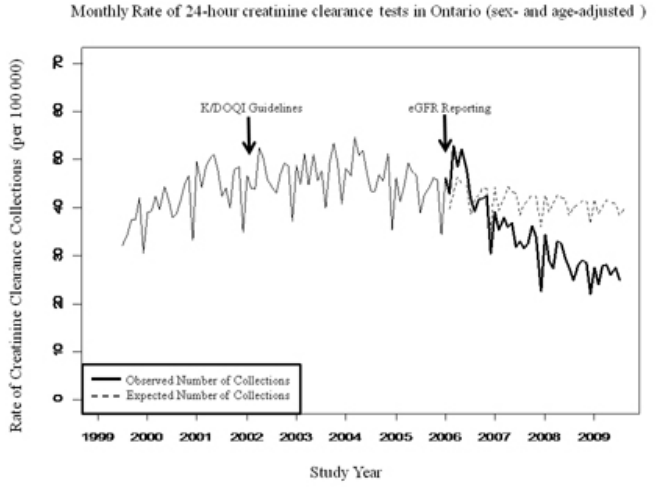
FR-PO1405

The Effects of Clinical Practice Guidelines and Estimated Glomerular Filtration Rate Reporting on Creatinine Clearance Testing Yoan K. Kagoma,[1] Amit X. Garg,[1,2,3,4] Lihua Li,[2] Arsh Jain.[1,2] *[1]Schulich School of Medicine and Dentistry, London, ON, Canada; [2]Division of Nephrology, University of Western Ontario, London, ON, Canada; [3]Department of Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada; [4]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada.*

Background: In 2002, the Kidney Disease Outcomes Quality Initiative (K/DOQI) suggested that renal function should be assessed using the estimated glomerular filtration rate (eGFR) and that the use of timed urine collection (creatinine clearance, CrCl) should be minimized. We sought to determine the effect of the K/DOQI guidelines and the province-wide introduction of eGFR reporting on requests for CrCl collection.

Methods: Time-series models with intervention analyses were used to model the impact of the following interventions on the rate of CrCl collection: 1)the publishing of K/DOQI guidelines and 2)the province-wide introduction of eGFR reporting. We collected sex- and age-adjusted CrCl collection rates and patient demographics monthly. Patients <25 years or on dialysis were excluded.

Results: The study period spanned August 1999 to July 2009. Data from 8.4 million patients per month were available.



The K/DOQI guidelines were not associated with a significant change in the sex- and age-adjusted rate of CrCl tests performed (p=0.82). eGFR reporting was associated with a significant decline (23.5% absolute change) in the sex- and age-adjusted rate of CrCl tests performed (p<0.0001).

Conclusions: In this setting, practice guidelines did not influence CrCl tests. Rather, the direct introduction of eGFR reporting into physician workflow resulted in a sudden and dramatic decrease in CrCl collection. These data suggest that changing physician practice patterns requires more than guidelines, rather it is more likely to occur through educational and structural changes to practice.

FR-PO1406

Clinical Phenotypes Associate with Outcome in ARVD James Ritchie, Constantina Chrysochou, Philip A. Kalra. *Vascular Research Group, MAHSC, University of Manchester, Salford Royal Hospital, United Kingdom.*

Background: ASTRAL showed unselected revascularization for atherosclerotic renovascular disease (ARVD) did not reduce mortality or cardiovascular events vs. medical therapy. Revascularization is still undertaken for some clinical presentations: flash pulmonary edema (FPE), refractory hypertension (RH) and rapidly declining renal function (RDF), though randomized data is lacking. By analyzing prospective data (1995-2010) on our ARVD cohort (n=819) we aimed to quantify effects of revascularization in these clinical settings.

Methods: Patients were categorized as receiving medical therapy (n=668) / revascularization (n=145). These groups were divided by presence / absence of indications to revascularize. Indications were divided by phenotype: FPE (identified from clinical records); RH (SBP >160mmHg despite ≥3 anti-hypertensives); RDF (creatinine at diagnosis >1.2x or 100µmol/L higher than result in previous 6 months). Effects of phenotype on death and dialysis events were assessed using Cox proportional hazards corrected for age, sex, BP, eGFR, vessel patency, proteinuria and angiotensin blockade. In the medical group patients with each indication were compared vs. those without. In the revascularization group each indication was compared for revascularized vs. medically treated patients.

Results: In the medical therapy group presence of an indication was associated with significant increases in hazards for both endpoints (p<0.005). This was not seen in the revascularized group.

In the medical group FPE had the greatest hazard for death and RDF for dialysis. Revascularization reduced hazard for death in patients with FPE but did not reduce hazard for dialysis for any phenotye.

		DEATH		DIALYSIS	
	n	HR (95%ci)	p	HR (95%ci)	p
	MEDICAL				
Indication	274	1.6 (1.2-2.2)	0.004	3.1 (1.7-5.7)	<0.001
FPE	44	2.4 (1.2-4.6)	0.01	2.7 (0.9-7.8)	0.08
RDF	146	1.5 (1.1-2.1)	0.02	3.5 (1.8-6.6)	<0.001
RH	124	1.2 (0.8-1.9)	0.47	2.6 (1.1-5.9)	0.02
	REVASCULARIZED				
Indication	71	0.7 (0.4-1.3)	0.27	1.2 (0.5-2.8)	0.76
FPE	15	0.08 (0.01-0.6)	0.01	*Insufficient events*	
RDF	33	1.3 (0.6-2.6)	0.49	1.9 (0.7-5.3)	0.19
RH	37	0.3 (0.1-1)	0.054	0.35 (0.06-2.2)	0.26

Conclusions: These findings emphasize the importance of the clinical presentation of the ARVD patient in deciding management.

FR-PO1407

Hepatitis C Virus Infection Increases Risk for End-Stage Renal Disease in Patients with Chronic Kidney Diseases Shang-Jyh Hwang,[1,2] Jia-Jung Lee,[1] Ming-Yen Lin,[1,3] H.C. Chen.[1,2] *[1]Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [2]Department of Renal Care, Kaohsiung Medical University, Kaohsiung, Taiwan; [3]Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung, Taiwan.*

Background: Current evidence regarding the relationship between hepatitis C virus infection and outcome of chronic kidney disease is controversial. We aim to explore whether hepatitis C virus infection increases risk for end stage renal disease in patient with chronic kidney disease.

Methods: Our study cohort was patients received multidisciplinary chronic kidney disease care in one medical center, southern Taiwan from 2002 through 5/31/2008. Subjects were traced until starting dialysis, death or end of 2008. Subjects with adequate register data and follow up for at least 90 days were included in our analysis. Survival analyses including Kaplan Meier and Cox proportional model were used to estimate the cumulative incident rate and risk of end stage renal disease and p-value <0.05 was considered as significant.

Results: A total of 2021 patients in our chronic kidney disease cohort were included in the final analysis.After adjusted age, sex, primary diseases, educational status, herb use, stage of chronic kidney diseases, body mass index, blood pressure, and hemoglobin, hepatitis C virus infection shows significantly increasing risk for end stage renal disease.

Table 1. The Cox-regression analysis of Hepatitis C virus infection to dialysis in patients with CKD

	Hazard Ratio	95% CI	p-value
Age	0.97	0.96-0.98	<0.001
Sex			
male	1.00	-	-
Female	0.46	0.34-0.61	<0.001
Primary diseases			
CGN	1.10	0.81-1.48	<0.001
Diabetes mellitus	2.23	1.66-3.00	<0.001
Others	1.00	-	-
Hepatitis C infection			
None	1.00	-	-
Yes	1.45	1.03-2.05	0.03

Model was adjusted by age, sex, primary diseases, educational status, herb use, stage of chronic kidney diseases, body mass index, blood pressure, hemoglobin.

Conclusions: Hepatitis C virus infection increased risk for end-stage renal disease in chronic kidney disease cohort in a hepatitis C endemic area. Our results encouraged further research to evaluate effect of antivirus drug on risk reduction for end stage renal disease in patient with chronic kidney disease.

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FR-PO1408

Urinary Level of Ngal Is Superior to Urinary Protein in Prediction of Chronic Kidney Disease Progression

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Background: Urinary neutrophil gelatinase-associated lipocalin (uNgal) level is a biomarker which enables early diagnosis of acute kidney injury. In this study, we evaluated the association of uNgal levels with clinical parameters in chronic kidney disease (CKD) patients, their changes by treatment, and the predictive ability of uNgal for the progression of CKD in comparison with urinary protein.

Methods: Urine samples were collected from 98 CKD patients admitted to our department of Kyoto University Hospital, and they were followed for a mean period of 12 months. uNgal levels were measured by chemiluminescence immunoassay and normalized to urine creatinine. We defined renal events as >50% increase in serum creatinine levels or progression to end-stage renal disease.

Results: uNgal levels were significantly correlated to serum creatinine and urinary protein levels, respectively. uNgal level was decreased by steroid therapy in IgAN or FSGS, and by percutaneous nephrostomy in hydronephrosis. uNgal levels on entry of patients who later developed renal events were significantly higher compared with those of patients without it. As to prediction of renal events, areas under the ROC curves for uNgal and urinary protein were 0.94 and 0.82, respectively (difference, p=0.06). Kaplan-Meier curves of renal event-free survival indicated that subjects with uNgal values above the optimal ROC cut-off level (112 μg/gCr) experienced much faster occurrence of renal events than ones below the cut-off ($P < 0.0001$).

Conclusions: uNgal was shown to be a useful biomarker of disease severity and treatment efficacy of CKD, and uNgal appears to be more powerful than urinary protein in anticipating deterioration of CKD.

FR-PO1409

Combined Association of Cystatin C-Based and Creatinine-Based Estimated Glomerular Filtration Rate with Mortality: The Atherosclerosis Risk in Communities (ARIC) Study

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Background: Estimates of GFR based on serum creatinine ($eGFR_{cr}$) can be biased by several non-GFR determinants, including muscle mass, resulting in misclassification of risk categories. Cystatin C-based GFR estimates ($eGFR_{cys}$) have a stronger and more linear association with mortality than $eGFR_{cr}$. More needs to be known about the combined association of these two markers with mortality.

Methods: We followed 10440 adults enrolled in the ARIC study for a median of 10.2 years. Participants were categorized as <30, 30-59, 60-89, 90-104 (reference) or ≥105 mL/min/1.73 m^2 determined by $eGFR_{cr}$ or $eGFR_{cys}$. We compared the association of $eGFR_{cr}$ and $eGFR_{cys}$ with mortality, individually as well as combined. Cox proportional hazards models were used with adjustment for demographics, diabetes, hypertension, cardiovascular disease, smoking, BMI and C-reactive protein.

Results: Mean age was 63 years with 22% blacks and 56 % women. 10.2% and 9.8% had eGFRcys and eGFRcr <60 mL/min/1.73 m2, respectively. Both reduced eGFRcys and eGFRcr were independently associated with mortality with a stronger association with eGFRcys than eGFRcr. Approximately half of the participants with eGFRcr 30-59 were reclassified up to >60 by eGFRcys. Participants reclassified to a higher GFR category by eGFRcys than eGFRcr had lower mortality risk and those reclassified to a lower category had higher mortality compared to those with concordant classification.

$eGFR_{cys}$	$eGFR_{cr}$ (ml/min/1.73 m^2)					
(ml/min/1.73m^2)	≥105	90 - 104	60 - 89	30 - 59	<30	Overall
≥105	1.2 (0.8, 1.8)	0.8 (0.5, 1.2)	0.5 (0.3, 0.8)	a	a	0.9 (0.7, 1.1)
N	[194]	[405]	[282]	[6]	[0]	[887]
90 - 104	1.3 (0.8, 2.1)	Reference	0.8 (0.6, 1.1)	0.3 (0.003, 1.9)	a	Reference
N	[139]	[848]	[1187]	[33]	[0]	[2207]
60 - 89	1.2 (0.7, 2.0)	1.2 (0.9, 1.5)	1.0 (0.8, 1.3)	1.0 (0.7, 1.4)	a	**1.2 (1.0, 1.4)**
N	[110]	[1219]	[4454]	[495]	[0]	**[6278]**
30 - 59	a	1.4 (0.7, 3.0)	**1.8 (1.3, 2.4)**	**1.9 (1.4, 2.5)**	a	**2.1 (1.8, 2.6)**
N	[3]	[51]	**[431]**	**[427]**	[11]	**[923]**
<30	a	a	**2.0 (1.2, 3.6)**	**4.7 (2.8, 8.1)**	**6.9 (4.1, 11.6)**	**4.1 (3.5, 5.0)**
N	[2]	[19]	**[69]**	**[28]**	**[27]**	**[145]**
Overall	1.2 (0.9, 1.6)	Reference	0.9 (0.8, 1.1)	**1.3 (1.1, 1.6)**	**5.8 (3.8, 8.8)**	
N	[448]	[2542]	[6423]	**[989]**	**[38]**	
Reclassified Up	NA	16%	22.90%	54%	28.90%	
Reclassified Down	56.70%	50.70%	7.80%	2.80%	NA	

N=sample size; a= sample size<25; **statistically significant results in bold**

Conclusions: Combining serum creatinine and cystatin C can improve mortality prediction replicating the results in the REGARDS cohorts suggesting this may provide a useful strategy when finer risk stratification is useful.

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FR-PO1410

Cost-Effectiveness of Chronic Kidney Disease Mass Screening Test in Japan

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Background: AlthoughCKD is a significant public health problem, strategy for its early detection is still a matter of controversy.

Methods: To assess the cost-effectiveness of mass screening for CKD, we performed cost-effectiveness analysis using a decision tree and Markov model to compare test modalities (dipstick test to check proteinuria only, serum creatinine (Cr) assay only, and both) in the context of the reform of Japan's mandatory annual health check-up for adults. In modelling, we carry out a deliberate literature survey to find out the best available evidence from Japan, while reports from other countries are excluded.

Results: Incremental cost-effectiveness ratios (ICERs) of mass screening compared to do-nothing are $12,660/QALY for urine dipstick test only, $90,250/QALY for serum Cr assay only, and $91,505/QALY for both, respectively. And ICERs associated with the reform are $103,618/QALY for mandating serum Cr assay in addition to currently mandatory dipstick test, and $100,016/QALY for mandating serum Cr assay and making dipstick test discretionary, respectively.

Conclusions: Taking a threshold to judge cost-effectiveness according to a World Health Organisation's recommendation, three times of Gross Domestic Product per capita, U.S.$128 thousand /QALY, a policy for reform that making serum Cr assay mandatory is cost-effective. And a choice of continuing current policy that keeping dipstick test mandatory is also cost-effective. Our results suggests that population strategy for CKD detection such as mass screening using dipstick test and/or serum Cr assay can be justified as an efficient use of health care resources in a population with high prevalence of the disease like Japan and Asian countries.

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FR-PO1411

Epidemiology of Pulmonary Hypertension in Different Stages of Chronic Kidney Disease Zhilian Li, Wei Shi, Shuangxin Liu, Xinling Liang, Wenjian Wang, Zhiming Ye, Yuan Han Chen, Lixia Xu. *Nephrology, Guangdong General Hospital.*

Background: Pulmonary hypertension(PH) has been reported to occur in a considerable proportion of patients with ESRD and has been regarded as an independent predictor of mortality in hemodialysis patients. However, the epidemiology of PH in non-ESRD patients remains unclear. The aim of this study is to evaluate the incidence of PH in different stages of chronic kidney disease (CKD) and the association of PH with cardiovascular status in this population.

Methods: We retrospectively evaluated the records of 1,600 in-patients between 2008 and 2010. Patients were divided into 6 groups: Group 1-4 for CKD stage 1-4; Group 5 for those were in stage 5 but still not or initiated dialysis <3 months; Group 6 for maintenance hemodialysis(MHD) patients(hemodialysis≥3 months). Systolic pulmonary artery pressure(SPAP) was evaluated using Doppler echocardiography and calculated using Bernoulli equation, a value of >40mmHg was defined as PH. History of cardivascluar events was recorded. Patients with chronic obstructive pulmonary disease(COPD), connective tissue disease, history of pulmonary embolism or chest wall or parenchymal lung disease, rheumatic heart disease, congenital heart disease and acute heart failure were excluded.

Results: PH was detected in 214(13.4%) of the total 1,600 CKD patients. Prevalence of PH in Group 1-6 was 0.5%, 3.17%, 3.77%, 6.92%, 12.84% and 30.70%, respectively. Chi-Square Test (linear-by-linear association) showed with renal function progressively declined, the incidence of PH (R2=0.772, P=0.000)and cardiovascular events (R2=0.959, P=0.000) increased. Patients with history of cardiovascular events had a higher incidence of PH than those without history of cardiovascular events (25.8% v.s 8.0%, P=0.000).

Conclusions: These results showed that incidence of PH was low in Stage 1-4 of CKD patients but elevated in ESRD especially in MHD patients. PH may be a risk factor of cardiovascular events in CKD patients.

FR-PO1412

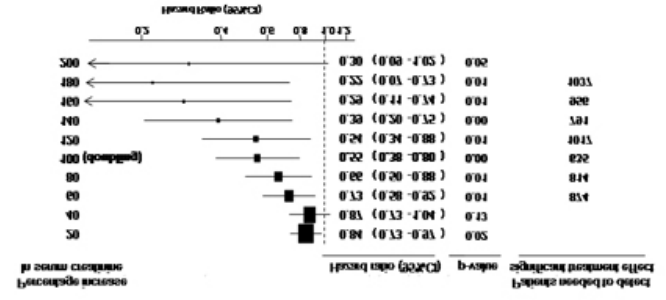
Is Doubling of Serum Creatinine the Right Endpoint in Nephrology Trials? Hiddo Jan Lambers Heerspink,[1] Ron T. Gansevoort,[1] Vlado Perkovic,[2] Giuseppe Remuzzi,[3] Julia Lewis,[4] Dick de Zeeuw.[1] *[1]UMCG, Groningen, Netherlands; [2]George Institute, Sydney, Australia; [3]Mario Negri Institute, Bergamo, Italy; [4]VanderBilt University, Nashville.*

Background: Doubling of serum creatinine (DSCR) is frequently used as an endpoint in nephrology trials. However, DSCR is reached by only few patients during trials. We aimed to assess whether adopting smaller increases in serum creatinine as endpoint may yield more endpoints while maintaining similar precision of treatment effects. If true, this would potentially allow fewer patients to be included in nephrology trials.

Methods: In a post-hoc analysis of the RENAAL and IDNT trial, the effects of Angiotensin Receptor Blockers (ARB) were estimated on the renal endpoint defined by different percentages increase in serum creatinine by means of Cox proportional hazard analysis. A post-hoc power calculation was performed to determine the minimal number of patients needed to obtain a significant treatment effect. The percentage increase in serum creatinine was assessed from month 3, thus excluding the initial hemodynamic effect.

Results: The effect of the ARB irbesartan decreased when smaller percentages increases in serum creatinine were used as endpoint (figure 1). However, the confidence interval of the treatment effect also decreased at smaller increases in serum creatinine as endpoint so that the least number of patients were required to detect a significant treatment effect at a 100% increase (doubling) in serum creatinine. Similar results were obtained in the RENAAL trial, or when the change in serum creatinine was calculated from baseline in both studies.

Conclusions: For drug trials in nephrology enrolling patients with type 2 diabetes and nephropathy, DSCR is an appropriate endpoint. Using smaller increases in serum creatinine as endpoint does not lead to fewer patients needed to detect a significant treatment effect.



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FR-PO1413

Decreased Kidney Function and Risk of Venous Thrombosis Gurbey Ocak,[1] Carla Y. Vossen,[1,2] Marion Verduijn,[1] Friedo W. Dekker,[1] Willem Lijfering,[1,3] Frits R. Rosendaal.[1,3] *[1]Department of Clinical Epidemiology, Leiden University Medical Center; [2]Department of Medical Genetics, University Medical Center Utrecht; [3]Department of Thrombosis and Haemostasis, Leiden University Medical Center, Netherlands.*

Background: Whether decreased kidney function is associated with venous thrombosis (VT) is unknown. We investigated whether decreased kidney function, assessed by estimated glomerular filtration rates (eGFR), is associated with an increased risk of VT. In addition, we investigated whether an increased risk of VT is a result of increased levels of hemostatic factors. Furthermore, we assessed the effect of decreased kidney function on VT in combination with other risk factors to identify high-risk groups.

Methods: MDRD based eGFR and hemostatic factors were determined in 2469 patients with VT and 2931 control participants enrolled in the MEGA case-control study. Odds ratios were calculated and adjusted for age, sex, and body mass index.

Results: Mildly decreased kidney function (eGFR 60-90 ml/min; n=3098) increased the risk of VT 1.1-fold (95%CI 1.0-1.2), moderately decreased kidney function (eGFR 30-60; n=235) 2.5-fold (95% CI 1.9-3.4), and severely decreased kidney function (eGFR <30; n=15) 5.8-fold (95% CI 1.6-21.0) as compared to persons with a normal kidney function (eGFR >90; n=2052). Nearly all hemostatic factors that we studied showed a shift to a procoagulant state with decreasing kidney function, most notably FVIII, with levels that compared with normal kidney function, were on average 6.4 IU/dL (95% CI 2.9-9.9), 34.5 (95% CI 24.5-44.4), and 135.7 (95% CI 90.0-181.4) higher for mildly, moderately, and severely decreased kidney function, respectively. The odds ratios for VT attenuated after adjustment for FVIII. The risk of VT was highly increased (odds ratios > 9) for moderately to severely decreased kidney function in combination with surgery, hospitalization, or non-O blood group.

Conclusions: Decreased kidney function was associated with VT and with a procoagulant state. The increased risk of VT was mediated by FVIII. The risk of VT was highly increased in CKD in the presence of other risk factors of VT.

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FR-PO1414

Low GFR after Kidney Donation Is Not CKD Laura V. de Vries,[1] H. Tent,[1] Johannes S. Sanders,[1] Hendrik Sijbrand Hofker,[2] Stephan J.L. Bakker,[1] Gerjan Navis.[1] *[1]Department of Nephrology, University Medical Center Groningen, Netherlands; [2]Department of Surgery, University Medical Center Groningen, Netherlands.*

Background: CKD staging is based on prognostic impact of GFR with/without proteinuria. Many kidney donors have an estimated (e)GFR<60 mL/min/1.73m² post-donation and thus meet with criteria of CKD stage 3 (CKD3). However, prognostic impact of a given GFR in two diseased kidneys may not be equivalent to the same GFR in one healthy kidney. To test this assumption, we compared renal function course in former kidney donors to CKD patients matched for GFR, age and gender.

Methods: We included 57 (63% male) kidney donors (baseline values 3 months after donation) and 57 CKD patients. All had repeated GFR (^{125}I-iothalamate) and ERPF (^{131}I-hippuran) measurements after 4.7±1.5 years.

Results: At baseline, 25% of donors met criteria for CKD3. In all donors GFR increased over time, with a slight fall in ERPF, while GFR and ERPF fell in all CKD patients. CKD stage improved to CKD 0-2 in 13 donors with CKD3 at baseline, whereas it worsened in 21 CKD patients. Change in GFR significantly differed between donors and CKD patients (see table), both for CKD with (n=31; -2.1±3.3) and CKD without (-1.1±3.2 ml/min/yr) proteinuria.

Conclusions: Thus, despite similar baseline GFR and CKD stage, renal prognosis is substantially different for donors and CKD patients. Although many donors initially meet criteria of CKD3 they should not be regarded as CKD patients. CKD staging is not applicable to kidney donors.

		Donors	CKD patients	P-value
Baseline	Age (yr)	48±12	48±11	1.00
	creatinine (mg/dl)	1.4±0.2	1.3±0.4	0.57
	UPE (g/24h)	0.0 [0.0-0.1]	0.7 [0.0-2.9]	<0.01
	GFR/BSA	67±11	71±18	0.21
	ERPF/BSA	264±48	304±128	0.03
	Mean arterial pressure	93±9	101±13	<0.01
	eGFR (CKD-EPI)	56±11	63±22	0.08
	% CKD 0-2	75	74	0.50
	% CKD 3	25	26	0.50
Follow-up	creatinine (mg/dl)	1.2±0.2	1.5±0.5	<0.01
	UPE (g/24h)	0.0 [0.0-0.2]	1.0 [0.0-2.5]	<0.01
	GFR/BSA	73±12	63±21	<0.01
	ERPF/BSA	247±49	258±96	0.45
	Mean arterial pressure	92±10	105±18	<0.01
	eGFR	63±13	54±20	<0.01
	% CKD 0-2	88	53	<0.01
	% CKD 3	12	42	<0.01
	% CKD 4	0	5	0.12
Change/ year	GFR/BSA	1.8±1.6	-1.4±3.4	<0.01
	ERPF/BSA	-3.2±6.6	-9.8±15.7	<0.01
	eGFR	1.2±1.6	-1.7±2.8	<0.01

UPE: urinary protein excretion; GFR, ERPF and CKD-EPI in ml/min/1.73m².

FR-PO1415

Chronic Kidney Disease (CKD), Hypertension Control, and Risk of Obstructive Sleep Apnea (OSA) Brian J. Mussio,[1,2] Loretta Simbartl,[2] Ralph Panos,[1,2] Charuhas V. Thakar.[1,2] *[1]Internal Medicine, University of Cincinnati, OH; [2]Medical Service, Cincinnati VA, Cincinnati, OH.*

Background: Hypertension is an important modifiable risk factor in patients with CKD. OSA is a known cause of secondary hypertension, however, there are limited studies examining its prevalence in CKD, and its effect on hypertension control

Methods: We conducted a prospective study in 211 patients at the Cincinnati Veteran's Administration Medical Center's CKD & Hypertension Clinic. All enrolled patients completed a Berlin Questionnaire and an Epworth Sleepiness Scale, validated screening tools for OSA and daytime sleepiness, respectively. Baseline demographics, comorbidities, laboratory data, and anti-hypertensive medications were recorded at the time of survey, whereas outpatient blood pressure readings were averaged for a time-period of one-year prior to the survey date. Univariate analyses were conducted using t-test, Wilcoxon test, and Chi-square tests.

Results: 99% of participants were male with a mean age of 63.7 (± 10.7), and mean creatinine of 2 mg/dl (± 1.3). Only 29% of patients had an average BP of < 130 or < 80 mm of Hg, and 80% of patients were taking ≥ 3 medications for hypertension. Based on the Berlin score, 142 (67%) participants were in the "high-risk" group for OSA, and 69 (33%) were in the "low-risk" group. Based on the 29 patients with a confirmed diagnosis of OSA, the sensitivity of the Berlin Questionnaire was 93%. Compared with the low-risk group, patients in high-risk group were younger (62.5±10.1 vs. 66.2±11.6, p = 0.02), had a higher prevalence of diabetes (51% vs. 36%, p = 0.04), and had higher Epworth scores (11±6 vs. 6±5, p < 0.001). Average blood pressures were similar in both groups, but the number of blood pressure medications required to achieve the blood pressures was higher in the high-risk group (3.2±1.3 vs. 2.7±1.2, p = 0.01).

Conclusions: OSA in veterans attending CKD/hypertension clinics may be under-recognized. Although blood pressure control was comparable, patients at high-risk for OSA required more antihypertensive medicines. Whether OSA is a modifiable risk factor in hypertension management in CKD needs prospective investigation.

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FR-PO1416

Development and Validation of a Model To Predict ESRD in Elderly Patients with Advanced CKD Puja Goswami,[1] Paul E. Drawz,[2,3] Denise C. Babineau,[4] Mahboob Rahman.[1,2,3] *[1]Medicine, University Hospitals Case Medical Center, Cleveland, OH; [2]Nephrology & Hypertension, Case Western Reserve University, Cleveland, OH; [3]Medicine, Louis Stokes Cleveland VAMC, Cleveland, OH; [4]Center for Clinical Investigation, Case Western Reserve University, Cleveland, OH.*

Background: KDOQI guidelines recommend preparation for renal replacement therapy (RRT) in all patients with a GFR<30. However, elderly patients are at increased risk for all-cause mortality and have a lower risk for ESRD. The goal of this study was to develop and validate a model to predict the 12 month risk for ESRD in elderly subjects with advanced CKD. Additionally, we assessed the ability of the model recently published by Tangri et al to predict ESRD in our elderly cohort.

Methods: We performed a retrospective cohort study at the Louis Stokes Cleveland VAMC. Eligible subjects were 65 years of age and older, had an outpatient GFR<30, and had not received RRT. The primary outcome was the need for RRT within 1 year of the index GFR. A Cox proportional hazards regression model was developed using backward step-down variable selection and boostrap sampling. The accuracy of the model to predict ESRD was assessed using Harrell's C index.

Results: Of the 1,866 patients included in the study, 77 patients developed ESRD. Risk factors for ESRD in the model were age (HR 0.47 (75th vs 25th quantile for continuous variables)), CHF (HR 4.72), elevated BP (HR 1.55), index GFR (HR 0.24), potassium (HR 0.67), and albumin (HR 0.69). There were significant interactions between GFR and CHF and between GFR and age. The bootstrap corrected C-index for the model was 0.85, indicating excellent predictive ability. The C-index for the Tangri model was 0.78, indicating good predictive ability.

Conclusions: A model using commonly available clinical measures showed excellent predictive ability for ESRD at 12 months in elderly subjects with advanced CKD. Additionally, we validated the ability of the Tangri model to predict ESRD in an external cohort. These risk prediction models can be utilized by patients and physicians to make more informed decisions regarding the need for preparation for ESRD, such as vascular access placement.

Funding: NIDDK Support

FR-PO1417

Risk Factors for Progression in DM and Non DM CKD Patients after CKD Integrated Program Mei-Chuan Kuo,[1,2] Chi-Chih Hung,[1] Shang-Jyh Hwang,[1,2] H.C. Chen.[1,2] *[1]Nephrology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [2]Faculty of Renal Care, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.*

Background: Taiwan has the highest incidence of end stage renal disease in the world. For decreasing the incidence of end stage renal disease and better care of chronic kidney disease patients, team of chronic kidney disease (CKD) was set up in our hospital since Dec 31, 2002. Despite of intensive intervention including education and diet modification, rapid progression was still found in many patients. This aim of this study is to evaluate the difference potential risk factors to influence the progression of DM and non DM CKD.

Methods: Patients that ever joined this program from Dec 31, 2002 to Dec 31, 2008 were enrolled. Those patients followed up for more than one year with at least 3 data of serum creatinine after joining this program were enrolled. After calculate estimated glomerular filtration rate (eGFR), we can further calculate the slope decline in each patient. The slope declines more than -1ml/min/1.73m^2/year was defined as progression group and -5ml/min/1.73m^2/year was defined as rapid progression (RP) group. By comparison with the demographic and laboratory data, we can get the potential risk factors for the progression of CKD. The statistic methods we used include chi-square test, student's t test and logistic regression.

Results: 1018 patients(female: 445, male: 573, age: 63±14 y/o) were enrolled in this study. 335 were DM patients. 211 patients were rapid progression and 100 were DM patients. The follow up days in rapid progression groups is shorter than others . The frequency for CKD nurse education and dietician education are higher in RP groups. By single variant analysis, older age, severity of proteinuria, lower hematocrit, higher serum cholesterol and phosphate level, rapid progression before CKD program and diabetes mellitus (DM) were risk factors for progression. After multivariant analysis, only severity of proteinuria are significant risk factors for CKD progression.

Conclusions: Severity of proteinuria is still the most significant risk factors for CKD progression despite of intensive CKD education program.

FR-PO1418

Therapeutic Plasma Exchange for Renal Indications in the Elderly: Ten Years Experience in One Center Emaad M. Abdel-Rahman, John S. Hayes, Jamison W. Chang, Rasheed A. Balogun. *Division of Nephrology, University of Virginia, Charlottesville, VA.*

Background: Elderly, above age 65 years, are growing in number. The structural and functional changes associated with aging place elderly at risk when challenged by extra corporal therapies as therapeutic plasma exchange (TPE). **Aim:** To compare renal indications and mortality associated with the use of TPE in elder versus younger patients.

Methods: We retrospectively analyzed data on all patients who underwent TPE for renal indications at the UVA between January 1 2000 and June 30, 2010. Beside demographic and comorbidity data, we collected therapy specific data; procedure access, indications, side effects and mortality data.

Results: During this period, 621 patients underwent 4722 sessions of TPE. Of them 191 patients were elderly (30.7%) and they underwent 1289 sessions (27.3%) of TPE. Total of 101 patients (16.3%) underwent 593 sessions of TPE because of renal- indications; 24 patients (92.3% white and 54% males) in the elderly group and 77 (90 % white, 58.1% males) in the younger. No statistical differences were observed in laboratory parameters, technique or comorbidies. Side effects of dyspnea and hypotension were documented in only two patients, both in the elderly cohort. Main indications of TPE in the elderly were glomerulonephritis (GN) followed by multiple myeloma (MM), with a trend towards more death in the elderly (p=0.07). The multivariable regression model which included age category, serum albumin and entrance serum creatinine was unable to predict mortality in this group of patients.

	Age (yrs)	# of treatment (Mean/pt)	GN	MM/Cast	Kidney TX rejection	HUS/TTP	Mortality
Elder (n=24)	73.5 +/- 6.2	109 (6.7)	70.8%	12.5%	8.3%	8.3%	42.3%
Young (n=77)	43.8+/-14.4	484 (6.2)	24.7%	6.5%	62.3%	6.5%	23.1%

Indications and Mortality Associated with TP in Elderly

Conclusions: In our experience, renal indications for TPE in elderly are different than for younger patients, with GN being the main indication .TPE used for renal indications in the elderly is relatively safe, with an increased occurrence of adverse events compared to younger patients. Trends towards death in the elderly may be multi factorial and not related to TPE alone.

FR-PO1419

Clinicopatholgical Manifestation in Japanese Patients of idiopathic Membranous Nephropathy with Each Era Shinji Kitajima,[1] Tadashi Toyama,[1] Kiyoki Kitagawa,[1] Kengo Furuichi,[1] Hitoshi Yokoyama,[2] Shuichi Kaneko,[3] Takashi Wada.[1] *[1]Division of Nephrology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; [2]Division of Nephrology, Kanazawa Medical University Hospital, Uchinada, Ishikawa, Japan; [3]Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Ishikawa, Japan.*

Background: The 20-year renal survival of idiopathic membranous nephropathy (IMN) in Japanese adults with nephrotic syndrome was reported around 60%. In this study, we evaluated the predisposing clinicopathological factors of patient survival with each era in Japan.

Methods: One hundred forty six Japanese patients (85 males and 61 females; mean age 46.2 years) with biopsy proven IMN from 1965 to 2007 in Kanazawa University Hospital were evaluated in this study. The patients were followed for more than three years, or until renal or patient death. The patients were divided into three groups with each era; group 1 (1963-1979, 74 cases), group 2 (1980-1989, 44 cases), and group 3 (1990-2007, 28 cases). Clinicopathological features were evaluated for rate of remission, renal death, and patient death.

Results: Age of onset was higher in group 3 (group 1; 37.7 years, group 2; 52.5 years, group 3; 58.6 years). The rate of nephrotic syndrome was higher in group 3 (group 1; 66.2%, group 2; 72.7%, group 3; 85.7%). Major immunosuppressive therapy at onset with each era were cyclophoshamide in group 1 (38.8%), immunoglobuline in group2

(59.3%), cyclosporine A in group 3 (60.7%) in addition to steroids. The remission rate at 100 months (group 1; 69.4%, group 2; 50.0%, group 3; 62.5%), the renal death rate at 100 months (group 1; 4.1%, group 2; 6.8%, group 3; 7.1%), and the patient death rate at 100 months (group 1; 4.1%, group 2; 9.1%, group 3; 7.1%) had no statistical difference. Based on the electron microscopic findings, the patients were assigned to two distinct groups, homogeneous type and heterogeneous type based on our previous report (Yoshimoto , et al. KI 2004). The rate of homogeneous type was high in group 3 (group 1; 57.8%, group 2; 50.0%, group 3; 82.1%).

Conclusions: Age, rate of nephrotic syndrome, and homogeneous type were higher in recent era.

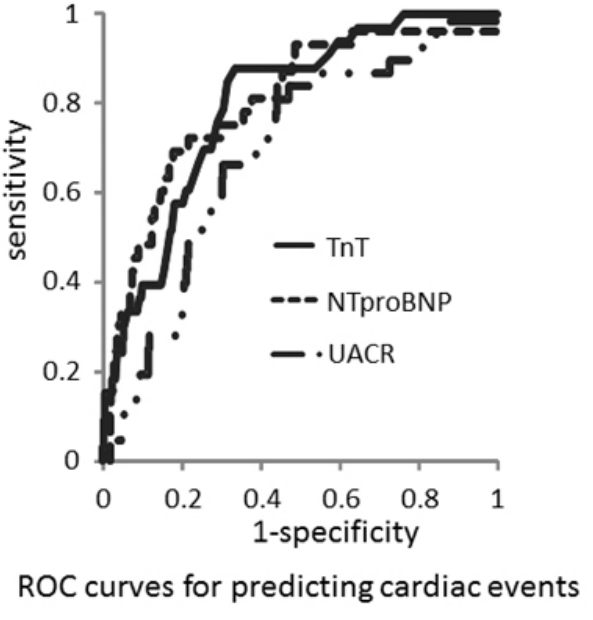
FR-PO1420

Cardiac Biomarkers in Patients with Chronic Kidney Disease Not on Dialysis Midori Hasegawa, Kyoko Kanayama, Yukio Yuzawa. *Nephrology, Fujita Health University School of Medicine, Toyoake, Aichi, Japan.*

Background: The purpose of this study is to assess N-terminal pro-brain natriuretic peptide(NT-proBNP), high sensitivity troponin T(hsTnT), and urinary albumin creatinine ratio (UACR) as predictors of cardiac events in patients with chronic kidney disease (CKD) not on dialysis.

Methods: The levels of serum NT-proBNP, hsTnT and UACR were measured in 413 ambulatory CKD patients not on dialysis whose estimated glomerular filtration rate was <60 ml/min/1.73 m^2. The patients underwent clinical follow-up for a median period of 17 months. Cardiac events were defined as cardiac death or hospitalization for acute coronary syndrome or for worsening heart failure.

Results: There were 33 cardiac events. The levels of NT-proBNP, hsTnT, and UACR were each divided into tertiles. Kaplan-Meier curves for cardiac events were clearly separated by the tertile of hsTnT, NT-proBNP, and UACR levels. Tertiles of NT-proBNP(HR4.79, 95%CI2.31-9.97), hsTnT (HR2.98, 95%CI 1.66-5.38), and UACR(HR2.71, 95%CI 1.64-4.48) were predictors of cardiac events by Cox regression analysis adjusted by age, gender, the presence of diabetes mellitus, and eGFR. Figure shows ROC curves for NT-proBNP (AUC 0.844; 95%CI, 0.782 to 0.907), hsTnT (AUC 0.795; 95% CI, 0.725 to 0.865), and UACR(AUC 0.714; 95%CI, 0.629 to 0.800) in predicting cardiac events. The best cut-off values were NT-proBNP of 1217pg/mL(sensitivity 72.7%, specificity 83.2%), hsTnTof 25.5 pg/mL (sensitivity 84.8%, specificity 68.2%), and UACR of 0.91g/gCr(sensitivity 66.7%, specificity 71.6%), respectively.



ROC curves for predicting cardiac events

Conclusions: NT-proBNP, hsTnT, and UACR are useful for risk stratification of cardiac events in CKD patients not on dialysis.

FR-PO1421

Risk Factors for Progression to CKD 3 in IgA Nephropathy Chung-Hoon Yu,[1,2] Jang-Hee Cho,[1,2] Mi-Kyung Jin,[1,2] Owen Kwon,[1,2] Kyung-Deuk Hong,[1,2] Ji-Young Choi,[1,2] Se-Hee Yoon,[1,2] Chan-Duck Kim,[1,2] Yong-Lim Kim,[1,2] Sun-Hee Park.[1,2] *[1]Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea; [2]Clinical Research Center for End Stage Renal Disease, Republic of Korea.*

Background: IgA nephropathy (IgAN) is the most common primary glomerulonephritis in Korea. To investigate the risk factors for progression, we retrospectively analyzed the data of IgAN from a single center in Korea.

Methods: Three hundred and twenty nine patients (M: F 173:156, mean age 34 ± 12) with biopsy-proven IgAN (January 2002 ~ December 2009) were available for analysis. Progression was defined as an development of CKD stage 3 (eGFR<60 mL/min/1.73m2 by MDRD) or start of renal replacement treatment (RRT) due to ESRD. Cox regression analysis was used and presented as odd ratio (OR; 95% CI).

Results: Number of patients with episodic gross hematuria, microscopic hematuria with proteinuria, nephritic syndrome and hypertension as an initial clinical presentation was 42 (12.8%), 203 (61.7%), 5 (1.5%) and 60 (18%), respectively. At presentation, mean creatinine and protein-creatinine ratio (PCR) by spot urine was 0.99±0.98 mg/dl and 970±130 mg/g. Number of patients with glomerulosclerosis on pathology was 151 (45%). During mean follow-up of 43 months (range 12~101), 14 (4.3%) patients had begun RRT and 28 (8.2%) patients were diagnosed as CKD stage 3 and above. With univariate analysis, age at diagnosis (p=0.014, OR=1.05 (CI 1.01-1.09)), glomerulosclerosis(p=0.02, OR= 7.89;(2.07-29.98)) and PCR > 500mg/g (p=0.013, OR=6.67 (1.508-29.67)) were associated with development of CKD stage 3. Glomerulosclerosis (p=0.01, OR=1.55 (1.07-2.26)) and hypertension (p=0.025, OR=3.55 (0.43-1.13)) were associated with start of RRT. With multivariate analysis, age at diagnosis, glomerulosclerosis and PCR ≥ 500mg/g were independent risk factors for development of CKD stage 3 and above.

Conclusions: This study suggests that age at diagnosis, hypertension, proteinuria more than 500mg/g and glomerulosclerosis on pathology are major risk factors for progression to CKD 3 and above in Korean patients with IgA nephropathy.

Funding: Government Support - Non-U.S.

FR-PO1422

Lower Estimated Glomerular Filtration Is Associated with Trajectories of Life-Space Mobility among Older Adults C. Barrett Bowling,[1] Patricia Sawyer,[2] Paul Muntner,[2] Paul W. Sanders,[2] Richard M. Allman.[1] *[1]Birmingham VAMC; [2]University of Alabama at Birmingham.*

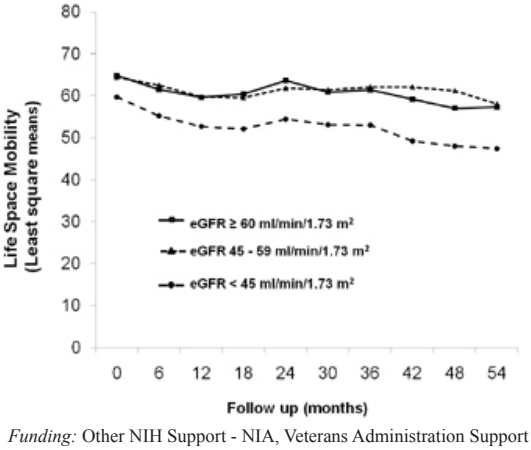
Background: While reduced estimated glomerular filtration rate (eGFR) is associated with decline in activities of daily living, less is known about the association of eGFR and mobility.

Methods: This analysis includes prospective, observational data from 400 community-dwelling Medicare beneficiaries (age >69) who had serum creatinine measured during an in-home assessment in 2004. eGFR was calculated using the CKD-EPI equation and categorized as ≥ 60, 45-59 and <45 ml/min/1.73 m^2. Life-space mobility was evaluated by telephone interviews every six months for up to 54 months. The Life-Space Assessment (LSA) reflects mobility and social participation by measuring where a person goes, how frequently they go there, and the degree of assistance needed. LSA scores range from 0-120 with higher scores indicating greater mobility.

Results: Participants had a mean age of 77.6 (SD=5.8, range 69-98), 42% were African American, 50% were female, and 29% and 20% had eGFR 45-59 and <45 ml/min/1.73 m2, respectively. Baseline life-space scores were 66.8, 64.6, and 53.7 for participants with eGFR levels of ≥ 60, 45-59 and <45 ml/min/1.73 m2, respectively (p<0.05 for those with eGFR < 45 versus ≥ 60 ml/min/1.73 m2). After adjustment for age, race, and sex, eGFR < 45 ml/min/1.73 m2 was associated with life-space trajectory (p=0.001; figure).

Conclusions: Among community-dwelling older adults, eGFR <45 ml/min/1.73 m^2 is associated with a lower baseline life-space mobility and longitudinal life-space mobility trajectory.

Figure. Trajectories of Life-Space Mobility among participants of the UAB Study of Aging by baseline level of estimated glomerular filtrate rate (eGFR) adjusted for age, sex, and race



Funding: Other NIH Support - NIA, Veterans Administration Support

FR-PO1423

Factors Associated with Impaired Urinary Albumin Excretion in Chinese Rural Population Xiaohong Fan, Jianfang Cai, Bixia Gao, Hang Li, Xuemei Li, Xue-Wang Li. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: Obesity may be measured by five anthropometric indicators such as waist circumference(WC), waist-to-hip ratio(WHR), waist-to-height ratio(WSR), and so on . We assess to investigate the relative factors associated with UAE in Chinese rural population, especially obesity and uric acid.

Methods: The investigated subpopulation derived from an epidemiological study in Pinggu district, Beijing(n=992, 51.7±10.8 years old) was analyzed in this

study. Measurements included overnight urine collection for UAE, blood pressure, anthropometric indicators, lipids, fasting glucose, uric acid, and hypersensitive C-reactive protein(hsCRP).

Results: In multiple logistic regression analysis, male, hypertension, diabetes, increased level of uric acid and hsCRP were significantly associated with albuminuria(UAE≥20ug/min); but five anthropometric indicators were not independently related to albuminuria. If stratified by gender, hyperuricemia and hsCRP were significantly potential risk factors for albuminuria in females, whereas the significant associations were not detected in males. And abdominal obesity measured by WC(OR=2.59 95%CI:1.26-5.33, P=0.03), WHR(OR=2.38, 95%CI:1.14-4.94, P=0.04) and WSR(OR=2.52, 95%CI:1.21-5.29, P<0.05) was significantly related to albuminuria in females after adjusted for age, diabetes and hypertension, but the association was not detected in males.

Logistic regression for relative factors associated with UAE

Population	relative factors	OR value (95%CI)	P value
Overall	male	1.68(1.15-2.47)	<0.01
	hypertension	2.29 (1.54-3.42)	<0.01
	diabetes	4.30 (2.75-6.73)	<0.01
	hyperuricemia	2.31 (1.15-4.68)	0.02
	elevated hsCRP	1.83(1.11-2.99)	0.02
Women(N=529)	diabetes	4.36 (2.34-8.15)	<0.01
	hyperuricemia	7.13(2.12-23.94)	<0.01
	elevated hsCRP	2.49 (1.24-5.00)	0.01
Men(N=463)	hypertension	3.20(1.86-5.51)	<0.01
	diabetes	4.83(2.50-9.35)	<0.01
	hyperuricemia	1.27 (0.52-3.14)	0.60
	elevated hsCRP	1.38 (0.68-2.82)	0.38

Conclusions: As the potential risk factors, hyperuricemia and elevated hsCRP had the genetic-specific effect on UAE, especially in females, abdominal obesity was also significantly related to impaired UAE in females.

Funding: Government Support - Non-U.S.

FR-PO1424

Self-Reported Chronic Kidney Disease Prevalence – Results from the 2009 Michigan Behavioral Risk Factor Surveillance System Lori Corteville,[1] Chris Fussman,[1] Elizabeth Hedgeman,[2] Jerry Yee,[3] Rajiv Saran.[2] *[1]Michigan Department of Community Health, Lansing, MI; [2]University of Michigan, Ann Arbor, MI; [3]Henry Ford Hospital, Detroit, MI.*

Background: The incidence of kidney failure in Michigan is among the highest in the nation. However, state-specific prevalence estimates for the earlier stages of chronic kidney disease (CKD) are lacking. We sought to estimate the prevalence of CKD as well as the frequency of testing for CKD in the Michigan population using the Michigan Behavioral Risk Factor Surveillance System (MiBRFSS).

Methods: The MiBRFSS is an annual, state-level, random-digit-dialed telephone survey of adults who are 18 years of age and older conducted in cooperation with the Centers for Disease Control and Prevention; goals include providing population-level estimates of health behaviors, knowledge and awareness. In 2009, >6,000 Michigan adults were asked four questions regarding their current kidney function and whether they had undergone a serum creatinine and/or urine albumin test in the past three years. Final survey results were weighted to adjust for selection probability and to approximate the Michigan population.

Results: Initial results of this first MiBRFSS CKD module suggest that testing-for and awareness-of CKD is low within the Michigan adult population. Overall, 3.5% of Michigan adults were aware they had been diagnosed with CKD, and fewer than half (38.6%) were aware of having been tested in the past three years. Among individuals with diabetes (the leading cause of ESRD in Michigan), 8.6% were aware of a CKD diagnosis and 63.3% reported having been tested. Similarly, adults with other known risk factors had higher odds of awareness of CKD and testing for CKD than the general population.

Conclusions: We report the first measurement of state-level CKD prevalence using the MiBRFSS. Our results indicate that self-reported prevalence and testing is low within the general population. We contend that state efforts to raise the levels of detection and awareness of CKD are warranted to reduce the number of adults in Michigan living with CKD-associated complications and kidney failure.

Funding: Other NIH Support - Centers for Disease Control and Prevention

FR-PO1425

Soluble RAGE, Glycated Hemoglobin and C-reactive Protein as Risk Factors for Incident Chronic Kidney Disease: The Atherosclerosis Risk in Communities (ARIC) Study Brad C. Astor,[1] Marc Halushka,[1] Ron C. Hoogeveen,[2] Christie Ballantyne,[2] Josef Coresh,[1] Elizabeth Selvin.[1] *[1]Johns Hopkins University; [2]Baylor College of Medicine.*

Background: Advanced glycation end products (AGEs) and their cell-bound receptors (RAGE) have been implicated in the pathogenesis of atherosclerosis and progression of chronic kidney disease (CKD). Circulating soluble RAGE (sRAGE) may act as a decoy to prevent the inflammatory processes initiated by RAGE activation. Alternatively, sRAGE level may be a marker of higher AGE levels, indicating increased risk of complications.

Methods: We conducted a case-control study to determine whether levels of sRAGE, glycated hemoglobin (HbA1c) and high-sensitivity CRP (hsCRP) are risk factors for incident CKD over 6 years. GFR was estimated by cystatin C ($eGFR_{Cystatin}$) and serum creatinine ($eGFR_{CKD-Epi}$) in ARIC Study participants (ages 47-68 years; 55% female; 19% black). A total of 1,942 participants with $eGFR_{CKD-Epi}$ and $eGFR_{Cystatin}$≥60 mL/min/1.73m^2 at baseline were included. CKD cases had a decline in eGFR≥25% over 6 years and a final eGFR<60 mL/min/1.73m^2.

Results: Higher HbA1c and CRP were significantly associated with incident CKD defined by eGFRCKD-Epi and eGFRCystatin. Higher sRAGE was associated with incident CKD by eGFRCKD-Epi only. Each of these associations was stronger in blacks (n=365) than whites (n=1,577).

Adjusted* Odds Ratio (95%CI) for Incident CKD, defined by:

	$eGFR_{CKD-Epi}$ (n=156)	$eGFR_{Cystatin}$ (n=433)
HbA1c category		
<5.7%	1.0 (Reference)	1.0 (Reference)
5.7-6.5%	1.1 (0.7-1.7)	1.1 (0.8-1.4)
>6.5% or diabetes†	2.1 (1.3-3.4)	1.9 (1.4-2.7)
sRAGE quartile		
<705 pg/mL	1.0 (Reference)	1.0 (Reference)
705-961 pg/mL	1.4 (0.8-2.3)	1.1 (0.8-1.5)
962-1266 pg/mL	1.8 (1.0-3.0)	0.9 (0.7-1.4)
>1267 pg/mL	1.9 (1.2-3.3)	1.1 (0.8-1.6)
8-fold higher hsCRP	1.8 (1.3-2.6)	1.4 (1.1-1.8)

***** Adjusted for age, sex, race, baseline eGFR, systolic blood pessure, anytihypertensive medication use, current smoking, BMI, LDL, HDL, triglycerides. †Self-reported diagnosis or medication use.

Conclusions: Higher HbA1c and CRP are risk factors for incident CKD in the general population. It remains uncertain whether sRAGE is an independent risk factor for CKD.

Funding: NIDDK Support

FR-PO1426

Solube RAGE, Glycated Hemoglobin,and hsCRP as Predictors of End-Stage Renal Disease: A Case-Control Analysis in the Atherosclerosis Risk in Communities (ARIC) Study Brad C. Astor,[1] Marc Halushka,[1] Ron C. Hoogeveen,[2] Christie Ballantyne,[2] Elizabeth Selvin,[1] Josef Coresh.[1] *[1]Johns Hopkins University; [2]Baylor College of Medicine.*

Background: Advanced glycation end products (AGEs) and their cell-bound receptors (RAGE) have been implicated in the pathogenesis of atherosclerosis and chronic kidney disease (CKD). Circulating soluble RAGE (sRAGE) may act as a decoy to prevent the inflammatory processes initiated by RAGE activation. Alternatively, sRAGE level may be a marker of higher AGE levels, indicating increased risk of these complications.

Methods: We conducted a case-control study nested within the ARIC Study to determine whether levels of sRAGE, glycated hemoglobin (HbA1c), and hsCRP predict incident ESRD over 18 years of follow-up. A total of 161 ESRD cases were frequency matched on sex, race, diabetes status and baseline eGFR (10 mL/min/1.73m^2) to 141 controls.

Results: Median (IQR) eGFR was 65.7 (47.4-92.3) mL/min/1.73m2. Cases had higher mean systolic blood pressure (138 vs. 130 mmHg; p=0.01), were more likely to be using antihypertensive medication (64 vs. 52%; p=0.03), and had higher mean sRAGE (1240 vs. 1030 pg/mL; p=0.02) than controls. Higher sRAGE was significantly associated with odds of ESRD in minimally adjusted analyses. Higher sRAGE and HbA1c were strongly associated with ESRD after full adjustment.

Odds Ratio (95% CI) of ESRD

	Minimally Adjusted*	Fully Adjusted†
HbA1c category		
<5.7%	1.0 (Reference)	1.0 (Reference)
5.7-6.5%	2.9 (1.1-7.2)	4.6 (1.6-13.2)
≥6.5% or diabetes‡	1.8 (0.3-12.4)	11.6 (4.5-30.0)
sRAGE quartile		
<620pg/mL	1.0 (Reference)	1.0 (Reference)
620-880 pgmL	1.9 (0.8-4.5)	1.4 (0.5-4.0)
880-1170 pgmL	1.4 (0.6-3.2)	1.6 (0.6-4.4)
≥1170pg/mL	3.2 (1.3-7.8)	4.8 (1.6-14.0)
8-fold higher hsCRP	1.2 (0.7-2.2)	1.1 (0.5-2.3)

*Adjusted for Matching criteria. †Adjusted for age, sex, race, baseline eGFR, systolic blood pressure, anytihypertensive medication use, current smoking, BMI, LDL, HDL, triglycerides and variables in the table. ‡Self-reported diagnosis or medication use.

Conclusions: Higher sRAGE and HbA1c are independent risk factors for ESRD.

Funding: NIDDK Support

FR-PO1427

Association of Serum Levels of and Genetic Variation in Inflammatory Genes with Decline in Renal Function: Results from the Chronic Renal Insufficiency Cohort (CRIC) Study Jayanta Gupta,[1] Peter A. Kanetsky,[1] Nandita Mitra,[1] Marshall M. Joffe,[1] Harold I. Feldman,[1] Muredach Reilly,[1] Nicolas Jose Guzman,[2] Maria R. Wing,[2] Vaidyanathapura S. Balakrishnan,[3] Vallabh O. Shah,[4] John W. Kusek,[5] Dominic S. Raj.[2] *[1]University of Pennsylvania; [2]George Washington University; [3]Tufts Medical Center; [4]University of New Mexico; [5]NIDDK.*

Background: Serum levels of inflammatory biomarkers and inherited variation in genes encoding these biomarkers previously have been associated with adverse renal outcomes. We investigated the association of serum levels of interleukin (IL) 6, IL1β, IL1 receptor antagonist (IL1RA), transforming growth factor (TGF) β, high sensitivity C-reactive protein (hsCRP) and fibrinogen and underlying genetic variation with progressive deterioration of renal function in CRIC study participants.

Methods: Biomarkers were determined in baseline serum samples. Genotypes for 266 corresponding SNPs were available from the ITMAT/Broad/CARE chip. For each participant, up to five measures of eGFR were available across study visits. Estimates were determined separately among 1,638 white and 1,651 African-American subjects

using a linear mixed effects model. Analyses were conducted with log-transformed measures. We adjusted for age, gender, and baseline eGFR in genetic models, and also for proteinuria, diabetic status, blood pressure and smoking status in addition to other covariates in biomarker models.

Results: Serum level of hsCRP was significantly associated with decline in eGFR in the African-American population after correction for the false discovery rate (FDR; p=0.04). We noted no other associations with baseline serum measures among either the white or African-American group. Although several variants in various genes were nominally associated with temporal decline in eGFR (p<0.05), none retained significance after FDR adjustment.

Conclusions: hsCRP predicts deterioration of renal function in the African-American subgroup of the CRIC cohort. Individual sequence variants in selected inflammatory pathway genes were not associated with progression of kidney disease; however, these findings need to be further investigated using pathway-based analysis.

Funding: NIDDK Support

FR-PO1428

Even Mild Chronic Kidney Disease Increases the Risk of Adverse Events after Elective Cholecystectomy: Analysis of National Data from the American College of Surgeons Sreedhar A. Mandayam,[1] Linda W. Moore,[2] Stephen L. Jones,[2] Edward Graviss,[2] Barbara Lee Bass,[2] William E. Mitch,[1] A. Osama Gaber.[2] *[1]Nephrology, Baylor College of Medicine, Houston, TX; [2]Weill Cornell Medical College, The Methodist Hospital, Houston, TX.*

Background: Cardiovascular risks from CKD are well described but whether CKD Stage 3 (CKD3) increases the risk of unanticipated adverse events in adults undergoing elective surgery is unknown. We assessed whether CKD3 increases unanticipated adverse events after elective cholecystectomy

Methods: We evaluated the ACS-NSQIP 2005-2007 database (a nationally representative dataset of surgical procedures and outcomes) for 30-day mortality and major complications following elective surgery. Pre-operative serum creatinine (SCr) and estimated GFR (CKD-Epi formula) were used to stratify patients into CKD3 (eGFR, 30-59) or NoCKD (eGFR>90)

Results: Non-emergency cholecystectomy occurred in 18,260 cases: median age was 47 (18-89years), 66.9% were white, 71% were women, 11.7% diabetic and 35.2% hypertensive. CKD3 was present in 5,542 (30.4%) with median SCr 1.2mg/dL; 0.9 to 2.5 compared to NoCKD (median Scr 0.7mg/dL, 0.4 to 1.3). Only 62 patients died but 638 had major complications. 30 day mortality occurred in 42 CKD3 pts vs 20 with NoCKD (p<0.0001) Major complications occurred in 5.9% of CKD3 and 2.5% of the NoCKD group (p<0.0001). Length of stay was also longer (2.2±0.4days vs 1.2±0.2days; p<0.0001). By multivariate nominal logistic regression, CKD3 was significant risk factor for major complications (p=0.0007). The proportional hazard for mortality was 2.6 (95% CI, 1.5-4.6; p=0.0007) with CKD3 of all pre-surgery risk factors.

The presence of CKD3 carries a significant risk of mortality and major complications plus a longer hospitalization even when the surgical procedure (e.g., cholecystectomy) is "low risk". Since the median serum Cr was only 1.2, most patients & surgeons are likely be unaware of preexisting kidney disease.

Conclusions: The presence of even mild renal insufficiency increases the risk(>2.5 fold) of major adverse outcomes following elective surgery. This raises the potential for more intensive evaluation in patients with CKD3.

Funding: NIDDK Support, Private Foundation Support

FR-PO1429

Progression of Risk Factors for Chronic Kidney Disease in Zuni Indians Antonia M. Harford,[1] Arlene Bobelu,[2] Vallabh O. Shah,[1] Donica M. Ghahate,[1] Jeanette Bobelu,[1] S. Paine,[1,2] Philip Zager.[1,2] *[1]Internal Medicine, UNMHSC, Albuquerque, NM; [2]Dialysis Clinic, Inc., Albuquerque, NM.*

Background: The Zuni Kidney Project (ZKP) has described the epidemic of kidney disease in the Zuni Indians in a population based cross-sectional study identifying high prevalence of incipient and overt albuminuria in both diabetic and non-diabetic subjects. The subsequent investigation of Genetics of Kidney Disease in Zuni Indians (GKDZI) described the heritability of kidney disease and its intermediate phenotypes in a study of extended Zuni families.

Methods: In order to test the hypothesis that the risk factors for CKD would progress over time, we performed an analysis of a subset of GKDZI subjects who participated in both ZKP and GKDZI.

Results: Five hundred and twenty nine individuals who participated in GKDZI were studied at 2 time points at a mean interval of 6.7 years (range 2.5-9.9 years). Forty eight percent of this cohort was female, mean age at the 1st study point was 30.8 years. The table shows the progression of CKD and its risk factors over time.

CKD RISK FACTOR PROGRESSION IN ZUNI INDIANS

	Time Point 1	Time point 2
ESRD	0	4
% w eGFR <60	1.7%	3.2%*
Mean BMI	27.3	29.0*
% Diabetic	12.6	19.3*

529 participants (* statistically significant p<0.05)

The development of ESRD in 4 individuals in this cohort underscores the high incidence of renal disease previously described by our group in this population. In addition decreased renal function was noted in 9 (1.7%) individuals at the time point I and 17 (3.2%)at time point II. 105 participants were teenagers at the first study point and young adults at the second time point. This group had particularly high rates of progression of obesity, 42.9% were overweight or obese at time point I, 63.7% at time point II.

Conclusions: This analysis of a cohort of individuals from the ZKP/GKDZI studied at 2 time points over up to 9 years shows a progression of CKD and its risk factors including diabetes and obesity. These findings reinforce the need for interventions to modify these risk factors for CKD progression in this high risk population, particularly amongst the young adult Zuni.

Funding: NIDDK Support, Clinical Revenue Support

FR-PO1430

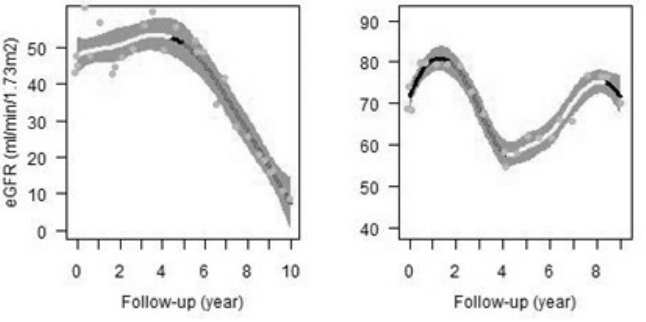
A Novel Within-Person Design To Study the Relationship between Concurrent Risk Factors and CKD Progression Liang Li,[1] Bo Hu,[1] Lawrence J. Appel,[2] Brad C. Astor,[2] Julia Lewis,[3] Michael S. Lipkowitz,[4] Robert D. Toto,[5] Xuelei Wang,[6] Jackson T. Wright,[6] Tom H. Greene.[7] *[1]Cleveland Clinic; [2]Johns Hopkins; [3]Vanderbilt; [4]Georgetown; [5]University of Texas Southwestern; [6]CWRU; [7]U Utah.*

Background: GFR decline in CKD is often assumed linear over time. We recently showed that over 9-12 yrs follow-up, a substantial portion of 1094 African American Study of Kidney Disease (AASK) pts had nonlinear eGFR patterns, which may include periods of rapid GFR decline or stable/improving GFR. Concurrent risk factors from the two periods on the same patient can be compared to study their relationship with CKD progression, removing confounding from baseline characteristics.

Methods: Penalized splines were used to estimate nonlinear eGFR trajectories. For each patient, we define a stable/improving period to be at least 3 yrs with eGFR slope no steeper than 3ml/min/1.73m^2/yr and total decline<4.5 ml/min/1.73m^2(red segment in figure) and a rapid decline period with slope steeper than 3 and total decline>12(yellow). Concurrent factors, measured every 6-12 months, are compared between the two periods with ANOVA.

Results: 105 AASK pts had both a stable/improving period and a rapid decline period. Rapid GFR decline was associated with higher serum sodium (p=0.021), serum CO2 (p=0.012), creatinine (p<0.001), urine protein/creatinine ratio (p<0.001), hospitalization rate (p=0.032), and lower urine urea nitrogen (p=0.0014), but not with urine sodium (p=0.69) and serum glucose (p=0.67).

Conclusions: This design is a new way to study relationship between concurrent risk factors and CKD progression, which avoids confounding from patient specific factors. It may yield useful insight into the pathophysiology of biomarkers and other concurrent risk factors with the acceleration/deceleration of CKD progression.



Funding: NIDDK Support

FR-PO1431

Hypoalbuminemia, Mortality and Chronic Kidney Disease (CKD) among Participants of the REGARDS Study Rebecca J. Schmidt,[1] Bethany S. Pellegrino,[1] Suzanne E. Judd,[2] Paul Muntner,[2] David G. Warnock,[2] Brian D. Bradbury,[3] Orlando M. Gutierrez,[2] William M. McClellan.[2] *[1]West Virginia University, Morgantown, WV; [2]University of Alabama, Birmingham, AL; [3]Amgen, Thousand Oaks, CA.*

Background: The predictive value of hypoalbuminemia in patients with end stage renal disease (ESRD) is well known. The prevalence and prognostic implications of low serum albumin (SA) in the general population and in patients with earlier stages of CKD are less clear.

Methods: The association between SA, CKD and income was examined among 20,106 subjects from REGARDS, a population-based study designed to identify factors leading to stroke in the Southeastern US.

Results: Hypoalbuminemia, defined as SA < 3.8 g/dl (10th percentile), was found in 10.2% of females (8.2% of males), 12.4% of blacks (7.3% of whites), 15.1% of diabetics and 10.9% of hypertensives. Mean (SD) SA was 4.17 g/dl (0.33).

Females were 28% more likely to have low SA than males, blacks 89% more likely than whites; diabetics 75% and hypertensives 23% more likely than those without either diagnosis. Low SA was 28% and 26% more likely in subjects without a high school education or with an annual income <$20,000, respectively. In both racial groups, the likelihood of having a low SA was >30% higher in those with low income or education. The prevalence of low SA rose with age reaching 17.3% for age >75.

Abnormal renal function associated with low SA; subjects with GFR <45 ml/min were twice as likely to have low SA; urine albumin-creatinine ratio (ACR) ≥ 30 mg/g posed

76% greater odds of having low SA. The association between low SA and both GFR and ACR persisted after adjusting for age, race, sex, comorbid illness and income. Low SA was associated with a mortality rate of 13.8% vs 5% among those with higher SA, HR (95% CI)=2.18 (1.90, 2.50). Higher mortality persisted after controlling for age, race, sex, hypertension, diabetes and income, adjusted HR (95% CI)=2.03 (1.77, 2.33).

Conclusions: Our findings suggest that hypoalbuminemia is associated with the presence of CKD and survival in patients with CKD. Hypoalbuminemia is worthy of further study as a biomarker of nutritional and socioeconomic status, as well as of inflammation in progressive CKD.

Funding: Pharmaceutical Company Support

FR-PO1432

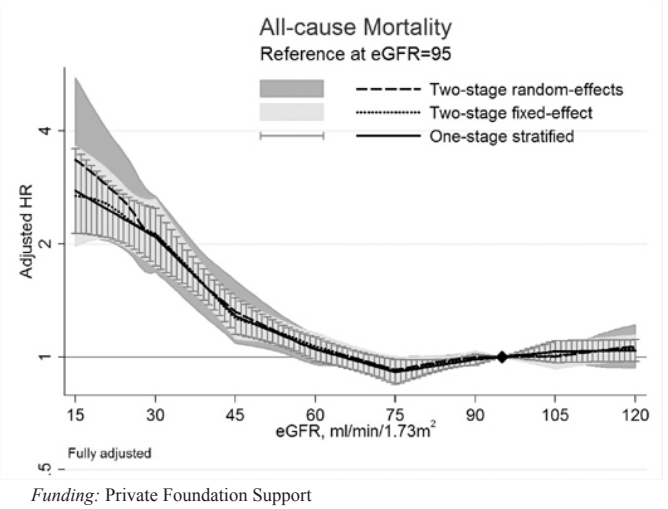
Comparison of Two-Stage and One-Stage Meta-Analyses: An Example of eGFR-Mortality Association (for CKD-PC Collaborators) Yingying Sang, Kunihiro Matsushita, B. Khan Mahmoodi, Brad C. Astor, Josef Coresh, Mark Woodward. *Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Background: Individual participant data (IPD) meta-analysis provides precise statistical estimates. Two approaches to meta-analyze IPD are currently used. The 1-stage approach fits a regression model to a pooled dataset including all studies. The 2-stage approach fits models in individual studies and meta-analyzes the estimates. However, their comparability is not well described. We compare these methods for eGFR-mortality association in the CKD Prognosis Consortium (CKD-PC).

Methods: For the 1-stage method, we fitted a Cox stratified model, allowing each study to have a unique baseline hazard but assuming a common hazard ratio (HR) for eGFR across studies. For the 2-stage method, we first fitted a Cox model in each study, and then meta-analyzed HRs using a fixed-effect (assuming one true HR for eGFR across studies) and a random-effects (allowing some variance of true HR) model. eGFR was fitted as linear splines in all models.

Results: In a sample of 10 of 46 cohorts joining CKD-PC (58,790 participants and 8,369 deaths), these methods gave nearly identical estimates except for eGFR <30 (figure). The difference in eGFR <30 for the random-effects 2-stage method results from relatively high weights to studies with unreliable estimates in this range. The 95% CIs were wider as methods made fewer assumptions – narrowest for 1-stage method, slightly wider for the fixed-effect 2-stage and wider for the random-effects 2-stage method.

Conclusions: The two-stage and one-stage meta-analyses provided nearly identical estimates for the eGFR-mortality relationship. The random-effects 2-stage method will provide conservative estimates with wider 95% CIs but this is necessary in the presence of heterogeneity.



Funding: Private Foundation Support

FR-PO1433

Kidney Function and Mortality in Octogenarians Shani Shastri, Ronit Katz, Dena E. Rifkin, Carmen A. Peralta, Michelle Odden, Michel B. Chonchol, Linda F. Fried, Michael Shlipak, Anne B. Newman, Mark J. Sarnak. *Nephrology, Tufts Medical Center, Boston, MA.*

Background: The clinical significance of chronic kidney disease has been questioned in elderly, particularly in the oldest old. We examined the association of kidney function with all-cause mortality among octogenarians in Cardiovascular Health Study (CHS) All Stars participants.

Methods: Kidney function was assessed using serum cystatin C and creatinine-based estimated glomerular filtration rate (eGFRCr) using the CKD-Epi equation in 1053 CHS All Stars participants. The association of kidney function with all-cause mortality was analyzed using unadjusted and adjusted Cox proportional hazards models in continuous and quintile-based analyses.

Results: Mean age was 86 years, 64% were females, 66% had hypertension, 14% had diabetes and 38.5% had prevalent cardiovascular disease. There were 154 deaths over median follow up of 2.63 years. After multivariable adjustment, the highest quintile of cystatin C as well as highest and lowest quintiles of eGFRCr were significantly associated with all-cause mortality.

Association of Mortality with Kidney Function

	Mortality Rate per 100 person yrs	Unadjusted Hazard Ratio (95% CI)	Adjusted* Hazard Ratio (95% CI)
Cystatin C Quintiles (mg/L)			
≤0.89	3.6	1.00 (ref)	1.00 (ref)
0.90 -1.02	4.8	1.37 (0.76, 2.49)	1.31 (0.72, 2.40)
1.03 - 1.15	4.8	1.33 (0.72, 2.45)	1.06 (0.57, 1.98)
1.16 - 1.39	5.5	1.44 (0.80, 2.59)	1.14 (0.62, 2.07)
≥ 1.40	11.1	3.12 (1.83, 5.30)	1.92 (1.09, 3.38)
CKD Epi eGFR Quintiles (ml/min/1.73 m2)			
≥75	6.9	2.32 (1.28, 4.20)	2.49 (1.36, 4.55)
64-75	3.1	1.00 (ref)	1.00 (ref)
55-63	4.1	1.25 (0.64, 2.43)	1.23 (0.63, 2.41)
44-54	6.4	1.99 (1.09, 3.66)	1.68 (0.91, 3.09)
≤ 43	9.0	2.86 (1.60, 5.09)	2.28 (1.26, 4.10)

*Adjusted for age, gender, race, hypertension, diabetes, smoking, body mass index, prevalent cardiovascular disease, prevalent heart failure, low density and high density lipoprotein, and C-reactive protein.

Conclusions: Decreased kidney function assessed using either cystatin C or eGFR based on creatinine is a risk factor for all-cause mortality in octogenarians. In adjusted analyses the relationships do no appear linear for either measure.

Funding: NIDDK SupportNIA

FR-PO1434

The Association of *klotho* Polymorphism with Disease Progression and Mortality in IgA Nephropathy Gang Jee Ko,[1] Eunah Lee,[1] Un Sil Jeon,[1] Heui Jung Pyo,[1] Ho Jun Chin,[2] Suhnggwon Kim,[2] Young-Joo Kwon.[1] *[1]Department of Internal Medicine, Korea University School of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Bundang, Gyeonggi-do, Korea; [3]Progressive Renal disease and Medical Informatics and gEnomics Research (PREMIER) members, .*

Background: IgA nephropathy is most common in primary glomerulonephritis causing end stage renal disease (ESRD), and vasculopathy is known to involve disease progression. *Klotho*, a gene related to aging, has been reported to play a role in atherosclerosis and endothelial dysfunction. We investigated whether *klotho* gene polymorphism affect clinical course of IgA nephropathy.

Methods: The data registered for the Progressive Renal disease and Medical Informatics and gEnomics Research (PREMIER) study which enrolled the patients with biopsy proven IgA nephropathy from 34 hospitals and clinics were analyzed. Two single nucleotide polymorphisms for *klotho* gene, G395A of promoter region and C1818T of exon 4, were examined using Taqman PCR assay, and investigated the association of genotypes of *klotho* with the progression of IgA nephropathy and patients survival.

Results: Among 1078 patients, clinical data from 978 patients confirmed whether alive or dead were analyzed. The allele frequency was 0.174 for A allele of G395A and 0.184 for T allele of C1818T complied with Hardy-Weinberg equilibrium. Death was observed more frequently in A allele carrier of G395A polymorphism (0.7 vs 2.6 % in GG vs GA+AA , p=0.04). Proportion of patients who were progressed to ESRD treated with dialysis also tended to be higher in A allele carrier of G395A polymorphisms (p=0.07), and renal survival was worse in same group (p=0.04). In subgroup analysis of CKD stage I to III patients at enrollment, more patients progressed to CKD stage IV and V in T allele carrier of C1818T polymorphism (6.5 vs 11.1% in CC vs CT+TT, p=0.04)

Conclusions: *Klotho* gene polymorphism was associated with patients' survival and disease progression of IgA nephropathy. The exact role and mechanism of Klotho protein in IgA nephropathy should be studied further in the future.

FR-PO1435

Factors Contributing to Suboptimal Initiation of Dialysis Despite Early Nephrologist Referral Stephanie A. Hughes,[4] Sheldon W. Tobe,[1] Philip McFarlane,[2] David C. Mendelssohn.[3] *[1]Medicine, Sunnybrook Health Sciences Centre, North York, ON, Canada; [2]Medicine, St. Michael's Hospital, Toronto, ON, Canada; [3]Medicine, Humber River Regional Hospital, Weston, ON, Canada; [4]Queen's University, Kingston, ON, Canada.*

Background: Early referral to a nephrologist improves dialysis outcomes. The STARRT study recently demonstrated that many patients still experience suboptimal dialysis starts, even when followed by nephrology for more than 12 months (NDT 2011). However, STARRT did not identify the factors associated with this, nor is there much information about this problem in the literature. The objectives of this study were to extend the results of the STARRT study by ascertaining the factors leading to suboptimal initiation of dialysis in patients who were referred to a nephrologist at least 12 months prior to commencement of RRT.

Methods: The methodology is a retrospective chart review. At each of three Toronto centers, charts of consecutive incident RRT patients were identified from January 1st to December 31st 2010. Information was collected from initial referral to a nephrologist until initiation of RRT. Preliminary data from a single center is presented.

Results: 88 incident RRT patients were studied. 52.2% were followed by a nephrologist for more than 12 months prior to initiation of dialysis. Of this group, 47.8% started dialysis with a central venous catheter, 37% with an arteriovenous fistula and 15.2% with a peritoneal catheter. Suboptimal starts occurred in 52.5% of patients receiving more than 12 months of

predialysis care. Factors contributing to suboptimal starts included patient-related delays (45.8%), acute-on-chronic kidney disease (29.2%), surgical delays (8.3%), late decision-making (8.3%), and other factors (8.3%).

Conclusions: The rate of suboptimal starts despite early referral remains high. This is due primarily to patient-related delays and urgent RRT initiation following an acute on chronic event. Especially the former would seem to be modifiable, and reducing the frequency of this occurrence would be expected to improve the quality of dialysis initiation and subsequent outcomes.

FR-PO1436

Use of an Electronic Medical Record, To Examine the Factors Associated with the Progression of Chronic Kidney Disease in Referred Patients in Australia Neil Boudville,[1, 2] Henry R. Moody,[2] Anna Kemp,[1] Robert G. Fassett,[2] Craig L. Nelson,[2] Eugenia Pedagogos,[2] Helen G. Healy,[2] George Jack Mangos,[2] Geoffrey S. Kirkland,[2] Troy D. Kay,[2] David A. Waugh.[2] *[1]University of Western Australia, Australia; [2]electronic Kidney Disease National Audit Alliance, Australia (eKiDNAA), Australia.*

Background: Despite the implementation of best practice guidelines, patients with chronic kidney disease (CKD) still progress. The aim of this study was to examine the utility of an electronic medical record (EMR), used by a number of Nephrology practices throughout Australia, to explore the factors that are associated with progression of CKD.

Methods: This was a retrospective study utilising *Audit4* (Software 4 Specialists, Australia), which is used by over 40 nephrology practices around Australia. Patients were included if they had a minimum of 2 serum creatinine measurements at least 90 days apart. Baseline was the time of the first entry of the patient into *Audit4*. Patients on renal replacement therapy at baseline were excluded. Rate of change in estimated Glomerular filtration rate (eGFR) was the primary outcome.

Results: 1327 patients were included, mean eGFR at baseline was 37.4±0.7mL/min/1.73m2 with a follow-up of 17.7months (range 7 to 26months). The change in eGFR was -0.84 ± 0.26mL/min/1.73m2/year. Univariate analysis demonstrated that women, smoking, erythropoeisis stimulating agent (ESA) use and high serum phosphate were associated with a greater decline in eGFR. Multivariate analysis showed that the factors associated with a more rapid decline in eGFR included: gender, age, ESA use, use of phosphate binders, and baseline eGFR (r2=0.11). Additional modelling demonstrated that hypertension (n=597, r2=0.15) and albuminuria (n=311, r2=0.22) were not predictors of progression in the Nephrologist-care setting, though inclusion of additional variables reduced the number of participants considerably due to missing data.

Conclusions: Our results demonstrate that the retrospective use of this EMR may result in inadequate data quality that could potentially bias the results. Mechanisms to increase the rigor of prospective data collection are required to enable EMRs to perform adequately for research.

FR-PO1437

Moderate Renal Dysfunction as an Independent Predictor of Impaired Preference-Based Health-Related Quality of Life: The 3rd Korean National Health and Nutrition Examination Survey Yun Jung Oh,[1] Chun Soo Lim,[2] Yon Su Kim,[1] Dong Ki Kim.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Internal Medicine, Seoul National University Boramae Hospital, Seoul, Korea.*

Background: Only a few large-scale studies have investigated the association between health-related quality of life (HRQOL) and renal function. This study aimed to assess the impact of renal function on the HRQOL of the general population.

Methods: We analyzed data for 5,555 adults, aged 19 years or older, from the Korean National Health and Nutritional Examination Survey 2005. The EuroQol-5D (EQ-5D) score was used to evaluate HRQOL. The population was stratified into 3 groups according to the estimated glomerular filtration rate (eGFR): group 1, eGFR ≥ 90; group 2, 90 > eGFR ≥ 60; group 3, 60 > eGFR ≥ 30 mL/min/1.73 m^2. Individuals with more advanced renal dysfunction were excluded from the analysis.

Results: In this study population, parameters concerning the subjects' socioeconomic status (marital status, educational status, occupation, income and residential space) and psychological status (degree of stress, and quality of sleep) were all deteriorated with declining eGFRs. Moreover, the score of EQ-5D in patients with eGFR less than 60 mL/min/1.73 m2 was significantly lower compared to the other groups. On multivariate analysis, eGFR less than 60 mL/min/1.73 m2 was an independent determinant of impaired HRQOL after adjustment for age, gender, health-related behaviors (smoking and alcohol intake), socioeconomic and psychological variables, and other medical comorbidities including diabetes, hypertension, metabolic syndrome, ischemic heart disease, and cerebrovascular disease (odds ratio, 1.455; 95% CI, 1.020-2.074 ; p = 0.038). In subgroup analysis for patients with eGFR less than 60 mL/min/1.73 m2, eGFR was indepedent determinants for reporting problems in the mobility dimension of EQ-5D questionnaire (odds ratio, 3.583; 95% CI, 1.579-8.131 ; p = 0.022).

Conclusions: This study strengthens evidence that moderate renal dysfunction is an important predictor of HRQOL and also suggests that even the patients with moderately decreased eGFR should be managed to improve its global health outcome.

Funding: Private Foundation Support

FR-PO1438

Visit to Visit Blood Pressure (BP) Variability and Cardiovascular Risk in CKD Patients Francesca Mallamaci, Daniela Leonardis, Giovanni Tripepi, Filippo Catalano, Anna Pisano, Giuseppe Enia, Maurizio. Postorino, Carmine Zoccali. *CNR-IBIM, Clin. Epid. and Physiopath. of Renal Dis. and Hypert., Reggio Calabria, Italy.*

Background: Blood pressure (BP) variability as measured by 24 ABPM is an independent risk factor for cardiovascular (CV) events in the general and in the hypertensive population. Recent observations in essential hypertensives indicate that also BP variability during consecutive visits predicts a high CV risk. No study investigated the relationship between visit to visit BP variability and the risk of CV events in CKD patients.

Methods: We investigated the relationship between visit to visit BP variability [expressed in terms of standard deviation (SD)] during consecutive visits (from 2 to 7 visits at 6-months intervals) in 792 patients with stage 3-4 CKD ($eGFR_{MDRD175}$:29±15 ml/min/1.73 m^2) over a an average follow-up of 31 months.

Results: During the follow-up, BP was on average 131/76 mmHg with a SD of 11±5/7±3 mmHg. The SDs of systolic BP (SBP) were directly related to the corresponding average values of SBP during the follow-up (r=0.31, P<0.001) and closely associated to the presence of CV comorbidities(P<0.001). During the follow-up, 104 patients had CV events. On univariate Cox regression analyses, the SD of SBP predicted a high risk of CV events [HR (5 mmHg increase): 1.34, 95% CI: 1.10-1.2.37, P<0.001] while no relationship was found between the SD of diastolic BP and the same outcome. In a multiple Cox model including the SDs [HR (5 mmHg): 1.22, 95% CI: 1.05-1.1.47, P=0.03] and the corresponding average values of SBP [HR (5 mmHg): 1.10, 95% CI: 1.05-1.16, P=0.03] both variables significantly predicted CV events. Data adjustment for Framingham risk factors did not modify the association between the SD of SBP and CV outcomes [HR (5 mmHg): 1.22, 95% CI: 1.02-1.47, P=0.03] while the average value of SBP lost its prediction power for these outcomes after multivariate data adjustment (P=NS).

Conclusions: Visit to visit variability of systolic BP is a stronger risk factor for CV events than average SBP in CKD patients. Assessment of visit to visit SBP variability may be useful for risk stratification in CKD patients.

*On behalf of the MAURO working group

Funding: Government Support - Non-U.S.

FR-PO1439

Five-Year Review of Disease Pattern Observed in Native Renal Biopsies in Children from a Single Center in Korea Byoung-Soo Cho, Jin-Soon Suh. *Department of Pediatrics, Kyung Hee University Hospital, Seoul, Korea.*

Background: In the absence of a national renal biopsy registry for children, there is a paucity of information on the pattern of renal disease observed in native renal biopsies in pediatric patients in Korea.

Methods: A retrospective review of native renal biopsies performed in pediatric patients was undertaken at the Kyung Hee University Hospital from January 2005 to December 2009. Renal biopsies were studied by light, immunoflourescence and electron microscopy. And renal biopsy diagnoses were divided into the following groups: primary glomerulonephronephritis(GN), secondary glomerulonephritis, tubulointerstitial disease (TID) and hereditary disease (HD).

Results: A total of 617 pediatric patients were included in the study. The mean age was 12.4 years old and a male predominance (1.3:1) was observed. The most common clinical syndrome leading to renal biopsy was asymptomatic urinary abnormalities (64.3%), followed by macroscopic hematuria (16.1%), systemic disease (8.9%) and nephrotic syndrome (4.7%). Among the histologic diagnoses, primary GN was the most commonest diagnosis representing 76.7%, followed by secondary GN representing 6.6%. Among primary GN, mesangial proliferative GN, IgA nephropathy, membranous GN and focal segmental glomerulosclerosis accounted for most. Among secondary GN, Henoch-Scholein purpura nephritis was the most commonest, followed by lupus nephritis. TID and HD comprised 4.1% and 0.5% of all renal biopsy diagnoses, respectively.

Conclusions: The pattern of biopsied renal pathology is somewhat different from that of reported recently from other parts of the world. This study provides valuable information for epidemiological information for childhood renal disease, and it can be the initial step for follow-up and prospective studies.

FR-PO1440

Serum 25-hydroxyvitamin D Level Was Associated with Albuminuria in Korean Adults: A Population-Based Study Youngki Lee, Young Rim Song, Jong-Woo Yoon, Ja-Ryong Koo, Jung-Woo Noh. *Department of Internal Medicine, Hallym Kidney Research Institute, Hallym University, Seoul, Republic of Korea.*

Background: Several observational studies and trials have suggested that vitamin D deficiency was associated with cardiovascular diseases. However, studies have included primarily white populations and a recent systemic review showed the association between vitamin D status and cardiometabolic outcome was uncertain. The present study was undertaken to analyze the serum levels of 25-hydroxyvitamin D and cardiovascular risk factors in the Korean populations.

Methods: Data for this study was obtained from the Fourth Korea National Health and Nutritional Examination Surveys (KNHANES IV) conducted by the Division of Chronic Disease Surveillance, Korea Centers for Disease Control and prevention from 2007 to 2009. We examined interview and laboratory data including 25-hydroxyvitamin D levels from 13,022 participants aged 20 years and older.

Results: In the whole population, the mean 25-hydroxyvitamin D concentration was 18.9 ng/mL. The serum levels of 25-hydroxyvitamin D were lower in women (male 20.6 ng/mL, female 17.6 ng/mL). The serum level was lowest at the age range of 20-39, and then increased, reaching its peak at the age of >60 years. Serum 25-hydroxyvitamin D level was lower in participants with albuminuria and higher in persons with obesity (BMI >25 kg/m2). But vitamin D status was not associated with hypertension, diabetes mellitus, eGFR and history of cardiovascular diseases. After adjustment for age, gender, BMI, hypertension, diabetes mellitus, dyslipidemia, participants with vitamin D deficiency had an increased risk of albuminuria (odds ratio 1.66 [95% confidence interval (CI) 1.36-2.02; p<0.001]).

Conclusions: A lower 25-hydroxyvitamin D level may be associated with higher risk of albuminuria, but cardiovascular diseases or eGFR was not associated with vitamin D status.

FR-PO1441

Development of Diagnostic Panel for Diabetic Kidney Disease Mysore Keshavmurthy Phanish,[1] Nileshkumar Shah,[1] Sarah Yates,[1] Paul J. Roderick,[2] Scott Harris,[2] Marta Lapsley,[1] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]Public Health, Southampton Unversity, Southampton, United Kingdom.*

Background: Albuminuria and eGFR are used to detect and monitor progression of diabetic kidney disease. A proportion of diabetic patients with CKD do not have albuminuria and progression may or may not occur with worsening albuminuria. In this work, we investigated urinary biomarkers in 400 patients with diabetes and correlated their levels with stages of CKD.

Methods: Urine samples were collected from 400 diabetic patients, 388 were analysed. eGFR<60= 204 patients, eGFR>60= 184; ACR (Albumin/Creatinine ratio) <3 =186 and ACR >3=202 patients. We measured markers and mediators of renal injury: inflammatory cytokines (IL6, IL1β, TNFα and MCP1), markers of proximal tubular injury (RBP and NAG) and matrix protein Fibronectin (Fn) using Luminex and ELISA.

Results: Urinary RBP and IL6 correlated (Kruskal-Wallis test, p<0.001, JT test for trend in the ordinal eGFR categories, p<0.001) with CKD stages but not NAG, IL1β, TNFα and MCP1. MCP1 showed significant correlation with CKD stages when albuminuria was included in the analysis. In a subset urinary Fn, cadherin 2 & 6 (proximal tubule markers) and TGF β 1, 2 & 3 were measured. Compared to the healthy controls (n=20, Fn 5.39±1.37 ng/ml), urinary Fn levels were elevated in diabetic patients without CKD (eGFR >60, ACR <3): 9.55± 2.6 ng/ml. There was progressive increase in excretion of Fn with increasing stages of CKD. TGFβ isoforms or cadherin 2 did not correlate with disease; however cadherin 6 was associated with severity of CKD. Cadherin 6 was undetectable in healthy controls (n=12) and was detected in the urine of all patients with progressive CKD.

Conclusions: Urinary levels of IL 6 and RBP correlate with CKD stages in diabetic patients both in the presence and absence of albuminuria. Urinary Fn excretion is elevated in diabetic patients without any other evidence of kidney disease suggesting that it could be an early marker of diabetic kidney disease. Cadherin 6 appears to be a sensitive marker to detect and monitor progression of kidney disease in diabetic patients. Further analysis is in progress.

Funding: Pharmaceutical Company Support

FR-PO1442

Prevalence of Comorbidities among Veteran Chronic Kidney Disease Patients Neha Nainani,[1] Nilang G. Patel,[1] James W. Lohr,[1,2] Pradeep Arora.[1,2] *[1]Department of Medicine, SUNY, Buffalo, NY; [2]Nephrology, VAMC, Buffalo, NY.*

Background: Understanding the relationship between CKD and other chronic diseases is important to develop a public health policy to improve outcomes. In this study, we sought to describe prevalence of comorbid conditions in a cohort of veteran patients.

Methods: We conducted a retrospective cohort study of 97,451 patients seen in primary care clinic in VISN 2 network over 7 years to determine the prevalence of CKD using the MDRD study equation and the CKD-EPI study equation based on single serum creatinine value (MDRD-I, CKD-EPI-I) . Prevalence comorbidities were calculated by both equations. We compared the prevalence of comorbidities in VA vs other data bases like NHANES, KEEP and Medicare

Results: Veterans have a much higher burden of coexisting comorbidities with CKD. On further analysis of patients above age of 65, Veterans have much higher prevalence of CKD and peripheral vascular disease compared to the KEEP and NHANES dataset. Among patients below 65 years of age, only 10.6 % of patients had CKD. 36% patients had vascular disease in the CKD group as compared to 13.88% in the non CKD group. The prevalence of myocardial infarction, coronary artery disease, heart failure and peripheral vascular disease was much higher in the patients with CKD vs the non CKD group.

Prevalence of Comorbidities among CKD patients >65 years

	VAMC CKD	VAMC Non-CKD	KEEP CKD	KEEP Non-CKD	NHANES CKD	NHANES non-CKD
Number of Patients	74.7%	25.3%	43.6%	56.4%	44.2%	55.8%
CAD	26.9%	18%	21%	14.6%	26.7%	16.8%
CHF	12.1%	3.4%	7.5%	4.2%	13.5%	4.2%
PVD	16.9%	9.7%	1.3%	1%	8.5%	4%
CVA	13.6%	8.7%	11.1%	7.8%	12.4%	5.7%
HTN	76.6%	64.7%	94.9%	84.9%	91.6%	69%
Cancer	22.4%	20%	20.3%	18.6%	13.2%	4%
DM	34%	22.6%	45.1%	37.4%	21.4%	12.1%
Dyslipedemia	62%	59.5%	38.8%	42.1%	50.5%	56%

Conclusions: Prevalence of comorbidities including cardiovascular disease is very high among Veterans. This is also true for patients who were younger than 65 years. Higher prevalence of CKD among Veterans can be partially explained by a higher load of vascular comorbidities.

FR-PO1443

Reversible Acute Kidney Injury Is Associated with Improved Survival Compared to Persistent or No Acute Kidney Injury in Septic Shock Manish M. Sood, Claudio Rigatto, Leigh Anne Shafer, Joe A. Bueti, Paul Komenda, Anand Kumar. *Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: AKI is common in septic shock being present in greater then 50% of cases. We set out to determine if reversible AKI alters survival compared to those with persistent AKI or no AKI.

Methods: Data was obtained from the Cooperative Antimicrobial Therapy of Septic Shock (CATSS) database comprising 28 centres in Canada, USA and Saudi Arabia. 7,390 individual case of septic shock occurring between Jan. 1996 and Dec. 2008 had baseline creatinine measurements. Cases with pre-existing chronic kidney disease (N=566), previous dialysis or dialysis during the current ICU admission (N=1,033) were excluded leaving 5,791 cases in the final analysis. Reversible AKI was defined by the presence then reversal of AKI according to RIFLE criteria during the first 24 hours of ICU admission. Persistent AKI was AKI over any two time points that did not reverse within the first 24 hours. Cox proportional hazards accounting for repeat creatinine measurements at baseline, 6 and 24 hours were used to determine the association between AKI status and in-hospital mortality.

Results: During the first 24 hours, AKI occurred in 79.3% of whom 11.8% recovered (reversible AKI). A total of 2,774 (47.9%) died in hospital, 49.6% of those with AKI (1,913) and 44.5% of those without AKI (861). Reversible AKI was associated with improved survival (HR 0.58 95%CI 0.48-0.71) compared to NO AKI (referent) and persistent AKI (HR 1.88, 95% CI 1.70-2.08). The improvement in survival in reversible AKI remained after adjustment for demographics, co-morbidities, illness severity (APACHE, number of failed organs, type of infection) and interventions (intubation, blood pressure support agents, appropriate antibiotics) with HR 0.61 (95%CI 0.50-0.74).

Conclusions: In septic shock, reversible AKI within the first 24 hours of admission confers a survival benefit compared to persistent or no AKI.

FR-PO1444

Site of Infection Impacts Presence and Severity of Septic Acute Kidney Injury Manish M. Sood, Keren Mandelzweig, Claudio Rigatto, Paul Komenda, Joe A. Bueti, Anand Kumar. *Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: Sepsis is a common cause of acute kidney injury (AKI) in the Intensive Care Unit (ICU) and its presence is an independent marker of adverse outcomes. We set out to determine if the site of infection in septic shock impacted the presence and severity of AKI.

Methods: Using a large multi-centre database that included ICUs from 28 ICU's in Canada, the United States and Saudi Arabia, we analyzed data from cases of septic shock between 1996 to 2008. AKI was classified by the RIFLE criteria. Multivariate logistic regression was used to determine the association between infection site and the outcomes of presence and severity of AKI. Further the association of site of infection stratified by the presence/absence of AKI and in-hospital mortality was investigated. Analyses were adjusted for demographics, illness severity, co-morbidities and ICU interventions.

Results: A total of 7,587 patients had available baseline creatinine values of which 1,115 (15%) patients were excluded due to pre-existing CKD or being on chronic dialysis. The remaining 6,482 (85%) patients were included in the study. Those with AKI were sicker (higher APACHE score; p<0.0001), had more organ failure (p<0.0001), and had more lab and hemodynamic abnormalities. There was considerable variability in both the presence and severity of AKI based on the site of infection. AKI most commonly occurring in GI pseudomembranous (81.2%) and ischemic colitis (79.2%) and UTI (78.8%). Severe AKI (those with RIFLE class fail) was most common in GI pseudomembranous colitis (26.7%) and UTI (22.1%). After multivariate adjustment, ischemic colitis (OR 1.44, CI 1.02-2.02), urinary tract infection (UTI) (OR 1.84, CI 1.38-2.47), and necrotizing fasciitis (OR 1.76, CI 1.19-2.59) were associated with the presence of AKI. In an adjusted analysis looking the site of infection and in-hospital mortality, stratified by AKI the presence of AKI made no impact on mortality in UTI, and most GI infections (biliary, pseudomembranous colitis, perforation, abscess).

Conclusions: This study demonstrates that the presence and severity of septic AKI varies significantly based on the site of infection.

FR-PO1445

Estimated Glomerular Filtration Rate Using Standardized Serum Creatinine, Proteinuria, and Mortality Risk for All Causes and Cardiovascular Diseases Sewon Oh,[1] Su Mi Lee,[2] Ki Young Na,[1,2] Suhnggwon Kim,[2] Ho Jun Chin.[1,2] *[1]Seoul National University Bundang Hospital; [2]Seoul National University College of Medicine, Republic of Korea.*

Background: Lower eGFR and proteinuria (PU) are associated with adverse outcomes. However, it has not been determined which level of eGFR increase the risk of adverse outcomes using standardized serum creatinine. In addition, PU is not used to refine risk estimation of adverse events in the current staging system.

Methods: Based on the data from routine health check-ups in tertiary university hospitals during 2003-2009, 112,115 adult subjects were identified. Serum creatinine levels were calibrated to an assay traceable to isotope-dilution mass spectrometry . The eGFR was calculated using MDRD equation. PU was determined by urine dipstick test and defined as being trace or more.

Results: The majority of subjects (96.9%) had an eGFR of 60mL/min/1.73m2 or greater and 79.7% of subjects were under 60 years of age. Over 39.9±20.7 months, 498 (0.4%) subjects died and 72 (0.1%) died for cardiovascular cause. Compared with those with eGFR of ≥105mL/min/1.73m2, subjects with eGFR 90-104 mL/min/1.73m2 did not have increased risk for all-cause mortality after adjusting for confounders. However, the hazard ratio (HR) for death was 1.67 for subjects with eGFR 75-89 (95%CI, 1.17-2.38); 1.98 for those with eGFR 60-74 (95%CI, 1.36-2.88); 3.65 for those with eGFR<60 (95%CI, 2.31-5.77). In addition, PU revealed as an independent factor for death (HR, 1.25; 95%CI 1.01-1.54). For cardiovascular death (CVD), subjects with eGFR 75-104 did not show increased risk compared with those with eGFR of ≥105. The HR for CVD was 6.04 for those with eGFR 60-74 (95% CI, 1.39-26.37), 17.01 for those with eGFR<60 (95%CI, 3.63-79.77). Also, PU was associated with CVD (HR, 1.81; 95%CI, 1.07-3.05).

Conclusions: By using MDRD equation with standardized serum creatinine, the adjusted rate of all-cause mortality was higher in subjects with eGFR 75-89 mL/min/1.73m^2 compared with those with eGFR of ≥105 mL/min/1.73m^2 and the adjusted risk of CVD was higher in subjects with eGFR 60-74 mL/min/1.73m^2. In addition, PU more than trace was an independent risk factor for death from all-cause and CVD.

FR-PO1446

GFR Variability and Death among Patients with Stage 3 CKD

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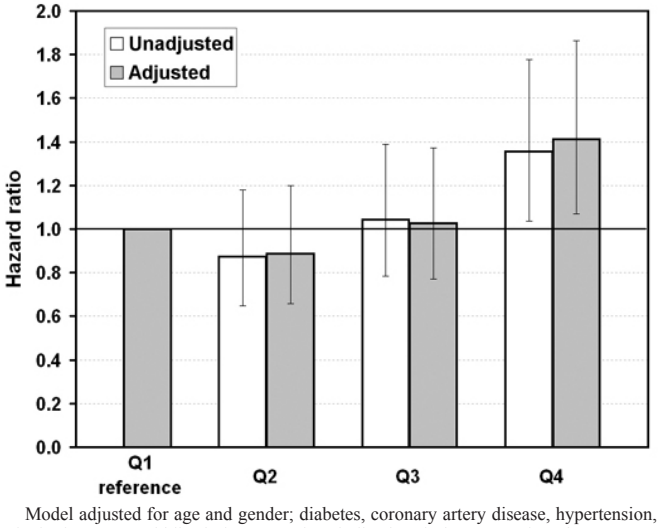
Background: Fluctuations in kidney function may herald hemodynamic compromise. We hypothesized that GFR variability predicts death among those with CKD.

Methods: All adults receiving primary care at Geisinger Health System between 2005 and 2007 who had stage 3 CKD and a minimum of 4 outpatient eGFR values within 24 months were enrolled. Subjects were excluded for any prior ESRD, CHF, metastatic cancer, or immunosuppressive therapy. The slope of eGFR change over time was calculated. Mean absolute residual value was calculated, where residual was the the difference between observed and predicted eGFRs. Quartiles of the mean absolute residual were used for stratification criteria. Subjects were followed until death or study end (March 31, 2011). An adjusted Cox proportional hazard model was developed to estimate the association between eGFR variability and death, the primary study outcome, while controlling for potential confounders.

Results: 3355 patients met entry criteria. Median (IQR) follow-up was 3.9 (3.5-4.1) years. Those patients with the highest (Q4) eGFR residual, relative to those with the lowest (Q1) were more likely to be younger and have diabetes, and to have been hospitalized in the prior 6 months; they were also more likely to have higher HDL and baseline eGFR levels. Unadjusted mortality rates (Q4 vs. Q1) were 40.8 vs. 30.0 deaths/1000 PY.

Figure. Multivariable adjusted Cox proportional hazard ratios for death by quartile of eGFR variability among patients with baseline stage 3 CKD



Model adjusted for age and gender; diabetes, coronary artery disease, hypertension, and Charlson co-morbidity index score; hospitalization during 6 months prior to entry; baseline eGFR, HDL, and serum albumin levels.

Conclusions: EGFR variability independently predicts death among patients with stage 3 CKD.

FR-PO1447

Clinical Impact of the Examination of the Ocular Fundus for CKD Patients without Atherosclerosis Risk Factors

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Background: In recent years, chronic kidney disease (CKD) has been increasingly highlighted as a risk factor for dialysis and cardiovascular diseases. Although arteriosclerosis plays an important role for CKD onset and progression, clinical significance of the examination ocular fundus (EOF), a marker for arteriosclerosis, has not elucidated yet. Thus we analyzed relationship between EOF and CKD, especially among CKD without arteriosclerosis risks.

Methods: The study subjects were 3,464 men and 3,251 women, who underwent health check including the EOF in Kasugai City Medical Care Center in 2008. Estimated GFR (eGFR) was calculated by the Japanese eGFR equation, and cases with eGFR less than 60 mL/min/1.73m² and/or with proteinuria were diagnosed as CKD. EOF abnormality was diagnosed by abnormal findings by Keith-Wagner and/or Scheie classifications. Multivariate odds ratios for CKD were calculated using logistic regression adjusted for age, sex. In 3,470 subjects without arteriosclerosis risks of hypertension, dyslipidemia or hyperglycemia, multivariate odds ratios and specificity for CKD were also calculated.

Results: EOF abnormalities were observed in 602 cases (8.97%), whose eGFR was significantly lower than those without. Multivariate analysis revealed that age, male, obese, dyslipidemia and EOF abnormality were significant factors for CKD. Among subjects without arteriosclerosis risks, EOF abnormalities were found in 138 cases (4.00%), and EOF was significant risk factor for CKD by multivariate analysis.

Multiple logistic regression analysis of the risk factor for CKD

Risk factors	OR	95% CI	p value
Age	1.07	1.06 - 1.08	< 0.01
Male	1.53	1.28 - 1.82	< 0.01
Obese	1.32	1.02 - 1.70	< 0.05
EOF	1.52	1.05 - 2.20	< 0.05

Specificity of EOF abnormality against CKD was 92.7%.

Conclusions: EOF abnormality would be useful screening tool for CKD, especially among patients without arteriosclerosis risks. EOF might predict subclinical nephrosclerosis by reflecting the sum total of various arteriosclerosis risks for life.

Funding: Other U.S. Government Support, Private Foundation Support

FR-PO1448

Mortality Risk Factors as a Function of CKD Cohort Definition: Implications for Outcomes Analyses

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Background: Most retrospective cohort studies examining associations between CKD co-morbidities and mortality have enrolled mixed prevalent and incident populations. The influence of differential length-biased sampling on estimates of mortality risk associated with various covariates is unknown.

Methods: We analyzed all adults receiving primary care in an integrated healthcare system in central Pennsylvania with at least one outpatient estimated GFR value between January 1, 2004 and December 31, 2009, and stratified them non-exclusively by incident (at least two CKD-EPI eGFR values < 60 ml/min/1.73m^2, separated in time by at least 90 but no more than 730 days, with at least one prior value >= 60 ml/min/1.73m^2); prevalent (at least two CKD-EPI eGFR values < 60 ml/min/1.73m^2, separated in time by at least 90 but no more than 730 days, with no prior values >= 60 ml/min/1.73m^2); and mixed incident-prevalent categories. Patients with AKI at baseline, ESRD, or metastatic malignancy were excluded. Separate adjusted Cox proportional hazard models for each cohort were developed to identify factors independently associated with death.

Results: 32,596 subjects met study criteria. 12,578 were unclassifiable, largely due to having only a single eGFR value. Median follow-up across stratified groups ranged from 3.5-5.2 years. Mortality rates in the incident, prevalent, and mixed groups was 30.4, 49.9, and 42.4 deaths per 1000 PY, respectively. No covariates demonstrated discrepant risk directionality; many (age, gender, smoking status, ACEI/ARB use, CHF, AKI, proteinuria, and serum albumin) demonstrated consistent and significant hazard estimates across all cohorts. While a history of diabetes and myocardial infarction each independently predicted death among the prevalent population, these covariates were not independently associated with an increased risk of death in the incident cohort. Uniquely in the incident cohort, baseline BMI and eGFR did not independently predict death.

Conclusions: CKD cohort definitions influence mortality rates and mortality risk estimates for traditional covariates, with implications for risk modeling and prognostication.

Funding: Pharmaceutical Company Support

FR-PO1449

Association between Hepatitis B Virus Infection with High Alanine Aminotransferase Levels and Low Renal Function: A Cross-Sectional Study in a Representative Sample of Chinese Jianfang Cai, Xiaohong Fan, Bixia Gao, Xuejiao Liu, Lili Liu, Hang Li, Xuemei Li, Xuewang Li. *Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China.*

Background: Population-based studies failed to ascertain the association between hepatitis B virus (HBV) infection and renal disease, which is clinically observed. Our study aimed to re-test this association by considering alanine aminotransferase levels (ALT).

Methods: We tested a representative sample of 6925 Chinese adults aged 30 to 75 years for levels of serum hepatitis B surface antigen, ALT, creatinine, urinary albumin-creatinine ratio, and potential risk factors for chronic renal dysfunction. Elevated ALT was defined as an ALT ≥ 1.25 times upper limit of normal proposed by AASLD.The participants were divided into HBV carriers with elevated ALT (HBV+/ALT+), HBV carriers with normal ALT (HBV+/ALT-), and non-carriers (HBV-/ALT±), then were reorganized into HBV+/ALT+, non-carriers with elevated ALT (HBV-/ALT+), and persons with normal ALT (HBV±/ALT-). General linear model was used to calculate and compare mean eGFR in different groups and odds ratios were estimated by using logistic regression.

Results: With Bonferroni correction for multiple comparisons (α = 0.017), group HBV+/ALT+ had a multivariate-adjusted estimated glomerular filtration rate (eGFR, mL/min/1.73 m2) that was 4.4 lower than that of HBV-/ALT± (95% CI, -7.6 to -1.1; P = 0.004), 4.8 lower than that of HBV-/ALT+ (95% CI, -8.1 to -1.4; P = 0.002), and 4.2 lower than that of HBV±/ALT- (95% CI, -7.5 to -1.0; P = 0.005). Estimated GFR didn't differ between HBV+/ALT- and HBV-/ALT±. Neither did that between HBV-/ALT+ and HBV±/ALT-. An eGFR less than 60 was 3.75 (95% CI, 1.11 to 12.73, P = 0.032) times as likely to occur in carriers with elevated ALT as non-carriers. HBV+/ALT+, HBV+/ALT-, and HBV-/ALT± didn't differ in the probability having albuminuria (10.9% vs. 7.5% vs. 10.8%, P = 0.39).

Conclusions: HBV carriage with elevated ALT levels was associated with reduced renal function. HBV infection may impair renal function paralleling liver injury and preceding HBV-associated nephropathy.

Funding: Government Support - Non-U.S.

FR-PO1450

Total Serum Free Light Chains Independently Predict Survival in Patients with Stage 3 Chronic Kidney Disease Lakhvir Assi,[1] Natasha J. McIntyre,[2] Colin A. Hutchison,[3] Richard Hughes,[1] Stephanie J. Stringer,[3] Richard J. Fluck,[2] Chris W. McIntyre,[2] Paul Cockwell,[3] Maarten W. Taal.[2] *[1]The Binding Site Group Ltd; [2]Royal Derby Hospital; [3]Renal Institute of Birmingham.*

Background: Approximately 50% of all chronic kidney disease (CKD) patients have moderate renal impairment (Stage 3, GFR 30-59ml/min/1.73m^2) and are generally managed in primary care practices. Tools which allow appropriate risk stratification of this population are required to identify those individuals at risk of progressive renal failure or cardiac events. This study evaluated the prognostic value of polyclonal free light chains (FLCs) in CKD stage 3 patients.

Methods: Patients were recruited from primary care practices, as part of the Renal Risk in Derby study. FLCs were measured with the Freelite™ assay and established normal ranges were used (κ: median 7.3mg/L, range 3.3-19.4mg/L, λ: median 12.7mg/L, range 5.71-26.3mg/L). Total FLCs and κ/λ ratios were also calculated. Time to death was assessed using Kaplan Meier and Cox regression analysis.

Results: At baseline, total FLCs were elevated in 383/1741 (23%) patients (>50mg/L). To date, 52 patients had died. Of these deaths, 23/52 (44%) had abnormally elevated FLCs (median: 46.65mg/L, IQR: 26.24) vs alive patients (median: 36.02, IQR: 19.12). Total FLCs were significantly associated with early mortality (p<0.001). Patients with FLCs >50mg/L had shorter overall survival compared to patients with <50mg/L (p<0.001). In a univariate analysis, the following markers were associated with death: FLCs >50mg/L, gender, CRP, age, calcium, and HDL. Multivariate analysis identified FLCs >50mg/L, age and CRP, to be associated with reduced time to death. Interestingly, eGFR and urinary albumin/creatinine ratio, two factors widely reported as predictors of death, were not associated suggesting FLCs may be a more sensitive predictor of death.

Conclusions: To conclude, total FLCs provide independent prognostic information on the survival of patients with CKD stage 3. Further work will determine how FLCs can be prognostically used to manage these patients.

Funding: Pharmaceutical Company Support

FR-PO1451

Are Chinese Herbs a Risk Factor for Progression to End-Stage Renal Diseases in Patients of Newly Diagnosed Chronic Kidney Disease?-- A Population-Based Study Ming-Yen Lin,[1,2] Yi-Wen Chiu,[1,3] H.C. Chen,[1,3] Shang-Jyh Hwang.[1,3] *[1]Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [2]Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung, Taiwan; [3]Department of Renal Care, Kaohsiung Medical University, Kaohsiung, Taiwan.*

Background: The impact of Chinese herbs on renal function in patients of chronic kidney disease remains controversial. We searched claimed data of National Health Insurance (NHI) to study the ESRD outcome in newly diagnosed CKD patients in Taiwan.

Methods: Newly diagnosed CKD patients, based on sets of ICD-9 codes were screened from 1997 to 2008. Patients, who took Chinese hers for over 90 days after first diagnosed of CKD were categorized as Herb-used group and else as Control group. Time period from diagnosed as CKD to first dialysis, date of withdraw from NHI or to the end of 2008 was traced in each patient. Patients who started dialysis were considered as event and else as censored. Survival analyses were used, and p <0.05 was considered as statistically significant.

Results: There were 39,620 newly diagnosed CKD patients in Herb-used group and 66,795 patients in Control group. After adjusted by age, sex, diabetes mellitus, index year, Charlson Index, and urbanization of residence, the Herb-used group significantly had a reduced risk to end-stage renal disease than Control group.

Table 1. Risk for ESRD in newly diagnosed CKD patients by Cox regression analysis(n=105,725)

	Hazard Ratio	95% CI	p-value
Age			
<45	1.00	-	-
45-64	2.52	2.29-2.78	<0.0001
≥65	2.19	1.97-2.43	<0.0001
Sex			
male	1.00	-	-
female	1.22	1.14-1.30	<0.0001
Chinese herb used			
control	1.00	-	-
used	0.56	0.52-0.61	<0.0001

All variables (age, sex, diabetes mellitus, index year, Chinese herb used, Chalson index, and urbanization of residence) was including in the model

Conclusions: The preliminary results of this study show a beneficial effect of Chinese herbs use for the outcome of ESRD, which is contrary to the detrimental effect of herbs containing Aristolochic acid. Since one-third of newly diagnosed CKD patients in Taiwan still took Chinese herbs, thus the research on the therapeutic effect of Chinese herbs on kidney disease is encouraged.

FR-PO1452

Long-Term Antiproteinuric Effect of Spironolactone Translates into Slowing in the Progression of Renal Failure Enrique Morales, Ana Huerta, Victor Gutierrez-Millet, Eduardo Gutierrez, Elena Gutierrez-Solis, Natalia I. Polanco Fernandez, Esther Gonzalez Monte, Manuel Praga. *Nephrology, H.12 de Octubre, Madrid, Spain.*

Background: The efficacy of spironolactone (SL) to reduce proteinuria has been confirmed in several studies. However, information about whether this effect is persistent or transient and its possible repercusions on renal outcomes is lacking. Aims of the study were 1) to analyze the influence of long-term treatment with SL on the slope of GFR in patients who maintain proteinuria >1g/d in spite of renin-angiotensin blockade with angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB) or ACEI+ARB combination, 2) to analyze if the short-term antiproteinuric effect of SL is sustained over time and 3) safety and side effects of long-term SL treatment.

Methods: Prospective observational single-center study was performed. 87 patients (59 m/28 f, mean age 58.4 ± 15.3 yr, 62% with GFR <60 ml/m) who maintain proteinuria > 1 g/d in spite of ACEI (16 p, 19.5%), ARB (49 p, 56.3%) or ACEI+ARB (21 p, 24.1%) were included. SL 25 mg/d (12.5-25) was added to previous treatment. Mean follow up was 24.5 ± 15.7 months (1-84).

Results: Baseline proteinuria (2.9 g/d, 1-10.4) significantly decreased by 41% (23-52) 1 mo after SL and 63% (39-79) at 12 months. At the end of follow-up proteinuria had decreased 61% from baseline (43-77), and 69% p had a proteinuria reduction >50%. GFR slope was -0.3 ml/m/mo in the 12 mo prior to SL treatment. In the first mo after SL, GFR significantly decreased (-4.7 ml/m), likely because of the diuretic effect of SL, but it tended to stabilize or improve thereafter. GFR slope after the first mo of therapy was 0.1 ml/m/mo, p 0.047 (in comparison with pre-SL period). Blood pressure showed a significant decrease and serum potassium increased from 4.6 ± 0.4 to 4.9 ± 0.6, p 0.00). 10 p (11.5%) discontinued SL because of hyperkalemia. Nine of them had GFR <60 ml/m at baseline.

Conclusions: SL induces an important and sustained antiproteinuric effect in patients with chronic proteinuric diseases. The incidence of hyperkalemia was low among patients with GFR>60 ml/m. GRF slope significantly improved after the introduction of SL.

FR-PO1453

Screening for CKD in Mexico. Targeting High-Risk Populations Guillermo G. Garcia,[1] Alfonso Gutierrez Padilla,[1] Alberto Barajas,[1] Martha Mendoza,[1] Ma del Mar Gonzalez,[1] Marcello Tonelli.[2] *[1]Nephrology, Hospital Civil de Guadalajara, Guadalajara, Mexico; [2]U of Alberta, Canada.*

Background: Chronic non-communicable diseases, such as obesity, diabetes mellitus, hypertension, and CKD, have become a major public health problem in Mexico. Since 2006, we pioneered screening people at risk for the presence of CKD using mobile units that travel to rural and urban communities of Jalisco.

Methods: Participants were informed of risk factors for CKD, but all consenting adults without known CKD were included. Trained personnel collected demographic and clinical data, and obtained blood and urine for serum chemistry and dipstick urinalysis. GFR was estimated with the MDRD formula. CKD was defined as per KDOQI guidelines. Between September 2006 and December 2009, 9,619 adults were screened in the mobile units. Results are compared with those of Mexico's National Health and Nutrition Survey (NHNS) 2006.

Results:

	Jalisco n=9619	NHNS 2006 n=33366	p
Age (y)	55.5±0.14	42.5±0.25	<0.000
Male, %	28.7	44.4	<0.000
Self-Reported DM, %	41.9	7.34	<0.000
DM2, %	56.1	14.42	<0.000
Self-Reported HTN, %	51.6	16.5	<0.000
% with SBP≥140 or DBP≥90	70.9	43.2	<0.000
BMI≥30, %	42.8	29.3	<0.000
S Chol≥200 mg⁄dl, %	54.7	43.6	<0.000
S Trig≥150⁄dl, %	58.5	31.5	<0.000
Waist >88 or >102 cm, %	61.2	43	<0.000
CKD, %	31.3	N⁄A	

Conclusions: We conclude that: 1) Our program has successfully targeted high risk populations for CKD; 2) the prevalence of CKD and its risk factors is higher in screenees served by these units as compared to the general Mexican population; 3) a program to use mobile units to deliver a protocol-driven care for this high-risk population will start shortly.

Funding: Private Foundation Support

FR-PO1454

A Simple Estimation of Serum Bicarbonate Concentration in CKD Stage 5 Patients Tetsuya Makiishi, Shinya Yamamoto, Sayako Maeda. *Internal Medicine, Otsu Red Cross Hospital, Otsu, Shiga, Japan.*

Background: To evaluate bicarbonate concentration is essential in daily clinical practice for CKD patients. Blood gas analysis is usually used in Japanese medical facilities for this pourpose, where to measure CO2 content of serum sample is uncommon. However, routine use of a blood gas analyzer at outpatient clinic bears some difficulties because it requires additional techniques and costs. Recently, Hirose et al. founded a linear correlation between values of anion gap (AG) and those of serum phosphate (iP) levels in maintenance hemodialysis (MHD) patients, and reported a simple estimation equation for MHD patients (J Jpn soc Dial Ther 2010;43:919-23). The purpose of the present study is to create a simple estimation equation of bicarbonate for CKD stage 5 (Pre-dialysis) patients.

Methods: Among total of 71 patients who started dialysis therapy at our institution between April 2008 and November 2010, 51 data sets which were simultaneously measured both with a blood gas analyzer and a central laboratory analyzer at their first day of dialysis therapy were collected and examined for a relationship between values of serum anion gap (AG) (mEq/L) and inorganic phosphate (iP) levels (mg/dL) to yield a regression equation. By substituting this into the equation of the definition of AG (AG=Na–Cl–bicarbonate), an estimation equation for bicarbonate was obtained. A relationship between values of estimated bicarbonate (eHCO3-) calculated with the equation and those from the blood gas analyzer was examined. Effects of serum albumin and iP levels on the relationship was also tested.

Results: AGs were well correlated with iP levels (AG=2.08xiP+2.04, r=0.88, p<0.001). With a simplified form of the equation, a new simple estimation equation for bicarbonate, eHCO3- = Na–Cl–2iP–2, was then obtained. Values of eHCO3- were well correlated with those measured with the blood gas analyzer (r=0.84, p<0.001). In either of the low and the high albumin group, and in either of the good (iP < 6) and the high (iP > 6) phosphate group, eHCO3- were well correlated with those from the blood gas analyzer.

Conclusions: This simple equation could be a useful tool to assess serum bicarbonate concentration in CKD 5patients.

FR-PO1455

How Reliable Is Estimation of Glomerular Filtration Rate in Type 2 Diabetes? Xun Liu, Cheng Wang, Tan-Qi Lou. *Division of Nephrology, Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.*

Background: Type 2 diabetes is a recognized public health problem worldwide. We compared both estimates with standard glomerular filtration rate (sGFR), measured by the ^{99m}Tc-DTPA method, in Chinese patients with type 2 diabetes.

Methods: A total of 207 type 2 diabetic patients were recruited. Serum creatinine (SC) level was measured by the enzymatic method. Average sGFR was 47.8 ± 26.3 ml/min per 1.73 m^2. In this study, GFR was predicted by Cockcroft-Gault-equation, 6-variable MDRD equation, 4-variable MDRD equation and MCQ equation.

Results: The agreement limits of all the equations exceeded the prior acceptable tolerances defined as 60 ml/min per 1.73 m2. Accuracies with a deviation less than 30% of all the equations were less than the prior acceptable tolerances defined as 70%. When compared the precision, bias as well as accuracy of estimated GFR (eGFR) with sGFR, GFR estimated by Cockcroft-Gault-equation showed better results. Detailed performances are listed in the table.

Overall performance between eGFR and sGFR

	Median of difference	Accuracy within 30%	Accuracy within 50%	Precision	slope of regression line with the X-axis	intercept of regression line with the Y-axis
Cockcroft-Gault equation	-1.0	52.7	72.0	99.8	0.44	-18.8
6-variable MDRD equation	-2.7*	45.9*	68.1*	91.7	0.47	-19.7
4-variable MDRD equation	0.5	45.9*	65.7*	90.8	0.53*	-20.8
MCQ equation	3.5*	34.3*	59.9*	102.8	0.54*	-16.7

*P<0.05 comparing with Cockcroft-Gault-GFR

Conclusions: When SC was measured by the enzymatic method, the performances of GFR estimation equations in Chinese type 2 diabetic patients were disappointing. At present, the Cockcroft–Gault equation may be more suitable in Chinese patients with type 2 diabetes.

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FR-PO1456

Performance of GFR Estimation Equations in a South Asian Population Saleem Jessani,[1] Andrew S. Levey,[2] Lesley Stevens Inker,[1] Rasool Bux,[1] Christopher R. Mariat,[3] Christopher H. Schmid,[2] Tazeen H. Jafar.[1] *[1]Aga Khan University, Karachi, Pakistan; [2]Tufts Medical Center, Boston, MA; [3]University de Saint-Etienne, France.*

Background: Ethnic differences in the performance of glomerular filtration rate (GFR) estimation equations have been observed, perhaps in part from non GFR determinants of serum creatinine. However, the existing equations have not been evaluated in people of South Asian descent.

Methods: We measured GFR (mGFR) using the gold standard of urinary clearance of inulin in 581 South Asian men and women in the communities of Karachi, Pakistan, including 40 patients from renal clinics. Standardized creatinine assay was used. The performance of the MDRD Study and CKD-EPI equations was assessed as bias (median difference between measured and estimated GFR), precision (interquartile range of the differences, IQR), accuracy (percent of estimated GFR values that are within 30% of measured values, P30), and the root mean square error (RSME) on the log scale. 95% CI were computed via the bootstrap method using 1000 replications.

Results: The mean (SD) age of subjects was 51 (10) years. The mean (SD) mGFR was 90 (33) ml/min/1.73 m2. The performance of the equations is presented in table.

Metrics (95% CI)	CKD-EPI Equation	MDRD Study Equation
Bias, ml/min/1.73 m2	-6.8 (-9.4, -6.0)	-8.5 (-9.8, -6.5)
IQR, ml/min/1.73 m2	-15.0 (-19.1, -10.4)	-19.9 (-25.1, -15.2)
P30 %	76.1 (72.2, 79.0)	68.0 (63.6, 71.5)
RMSE	0.265 (0.240, 0.291)	0.295 (0.268, 0.322)

Conclusions: The CKD-EPI has significantly greater accuracy (P30) than the MDRD Study equation in this largely unselected South Asian population in Karachi. The overall fit of the equations is similar to reports in the US and European populations, however both equations over-estimate measured GFR at the high levels observed in this Pakistani population. The difference in bias between the Pakistani and US and European populations may be secondary to differences in GFR measurement methods, calibration of the creatinine assay, or non GFR determinants of serum creatinine. Possibly GFR estimation may be improved by modification of existing equations, development of new equations, or use of other endogenous filtration markers.

Funding: Other NIH Support - Fogarty International Center

FR-PO1457

CKD-EPI without Adjustment for Black Race Is the Best Method for Estimating Glomerular Filtration Rate in Adult Patients with Sickle Cell Disease Marie Courbebaisse,[1] Jean-Antine Ribeil,[2] Gilles Chatellier,[5] Dominique Prie,[3] Dominique Eladari,[3] Jacques Pouchot,[4] Gerard Friedlander,[3] Jean-Benoit Arlet.[4] *[1]Néphrologie et Dialyses, Hopital Tenon, APHP, Paris, France; [2]Biothérapie, Hopital Necker, APHP, Paris, France; [3]Explorations Fonctionnelles, Hopital Necker, APHP, Paris, France; [4]Médecine Interne, Hôpital Européen Georges Pompidou, Paris, France; [5]Informatique Hospitalière, Hôpital Européen Georges Pompidou, Paris, France.*

Background: The aim of our study was to determine the best equation to estimate glomerular filtration rate (GFR) in adult sickle cell disease (SCD) patients.

Methods: Since 2007, all adult SCD patients on a steady state had GFR measurement by iohexol plasma clearance. Five equations were tested to estimate GFR: Cockcroft and Gault, MDRD-v4 and CKP-EPI equations with and without adjustment for black race. Measured GFR and estimated GFRs were compared according to Bland and Altman method.

Results: Sixty-four adult SCD patients (16 men, median age 27.5 (18-67.5), 41SS and 23 non SS) were evaluated.They were lean (median body mass index (BMI): 22 kg/m2 (16-33)) and mainly native of Sub-Saharan Africa and of French West Indies. Hyperfiltration (defined as GFR above110 ml/min/1.73m2) was detected in 53% of our patients and chronic

renal failure (CRF) in 18.8%. Micro or macroalbuminuria were detected in 65%, 50% and 28% of patients with hyperfiltration, CRF and normal GFR, respectively (p=0.04). Among the 5 equations tested, the CKD-EPI equation without adjustment for racial group had both the lowest biais and the greatest precision and the difference with the gold standard decreased with increasing GFR values, whereas it increased with the Cockcroft and Gault and MDRD-v4 equations.

Conclusions: SCD adult patients have a high rate of true glomerular hyperfiltration which is frequently associated with albuminuria. In this population, CKD-EPI equation without adjustment for black race is the best method for estimating GFR. The validity of this equation in black people of non American origin and in Black-Americans with low BMI should be evaluated.

FR-PO1458

Performance of GFR Estimating Equations in an HIV Population Lesley Stevens Inker,[1] Zipporah Krishnasami,[2] Hiba Graham,[4] James Hellinger,[1] Maia Leppo,[1] Andrew S. Levey,[1] Aghogho A. Okparavero,[1] Christopher H. Schmid,[1] Hocine Tighiouart,[1] Christina M. Wyatt.[3] *[1]Tufts Medical Center; [2]University of Alabama at Birmingham; [3]Mt Sinai Hospital; [4]Gilead Sciences Inc.*

Background: The performance of GFR estimating equations using serum creatinine (cr) or cystatin C (cys) has not been extensively tested in people with HIV.

Methods: We evaluated the performance of CKD-EPI cr, cys, and cr-cys GFR estimating equations compared to measure GFR (mGFR) using plasma clearance of iohexol in 200 HIV- positive patients on stable antiretroviral therapy. Assays for cr and cys are standardized to certified high-level reference materials. Performance was evaluated by bias (median difference between measured and estimated GFR), precision (interquartile range, IQR, of the difference), and accuracy (percentage of estimated GFR that are greater than 30% of the mGFR, 1-P_{30}).

Results: Of the 200 patients, 125 were on tenofovir disoproxil fumerate (TDF) and 75 were not. Mean age was ± 8 and 73% were male. Mean CD4 cell count was 583 ± 352 cells/mm3 and mean mGFR was 87 ± 26 (range 23-175) ml/min per 1.73 m2. There was no difference in clinical characteristics between patients on or off TDF. There was no difference in bias, precision and accuracy between patients on and off TDF for cr and cr-cys based equations, but a large difference in precision and accuracy for the cys equation.

Performance of GFR Estimating Equations, Overall and by Tenofovir Status

		Overall	Tenofavir	
			Y	N
Creatinine	Median Difference	5.4	5.1	6.6
	IQR	22.7	22.5	22.8
	1-P30	15	13,6	17.3
Cystatin C	Median Difference	4.3	3.2	5.1
	IQR	25.7	28.7*	17.09*
	1-P30	17.5	21.6*	10.7*
Creatinine-Cystatin C	Median Difference	6.4	6.3	6.5
	IQR	21.7	24.1	18.0
	1-P30	10	9.6	10.7

*p-value < 0.05. Units for median difference and IQR are ml/min/1.73m²

Conclusions: The CKD-EPI cr and the cr-cys estimating equations are reasonably accurate and can be used in patients with HIV. Cys based estimating equations are less accurate in patients on TDF due to some people having large under and overestimates of mGFR. It is not known whether this is due to differences in cys generation, kidney handling, or extra-renal elimination.

Funding: Pharmaceutical Company Support

FR-PO1459

Combination Biomarkers for Glomerular Filtration Rate Estimating Equations May Obviate Ethnicity Adjustments in a Multi-Ethnic Asian Population Boon Wee Teo, Evan J.C. Lee. *Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.*

Background: Estimates of glomerular filtration rates (eGFR) are improved using serum creatinine (Scr), serum cystatin C (CysC) and/or beta-trace protein (BTP) in combination with demographic data. We hypothesize that ethnicity is not a significant factor when multiple biomarkers are used for eGFR prediction. We assess eGFR using standardized Scr, CysC, and BTP in different combinations with demographics in a multi-ethnic Asian population of healthy and chronic kidney disease (CKD) patients.

Methods: We prospectively recruited 232 CKD patients and 103 healthy participants (52% male, Chinese 38.5%, Malay 29.6%, Indian and others 31.9%). We measured Scr by an enzymatic method, and cysC and BTP by nephelometry. We measured glomerular filtration rate (mGFR) using 3-sample plasma clearance of ^{99m}Tc-DTPA, calculated by the slope-intercept method, with body surface area normalization (du Bois) and Brochner-Mortensen correction. We fitted models developed using linear regressions of combinations of Scr, CysC, BTP and demographics. We use Akaike Information Criterion (AIC) and Bayesian Information Criteria (BIC) to select the best models. We assess the equations by considering the median bias between eGFR minus mGFR, precision (inter-quartile range) root mean square error (RMSE), and percentage accuracy (P15) to within 15% mGFR.

Results: Population means: age 53.5±15years, Scr 1.44±0.97mg/dL, CysC 1.26±0.66mg/L, BTP 1.34±0.86 mg/L, mGFR 66.7±33.3mL/min/1.73m2. In all models, ethnicity is not significant.

Models

Model	Criteria for model selection		
	R2	AIC	BIC
CysC, Scr, Age, Gender	0.893	-126.9	-104.0
BTP, Scr, Age, Gender	0.889	-114.5	-91.7
BTP, CysC, Age, Gender	0.874	-70.3	-47.5
BTP, CysC, Scr, Age, Gender	0.899	-143.8	-117.1
BTP, CysC, Scr, Age, Gender, Interaction*	0.902	-151.1	-120.6

*Interaction of age and cystatin C

The best equation included all biomarkers with demographics. The bias is -0.8, precision is 12.2, and RMSE is 12.3 (all mL/min/1.73m²). The P15 is 64.2%.

Conclusions: Using a combination of biomarkers with demographics may eliminate ethnicity as a significant factor in GFR prediction equations.

Funding: Government Support - Non-U.S.

FR-PO1460

Estimation of Glomerular Filtration Rate in Lupus Nephritis: Which Formula More Accurately Reflects Actual GFR? Duangrutai Jitprawat,[1] Pongpija Tuchinda,[2] Thonnapong Thongpraparn,[2] Somkiat Vasuvattakul.[1] *[1]Division of Nephrology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand; [2]Division of Nuclear Medicine, Department of Radiology, Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

Background: Estimation of glomerular filtration rate (GFR) is widely used in clinical practice. Creatinine is hypersecreted by injured tubules of lupus nephritis (LN). Routine measurement of creatinine clearance may over estimate GFR. We, therefore, assessed the performance of the formulas for GFR estimation: Modification of Diet in Renal Disease (4-v MDRD); 4-v MDRD with the ethnicity factor as established for Chinese population [4-v MDRD (Chinese)]; Cockcroft–Gault (CG); and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas, to determine the best formula reflecting actual GFR of LN patients.

Methods: In this cross-sectional study,we enrolled sixty-two LN patients at our renal clinic. 99mTc-DTPA renogram was used as a gold standard to measure GFR. Estimated GFR calculated by the 4-v MDRD, 4-v MDRD (Chinese), CG, and CKD-EPI formulas were compared with the measured GFR using 3 modalities: the intraclass correlation coefficient, bias, and proportion of estimated GFR within 30 % of measured GFR (P30).

Results: The intraclass correlation coefficients between measured GFR and estimated GFR were 0.88 for 4-v MDRD, 0.81 for CG, 0.87 for CKD-EPI, and 0.77 for 4-v MDRD (Chinese) formula. All of the estimated GFR formula overestimated measured GFR by 3.4, 9.6, 11.9, and 17.7 mL/min/1.73 m², for 4-v MDRD, CG, CKD-EPI, and 4-v MDRD (Chinese) formulas respectively). There were statistically significant difference of bias between the 4-v MDRD formula and the others. The 4-v MDRD formula also had the most accuracy (P30 = 93.5%). All formulas lacked precision in estimating GFR in the patients with measured GFR more than 60 mL/min/1.73 m².

Conclusions: The 4-v MDRD, 4-v MDRD (Chinese), CG, and CKD-EPI formulas can be used for estimating GFR in lupus nephritis, especially among patients with the GFR less than 60 mL/min/1.73 m². The 4-v MDRD formula is the most reliable and accurate method to reflect actual GFR.

FR-PO1461

Prevalence of Chronic Kidney Disease in England: Findings from the 2009 Health Survey Paul J. Roderick,[1] Jenny Mindell,[1] Marilyn Roth,[1] Beverley Matthews,[2] Donal O'Donoghue.[3] *[1]University of Southampton, United Kingdom; [2]NHS Kidney Care; [3]Salford Royal Foundation NHS Trust.*

Background: Chronic kidney disease is a global public health problem because it is common and associated with cardiovascular risk. Prevalence estimates from national health or research surveys have been obtained from several developed countries but no such survey undertaken in the UK. This paper presents nationally-representative general population data from the Health Survey for England 2009 on the prevalence of CKD in adults in England.

Methods: The HSE 2009 was one of an annual series of national cross sectional surveys which uses multistage probability sampling to obtain nationally representative estimates. Sampling was stratified by region, and used postcode sectors and postcode address file to sample households. 4680 households were invited and 2832 (61%) participated; in these all adults (age 16+) were invited and 4645 individuals took part and were interviewed. 3261 (71%) had a nurse visit, 2446 (75% of nurse visit) had a single blood test and 2864 (86% of nurse visit) provided a single urine sample. Serum creatinine was measured in one laboratory by an enzymatic method calibrated to IDMS. eGFR was calculated by the MDRD equation. Albuminuria was any albumin in the urine >2.5 mg/mmol in males and > 3.5mg/mmol in females.

Results: The prevalence of CKD stage 3-5 was 6% (7% in females, 5% in males). It varied with age ranging from 1% of males and 2% of females aged 16-54 to 31% of males and 36% of females aged 75 and over. It was commoner in females and there was an inverse socio-economic gradient. CKD stage 4 -5 was rare. Prevalence of albuminuria was 9%, higher in males (10% vs 8%) and with a strong inverse socio-economic in males. The overall prevalence of CKD stages 1-5 was 14% in males and 13% in females. Only 1.5% of males and 1.3% of females reported being told by a doctor they had CKD); 7.6% males and 7.9% females reported they had been tested for CKD.

Conclusions: These are the first nationally representative, population-based data on the prevalence of CKD in England. CKD is common, though heavily age–related. Like other vascular conditions, it has an inverse socio-economic gradient.

FR-PO1462

Comparison of Creatinine- and Cystatin C-Based GFR Estimating Equations in Children with the Diagnosis of Systemic Lupus Erythematosus

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Background: There is no data about glomerular filtration rate, measured through plasma clearance of the exogenous markers, in the systemic lupus erythematosus (SLE) children. The objective of this study was to measure GFR and validate of the GFR estimating equations in the SLE children.

Methods: Children diagnosed as SLE were enrolled. Single-compartment plasma clearance of ^{99m}Tc-DTPA was measured and transformed into two-compartment clearance (mGFR). mGFR was normalized (nGFR) by BSA. eGFR was calculated using 9 different equations. Creatinine and cystatin C were measured using kinetic Jaffe method and immunoturbidimetry, respectively. Correlation and agreement between eGFR and nGFR and the accuracy of GFR estimation were compared.

Results: 23 children diagnosed as SLE were enrolled. The original Schwartz equation and CKiD equation, compared with the other seven equations, produced eGFR with better correlation with nGFR (γPearson was 0.567 and 549, respectively), smaller bias (1.8 and 4.12 ml/min×1.73m2), narrower 95% LOA ([-61, 64] and [-40,47]), better performance in Bland-Altman analysis, higher intraclass correlation coefficient (0.56 and 0.52) and concordance correlation coefficient (0.55 and 0.51), higher ratio of eGFR within nGFR±10% (30.4% and 40%) and within nGFR±30% (82.6% and 80%), higher ratio of correct CKD staging (56% and 70%) and better agreement in CKD staging between eGFR and nGFR (Kappa value was 0.44 and 0.46).

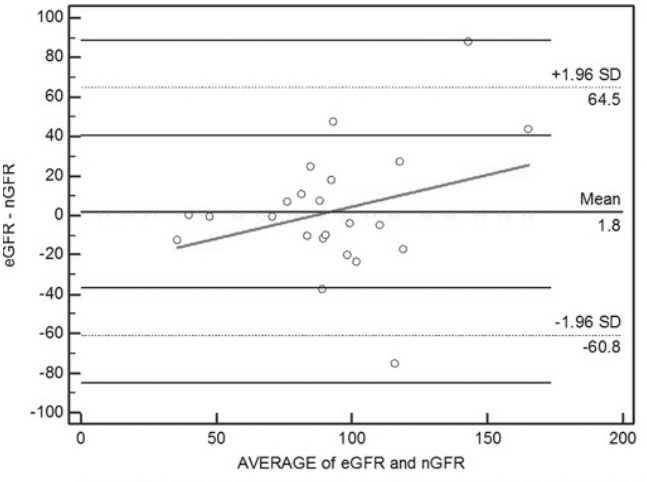

Figure 1 Bland-Altman Analysis of the original Schwartz equation in SLE children

Conclusions: Though the equations with better performance of GFR estimation in SLE children were the original Schwartz equation and the CKiD equation, neither equation is validated to be used for GFR estimation in SLE children.

Funding: Government Support - Non-U.S.

FR-PO1463

Accurate Assessment of Kidney Function in Indigenous Australians: The eGFR Study

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Background: Differences in body builds and compositions raise the possibility that creatinine-based estimates of glomerular filtration rate (GFR) derived for use in European populations may not be appropriate for Indigenous Australians. The aim of this study was to develop a validated and practical measure of GFR suitable for Indigenous Australians.

Methods: We measured GFR (mGFR) by plasma disappearance of iohexol over 4 hours and estimated GFR (eGFR) using the 4 variable modification of diet in renal disease (MDRD) equation.

Results: In 539 Indigenous Australians aged 44±15years, 37%males, 39% with diabetes (DM), BMI 30±7kg/m2, participants were classified by DM status and kidney function (eGFR, ml/min/1.73m2) into 5 strata: healthy, DM or albuminuria with eGFR >90, eGFR 60-89, eGFR 30-59, eGFR<30.

Indigenous Australians

	Healthy	DM or albuminuria with eGFR >90	eGFR 60-89	eGFR 30-59	eGFR<30
n	175	130	165	47	22
Mean eGFR	110	108	78	45	18
Mean mGFR	115	116	95	52	23
Bias (mGFR-eGFR, ml/min/1.73m²)	7.4	8.6	17.8	8.4	4.6
Precision (interquartile range of bias, ml/min/1.73m²)	24.8	28.5	24.7	14.0	7.8
Accuracy (% eGFR within 30% of mGFR)	99%	94%	82%	81%	77%

Data are geometric mean or median (bias). Units of eGFR, mGFR are ml/min/1.73m²

Bias did not differ by DM status. When compared to 42 Caucasian participants: bias and accuracy were not significantly different when eGFR<60mls/min/1.73m²; however bias (mls/min/1.73m²) was higher in Indigenous than Caucasian participants when eGFR≥60mls/min/1.73m² (11.7 vs 2.6, p=0.044), but improved with the Chronic Kidney Disease Epidemiology Collaboration equation (4.0 vs -6.8, p=0.1).

Conclusions: In summary, eGFR using the MDRD equation provides a reasonably unbiased and accurate estimation of GFR in Indigenous Australians. Detailed assessment of fat free mass may enhance accuracy of eGFR and is in progress.

Funding: Government Support - Non-U.S.

FR-PO1464

Performance of GFR Prediction Formulas in ADPKD and Type 2 Diabetes Patients: Role of Kidney Function and Demographic, Anthropometric and Clinical Patient Characteristics

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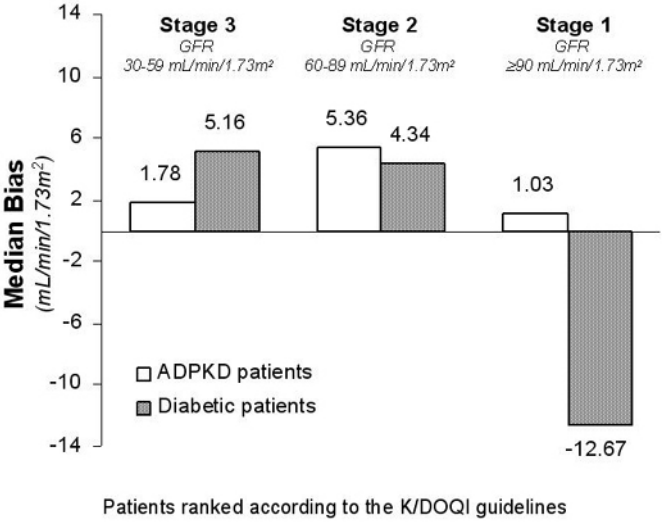
Background: Prediction formulas have been developed for glomerular filtration rate estimation (eGFR) in subjects with reduced renal function. Whether and to which extent their performance is affected by kidney function, underlying renal disease and patient demographic, anthropometric and biochemical characteristics is poorly understood.

Methods: We evaluated the precision of GFR estimations by 14 formulas versus GFR measured by iohexol plasma clearance technique (mGFR) in 2 cohorts of ADPKD and type 2 diabetic patients matched by gender and GFR (difference between matched patients <±1 mL/min/1.73m²). Performance was assessed considering bias, mean percent error (MPE) and accuracy.

Results: In 97 ADPKD and 97 matched diabetic patients, mGFR (81.4±26.4 vs. 81.7±26.2 mL/min/1.73m²) and serum creatinine (1.15±0.43 vs. 1.04±0.40 mg/dL) were similar. Compared to ADPKD patients, however, diabetics were significantly (p<0.05) shorter, heavier, older, more dyslipidemic and hypertensive. In the whole study group accuracy within ±10% error of all formulas ranged from 14.4 to 49.5%. In diabetics with GFR ≥90mL/min/1.73m² median accuracy was as low as 31%. Both bias (see Figure) and MPE showed a large and systematic GFR underestimation in this subgroup.



Conclusions: Performance of prediction formulas was similarly poor in ADPKD patients independent of kidney function and in diabetic patients with GFR <90mL/min/1.73m². In diabetics with higher GFR all formulas were fully unreliable, possibly because of the confounding effect of demographic, anthropometric and clinical parameters in this population.

Funding: Private Foundation Support

FR-PO1465

Epidemiologic Investigation of Chronic Kidney Disease in the District of Hulunbeir of Inner Mongolia Autonomous Region Xiao-Yi Xu,[1] Jing-Hua Duo,[3] Yang Luo,[2] Hong-Liang Rui,[1] Guo-Bing Xu,[4] Hong Cheng,[1] Yi-Pu Chen.[1] *[1]Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China; [2]Division of Nephrology, Tiantan Hospital Affiliated to Capital Medical University, Beijing, China; [3]Division of Nephrology, People's Hospital of Hulunbeier, Inner Mongolia Autonomous Region, China; [4]Department of Clinical Laboratory, Peking University First Hospital, Beijing, China.*

Background: To investigate the prevalence and risk factors of chronic kidney disease (CKD) in the general adult population in the Hulunbeir Prefecture, Inner Mongolia Autonomous Region where many minorities of north China live.

Methods: Sampling survey was performed in the residents aged 20 years and older in the Hulunbeir Prefecture. All investigated subjects were tested for urinary albumin to creatinine ratio (ACR); hematuria by microscopy of urinary sediment; and GFR estimated by modified MDRD equation for Chinese adults (eGFR). The related risk factors of CKD were also investigated.

Results: Total 4522 subjects were enrolled in the study. The prevalence of albuminuria was 7.11%; hematouria was 2.64% and reduced eGFR [<60ml.min-1.(1.73m2)-1] was 2.75%. The prevalence of hypertension was 38.90%; hyperglycemia 6.61%; hyperlipidemia 2.72%; increased waist 24.79% and metabolic syndrome 15.02%. After the subjects with combined microalbuminuria, hematuria and reduced eGFR were excluded, the prevalence of CKD was 12.95%. Logistic regression analysis and stratified analysis showed, increased waist, elevated systolic pressure, hyperglycemia, hypertriglyceridemia and metabolic syndrome were independent risk factors associated with albuminuria; increased age, elevated systolic pressure and hyperglycemia were independently associated with reduced eGFR; increased age was independently associated with hematuria.

Conclusions: The prevalence of CKD is 12.95% in the Hulunbeir Prefecture, Inner Mongolia Autonomous Region. Independent risk factors of CKD include increased age, increased waist, hypertension, abnormal blood glucose or lipid, and metabolic syndrome.

FR-PO1466

Does Hemi-Nephrectomy Aggravate Residual Renal Function? Shohei Ishida,[1] Yoshinari Yasuda.[2] *[1]Department of Urology, Chukyo Hospital, Nagoya, Japan; [2]Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: Partial nephrectomy has been reported overseas to keep renal function better than hemi-nephrectomy. In japan, the influence of hemi-nephrectomy is unclear. The aim of this study is to assess of hemi-nephrectomy on long term postoperative renal function.

Methods: Among patients who underwent hemi-nephrectomy from January 2004 to December 2005, 66 patients could be followed at least five years and their pre-operative estimated glomerular filtration rate (eGFR) were more than 30ml/min/1.73m^2. For these 66 patients, the changes in eGFR were retrospectively analyzed over five years after the expiration of six months from hemi-nephrectomy.

Results: 49 cases of men and women in 17 cases, mean age at hemi-nephrectomy was 57.6 (30-79) years old. eGFR (mean±SD) before hemi-nephrectomy was 75.3±18.7ml/min/1.73m2, at six months after hemi-nephrectomy 49.7±12.4ml/min/1.73m2, at five years and six months after hemi-nephrectomy 51.9±11.4 ml/min/1.73m2. The rate of decrease in eGFR for five years is -0.4±1.7 ml/min/1.73m2/year (-0.7±4.5%/year), and renal function was slightly increased . Of these five years the rate of decrease in eGFR 50% or more (10%/year or more) patients were only two. These two patients showed proteinuria with qualitative analysis before and sx months after hemi-nephrectomy, and their eGFR at six months after hemi-nephrectomy were less than 30ml/min/1.73m2.

Conclusions: Preoperative view of the overall eGFR than patients 30ml/min/1.73m^2 (CKD stage 1-3), the hemi-nephrectomy specimen and should not cause deterioration of renal function. On the other hand, in some cases worsening renal function after hemi-nephrectomy, suggesting its potential as an indicator of the presence or absence of proteinuria before and after hemi-nephrectomy.

FR-PO1467

A Comparison of Equations To Estimate Glomerular Filtration Rate in Living Kidney Donor Candidates Nishant Fozdar, Charmaine E. Lok, Olusegun Famure, Joseph Kim. *Medicine (Nephrology), Toronto General Hospital, University Health Network, Toronto, ON, Canada.*

Background: Glomerular filtration rate is routinely estimated by equations. This study aims to validate and compare the performance of the Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD), Mayo Clinic (MC), and CKD-EPI equations for calculating estimated GFR (eGFR) in Canadian living kidney donor candidates.

Methods: A total of 440 individuals assessed for kidney donation at the Toronto General Hospital from 2006 to 2010 were included. Measured GFR (mGFR) from 99mTc-DTPA scans were compared to each equation using measures of bias (median difference between mGFR and eGFR), precision (interquartile range), accuracy (% of eGFR within 30% of mGFR), and classification (sensitivity and specificity) at mGFR cutoffs relevant for living kidney donation.

Results: The CKD-EPI equation performed well overall (see table). While CG had the lowest bias, it had relatively low precision and accuracy. MC and CG performed well at higher mGFR while CKD-EPI and MDRD performed well at lower mGFR. CKD-EPI had the most favorable operating characteristics at an mGFR cutoff of 90 ml/min (correctly identifying an mGFR below the cut-off as "positive") while MDRD performed well at mGFR cutoffs of 80 and 85 ml/min. MC had the greatest specificity in detecting GFRs below the cut-off values.

Conclusions: The CKD-EPI equation generally performed well, especially in terms of precision, accuracy, and clinically relevant classification but MC may be the most useful equation in correctly identifying living kidney donor candidates with inappropriately low GFR.Performance of GFR Equations in Living Kidney Donor Candidates

Characteristic	MDRD	MC	CG	CKD-EPI
Bias (95% CI)	13.4 (11.4, 15.4)	-5.5 (-7.7, -3.2)	0.5 (-2.9, 3.8)	8.9 (7.3, 10.5)
% Bias (95% CI)	13.1 (11.2, 15.0)	-5.6 (-7.8, -3.4)	0.5 (-3.0, 3.9)	8.6 (7.1, 10.1)
Precision (95% CI)	22.5 (10.9, 25.2)	21.9 (20.2, 23.6)	34.1 (30.5, 37.7)	20.3 (17.7, 22.9)
Accuracy (95% CI)	89.1 (85.8, 91.8)	89.1 (85.8, 91.8)	77.1 (72.8, 80.9)	92.1 (89.1, 94.4)
Sens/Spec at 80 ml/min	72%/75%	8%/99%	44%/87%	64%/84%
Sens/Spec at 85 ml/min	78%/70%	10%/98%	51%/82%	71%/78%
Sens/Spec at 90 ml/min	86%/59%	18%/97%	50%/75%	76%/73%

FR-PO1468

Assessment of Renal Function in African Americans (AAs) after Kidney Donation by Cystatin C and Creatinine Based Formulas Compared to ^{125}I iothalamate Clearance Sunil Kumar Jain,[1] John M. Arthur,[1] Milos Budisavljevic.[1] *[1]Division of Nephrology, Dept of Medicine, Medical University of South Carolina, Charleston, SC; [2]Division of Biostatistics and Epidemiology, Medical University of South Carolina, Charleston, SC; [3]Division of Biostatistics and Epidemiology, Medical University of South Carolina, Charleston, SC; [4]Division of Nephrology, Dept of Medicine, Medical University of South Carolina, Charleston, SC; [5]Division of Nephrology, Dept of Medicine, Medical University of South Carolina, Charleston, SC.*

Background: AAs have 4 times higher prevalence of ESRD than Caucasians. Therefore, assessment of kidney function is of considerable importance in this patient population. Kidney function is currently assessed most commonly by estimated glomerular filtration rate (eGFR) using creatinine based equation. Some studies indicate that measuring Cystatin C levels provide better estimate of GFR. We compared cystatin C (Cys C) and creatinine based formulas for GFR estimation with measured GFR in 33 AAs who donated their kidneys 5-27 years (mean=11.1 years) previously.

Methods: GFR was measured by ^{125}I iothalamate clearance. Cystatin C was measured by Bio Vendor Human Cystatin C ELISA Kit. Pearson correlation was used for statistical analyses.

Results: The mean measured GFR was 76.18 ml/min/1.73m2. The correlation between measured GFR and cystatin C and creatinine based equations is presented in Table 1.
Table 1

Variable	GFR ml/min	SD	Pearson correlation	p-value
Iothalamate GFR	76.18	13.11		
eGFR Cys C				
EPI unadjusted	85.3	19.3	0.4838	0.0058
EPI adjusted*	90.04	18.03	0.5718	0.0008
eGFR Creatinine				
MDRD	86.56	16.11	0.6370	<0.0001
AASK Study	92.19	16.88	0.6409	0.0003
Cockcroft - Gault	115.08	33.55	0.4990	0.0677

SD- Standard Deviation, *Adusted for age, gender and race

Conclusions: In AAs with a single kidney, both creatinine and cystatin C based equations overestimate GFR compared to measured GFR. The correlation of estimated GFR with measured GFR was statistically significant but not strong. Creatinine based formulas appear to be more accurate than cystatin C based formulas. As reported for subjects with two kidneys the MDRD equation estimated GFR better than Cockcroft-Gault.

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FR-PO1469

Idiopathic Hypercreatininemia; Limitation of Creatinine Based eGFR and Utility of Cystatin C Based eGFR Masahiko Nagahama, Yasuhiro Komatsu. *Nephrology, St. Luke's international hospital, Tokyo, Japan.*

Background: The number of nephrology referral has increased as CKD classification by serum Cr based eGFR is accepted globally. However, some populations show disproportionally higher level of Cr compared to true GFR without apparent causes such as large muscle mass, rhabdomyolysis, excessive protein intake or medications which affects urinary Cr excretion such as Bactrim or Cimetidine. Those who exhibit higher serum Cr without any particular reason can be defined as "Idiopathic hypercreatininemia (IHC)". The aim of the present study is to clarify the prevalence and diagnostic test to prevent overdiagnosis of CKD leading to unnecessary diagnostic procedure and psychological burden of the patients.

Methods: Among 1,482 patients who were consulted to our nephrology clinic between 2005 and 2010, 103 patients (Group A, n=103) had their true GFR determined by 24hr urine collection (the average of Cr and urea clearance). Patients with Cr-based e GFR < 60 ml/min/1.73m^2 are categorized as IHC if they have; 1) true GFR > 60 ml/min/1.73m^2, 2) absence of large muscle mass (24hr urine Cr/Wt > 30), 3) CPK > 100mg/dl, and 4) no particular medications changing urinary Cr secretion. Fifteen patients out of 103 (14.5%) were diagnosed with IHC (IHC, n=15).

Results: Cystatin C based eGFR is normal, greater than 60ml/min/1.73m2, in majority of IHC (normal ratio 0.31 vs. 0.93, Group A vs. IHC, p<0.05), so as to urinary protein excretion (24hr urinary protein excretion 1904+/-2378mg vs. 38+/- 23mg Group A vs. IHC, P<0.05). The female ratio in IHC was significantly higher (female ratio 0.30 vs 0.60, Group A vs. IHC, P<0.05) and BSA tends to be smaller (BSA 1.68+/-0.2m2 vs. 1.64+/- 0.2 m2, Group A vs. IHC, p=0.16).

Conclusions: In conclusion, prevalence of IHC is high (14.5%) among patients referred to renal clinic. Cystatin C based eGFR can effectively rule out IHC. Higher female ratio and relatively smaller BSA imply that Cr metabolism rather than Cr production is related to this condition.

FR-PO1470

Prevalence of Chronic Kidney Disease in the Adult Population of Lausanne-Switzerland Belen Ponte,[1] Menno Pruijm,[2] Pierre-Yves F. Martin,[1] Michel Burnier,[2] Vincent E. Mooser,[3] Gerard Waeber,[2] Murielle Bochud.[2] *[1]University Hospital of Geneva; [2]Centre Hospitalier Universitaire Vaudois; [3]GlaxoSmithKline.*

Background: Chronic kidney disease (CKD) represents an important burden in the general population with increased cardiovascular morbidity and mortality. Population-based data are available in US and some countries of Europe but are lacking in Switzerland. We aimed to determine the risk factors and prevalence of CKD in the population of Lausanne, Switzerland.

Methods: This population-based study included 6184 Caucasians aged 35-75 years old between 2003 and 2006, of whom 2821 men and 3158 women had data for the present analysis. CKD was defined using KDOQI stages 1-5 according to estimated glomerular filtration rate (eGFR) and microalbuminuria. We compared CKD-EPI and MDRD equations to calculate eGFR and classify CKD.

Results: The prevalence of CKD using MDRD was 2.1%, 3.5%, 4.7% and 0.17% for stages 1, 2, 3 and 4-5, respectively. The corresponding prevalence using CKD-EPI was 2.3%, 3.2%, 4.5% and 0.17%. The prevalence of CKD (stages 1-5) was 10.4% using MDRD and 10.2% using CKD-EPI. Overlap between the two equations was 91% for stage 3 and 100% for stages 4-5. The prevalence of CKD (CKD-EPI) was 8.7% in non-diabetic and 28.9% in diabetic, 5.9% in normotensive and 17.4% in hypertensive subjects. It was 4.9%, 5.6%, 11.3% and 25.1% in persons aged 35-45, 45-55, 55-65 and 65-75 years, respectively. According to the Body Mass Index (BMI) prevalence was 7%, 10.7% and 17.2% for BMI<25, BMI 25-30 and BMI≥30kg/m2 respectively.

In multiregression analysis, determinants of CKD were age per year (OR 1.06; 95% CI 1.05-1.07), female sex (OR 1.22; 95% CI 1.02-1.47), hypertension (OR 1.78; 95% CI 1.45-2.18), diabetes (OR 2.32; 95% CI 1.78-3.03) and BMI≥30kg/m^2 (OR 1.33; 95% CI 1.03-1.72).

Conclusions: The prevalence of CKD in the adult population of Lausanne is substantial, although lower than in US. CKD prevalence sharply increases after 55 years of age and is particularly high in diabetic, hypertensive and obese subjects. Our results suggest that screening strategies aiming at detecting CKD should focus on higher risk patients such as elderly, diabetic, obese and hypertensive ones.

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FR-PO1471

Annual Incidence of Kidney Damage in General Population Kei Nagai, Chie Saito, Kunihiro Yamagata. *Nephrology, Clinical medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Background: Several reported studies on the prevalence of proteinuria in mass screening were based on single-screening data. Therefore, we investigated consecutive screening results to determine the annual incidence of kidney damage in the general population.

Methods: The subjects were participants in an annual health examination held in Ibaraki, Japan, between 1993 and 2003. 314,354 males and 649,533 females, who underwent serial urine studies for 3 years and were confirmed as negative for proteinuria in the initial year, were enrolled. Those subjects whose eGFR (estimated glomerular filtration rate) was less than 60 ml/min/1.73 m^2 were excluded. We calculated the incidences of proteinuria in the 2nd year and sustained proteinuria in the 3rd year, with separation by clinical condition and categorization by age. In addition, we evaluated risk factors and the effects of co-morbid conditions on the incidence of kidney damage.

Results: The positive rate of proteinuria was 2.51% in diabetic men, 1.81% in hypertensive men, and 0.89% in non-diabetic and non-hypertensive men. Among them, the population with sustained proteinuria in the 3rd year was 1,608/314,354 (0.51%) in all men, and 1,290/649,533 (0.20%) in all women. In non-diabetic and non-hypertensive subjects, the values were 351/145,256 (0.24%) in men and 337/380,743 (0.09%) in women. Age distribution of the positive rate of proteinuria and kidney damage in the diabetic population showed almost constant in all age groups, whereas those in non-diabetic populations increased with age. For the risk factor of newly developed kidney damage, severe hypertension was a significant positive correlation with the incidence of proteinuria.

Conclusions: Annual incidence of CKD stage I and stage II was 0.30% in the Japanese general population in both sexes. That of non-hypertensive non-diabetic subjects were 0.24% in men and 0.09% in women among Japanese adults aged 40 and over. These subjects would be missed without universal urinalysis screening in Japan.

FR-PO1472

Estimation of Glomerular Filtration Rate in Chinese Patients with Chronic Kidney Disease Xun Liu, Zhujiang Chen, Cheng Wang, Tan-Qi Lou. *Division of Nephrology, Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.*

Background: Glomerular filtration rate (GFR) is the best index for the assessment of renal function. The applicability of formulas based on serum creatinine (SC) levels in Chinese patients with chronic kidney disease (CKD) is, however, less well studied.

Methods: 831 CKD patients were enrolled. SC was measured by the enzymatic method. Average sGFR measured by ^{99m}Tc-DTPA GFR estimation was 45.4±27.3 (3.3-137.7) ml/min/1.73 m^2. The patients' GFRs were estimated by Cockcroft-Gault-equation, MDRD1-equation, abbreviated MDRD-equation, reexpressed 6-variable MDRD equation, reexpressed 4-variable MDRD equation, CKD-EPI-equation, Chinese-equation, previously Japanese equation and new Japanese equation, and the results were analyzed.

Results: Bland-Altman analysis showed that previously Japanese equation, new Japanese equation and reexpressed 6-variable MDRD equation were better than the other equations. However, the precisions of all the equations exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m2. Linear regressions demonstrated that the slopes of previously Japanese equation, new Japanese equation and reexpressed 6-variable MDRD equation were closer to the identical line. The median of difference of Cockcroft-Gault-equation, abbreviated MDRD-equation and reexpressed 6-variable MDRD equation were smaller. The median % absolute difference of Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation and Chinese-equation were smaller. Accuracy of Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation and MDRD1-equation were higher than those of the other equations. However, accuracies with a deviation less than 30% of all the equations were less than 70%. When compared the performance between eGFR and sGFR in different stages of CKD, GFR estimated by Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation, MDRD1-equation and reexpressed 4-variable MDRD equation showed better results.

Conclusions: When SC was measured by the enzymatic method, this study highlights a limitation in the use of GFR estimation equations in Chinese CKD patients.

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FR-PO1473

Prevalence of Chronic Kidney Disease in China Luxia Zhang,[1] Li Wang,[2] Wenke Wang,[3] Bicheng Liu,[4] Jian Liu,[5] Menghua Chen,[6] Qiang He,[7] Yunhua Liao,[8] Xueqing Yu,[9] Nan Chen,[10] Jianer Zhang,[11] Zhao Hu,[12] Fuyou Liu,[13] Haiyan Wang.[1] *[1]Peking University First Hospital; [2]Sichuan Provincial People's Hospital; [3]Chifeng Second Hospital; [4]Zhongda Hospital; [5]First Hospital of Xinjiang Medical University; [6]Affiliated Hospital, Ningxia Medical University; [7]First Affiliated Hospital, Zhejiang University; [8]First Affiliated Hospital, Guangxi Medical University; [9]First Affiliated Hospital, Sun Yat-sen University; [10]Ruijin Hospital; [11]Taihe Hospital; [12]Qilu Hospital; [13]Second Xiangya Hospital.*

Background: Previous studies revealed a high prevalence of CKD among developing countries. However, there is no national survey of CKD incorporating both estimated glomerular filtration rate (eGFR) and albuminuria in a developing country with marked heterogeneity like China.

Methods: The present study is a cross-sectional survey among a representative sample of 47 204 participants in China. Participants were interviewed and were tested for albuminuria and reduced renal function. The crude and adjusted prevalence of indicators of kidney damage were reported.

Results: The adjusted prevalence of eGFR<60mL/min/1.73m2 and albuminuria was 1.7% (95% CI 1.5%-1.9%) and 9.3% (95% CI 8.7%-9.8%), respectively. The overall prevalence of CKD was 10.6% (95% CI 10.1%-11.2); therefore the number of patients with CKD in China is estimated to be 117.3 million. In rural area, the prevalence of eGFR<60mL/min/1.73m2 did not vary markedly with levels of economic development, while higher prevalence of albuminuria was observed in higher tertiles of GDP per capita. In urban area, lower prevalence of both eGFR<60 mL/min/1.73m2 and albuminuria was observed in sites with higher level of economic development.

Conclusions: Our study revealed that China is going to experience an enormous increase in the prevalence of CKD, especially for the rural residents, who comprised more than half of the population in China. The rapid surge in diabetes and hypertension, both of which are predicted to drive epidemics in CKD, will have profound socioeconomic and public health consequences in developing countries such as China.

FR-PO1474

Frequency of Mild Kidney Disease and Associated Risk Factors in Apparently Healthy Mexican Subjects Carlos Kornhauser. *Department of Medical Sciences, Universisty of Guanajuato, Leon, Guanajuato, Mexico.*

Background: Chronic kidney disease (CKD) associates with a wide rank of complications leading to a decreased quality of life. Mild kidney disease comprise the first three stages of CKD, being over 50 times more frequent than terminal kidney disease.

Objective: We evaluated the frequency of CKD in stages 1, 2, and 3, by assessing GFR according to the MDRD equation, and the presence of microalbuminuria in apparently healthy people.

Methods: We did an epidemiological, cross-sectional study in 1160 apparently healthy adult subjects of both sexes, in the city of Leon, Mexico. Subjects were randomly selected. Clinical and anthropometric data were collected. Glucose, creatinine, uric acid,

total cholesterol, triglycerides, and HDL, LDL cholesterol fractions were measured in serum. Urinary creatinine and albumin were also assessed. Descriptive statistics, Anova, Kruskal Wallis, multiple regression and logistic regression test were performed. p< 0.05 was considered significant.

Results: Male and female genders were evenly distributed. Male's average age was 40.5±8.9 years and 45±11 for women. 38.9% of the total population had overweight (BMI = 26-29.9), 31.5% were obese (BMI >; 30). 25% of the subjects were active cigarette smokers, 53% of the total population had high serum cholesterol and triglycerides levels. 9.4% of the total population was found with CKD: stage 1: 0.8%, stage 2: 3.8%, stage 3: 4.7%, and stage 5: 0.08%. Factors associated with CKD found were: Serum uric acid (> 5.0 mg/dl) with an OR of 1.9 (CI 95% 1.1-1.6, p= 0.009). Serum cholesterol (> 200 mg/dl), OR 1.5 (CI 95% 1.25–1.7, p= 0.01), serum triglycerides (> 150 mg/dl) OR 1.1 (CI 95% 0.93–1.3, p = 0.01), and obesity (BMI ≥ 30), OR 1.3 (CI 95% 1.1–1.6, p = 0.009).

Conclusions: The frequency of kidney disease stages in our population studied was similar to the frequency observed in other countries. eGFR and microalbuminuria have a negative association with age, female sex, hyperuricemia, hypertriglyceridemia, hypercholesterolemia and amount of body fat mass. High levels of uric acid, triglycerides, cholesterol, and obesity constitute factors associated with CKD.

FR-PO1475

The Prevalence of Chronic Kidney Disease in the ARV-Naive HIV-Infected Adults in Mainland China: Data from a National Multicenter Prospective Study

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Background: The prevalence of both chronic kidney disease and HIV infection are increasing in China, yet no data existed describing the epidemiologic characteristics of CKD in HIV infected adults in mainland China. The objective of this study is to determine the prevalence of CKD in the antiretroviral therapy-naïve HIV infected adults in mainland China.

Methods: This prospective study was conducted in 12 clinical trial units that geographically and socioeconomically covered major areas of mainland China. Demographic variables, medical information was recorded at the entry of the study. Spot urine tests and creatinine measurement (Jaffe method) were performed in local laboratories. Plasma HIV-1 RNA was evaluated using theAmplicor system (RocheDiagnostics and Abbott). eGFR was calculated using the MDRD equation. Patients with abnormal urine test or a decreased eGFR lower than 90ml/min*1.73m^2 were followed and spot urine test and creatinine test were repeated 12 weeks after the screening to confirm the diagnosis of CKD.

Results: 541 patients were enrolled and most of them were male (74.2%), with a mean age of 37.3 years (Table 1) . All of the patients were ARV-naive and the mean time since HIV diagnosis was 1.93 years. Baseline mean CD4 cell counts were 172, with a majority (99.6%) below 350 cells/mm3. The log value of viral load was on average 4·58 ± 0·04, with 58% under 50copies/uL.The prevalence of CKD in the Chinese ARV-naive HIV-infected patients was (16.0 ±1.7) %. Among the CKD patients, the majority were in CKD1 or CKD2.

Table 1 Prevalance of impaired renal function and kidney injury markers at baseline and 12-week follow-up

	Baseline	12-week
Proteinuria	9.4%	4.2%
Hematuria	10.7%	11.4%
Mean eGFR	109.9 ±1.1	111.7 ±1.2
eGFR≤90*	20.4%	20.5%
eGFR≤60*	0.4%	0.6%

* unit: mL/min×1.73m2

Conclusions: CKD is prevalent in the Chinese ARV-naive HIV-infected adults and the majority of CKD patients have mildly impaired renal function.

Funding: Government Support - Non-U.S.

FR-PO1476

Chronic Kidney Disease in Patients on Lithium Therapy in Iceland

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Background: Long-term lithium treatment may lead to chronic kidney disease (CKD) but the magnitude of this risk has not been well characterized. The purpose of this study was to examine CKD associated with lithium therapy in Iceland.

Methods: In this retrospective study, we obtained information on all adult subjects taking lithium during the years 2003-2010 by examining records of serum lithium measurements performed at the Univeristy Hospital in Reykjavik and prescriptions for lithium in the National Drug Prescription Database. IDMS-standardized serum creatinine (SCr) was obtained from medical records and eGFR calculated from the lowest SCr value in the last year of measurement for each subject using the MDRD equation. CKD was defined as eGFR <60 mL/min/1.73m^2. A group of randomly selected community-dwelling adults was used as controls.

Results: A total of 1577 subjects received at least one lithium prescription during the study period, 628 (39.8%) of whom had no recorded measurements of serum lithium or SCr at the University Hospital. The remaining 949 subjects had a median (range) age of 50 (19-98) years, and 558 were female (58.8%). The control group comprised 1630 subjects with a median age of 60 (29-87) years, 1039 (63.7%) were female. The prevalence of CKD was 13.6% in the lithium group and 5.3% in the control group.

Prevalence of CKD in the lithium and control groups, by age

Agegroups	Lithium group	Control group	P-value	OR (95% CI)
18-30	0%	0%	-	-
30-40	2.6%	0%	0.3	-
40-50	3.5%	0.6%	0.016	6.1 (1.2-29.5)
50-60	14.4%	0.3%	<0.001	54.7 (7.4-405.2)
60-70	21.1%	2.3%	<0.001	11.5 (5.1-25.8)
>70	44%	15.5%	<0.001	4.4 (2.9-6.7

The duration of lithium treatment was independently associated with CKD with OR = 1.34 (95% CI, 1.28-1.41) for each year of therapy.

Conclusions: Lithium markedly increases the risk of CKD, particularly in the younger age groups. This may eventually lead to kidney failure but more importantly places these patients at increased risk of cardiovascular disaease.

FR-PO1477

A Prospective Population Screening Study of Prevalence of CKD and Its Risk Factors: The Texas CKD Study

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Background: Recent reports indicate a high prevalence of CKD in general population in the US (16.8%, NHANES 2006). However most such reports are based on retrospective sampling and the true prevalence of CKD remains unclear. Texas is estimated to have disproportionately high prevalence of CKD and ESRD and related healthcare costs. The Texas CKD study is an initiative funded by the Texas Department of Health to address the same.

Methods: The study was organized by Texas Tech University and conducted in the West Texas population with a goal to screen a total of 1000 adult subjects (age >21 yrs). The subjects were recruited by a random digit dialing (RDD) methodology with phone interviews and scheduling administered by the University of North Texas Survery Research Center. Appriopriate approvals were obtained to comply with IRB and HIPAA regulations. The sample was adjusted to conform to rural urban mix, as well as age, race and sex ratios in the Texas adult population. Risk factors for CKD were noted by personal questionnaire while GFR was estimated using both MDRD and CKD-EPI formulae from serum creatinine values. Urine microalbumin and urine prot/creat ratio were also determined. CKD was defined using KDOQI guidelines. Individual results were discussed with the subjects to be relayed to their primary physicians for follow up as needed.

Results: Of a total of 931 subjects who completed evaluation, 172 met the critera for CKD using the MDRD formula giving an overall prevalence of 18.5%. Of them, 108 had stage 3 or worse CKD (11.6%). Only 21 of the 172 (12.2%) were aware that they had CKD. Hypertension was present in 389 subjects(41.8%), while diabetes was present in 155 (16.7%) and smoking in 17.4%. Furthermore 729 (78%) were overweight (BMI>25 Kg/m2) with 437(49%) being clearly obese (BMI>30 Kg/m2). Using the CKD-EPI formula the prevalence was slightly lower (155 or 16.7%).

Conclusions: CKD is very common in West Texas with a prevalence of 18.5% with a high prevalence of risk factors for CKD especially obesity. Awareness of the condition is dismally low underscoring the need for widespread screening and prevention by addressing the risk factors.

Funding: Government Support - Non-U.S.

FR-PO1478

Pre-Dialytic Chronic Kidney Disease in Children: A Nationwide Epidemiologic Survey in Japan

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Background: Chronic kidney disease (CKD) in children is a progressive and intractable condition that may severely impair patients' growth, development, and quality of life; however, there is little epidemiologic information available on the disease, particularly in Asian children.

Methods: We performed a nationwide epidemiologic survey of children (3 months to 15 years old) with pre-dialytic CKD. CKD staging was classified as stages 3 to 5 according to new criteria we developed on the basis of new reference levels for serum creatinine (S-Cr) according to age and sex of Japanese children (Uemura O et al. Clin Exp Nephrol 2011 [Epub ahead of print]). CKD stages 3 to 5 were defined as follows: CKD stage 3, S-Cr more than twice the median level; CKD stage 4, S-Cr more than 4 times the median level; and CKD stage 5, S-Cr more than 8 times the median level. Questionnaires were then sent to 1190 institutions, which treat most of the children with pre-dialytic CKD in Japan.

Results: A total of 925 institutions (77.7%) responded. Information on 440 children with CKD in 112 institutions was collected as of April 1, 2010: 331 in stage 3, 103 in stage 4, and 26 in stage 5. The median age was 8.7 years, and 265 (60.2%) were male. The total number of children with CKD stage 3 to 5 in Japan was estimated to be 528.5 (95% confidential interval, 486.1-570.9). The prevalence was estimated to be 29.0 per 1,000,000 children aged 15 years or younger. Of the total number of patients, 90.7% had non-glomerular diseases. Among the children with non-glomerular diseases, 68.4% had congenital anomalies of the kidney and the urinary tract (CAKUT). Only 2.0% and 1.8% of all children had focal segmental glomerulosclerosis and chronic glomerulonephritis, respectively.

Conclusions: In conclusion, this first nationwide survey of children with CKD stage 3 to 5 in Japan indicated that most of the children had non-glomerular underlying diseases, mainly CAKUT. Improved management of CAKUT, including renoprotective treatment and urological interventions, is required.

Funding: Government Support - Non-U.S.

FR-PO1479

Endocrine-Metabolic Disorders in Patients with Chronic Kidney Disease

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Background: Endocrine-metabolic disorders are common in patients with chronic kidney disease. Several studies showed that the prevalence of endocrine-metabolic disorders is increased in patients with chronic kidney disease, and that could have important role in the prognosis of chronic kidney disease. In this study, we investigated the prevalence of endocrine-metabolic disorders in healthy personS according to the renal function.

Methods: We retrospectively reviewed 948 adults selected from the Health Promotion Center at Chung-Ang University Hospital. Age, sex, height, weight, waist circumference, blood pressure, fasting glucose, lipid profile, serum creatinine and bone mineral density were evaluated. The glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) formula. Decreased renal function was defined as an estimated GFR under 60 ml/min/1.73m^2 and metabolic syndrome was defined as per the International Diabetes Federation (IDF) 2006 criteria.

Results: The mean age was 47.7±9.3 years in men (276, 29.1%) and 50.8±9.7 years in women (672, 70.9%). There were 918 persons with normal renal function and 30 persons with decreased renal function. The prevalences of metabolic syndrome and osteoporosis were 13.8% (10.1% in men, 11.1% in premenopausal women and 27.0% in postmenopausal women) and 14.2% (9.8% in men, 11.1% in premenopausal women and 29.8% in postmenopausal women), respectively. The percentages of persons with metabolic syndrome and osteoporosis were increased in persons with decreased renal function (p=0.002 and 0.0048). Subgroup analysis was conducted for men, premenopausal women and postmenopausal women groups. In premenopausal women, we found similar results, but in men and postmenopausal women, we found no difference of prevalence between persons with normal renal function and persons with decreased renal function.

Conclusions: Metabolic syndrome and osteoporosis were increased in persons with a decreased renal function in premenopausal women. However, there was no association between endocrine-metabolic disorders and decreased renal function in men and postmenopausal women.

FR-PO1480

Estimated Glomerular Filtration Rate Does Not Progressively Decline in the Healthy Elderly, Particularly in Females and Those with High Cholesterol Levels

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Background: Estimated GFR (eGFR) seems to be inappropriately underevaluated in the healthy elderly. Consequently, in a sweeping assumption, many of the elderly have been grouped as CKD stage 3 and considered as would-be candidates for HD in the future. Therefore, this study was performed to discern whether or not eGFR does progressively decline in the elderly with aging.

Methods: A total of 574 elderly people (286 males, 288 females) at the age of 60 or older (60 to 94 years old) without serious diseases were enrolled. They were living in one local community in central Tokyo. Clinical data including eGFR, BP, BUN, total cholesterol (TC), triglyceride, uric acid and urinalysis between 2008 and 2010 were annually collected and analyzed. The presence of DM, hypertension, hyperuricemia, past history for coronary events and apoplexy as well as the use of anti-hypertensives were examined. Those who had serum creatinine level of 1.5 mg/dL or more, and had been diagnosed as CGN, hemodialyzed or hospitalized during the study period were excluded from the study.

Results: eGFR did not markedly change for 2 years (ΔeGFR/2 yrs: -0.39 ± 7.86 mL/min). However, eGFR was slightly decreased in men (ΔeGFR/2 yrs: -1.98 ± 7.91 mL/min, P<0.001) and slightly increased in women (ΔeGFR/2 yrs: 1.18 ± 7.49 mL/min, P=0.027). It was confirmed by a multiple regression analysis that male sex, higher BUN (>21 mg/dL) and lower TC (<220 mg/dL) significantly contributes to the decline of eGFR. Past history for coronary events, hyperuricemia, proteinuria and higher BUN levels were also confirmed to be significant risk factors for an eGFR of lower than 50 mL/min in the elderly.

Conclusions: In contrast to the previous assumption, eGFR did not greatly decline year by year in the healthy elderly. Interestingly, female sex and relatively high TC levels might be the favorable factors to slow the decline of eGFR. Further study will be necessary to precisely comprehend the meaning of these results and to confirm the validity of the MDRD equation for calculating eGFR.

FR-PO1481

Validation of MDRD and Japanese eGFR Equation among Taiwanese and Korean: Approach To Set the Fundamental Scheme for eGFR Evaluation in Asia

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Background: Glomerular filtration rate (GFR) is essential for CKD diagnosis and staging. The Modification of Diet in Renal Disease (MDRD) Study equation is globally well used, however ethnicity coefficient is not available for Asians. Japanese society of nephrology develop Japanese coefficient of 0.808 for MDRD equation, whereas Chinese and Korean coefficients were reported to be 1.233 and 1.09285, regardless of similar genetic and cultural background among Asians, probably due to different GFR measurement methods and lack of creatinine (Cr) standardization. Thus we validate MDRD and Japanese eGFR equations among Taiwanese and Koreans by inulin clearance (Cin), a gold standard for GFR, under the same protocol as in Japan and accurate sCr values.

Methods: Cin was evaluated among198 Taiwanese and 157 Koreans. All samples were measured in a single center, and sCr values were IDMS-traceable. This study was a part of Asian Collaborative Study for Creation of GFR Estimation Equation (ACOS-CG-FREE). Urinary Cr excretion rate divided by body weight was compared to that of Japanese.

Results: Performance of MDRD and Japanese equations are shown in table.

Performance of Japanese and MDRD GFR equations for Taiwanese and Korean

	Japanese equation	MDRD equation	Japanese equation	MDRD equation
	Taiwanese	Taiwanese	Korean	Korean
20% accuracy	40 %	57 %	44 %	42 %
30% accuracy	64 %	75 %	64 %	54 %
R	0.922	0.922	0.872	0.861
RMSE	17.85	16.51	22.3	26.0
Bias	-10.2±14.7	1.3±16.5	-2.4±22.2	10.3±23.9

Taiwanese and Korean coefficient for IDMS-MDRD equation were 1.054 and 0.916, respectively. Creatinine excretion was high among Taiwanese than Japanese or Korean.

Conclusions: Better accuracy was demonstrated in Japanese eGFR equation for Korean but not for Taiwanese, probably due to different body muscle mass.

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FR-PO1482

Risk Factor Profiles Based on Estimated Glomerular Filtration Rate and Dipstick Proteinuria among Participants of the Specific Health Check and Guidance System in Japan 2008

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Background: Both estimated glomerular filtration rate (eGFR) and albuminuria (proteinuria) are important determinants of the risk of cardiovascular disease (CVD), end-stage renal disease (ESRD), and mortality. Few studies, however, have examined the risk factor profiles based on eGFR and proteinuria among the general population.

Methods: Data of the newly developed nationwide screening program of the Specific Health Checkups and Guidance System (Tokutei-Kensin) initiated in 2008 were used in this study. The aim of this screening, targeting people 40 to 74 years of age, was to detect those with metabolic syndrome and to offer those services regarding lifestyle modifications that will lead to the reduction of diabetes mellitus (DM) and DM-related ESRD. Individual records of 580,000 participants in 69 cities and towns and 3 unions' cohorts throughout Japan were anonymously provided and included in the present study.

Results: Details of 332,174 (57.3% of the total) participants with both serum creatinine and dipstick urine test data were analyzed. Mean (SD) age was 63.6 (8.3) years and 40.6% were men. The mean (SD) eGFR was 67.2 (17.7) ml/min/1.73m2 and 5.4% had proteinuria. The prevalence of CKD stage 3, 4, and 5 was 34.5%, 0.5%, and 0.1%, respectively. The prevalence of DM, hypertension, and history of stroke and heart disease was correlated with the combination of eGFR and degree of proteinuria.

Conclusions: The findings of the present study indicate that CKD and risk factors for CVD are quite common among middle-aged Japanese. CKD classification based on eGFR and proteinuria may be useful for predicting CVD, mortality rate, and ESRD in the Japanese.

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FR-PO1483

The Choice of the CKD Definition and the Impact on the Chronic Kidney Disease Prevalence

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Background: CKD definition is notably based on an estimated GFR fixed cut-off at 60 mL/min/1.73 m². Such a fixed knot has already been criticized in the literature because GFR physiologically decreases with aging. In this work, we will illustrate the consequences of this fixed reference value on the CKD epidemiology.

Methods: Over a two years period, we tested 4208 voluntary subject aged of more than 50 years old for CKD with the measurement of an IDMS traceable creatinine. GFR was estimated with the MDRD study equation. CKD was defined by two ways: either with a reference range fixed at 60 mL/min/1.73 m² or according to age as defined in the Nijmegen

epidemiologic study (which also used the MDRD study equation)(Wetzels JF, Kidney Int, 2007). Vast majority of subjects were Caucasian. Subjects were classified according to their age, in 5 years-lengths categories. Older than 85 years subjects were excluded (n=4).

Results: Using the fixed definition, CKD was found in 13% of our subjects. Using the Nijmegen criteria, the CKD prevalence decreased to 5.9%. Therefore, according to the CKD definition used, the CKD prevalence can vary from simple to double. Among subject with discordant results, 73% were women. As expected, the discrepancies increased according to age (0.9% of subjects with discrepant results in the 50-55 years old bracket and up to 19% in the 75-79 years old one).

Conclusions: More than half of subjects with CKD as defined by estimated GFR under 60 mL/min/1.73 m² will become "healthy" if the CKD definition is modified according to age. This is particularly relevant in women and older population. This seems logical because both populations have physiologically lower GFR values. Not considering age in the CKD definition will overestimate the number of CKD patients, especially in women and older subjects.

FR-PO1484

The Effects of Ethnicity on C-reactive Protein Level in Patients with Diabetic Nephropathy Satyesh K. Sinha, Susanne B. Nicholas, Magda Shaheen, Deyu Pan. *Research, Charles R Drew University of Medicine and Science, Los Angeles, CA.*

Background: Studies show that ethnic differences alter the rate of progression of diabetic nephropathy (DN) and that African Americans (AA) have a more rapid course than Whites. We postulated that elevated indicators of inflammation in AA may potentially explain this disparity possibly reflected by levels of inflammatory marker C-reactive protein (CRP). We examined CRP and other clinical indicators of DN in different ethnic groups in the data from the National Health and Nutrition Examination Surveys (NHANES) 1999-2004.

Methods: We analyzed data for 1,637 adults aged =>20 years who were told had diabetes or had fasting blood sugar >125mg/dl. Descriptive statistics characterized the subjects. We use Chi-square for categorical variables and t-test or ANOVA for continuous variables to test the statistical differences between the groups.

Results: The data indicate that CRP, urinary albumin, serum creatinine, HbA1c and fasting glucose were significantly higher in diabetic patients with albuminuria compared to patients without albuminuria (0.75±0.06mg/dl, 455±48ug/ml, 1.04±0.04mg/dl, 7.83±0.11% and 187±5mg/dl, and 0.62±0.04, 9.60±0.31, 0.86±0.01, 7.24±0.09, 156±3.57 respectively; p≤0.05). Estimated GFR was significantly reduced in diabetic patients with albuminuria (75±1ml/min/1.73m2) compared to those without it (79±1ml/min/1.73m2) (p=0.004). There was no ethnic difference in the level of CRP and urinary albumin in patients aged 20-44 and ≥65, except among those without albuminuria, where AA had higher CRP compared to Whites (1.05±0.14 and 0.46±0.08 respectively) (p<0.01). However, in age group 45-64, for those without albuminuria, CRP and urinary albumin were significantly elevated in AA compared to Whites (p<0.01) but for those with albuminuria, only urinary albumin was significantly higher in AA compared to Whites (p<0.01). However, CRP was trended higher in AA compared to Whites (p=0.05).

Conclusions: Concentration of CRP is higher in AA compared to Whites aged 45-64. The results suggest that more advanced inflammatory processes might explain the more rapid progression of type 2 DN in AA patients in this age group and should be further examined.

FR-PO1485

Chronic Kidney Disease: Still an Unrecognized Health Issue? Claudia Friedl,[1] Alexander R. Rosenkranz,[1] Astrid Fahrleitner-Pammer.[2] *[1]Department of Internal Medicine, Division of Nephrology and Hemodialysis, Graz, Austria; [2]Department of Internal Medicine, Division of Endocrinology and Metabolism, Graz, Austria.*

Background: Chronic kidney disease (CKD), especially end stage renal disease (ESRD), is not only associated with a higher morbidity and mortality but also with extremely high cost for the healthcare system. Early diagnosis and therapy of CKD could slow down the progression of the disease and minimize the prevalence of cost-intensive ESRD. Aim of this present study was to determine the prevalence of CKD in inpatients at a general internal ward and to evaluate the frequency of documented CKD according to ICD-10 classification at discharge.

Methods: Over a period of 4 months the data of 238 patients admitted to a general internal ward of the Department of Internal Medicine, Medical University Graz, Austria, were collected. We analyzed the prevalence of CKD, defined as eGFR<60ml/min/1.73m². eGFR was derived from the four-variable MDRD. Furthermore discharge letters were reviewed and the frequency of documented ICD-10 CKD was analyzed. Patients on dialysis (n=7) and those with acute renal failure (n=3) were excluded from analysis. Continuous variables are presented as median (range).

Results: We included 228 patients, 110 (48%) female and 118 (52%) male, in the present analysis. Median age of the study population was 76 years [20-99], whereby with a median of 80 [20-99] women were significantly older (men: 70 [25-97] (p<0.001). eGFR was the same for both sexes with a median of 54,5 [15.8-149.0] in women and 63.6 [10.4-171.9] in men (n.s.). Nearly half of the study population at least had a eGFR<60 ml/min/1.73m². According to CKD stages the prevalence was 39.9% for stage 3, 7.9% for stage 4 and 1.3% for stage 5. The analysis of the related discharge letters showed a documentation of ICD-10 CKD diagnosis of 21% (n=48) in the total study population. Of those with an eGFR < 60ml/min/1,73m² just 38% were diagnosed as patients with CKD.

Conclusions: The present analysis shows a high rate of undiagnosed CKD in hospital setting even at an internal ward. This could have dramatic implications on the care, treatment and prevention of CKD and associated complications.

FR-PO1486

Survival in Patients Entering Renal Replacement Therapy in Their First Month of Life – A World-Wide Collaborative Study Karlijn J. Van Stralen,[1] Dagmara Borzych-Duzalka,[6] Kitty J. Jager,[1] Enrico Verrina,[2] Sean E. Kennedy,[5] Franz S. Schaefer,[3] Pierre Cochat.[4] *[1]ESPN/ERA-EDTA registry, Netherlands; [2]Gaslini Children's Hospital, Italy; [3]University of Heidelberg Childrens Hospital, Germany; [4]Université de Lyon, France; [5]Sidneys Childrens Hospital, Australia; [6]Medical University of Gdansk, Poland.*

Background: End-stage renal disease requiring RRT from the neonatal period is a very rare condition. Hence, little information is available regarding long-term treatment outcomes in these challenging patients.

Methods: Neonates who started RRT in their first month of life were included in the present study. patients starting RRT since 2000 who were registered prospectively in the ESPN/ERA-EDTA (122 patients), IPPN registry (42 patients) or ANZdata (4 patients) were considered, 8 patients were included in multiple registries. Patients were censored after 2 years of follow-up. Survival was estimated using the Kaplan-Meier method.

Results: A total of 161 patients from 30 countries started RRT during the first month of life. They were followed for a median of 31 (IQR 8-47) months. Nearly all children started on PD (90%), while 15 started on HD and one patient with a transplant. Half of them started in their first week of life. The most important causes of renal failure were congenital anomalies of the kidney and urinary tract (62%), followed by cystic kidneys (14%) and tubular necrosis (9%). Within the first 2 years after start of RRT, there were 99 changes in treatment modality among 75 children, including 26 transitions from PD to HD, 19 from HD to PD, 22 transplants, and 3 recoveries of renal function, of whom 2 were only temporary. Sixteen children died after a median of 6 months, resulting in an estimated two-year survival of 86%.

Conclusions: While we cannot exclude potential selection bias from excluding patients in whom RRT was electively not initiated, this study demonstrates that with current RRT technology remarkably good medium-term patient survival is achieved in those neonates in whom a decision for RRT is made. In addition to neurodevelopmental issues, this study might help physicians in deciding whether RRT is an option in neonates with severe renal failure.

FR-PO1487

Foxd1 Is an Upstream Regulator of the Renin-Angiotensin System (RAS) during Metanephric Kidney Development Renfang Song,[1] Graeme James Preston,[1] Maria Luisa S. Sequeira Lopez,[2] Ihor V. Yosypiv.[1] *[1]Pediatrics, Tulane University, New Orleans, LA; [2]Pediatrics, University of Virginia, Charlottesville, VA.*

Background: The RAS is critical in ureteric bud (UB) branching and metanephric kidney development. Mutations in the RAS genes in mice or humans cause a spectrum of congenital anomalies of the kidney and urinary tract (CAKUT). However, the mechanisms by which RAS gene mutations result in CAKUT are poorly understood. In this study, we tested the hypothesis that Foxd1, a forkhead box transcription factor essential for normal kidney development, is an upstream regulator of the RAS during UB morphogenesis.

Methods: The effect of genetic inactivation of Foxd1 on UB branching and RAS gene and protein expression was examined in embryonic (E) day E13.5 Foxd1+/+ and -/- kidneys in vivo and in mesenchymal (MK4) cells transfected or not with Foxd1 expression vector (1.0 μg plasmid DNA) in vitro. Angiotensinogen (AGT), renin, angiotensin I-converting enzyme (ACE), angiotensin (Ang) II receptor type 1 (AT1R) mRNA levels were determined by real-time qRT-PCR. Cellular distribution of AGT and renin proteins was examined by immunohistochemistry.

Results: The number of UB tips was decreased in Foxd1-/- (n=4) compared with Foxd1+/+ (n=6) metanephroi (14±2.1 vs. 28±1.3, p<0.05). Renin, ACE, AT1R mRNA levels as well as AGT and renin protein expression was decreased in Foxd1-/- compared with Foxd1+/+ E14.5 metanephroi (mRNA: renin: 0.23±0.01 vs. 1.0±0.02, p<0.01, ACE: 0.37±0.02 vs. 1.0±0.01, p<0.01, AT1R: 0.41±0.06 vs. 1.0±0.02, p<0.01), whereas AGT mRNA levels did not change. Foxd1 overexpression in MK4 cells increased renin, ACE, AT1R and did not alter AGT mRNA levels. Treatment of E13.5 Foxd1-/- kidneys with exogenous Ang II (10-5 M) for 24 hours increased the number of UB tips compared with media (control) (42±2.0 vs. 33±2.5, p<0.05).

Conclusions: In summary, RAS gene expression during metanephric development is differentially regulated by Foxd1 at the transcriptional level. We conclude that the cross-talk between the RAS and Foxd1 plays an important role in UB morphogenesis and pathophysiology of CAKUT.

Funding: NIDDK Support

FR-PO1488

Association between Genetic Variation at FOXP3 Gene Locus and Renal Transplant Outcomes Jennifer A. McCaughan,[1,2] Aisling E. Courtney,[1] A.J. McKnight,[2] Alexander P. Maxwell.[1,2] *[1]Department of Nephrology, Belfast City Hospital, Belfast, United Kingdom; [2]Nephrology Research Group, Queen's University, Belfast, United Kingdom.*

Background: T regulatory cells have emerged as key coordinators of immune tolerance. A unique feature of these cells is the stable expression of the forkhead box transcription factor gene *FOXP3* (Xp11.23). It is reported that transplant recipients with longterm graft survival demonstrate significantly higher *FOXP3* expression than those with graft loss from chronic immunological injury. This study is the first to investigate whether genetic variation at the *FOXP3* locus is associated with allograft or transplant recipient survival.

Methods: Genomic DNA was prospectively collected from recipient and donor pairs in first deceased donor kidney transplants. A total of 575 recipients and 516 donors were included with 354 (61%) recipients and 301 (58%) donors being male. Over 99% of both populations were White.

Genotyping data for *FOXP3* single nucleotide polymorphisms (SNPs) with a minor allele frequency > 5% were downloaded from the International HapMap Project for a White population. Five SNPs met the criteria for a Hardy Weinberg cutoff 0.001 and genotype rate > 95%. Four Tag SNPs were chosen using the pair-wise approach implemented in Haploview. Five potentially functional SNPs were downloaded from the Ensembl Genome Browser.

Eight SNPs were genotyped using Sequenom iPLEX and called using MassARRAY TYPER. A single SNP was genotyped using Taqman technology. Genotypes and allele frequencies were analysed on a gender-specific basis using SPSS.

Results: One FOXP3 SNP within the donor genome was significantly associated with allograft survival [rs2280883, p=0.038] and one with recipient survival [rs2294021, p=0.031]. FOXP3 gene variants in the recipient genome were not significantly associated with either allograft or recipient survival.

Conclusions: Recipient *FOXP3* expression post transplantation appears to be associated with long term allograft survival. It was an unexpected finding that *FOXP3* variants in the donor genome were associated with recipient and allograft outcomes. Replication of this study in another cohort of donor-recipient pairs will establish if this association is robust.

FR-PO1489

Genetic Variation at Caveolin-2 Locus and Renal Transplant Outcomes Jennifer A. McCaughan,[1,2] Aisling E. Courtney,[1] A.J. McKnight,[2] Alexander P. Maxwell.[1,2] *[1]Dept. of Nephrology, Belfast City Hospital; [2]Nephrology Research Group, Queen's University, Belfast.*

Background: Caveolae are invaginations of the plasma membrane which are formed from a stable complex of proteins caveolin-1 and caveolin-2. Caveolae have a key role in intracellular drug transport, cell growth, transmembrane signalling, and apoptosis. Single nucleotide polymorphisms (SNPs) within the caveolin-1 gene are significantly associated with renal allograft fibrosis and survival. The caveolin-2 gene (*CAV2*), located at 7q31.1, is also a plausible candidate gene implicated in renal allograft survival.

Methods: We performed SNP analysis on genomic DNA from 575 recipients and 516 donors in paired, first deceased kidney transplants. Prospective clinical data has been recorded from 1986 on all recipients. Over 99% of both populations were White.

Genotyping data for *CAV2* SNPs with a minor allele frequency > 5% were downloaded from the International HapMap Project for a White population. Thirteen SNPs met the criteria for Hardy Weinberg cutoff 0.001 and genotype rate >95%. Seven Tag SNPs were chosen using the pair-wise approach implemented in Haploview. Three potentially functional SNPs were downloaded from the Ensembl Genome Browser.

Eight SNPs were genotyped using Sequenom iPLEX and called using MassARRAY TYPER. Two SNPs were genotyped using Taqman technology. Genotypes and allele frequencies were analysed using SPSS.

Results: Investigating all common variants in CAV2 revealed statistically significant associations between two recipient SNPs and allograft survival [rs11980719, p = 0.017 and rs 13221869, p = 0.015]. rs13221869 is a potentially functional SNP resulting in an amino acid change: NP_937855.1:p.Phe13Leu.

Conclusions: This study provides further evidence that genetic variation in caveolin genes may play a role in renal allograft fibrosis and survival.

FR-PO1490

Genome Wide Methylation Analysis of 485,577 Features in a Renal Transplant Population Jennifer A. McCaughan,[1,2] Aisling E. Courtney,[1] Alexander P. Maxwell,[1,2] A.J. McKnight.[2] *[1]Regional Nephrology Unit, Belfast City Hospital, Belfast, NI, United Kingdom; [2]Centre for Public Health, Queen's University of Belfast, NI, United Kingdom.*

Background: Studies have highlighted that renal (dys)function is influenced by differential methylation at several loci. We investigated the DNA methylome by conducting an epigenome-wide association study in peripheral blood leukocytes.

Methods: Ninety-six individuals (recipients vs. donors) were matched for age and gender. Comparisons were performed for end stage kidney disease (ESKD), IgA nephropathy, chronic allograft nephropathy and survival outcomes.

Methylation status was profiled in individuals by hybridisation to 450K Infinium methylation beadchips (Illumina Inc, USA). 90 samples and arrays passed stringent quality control; raw data were normalised and beta values calculated. Significantly up and down regulated genes for each comparison (adjusted p<0.0001) were considered for biological relevance by functional enrichment analysis using KEGG pathways.

Results: Quantitative methylation values were obtained at a single-CpG site level for 485,577 features, encompassing coverage of all designable RefSeq genes, including promoter, 5', and 3' regions, CpG islands outside coding regions, and miRNA promoter regions. Experimentally defined genders matched each individual submitted for analysis and >99% duplicate concordance was observed.

Significant association ($P<10^{-10}$) was observed for ESKD, including multiple hits in biological candidate genes such as *NOSIP*, *JAG2*, *SMAD3*, *TBCD*, and *AFF3*. Genome-wide significance was not observed for IgA nephropathy or chronic allograft nephropathy; top ranked genes include *CALM2*. Multiple genes reached $P<10^{-8}$ for survival outcomes, top ranked members include the ADAM family. WNT, Jak-STAT signalling, tight junction and apoptotic pathways were highlighted by all comparisons.

Conclusions: We have identified differences in methylation profiles both globally, and at individual CpG sites, which are associated with ESKD and renal transplant outcomes.

Funding: Private Foundation Support

FR-PO1491

Comprehensive Genetic and Epigenetic Investigations for Association of the Major Histocompatibility Complex Region in a Renal Transplant Population A.J. McKnight,[1] Jennifer A. McCaughan,[1,2] Aisling E. Courtney,[2] Alexander P. Maxwell.[1,2] *[1]Centre for Public Health, Queen's University of Belfast, NI, United Kingdom; [2]Regional Nephrology Unit, Belfast City Hospital, Belfast, NI, United Kingdom.*

Background: The prevalence of end-stage kidney disease (ESKD) continues to rise so that strategies to delay the onset of ESKD and improve long-term survival of renal grafts and transplant recipients are important goals. Genetic variation within the major histocompatibility complex (MHC) results in phenotypic variation in expressed human leukocyte antigens (HLA) that, along with non-HLA targets, affect renal function and transplant outcomes. We have previously reported association of MHC-related genes with diabetic nephropathy.

Methods: We have genotyped >3,000 maximally informative SNPs in the MHC region and MHC-related genes in 900 matched, White European, kidney transplant donors and recipients (maximum follow-up 238 months; median 69 months) using a combination of dedicated MHC panels (Illumina Inc, USA) and Sequenom-based genotyping. Complementary to this approach, next generation sequencing technology (Illumina) was performed to individually fine-map the entire MHC region for 10 participants. Association of variants with ESKD was evaluated using PLINK while short and long-term graft and recipient survival was evaluated using Kaplan-Meier plots and Cox regression models. Methylation status of CpG islands within 6p21 was available for 90 of the individuals genotyped.

Results: Greater than 97.5% completion was observed with >99% duplicate concordance. Genome-wide significant association (adjusted P<10-8) was identified for several loci. We also provide detailed coverage of the technically challenging 5 Mb region that includes HLA-A, B, C, DMA, DMB, DOA, DOB, DPA1, DPB*, DQA*, DQB*, DRA, DRB* E, F G, H, J, K, L, U and HLA-W genes.

Conclusions: Our comprehensive study highlights the complexity of the MHC region and provides important information for genes that are primarily involved in innate and adaptive immune systems. While we have identified several key loci that influence renal function, these need validated in larger cohorts followed by functional experiments to elucidate the mechanisms.

Funding: Private Foundation Support

FR-PO1492

APOL1 Risk Genotypes Are Enriched in African American Study of Kidney Disease and Hypertension (AASK) Participants, Particularly among Those with Kidney Disease Progression Cheryl Ann Winkler,[1] Barry I. Freedman,[2] Wen Hong Linda Kao,[3] Carl D. Langefeld,[2] Brad C. Astor,[3] George W. Nelson,[1] Mary E. Comeau,[2] Donald W. Bowden,[2] Jeffrey B. Kopp,[4] Michael S. Lipkowitz.[5] *[1]SAIC, NCI, Frederick, MD; [2]Wake Forest University, Winston-Salem, NC; [3]Johns Hopkins University, Baltimore, MD; [4]NIDDK, Bethesda, MD; [5]Georgetown University Medical Center, Washington, DC.*

Background: Chromosome 22 variation explains much of the increased kidney disease risk in African descent individuals. The AASK study enrolled 1094 subjects with hypertension and reduced glomerular filtration rate and examined the effect of blood pressure control on nephropathy progression, defined as serum creatinine >3 mg/dL (61 events) or ESKD (158 events).

Methods: We compared APOL1 and MYH9 genotypes in 675 AASK subjects and 618 African American population controls. The control group, of whom 43% had hypertension, was recruited in southeastern USA and lacked a personal or family history of kidney disease. Mean African admixture was 0.89 in both cases and controls.

Results: APOL1 risk allele frequencies (G1 and G2) were 23.2% in cases compared to 11.6% in controls, OR 2.31, P= 7.1X10E-8. After adjusting for age and gender, the OR associated with having two risk alleles were as follows: for hypertensive kidney disease (all AASK cases) versus all controls, 2.3 (95% CI 1.7, 3.1), P = 6 X10E-8); for hypertensive kidney disease (all AASK cases) versus hypertensive controls, 2.2 (1.4, 3.7), P<0.002; for AASK cases with baseline urine protein/creatinine ratio >0.2 g/g vs controls, 4.3 (3.0, 6.2), P=1X10E-14; and for AASK cases with progression versus controls, 4.1 (2.9, 5.9), P= 1.8X10E-14. After adjusting for APOL1, the presence of two MYH9 E1 haplotype copies

was associated with OR 1.7 (1.1, 2.4), P=0.01 for progression. There was no interaction between APOL1 risk allele status and ACE inhibitor use with regard to progression.

Conclusions: APOL1 risk allele prevalence is elevated among AASK participants with hypertension-attributed kidney disease. Two APOL1 risk alleles markedly increase the risk of kidney disease progression.

Funding: NIDDK Support, Other NIH Support - NCI

FR-PO1493

The Use of Homozygosity Mapping To Identify the Responsible Gene for Diffuse Mesangial Sclerosis, an Entity with Genetic Heterogeneity Yaacov Frishberg, Efrat Ben Shalom, Rachel Becker-Cohen, Choni Rinat, Sofia Feinstein, Ruth Belostotsky. *Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel.*

Background: Many hereditary kidney diseases display genetic heterogeneity manifesting as a common phenotype derived from mutations in a number of different genes. Diffuse mesangial sclerosis (DMS), a histologic variant of steroid-resistant nephrotic syndrome (SRNS) in the pediatric age group represents an example of this phenomenon, with 4 genes (NPHS1, NPHS2, PLCE1 and WT1) responsible for a third of all cases. Given that these 4 genes contain together 79 exons, diagnostic sequencing is expensive, and remains ineffective in about 70% of all cases.

Methods: Detecting the genetic basis of the disease in patients from consanguineous or ethnically-related families can be accomplished by the implementation of homozygosity mapping based on SNP microarray analysis. Localization of homozygous genomic loci "identical-by-descent" allows to restrict the number of known genes which may be associated with the disease in particular cases.

Results: We performed homozygosity mapping in an attempt to study a consanguineous Arab family with 6 offspring affected with SRNS, 4 of whom succumbed to the disease. In one child, the histologic diagnosis of DMS was made when he already had ESRD. Homozygosity mapping revealed 8 homozygous regions of 10 to 2 Mbp in length, each. The only gene that has previously been associated with DMS or SRNS within these regions was PLCE1. Direct sequencing revealed the novel homozygous deletion c.4977_4982delCAGA leading to a frame shift p.Q1660LfsX9. The same mutation was later detected in an unrelated family of the same ethnic background with two affected children, pointing to a founder effect.

Conclusions: This approach is economically sound when the total number of exons of the potentially relevant genes exceeds 40-50. We recommend the implementation of homozygosity mapping in complex consanguineous families affected by diseases characterized by genetic heterogeneity.

Funding: Clinical Revenue Support

FR-PO1494

Glucocorticoid Receptor Sensitivity in Patients with Focal Segmental Glomerulosclerosis Nataliya Chorny,[1] Steven Ghanny,[3] Shella Mongia,[2] Amrit Bhangoo,[3] Anil K. Mongia.[1] *[1]Peds Nephrology, SUNY, Brooklyn, NY; [2]Peds Endocrinology, SUNY, Brooklyn, NY; [3]Pathology, SUNY, Brooklyn, NY.*

Background: Glucocorticoids are the primary therapy used to treat FSGS though neither the target cell nor their mechanism of action in FSGS are known.

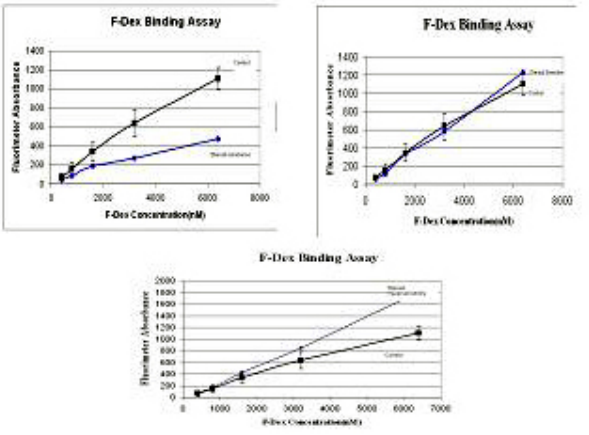
The aim of this study was to compile the clinical data, NPHS2 genes mutation and Glucocorticoid Receptor sensitivity in our patients with FSGS.

Methods: We evaluated 14 patients with FSGS. There were 4 Hispanics and 10 African Americans. 4 had ESRD. 11 patients were evaluated for NPHS2 mutations. Monocytes Glucocorticoid receptors sensitivity was studied in 5 patients. For monocytes testing cell suspension was centrifuged, resuspended and incubated. The next day the cells were reincubated at 37oC for 1 hour with F-Dex in concentration range from 400 to 6400nm. After washing, the absorbance was detected by Microplate Fluorometer Series 7600. Clinical data were compared with GR sensitivity results. NPHS2 mutation sequencing is in progress. IRB approved the study.

Results: 2 patients with no responce to Steroids and Immunosupression, who progressed to ESRD had Hypersensitive GR response. One patient who is steroid dependent had normal GR sensitivity.

Clinical features and GR sensitivity

Patient Ethnicity	Immune deposits	Response to Steroid	Response to Prograf/ Cellcept	ACE Tx	ESRD	GR sensitivity
Hisp	+	Steroid dependent	+	+	-	Normal
Hisp	+	-	-	-	+	Hyper
AA	+	-	-	no	+	Hyper
AA	+	never Tx	never Tx	+	-	Normal
AA	+	never Tx	never Tx	+	-	Resistant



Conclusions: We conclude that GR sensitivity testing may provide predictive value prior to deciding on starting steroid treatment and dose of steroids.

Funding: Private Foundation Support

FR-PO1495

Replication and Validation of MYH9/APOL1 Chronic Kidney Disease Risk Alleles in an Urban Tertiary Care Center: Implications for Personalized Medicine Omri Gottesman, Marie Teil, Vaneet Lotay, Rajiv Nadukuru, Bernadette Liggayu, Kash Patel, Stephen B. Ellis, Erwin P. Bottinger. *Charles R. Bronfman Institute for Personalized Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Genetic variants in a region of chromosome 22 have consistently associated with nephropathy in African Americans and have been replicated in other populations. These have potential utility for genomic prediction of CKD risk. The Institute for Personalized Medicine Biobank is an electronic medical record-linked biorepository with up to 20,000 participants. Self-reported populations include ~40% Hispanic Americans (HA), ~30% African Americans (AA) and ~25% European Americans (EA). The prevalence of CKD stage 3 or higher (KDOQI definition) is ~15%.

Purpose: To replicate previous genotype-phenotype associations and validate risk loci in our heterogeneous local population prior to potential clinical decision support implementation.

Methods: We are directly genotyping all Biobank participants for 12 SNPs in the APOL1/MYH9 region that have been associated with CKD. Preliminary genotyping was undertaken on 2052 participants (900 cases (≥CKD3), 1152 controls). Logistic regression analysis was performed in each population for CKD and CKD +/- diabetes using age, sex, age^2 and the first two principal components (from genome-wide data) as covariates. Levels of statistical significance were determined by the Bonferroni correction or at $p<0.05$ for previously replicated loci.

Results: We have demonstrated that previously validated risk variants can be replicated within our local populations. In particular, the G1 alleles of APOL1 (rs60910145, rs73885319) are strongly associated with non-diabetic CKD in our AA population (OR's 2.6 and 3.8 respectively, $p<0.01$) with a recessive model of inheritance, consistent with previous reports. We also replicated a previous association of an MYH9 E1 allele (rs4821480) with non-diabetic CKD in our HA (OR 2.43, $p<0.05$).

Conclusions: We anticipate that extended results from ~5000 AA and ~7000 HA patients will provide a compelling rationale for future studies to evaluate the impact of genomic prediction tools in the management of modifiable CKD risk factors.

FR-PO1496

A Combined Deletion of CFHR1 and CFHR3 Conveys Protection Against IgA Nephropathy Jingyuan Xie,[1,2] Krzysztof Kiryluk,[2] Yifu Li,[2] Ping Hou,[3] Simone Sanna-Cherchi,[2] Zhaohui Wang,[1] Weiming Wang,[1] Hong Zhang,[3] Nan Chen,[1] Ali G. Gharavi.[2] *[1]Renal Department, Shanghai Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China; [2]Division of Nephrology, Columbia University, New York City, NY; [3]Renal Division, Peiking University First Hospital, Institute of Nephrology, Peiking, China.*

Background: In a recent GWAS, we detected a major susceptibility locus for IgAN within the complement factor H (CFH) gene cluster on chr.1q32. The top SNP, rs6677604, tags a common deletion of CFHR1 and CFHR3 genes (CFHR1,3Δ). The goal of this study was (1) to directly type CFHR1,3Δ to determine whether it accounts for the association a the CFH locus, and (2) to identify additional rare CNVs in this region.

Methods: We studied a total of 1,929 biopsy-diagnosed primary IgAN cases and 1,652 healthy controls of Chinese ancestry. The CFHR1 and CFHR3 region was screened with multiplex ligation-dependent probe amplification (MLPA) and quantitative PCR. Association analyses were performed with PLINK v1.07 and UNPHASED 3.1.3.

Results: The CFHR1,3Δ and rs6677604 variants were in strong LD (r2=0.90, D'=0.98) and both had a strongly protective effect on IgAN (rs6677604 OR=0.61, p=1*10-6; CFHR1,3Δ OR=0.58, p=2*10-7). After conditioning on rs6677604, CFHR1,3Δ had an independent protective effect (OR=0.51, 95%CI: 0.26-1.00, p=0.05). In contrast, after conditioning on CFHR1,3Δ, rs6677604 was no longer significant (95%CI: 0.59-2.17, P=0.7). We also identified 3 rare single-gene CNVs (CFHR3Δ, CFHR1Δ and CFHR1 duplication, freq=0.8-2%), but these CNV's were not associated with risk of IgAN.

Conclusions: The CFHR1,3Δ variant explains the rs6677604 association signal, strongly suggesting that this deletion is the functional allele at the CFH locus. The absence of association of single-gene CNV's suggests a synergistic effect of the CFHR1 & CFHR3 deletions on the alternative complement pathway.

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FR-PO1497

Genome-Wide Linkage Scan of Japanese Families with IgA Nephropathy

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Background: A genetic predisposition of IgA nephropathy (IgAN) has been suggested by the familial clustering of the disease. Previously, genome-wide linkage analysis of IgAN have revealed several susceptibility loci including 6q22-23 (IGAN1), 4q26-31, 17q12-22, and 2q36. However, no causative gene mutations underlying these linkage loci has been identified. From the point of view of genetic heterogeneity of familial IgAN, oligo/polygenic and multiple susceptibility gene model for the disease are proposed.

Methods: In this study, we investigated 10 Japanese multiplex families in which multiple members are affected by biopsy proven IgAN. A total of 62 subjects (24 affected) were genotyped using Genome-Wide Human SNP Array 6.0. Multipoint linkage analysis was performed using SNP HitLink, which is a program providing a useful pipeline to directly connect SNP data and linkage analysis program, enabling a high-throughput analysis.

Results: The genotypes of each sample were determined with a high overall call rate of over 99.5%. Parametric analysis with assumption of an autosomal dominant mode of inheritance, with estimated penetrance of 75%, yielded maximum heterogeneity LOD score of 1.84 on 1p36. By nonparametric analysis, eight regions of potentially interesting level of nonparametric LOD (NPL > 3.0) were identified including 1p36 (NPL score 3.88).

Conclusions: These results provide a support for genetic heterogeneity among families with IgAN, and novel candidate loci responsible for familial IgAN in Japan.

FR-PO1498

Coding Polymorphisms of Interleukin-22 Receptor Alpha-1 Contributing to the Development of Childhood IgA Nephropathy in Korean Population

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Background: Interleukin (IL)-22 is a member of the IL-10 cytokine family and represents an important effector molecule of activated immune cells. It mediates its effects via two receptors, IL-22R1 and IL-10R2 and subsequent Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathways. Because of the restricted expression of IL-22R1, the main receptor of IL-22, its target cells are not immune cells but certain tissue cells including kidney. In the present study, we investigated the associations between polymorphisms of *IL-22R1 and IL-10R2* and childhood IgA nephropathy in Korean children.

Methods: We evaluated 194 pediatric patients with biopsy-proven IgAN and 287 healthy controls. Two single nucleotide polymorphisms (SNPs) in the coding region of the IL-22R1 gene [rs rs3795299 (missense, Arg518Gly) and rs10903022 (synonymous, Pro45Pro)], and one SNP in the IL-10R2 gene [rs2834167 (missense, Lys47Glu)] were selected and genotyped by direct sequencing methods.

Results: Our case-control analysis showed that genotypes of rs3795299 were associated with childhood IgAN in the codominant model [p=0.0028, OR(95% CI)=1.33 (0.91-1.95)] and in the recessive model [p=0.002, OR(95% CI)=0.28(0.11-0.69)]. After Bonferroni correction, this association of rs3795299 with IgAN risk remained significant.

Conclusions: Polymorphisms in IL22R1 may be associated with the development of childhood IgAN. Replication and functional studies are involved to better understand themechanism by which these polymorphisms may contribute to the pathogenesis of IgAN.

FR-PO1499

APOL1 Genetic Variants Significantly Increase Susceptibility to Focal Segmental Glomerulosclerosis and HIV-1 Associated Nephropathy but Not IgA Nephropathy in African Americans

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Background: A chromosome 22q13 locus, encompassing the MYH9 and APOL1 genes, has been strongly associated with increased risk of idiopathic focal segmental glomerulosclerosis (FSGS), HIV-1-associated nephropathy (HIVAN) and hypertensive end-stage kidney failure among individuals of African descent.

Methods: In this replication study, we examined the six top-most associated variants in *APOL1* and *MYH9* in an independent cohort of African-Americans with various nephropathies [FSGS (N=44), HIVAN (N=21), IgA nephropathy, (IgAN, N=32), vs. healthy controls (N=74)].To assess the role of MYH9 deficiency in nephropathy, we examined M*yh9* haploinsufficient mice (*Myh9*$^{+/-}$) and crossbred them with HIV-1 transgenic mice.

Results: All six variants were associated with FSGS and HIVAN (additive ORs 1.8-3.0, P-values 3 x 10-2 – 5 x 10-5), but not with IgAN. In conditional and haplotype analyses, two APOL1 haplotypes (tagged by rs60910145 and rs71785313) accounted for virtually all the association with FSGS and HIVAN on Chr. 22q13 (haplotype P-value = 5.6 x 10-8). Myh9+/- mice are healthy and devoid of proteinuria or nephropathy, irrespective of the presence of the HIV-1 transgene.

Conclusions: These data further support the strong association of genetic variants in *APOL1* with susceptibility to FSGS and HIVAN among African-Americans.

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FR-PO1500

Genome-Wide Association Study Identified Multiple Susceptibility Loci for IgA Nephropathy in Chinese Population

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Background: IgA nephropathy (IgAN) is the most common primary glomerulonephritis among patients undergoing renal biopsy and has a higher prevalence in Asian than western countries. There are multiple lines of evidence for the involvement of genetic risk factors, but only a few susceptibility genes have been identified.

Methods: Two-stage genome-wide association study (GWAS) was performed in the Chinese population, where the genome-wide discovery analysis was done in 1434 cases and 4270 controls by using the Illumina 660K SNP chips, and the replication analysis of the top 60 SNPs from GWAS was done in an additional 2703 cases and 3464 controls by using the iPLEX system and TaqMan assay.

Results: We found the most significant signal is in the MHC region on Chr.6 (P=4.92×10-21, OR=1.35), where several HLA alleles and haplotypes were implicated. Also, we discovered other two independent loci with genome-wide significance associated with IgAN, where multiple genes within these loci involved in the immune response. Furthermore, we confirmed the locus 22q12 of previous study (P=1.17×10-11, OR=0.78). Our study did not yield any supporting evidence for the association on 1q32 locus reported by the previous study (rs6677604, P=0.52, OR=0.94), although our discovery sample has sufficient power for detecting the association (power=0.86 at a significance of 0.01).

Conclusions: By identifying several susceptibility loci, our study may reveal the new insight into the biological variability in the development of IgAN.

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FR-PO1501

APOL1 and MYH9 Genetic Variants Are Independently Associated with Kidney Disease Risk in African Americans

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Background: Admixture mapping identified a locus centered on MYH9 as a risk locus for focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN), and non-diabetic hypertension-attributed kidney disease in African Americans (AAs). Although MYH9, encoding myosin heavy chain 9, was a plausible candidate gene, a subsequent GWAS for FSGS, and results of the 1000 Genome project, revealed that coding variants of APOL1 accounted for the chromosome 22 kidney disease susceptibility; regression analysis suggested that all of the increased risk was due to two APOL1 mutations.

Methods: We re-examined this question using additional AA cases and controls. First, 271 FSGS and HIVAN cases and 939 controls were studied to determine the effect of MYH9 in each of six possible APOL1 genotype combinations in a stratified analysis. Second, 885 non-diabetic ESRD cases and 1259 population controls recruited at Wake Forest were evaluated, adjusting for APOL1 G1/G2 alleles.

Results: In the FSGS/HIVAN cohort, the risk-associated MYH9 SNP rs2413396 was tested by Fisher exact test and reached significance for two APOL1 genotypes (G1 = AA, G2 = DI, p = 0.006; G1 = AG, G2 = DI, p = 0.04). Considering all AA subjects in an analysis stratified on the APOL1 genotype, the MYH9 SNP was associated with renal disease (p=0.006, odds ratio 2.0, 95% confidence interval 1.4, 3.1). In the hypertension-attributed ESKD cohort, residual MYH9 effects were detected for three E1 haplotype SNPs under a recessive model: rs4821480 (P=0.029, OR=1.26), rs2032487 (P=0.036, OR=1.32), and rs4821481 (P=.012, OR=1.40).

Conclusions: These results suggest that additional independent loci within the MYH9/APOL1 extended linkage disequilibrium region on chromosome 22 are associated with renal disease.

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FR-PO1502

Global Histone 3 Lysine 27 Trimethylation Status of Neutrophils and RUNX3 Expression in Anti-Neutrophil Cytoplasmic Autoantibody Vasculitis Kerry R. Colby, Meghan E. Free, Jia Jin Yang, Donna O. Bunch, Gloria A. Preston, J. Charles Jennette, Ronald J. Falk, Dominic J. Ciavatta. *UNC Kidney Center, University of North Carolina at Chapel Hill, NC.*

Background: The genes encoding two anti-neutrophil cytoplasmic autoantibody (ANCA) autoantigens, proteinase 3 (PR3) and myeloperoxidase (MPO), are aberrantly expressed in neutrophils of patients with ANCA disease. We have shown that both *PR3* and *MPO* genes in neutrophils of patients with ANCA disease, as compared to healthy controls, have decreased levels of histone H3 lysine 27 trimethylation (H3K27me3), a histone modification associated with epigenetic gene silencing at the *MPO* and *PR3* loci. This epigenetic difference may be a consequence of active demethylation via a H3K27me3 specific demethylase. Differences in H3K27me3 levels could also result from a failure to recruit the H3K27 methyltransferase. We have described a mechanism whereby RUNX3 recruits the H3K27 methyltransferase to *MPO* and *PR3* loci, and we have shown a decrease in the amount of *RUNX3* mRNA expression in total blood leukocytes of ANCA patients as compared to controls.

Methods: Total blood leukocytes were labeled with cell surface markers. Cells were incubated with primary antibodies recognizing either UTX, H3K27me3, or RUNX3. Levels of H3K27me3 and amount and distribution of UTX and RUNX3 among leukocytes were determined by flow cytometric analysis.

Results: Preliminary data show global H3K27me3 levels are reduced in patients with ANCA disease as compared to controls. We identified some patients with active ANCA disease that have increased UTX protein in subset of cells. Finally, our preliminary results show RUNX3 protein is decreased in monocytes of patients with ANCA disease as compared to healthy controls.

Conclusions: The global deficit in H3K27me3 may underlie altered expression of multiple neutrophil genes. This deficit could be due to both active demethylation in patients and the failure to recruit methyltransferases. These fundamental analyses to identify the molecular differences in peripheral blood cells between ANCA patients and healthy controls are crucial to understanding disease mechanisms of ANCA vasculitis.

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FR-PO1503

Common Serum Urate – Associated Genetic Variants in over 100,000 Individuals Are Associated with Gout and Renal Urate Excretion Anna Kottgen,[1,10] Eva Albrecht,[2] Alexander Teumer,[3] Veronique I. Vitart,[4] Claudia Hundertmark,[1] Giorgio Pistis,[5] Daniela Ruggiero,[6] Toomas Haller,[12] Toshiko Tanaka,[7] Qiong Yang,[8] Murielle Bochud,[9] Wen Hong Linda Kao,[10] Caroline S. Fox,[11] Christian Gieger.[2] *[1]Freiburg University; [2]Helmhotz Center Munich, ; [3]Greifswald University, ; [4]MRC Human Genetics Unit, Edinburgh; [5]San Raffaele Scientific Institute; [6]IGB-ABT, CNR, Naples; [7]National Institute on Aging, Baltimore; [8]Boston University; [9]Lausanne University; [10]Johns Hopkins University; [11]National Heart, Lung, and Blood Institute; [12]University of Tartu.*

Background: Using genome-wide association studies and meta-analysis among 110,238 population-based European ancestry study participants, we identified 26 independent genomic loci, 16 of them novel, associated with serum urate concentrations ($p<5*10-8$).

Methods: To quantify associations with gout risk and highlight physiological mechanisms underlying the serum urate association, the single nucleotide polymorphism (SNP) with the strongest association at each genomic locus was related to gout among 73,236 participants (2,942 cases). Urate excretion was assessed as fractional excretion of urate (FEUA, n=3087), calculated from serum and urinary urate and creatinine.

Results: The urate-increasing allele at each of the 26 SNPs also associated with increased gout risk. The odds ratios for 15 SNPs associated at $p<0.05$ with gout ranged from 1.08 (rs675209 near RREB1, p=0.04) to 1.71 (rs2231142, ABCG2, p=10-32) per copy of the allele associated with higher urate levels, including novel loci such as near NFAT5 (OR=1.10). Association with FEUA ($p<0.05$) was observed for 7 SNPs, with the strongest association for rs12498742 in SLC2A9 (lowering the median FEUA of ~4% by 0.2% per urate increasing allele, p=10-7). For each of these SNPs including the one near NFAT5, the urate increasing allele was associated with lower FEUA, consistent with lower renal urate excretion.

Conclusions: Genome-wide association studies of serum urate identify novel genomic risk loci for gout. Association with FEUA suggest that some, but not all, SNPs may be associated with higher serum urate levels by altering renal urate excretion.

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FR-PO1504

Association of SLC2A9 with Serum Uric Acid and Renal Phenotypes in Zuni Indians V. Saroja Voruganti,[1] Sandra L. Laston,[1] Karin Haack,[1] Jean W. Maccluer,[1] Shelley A. Cole,[1] Vallabh O. Shah,[2] Arlene Bobelu,[2] Jeanette Bobelu,[2] Antonia M. Harford,[2] S. Paine,[3] Philip Zager,[2,3] Anthony Comuzzie.[1] *[1]Genetics, Texas Biomedical Research Institute, San Antonio, TX; [2]University of New Mexico, Albuquerque, NM; [3]Dialysis Clinic Inc, Albuquerque, NM.*

Background: Elevated serum uric acid (UA) levels are associated with gout, metabolic syndrome, heart and renal disease. UA levels are heritable in Caucasian, African American and Asian populations. Genome-wide association studies (GWAS) have demonstrated an association of SNPs in the solute carrier protein 2 family, member 9 (SLC2A9) gene with UA. The Zuni Indians are a small, relatively endogamous, tribe in NM.

Methods: We studied 1016 members of extended families who participated in the NIH funded, Genetics of Kidney Disease in Zuni Indians (GKDZI) study. The GKDZI seeks to identify genetic factors, which modulate susceptibility to renal disease and intermediate phenotypes. We conducted a GWAS using the Illumina Human1M-Duo v3.0 BeadChips, read on the Illumina BeadStation 500GX and analyzed with Genome Studio software. We used a linear regression-based association test under an additive model of allelic effect. We accounted for the non-independence of family members using a kinship variance component. Analyses were done in SOLAR.

Results: There was strong heritability of UA levels ($h2 = 0.32 \pm 0.07$) ($p <0.001$) and strong association of UA levels with solute carrier family 2 (facilitated glucose transporter), member 9 (SLC2A9) SNPs, rs6449213, rs938555, rs16890979, rs12499857, rs734553 and rs6832439 ($p < 10-08$). SLC2A9 encodes a UA transporter that mediates renal urate flux from proximal tubules. Minor allele frequencies of SNPs ranged from 32 to 49% and the mean effect sizes ranged from 3.6 to 4.3%. All SNPs except rs6449213 and rs12499857 were associated with higher UA levels. There were associations of SLC2A9 SNPs with urine albumin-creatinine ratio, serum creatinine and glomerular filtration rate (eGFR) ($p < 0.05$).

Conclusions: The strong associations of SLC2A9 variants with UA in Zuni Indians replicate findings in other populations. The association of SLC2A9 with renal phenotypes is a novel finding.

Funding: NIDDK Support

FR-PO1505

Genetic Correlation between Urinary Calcium and Heritable Mandibular/Maxillary Hypoplasia in a Congenic Rat Line Krista L. Lewandowski,[1] Guy M.L. Perry,[1] Robert J. Reid,[1] Jyotirmoy Nandi,[1] David A. Bushinsky,[2] Steven J. Scheinman.[1] *[1]Medicine, SUNY Upstate Medical University, Syracuse, NY; [2]Medicine, University of Rochester, NY.*

Background: In a hypercalciuric congenic rat line derived from Genetic Hypercalciuric Stone-forming rat, we detected a de novo mutation (*pug*) with marked facial abnormalities (Figure 1), including: i) fusion of the coronal and sagittal sutures and ii) hypoplasia of the mandible, premaxilla and maxilla. These features have sometimes been associated with craniosynostosis and elements of calcium physiology in humans. We investigated possible genetic associations between the penetrance of this new mutation and urinary calcium excretion in our pedigree.

Figure 1. Facial abnormalities seen in *pug* (below), versus normal (above) rats

Methods: The rat pedigree consisted of 1589 rats over 14 generations, including 170 F_2 *pug*-normal heterozygotes to test possible bias from assortative breeding. Weight at 8 weeks was measured, followed by 4 days of urine collection. The *pug* phenotype was scored as a binary trait by multiple observers.

Results: Heritability for all three traits was high and pug was highly genetically correlated with calcium excretion (Table 1). There was no difference in the correlation of urinary calcium with pug in F2 pug-normal hets compared to the complete population (ß = 0.25±0.0039; P<0.05).

Table 1. Additive and dominant heritabilities (diagonal), and genetic correlations (above diagonal) for pug, calcium excretion, and 8-week weight (95% CI).

Additive	***pug***	**8-wk. wt.**	**Ca**
pug	0.63 (0.04)	-0.10 (0.03)	0.96 (0.04)
Ca		0.61 (0.04)	0.17 (0.04)
8-wk. wt.			0.31 (0.05)
Dominant	***pug***	**8-wk. wt.**	**Ca**
pug	0.23 (0.02)	0.37 (0.01)	0.50 (0.01)
Ca		0.34 (0.02)	-0.21 (0.04)
8-wk. wt.			0.15 (0.02)

Conclusions: Our results suggest the genetic association or linkage of calcium excretion with *pug*. Such a link could be clinically important for the genetic basis of craniosynostosis, and for renal complications of this and related disorders.

Funding: Private Foundation Support

FR-PO1506

Next Generation Resequencing of 40 Candidate Genes for Calcium-Based Kidney Stones in 810 Subjects Identifies Novel Allelic Variants and Shows Association of Urinary Calcium Excretion with Claudin14

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Background: Nephrolithiasis is a major cause of morbidity and a complex, multi-factorial condition involving multiple genes along with environmental influences determining the likelihood of stone formation. Higher urinary calcium excretion is associated with increased risk and can be considered as a strong risk factor.

Methods: This study was designed to investigate the role of rare, functionally significant allelic variants affecting urinary calcium excretion and other relevant urinary solutes. N = 40 known candidate genes were sequenced in 810 participants recruited from the Nurses Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). Subjects were selected based on availability of validated phenotypical data and 24-hour urine collection data. Novel approaches were utilized which included barcoding and pooling strategies, target gene enrichment with RainDance technology and next generation sequencing.

Results: Most samples showed excellent sequencing data with deep coverage of 20x or more. Over 1600 potential SNPs were detected. Several strategies were applied to remove false positive variants. None of the novel variants showed statistically significant association between the high and the low urinary calcium group and were observed mostly in single subjects only. Analysis of more common, known variants suggested an association of Claudin14 (CLDN14) with low urinary calcium excretion. Although, this effect of CLDN14 was previously not observed in our GWAS study using samples from both, NHS and HPFS (unpublished data), an Icelandic study has previously shown association of CLDN14 with kidney stones.

Conclusions: Validation studies in larger sample sets will be necessary to confirm the association of CLDN14 with urinary calcium excretion. Further phenotypical analysis and functional studies of selected novel allelic variants identified in this study may shed light on molecular mechanisms leading to nephrolithiasis in a subset of patients in the general population.

Funding: Other NIH Support - Program Project

FR-PO1507

A Single Nucleotide Polymorphism in the Aqp11 Gene Is Associated with an Increased Risk for Chronic Kidney Disease in Patients with Diabetes

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Background: Aquaporin-11 is a novel aquaporin family member. Disruption of the murine Aqp11 gene causes severe proximal tubular injury and renal failure. A G662A single nucleotide polymorphism (SNP) in the human Aqp11 gene results in Gly102Ser substitution in a functionally important domain. The purpose of this study was to determine if individuals carrying the Aqp11 G662A SNP are at higher risk for developing chronic kidney disease (CKD).

Methods: This was a retrospective case control study. Patient data and DNA samples were obtained from the Vanderbilt DNA Databank (BioVU) and associated de-identified medical record. Caucasian patients greater than 18 years of age with exposure to intravenous hyperosmolar contrast by either ICD9 or CPT code were identified as potential study patients. Cases were defined as having AKI by creatinine elevation or ICD9 code. Control patients were those not having an AKI event. Covariates included age, gender and diabetes. Patients were defined as having CKD if the patient had at least 2 creatinine values separated by at least 90 days that were greater than or equal to 1.5mg/dl. Risk for either AKI or CKD was assessed as an odds ratio.

Results: Patients with diabetes carrying the SNP were at increased risk for any AKI event (OR 1.834; 95% CI 1.047-3.212; chi2 4.507; p=0.034) and for AKI within 7 days following contrast exposure (OR 1.412; 95% CI 0.599-3.329; chi2 0.623; p=0.435). An increased risk for any AKI was not observed in patients without diabetes (OR 1.087; 95% CI 0.836-1.414; chi2 1.081; p=0.582). There was a strongly significantly increased risk for CKD in patients with diabetes associated with the SNP (OR 2.778; 95% CI 1.254-6.152; chi2 6.620; p<0.02) that was not seen in non-diabetes CKD and control subpopulations.

Conclusions: Patients with diabetes and whom carry the Aqp11 SNP are at higher risk for an AKI event and for developing CKD. These data suggest the Gly102Ser substitution in the Aqp11 protein results in altered function and susceptibility to chronic renal injury in stress-induced conditions present in diabetes.

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FR-PO1508

Modeling Study of Human Chloride Channel 5 Mutations in Japanese Families with Dent's Disease Suggests a Structure-Function Relationship

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Background: Dent's disease, an X-linked renal tubular disorder characterized by low molecular weight proteinuria, hypercalcinuria, and nephrolithiasis is caused mainly by inactivating mutations in the human chloride channel 5 (hCLC-5) gene. Molecular models of the extracytoplasmic domain and transmembrane domain of the protein have been deduced from the crystal structures of two bacterial chloride channels. Additionally, the X-ray crystal structure of the cytoplasmic domain of hCLC-5 has been established, thereby allowing us to construct a model of this domain and examine the role of its mutations.

Methods: We examined 114 Japanese cases of Dent's disease and identified hCLC-5 mutations in 69 cases, among which 26 missense mutations were subjected to molecular modeling analysis.

Results: The locations of the mutated residues were distributed around the three structural sites: 1) around the chloride or proton conduction pathway (12 mutated residues), 2) the subunit interface that would be buried during dimer formation (7 mutations), 3) the cytoplasmic domain that would regulate transport by binding to ATP (4 mutations).

Conclusions: The missense mutations identified in Japanese families can be classified into three clusters according to the structural sites at which they occur, each playing an important role in the Cl^-/H^+ activity of CLC-5.

FR-PO1509

Sickle Cell Trait Is Not Associated with ESRD Susceptibility in African Americans Barry I. Freedman,[1] Pamela J. Hicks,[2] Carl D. Langefeld,[3] Lingyi Lu,[3] Anthony J. Bleyer,[1] Jasmin Divers,[2] Patrick H. Nachman,[4] Vimal K. Derebail,[4] Donald W. Bowden.[2] *[1]Department of Internal Medicine-Nephrology, Wake Forest School of Medicine; [2]Department of Bioochemistry, Wake Forest School of Medicine; [3]Department of Public Health Sciences, Wake Forest School of Medicine; [4]Department of Internal Medicine-Nephrology, University of North Carolina at Chapel Hill.*

Background: Conflicting reports exist as to whether sickle cell trait (HbAS) is a risk factor for development and progression of nephropathy. To determine whether African Americans with HbAS are at increased risk for nephropathy, genetic association was assessed between HbAS and end-stage renal disease (ESRD).

Methods: Hemoglobin S (HbS), non-muscle myosin heavy chain 9 (*MYH9*) and apolipoprotein L1 (*APOL1*) nephropathy risk variants were genotyped in 3258 unrelated African Americans; 1085 with non-diabetic ESRD, 996 with type 2 diabetes (T2D)-associated ESRD, and 1177 non-nephropathy controls. Interactions between *APOL1* and *MYH9* risk variants and HbS were assessed using case-only and case-control centered two-way logistic regression interaction analyses.

Results: HbS genotypes met Hardy Weinberg Equilibrium expectations in both cases and controls. HbAS genotype frequencies were 8.7% in non-diabetic ESRD cases, 7.1% in T2D-ESRD cases, and 7.2% in non-nephropathy controls. Age-, gender- and admixture-adjusted p-values for HbAS association with non-diabetic ESRD were p=0.34 (odds ratio [OR] 1.16; 95% confidence interval [CI] 0.85-1.60, dominant); p=0.96 for T2D-ESRD (OR 1.01; 95% CI 0.70-1.50, dominant); and p=0.74 for all-cause ESRD (combined non-diabetic and T2D-ESRD cases versus controls; OR 1.05; 95% CI 0.79-1.40, dominant). No evidence of APOL1 or MYH9 interactions with HbAS was detected.

Conclusions: Sickle cell trait was not associated with diabetic or non-diabetic etiologies of ESRD in a large sample of African American residing in the southeastern U.S. Sickle cell trait does not appear to predispose to progressive nephropathy.

Funding: NIDDK Support

FR-PO1510

Association of Caveolin-1 Single Nucleotide Polymorphism on Clinical Outcomes in Vasculits Sourabh Chand,[1] Peter Hewins,[2] Matthew David Morgan,[1] Lorraine Harper,[1] Richard Borrows.[2] *[1]Centre for Translational Inflammation Research, University of Birmingham Research Laboratories, Birmingham, West Midlands, United Kingdom; [2]Renal Department, University Hospital Birmingham NHS Foundation Trust, Birmingham, West Midlands, United Kingdom.*

Background: Caveolin-1 (CAV1) is an essential structural component of caveolae and has been implicated in the pathogenesis of fibrosis, cancer, vascular disease, and the response to sepsis; these phenomena are important in systemic vasculitis.

Our group have identified a single nucleotide polymorphism (SNP) within the CAV1 gene (rs4730751) to be associated with renal allograft failure and fibrosis (Moore et al. JAMA 2010). The purpose of this study was to investigate the role of this gene variant in systemic vasculitis.

Methods: DNA from a prevalent cohort of 195 Caucasian patients with ANCA associated vasculitis, was genotyped for SNP rs4730751 of CAV1 using the Taqman® method.

The primary composite endpoint of time to all-cause mortality/renal replacement therapy (RRT) was chosen. Secondary endpoints were time to RRT, all-cause mortality, infective death and cancer incidence. The association between genotype and outcomes was assessed by survival and regression analyses.

Results: Genotype distribution across the cohort met with the Hardy-Weinberg law. Patients with genotype AA (24), AC (74) and CC (97) were identified.

For the primary composite endpoint, survival analysis suggested a protective effect of the CC from non-CC genotype (p=0.004). A protective effect of CC was also seen for all-cause mortality and for infective death (p=0.05 and p=0.013 respectively), but not time to RRT (p=0.112).

A regression model (adjusted for gender, age at presentation, creatinine at presentation, type of ANCA and diagnosis) revealed an independent protective effect of CC genotype for RRT/all-cause mortality, infective death and cancer incidence.

Conclusions: *CAV1* SNP rs4730751 genotype CC appears to be protective in vasculitis for the composite endpoint of time to all-cause mortality and RRT, with some suggestion that it also influences death due to sepsis as well as cancer incidence. Further work is warranted to identify the underlying mechanism for these findings.

FR-PO1511

The Influence of Some Polymorphisms on Development of AA Amyloidosis Zuzana Potysova,[1] Romana Rysava,[1] Jitka Stekrova,[2] Vladimir Tesar.[1] *[1]Department of Nephrology, Charles University in Prague, First Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic; [2]Department of Genetics, Charles University in Prague, First Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic.*

Background: Available data suggest an association between presence of AA (secondary) amyloidosis and MCP-1 (monocyte chemoatracttant protein-1) and MIP-1α (macrophage inflammatory protein-1 alpha) genes polymorphisms and an impact of polymorphisms in exon 3 of SAA 1 (serum amyloid A 1) gene on the incidence of AA amyloidosis in different populations.

Methods: DNA and serum specimens of patients with AA amyloidosis (43), rheumatoid arthritis (RA) without amyloidosis and healthy control group (100) were investigated by using PCR, RFLP and ELISA methods. Kruskal-Wallis and χ-square tests were used for statistical data evaluation.

Results: Significantly more frequent occurrence of 1.1/1.1 genotype in SAA 1 was recorded in AA amyloidosis group compared to RA group as well as in control group ($p<0.001$). Distribution of neither 1.1/1.1 genotype nor another ones did not vary among RA and control group. No significant difference in distribution of another genes was recorded among all three groups. Serum concentration of SAA was statistically significantly higher in AA amyloidosis group and also in RA group compared to healthy controls ($p<0.001$). Serum concentration of MCP-1 was statistically significantly higher in AA amyloidosis group compared to RA group ($p<0.05$). Concentrations of MIP-1α were markedly higher in both groups of patients compared to healthy controls (borderline to statistical significance).

Conclusions: Homozygosity of the 1.1 haplotype in SAA1 gene could be a risk factor for development of AA amyloidosis in Caucasian population. Our unique findings of higher serum concentration of MCP-1 in the AA amyloidosis group compared to RA group could advert to riskiness of another factors. This could have therapeutic consequence – earlier and more assertive therapy of underlying diseases in patients with appropriate genotype in order to prevent or interfere with occurrence of AA amyloidosis.

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Funding: Government Support - Non-U.S.

FR-PO1512

Clinical Characteristics of *PKD2* Gene-Linked Families Characterized by the Same Germ-Line Mutation Valentina Corradi,[1,3] Fiorella Gastaldon,[1] Grazia Maria Virzi,[1,3] Armando Vazquez,[3] Manish Kaushik,[3] Dinna N. Cruz,[1,3] Maurizio Clementi,[2] Claudio Ronco.[1,3] *[1]Nephrology, St Bortolo Hosp, Italy; [2]Clinical Genetics and Pediatrics, University of Padova, Italy; [3]International Renal Research Institute Vicenza, Italy.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common inherited renal cystic disease. It is genetically heterogeneous (*PKD1* and *PKD2*) with a significant intra-familial variability. ADPKD is characterized by "private mutations": it is rare to identify the same germ-line mutation in different families. The aim of the study was to evaluate the clinical characteristics of *PKD2*gene-linked families characterized by the same germ-line mutation.

Methods: Patients (pts) with ADPKD, by ultrasound criteria, were enrolled and followed prospectively. Complete clinical details were recorded, including family history and time of ADPKD diagnosis (t0). We applied linkage analysis to identify the gene involved. We used microsatellite markers (STR) flanking the 2 genes. Furthermore, we performed sequencing to identify mutations in PKD2 families. The eGFR was calculated with the 4-variable standardized-MDRD equation. The progression of CKD was determined by the change in eGFR per year. Data were shown as median (min;max).

Results: We identified 8 PKD2gene-linked families characterized by the same disease haplotype and the same germ-line mutation (2533C>T) in all affected individuals (16 pts). At t0 their age was 33.5 (19;53) yrs and eGFR 78.5 (32;94) mL/min/1.73 m². After a median follow-up of 10.73 (0.86;32.25) yrs, eGFR was 70 (14;94) mL/min/1.73 m² . Hypertension was present in 87.5% of pts, DM in 6.25%, kidney stones in 37.5%, and hepatic cysts in 62.5%. The change in eGFR per year was -0.70 (-7.01;+2.15) mL/min/1.73m².

Conclusions: The identification of the same germ-line mutation in 16 pts belonging to 8 families indicate the presence of a common ancestral founder in our geografical area. We observed a considerable variability in CKD progression, within the same mutation. This could be explained by other clinical or genetic factors. We plan to analyze for other possible candidate genes that may be contributing to the observed variability in the ADPKD progression.

FR-PO1513

Gender-Based Randomization in Renal Phenotype Guy M.L. Perry,[1] Steven J. Scheinman,[1] John R. Asplin.[2] *[1]SUNY Upstate Medical University, Syracuse, NY; [2]Litholink Corporation, Inc., Chicago, IL.*

Background: Models of mean effects on renal physiology may not account for inherent residual variance. Our work in a rodent model of heritable hypercalciuria indicates the existence of sex-linked loci for residual variation in urinary calcium. Inherent residual variation would be of great significance, potentially assorting individuals randomly among diagnostic and prognostic categories and contributing type II error to medical genetic assays of renal disease.

Methods: Since sex is a common element in this phenomenon, we hypothesized the existence of gender differences in coefficients of variation (CV) for paired 24-hour creatinine (Cr)-corrected measurements of urinary calcium (Ca), oxalate (Ox), citrate (Cit), uric acid (UA), sodium (Na), potassium (K), magnesium (Mg), phosphorus (P), ammonium (NH), chloride (Cl), urea nitrogen (UN) in 6,830 females and 9,135 males collected by the Litholink Corporation, Inc.

Results: Females had significantly ($P<0.05$) higher CVs than males for Na, Mg, P, Cl, K and NH post-Bonferroni correction (Tab. 1).

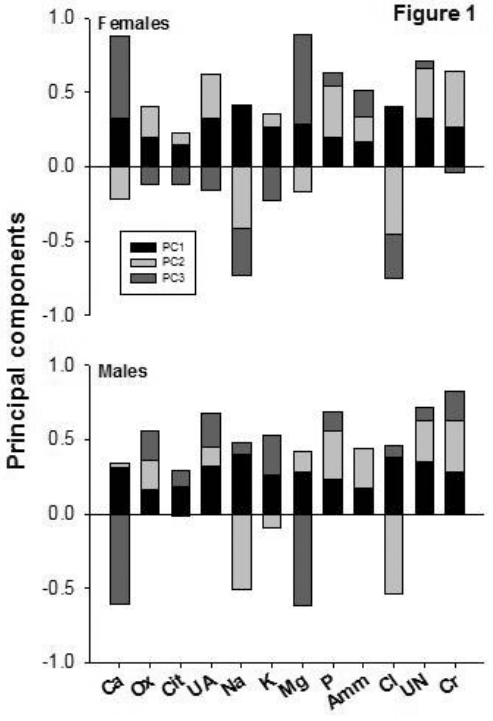
Table 1. Gender differences in mean coefficients of variation (CV) from paired 24-h urines in 6,830 females and 9,135 males.

Product	CV male (SE)	CV female (SE)	P
Na	0.19 (0.004)	0.18 (0.003)	0.001
Mg	0.16 (0.003)	0.15 (0.003)	0.001
P	0.14 (0.003)	0.13 (0.002)	0.001
Cl	0.19 (0.004)	0.18 (0.003)	0.001
K	0.13 (0.003)	0.13 (0.002)	0.008
NH	0.19 (0.004)	0.18 (0.003)	0.037

SE = standard error

There was no univariate effect of sex on Ca CV ($P>0.2$). Male and female loadings were similar over the first principal component ($\lambda_1=2.94$), but diverged over PC2 ($\lambda_2=1.55$) and PC3 ($\lambda_3=1.33$) largely for Ca, Mg, Na and Cl (Fig. 1)

Figure 1



Conclusions: Our findings support the hypothesis of sexual differences in residual variability in human renal phenotype. A full understanding of the genetics of urinary physiology needs to account for sexual and inherited variability and the factors underlying them.

Funding: Clinical Revenue Support

FR-PO1514

Role for Mammalian Target of Rapamycin in Mediating Expression and Activity of a Disintegrin and Metalloproteinase 17 in Diabetic Kidney Disease

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Background: Diabetic kidney disease (DKD) is characterized by extracellular matrix (ECM) accumulation. However, the mechanisms involved have not been completely identified. A disintegrin and metalloproteinase 17 (ADAM 17) cleaves growth factors involved in matrix accumulation. This study examined the role of ADAM 17 in matrix accumulation in the kidney cortex (KC) of a type 1 diabetic rat model.

Methods: Diabetes was induced in Sprague Dawley rats with streptozotocin and tissue was isolated at six weeks after the induction of diabetes for western blot analysis, immunoperoxidase staining, and a fluorometric-based assay for ADAM 17 activity . A subgroup of diabetic rats was treated with 1.0mg/kg of the mTORC1 inhibitor rapamycin, administered by intraperitoneal injection three times per week.

Results: MTOR phosphorylation on serine residue 2448 was enhanced in the KC of diabetic rats. ADAM 17 protein expression was significantly increased in the KC of type 1 diabetic rats by immunoperoxidase staining and western blot analyses. ADAM 17 enzyme activity in the KC and in proximal tubules (PT) isolated from the KC of the diabetic animals was also significantly increased suggesting a role for ADAM 17 in DKD. Collagen type IV expression was enhanced in the KC of diabetic animals. ADAM 17 protein expression, enzymatic activity, mTOR phosphorylation and collagen IV expression in the KC and in isolated PT were abolished with rapamycin treatment. In contrast to the findings in KC and in PT, the enzymatic activity of ADAM 17 was significantly decreased in isolated glomeruli of diabetic animals and was not affected by rapamycin treatment.

Conclusions: Collectively these data suggest a mechanism whereby mTORC1 mediates the activity and expression of ADAM 17 and collagen IV accumulation in the kidney cortex/proximal tubular kidney compartment. Moreover, the data suggest a differential role for ADAM 17 activity in kidney cortex and in proximal tubules as compared to the glomerular compartment.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1515

Reversibility of Microvascular Tortuosity after Simultanous Pancreas-Kidney Transplantation

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Background: Simultaneous pancreas-kidney transplantation (SPK) has been an advanced treatment option for type I diabetes patients with extensive microvascular (MV) disease and nephropathy. However, whether or not this treatment results in stabilization or even reversal of systemic diabetic MV disease is unknown, while such an effect may be relevant to the risk for future diabetic complications.

Methods: Mean capillary density and microvascular morphology were visualized using sidestream dark-field (SDF) imaging of the oral mucosa of diabetic nephropathy (DN; N=26) and SPK patients (N=38) and compared with healthy controls (N=20). In addition, 10 patients were studied longitudinally before (DN), 1 and 6 months after SPK. Furthermore circulating levels of growth factors that control MV structure, such as Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2) and Thrombomodulin (TM) were measured using ELISA.

Results: Both the cross-sectional as well as the longitudinal study showed no difference in capillary density. However, we found more capillary tortuosity in the DN group (mean 1.83 ±0.42 SEM vs 1,15 ±0.18 in controls) and normalization after SPK both in the cross-sectional study (1.31± 0.32, $p<0.05$) and after 6 months in the longitudinal study (1.53± 0.08, $p<0.05$). In line with these findings, markers of endothelial destabilization including Ang-2 and TM, were significantly increased in DN patients as compared to the SPK recipients in both studies and controls, while the Ang-1/Ang-2 ratio was normalized after SPK (from 0.16 ±0.04 to 0.08 ±0.02, $p<0.05$, controls 0.10 ±0.02).

Conclusions: SPK is effective in reversing the systemic MV derangements of type I diabetes patients with nephropathy.

Funding: Government Support - Non-U.S.

FR-PO1516

Netrin-1 a Promising Diagnostic Biomarker of Chronic Kidney Diseases Is Increased in Diabetic and Hypertensive Rats

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Background: Currently there are no validated early diagnostic biomarkers available for chronic kidney diseases (CKD). Microalbuminuria is insensitive and inaccurate for the diagnosis of CKD. Netrin-1 was recently identified as an early diagnostic biomarker of acute kidney injury. However, its usefulness for early diagnosis of CKD is unknown.

Methods: The current study evaluated whether netrin-1 is increased in urine from diabetic and salt sensitive hypertensive rats and correlated netrin-1 with development of nephropathy. Type 1 diabetes was induced using streptozotocin and salt sensitive hypertension was induced in uninephrectomized rats by implantation of a DOCA pellet with normal saline in drinking water. Netrin-1 and albumin concentrations in urine were measured by ELISA.

Results: Urinary protein excretion was not different between diabetic and control rats at 4 wks, but was increased in hypertensive rats (29 ± 7.6 vs. 25.1 ± 7.7 vs. 315.3 ± 51.8 mg/24h). By 10 weeks, urinary protein had increased in diabetic rats as compared to control rats (77.5 ± 13.4 vs. 32.3 ± 6.4 mg/24h). The urinary netrin-1 levels were significantly ($p<0.001$) higher in diabetic group at 4 (184±32 ng/24h urine) and 10 weeks (260±46 ng/24h urine) as compared to control group (15±1 ng/24h urine). Similarly, netrin-1 was increased significantly ($p<0.001$) in hypertensive Rat urine (141±20 ng/24h urine) at 4 weeks as compared to controls. To translate our animal findings into human disease, we analyzed urine samples from patients with different stages of diabetic nephropathy (normoalbuminuria, microalbuminuria, macroalbuminuria and renal dysfunction). Netrin-1 levels increased in a stage-dependent manner and were highest in patients with microalbuminuria and renal dysfunction.

Conclusions: In conclusion, netrin-1 can be detected in urine from diabetic and hypertensive rats and human diabetic patients and may serve as a useful diagnostic biomarker for CKD.

Funding: NIDDK Support

FR-PO1517

Nephronectin Is a Novel Protein Associated with Diabetic Nephropathy --Proteome Analysis of Isolated Glomeruli from Autopsy and Immunochemical Study Shinya Nakatani,[1] Eiji Ishimura,[1] Min Wei,[2] Katsuhito Mori,[1] Masaaki Inaba,[1] Hideki Wanibuchi.[2] *[1]Nephrology, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Metabolism, Endocrinology, and Molecular, Osaka City University Graduate School of Medicine, Osaka, Japan.*

Background: Proteome analysis of glomeruli of renal biopsy is difficult, since sufficient quantities of glomeruli are usually unavailable. Formalin-fixed paraffin-embedded (FFPE) kidney tissues of autopsies contain enough amounts of glomeuruli for proteome analysis. In order to identify a novel protein expression reflecting diabetic glomeruli, proteome analysis was performed, using FFPE kidney tissues of autopsy.

Methods: We conducted proteome analysis of lasermicrodissected glomeruli from FFPE kidney tissues of patients with diabetic nephropathy (n=10) and those of non-diabetic patients (n=10), using the isobaric tagging reagent iTRAQ, QSTAR Elite LC-MS/MS system, and Ingenuity Pathway Analysis (IPA). To validate the results of proteome analysis, we performed immunohistochemistry of 93 autopsies of type 2 diabetic patients.

Results: There were a total of 100 proteins that were differently expressed in glomeruli of diabetic patients, compared to those of non-diabetic patients. Based on the results of IPA, 31 renal and urological disease-related proteins were detected. Among them, nephronectin, an integrin α8β1 ligand which functions as assembly of extracellular matrix, was up-regulated in diabetic glomeruli (1.25 folds increase). Immunohistochemical analysis revealed that nephronectin was highly expressed in mesangial expansion and nodular glomerulosclerosis of diabetic patients, but not in glomeruli of non-diabetic patients. There was a significant positive correlation between glomerular sclerosis index and the percentages of nephronectin-positive glomeruli of diabetic patients ($r=0.89$, $p<0.0001$, $n=93$).

Conclusions: The present study demonstrated, for the first time, increased nephronectin expression in diabtic glomeruli, suggesting an important role of nephronectin in the development of diabtic glomerulosclerosis. Our study also showed that proteome analysis with FFPE kidney tissues is a useful tool for investigating glomerular disease.

FR-PO1518

Advanced Glycation End Products (AGEs) Activating Endoplasmic Reticulum Stress (ERS) Induces Renal Tubuloepithelial Cell Senescence Yani He, Jun Liu, Wei-Wei Zhang, Jurong Yang, BenGang Huo, Lirong Lin, Jun Zhan. *Department of Nephrology, Daping Hospital, the Third Military Medical University, Chongqing, China.*

Background: Renal tubuloepithelial cell (RTEC) senescence plays a critical role in the development of diabetic nephropathy(DN). Our study focuses on the role of ERS in RTEC senescence induced by AGEs in DN.

Methods: In vivo: Eighteen diabetic patients(60 to 75 yr)were classified into two groups:Early clinical DN(n=8) and advanced clinical DN (n=10), and renal carcinoma as normal control (n=10). All samples were subjected to examining SA-β-Gal of RTECs, also to immunohistochemical staining to analyze the expression of GRP78, ATF4, p16, p21. Double immunofluorescent staining was performed to study the expression of ATF4 and p16 or p21.

In vitro: RTECs were stimulated by AGE-BSA or ERS inducers tunicamycin(TM) or thapsigargin (TG) to stuy their influence on ERS and cell senescence. We assayed the ratio of SA-β-Gal and senescence-associated heterochromatic foci(SAHF).Cell proliferation and cell cycle were also examined by flow cytometry. The protein expression of GRP78, ATF4, p16, p21 was evaluated by western blot. Furthermore, the expression of p16 and GRP78 or ATF4 was assayed by double immunofluorescent staining.

Results: In vivo: We found GRP78, ATF4, p16, p21 and SA-β-Gal staining were significantly up-regulated in patients with DN ($P<0.01$). Immunofluorescent study showed that most ATF4 nuclear positive RTECs were co-stained with p16 or p21.

In vitro: The ratio of SA-β-Gal and SAHF, and the expression of GRP78, ATF4, p16, p21 were up-regulated by AGE-BSA with dose- and time-dependent manner, which associated with cell cycle arrest. Immunofluorescent staining showed that positive of SAHF in RTEC associated with elevated GRP78 or ATF4 expression. The ratio of SA-β-Gal and SAHF, and the expression of GRP78, ATF4, p16, p21 were also up-regulated by TM or TG with time- dependent manner. Furthermore, we found that GRP78 cytoplasm positive or ATF4 nuclear positive in RTECs were co-stained with p16.

Conclusions: Our study demonstrated that activation of ERS by AGEs could be a new pathophysiology mechanism of RTEC senescence in diabetic nephropathy.

FR-PO1519

Aggravation of Diabetic Nephropathy by Hyperlipidemia Is Mediated by MRP8/TLR4 Signaling in Macrophages Takashige Kuwabara,[1] Kiyoshi Mori,[1] Masashi Mukoyama,[1] Masato Kasahara,[1] Hideki Yokoi,[1] Yoko Saito,[1] Hirotaka Imamaki,[1] Tomoko Kawanishi,[1] Akira Ishii,[1] Kenichi Koga,[1] Keita P. Mori,[1] Yukiko Kato,[1] Akira Sugawara,[2] Kazuwa Nakao.[1] *[1]Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Nephrology, Osaka Red Cross Hospital, Osaka, Japan.*

Background: We have identified toll-like receptor 4 (TLR4) and myeloid-related protein 8 (MRP8) as commonly upregulated genes in glomeruli of insulin-dependent and -resistant diabetic mice using cDNA microarray. MRP8 is a high-affinity ligand for TLR4 that plays a key role in acceleration of inflammation mediated by TLR4. Since we have observed that high-fat diet (HFD) further enhanced glomerular expressions of these genes in streptozotocin (STZ)-induced diabetic mice along with doubling of albuminuria, we performed this study to clarify the role of MRP8/TLR4 signaling in worsening of diabetic nephropathy by hyperlipidemia (HL).

Methods: Diabetes was induced in wild-type (WT) and TLR4 knockout (KO) mice by ip injection of STZ. Normal diet was substituted with HFD at 2 weeks and all analyses were performed at 9 weeks after STZ.

Results: In diabetic WT and KO mice, addition of HFD similarly enhanced hypertriglyceridemia and renal lipid accumulation. Although HFD aggravated diabetic nephropathy in STZ WT mice, as indicated by marked increase in albuminuria, mesangial expansion, infiltration of macrophages (Mø) and upregulation of pro-inflammatory and pro-fibrotic genes in glomeruli and renal interstitium, and by decrease in glomerular podocin expression, synergistic effects of HFD upon renal lesions were almost completely abolished in STZ KO mice. MRP8 positive cells abundantly found in glomeruli of STZ-HFD-treated WT mice were mostly colocalized with Mø marker Mac2. In cultured bone marrow-derived Mø of WT mice, fatty acid enhanced MRP8 expression only under high-glucose conditions. Furthermore, phosphorylation of renal IRF3 in WT STZ mice was enhanced by HFD, suggesting the activation of TLR4-downstream, TRIF-dependent pathway.

Conclusions: These findings suggest that HL complicated with diabetes activates TLR4 on Mø, induces TLR4 ligand MRP8, enhances Mø infiltration and deteriorates glomerular lesions.

FR-PO1520

Deletion of *p47^{phox}* Reduces Progression of Diabetic Nephropathy in the Akita Mouse George Chu Liu,[1] Fei Fang,[1] Xiaohua Zhou,[1] Stuart Yang,[2] Heather N. Reich,[2] Rohan John,[3] Gavin Oudit,[4] James W. Scholey.[1] *[1]Institute of Medical Science, University of Toronto, ON, Canada; [2]Division of Nephrology, University of Toronto, ON, Canada; [3]Department of Pathology, University of Toronto, ON, Canada; [4]Division of Cardiology, University of Alberta, Edmonton, AB, Canada.*

Background: The role of NADPH oxidase-dependent superoxide generation in diabetic nephropathy has not been fully elucidated. Accordingly we examined the effect of deletion of the gene for the NADPH oxidase subunit, *p47^{phox}* on the progression of diabetic kidney injury in the Akita mouse ($Ins2^{WT/C96Y}$).

Methods: Four groups of mice were studied: non-diabetic *$Ins2^{WT/WT}$/p47$^{phox+/+}$* mice, non-diabetic *$Ins2^{WT/WT}$/p47phox$^{-/-}$* mice, diabetic *$Ins2^{WT/C96Y}$/p47$^{phox+/+}$* mice, and diabetic *$Ins2^{WT/C96Y}$/p47$^{phox-/-}$*. NADPH oxidase activity and gene expression levels were measured in isolated glomeruli at 8 weeks. Albumin excretion rates and kidney histomorphometry measures were assessed at 16 weeks of age. NADPH oxidase activity was examined in primary mesangial cell (MC), derived from $p47^{phox+/+}$ and $p47^{phox-/-}$ mice, and treated with 5.6mM glucose and 30mM glucose. The relationship between $p47^{phox}$ and collagen Iα1 mRNA expression was determined in kidney biopsies from normal and diabetic subjects.

Results: Deletion of the gene for p47phox reduced NADPH oxidase activity, superoxide generation, oxidative stress, and profibrotic gene expression in glomeruli from diabetic mice and led to a reduction in urinary albumin excretion, renal and glomerular hypertrophy, and mesangial matrix expansion in the diabetic mice. High glucose-induced NADPH oxidase activity and pro-fibrotic gene expression was attenuated in primary MC from mice with a deletion in the p47phox gene. There was a positive correlation between p47phox and collagen Iα1 mRNA levels in renal biopsy samples from control subjects and subjects with diabetic nephropathy.

Conclusions: Deletion of the gene for $p47^{phox}$ attenuates diabetic nephropathy in the Akita mouse, due in part to an effect on the mesangial cell response to high glucose.

Funding: Government Support - Non-U.S.

FR-PO1521

Aliskiren in Combination with Valsartan Improve Type 1 Diabetic Nephropathy in Mice Weidong Wang, Xiaoxin Wang, Nathaniel L. Solis, Liru Qiu, Hannah Danielle Santamaria, Moshe Levi. *School of Medicine, University of Colorado Denver, Aurora, CO.*

Background: The current study was undertaken to investigate if combination therapy with aliskiren, a direct renin inhibitor, with valsartan, an angiotensin type 2 receptor blocker, provides additive protective effects in 1 diabetic nephropathy in mice.

Methods: Insulin deficiency and hyperglycemia were induced with streptozotocin (STZ, 40mg/kg/day) injection in DBA/2J mice for five days. The mice were treated with either aliskiren (25 mg/kg/day), valsartan (8 mg/kg/day), or combined aliskiren and valsartan, for 4 weeks. Western blots, immunofluoresence and qPCR was used to examine protective effects of the treatment against diabetic nephropathy.

Results: Combined treatment with aliskiren and valsartan significantly attenuated albuminuria (125 ± 10 in controls, 209 ± 29 in diabetes, and 128 ± 20ug/mg in combination, $p<0.05$) and urine nephrin excretion (81 ± 16 in controls, 189 ± 23 in diabetes, and 112 ± 30ng/mg in combination, $p<0.05$). This was associated with prevention of the reduced protein abundance of podocyte markers nephrin, WT1, podocin, and synaptopodin in glomeruli of diabetic mice. The combination also markedly decreased 1) profibrotic growth factors: renal transforming growth factor-beta and PAI-1 expression, 2) proinflammatory cytokines: tumor necrosis factor-alpha, MCP-1, and CD68), and 3) neutral lipid (oil red o) accumulation. Single treatment with either aliskiren or valsartan provided marked, but less beneficial effects on all the above-mentioned parameters than combination.

Conclusions: The combination of aliskiren and valsartan therefore protects against diabetic kidney disease through multiple mechanisms, and seems to be a promising therapeutic strategy for diabetic nephropathy.

Funding: Pharmaceutical Company Support

FR-PO1522

Amelioration of Albuminuria and Tubulointerstitial Inflammation in STZ-Induced Diabetic TLR4-Deficient Mice Miao Lin, Wai Han Yiu, Hao-Jia Wu, Loretta Y.Y. Chan, Joseph C.K. Leung, Kar Neng Lai, Sydney C.W. Tang. *Medicine, Hong Kong University, Hong Kong, China.*

Background: We recently showed that tubular Toll-like receptor 4 (TLR4) expression was elevated in renal biopsies of histologically proven diabetic nephropathy (DN) and correlated with interstitial macrophage infiltration and HbA1c level. But the role of TLR4 in DN remains speculative. This study aims to study the role of TLR4 by using STZ-induced diabetic TLR4-deficient mice.

Methods: TLR4-/- mice and their wild type littermates (TLR4+/+) on C57BL6 background at 10-12 weeks old underwent uninephrectomy (Unx) or sham operation, and were then rendered diabetic by intraperitoneal injections of STZ. In vitro, the effect of an anti-TLR4 neutralizing antibody on high glucose (HG)-induced human proximal tubular epithelial cell (PTEC) inflammation was examined.

Results: At 12 weeks of diabetes, TLR4-/- mice demonstrated significantly ameliorated albuminuria and serum creatinine independent of blood glucose levels. This functional improvement was accompanied by substantially decreased F4/80+ macrophage infiltration into the tubulointerstitium and downregulation of cortical CCL-2, ICAM-1, and IL-1beta expression. At the signaling level, tubulointerstitial phosphorylated NF-κB/p65 activation was reduced in diabetic TLR4 -/- vs WT mice. The role of tubular TLR4 was confirmed in vitro. Pretreatment of PTEC with an anti-TLR4 neutralizing antibody significantly attenuated HG-induced NF-κB nuclear translocation and the downstream CCL-2 over-expression.

Conclusions: Our data suggest a pathogenic role of TLR4 in tubulointerstitial inflammation of diabetic kidney injury, and TLR4 may be a promising therapeutic target for further investigation.

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FR-PO1523

Podocyte Specific Inducible Overexpression of Angiopoietin-1 Ameliorates Albuminuria in Diabetic Tansgenic Mice Luigi Gnudi,[1] Cecile Dessapt-Baradez,[1] Kathryn E. White,[2] Anthea Elaine Hayward,[1] Adrian S. Woolf,[4] David A. Long.[3] *[1]Cardiovascular Division, Kings College London, London, United Kingdom; [2]Biomedical Electron Microscopy Unit, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; [3]Nephro Urology Unit, Institute of Child Health, University College London, London, United Kingdom; [4]Biomedical Research Centre, Medical School, Manchester University, Manchester, United Kingdom.*

Background: Diabetic glomerulopathy is characterised by increased glomerular angiopoietin (Ang)-2 and reduced Ang-1 levels. Moreover, we showed that targeted glomerular Ang-2 overexpression increases albuminuria in non-diabetic mice. Ang-2 is a natural antagonist of Ang-1 by competitively inhibiting its binding to the Tie-2 receptor. We hypothesised that increasing Ang-1/Ang-2 balance *via* podocyte-specific Ang-1 overexpression would ameliorate diabetic glomerulopathy.

Methods: We generated transgenic mice with inducible overexpression of Ang-1 specifically in podocytes (*Tet-on*). 5 week-old transgenic and control mice were rendered diabetic by daily intraperitoneal streptozotocin injections for 5 days. Eight weeks old mice with glycemia >22 mmol/l were randomised to receive either doxycycline (DOX; 2 mg/ml) or vehicle (VEH) in drinking water. 24 hour urine collections were performed at baseline, during 10 weeks of DOX or VEH administration and immediately before sacrifice. Tissue was collected for molecular determinations and electron microscopy. Urine albumin was measured by ELISA, and plasma and urine creatinine measured by mass spectrometry.

Results: Diabetes caused significant increases in albuminuria at 10 weeks compared with non-diabetic animals ($p<0.05$). Experimental Ang-1 induction significantly reduced but did not normalise diabetic albuminuria ($p<0.05$). Mesangial expansion and glomerular basement membrane (GBM) thickening was increased in diabetic mice compared with non-diabetic animals but was not ameliorated by Ang-1 overexpression.

Conclusions: Podocyte-specific Ang-1 treatment ameliorates albuminuria in experimental diabetes without affecting mesangial and GBM dysmorphology.

FR-PO1524

An In-Vitro Model of Hepatic Insulin Resistance in Uremia Via 11 beta Hydroxy-Steroid Dehydrogenase 1 (11HSD1) Ananda Chapagain, Julius Edward Kieswich, Steven Michael Harwood, Martin J. Raftery, Magdi Yaqoob. *Translational medicine and Therapeutics, WHRI, London, United Kingdom.*

Background: Insulin resistance (IR) is a major contributor to cardiovascular morbidity and mortality in uremic patients. The enzyme 11b-hydroxysteroid dehydrogenase type 1 (11bHSD1) catalyses intracellular conversion of the inactive glucocorticoid cortisone to active cortisol and promotes gluconeogenesis in the liver. We investigated the role of 11bHSD1in development of IR in uremia using an *in vitro* model of IR using H4IIEC3 cells.

Methods: Confluent cells were starved overnight,washed with warm PBS , then treated with 1.Earl's balanced salt solution (EBSS) 2.EBSS with 15% control serum (EBSS+SS) 3.EBSS with 15 % uremic serum (EBSS+US) and 4.EBSS with uremic serum treated with 10-5 M carbenoloxone (CBX,Sigma). (EBSS+US+CBX).All results were expressed as mM glucose produced by the cells. To assess gluconeogenesis, phosphoenolpyruvate carboxykinase (PCK1) and HSD1 mRNA was measured and PCK1 activity was measured using an in-house flurometric method.All experiments and measurements were performed in triplicate.

Results: H4IIEC3s showed a significantly high glucose production in EBSS+SS and EBSS+US, compared to EBSS alone or EBSS+US+CBX groups (Glucose production , EBSS alone, 0.0033 ± 0.0009 mM vs 0.018 ±0.02 for EBSS+SS , 0.0275 ± 0.008 mM for EBSS+US and 0.028 ±0.0013 mM, $p<0.001$ RM ANOVA). This was associated with an up-regulation of mRNA transcription for 11bHSD1 (10-fold) and PCK1 (20-fold), which normalized in response to CBX treatment. Furthermore, there was no difference in supernatant lactate measured in any of the treatment groups (mean 20 ± 2.10, P=NS). Incubation with IL-1b at 10,20,30 ng/ml and corticosterone at 0.1 and 0.5 micromolar concentrations yielded a dose-dependent increase in PCK1 activity and glucose production.

Conclusions: These results suggest that IR in uremia occurs partly through abnormally elevated GC-directed gluconeogenesis as a result of increased 11HSD. We also demonstrate a novel and easily reproducible *in vitro* model of IR in uremia.

Funding: Government Support - Non-U.S.

FR-PO1525

Intervention with JNK Blockade in the Early Phase of Type 1 Diabetic Nephropathy David J. Nikolic-Paterson,[1,2] Andy Lim,[1] Frank Yuanfang Ma,[1,2] Elyce Ozols,[1] Morag Young,[3] Brydon Bennett,[4] Glenn Friedman,[4] Gregory H. Tesch.[1,2] *[1]Nephrology, Monash Mediacl Centre, Clayton, Victoria, Australia; [2]Medicine, Monash University, Clayton, Victoria, Australia; [3]Prince Henry's Institute of Medical Research, Clayton, Victoria, Australia; [4]Celgene, San Diego, CA.*

Background: The c-Jun amino-terminal kinase (JNK) signalling pathway is activated in human kidney diseases, including diabetic nephropathy. Recent studies in animal models have shown that blockade of JNK signaling can suppress the development of renal injury in experimental glomerulonephritis and acutely reduce mean arterial pressure and vascular resistance in normal rats. The aim of this study was to determine whether JNK signalling plays a role in the development of diabetic nephropathy and in regulating hypertension, which exacerbates diabetic renal injury.

Methods: Diabetes was induced in spontaneously hypertensive rats (SHR) using streptozotocin. After 16 weeks of diabetes, rats with equivalent hyperglycaemia and albuminuria were randomised into groups which received no treatment, vehicle alone or a selective JNK inhibitor (CC-930, 60mg/kg/bid) for 10 weeks. These rats were assessed for hypertension and progression of renal damage.

Results: At week 16, diabetic rats showed increased kidney JNK activation compared with non-diabetic controls. Effective JNK inhibition was demonstrated at week 26 by reductions in c-Jun phosphorylation. CC-930 did not affect blood pressure, kidney hypertrophy, glomerular hyperfiltration, podocyte loss, glomerular fibrosis or tubulointerstitial injury in diabetic SHR. However, CC-930 reduced macrophages and ccl2 mRNA levels in diabetic kidneys. In contrast, CC-930 exacerbated albuminuria at week 26, which was associated with reduced glomerular mRNA levels of the podocyte-specific molecules, nephrin and podocin.

Conclusions: JNK inhibition does not prevent the progression of early diabetic renal injury in hypertensive rats, which contrasts with the ability of JNK inhibition to suppress albuminuria and injury in experimental glomerulonephritis.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1526

Serum and Urine Metabonomic Profiling Reveals the Metabolic Feature of db/db Mice with Diabetic Nephropathy Yongchun Ge,[1] Mengjie Li,[2] Jiye A,[2] Xufang Wang,[1] Guangji Wang,[2] Zhi-Hong Liu.[1] *[1]Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China; [2]Laboratory of Metabolomics, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, China.*

Background: Metabonomics is a systematic tool for quantitative measurement of metabolites and has the potential to identify novel markers that characterize the development of diabetic nephropathy (DN) and are involved in metabolic perturbation.

Methods: We utilized diabetic db/db mice (n=14) as a DN model and non-diabetic db/m mice (n=14) as normal control, and profiled their serum and urinary metabolites using GC/TOF-MS based metabonomic platform. Principal component analyses of the GC/TOFMS data revealed distinct metabonomic profiles of db/db mice and db/m controls.

Results: The identified discriminatory metabolites between db/db and db/m suggested a perturbed TCA cycle (malate, citrate, succinate, aconitate), lipid metabolism, glycolysis, urea cycle (urea, creatinine, glycine, alanine) and amino acids turn over. During the development of diabetes and DN (i.e., 6, 8, 10, 12 and 16 weeks of age), db/db showed clearly trajectory metabolic pattern of their scores plotsting away from the controls. The db/db mice were characterized with extremely high level of TCA intermediates at 6 weeks, followed by a sharp decline, and prominent elevation of free fatty acids in serum from 8 to16 weeks of age. The drop of TCA intermediate level in serum at 8 weeks of age (compared with 6 weeks) indicated insulin resistance and a marked down-regulation of glycolysis; In contrast, TCA intermediates in urine did not change accordingly. We have also found

serum lysine, and, to less extent, several other amino acids, were increased significantly at 8 weeks of age, in parallel with urinary albumin excretion.

Conclusions: In conclusion, diabetic db/db mice manifested a significant decrease of TCA intermediate and a significant increase of Lysine in serum at 8 weeks of age. Further studies would be important to determine if these changes also occur in DN patients.

FR-PO1527

Comparison of Renoprotective Effects of Alternative Medication Versus Enalapril Sudha Chennasamudram,[1] Shashi Kudugunti,[2] Majid Moridani,[2] Tetyana L. Vasylyeva.[1] *[1]Dept of Pediatrics, Texas Tech University Health Science Center, Amarillo, TX; [2]Dept of Pharmaceutical Sciences, Texas Tech Unviersity Health Sciences Center, Amarillo, TX.*

Background: To investigate the renoprotective effects of alternative medication, (+)-catechin (C) using *in vivo* and *in vitro* models of diabetes.

Methods: ***In vivo:*** Five groups of rats were used in the study that lasted 12 weeks. Group 1 and 2 were the non-diabetic rats (n=12/group) treated with and without C respectively. Group 3 and 4 (n=12/group) were diabetic rats treated with and without C respectively. Group 5 (n=5) were diabetic rats treated with ACEi, enalapril. The dose of C was 30 mg/day and ACEi was 10 mg/kg. Urine was collected and analyzed for albumin and endothelin-1 (ET-1). Plasma was collected and analyzed for creatinine. Protein expression of fibronectin in kidney was examined by western blotting. ***In vitro***: Effects of C in the formation of reactive oxygen species (ROS) in mesangial cells and apoptosis in endothelial cells were investigated under normal (5 mM) and high glucose (25 mM) by flow cytometry.

Results: ***In vivo:*** After 12 weeks of C treatment, urinary albumin excretion was reduced by 36% and the plasma creatinine by 40% compared to values in diabetic rats with no treatment. ET-1 in urine was also decreased by 56% with C treatment. Similar results were achieved using ACEi in albumin and ET-1 excretion. But the plasma creatinine was 26% more in ACEi treated rats compared to rats treated with C. In kidney, the protein expression of fibronectin was decreased by two-fold in diabetic rats treated with C compared to the diabetic rats with no treatment. In vitro: High glucose increased ROS in mesangial cells compared to cells in low glucose. Treatment with 50 μM C significantly reduced high glucose induced ROS. Also, in endothelial cells, C treatment significantly reduced high glucose induced apoptosis.

Conclusions: Our findings suggest that C has strong renoprotective properties in diabetic nephropathy. *In vivo* studies show that C decrease albumin and ET-1 excretion in diabetic rats. *In vitro* studies show that the mechanisms of C action might be related to antioxidant properties leading to reduced ROS and apoptosis.

FR-PO1528

ONO-1301, a Sustained-Release Prostacyclin Analog, Ameliorates Renal Alterations in a Mouse Type 2 Diabetes Model through Its Direct Protective Effects on Mesangial Cells Hiroko Yamasaki, Yohei Maeshima, Daisuke Saito, Norikazu Hinamoto, Hiroyuki Watatani, Haruyo Ujike, Hitoshi Sugiyama, Hirofumi Makino. *Okayama Univ. Med. School., Okayama, Japan.*

Background: Diabetic nephropathy is the most common pathological disorder predisposing ESRD, and novel therapeutic approaches are required. ONO-1301 is a novel sustained-release prostacyclin analog possessing thromboxane A2 synthase inhibitory activity. Therapeutic efficacies of ONO-1301 in experimental models of pulmonary hypertension, pulmonary fibrosis and myocardial ischemia has been reported, and we recently reported the therapeutic efficacies of slow-release ONO-1301(SR-ONO) in experimental rat type 1 diabetic nephropathy model. Here, we examined the therapeutic effects of intermittent administration of SR-ONO on diabetic nephropathy in the obese type 2 diabetes mouse as well as its direct effects on mesangial cells.

Methods: Db/db mice, a model of obese type 2 diabetes, received subcutaneous injections of either SR-ONO(3mg/kg) or vehicle buffer every 3 weeks. Animals were sacrificed at 16 weeks of age. Cultured mouse mesangial cells(Mes13) were stimulated with high ambient glucose(HG; 25 mM) in the presence of ONO-1301(1-100 nM) for 6hrs or 24 hrs. Clinical parameters, kidney weight, glomerular volume and mesangial matrix index were examined, and immunohistochemistry, immunoblot and real-time PCR was performed.

Results: SR-ONO treatment did not affect obesity or hyperglycemia, but significantly ameliorated albuminuria, glomerular hypertrophy, the increase of mesangial matrix index, glomerular accumulation of type IV collagen, F4/80+ monocyte/macrophage, TGF-beta1, alpha-SMA and MCP-1 in db/db mice compared with vehicle treatment. SR-ONO treatment reduced the increase of oxidative stress(nitrotyrosine and MDA) in db/db mice. In Mes13 cells, ONO-1301 suppressed the increase of TGF-beta, type IV collagen, alpha-SMA, MCP-1 and fibronectin induced by HG(immunoblot and real-time PCR).

Conclusions: Taken together, these results suggest the potential therapeutic efficacy of intermittent administration of SR-ONO in diabetic nephropathy through its direct protective effects on mesangial cells.

FR-PO1529

Effects of VEGFR-1 or VEGFR-2 or Both VEGFR-1 and VEGFR-2 Inhibition on Diabetic Nephropathy in db/db Mice Sungjin Chung, Ji Hee Lim, Min-Young Kim, Hyun Wha Chung, Hyung Wook Kim, Yong-Soo Kim, Yoon-Sik Chang, Cheol Whee Park. *Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: Interventions to manipulate vascular endothelial growth factor (VEGF)-VEGF receptors axis may be therapeutic tools in diabetic nephropathy. In this study, we examined the renal effects of anti-flt 1 hexamer (GNQWFI; VEGFR-1 inhibitor) or anti-flk 1 heptamer (ATWLPPR; A7R, VEGFR-2 inhibitor) or both of them in *db/db* mice.

Methods: The *db/m* and *db/db* mice were treated with GNQWFI or A7R peptide or both of them for 12 weeks.

Results: There were no differences in FBSand HbA1c levels in all db/db groups. Diabetes significantly suppressed the VEGFR-1 and increased VEGFR-2 expressions in the kidneys. VEGFR-1 and VEGFR-2 expressions were completely inhibited by GNQWF1 and A7R, respectively. In the db/db mice treated with GNQWF1 or A7R, albuminuria, glomerular mesangial matrix expansion and inflammatory cell infiltration, and profibrotic growth factors' expressions were more prominent than those of diabetic control db/db mice. They also exhibited increases in the number of apoptotic glomerular cells with no change in Ki-67 positive cells. Urinary 8-isoprostane and 8-OH-deoxyguanosine concentrations increased in the db/db mice treated with GNQWF1 or A7R compared with those in diabetic control db/db mice. More severe albuminuria and renal lesions were noted in the db/db mice treated with both of them compared with those of db/db mice treated with either GNQWFI or A7R. These changes were associated with inactivation of PI3K-Akt-eNOS pathway. In contrast, GNQWFI- and A7R-induced albuminuria and histopathological changes were not observed in any db/m groups. In HUVECs, high glucose media containing VEGFRs inhibitors induced more apoptotic cell death than did high-glucose media without VEGFRs inhibitors, in association with inactivation of PI3K-Akt-eNOS pathway.

Conclusions: The blockade of VEGFR-1 or VEGFR-2 or both using GNQWF1 or A7R peptide caused glomerular injury related to the inactivation of PI3K-Akt-eNOS pathway resulting in the oxidative stress-induced apoptosis in type 2 diabetic nephropathy.

FR-PO1530

Metabolic Syndrome Due to Deletion of the Gene Encoding Canonical Transient Receptor Potential 1 (TRPC1) Channel: A Novel Model Induced by Hyperphagia & Associated with Key Organ Dysfunctions Bonnie Eby,[1] Richard Matthew Atkins,[1] Chris Skaggs,[1] Jian Xu,[1] E-Ching Ong,[2] Joel Abramowitz,[3] Leonidas Tsiokas,[2] Lutz Birnbaumer,[3] Kai Lau.[1] *[1]Medicine, University of Oklahoma, Oklahoma City, OK; [2]Cell Biology, NIEHS University of Oklahoma, Oklahoma City, OK; [3]Intramural Research, NIEHS, Research Triangle Park, NC.*

Background: TRPC superfamily of cation channels includes certain members implicated in obesity & diabetes. TRPC1 expression is reduced in diabetes, but the relationship is unclear. Since null mice are fatter, we asked if TRPC1 deficiency produces insulin resistance & glucose intolerance & if hyperphagia is vital.

Methods: Metabolic studies were done on male mice at 2 mon & 4-22 mon, using glucometer, insulin ELISA, & lipids enzymatic assays.

Results: At 2 mon, null mice were 20% fatter, non-fasting glucose (171 vs 98 mg%) higher, & liver 36% heavier. At 7 mon, liver echo was 50% denser, suggesting steatosis. Random glucose (135 vs 100 mg%) at 9 mon & fasting glucose (109 vs 72 mg%) at 10 mon were higher. Null mice ate more food (3.3 vs 1.4 g/d) & calorie (19 vs 12 kcal/d). By 1 yr, they stayed 20% fatter as hyperglycemia & liver hyperdensity persisted. Glucose tolerance test (IP 2 mg/g) showed sustained hyperglycemia, 2-fold higher for 3 h & 65% higher by the 6th. Plasma insulin was 2.5-fold higher throughout. Fasting insulin (23 vs 7 μU/ml) & glucose (7 vs 4 mM) were elevated, due to severe insulin resistance (IR) (8 vs 1) by homeostatic model assessment (HOMA). HOMA beta cell function (β) (90 vs 99%) was normal. Null mice had elevated fasting total (153 vs 118 mg%) & LDL cholesterol (94 vs 59 mg%) & triglyceride (111 vs 36 mg%). At 13 mon, HOMA IR (5 vs 1) stayed high & HOMA β (84 vs 105%) normal. Hypertension was absent from 6 to 20 mon by tailcuff or intraarterial readings. Present were cardiomopathy, renal failure & endothelial dysfunction. Caloric restriction corrected excess weight, elevated glucose & cholesterols, implicating hyperphagia.

Conclusions: 1. TRPC1 deficiency produces all the features of metabolic syndrome except hypertension. 2. Hyperphagia is pathogenic. 3. The null mice are useful in studying the associated complications.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support, Clinical Revenue Support

FR-PO1531

Glomerular Immunoglobulin Deposition Is a Feature of Human Diabetic Glomerular Injury and Immunoglobulin Deficient Mice Are Protected from Diabetic Albuminuria David J. Nikolic-Paterson,[1,2] Andy Lim,[1] Frank Yuanfang Ma,[1,2] Yingjie Han,[1] A. Richard Kitching,[2] Peter G. Kerr,[1,2] Gregory H. Tesch.[1,2] *[1]Nephrology, Monash Medical Center, Clayton, Victoria, Australia; [2]Medicine, Monash University, Clayton, Victoria, Australia.*

Background: Glomerular immunoglobulin (Ig) deposition is observed in many animal models of diabetic nephropathy; however, its significance and relevance to human disease is unknown. This study aims to establish whether glomerular Ig deposition is a feature of human diabetic glomerulopathy and if it has a role in diabetic renal injury in mice.

Methods: Biopsy reports with assessment of Ig and complement deposition were examined from 82 patients diagnosed with diabetic glomerulosclerosis (90% type 2 diabetes) at the Monash Medical Centre (1996-2009). These diabetic patients had abnormal albuminuria but showed no signs of other glomerular diseases on biopsy. In addition, we established a model of streptozotocin-induced diabetes in wild type and B-lymphocyte deficient (mu-chain-/-) mice and compared the development of albuminuria (weeks 12-24) and renal injury (week 24).

Results: Immunostaining identified that >90% of patient biopsies had glomerular deposition of IgM, C1q and C3, being seen in the mesangium, arterioles, capillary loops and some nodules. In comparison, IgG showed a similar pattern, but with weaker intensity, and was detected in 61% of cases. All diabetic wild type mice displayed glomerular deposits of Ig and C3, which were absent in the diabetic mu-chain-/- mice. Compared to non-diabetics, diabetic wild type mice had a 5 to 6 fold increase in albuminuria at 18-24 weeks of diabetes, which was reduced by 50% in diabetic mu-chain-/- mice ($p<0.01$). Histological analysis showed no difference in the glomerular matrix fraction or tubular damage between diabetic groups; however, mRNA levels of TGF-β1 and PAI-1 were reduced ($p<0.001$) in the diabetic kidneys of mu-chain-/- mice compared to wild type.

Conclusions: Glomerular deposition of Ig is a feature of patients with diabetic glomerulosclerosis and severe albuminuria. Our findings in Ig-deficient mice suggest a pathologic role for Ig in diabetic albuminuria.

Funding: Government Support - Non-U.S.

FR-PO1532

Bis-Halpoinsufficiency Aggravates Diabetic Nephropathy by Increasing Oxidative Stress Sungjin Chung, Ji Hee Lim, Sun Ryoung Choi, Hoon Suk Park, In O Sun, Min-Young Kim, Yong-Soo Kim, Yoon-Sik Chang, Cheol Whee Park. *Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: *Bis* gene is ubiquitously expressed in cells and has anti-stress and anti-apoptotic activity by enhancing Bcl-2 activity in a synergistic manner. Total Bis-insufficient mice showed early lethality associated with malnutrition. Notably, oxidative stress-induced apoptosis contributes the pathogenesis of diabetic nephropathy. We investigated the role of Bis expression on the diabetic nephropathy using Bis haploinsufficiency (Bis-HT) mice after 20 weeks of diabetes induced by streptozotocin.

Methods: We treated anti-oxidant tempol starting after 12 weeks of diabetes for 8 weeks to see the anti-oxidant effect on the retardation of the progress of renal damage and restoration of the renal phenotypes associated with Bis expression.

Results: After 20 weeks of diabetes, there was an increase in Bis levels in diabetic Bis-wild type (Bis-WT DM) mice compared to Bis-WT mice in the kidneys. In contrast, there was a significant decrease in Bis expression in diabetic Bis-HT (Bis-HT DM) mice compared to Bis-WT DM mice even under the same degree of hyperglycemia. Serum creatinine and albuminuria were increased in the Bis-HT DM mice compared to those of Bis-WT DM mice. More glomerular matrix expansion, TGF-β1 and HIF-1α expression, and tubulointersitial fibrosis were also noted in the Bis-HT DM mice related to increases in apoptotic glomerular and tubular epithelial cells, accompanying with decreases in Bis and Bcl-2 expressions. SOD1 and SOD2 expressions were significantly decreased in the Bis-HT DM mice compared to those of Bis-WT DM mice, resulting in increased oxidative stress. Interestingly, anti-oxidant tempol treatment starting after 12 weeks of diabetes for 8 weeks reversed renal damages in the Bis-HT DM animals associated with increasing Bis and Bcl-2 expressions and decreasing oxidative stress.

Conclusions: Our results demonstrated that Bis protein has a protective effect on diabetic nephropathy and that its effect may be explained, at least in part, by preserving of anti-oxidative function.

FR-PO1533

Toll Like Receptor 4 Activation Mediates Injury in the Early Diabetic Kidney Jin Ma,[1,3] Huiling Wu,[1,3] Usha Panchapakesan,[2,3] Carol A. Pollock,[2,3] Steven J. Chadban.[1,3] *[1]Renal Medicine RPAH and Collaborative Transplant Research Group; [2]Kolling Institute and Royal North Shore Hospital; [3]University of Sydney, Australia.*

Background: We have reported that high glucose promotes release of endogenous TLR ligands, which activate TLR4 resulting in inflammation in early DN. Here we tested the hypothesis that TLR4 signalling is required for the development of DN.

Methods: DN was induced in WT and TLR4$^{-/-}$ mice by intraperitoneal injection of STZ. Samples were harvested at week 6, 12 or 24 post-induction. Renal tubular epithelial cells (TEC) exposed to 25mM glucose for 12-hours *in vitro* were also examined.

Results: WT and TLR4-/- mice developed equivalent diabetes. WT diabetic mice developed significant albuminuria versus normals (ACR:182.3±80.1 vs 34.1±9.2mg/mmol (wk6), 402.4±145.9 vs 37.1±8.8mg/mmol (wk12), 377.5±118.8 vs 41.3±10.2mg/mmol (wk24), $p<0.01$), which was reduced in TLR4-/- diabetic mice (117.0±33.4mg/mmol (wk6), 323.5±102.2mg/mmol (wk12), 224.9±99.3mg/mmol (wk24), $p<0.01$). WT diabetic mice developed progressive glomerular hypertrophy versus TLR4-/- diabetic mice [glomerular volume 99.3±9.5 vs 80.3±8.7μm3x103 (wk6), 74.3±6.4 vs 52.6±6.4μm3x103 (wk12), 102.1±16.4 vs 56.1±5.6μm3x103 (wk24). $p<0.001$]. Deposition of collagen was evident in WT diabetic kidney from wk12 versus TLR4-/- diabetic and non-diabetic kidneys. Macrophage accumulation was evident in WT diabetic kidneys, though not in TLR4-/- diabetic mice, as compared to age-matched normals [19.4±3 vs 5.7±0.8 vs 5.7±1.5 cells/HPF (wk6), 16.2±0.7 vs 6.4±1.0 vs 5.8±0.5cells/HPF (wk12), 13.4±1.8 vs 4.6±0.7 vs 5.5±1.5cells/HPF (wk24), $p<0.0001$). mRNA expression of TLR4-downstream cytokine, chemokine and fibrotic-genes was up-regulated in WT diabetic kidney versus control, with significant attenuation in TLR4-/- diabetic kidneys. In vitro, gene expression of key pathogenic cytokines, chemokines and fibrotic-genes was increased after high-glucose exposure, while increases were attenuated in TLR4-/- TECs.

Conclusions: TLR4$^{-/-}$ mice were protected from injury in DN, suggesting a pathogenic role for TLR4.

Funding: Government Support - Non-U.S.

FR-PO1534

Toll like Receptor 2 Activation Mediates Injury in the Early Diabetic Kidney Jin Ma,[1,3] Steven J. Chadban,[1,3] Usha Panchapakesan,[2,3] Carol A. Pollock,[2,3] Huiling Wu.[1,3] *[1]Renal Medicine RPAH and Collaborative Transplant Research Group; [2]Kolling Institute and Royal North Shore Hospital; [3]University of Sydney, Australia.*

Background: We have reported that high glucose promotes release of endogenous TLR ligands, which activate TLR2 resulting in inflammation in early DN. Here we hypothesize that TLR2 signalling is integral to the development of DN.

Methods: DN was induced in WT and TLR2$^{-/-}$ mice by intraperitoneal injection of STZ. Samples were harvested at week 6, 12 and 24 post-induction. Renal tubular epithelial cells (TEC) exposed to 25mM glucose for 12 hours in culture were also examined.

Results: WT and TLR2-/- mice developed equivalent diabetes. WT diabetic mice developed significant albuminuria from week 6 versus controls (ACR: 182.3±80.1vs34.1±9.2mg/mmol at 6wk, 402.4±145.9vs37.1±8.8mg/mmol at 12wk, 377.5±118.8vs41.3±10.2mg/mmol at 24wk, $p<0.01$), which was reduced in TLR2-/- diabetic mice (ACR: 43.3±7.8vs182.3±80.1mg/mmol at 6wk, 181.6±73.3vs402.4±145.9mg/mmol at 12wk & 124.3±35.2vs377.5±118.8mg/mmol at 24wk, $p< 0.01$). WT diabetic mice developed histological damage including glomerular hypertrophy from week 6 ($p<0.001$), interstitial collagen deposition from 12 weeks ($p<0.01$) and macrophage accumulation from week 6 ($p<0.0001$). These findings were significantly attenuated in TLR2-/- diabetic mice. Pathogenic cytokine (TNFa), chemokine (MCP1) and pro-fibrotic (TGFb&fibronectin) mRNA expression was significantly up-regulated in WT diabetic kidney compared to normals but was decreased in TLR2-/- diabetic kidneys. In vitro, WT TEC produced key cytokines, chemokines and fibrotic-genes after high-glucose exposure, which was attenuated in TLR2-/- TECs.

Conclusions: TLR2$^{-/-}$ mice were protected from injury in DN, suggesting TLR2 contributes to the development of DN.

Funding: Government Support - Non-U.S.

FR-PO1535

Histopathologic Changes of Diabetic Nephropathy in the db/db Mouse Following Iron Chelation with Deferiprone Ahmad Bilal Malik,[1] Syed M. Ali,[1] R. Stafford Justus,[2] Songthip Ounpraseuth,[1] Neriman Gokden.[1] *[1]University of Arkansas for Medical Sciences; [2]Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: Catalytic iron (CatFe) participates in the generation of powerful reactive oxidant species that propagate diabetic nephropathy (DN). Chelation may stabilize these histopathologic lesions.

Methods: Using a prototypical mouse model of human diabetes mellitus type 2 (Lepr^{db}/Lepr^{db}), we tested the efficacy of the iron chelator deferiprone in stabilizing mesangial matrix expansion and glomerular basement membrane (GBM) thickening. Using male mice from two batches of 6 animals for the control and treatment groups each, the latter received deferiprone dissolved in drinking water (125mg/kg body weight) starting at age 4.5 weeks till euthanasia at 28 weeks.

Results: While the mean blood glucose was lower in the treatment compared with the control group (569.38 ± 98.49 vs. 685.04 ± 31.31mg/dL) at euthanasia, body weight was better preserved (59.2 ± 2.29g vs. 54.15 ± 8.81g). Deferiprone predictably decreased the 24-h urinary CatFe excretion (CatFe/Creatinine ratio) which after adjustment for treatment group, batch, time, and their two-way interactions in a repeated measures analysis of variance (ANOVA), using Least Squares Means (LSM) gave values of 50.10 ± 233.25 vs. 942.75 ± 233.01μmol/mmol in the treatment and control groups respectively ($p= 0.0163$ at week 6). By 28 weeks excretion remained considerably less in the treatment group but became non-significant. A nephropathologist who was blinded to the treatment adjudication interpreted the light (LM) and electron microscopy (EM) findings. Using 2- way ANOVA, and determining the effect of treatment with deferiprone, time and batch by LSM, the area of the glomerular tuft occupied by the mesangial matrix was 26.25 ± 3.38% vs. 27.5 ± 3.05% in the treatment and control groups respectively. By EM, the mean GBM thickness was 0.099 ± 0.01μm vs. 0.14 ± 0.02μm with a representative area of absolute mesangial thickness of 11.2μm vs. 12.6μm in the treatment and the control groups respectively.

Conclusions: CatFe chelation with deferiprone in the db/db mouse model resulted in stabilization of the classic histopathologic lesions of DN.

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FR-PO1536

Ets-1 Acetylation Maintains Persistent Expression of microRNA-192 in Kidney Glomerular Mesangial Cells after Transforming Growth Factor-beta1 Treatment Mitsuo Kato, Mei Wang, Jung Tak Park, Sumanth Putta, Linda L. Lanting, Rama Natarajan. *Diabetes, Beckman Research Institute of City of Hope, Duarte, CA.*

Background: microRNAs (miRNAs) are involved in accumulation of extracellular matrix proteins in diabetic kidney glomerular mesangium. microRNA-192 (miR-192) is upregulated by Transforming Growth Factor beta1 (TGF-b) in a Smad3 and p53 dependent manner in early stages and plays a role in renal fibrosis. Although potential Ets-1 binding sequences are found in the promoter region of miR-192 and Ets-1 is an important factor in kidney injury, the role of Ets-1 has not been fully studied.

Methods: RNA levels were examined by Realtime PCR, protein, phosphorylation or acetylation levels by Western blotting, staining of phospho-p300 in mouse glomerui by immunohistochemistry, promoter activities by luciferase reporter assay, binding of Ets-1 and acetylation of histone H3 on the miR-192 promoter by Chromatin-immunoprecipitation assay.

Results: TGF-b activated Akt kinase which phosphorylates p300 (phospho-p300S1834) that acetylates Ets-1 and Histone H3K9/14 to regulate miR-192 expression through Ets-1 binding sites. This lead to persistent expression (>24hr) of miR-192 in mouse kidney glomerular mesangial cells. Luciferase reporter assays with miR-192 promoter constructs showed that the Ets-1 site is critical for the TGF-b response. Significant increase of phospho-p300S1834 levels were detected in glomeruli from the type 2 diabetes mice (db/db). Interestingly, in glomerular mesangial cells derived from Ets-1 knockout mice, basal miR-192 levels were higher and the persistent increase in TGF-b-induced miR-192 was lost. Furthermore, Ets-1 siRNA treatments increased miR-192 levels and inhibited the TGF-b-induced persistent increase of miR-192.

Conclusions: These results demonstrate a novel negative regulatory role for Ets-1 in miR-192 expression and demonstrate that the dissociation of acetylated Ets-1 (via TGF-b activated Akt/p300 pathway) from the miR-192 promoter region may allow a sustained and persistent expression of miR-192 in glomerular fibrosis. Thus Ets-1 may be a key regulator of fibrosis associated with diabetic nephropathy by controlling the expression of miR-192.

Funding: NIDDK Support

FR-PO1537

Insulin Modulates TRPC6 Channels in Podocytes: Possible Role in Stabilizing the Glomerular Filtration Barrier Stuart E. Dryer,[1] Jochen Reiser,[2] Alessia Fornoni.[2] *[1]University of Houston, Houston, TX; [2]University of Miami, FL.*

Background: Insulin signaling to podocytes is essential for normal function of the glomerular filtration barrier, but the effects of insulin on podocyte physiology are not well understood. TRPC6 channels are expressed in podocyte foot processes, and mutant forms of these channels can lead to glomerular disease.

Methods: Insulin modulation of TRPC6 channels was assessed by whole-cell recordings and cell-surface biotinylation assays in immortalized podocyte cell lines. Reactive oxygen species (ROS) were measured using fluorometric assays. Urinary albumin/creatinine ratios and glycemia were measured following tail-vein injections of glucose.

Results: Insulin caused a robust increase in macroscopic SKF96365- and La3+-sensitive cationic currents in podocyte cell lines. Insulin also increased steady-state surface expression of TRPC6 channels. These effects occurred in less than 15 min but were maximal after 24 hr. The effects of insulin on TRPC6 trafficking were blocked by siRNA against the NADPH oxidase NOX4 or by NADPH oxidase inhibitors. Insulin increased the generation of ROS in podocytes, and the effects of insulin on TRPC6 trafficking were blocked by manganese (III) tetrakis (4-benzoic acid) porphyrin chloride, a membrane-permeable scavenger of ROS. We also examined urinary albumin excretion following intravenous glucose in wild-type and TRPC6-knockout mice sufficient to produce a peak glycemia of 550 mg/dl. Glucose-induced albuminuria was greater in TRPC6 knockout mice compared to control, but glycemic control was not different.

Conclusions: Insulin modulates TRPC6 channels in podocytes, in part by increasing their steady-state surface expression. This effect requires generation of ROS, which appear to act as a type of second messenger for this effect. Modulation of podocyte TRPC6 channels, by increasing calcium influx and thereby stiffening the capillary wall, may be part of a mechanism to maintain stability of the glomerular filter in the face of stimuli that increase glomerular filtration rate (GFR). Insulin modulation of TRPC6 channels in particular may prepare podocytes to cope with the increased GFR evoked by a glucose load.

Funding: NIDDK Support

FR-PO1538

Increased Accumulation of Iron in Lysosomes of Proximal Tubule Cells of Hp 2-2 Diabetic Mice Farid M. Nakhoul,[1,3] Rachel Miller-Lotan,[3] Nakhoul Nakhoul,[3] Hoda Awad,[3] Andy P. Levy.[3] *[1]Nephrology, Poria Med Ctr, Lower Galilee; [2]Vascular Med.Lab, Technion-Faculty of Medicine, Haifa, Israel.*

Background: 30% of Patients with diabetes mellitus (DM) will develop ESRD. Its well know that diabetic patients with the Haptoglobin 1-1 (Hp 1-1) are more protected than Hp 2-2 in developing diabetic nephropathy(DN). Iron is a metal oxidant capable of generating ROS and has been postulated to contribute to progression of DN.Iron accumulates within proximal tubular (PCT) lysosomes in several models of renal disease. Vit.E may play a protective role in DN.

Methods: Samples of kidney tissue from the diabetic mice(Hp 1-1 DM, Hp 2-2 DM) with and without treatment with vit. E, were prepared for transmission electron microscopy. Areas showing positive Perls' stain were considered as containing more iron compounds and were ultra-thin cut and viewed through a JEOL 100SX EM. At least 4 glomeruli per sample plus the adjacent tubuli were examined. Clusters of iron-containing lysosomes (siderosomes) were seen in the PCT. The siderosomes were identified by the presence of typical electron-dense ferritin and hemosiderin within single-membrane bound bodies. To confirm the presence of iron in these organelles, Energy dispersive x-ray spectroscopy was used.

Results: LM examinations of glomeruli and PCT, disclosed increased in glomerular area in in Hp2-2 DM vs Hp1-1 DM, $p<0.05$), which decreased significantly by Vit.E in Hp2-2DM. Oxidative stress was measured by 4HNE immunostaining in glomeruli and PCT and was significantly increase in Hp 2-2 DM vs Hp1-1 DM and decreased by Vit. E in Hp2-2 DM. There was increase in ferritin in Lysosome of PCT cells of Hp2-2DM vs Hp1-1DM(0.194±0.01vs 0.116±005% area, $P<0.05$) and decreased by vit.E in Hp2-2DM(0.194 ±0.01 vs 0.13±0.007,$p<0.05$). Total iron content in the kidneys was increased in the Hp 2-2 DM vs Hp 1-1 DM (11.4 ±0.7 vs 9.4± 1.3 mg Fe/mg kidney) as measured by atomic absorption spectroscopy.

Conclusions: Increased iron accumulation within lysosomes of PCT cells of diabetic Hp 2-2 mice.

Increased iron accumulated as ferritin in Hp 2-2 DM, is associated with increased oxidative stress and Tubulo glomerular damage.

Funding: Other NIH Support - Israel Science Foundation (ISF), Government Support - Non-U.S.

FR-PO1539

Implications of Oxidative Stress Derived from Endothelial NAD(P)H Oxidase in the Development of Diabetic Nephropathy Hajime Nagasu, Minoru Satoh, Atsunori Kuwabara, Kengo Kidokoro, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Matsushima Kurashiki, Okayama, Japan.*

Background: Increased generation of reactive oxygen species (ROS) is a common pathogenic mechanism underlying vascular and renal complications in diabetes. Endothelial NAD(P)H oxidase is a major source of vascular ROS and plays important role in endothelial dysfunction. We hypothesize that activation of endothelial NAD(P)H oxidase would initiate and accelerate diabetic nephropathy, especially development of albuminuria, in diabetic milieu.

Methods: We used endothelial specific NOX2 transgenic (NOX2TG) mice, AKITA type1 diabetic (AKITA) mice, NOX2Tg crossbred with AKITA mice, and wild type (WT) mice. NOXTG was generated in which NOX2, gp91 phox of NAD(P)H oxidase, under the control of Tie2 promoter, was overexpressed in the endothelium. All mice were back-crossed into C57BL/6J. These mice were sacrificed at 6 and 12-week-old of ages for molecular and histological analysis. We applied the in vivo live imaging techniques with multi-photon laser microscopy and various sizes of FITC labeled dextrans to analyze alterations in permeability of glomerular capillary walls in disease conditions.

Results: Urinary albumin excretion was increased only in NOXTG-AKITA but not in WT and AKITA at 6-week-old. At 12-weeks-old, serum creatinine level was significantly elevated only in NOXTG-AKITA but not AKITA and WT. No significant morphological changes were detected in glomeruli from all groups by light microscopic examinations. But slight degree of structural changes in podocytes and mesangial cells were observed only in NOXTG-AKITA under the electron microscope. The in vivo live imaging techniques revealed increased filtration of 40kDa dextran in glomeruli in AKITA and NOXTG-AKITA, but not in WT. Moreover, increased permeability of larger molecules, 70KDa dextran, were detected in NOXTG-AKITA. Lectin staining was decreased along glomerular endothelium in NOXTG-AKITA.

Conclusions: Activation of endothelial NAD(P)H oxidase in hyperglycemic milieu initiated and accelerated diabetic nephropathy characterized by development of albuminuria and hyperfiltration of macromolecules.

FR-PO1540

Maxacalcitol Prevents Progression of Endothelial Dysfunction in Rats with Diabetic Nephropathy Michinori Hirata,[1] Ken Aizawa,[1] Ken-Ichi Serizawa,[1] Kenji Yogo,[1] Yoshihito Tashiro,[1] Yoshiyuki Moriguchi,[1] Masafumi Fukagawa.[2] *[1]Product Research Laboratory, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan; [2]Division of Nephrology, Endocrinology and Metabolism, Tokai Unicersity School of Medicine, Isehara, Kanagawa, Japan.*

Background: Endothelial function is a powerful surrogate marker of cardiovascular risk in patients with end-stage renal disease (ESRD) or diabetic nephropathy, and can be evaluated by flow-mediated dilatation (FMD). FMD is reportedly correlated with serum 25-hydroxyvitamin D levels in ESRD patients, and restored by nutritional vitamin D in patients with type 2 diabetes. There is evidence that vitamin D receptor activators (VDRAs) might protect against cardiovascular event directly or through protecting against endothelial dysfunction, but the mechanisms remain unclear.

Methods: This study aimed to assess whether the VDRA maxacalcitol (MXA) could prevent decrease of FMD in spontaneously diabetic Torii (SDT) rats, a non-obese, type II diabetes rat model with hyperglycemia and proteinuria (from 20 weeks of age). FMD was measured in rats as changes in femoral arterial diameter after 5 min ischemia.

Results: FMD was lower in SDT rats than in Sprague-Dawley (SD) rats (% diameter change [mean±S.E.]: SD, 12.8±2.1%; SDT, 8.3±1.2%; n=6). Treatment with MXA (0.2 or 0.6 μg/kg/day, i.p. 3 times/week for 10 weeks) significantly prevented the decline of FMD without hypercalcemia or decreased blood glucose level (MXA0.2, 15.4±2.4%; MXA0.6, 16.7±2.4%; n=4 or 6); with insulin, FMD also returned to normal level (17.6±3.4%; n=6) but with decreased glucose level.

To clarify the mechanism of MXA, we evaluated the effects of anti-reactive oxygen species (ROS) on human coronary artery endothelial cells (HCAECs). High glucose significantly increased ROS generation in HCAECs, and MXA significantly inhibited ROS generation by suppressing $p22^{phox}$ expression. Supporting such a mechanism, $p22^{phox}$ subunit of NADPH oxidase, related to oxidative stress, were increased in femoral arteries of SDT rats.

Conclusions: In conclusion, in rats with diabetic nephropathy, MXA prevented endothelial dysfunction without hypercalcemia or decreased blood glucose by ameliorating oxidative stress.

FR-PO1541

Low Nitric Oxide Bioavailability Upregulates HB-EGF Expression in eNOS Knockout Diabetic Mouse Kidney Fenghua Zeng, Tomoki Miyazawa, Suwan Wang, Xiaofeng Fan, Huifang Cheng, Raymond C. Harris. *Department of Medicine, Vanderbilt University, Nashville, TN.*

Background: There is strong evidence that decreased Nitric oxide (NO) bioavailability in the diabetic state plays a central role in endothelial dysfunction. Studies also indicate that factors contributing to low NO bioavailability, such as hyperglycemia, AGEs and oxidative stress, also cause endothelial cell (EC) damage directly or indirectly through the actions of HB-EGF, suggesting that HB-EGF expression may also being regulated by NO levels and serve as a mediator of EC dysfunction in diabetes.

Methods: eNOS knockout diabetic mouse (*eNOSKO db/db*) was used in this study. Renal HB-EGF expression was measured by western blot and immunostaining. Heparin-binding assay was used to test urinary HB-EGF excretion. NOS inhibitor, L-NAME, was used in both *in vitro* and *in vivo* studies of the interaction of NO and HB-EGF level.

Results: We found that increased kidney HB-EGF expression can be detected as early as 8 weeks of age, and is further elevated to parallel the progression of glomerulopathy in eNOSKO db/db mice. Strong HB-EGF expression was seen in both EC and podocytes of glomeruli, EC and smooth muscle cells of the blood vessels as well as in tubular epithelial cells of thick ascending limb and collecting ducts in both eNOSKO and eNOSKO db/db mouse kidney. Even though HB-EGF expression levels in the kidney were increased in both eNOSKO and eNOSKO db/db mice at 8 weeks, dramatically increased HB-EGF levels were seen at later time points in eNOSKO db/db mice, and urinary HB-EGF excretion was detected exclusively in eNOSKO db/db mice. In cultured glomerular endothelial cells, administration of the NOS inhibitors L-NAME or L-NIO increased HB-EGF mRNA and protein expression. Furthermore, in vivo administration of L-NAME dramatically increased renal HB-EGF expression and urinary HB-EGF excretion in db/db mice, but not in non-diabetic control mice.

Conclusions: In conclusion, our results suggest that decreased NO bioavailability in *eNOSKO db/db* mice leads to increased HB-EGF expression, which may be an important mediator of the resulting glomerular damage in the progressive diabetic nephropathy in this model.

Funding: NIDDK Support

FR-PO1542

RAGE-Dependent Apoptosis of Podocytes in a Model of Diabetes and/or High Protein Diet Rick L. Meek,[1] Sandeep Ajoy Saha,[1,2] Sheryl K. Cooney,[1] Robert J. Anderberg,[1] Katherine R. Tuttle.[1,2] *[1]Providence Medical Research Center, Sacred Heart Medical Center; [2]University of Washington School of Medicine, Spokane, WA.*

Background: Albuminuria is an early manifestation of diabetic kidney disease (DKD). Podocyte loss is a central mechanism for albuminuria. The study aim was to determine if metabolic stressors characteristic of diabetes and/or high protein diet, specifically high levels of glucose (HG), amino acids (AA), and/or advanced glycation end products (AGE), promote podocyte loss through apoptosis mediated by the receptor for AGE (RAGE).

Methods: Immortalized mouse podocytes were exposed to metabolic stressors: 1) hyperglycemia (HG, 30.5 mM glucose); 2) an amino acid mixture designed to mimic high protein diet (AA); 3) the combination (AA/HG); 4) AGE-bovine serum albumin (AGE). Anti-serum to RAGE and non-immune control serum were added to determine RAGE-dependent responses. Both early (caspase 3/7 activity, day 1) and later (TUNEL staining, day 2) markers of apoptosis were measured in podocytes. Expression of RAGE protein was assessed (immunocytochemistry, day 2), and levels of carboxymethyllysine (CML), an AGE biomarker, were determined in conditioned media (ELISA).

Results: Caspase 3/7 activity and TUNEL-positive podocyte number strikingly increased after exposure to metabolic stressors of HG, AA, AA/HG, and AGE (150-300 %). RAGE immunostaining on podocytes and CML in conditioned media also increased after exposure to these metabolic stressors. Anti-RAGE antiserum effectively reduced caspase 3/7 activity and TUNEL-positive podocytes numbers to control levels in response to HG, AA, AA/HG, and AGE.

Conclusions: Metabolic stressors characteristic of diabetes and/or high protein diet promote apoptosis in podocytes by RAGE-dependent mechanisms. In addition, increased AGE ligands produced in response to these conditions may augment RAGE binding, and thereby, perpetuate podocyte apoptosis.

Funding: Private Foundation Support

FR-PO1543

Exacerbated Diabetic Renal Alterations in Mice Lacking Vasohibin-1 Norikazu Hinamoto,[1] Yohei Maeshima,[1] Daisuke Saito,[1] Hiroko Yamasaki,[1] Hiroyuki Watatani,[1] Haruyo Ujike,[1] Hitoshi Sugiyama,[1] Hikaru Sonoda,[2] Yasufumi Sato,[3] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Discovery Research Laboratories, Shionogi, Settsu, Osaka, Japan; [3]Vascular Biology, Development, Aging, and Cancer, Tohoku Univ., Sendai, Miyagi, Japan.*

Background: Diabetic nephropathy is the leading cause of end-stage renal disease, and the involvement of proangiogenic factors such as VEGF-A had been reported. We previously reported the renoprotective role of exogenous Vasohibin (VASH)-1, a negative feedback regulator of angiogenesis and vascular maturation factor, in mouse models of diabetic nephropathy (Diabetes, 2009, AJPhys-Renal, 2011). VEGF-A and decreased angiopoietin (Ang)-1/2 ratio are associated with inflammation. In the present study, we aimed to evaluate the potential role of endogenous VASH-1 to regulate diabetic renal alterations.

Methods: Type 1 diabetes was induced in male VASH-1 heterozygous knockout mice (VASH1+/-) or wild-type (VASH1+/+) littermates (C57/BL6J) by intraperitoneal injections of streptozotocin (STZ, 50 mg/kg) for 5 consecutive days. Mice were sacrificed on week 16 after inducing diabetes.

Results: Renal histological alterations were not observed in non-diabetic VASH1+/- mice. Although hyperglycemia, blood pressure or glomerular hyperfiltration were not altered, renal hypertrophy, glomerular hypertrophy, albuminuria, glomerular accumulation of type IV collagen and mesangial matrix, and glomerular monocyte/macrophage infiltration, were significantly exacerbated in the diabetic VASH1+/- mice compared with diabetic VASH1+/+ mice. Renal levels of transforming growth factor (TGF)-beta1, VEGF-A and Ang-2 (immunoblot) were significantly increased, and the levels of Ang-1 was reduced in the diabetic VASH1+/- mice compared with the diabetic wild-type mice.

Conclusions: These results suggest that endogenous VASH-1 may exert renoprotective effects in type 1 diabetes, via suppressing inflammation and fibrosis partly through regulating VEGF-A and Ang-1/2 ratio, thus implicating its potential to serve as a novel therapeutic reagent for diabetic nephropathy.

FR-PO1544

Sepiapterin Improves eNOS Function and Attenuates Renal Injury in db/db Mice Huifang Cheng, Xiaofeng Fan, Raymond C. Harris. *Nephrology/Medicine, Vanderbilt University Medical School, Nashville, TN.*

Background: Our previous studies have demonstrated a role for impaired eNOS activity in the development of diabetic nephropathy (DN).

Methods: To investigate the effect of sepiapterin (Sep), a cofactor for endothelial nitric oxide synthase and a stable precursor of tetrahydrobiopterin (BH4), on DN, we administrated Sep (10 mg/kg/day by gavage) or the NO precursor, L-arginine (L-arg) (100 mg/kg/day) in a type II diabetic model, db/db (BKS) mice for 8 weeks (from 26 to 34 weeks).

Results: Neither Sep nor L-arg significantly affected the hyperfiltration (GFR: 290±20 μl/min. in control; 394±92 in untreated db/db; 431±52 in Sep and 394±19 in L-arg respectively), but both of the treatments reduced urine albuminuria (alb/cre:untreated: 759±281, Sep: 40±7 and L-arg: 163±37 μg alb /mg Cr respectively, n=6-8, $p<0.05$). Mesangial expansion was not affected by treatment, but Sep or L-arg decreased GBM thickness (control: 154±6; db/db: 335±23; Sep: 180±13; L-arg: 219±13 nm, n=3). After Sep or L-arg, urinary isoprostanes, a marker of oxidative stress, were significantly less (2.6±0.2 and 3.4±0.2 ng 8-iso-PGF2α /mg Cr), compared with untreated db/db (5.6±0.05), although they were still higher than control (1.4±0.1).

Neither immunohistochemistry nor immunoblotting indicated any significant alteration of glomerular eNOS monomer expression, but impaired eNOS dimerization was partially reversed by either Sep or L-arg (dimer/monomer ratio (wild type: 0.53±0.14; db/db: 0.23±0.08; Sep: 0.47±0.07 and L-arg: 0.40±0.13 respectively), indicating recovery of eNOS uncoupling after treatment. In addition, there was decreased phosphorylation of eNOS at Ser 1179 in db/db mice, which was partially restored by Sep or L-arg.

Conclusions: In summary, the current study further supports the important role of BH4 deficiency in eNOS dysfunction and suggests that sepiapterin supplementation might have therapeutic potential in diabetic nephropathy.

Funding: NIDDK Support

FR-PO1545

CTGF Is Overexpressed in BTBR *ob/ob* Mutant Mice and Inhibited upon Reversal of Diabetic Nephropathy Tri Q. Nguyen,[1,2] Tomasz A. Wietecha,[1] Roel Goldschmeding,[2] Kelly L. Hudkins,[1] Charles E. Alpers.[1] *[1]Dept of Pathology, University of Washington, Seattle, WA; [2]Dept of Pathology, University Medical Center Utrecht, Netherlands.*

Background: BTBR mice with the *ob/ob* leptin deficiency mutation develop progressive diabetic nephropathy (DN) that resembles its human counterpart (JASN 2010;1533-42). Connective tissue growth factor (CTGF; CCN2) is strongly upregulated in renal fibrosis and is an important factor in the progression of DN. It is not known if renal CTGF expression is dependent on the diabetic state or if it is regulated by other factors. We investigated CTGF expression in BTBR *ob/ob* mice both during progression of DN and during reversal of diabetes and DN.

Methods: Cohorts of female diabetic BTBR *ob/ob* mice (n=6) and normoglycemic BTBR wild-type (WT) mice (n=6) were followed for 24 wks. In a third cohort, leptin was administered by osmotic minipumps for 6 wks, starting at age 18 wks (n=6). In a fourth cohort, enalapril was given orally for the same period (n=6). CTGF expression was assessed by immunostaining and quantified by digital image analysis.

Results: In the kidneys of BTBR WT mice, CTGF was only focally present in podocytes and mesangial cells (mean positive glomerular area 1.7% ± 1.2). In BTBR ob/ob mice, glomerular CTGF was strongly increased (18.8%±4.6; P<0.001) and localized in podocytes, endothelial cells, mesangial cells and - matrix, and parietal cells. Treatment with leptin resulted in remission of diabetes and reversal of DN as reported previously (ASN abstract 2010) with corresponding decrease of CTGF (7.4%±1.9; P<0.05). Treatment with enalapril did not produce reversal of DN or decreased CTGF (20.5%±10.4; P=0.71). No significant CTGF staining was observed in the tubulointerstitium of BTBR WT or BTBR ob/ob mice.

Conclusions: CTGF is upregulated in glomeruli of BTBR *ob/ob* mice with DN. Treatment with leptin, but not enalapril, inhibits CTGF in association with reversal of the functional and structural kidney damage of DN. These data indicate that the expression of CTGF in DN may be closely linked or even dependent on the presence of a diabetic milieu, and that achieving normoglycemia may prevent the deleterious fibrosis mediated by CTGF.

Funding: Other NIH Support - MMPC

FR-PO1546

Mechano-Growth Factor Induces the Mesangial Cell GLUT1 Glucose Transport System Minghui Xiang,[1] Kathleen O. Heilig,[1] Leighton R. James,[1] Joana Panni,[2] N. Stanley Nahman,[3] Charles W. Heilig.[1] *[1]Medicine, University of Florida College of Medicine-Jacksonville, FL; [2]Anesthesiology, University of Florida College of Medicine-Jacksonville, FL; [3]Medicine, Georgia Health Sciences University, Augusta, GA.*

Background: Recently we reported in preliminary form the expression of Mechano-Growth Factor (MGF) in mouse glomeruli and primary culture mesangial cells (MC). Glomerular MGF was increased in the glomerular mesangium of both Type 1- and Type 2 diabetic mice. Here we describe responses of cultured mouse MC to MGF-overexpression and to 20mM high glucose, including GLUT1 glucose transporter expression, GLUT1 transcription, glucose uptake rates, and extracellular matrix (ECM) protein expression.

Methods: MGF overexpression in primary culture mouse MC via a MoMuLV retroviral vector; 3H2-Deoxyglucose (3H2-DOG) uptake rates; Western analyses for GLUT1 and ECM proteins; MC exposure to 8 vs 20mM high glucose x 5d; GLUT1-luciferase reporter assays for transcription.

Results: MGF was detectable in control MC (MC-EV) transduced with the empty MoMuLV vector. Multiple clones of MGF-sense transduced MC (MGF-S) were obtained with overexpression of MGF protein at 3-fold control, P < .0005. In comparison, 20mM high glucose treatment of MC-EV increased MGF protein 2.6-fold . In 8mM glucose, immunolabelling of cells revealed diffuse expression of MGF in MGF-S, as opposed to perinuclear localization of MGF in MC-EV. GLUT1 transcription via the promoter + Enhancer-2 was increased 2.3-fold in MGF-S, with 2.3-fold increased GLUT1 protein (P < .05), and 3-fold increased glucose uptake (P < .o5). Fibronectin (FN) protein was increased 1.6-fold in MGF-S.

Conclusions: 1.MGF-overexpression in MC induced GLUT1 transcription, GLUT1 protein, glucose uptake, and FN ECM protein. 2.High glucose similarly increased MC MGF protein. 3. MGF is a MC protein induced by high glucose and diabetes, and may play a role in diabetic glomerulosclerosis in vivo.

Funding: Private Foundation Support

FR-PO1547

Lowering Serum Uric Acid Attenuated NLRP3 Inflammasome-Induced Renal Inflammation in High Fructose Fed OLETF Rat Ju-Young Moon, Su-Mi Kim, Eun Young Kim, Yang Gyun Kim, Sul-Ra Lee, Kyung-Hwan Jeong, Sang-Ho Lee, Tae Won Lee, Chun-Gyoo Ihm. *Division of Nephrology, Department of Internal Medicine, Kyung Hee University, Seoul, Korea.*

Background: The NLRP3 inflammasome is a molecular platform activated upon signs of cellular danger to trigger innate immune defences though the maturation of pro-inflammatory cytokines such as interleukin-1β (IL-1β). Uric acid crystals have recently gained widespread attention due to their role as a natural endogenous adjuvant. In this study, we examined the hypothesis that soluble uric acid activates NLRP3 inflammasome-induced renal inflammation in type 2 diabetic rat.

Methods: The LETO and OLETF rats were divided into four groups: (1) LETO group; (2) OLETF group; (3) OLETF + high fructose diet (HFD) group; (4) high fructose fed OLETF with allopurinol treatment (HFD + allopurinol) group. Human renal proximal tubular epithelial cells (HK-2) and THP-1 cells were also cultured and stimulated with 15mg/dL of uric acid with or without allopurinol.

Results: HFD group showed a higher serum uric acid and urinary albumin creatinine ratio than OLETF group. HFD group showed marked increase of NLRP3 and IL-1β in kidney cortex. Immunohistochemical staining of ED-1 showed significant increase in HFD group compared to OLETF group. Allopurinol attenuated HFD induced hyperuricemia and NLRP3 activation-related renal inflammation. Uric acid also stimulated NLRP3 by reactive oxygen species (ROS) production in HK-2 and THP-1 cell.

Conclusions: The soluble uric acid could stimulate NLRP3 and IL-1β by ROS production in diabetic kidney. It may be related with hyperuricemia induced inflammation and progression of diabetic nephropathy.

FR-PO1548

Regulation of Stress Granule Formation through Diabetes and RACK1 Expression Michael Merchant, Michelle T. Barati. *Medicine-Nephrology, University of Louisville, KY.*

Background: Stress granules are transient cytoplasmic aggregations of protein and RNA, formed following stressors including heat, osmotic, and oxidant stress. They represent a compensatory attempt of the cell to triage and sequester important proteins and messenger RNA during stress but preceding ERAD. Previous studies indicated alteration to stress granule metabolism in parenchyma of patients with early diabetic nephropathy (DN). This current study addressed the hypothesis that elevated urinary glucose and/or protein may alter stress granule proteome.

Methods: Cultured immortalized human proximal tubules (HK2) cells were exposed to high glucose and/or albumin concentrations, then fractionated to enrich stress granules. Differentially abundant proteins were identified using electrophoresis and mass spectrometry. Soluble and granule-enriched HK2 lysate fractions, human renal biopsies, and isolated mouse tubules were subjected to immunoblot or confocal analysis for constituitive and conditionaly associating stress granule proteins. HK2 cells were treated with thapsigargin, a pharmacologic inducer of ER stress, to characterize the recruitment of proteins into stress granule structures.

Results: The receptor for activated protein C kinase-1 (RACK1) was recruited into stress granule containing fractions by diabetic stressors. Expression of RACK1 in renal biopsies from diabetic patients transiently increase with early DN and then decreased with advanced DN. RACK1 expression in isolated mouse tubules of non-diabetic and diabetic mice decreased with diabetes and with age. Treatment of HK2 cells with the ER stress inducer, thapsigargin, resulted in 1) constitutive TIA-1 and G3BP co-localization to granular structures and also a transient and significant re-localization of RACK1 to granule structures.

Conclusions: Sequestration of RACK1 into stress granules has been shown to inhibit cell apoptosis, by scaffolding key signaling proteins (eg protein kinase C). Stress granule triage of RACK1 by HK2 cells in diabetic conditions suggests an early attempt of the cell to survive the diabetic stressors. Data from human biopsy and isolated mouse tubules suggests this role of RACK1 may be lost with disease progression and/or age.

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FR-PO1549

Impact of CD45+/Col1+ Cells through CCR2 Signaling on the Pathogenesis of Diabetic Nephropathy Akinori Hara,[1] Norihiko Sakai,[1] Kiyoki Kitagawa,[1] Kengo Furuichi,[1] Shuichi Kaneko,[1] Takashi Wada.[2] *[1]Disease Control and Homeostasis, Kanazawa University, Kanazawa, Ishikawa, Japan; [2]Department of Laboratory Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan.*

Background: Recent evidence has revealed that migration and infiltration of bone marrow-derived cells, via the chemokine/chemokine receptor system, have been implicated in the pathogenesis of kidney fibrosis. In this study, the involvement of CD45 and type 1 collagen (Col1) dual-positive (CD45+/Col1+) cells through monocyte chemoattractant protein (MCP)-1/CCL2/CC chemokine receptor 2 (CCR2) signaling was examined in the pathogenesis of diabetic nephropathy.

Methods: Eight-week-old male db/db mice and db/+ control mice were fed with propagermanium (PG), a CCR2 inhibitor, or vehicle for 16 weeks. Human CD45+/Col1+ cells were isolated from healthy volunteers and incubated with normal or high concentrations of glucose.

Results: In flow cytometric and immunohistochemical analysis, CD45+/Col1+ cells were detected in the diabetic kidneys, especially in the interstitium, and the number of the

cells was reduced in PG-treated mice. Blockade of CCR2 signaling by PG reduced the extent of glomerulosclerosis and interstitial fibrosis accompanied with the decreased renal mRNA expression of transforming growth factor (TGF)-beta1, pro-alpha1 chain of Col1 (COL1A1), and MCP-1 in PG-treated mice. Urinary albumin excretion was reduced in PG-treated mice compared with that in vehicle-treated mice. In vitro experiments revealed that high glucose enhanced the synthesis of TGF-beta1, COL1A1 and MCP-1 in human isolated CD45+/Col1+ cells, and up-regulated expression of these molecules was blocked by PG treatment.

Conclusions: These findings suggest that CD45+/Col1+ cells may be involved in the progression of diabetic nephropathy through MCP-1/CCR2 signaling.

FR-PO1550

Functional Analysis of miR-30c, miR-26a and miR-379 in Podocytes and Their Potential Roles in Diabetic Nephropathy Kenichi Koga,[1] Hideki Yokoi,[1] Masashi Mukoyama,[1] Kiyoshi Mori,[1] Masato Kasahara,[1] Takashige Kuwabara,[1] Hirotaka Imamaki,[1] Tomoko Kawanishi,[1] Akira Ishii,[1] Keita P. Mori,[1] Yukiko Kato,[1] Moin Saleem,[2] Akira Sugawara,[1] Kazuwa Nakao.[1] *[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Academic and Children's Renal Unit, University of Bristol, Bristol, United Kingdom.*

Background: MicroRNAs (miRNAs) are small non-coding RNAs that downregulate mRNA levels. We have reported that connective tissue growth factor (CTGF) is involved in the progression of diabetic nephropathy, and that the natriuretic peptide/guanylyl cyclase-A (GC-A) pathway has a protective role against diabetic nephropathy. Recently, we showed that 48 miRNAs are changed with the stimulation of TGFβ1 on immortalized human podocytes by microarray analysis. Among them, we focused on miRNAs targeting CTGF (i.e., miR-30c and miR-26a) and GC-A (i.e., miR-379).

Methods: We transfected their mimics or inhibitors into podocytes by using nucleofection and examined target mRNA expression. We studied the role of miR-26a using its mimics in inhibiting TGFβ-CTGF pathway on podocytes with the stimulation of TGFβ1. Finally we examined expression of these miRNAs in glomeruli in type 2 diabetic db/db mice.

Results: Transfection of miR-30c mimic into podocytes tended to inhibit CTGF mRNA. miR-26a inhibitor upregulated CTGF mRNA by 1.3-fold. miR-379 mimic downregulated GC-A mRNA by 47%. TGFβ1 stimulation significantly increased col1a1 and col4a3 mRNA 2.5- and 1.8- fold in control group, respectively and transfection of miR-26a mimic significantly suppressed expression of col1a1 and col4a3 mRNA by 33% and 43% in TGFβ1-stimulated podocytes, respectively ($p < 0.05$). In glomeruli of db/db mice, miR-30c, miR-26a and miR-379 were significantly increased by 9.2-, 20.8- and 10.6-fold compared with those of db/m mice, respectively ($p < 0.05$).

Conclusions: These results indicate that miR-30c, miR-26a and miR-379 can be negative regulators of CTGF or GC-A in human podocytes, and suggest that these miRNAs may be involved in the progression of diabetic nephropathy.

FR-PO1551

Activated Local Renin-Angiotensin System Plays a Role in Albumin Permeability in Glomerular Endothelial Cells under High Glucose Conditions Jisun Paeng, Sun Ha Lee, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. *Dept. of Int. Medicine, Collge of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Local renin-angiotensin system(RAS) is present in mesangial cells and podocytes, and their activation has been demonstrated to play an important role in the pathogenesis of diabetic nephropathy. However, little is known on local RAS within glomerular endothelial cells(GEC). This study was undertaken to investigate the existence of RAS components in GEC, their changes under high glucose conditions, and the role of local RAS in morphological and functional changes in high glucose-stimulated GEC.

Methods: GEC were exposed to 5.6 mM glucose(NG) or 30 mM glucose(HG) with or without 10^{-7} M losartan for 24 hours. Real-time PCR and Western blot analysis were performed for all components of RAS. Angiotensin I(AI) and AII concentrations in conditioned media were measured by ELISA. Renin activities were defined as the formation of AI in the presence of porcine angiotensinogen(AGT). Morphological changes were examined by scanning electron microscopy(SEM) and a Transwell assay was performed to determine FITC-tagged albumin permeability.

Results: AGT mRNA and protein expression were significantly increased in HG-stimulated GEC compared to NG cells. AI and AII concentrations were also significantly higher in HG-conditioned media. In contrast, there were no differences in renin activities, and angiotensin converting enzyme and AII type 1 and type 2 receptor expression among the groups. On SEM examination, the diameter of fenestrae in HG-stimulated GEC was significantly greater compared to NG cells. The number of fenestrae tended to be increased in GEC exposed to HG, but did not reach statistical significance. A Transwell assay revealed that FITC uptakes in filtered media through HG-stimulated GEC were 3.3-fold higher compared to NG cells($p<0.05$). The increase in fenestrae diameter and enhanced albumin permeability through GEC under HG conditions were significantly abrogated by losartan pretreatment.

Conclusions: These findings suggest that local RAS is activated in GEC under HG conditions and this activated RAS may play an important role in the development of albuminuria in diabetic nephropathy.

FR-PO1552

miR-200b Prevents TGFβ Induced Epithelial To Mesenchymal Transition and Represses Fibronectin by Targeting Its 3'UTR Owen Tang, Xinming Chen, Michael A. Hahn, Sylvie Shen, Carol A. Pollock. *Kolling Institute of Medical Research, St Leonards, NSW, Australia.*

Background: Data suggest that miR-200b suppressed the fibronectin by binding to its 3'UTR and we have also confirmed the site using site directed mutagenesis. Given this, miR-200b may have future therapeutic potential in progressive kidney fibrosis. MicroRNAs comprise a novel class of endogenous small noncoding RNA that control the expression of target genes. Our previous work had indicated miR-200b prevented transforming growth factor β1 induced epithelial to mesenchymal transition in human proximal tubular cells. Fibronectin which is a main constituent of extracellular matrix in renal fibrosis was also significantly reduced at transcription, translation level as well as secreted form when transfected with miR-200b. Hence this study was designed to explore the molecular mechanism in which miR-200b regulates fibronectin expression.

Methods: Potential miR-200b binding site on fibronectin 3' untranslated region was identified and cloned into downstream of luciferase gene in pMIR luciferase reporter plasmid. Another identical plasmid containing 4bp mutation in potential binding site was made using site directed mutagenesis. miR-200b was co-transfected with these reporter plasmids respectively into immortalised human proximal tubular cells. A non-specific miR was also used to serve as a negative control. Luciferase activity was measured 48hr post transfection.

Results: miR-200b reduced luciferase activity ($p<0.001$) by at least 2.5fold when co-transfected with reporter plasmid containing non-mutated fibronectin 3'UTR comparing to negative control. On the other hand, cells co-transfected with miR-200b and mutated fibronectin 3'UTR maintained high luciferase activity. Lentiviral construct had also been developed to achieve sustained over expression of miR-200b in fibrotic kidney mice model.

Conclusions: Data suggest that miR-200b suppressed the fibronectin by binding to its 3'UTR and we have also confirmed the site using site directed mutagenesis. Given this, miR-200b may have future therapeutic potential in progressive kidney fibrosis.

Funding: Government Support - Non-U.S.

FR-PO1553

Protein Kinase DLK Is Necessary for Cell Autonomous Regulation of Insulin Sensitivity Hetty N. Wong,[1] Nathan Qi,[2] Deepak Nihalani,[1] Lawrence B. Holzman.[1] *[1]Medicine/Renal, Univ of Penn, Philadelphia, PA; [2]Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: The protein kinase DLK (MAP3K12) is a component of a JIP1-JNK signaling module. Because we had previously observed that the DLK-JIP1-JNK1 complex is activated by insulin in cultured cells and because proximal components of the JNK1-JIP1-IRS negative feedback loop that in part determine insulin sensitivity and energy expenditure remain incompletely defined, we investigated the hypothesis that DLK is the MAP3K that functions proximal to JNK1 in determining cell autonomous insulin sensitivity in peripheral tissues and in regulating whole animal energy expenditure.

Methods: We generated a mouse line that carries a hypomorphic allele of Dlk (Dlk neo/neo). These mice were evaluated by indirect calorimetry and activity monitoring to determine their energy expenditure and hyperinsulinemic euglycemic clamp to determine whole body insulin sensitivity. Furthermore, we used ex vivo muscle strips, adipocytes and mouse embryonic fibroblast to study the cell autonomous role of DLK in regulation of insulin sensitivity.

Results: Dlk neo/neo mice are protected from age- and diet-induced obesity primarily because they expend more energy than control mice. This increased energy expenditure appears to result from increased resting metabolic rate and from increased energy consumption associated with increased motor activity. Relative to control mice, the Dlk neo/neo mice exhibit increased insulin sensitivity independently of obesity. Indeed, DLK regulates insulin sensitivity in peripheral tissues including white adipose and skeletal muscle in a cell autonomous fashion via a JNK-dependent pathway.

Conclusions: 1) DLK hypomorphic mice are protected from diet- and age-induced obesity.

2) DLK hypomorphic mice exhibit higher energy expenditure and increased motor activities.

3) Independent of fat mass, by using fat mass percentage matched young mice, we showed that DLK hypomorphic mice have altered glucose homeostasis and improved insulin sensitivity.

4) Using isolated muscle strips, white adipocytes and MEF, we showed that DLK has a cell autonomous role in insulin sensitivity regulation.

Funding: NIDDK Support

FR-PO1554

Renoprotection with Sodium Glucose Cotransporter 2 Inhibition Amanda J. Mather, Katherine Jane Pegg, Harshini Mudaliar, Carol A. Pollock, Usha Panchapakesan. *Renal Laboratory, Kolling Institute, Sydney, NSW, Australia.*

Background: This paper aims to examine the renoprotective effects of sodium glucose cotransporter 2 inhibitors (SGLT2i) using an in vitro model of proximal tubular cells (PTC). SGLT2i are oral hypoglycaemic agents used to treat patients with diabetes mellitus. SGLT2i block the reuptake of filtered glucose by inhibiting SGLT2, the primary glucose transporter in the PTC, leading to glycosuria and reductions in serum glucose. As a third of patients with

diabetes have diabetic nephropathy (DN), oral hypoglycaemic agents that have additional renoprotective effects beyond glucose lowering would be highly desirable

Methods: HK2 cells (a human kidney PTC line) were exposed to control (5mM) or high glucose (30mM), 0.5 ng/ml transforming growth factor beta (TGFβ) +/- the SGLT2i BI10773 for up to 72h. Cells were harvested for nuclear extract, RNA/protein and supernatants were collected. SGLT2, SGLT1 as well as various inflammatory/fibrotic markers relevant to DN were measured.

Results: TGFβ but not HG increased SGLT2 expression. SGLT2 inhibition with BI10773 reduced HG induced toll like receptor 2 and 4 as well as the transcription factors nuclear factor kappa B and activator protein 1 which promote inflammation and fibrosis in DN. Furthermore, BI10773 lowered HG induced collagen IV, an extracellular matrix protein as well as interleukin 6.

Conclusions: The SGLT2 inhibitor BI10773 reduces high glucose induced inflammatory and fibrotic markers by blocking glucose transport into the PTC. SGLT2 inhibition didn't increase compensatory glucose transport through SGLT1. Although HG did not regulate the expression of SGLT2, the presence of TGFβ, a cytokine intrinsic to the development of diabetic nephropathy, may potentiate the ill effects of HG through upregulating SGLT2.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1555

Effects of Probiotic Ingestion on Renal Function and Oxidative Stress in Diabetic Rats Elisa M.S Higa,[1,4] Giovana Rita Punaro,[1] Fabiane Maciel,[1] Adelson Marçal Rodrigues,[1] Cristina S.B. Bogsan,[2] Marice Oliveira,[2] Marcelo Rogero.[3] *[1]Nephrology, UNIFESP, Brazil; [2]Biochemical Pharmaceutical Technology, USP, Brazil; [3]Nutrition Department, USP, Brazil; [4]Emergency Division, UNIFESP- EPM, Sao Paulo, Brazil.*

Background: Probiotics are defined as live microorganisms which could confer a health benefit, perhaps by interacting with the antioxidant system. Previous study in our Laboratory showed that after 8 weeks of DM induction in rats, the treatment with probiotic reduced oxidative stress and increased nitric oxide (NO), without significant changes in renal function.

The aim of this study is to assess the effects of early probiotic ingestion on the renal function and oxidative stress in diabetic rats.

Methods: DM was induced in male Wistar rats, with streptozotocin (45mg/kg, iv). The animals received probiotic (P) or its vehicle at 1.8 mL/d by gavage, in the 5th day of DM, during 8 weeks, being distributed in 4 groups (n=4 each): CTL; CTLP; DM; DMP. Before and after the treatment, 24 hour urine was collected to determine thiobarbituric acid reactive substances (TBARS) (nmol/24h), NO (µmol/24h) and proteinuria (mg/24h); plasma was obtained for urea (mg/dL). The results are presented as mean ±SEM, analyzed by one way ANOVA with Newman-Keuls post-test; significant for $P<0.05$.

Results: DM x CTL presented increased TBARS (293±19 x 81±2) and proteinuria (32 ±8 x 9±.7) and decreased NO (4±3 x 23±2); plasmatic urea was higher in DM (61±8 x 32±2). All P<0.05. After probiotic treatment in DM group there was a reduction of TBARS (247±17), proteinuria (16±2) and plasmatic urea (47±3) with increased NO (32±5), all P<0.05.

Some studies suggest the oxidative stress as well as the NO in the pathophysiology of diabetic nephropathy.

In fact, in this study, TBARS, an indicator of lipid peroxidation, was increased and NO was reduced in DM group. The use of probiotic attenuated these effects and, at the same time, reduced plasmatic urea in these animals.

Conclusions: Our study suggests that the early utilization of probiotic can protect against the DM deleterious effects on kidneys, by controlling the oxidative stress and recovering NO levels.

Funding: Government Support - Non-U.S.

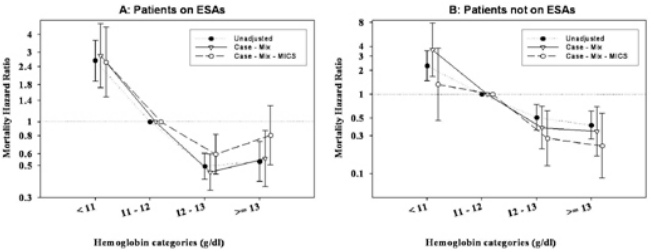
FR-PO1556

Hemoglobin Level and Survival in Hemodialysis Patients with and without Polycystic Kidney Disease: The Role of Administered Erythropoietin Anuja P. Shah,[1] Miklos Z. Molnar,[1,2] Lilia R. Lukowsky,[1] Joshua Zaritsky,[3] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]Salem VA Medical Center, Salem, VA.*

Background: Interventional trials indicate adverse outcomes when hemoglobin >13 g/dL is targeted in CKD patients who receive erythropoiesis stimulating agents (ESAs). It is not clear whether high achieved hemoglobin with minimal to no ESA such as in some polycystic kidney disease (PKD) patients (pts) is also associated with poor outcomes.

Methods: Survival models were examined to assess the hemoglobin-mortality association in a 6-year cohort of 110875 non-PKD and 2402 PKD hemodialysis pts across infrequent versus regular ESA therapy defined as ESA <25% of cohort time vs. otherwise, respectively.

Results: PKD and non-PKD pts were 58±13 & 62±15 years old & included 46% & 45% women. PKD pts had lower mortality than non-PKD within each hemoglobin level above 12 g/dL. In PKD pts, fully adjusted death HRs (95% confidence interval) of time-averaged hemoglobin increments <11.0, 12.0-<13.0, & ≥13.0 g/dL (reference: 11.0-<12.0 g/dL) for regular ESA therapy were 2.57 (1.48-4.48), 0.60 (0.43-0.82) & 0.81 (0.50-1.29); & for infrequent ESA therapy were 1.33 (0.47-3.78), 0.28 (0.13-0.61) & 0.22 (0.09-0.57), respectively.



In non-PKD pts, a similar trend towards higher death with hemoglobin>13g/dl was noticed with regular ESA dosing, although infrequent ESA therapy level did not modify the U-shaped Hb-death associations.

Conclusions: Achieved hemoglobin >13.0 g/dL exhibits a trend towards higher mortality in hemodialysis pts, which is not observed in PKD patient subgroup with infrequent ESA therapy. Whether ESA therapy leads to mortality of high hemoglobin warrants additional studies.

Funding: NIDDK Support

FR-PO1557

Sustained Erythropoiesis (6-30 Months) by the EPODURE Biopump in Patients with Chronic Kidney Disease: Further Results of Phase I/II Proof of Concept Trial Anatole Besarab,[1] Michal Elhalel,[2] Doron Schwartz,[3] Ehud Shoshani,[4] Andrew L. Pearlman,[4] Baruch Stern,[4] Philip Ng,[5] Allen R. Nissenson.[6] *[1]Henry Ford Hospital, Detroit, MI; [2]Hadassah Hospital, Jerusalem, Israel; [3]Sourasky Medical Center, Tel Aviv, Israel; [4]Medgenics, Inc, Misgav, Israel; [5]Baylor College of Medicine, Houston, TX; [6]DaVita, El Segundo, CA.*

Background: Sustained delivery of EPO maintaining levels 1-5 fold of normal could reduce risks of hemoglobin variability yet achieve recommended Hb targets, avoid supraphysiologic EPO concentrations, and increase patient compliance. The goal of EPODURE is to provide > 6 months of sustained EPO delivery from a single treatment using autologous 30mm x 2mm dermis core biopsies excised from the patient's skin under local anesthetic and converted in days into "biopump" EPO production units by introducing the EPO gene into cells of the intact explant. We reported (ASN 2010) the results in 12 patients treated up to 12 months with EPODURE Biopumps.

Methods: We now report results in 16 (8 EPO-naïve , 8 EPO-dependent) of a planned 18 CKD patients treated 6-30 months by 20, 40, or 60 IU/kg/day EPODURE implanted dose in an open label, dose ranging Phase I-II study in anemic CKD patients.

Results: SAFETY: No related SAEs were reported, EPO serum levels never exceeded 70 mU/ml, and all tests for anti-EPO antibodies were negative. Clinical feasibility was demonstrated, the brief procedure well tolerated.

EFFICACY: A single EPODURE administration elevated Hb levels for >3 mo in 14/16 and >6 mo in 10 /16, maintained Hb between 10-12 g/dl in 14/16 for >3 mo and 9/16 for >6mo, with longest >30 mo.

Where Hb declined it correlated with decreasing EPO levels, which peaked at 3 days post implantation. We suspect possible decline in EPO output in some biopumps, possibly due to suboptimal implantation. Improved implantation methods are now under study.

Conclusions: EPODURE is safe at doses up to 65 IU/kg/day is safe, a single administration in most patients can elevate Hb levels for 3-30 months and in appropriate dose maintain Hb in 10-12 g/dl range for up to 30 months. Further refinement in implantation methods to further increase average duration are underway.

Funding: Pharmaceutical Company Support

FR-PO1558

Effect of Vitamin B12 and Folic Acid Supplementation on Erythropoietin Requirements To Maintain Hemoglobin Concentrations – Clinical and Economic Outcomes at 2 Years John P. Killen. *Department of Renal Medicine, Cairns Base Hospital, Cairns, Queensland, Australia.*

Background: The deficiency of vitamin B12 is known to cause erythropoietin resistance in hemodialysis (HD) patients. B12 is a middle molecule that is effectively removed by modern dialysis membranes, leading to an increased risk of B12 deficiency in HD patients. The aim of this prospective observational study was to assess the effect of intramuscular B12 and oral folic acid supplementation on hemoglobin concentrations and erythropoietin requirements in HD patients over 2 years.

Methods: All HD patients were assessed during the study period of January 2009 to January 2011 for erythropoietin requirement. Hemoglobin, B12, red cell folate and ferritin concentrations and transferrin saturation were also assessed. From January 2009 to January 2010 all HD patients with a plasma concentration of vitamin B12 less than 300 pmol/L were offered weekly injections of hydroxocobalamin 1000 micrograms (Neo-B12®, Hospira Australia) intramuscularly for 3 weeks and oral folic acid supplementation 0.5 mg daily for the course of the study. The B12 treatment was repeated if levels again fell below 300 pmol/L during the study period. No change in intravenous iron protocols occured during the study. Exclusion criteria included transplantation, change to peritoneal dialysis, death and loss to follow up during the study period.

Results: 48 HD patients were eligible. Average B12 concentration rose from 216 to 487 pmol/L (p<0.0001) and red cell folate from 928 nmol/L to 1669 nmol/L (p<0.0001). Average erythropoietin usage reduced from 11300 IU/week to 6300 IU/week (p<0.0005) to maintain an average haemoglobin of 116 g/L. The average transferrin saturation did not change during the study. The average ferritin rose from 670 to 880 microg/L.

Conclusions: In this 2 year observational study, intramuscular vitamin B12 with oral folic acid supplementation reduced the average erythopoitin requirements in HD patients with a serum B12 concentration less than 300 pmol/L by 44% during the study period without a significant change in the average hemoglobin concentration. This translates to an annual cost saving of approximately $AUD4600 per HD patient in this group.

FR-PO1559

An Open, Randomized, Parallel Group, Multi-Center Study on the Prognosis of Hemodialysis Patients in Anemia Treatment by Combination Therapy with Iron and Vitamin C and Erythropoietin (ACTIVE Study)
Takahiro Kuragano, Takeshi Nakanishi. *Hyogo College of Medicine, Department of Internal Medicine, Division of Kidney and Dialysis, Nishinomiya, Hyogo, Japan.*

Background: Clinical trials demonstrated that higher levels of hemoglobin (Hb) do not necessarily translate into improved mortality patients with chronic kidney disease. As such, there has been an increasing focus on individualized therapy in the renal anemia treatment. We evaluated the effect of newly proposed protocol for anemia therapy on adverse events in patients undergoing maintenance hemodialysis (MHD).

Methods: Study design: Randomized parallel group multi-center study. Study period: 3 years. Patients: 266 MHD patients. Intervention group: For obtaining target Hb (10≤Hb<12) and ferritin (300>), doses of erythropoietin (EPO), iron and vitamin C (VC) were changed every month based on ferritin and Hb levels according to the ACTIVE protocol. Non-intervention group: Attending physician determined the doses of EPO and iron. Primary outcomes: Survival rate, hospitalization rate, infection and cardiovascular disease (CVD). Secondary outcomes: Comparison of Hb, ferritin, TSAT, and dose of EPO between the groups.

Results: A total of 45 composite events occurred (4 deaths, 31 hospitalizations, 3 infections, 7 CVD) during the period. There was no significant difference between the groups in the frequency of adverse events. The percentage of the patients who could maintain target Hb (78±6% vs. 57±13%, p<0.001) and ferritin (72±12% vs. 59±14%, p=0.048) levels was significantly higher in the intervention group than in the non-intervention group. The frequency of adverse events was significantly lower (p=0.023, Hazard risk: 0.296) in patients maintaining the target Hb (≥75%) during the period. In the intervention group, Hb, TSAT levels, and dose of EPO of the intervention group were significantly higher, and ferritin level was lower than that of the non-intervention group.

Conclusions: The ACTIVE protocol modifying the doses of EPO, iron and VC according to monthly Hb and ferritin levels of individual MHD patient could stabilize Hb and ferritin levels within the target range, which in turn could contribute to the lower frequency of adverse events.

FR-PO1560

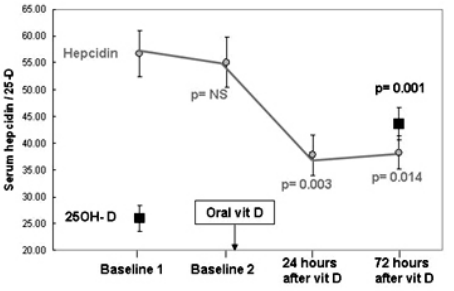
Vitamin D as a New Regulator of Iron Metabolism: Vitamin D Suppresses Hepcidin In Vitro and In Vivo
Justine Bacchetta,[1] Joshua Zaritsky,[1] Thomas S. Lisse,[1] Jessica L. Sea,[1] Rene Chun,[1] Elizabeta Nemeth,[1] Tomas Ganz,[1] Mark E. Westerman,[2] Isidro B. Salusky,[1] Martin Hewison.[1] *[1]David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Intrinsic LifeSciences, La Jolla, CA.*

Background: Recent reports have shown improved hemoglobin levels and decreased ESA doses with vitamin D repletion in CKD patients. We examined the effects of vitamin D on hepcidin (Hep), the iron-regulatory peptide hormone responsible for iron sequestration and anemia by posttranslational downregulation of ferroportin (Fp), the sole known exporter of iron from cells to the systemic circulation. In CKD, decreased renal clearance and inflammation increase Hep levels.

Methods: We utilized rtPCR techniques to assess Hep mRNA production and immunohistochemistry to stain for FP in peripheral blood mononuclear cells (PBMC) isolated from healthy donors and in monocytes (PDM) isolated from the dialysate of patients undergoing peritoneal dialysis.

Results: When treated with active 1,25-vitamin D (5 nM) or with precursor 25OH-vitamin D (100 nM) for 6 hours, PBMC and PDM showed decreased expression of Hep (fig). Chromatin immunoprecipitation revealed decreased recruitment of the RNA polymerase II within the promoter of the human Hep gene after treatment with 1,25D, pointing to direct effects of 1,25D on Hep transcription. Immunohistochemistry showed that PBMC and PDM expressed Fp, with membrane enhancement after treatment with 1,25D. Finally we observed a 50% decrease in serum hepcidin (ELISA) which persisted for 72 hours in 7 healthy human subjects after a single oral dose of vitamin D (100,000 IU).

Studied cells	Treatment	Fold-change hepcidin mRNA	P compared to vehicle
PBMC	25-D; 100 nM	0.87	NS
PBMC	1-25 D; 0.5 nM	0.42	< 0.05
PBMC	1-25 D; 5 nM	0.19	< 0.05
PD cells	25-D; 100 nM	0.57	< 0.05
PD cells	1-25 D; 5 nM	0.52	< 0.05



Conclusions: For the first time, these results in vitro and in vivo indicate that vitamin D is a potent suppressor of Hep in humans. These findings provide a clinically relevant mechanism by which vitamin D supplementation can improve anemia management in CKD.

Funding: NIDDK Support, Private Foundation Support

FR-PO1561

Early L-Carnitine Treatment in Incident Hemodialysis Patients: Randomized, Double Blind, Placebo-Controlled Trial. CARNIDIAL NCT 00322322
Lucile Mercadal,[1] Mathieu Coudert,[2] Anne Vassault,[3] Laurence Pieroni,[3] Messaoud Ouziala,[4] Hélène De Préneuf,[5] Aude Servais,[6] Nader Bassilios,[7] Ubald Assogba,[8] Gilbert Deray.[1] *[1]Nephrology, AP-HP, Pitie Salpetriere, Paris, France; [2]Biostatistics, AP-HP; [3]Biology, AP-HP; [4]CMC Pantin; [5]AURA, Paris; [6]Nephrology, AP-HP, Necker; [7]Clinique de Turin; [8]Clinique des Mousseaux.*

Background: Carnitine level prematurely decreases with hemodialysis vintage. A supplementation has been recommended in hemodialysis patients with carnitine deficiency and erythropoietin (rHuEPO) hypo responsiveness. Our hypothesis was that an early L-carnitine (LC) supplementation before the occurrence of a carnitine deficiency, could improve rHuEPO responsiveness.

Methods: Patients hemodialyzed for less than 6-months were randomized to receive either placebo or 1 gr LC after each dialysis session for a 1 year inclusion period. Primary outcome compared rHuEPO resistance index (RI) defined as weekly rHuEPO (UI/kg body weight) / hemoglobin level (g/dL) between groups. Study was double blinded for treatment and carnitine level.

Results: 92 patients hemodialyzed since 39 ± 27 days were randomized. At baseline, LC group had a higher RI partly explained by a higher CRP and a lower albuminemia in this group. Total plasmatic carnitine levels rose from 79 ± 51 to 258 ± 137 µmol/L at month 12 in the LC group whereas it fell from 68 ± 25 to 53 ± 24 µmol/L in the placebo group (x 0.77 placebo group, x 3.01 LC group; p<0.0001). Difference reached statistical significance at month 3. Free/total carnitine ratio was similar between groups and didn't vary during the study period(0.78 ± 0.09 to 0.77 ± 0.09 placebo group; 0.76 ± 0.10 to 0.73 ± 0.08 LC group). RI steadily improved in both groups from 15.8 ± 11.3 to 9.5 ± 5.8 UI/kg per g/dL in placebo group, and from 20.6 ± 12.8 to 15.6 ± 15.6 UI/kg per g/dL in the LC group (baseline-month12 difference between groups, ns). After adjustment for baseline characteristics, both groups had similar RI during the study period.

Conclusions: We confirm a 30% decline of carnitine level during the first dialysis year. An early treatment by L-carnitine does not improve rHuEPO responsiveness.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1562

Novel Approaches To Treat Anemia in Chronic Kidney Disease
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Background: Anemia is prevalent in patients with chronic kidney disease (CKD). An excess of the iron regulatory hormone hepcidin may contribute to the anemia of CKD by limiting iron availability for red blood cell production. Hepcidin decreases iron absorption from the diet and promotes iron sequestration in macrophage stores by downregulating the iron exporter ferroportin. Hepcidin is thought to accumulate in CKD patients due to reduced renal clearance and inflammation.

We have demonstrated a central role for the bone morphogenetic protein (BMP) signaling pathway, via the ligand BMP6 and the co-receptor hemojuvelin (HJV), in regulating hepcidin expression and systemic iron balance. While cell-surface HJV acts as a BMP co-receptor to increase hepcidin, soluble HJV (sHJV) inhibits BMP signaling

and hepcidin expression, presumably by sequestering BMP ligands. Importantly, proinflammatory cytokines require BMP signaling to upregulate hepcidin expression, suggesting that BMP inhibitors may be promising candidates as hepcidin lowering agents to treat anemia of inflammation, including anemia of CKD.

Methods: Here, we investigate the selectivity of sHJV as a BMP inhibitor by quantitating its binding affinity for various BMP ligands using Surface Plasmon Resonance, and we test the effects of BMP inhibitors in 3 rodent anemia models.

Results: We show that sHJV has the highest binding affinity for BMP6, while it has lower affinity for BMP7 and does not bind BMP9. sHJV and the small molecule BMP inhibitor LDN-193189 lower hepcidin expression, mobilize macrophage iron stores, and improve anemia in a PG-APS rat model of anemia of inflammation and a genetic model of anemia due to hepcidin excess (Tmprss6-/- mice). Finally, we investigate the use of these BMP inhibitors in an adenine rat model of anemia of CKD.

Conclusions: Together, our data suggest the possible utility of BMP inhibitors as hepcidin lowering agents to treat anemia of CKD.

Funding: NIDDK Support

FR-PO1563

Impact of Cholecalciferol Repletion on Erythropoietin Requirements in Vitamin D-Deficient Hemodialysis Patients: Pilot Data from a Randomized Controlled Trial Anita Mehrotra, Maria Krassilnikova, Brian D. Radbill, Peter S. Heeger. *Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Vitamin D deficiency is common in hemodialysis patients. Uncontrolled studies suggest that correction of Vit D deficiency with ergocalciferol (D2), cholecalciferol (D3), and calcitriol is associated with decreased erythropoietin (EPO) requirements.

Methods: To better characterize the relationship between Vit D deficiency/repletion and EPO requirements, we examined the impact of D3 repletion on EPO requirements in 79 Vit D-deficient (25OH-D <25 ng/mL) hemodialysis patients randomized to receive D3 (n=51) or standard of care (no repletion, n=28) in a 2:1 ratio. Patients randomized to treatment with D3 received 50,000 IU/wk to a goal 25OH-D of >35ng/mL, followed by 10,000 IU/wk. Changes in 25OH-D, hemoglobin (Hb), and EPO requirements were assessed at 3 months. EPO (Darbepoetin) doses were adjusted by the nursing staff as per the dialysis unit protocol (for target Hb 10-12 g/dL) independent of D3 administration.

Results: Baseline demographic characteristics (age, race, sex) were similar between both groups, as were baseline Vit D levels (median 13.5 in treatment group vs 13.1 in control group, p=0.623), baseline Hb (mean Hb 11.8 g/dL in treatment group vs 11.4 g/dL in control group, p=0.155), and baseline EPO requirements (median Darbepoetin dose 40 units/wk in treatment group vs 50 units/wk in control group, p=0.262). 45 patients had 3 month follow-up data available. Patients randomized to D3 treatment had a rise in 25OH-D at 3 months (11.9 to 44.1 ng/mL, p<0.001, n=30), with a corresponding fall in EPO requirements (50.00 to 40.32 units/wk, p=0.029, n=30) despite no change in Hb (11.9 to 11.5 g/dL, p=0.180, n=30). No change in Vit D level, Hb, or EPO dose was observed in control patients at 3 months (n=15). Patients randomized to D3 did not experience hypercalcemia or other adverse events.

Conclusions: Our preliminary data from this ongoing randomized controlled trial suggest that treatment of Vit D deficient dialysis patients with D3 is safe, effective, and may result in lower EPO requirements. If these results are confirmed, the cost-savings may be significant.

Funding: Other NIH Support - T32 DK007757-12, Private Foundation Support

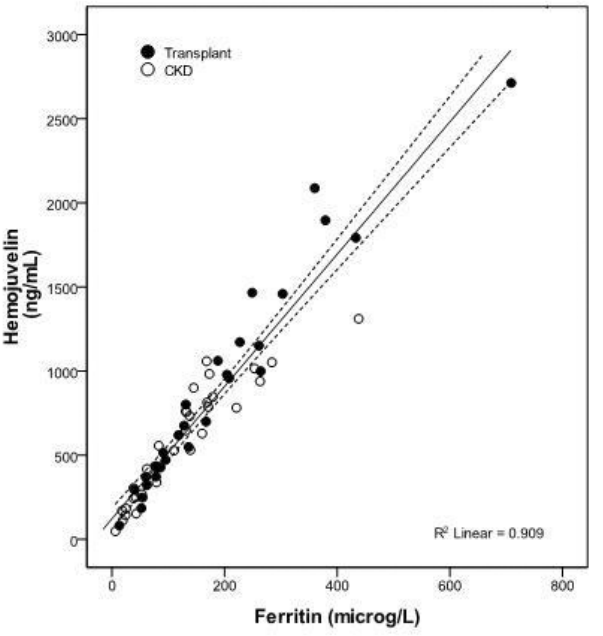
FR-PO1564

Serum Hemojuvelin and Ferritin Levels Are Tightly Correlated in CKD Patients Adam Rumjon,[1] Pantelis Sarafidis,[1] Jolanta Malyszko,[2] Iain C. Macdougall.[1] *[1]King's College Hospital; [2]Medical Academy, Bialystok.*

Background: Hemojuvelin (HJV) is a recently discovered protein involved in the cellular regulation of hepcidin. Both HJV and hepcidin are emerging as crucial players in the complex biology of iron homeostasis. It is known that membrane-bound and soluble forms of HJV exist, but their exact actions remain uncertain. Hepcidin levels are known to be elevated in CKD and dialysis patients, but this is the first report of serum HJV levels in a renal population.

Methods: 93 patients were studied (31 HD, 31 transplant, and 31 CKD; age- and gender-matched). Blood samples were taken for measurement of serum HJV, hepcidin, ferritin, IL-6, CRP, and standard hematological parameters. HJV levels were measured using an ELISA (USCN Life Science Inc, Wuhan, China) by an operator blinded to the clinical details. Hepcidin levels were determined by mass spectrometry, using hepcidin-25 as an internal standard.

Results: HJV levels (ng/ml) were highest in HD (2619±1445) followed by transplant (870±638), and then CKD patients (590±344). HJV and hepcidin were moderately correlated in CKD (r=0.641, p<0.001) and transplant (r=0.569, p=0.001), but not in HD patients (r=0.112, p=0.570). The correlation of HJV with ferritin was very tight in all 3 groups; CKD (r=0.918, p<0.001), transplant (r=0.969, p<0.001), HD (r=0.794, p<0.001). A correlation between HJV and Hb was seen only in transplant patients (r=-0.407, p=0.028); HJV did not correlate with IL-6 or CRP.



Conclusions: This is the first time that serum hemojuvelin levels have been examined in a CKD population. The tight correlation between HJV and ferritin was unexpected, but consistent across all 3 sub-populations studied. Although a role for HJV in the cellular regulation of hepcidin has been established, clinical data are lacking. The results presented here suggest that this is worthy of further study.

FR-PO1565

Similar Mortality in Hemodialysis Patients with Hemoglobin 9 to 9.9 Versus 10 to 10.9 A. Gul,[1] O. Myers,[1] Bruce L. Horowitz,[1] Edward J. Bedrick,[1] Antonia M. Harford,[1] Philip Zager.[1,2] *[1]Internal Medicine, UNMHSC, Albuquerque, NM; [2]Dialysis Clinic, Inc., Albuquerque, NM.*

Background: High epoetin (EPO) doses have been associated with increased mortality in hemodialysis (HD) patients. This finding, in conjunction, with the 'bundle', have led opinion leaders to recommend reducing the target hemoglobin (Hb) from 10-12 to 9-11. However, prior to implementing a randomized controlled trial to assess the safety and efficacy of the proposed change, a preliminary comparative effectiveness study comparing outcomes in patients with Hb of 9-9.9 versus 10-10.9 is needed. The present study explored the hypothesis that all-cause mortality is similar in patients with Hb levels of 9-9.9 versus 10-10.9.

Methods: We studied an incident cohort of 8365 patients, who began HD in DCI facilities between 2006-2009 and survived ≥150 days. We used Cox models to assess the relationship between the most recent Hb value, excluding a 30-day lag, and mortality. Baseline covariates included age, sex, race, cause of ESRD and vintage. Time-varying covariates included pre-dialysis systolic blood pressure, albumin, creatinine, Kt/V, TSAT, ferritin, BMI, vascular access and iron dose.

Results: There were 1867 deaths. Mortality in patients with Hb 9.0–9.9 (HR = 0.97; 95% CI 0.79-1.19) was similar to the referent group (Hb 10.0-10.9). Mortality was lower among patients with Hb ≥ 11-11.9 (HR 0.76; 95% CI 0.67-0.87) versus the referent group. Conversely, mortality tended to be higher in patients with Hb <9.0 versus the referent group (HR = 1.20; 95% CI 0.92-1.57). In a sensitivity analysis, addition of EPO dose to the model did not significantly change the hazard ratios. EPO doses were highest in patients with Hb values < 9.0. EPO doses ≥20,000 units/week, were associated with increased mortality versus the EPO referent group (8,000- 12,499 units/week) (HR = 1.18 95% CI 1.02 – 1.36).

Conclusions: Mortality among patients with Hb 9-9.9 was similar to that in the referent group (Hb 10-10.9). These observational data suggest that it may be safe and feasible to conduct a pilot study comparing Hb targets 9-11 versus 10-12. EPO resistance may be a significant risk factor for mortality.

Funding: Clinical Revenue Support

FR-PO1566

TSAT and Serum Ferritin Increases Observed in Ferric Citrate Clinical Trials May Lead to Dialysis Cost Savings Jaime Rubin,[1] T. Christopher Bond,[1] Steven Wang,[1] Robert M. Niecestro,[2] Enrique Poradosu,[2] Tracy Jack Mayne.[1] *[1]DaVita Clinical Research, Minneapolis, MN; [2]Keryx Biopharmaceuticals, New York, NY.*

Background: Ferric citrate, a novel, investigational phosphate binder for the treatment of hyperphosphatemia in dialysis patients (pts), has been shown in clinical trials to increase serum ferritin (SF) and saturated transferrin (TSAT) and reduce erythropoietin stimulating agents (ESAs) and intravenous (IV) iron (Fe) use. We developed a cost-offset model

quantifying potential cost-savings associated with ESA and Fe reductions observed in moderate (M)/high (H) ESA use-pts experiencing equivalent increases in Fe markers.

Methods: We constructed a cost-offset model of M(4500 to <9000U/session) and H(≥9000U/session) ESA users over a 2 mo time horizon from a payor perspective. Unit costs for phosphate binders (lanthanum carbonate, sevelamer, calcium acetate), ESAs and IV Fe were derived from 2011 published sources. Monthly ESA (M=10,400U; H=27,480U) and Fe (M=132mg; H=61mg) dose reductions resulting from concurrent TSAT (≥ 10%) and SF (15-25%) increases were derived from a DaVita database. We assumed equal phosphorus outcomes and price for ferric citrate and comparator binders.

Results: Given equivalent phosphorus outcomes and price, ferric citrate would potentially generate monthly cost savings of M:$123 and H:$315 due to reductions in ESA (M:$102; H:$268) and IV Fe (M$22; H:$47); reducing ESA costs by 15% and 21% per mo, respectively. Ferric citrate would potentially save $630/high-use pt/2 mo. For the average dialysis clinic with 80 pts and 50% M/H ESA users, the monthly savings would be $17,500 for these pts. When the expected per-session reduction in ESA and IV Fe dose was reduced by half, the expected cost savings would be reduced proportionally.

Conclusions: These results indicate that the rises in TSAT and SF observed with the investigational drug ferric citrate in clinical trials may produce significant reductions in monthly ESA and IV Fe costs, generating meaningful savings under the Medicare bundled dialysis payment. Sustained rises in TSAT and SF over a longer duration would potentially lead to considerable annual cost-savings.

Funding: Pharmaceutical Company Support

FR-PO1567

The Impact of Frequent In-Center Versus Conventional Hemodialysis on Anemia: The Frequent Hemodialysis Network Trial

Daniel B. Ornt,[1] Alan S. Kliger,[2] Rita Suri,[3] Mohamad Akram Rashid,[4] Anjay Rastogi,[4] Manjula Kurella Tamura,[6] Brett Larive,[7] John T. Daugirdas,[8] Tom H. Greene,[9] Nathan W. Levin,[10] The FHN Trial Group.[11] *[1]CaseWestern Reserve U.; [2]St. Raphael and Yale U.; [3]U. of Western Ontario; [4]UCLA; [5]UCLA; [6]Stanford U.; [7]Cleveland Clinic; [8]U. of Illinois; [9]U. Utah; [10]Renal Research In.; [11]NIDDK.*

Background: Management of anemia in hemodialysis (HD) patients remains complex and costly. The Frequent HD Network (FHN) Daily prospective, randomized trial demonstrated significantly reduced left ventricular hypertrophy and improved physical-health composite scores for patients receiving 1 year of 6 times/week HD (6X) compared to 3 times (3X). We also hypothesized that 6X HD would improve management by reducing use of erythropoietin-stimulating agents (ESA) (pre-specified main secondary outcome) and/or improving hemoglobin (Hb) levels.

Methods: Hb was measured monthly and other anemia related measures (iron (Fe), transferrin saturation, and ferritin), were obtained at baseline (B) and follow-up months 4, 8 and 12. ESA and IV Fe administered were reported as total 4-week dose. We report treatment comparisons of changes from B to month 12 obtained using mixed effects models, with log transformations applied to ESA and IV Fe.

Results: Hb increased slightly in the 6X (n=125) group vs. 3X (n=120) (0.3; CI 0.0 to 0.6, p=.02), but both groups had mean Hb levels in the appropriate clinical range at month 12 (11.7 to 12.0 mg/dL). Geometric mean ESA-equivalent dose declined 16% more in the 6X group vs. 3X, but this difference was not statistically significant (95% CI: 35% decrease to 8% increase, p = 0.18). The change in mean ESA/Hb also did not differ significantly between the 6X and 3X groups (19% greater decrease in 6x, 95% CI: 38% decrease to 6% increase, p = 0.12). There were no significant differences in IV Fe or Fe stores between the groups.

Conclusions: In the FHN Daily trial, the frequent HD intervention had no significant effect on either ESA or ESA/Hb. Consistent with some observational studies; the 6X intervention did lead to a small increase in Hb level. The implications of this finding remain under investigation.

Funding: NIDDK Support, Other U.S. Government Support

FR-PO1568

Patterns of Anemia Management in U.S. Hemodialysis Patients (2002-2008)

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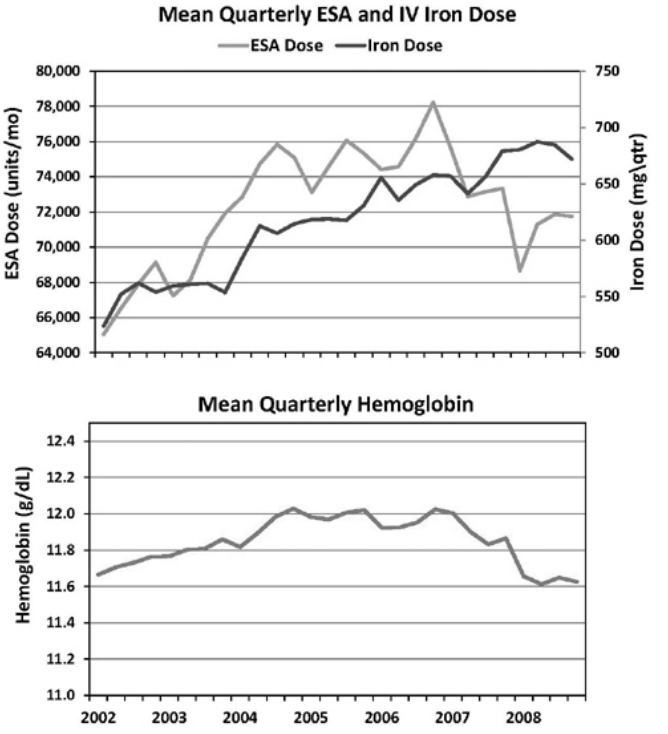
Background: Current data on patterns of anemia management, particularly in regard to iron use, are lacking in the hemodialysis (HD) population. Such information will further our understanding of changes in anemia management in response to changing clinical guidelines and safety concerns raised from high hemoglobin (Hb) target studies using erythropoiesis-stimulating agent (ESA) therapy; and will provide baseline data for future studies examining the effect of the new dialysis bundled payment system on anemia management.

Methods: USRDS data (2002-2008) on prevalent, Medicare HD patients receiving ESA therapy were examined. For each patient, receipt of intravenous (IV) iron, total IV iron dose, total ESA dose/month, and Hb values were determined. These data were then summarized by calendar quarter (percentage or mean) and plotted for the entire sample and by demographic (sex, age, race) and clinical (cause of ESRD, dialysis vintage) subgroups.

Results: Among ~250,000 HD patients/year, quarterly iron use increased from approximately 65% in 2002 (Q1) to 78% in the 2008 (Q4). Mean quarterly iron dose increased from approximately 525 mg in 2002 to 675 mg in 2008. Mean quarterly ESA dose/month increased from 2002 to 2006 and then began to decline. Similar patterns were observed for Hb values.



The same patterns in iron and ESA doses and Hb were observed across demographic and clinical subgroups, but there were important differences between subgroups in the amount of iron and ESA received. These differences were most notable among black versus white and shorter versus longer dialysis vintage subgroups.

Conclusions: Anemia management patterns have changed markedly between 2002-2008 with a steady increase in IV iron use even after declines in ESA dose and Hb. The clinical impact of these changes need further study.

Funding: Pharmaceutical Company Support

FR-PO1569

Treatment with Hemodiafiltration (HDF) Improves ESA Responsiveness over 12 Months in Patients with High ESA Resistance without a Concomitant Reduction in Predialysis Hepcidin-25 (Hep25) Levels

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Background: In chronic Hemodialysis (HD) patients with high resistance to erythropoiesis stimulating agents (ESA; expressed as an ESA index [ESA dose/weight/Hct/wk]), treatment with online HDF may improve ESA responsiveness. One of the possible mechanisms is lowering of Hep25 by HDF. The aim of this analysis was to investigate whether the decreased ESA resistance in ESA resistant patients treated with online HDF is correlated with a change in predialysis Hep25 levels, as compared to those treated with conventional low-flux HD.

Methods: Baseline and 12 months' data from 82 prevalent HD patients in the highest tertile of ESA resistance who were included in the randomized controlled CONvective TRAnsport STudy (CONTRAST; NCT00205556), were analyzed (50% male, age 63.2±13.7 [mean±SD]). Predialysis levels of Hep25 were measured with mass spectrometry.

Results: In the HDF group (n=40), both the ESA index (-0.27 DDD/kg/Hct/wk [-0.38 to -0.15]; mean [95%CI]) and the soluble transferrin receptor (sTfR, -0.33 mg/L [-0.56 to -0.10]) decreased significantly over 12 months, with no changes in the HD group. Cumulative doses of iron supplements and ferritin were similar in both groups. In the HDF group, the transferrin saturation ratio increased over time with 0.43 (0.06 to 0.80). Hep25 increased non-significantly in both groups (HDF: 4.3 mg/L [-0.1 to 8.7]); HD: 1.4 [-3.9 to 6.6]; p=0.40). The Hep25/sTfR ratio increased in the HDF group (p=0.02) and remained unaltered in HD patients.

Conclusions: These data suggest either that ESA resistance is not primarily mediated by Hep25, or that treatment with HDF results in a temporarily decrease in Hep25 levels resulting in improved iron availability, ultimately leading to a new balance between Hep25 and sTfR .

FR-PO1570

Rate of Hemoglobin (Hb) Decline Following Peginesatide Dose Interruption

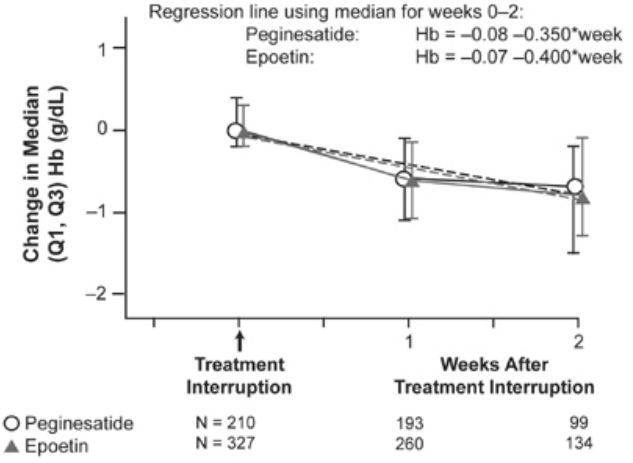
Anatole Besarab,[1] Francesco Locatelli,[1] Steven Fishbane,[1] Nathan W. Levin,[1] Carol Francisco,[2] Hong-Ye Gao,[2] Vandana S. Mathur,[2] Alex Yang,[2] Anne-Marie Duliege,[2] Krishna R. Polu.[2] *[1]AFX-01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc., Palo Alto, CA.*

Background: The rate of Hb decline after ESA dose interruption is likely due to intrinsic factors such as RBC lifespan rather than the effect of the ESA used on erythropoiesis (Locatelli, 2001). Similar rates of Hb decline are reported for various ESAs (Barany, 2007), despite differences in pharmacokinetics. Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based ESA that is designed to specifically stimulate the erythropoietin receptor. This analysis characterizes Hb decline after ESA dose interruption in patients on dialysis.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials (N=1608) assessing safety and efficacy of peginesatide (once monthly) compared with epoetin alfa/beta (epoetin; 1-3 times weekly) in hemodialysis patients (EMERALD 1 and 2). Doses were titrated to Hb levels of 10-12 g/dL. Protocol-specified thresholds for dose interruption (delay by ≥1 week) due to elevated Hb levels were: Hb ≥13.0 g/dL for peginesatide and ≥12.5 g/dL for epoetin. The rate of Hb decline was estimated from linear regression after a dose interruption occurred.

Results: This analysis included 210 of 1066 patients on peginesatide and 327 of 542 patients on epoetin who had ≥1 protocol-specified dose interruption (median Hb = 13.3 g/dL vs 12.9 g/dL at time of dose hold, respectively). The rate of median Hb change was -0.35 g/dL/wk for peginesatide and -0.40 g/dL/wk for epoetin (Figure). Mean time from dose interruption until resumed dosing was ~3 wks for both groups (>75% of patients reinitiated the ESA within 4 wks).

Figure. Change in Median Hb After First Dose Interruption



Conclusions: Rates of Hb decline after dose interruption were similar for peginesatide and epoetin.

Funding: Pharmaceutical Company Support

FR-PO1571

Safety Results from Two Phase 3 Studies of Peginesatide Treatment for Anemia in Hemodialysis (HD) Patients

Francesco Locatelli,[1] Steven Fishbane,[1] Iain C. Macdougall,[1] Andrzej Wiecek,[1] Adrian Constantin Covic,[1] Hina Patel,[2] Daniel S. Cooper,[2] Helen Tang,[2] Minjia Chen,[2] Anne-Marie Duliege,[2] Martha Mayo,[2] Krishna R. Polu.[2] *[1]AFX-01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc, Palo Alto, CA.*

Background: Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that acts via stimulation of the erythropoietin receptor. Key adverse events (AEs) including those associated with the ESA class in HD patients are reported here.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials evaluating the safety and efficacy of peginesatide (once monthly) compared with epoetin alfa/beta (epoetin; 1-3 times weekly) in HD patients (EMERALD 1 and 2). A primary analysis of cardiovascular (CV) events adjudicated by an independent Event Review Committee showed similar rates in the peginesatide and epoetin groups; here nonadjudicated CV events and ESA class AEs were evaluated.

Results: A similar number of patients in the peginesatide and epoetin groups had AEs (94.6% vs 93.0%), serious AEs (53.7% vs 57.0%), nonadjudicated CV AEs, and ESA class AEs (Table). Adjusted on-study mortality rates were also similar for the two groups (8.7 vs 9.5 deaths per 100 patient follow-up years). No clinically relevant differences in laboratory parameters, including platelet counts, or blood pressure levels were observed between treatment groups.

Event, n (%)	Peginesatide (n = 1066)	Epoetin (n = 542)
Cerebrovascular Disorders	45 (4.2%)	40 (7.4%)
Cardiac Failure	229 (21.5%)	116 (21.4%)
Cardiac Arrhythmias	210 (19.7%)	123 (22.7%)
Ischemic Heart Disease*	118 (11.1%)	67 (12.4%)
Hypertension	208 (19.5%)	101 (18.6%)
Thromboembolic Events		
Arterial	71 (6.7%)	48 (8.9%)
Venous	21 (2.0%)	9 (1.7%)
Vascular access complications	193 (18.1%)	107 (19.7%)
Convulsions	23 (2.2%)	11 (2.0%)
Infusion/Injection-related Reactions	32 (3.0%)	11 (2.0%)
Gastrointestinal Hemorrhage	71 (6.7%)	47 (8.7%)
Malignancy	41 (3.8%)	23 (4.2%)

*Includes myocardial infarction and unstable angina.

Conclusions: Once-monthly peginesatide had similar frequencies of AEs including CV and ESA class AEs compared to epoetin in HD patients.

Funding: Pharmaceutical Company Support

FR-PO1572

Greater Rise in Percent Hemoglobin < 10 g/dL among Black Patients with Implementation of the New US Bundled Dialysis Payment System: Initial Results from the DOPPS Practice Monitor

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Background: A new bundled Medicare ESRD prospective payment system (PPS) for dialysis services was implemented in the U.S. in Jan 2011. The US Government Accountability Office (GAO) urged prompt monitoring of the PPS, cautioning that some patients may be adversely affected by provider responses to the PPS. We report initial trends in anemia management in blacks vs. non-black with PPS implementation.

Methods: The Dialysis Outcomes and Practice Patterns Study (DOPPS) launched the nationally representative DOPPS Practice Monitor (DPM; www.dopps.org/dpm) for timely, public reporting of US hemodialysis (HD) practice trends during implementation of the PPS and quality incentive program (QIP). Linear regression analyses were based on monthly cross-sectional data from US dialysis units between July 2010 to Feb 2011.

Results: During the 8 months, mean Hgb level fell by 0.4 g/dL in blacks vs 0.1 g/dL in other patients (p=0.01). The proportion of patients with Hgb <10 g/dL rose from 6.6% to 11.3% in blacks vs little change in other patients (p=0.02). Mean EPO dose declined similarly among black vs other patients (-204 vs -164 U/mo; p=0.76). The proportion of patients prescribed IV iron use rose 1.3-1.5%/mo in both groups; mean IV iron dose remained steady and did not differ by race. >98% of EPO was given by IV during each month.

Distribution of monthly anemia measurements in US-DOPPS

Month	Mean Hgb (g/dL)		Hgb <10 g/dL (%)		IV iron use (%)		IV epoetin (U/wk)	
	Black	Non-Black	Black	Non-Black	Black	Non-Black	Black	Non-Black
Mean N	854	1,849	854	1,849	642	1,373	691	1,393
Jul 2010	11.66	11.46	6.6	9.2	73.8	72.9	18,815	16,495
Aug 2010	11.61	11.46	8.0	8.5	75.1	73.5	18,802	16,841
Sep 2010	11.46	11.51	9.0	8.2	72.4	74.8	19,133	17,651
Oct 2010	11.48	11.47	10.1	9.0	74.1	74.4	17,946	16,204
Nov 2010	11.45	11.44	8.5	8.4	79.5	77.5	17,719	15,372
Dec 2010	11.35	11.44	9.2	9.4	78.0	78.9	17,285	15,320
Jan 2011	11.28	11.37	10.1	8.3	80.3	82.1	17,828	16,034
Feb 2011	11.22	11.36	11.3	8.4	83.0	83.1	17,805	16,207

Hgb=based on single measurement during month; IV iron use=any IV iron use during the 3-month period ending with the month shown; IV epoetin=actual EPO administered over the prior 30 day period expressed as a weekly average. Trend for "Mean Hgb (g/dL)" in blacks=-0.047/mo (95% CI: -0.071, -0.024); in non-Blacks=-0.018/mo (95% CI: -0.03, -0.005). Trend for "Hgb <10 g/dL (%)" in blacks=0.49/mo (95% CI: 0.07, 0.91); in non-Blacks=-0.04/mo (95% CI: -0.33, 0.25).

Conclusions: During this PPS transition period, changes in anemia management have led to a greater rise in % of patients with Hgb <10 g/dL and larger decline in mean Hgb in black vs other patients. Continued monitoring of these practice trends and effect on transfusion rates and other clinical outcomes is warranted.

Funding: Pharmaceutical Company Support

FR-PO1573

The Safety of Feraheme® (ferumoxytol) in Hemodialysis Patients at Three Dialysis Chains over a One Year Period

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Background: Feraheme® (ferumoxytol) is an IV iron approved in June 2009 for the treatment of iron deficiency anemia (IDA) in adult patients with chronic kidney disease (CKD). The aim of this study was to characterize the safety profile of ferumoxytol as administered in a real-world setting.

Methods: Adverse events (AE) following treatment were tracked by the medical staff at each of the sites across the three dialysis chains. Any patient who received any dose of ferumoxytol from January through December 2010 was included in this analysis. To standardize the reported AE terms, AEs were coded using a standard drug safety coding

convention (MedDRA Version 13). All AEs were cross-checked against post-marketing safety reports received by AMAG to ensure all AEs were captured.

Results: Overall, 8,666 CKD patients were administered a total of 33,358 doses of ferumoxytol at these three dialysis chains. The table below displays the rates of three AE categories calculated on a per-patient and on an event-per-exposure basis.

	Total Subjects (N=8,666)	Total Exposures (N=33,358)
	Subjects (%)	Events (%)
AEs	108 (1.25)	330 (0.99)
Serious AEs	18 (0.21)	45 (0.13)
AEs leading to drug discontinuation	49 (0.57)	NA

The overall rates of AEs, SAEs, and AEs leading to ferumoxytol discontinuation were low. The most common SAEs (>2 subjects) were hypotension (0.12%), hypersensitivity (0.06%), dyspnea (0.05%), and loss of consciousness (0.03%). The frequency or severity of AEs did not increase among patients receiving two or more courses of ferumoxytol.

Conclusions: Based on a one year observation period across three dialysis chains involving 8,666 patients treated with 33,358 doses of ferumoxytol, the AE profile was consistent in frequency and severity with data from clinical trials. These long-term data with repeat dosing in a large number of hemodialysis patients confirm the safety for the treatment of IDA in patients with CKD on hemodialysis.

FR-PO1574

Hepcidin-25 (Hep25) Is a Biomarker of Iron Stores and Erythropoiesis in Chronic Hemodialysis (HD) Patients, with an Important Role of Residual Kidney Function Neelke C. Van Der Weerd,[1] Muriel Grooteman,[1] Peter J. Blankestijn,[2] Michiel Bots,[2] Marinus A. Van Den Dorpel,[3] Claire H. Den Hoedt,[3] Albert H. Mazairac,[2] Menso Nube,[1] Erik L. Penne,[2] Jack F. Wetzels,[4] Dorine W. Swinkels,[4] Pieter M. Ter Wee.[1] *[1]VU Medical Center, Amsterdam, Netherlands; [2]University Medical Center, Utrecht, Netherlands; [3]Maasstad Hospital, Rotterdam, Netherlands; [4]Radboud University Medical Center, Nijmegen, Netherlands.*

Background: Hep25, the active form of hepcidin, is a key regulator of iron homeostasis and is increased in chronic HD patients. Little is known about patient-, laboratory- and treatment related factors that influence Hep25 levels. Therefore, in the current study, potential determinants of Hep25 were assessed in a large cohort of stable, chronic HD patients.

Methods: Baseline data were studied from 405 patients (62% male; age 63.7 ± 13.9 [mean ± SD]) enrolled in the CONvective TRAnsport STudy (CONTRAST; NCT00205556), from whom additional blood samples were available. Hep25 was measured with mass spectrometry. Patient- (gender, age, dialysis vintage, diabetes, body mass index, residual kidney function [eGFR]), laboratory- (hemoglobin, ferritin, transferrin saturation, soluble transferrin receptor [sTfR], albumin) and treatment related characteristics (spKt/V, dose of erythropoiesis stimulating agents [ESA], use of iron therapy) were entered in a multivariable linear regression model if they showed a univariable relation ($p<0.15$) with log-transformed Hep25. All models were adjusted for participating center.

Results: Hep25 levels were independently positively associated with ferritin (per 10 ng/mL; B=0.02; $p<0.001$) and negatively with eGFR (per ml/min/1.73m2; B=-0.04; p=0.003) and sTfR (per mg/L; B=-0.33; $p<0.001$). An inverse relation with ESA dose was present only in the univariable analysis (p=0.05).

Conclusions: These findings confirm the vital role of hepcidin in the iron mobilization in chronic HD patients and underscore the importance of residual kidney function in these patients.

FR-PO1575

Treatment of Confirmed B12 Deficiency in Hemodialysis Patients Improves Epogen Requirements Norbert Shtaynberg, Majed Samarneh, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: Vitamin B12 deficiency may have deleterious effects on end stage renal disease (ESRD) patients on maintenance hemodialysis, and may increase erythropoietin stimulating agent (ESA) resistance, yet little is known about its' prevalence in this population.

Methods: Serum Vitamin B12 and MMA levels were drawn from ESRD patients prior to hemodialysis. All patients with MMA levels greater than 800nmol/L had peripheral smears evaluated for B12 deficiency. Those with confirmatory smears were considered to be deficient and received intramuscular vitamin B12 injections for four months. Post treatment MMA levels and smears were obtained. Erythropoietin dosages were monitored throughout the treatment period.

Results: There was a 58% (60/103) prevalence of vitamin B12 deficiency as defined by a positive MMA level and a positive blood smear. Out of 52 patients with positive smears, 36 (69.2%) were negative on repeat analysis after B12 treatment.

Mean epogen (EPO) dosages significantly decreased by 16,572 ± 41,902 units per month from baseline to the post-B12 treatment period (p=.0082, Wilcoxon Signed Rank test). Three months prior to treatment, the mean monthly EPO dose was 82,067 ± 47,906 and post, the mean EPO usage was 65,495 ± 39,691. Post treatment hemoglobin levels were not significantly different from baseline. Supplementation was also noted to improve social function (p=0.047).

Conclusions: Vitamin B12 supplementation was associated with a decrease in the mean dose of ESA administration while maintaining a stable hemoglobin level. Maintaining serum vitamin B12 levels improves functionality, and may allow a decrease in the use of ESA's, avoiding their toxicities and significant costs.

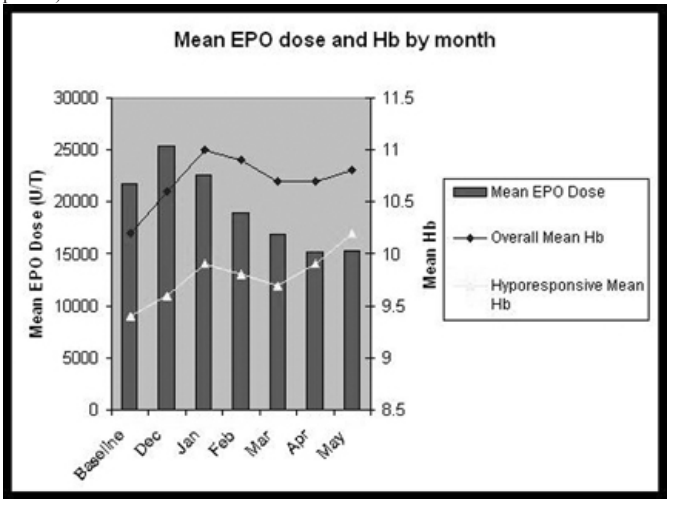
FR-PO1576

Erythropoietin Maximum Dose Reduction Protocol for Hemodialysis Amret T. Hawfield, Gregory B. Russell, John M. Burkart, Vidhya Chandramohan. *Wake Forest School of Medicine, Winston-Salem, NC.*

Background: Clinical trials using erythropoietin stimulating agents (ESAs) failed to demonstrate benefit from high hemoglobin targets in ESRD and CKD. The CHOIR trial demonstrated higher mortality and cardiovascular events in those unable to meet hemoglobin targets on high dose erythropoietin (EPO).

Methods: A protocol to reduce the maximum EPO dose at 17 dialysis facilities was initiated in December 2010. EPO dose was tapered from 28,500 Units/Treatment (U/T) to 20,000 U/T for patients on thrice weekly in-center hemodialysis. Hemoglobin (Hb) was checked twice monthly. Iron deficiency and hyperparathyroidism were managed per standardized protocols. Fifty six of 1223 prevalent hemodialysis patients were on EPO >20,000 U/T in December 2010 and 43 of them had 6 month data in May 2011. Seven were considered EPO hyporesponsive (Hb < 11 mg/dL for the prior 3 months despite maximum dose EPO). Baseline values were obtained from the mean EPO dose and Hb value for the 3 months prior to the protocol change. Monthly frequencies and means for EPO dose and Hb were calculated monthly for 6 months following the protocol change. A paired t-test was used to calculate significant changes.

Results: Mean baseline EPO dose and Hb were 21,714 U/T and 10.2 mg/dL, respectively. The mean EPO dose was reduced to 15,188 U/T over 6 months and mean Hb remained stable at 10.8 mg/dL. Among the 43 patients with 6 month paired data, the mean EPO dose reduction was 10,256 U/T (SD=5605; $p<0.0001$), while the mean Hb increased by 0.2 mg/dL (SD=1.6, p=0.41). Among EPO hyporesponsive patients a 7875 U/T EPO decrease (SD= 2023; p=0.0002) and with a 0.6 mg/dL Hb increase (SD= 1.0; p=0.20) were observed.



Conclusions: These data suggest that protocols gradually reducing maximum EPO doses in patients on hemodialysis can be safely implemented without significant short-term worsening of anemia.

FR-PO1577

Abstract Withdrawn

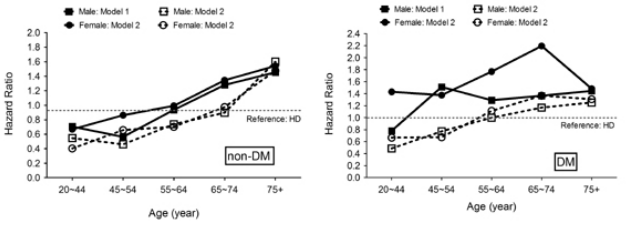
FR-PO1578

Nutrition and Anemia Status Affect the Survival of Older and Diabetic Peritoneal Dialysis Patients Wu-Chang Yang,[1] Yee-Yung Ng,[1] Shang-Jyh Hwang.[2] *[1]Division of Nephrology, Taipei Veterans General Hospital, National Yang-Ming University, Taipei, Taiwan; [2]Kaohsiung Medical University, Kaohsiung, Taiwan.*

Background: While the prevalent and incident ESRD patients are increasing worldwide, the penetration rate of peritoneal dialysis (PD) is decreasing in many countries. One of major reasons for this trend is a concern for a reduced survival in older and diabetic PD patients, especially in the females.

Methods: A nation-based comparison of survival between hemodialysis (HD) and PD incident patients, collected from Jan 1998 through Jun 2007 and followed up to Dec 2007, which included 66,678 HD and 4,680 PD patients who did not have a switch in dialysis mode during the follow-up period.

Results: PD utilization tends to be lower in older or diabetic patients (p= 0.055). As compared with HD patients at each age-specific group, Log-Rank and Cox analysis adjusted by sex, age, and diabetes (Model 1) did show a significantly unfavorable outcome for non-DM PD patients if age ≥65, and for diabetic PD patients if age ≥45, respectively.



The averaged levels of albumin and hematocrit during their follow-up period were significantly lower in patients who were diabetic, older or doing PD. If these averaged values of albumin and hematocrit were included in Cox analysis (Model 2), however, the survival of PD patients became comparable in non-DM patients until age of ≥75, and in diabetic patients until age of ≥65.

Conclusions: One of the major obstacles for PD utilization in older or diabetic ESRD patients is a relatively reduced survival than HD. Significantly lower levels of albumin and hematocrit are prevalent in these PD subgroups due to their co-mobilities, which could jeopardize and therefore, partly account for their reduced survival on dialysis. Thus, disorders in nutrition and anemia should be aggressively corrected in these patients doing peritoneal dialysis.

Funding: Government Support - Non-U.S.

FR-PO1579

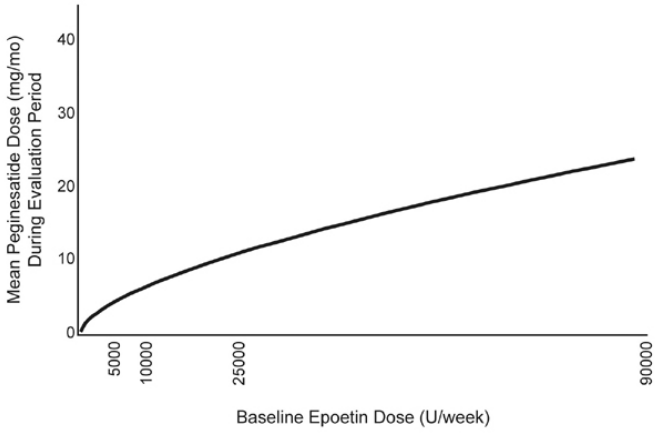
Relationship between Peginesatide and Epoetin Doses in Hemodialysis (HD) Patients Steven Fishbane,[1] Robert Provenzano,[1] Mark Kaplan,[1] Brigitte Schiller,[1] Anatole Besarab,[1] Carol Francisco,[2] Hong-Ye Gao,[2] Richard Daley,[2] Sandra Tong,[2] Martha Mayo,[2] Alex Yang,[2] Krishna R. Polu.[2] *[1]AFX-01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc., Palo Alto, CA.*

Background: Use of higher erythropoiesis-stimulating agent (ESA) doses may be associated with higher cardiovascular-related mortality (Regidor 2006), a risk likely confounded by patient factors such as health status (Besarab 2009). Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based ESA that is designed to specifically stimulate the erythropoietin receptor.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials assessing safety and efficacy of peginesatide (once monthly; N=1066) compared with epoetin alfa/beta (epoetin; 1-3 times weekly; N=542) in HD patients previously on stable epoetin doses (EMERALD 1 and 2). The relationship between baseline (BL) epoetin dose and mean evaluation period (EP; wks 29-36) ESA dose was evaluated. The dose ratio (BL epoetin [U/wk]: EP peginesatide [mg/mo] or EP epoetin [U/wk] dose) was calculated for each patient and tabulated by quartile.

Results: The relationship between epoetin and peginesatide dose was nonlinear (Figure). Median dose ratios by quartile for patients on peginesatide compared with BL epoetin doses ranged from 1040:1 in the first quartile (BL epoetin ≤4800 U/wk) to 2150:1 in the fourth quartile (BL epoetin ≥16,400 U/wk). Median dose ratios for patients who remained on epoetin were ~1:1 for all quartiles. The treatment groups were similar with respect to iron use, ferritin, TSAT, CRP, and hemoglobin levels at BL and during the EP.

Figure. Dose Relationship



Conclusions: The relationship between epoetin and peginesatide dose was nonlinear, suggesting that HD patients requiring more epoetin at baseline tend to require relatively less peginesatide to achieve similar hemoglobin levels.

Funding: Pharmaceutical Company Support

FR-PO1580

Efficacy of Oral Iron Supplementation in ESA-Treated Patients on Hemodialysis Whose Serum Hepcidin Levels Are Not Elevated Shinichi Suga,[1] Hironobu Kawai,[2] Keiju Hiromura,[3] Yoshihisa Nojima,[3] Naohisa Tomosugi.[4] *[1]Saiseikai Maebashi Hosp, Maebashi, Gunma, Japan; [2]Fujioka General Hospital, Fujioka, Gunma, Japan; [3]Gunma University, Graduate School of Medicine, Maebashi, Gunma, Japan; [4]Medical Research Institute, Kanazawa Medical University, Kahoku, Ishikawa, Japan.*

Background: Several factors including increased body's iron storage, decreased erythropoiesis and chronic inflammation result in an elevation of serum hepcidin levels in hemodialysis (HD) patients, which may prevent intestinal iron absorption. We hypothesized that hepcidin is not elevated in iron-deficient maintenance HD patients treated with ESA, and that oral iron supplementation can save ESA doses.

Methods: HD patients (serum ferritin < 100 ng/mL and transferrin saturation (TSAT) < 30%, n=25) treated with darbepoetin α, received 105 mg oral ferrous sulfate once a day for 24 weeks. The dose of darbepoetin was adjusted to maintain a Hgb level of 10 to12g/dL. We used mass spectrometry assay to measure serum hepcidin-25 (Hep-25).

Results: Before the iron administration, mean serum Hep-25 in the HD patients was comparable with that of healthy controls (22±12 ng/mL). Hep-25 positively correlated with serum ferritin (ρ=0.71, $p<0.01$), but not with TSAT. Oral iron administration resulted in the elevation of serum ferritin, TSAT and Hep-25. Although mean Hgb remained relatively constant throughout the study period, mean darbepoetin α dose decreased by 17% at week 12 and by 37% at week 24. In patients whose darbepoetin α dose was unchanged during the first 4 weeks, elevation of Hgb in the 4 weeks negatively correlated with Hep-25 at week 0 (ρ= -0.48, P = 0.048), but not with ferritin or TSAT.

	week 0	week 12	week 24
Hgb g/dL	10.5 ± 0.3	11.8 ± 0.2*	10.6 ± 0.2
Hep-25 ng/mL	12.3 ± 3.5	29.7 ± 5.5*	43.5 ± 5.3*
ferritin ng/mL	31.4 ± 3.4	54.2 ± 6.2*	97.5 ± 10.2*
TSAT %	20.4 ± 1.9	34.0 ± 2.4*	35.1 ± 3.3*
DA µg/week	21.1 ± 3.6	17.5 ± 1.9*	13.3 ± 1.8*

Values are mean ± SE. *p<0.05 vs week 0

Conclusions: We found that oral iron administration could effectively stimulate erythropoiesis in iron-deficient HD patients with ESA, if their serum Hep-25 levels are not elevated. Hep-25 may be a useful biomarker for the response to oral iron supplementation.

FR-PO1581

Effects of the ESRD Medicare Bundling Rule on Anemia Management in Private Dialysis Units Katie E. Cardone,[1,2] Brian Brian Fox,[1] Shari A. Meola,[3] Christopher D. Hoy,[3] Amy B. Pai.[1,2] *[1]Albany College of Pharmacy and Health Sciences, Albany, NY; [2]ANephRx, Albany, NY; [3]H&L Rubin Dialysis Center, Troy, NY.*

Background: Reform of the CMS ESRD payment policy in Jan 2011 required bundled payments for previously separately billable drugs. Given the cost difference between erythropoiesis stimulating agents & IV iron, this study evaluated implications of the new bundling rule on anemia management.

Methods: This was a retrospective cohort study of in-center hemodialysis (IHD), home hemodialysis (HHD) & peritoneal dialysis (PD) patients at 2 private, nonprofit dialysis centers in Upstate New York. Medical record & laboratory data were pooled and evaluated for time periods Jan 2010 - April 2010 (pre-bundle) and from Jan 2011 - April 2011 (post-bundle). All patients with available anemia medication use data were included. Monthly epoetin alfa (EPO) and intravenous iron sucrose (IVFe) doses were analyzed pre- and post-bundling. All available monthly [hemoglobin] for study patients were collected.

Results: A total of 1470 patient-months were evaluated, IHD=1061 mo, HHD=288 mo, PD=121 mo. Among IHD patients receiving EPO, mean(SD) monthly doses significantly decreased after the bundle was imposed, 62,758 (69,034) vs. 44,140 (45,454) units respectively ($p<0.001$). For those on IVFe, mean monthly doses significantly increased from 306 (221) to 453 (290) mg ($p<0.001$). Mean hemoglobin concentrations were significantly lower after implementation of the bundle 11.1(1.4) compared to 11.6(1.4) g/dL pre-bundle ($p<0.001$). Similar drug use shifts were observed in both the HHD and PD patients. Both the HHD and PD groups had a 0.5 g/dL reduction in Hb concentrations in the post-bundle observation period ($p<0.001$ for both groups).

Conclusions: Since revision of the ESRD Conditions for Coverage, we observed significant decreases in EPO and increases in IVFe doses. Hemoglobin concentrations were significantly reduced, but remained within the target range. Given long-term safety concerns with EPO and IV iron, practice pattern changes related to the bundled drug coverage policy should continue to be closely evaluated with regard to patient outcomes.

FR-PO1582

Four-Year Follow-Up of the Recombinant Human Erythropoietin (rHuEPO) Bundling Policy in Japan: Results from the Japan Dialysis Outcomes and Practice Patterns Study (DOPPS) Takeshi Hasegawa,[1] Jinyao Zhang,[2] Yun Li,[3] Ronald L. Pisoni,[2] Tadao Akizawa,[4] Bruce M. Robinson.[2,3] *[1]Showa University Fujigaoka Hospital; [2]Arbor Research Collaborative for Health; [3]University of Michigan; [4]Showa University.*

Background: A recombinant human erythropoietin (rHuEPO) bundling policy for hemodialysis (HD) patients in the Japanese health insurance system was implemented in April 2006. We previously reported short-term changes in anemia management following this policy change [Hasegawa, KI 2010]. Understanding longer term changes is important for understanding the possible impact of bundled payment policy in other health care systems.

Methods: Anemia management variables were determined in four cross-sections of chronic HD patients in Japan-DOPPS. One cross-section (Jan 2006) was prior to the rHuEPO bundling policy, with the others in 2007, 2009, and 2010.

Results: Since policy implementation, mean ESA dose has declined but % ESA use has increased slightly. Both IV iron use and dose over a 4 month period has increased (Table). There was little change in % with transferrin saturation (TSAT) <20% but a substantial decline in the % with serum ferritin <100 ng/mL. Hemoglobin (Hgb) levels increased modestly.

Conclusions: This 4-year follow-up after the rHuEPO bundling policy in Japan indicates an initial rise then stable IV iron use, an initial drop then stable ESA dose, plus a small rise in Hgb levels. Hemoglobin levels, average ESA doses, and IV iron use remain lower in Japan than in the US, and this may limit inference from these Japanese findings about the likely impact of ESA bundling in the US.

	Cross-sections			
	Before bundling	After bundling		
	Jan 2006	Jan 2007	Sep 2009	Sep 2010
Number of patients	1584	1622	1684	1631
Number of facilities	53	53	59	57
ESA use (%)	81.9	82.2	83.8	84.1
Darbepoetin use (as % of ESA)	0	0	46.4	48.5
Mean ESA dose (units/week)*	5266	4645	4681	4707
IV iron use (%)**	31.8	41.2	36.1	39.7
Mean IV iron dose (mg/month)**	105	111	134	125
Hemoglobin (g/dL), median	10.40	10.40	10.45	10.50
Hgb <10 g/dL, %	34.9	34.9	32.1	29.4
Hgb ≥12 g/dL, %	8.9	10.1	6.3	7.1
Transferrin saturation (%), mean	26.0	27.9	27.0	26.3
TSAT <20%, %	36.0	28.8	29.8	30.5
TSAT ≥40%, %	12.6	17.3	10.7	10.3
Serum Ferritin (ng/mL), mean	222	224	288	288
Ferritin <100 ng/mL, %	34.6	23.9	8.8	7.3
Ferritin ≥500 ng/mL, %	9.9	9.3	14.9	15.0

Data are from Japan-DOPPS phases 3 and 4; *ESA use was any use during the past month; ESA dose was the mean weekly dose during the past month; Aranesp dose was converted to estimated erythropoietin dose using a conversion factor of 1:200 (aranesp [mcg/week] : erythropoietin [units/week]); **IV iron use was any use during prior 4 months; IV iron dose was the mean monthly dose during the prior 4 month period among patients prescribed IV iron.

Funding: Pharmaceutical Company Support

FR-PO1583

Validation of an Intelligent Decision Support Tool for Anemia Management Adam E. Gaweda,[1,2] Michael E. Brier,[1,2,3] George R. Aronoff.[1,2] *[1]University of Louisville, KY; [2]Pharos Medicine, Inc., Louisville, KY; [3]Robley Rex VA Medical Center, Louisville, KY.*

Background: Erythropoiesis Stimulating Agents (ESA) are dosed by protocols derived from NKF/KDOQI guidelines and ESA package information. We developed an intelligent decision support system for individualized ESA dosing based on model predictive control called Smart Anemia Manager™ (SAM). We benchmark this tool against two standard protocols through in silico simulation.

Methods: We represented ESRD patients with a mathematical model of erythropoiesis relating weekly Hgb to weekly ESA dose received. To account for inter-patient variation in ESA response, we created a pool of models with different (fixed) parameters including ESA sensitivity from 0.1 to 0.9 g/dL per 1,000 ESA Units per week, RBC lifespan between 60 and 120 days, body mass 50 to 150 kg, and baseline Hgb 7 to 9 g/dL. We represented intra-patient Hgb variability by normally distributed random noise with zero mean and standard deviation 0.0 to 1.0 g/dL. All the simulation parameters other than Hgb, ESA dose and body mass were not "visible" to SAM nor the benchmark protocols. We simulated Hgb response over 12 months and used the following performance metrics: percent Hgb levels within target range (10-12 g/dL), and mean ESA dose per patient-week.

Results: Results of the comparison are shown in the table for three different levels of intra-patient variability. SAM outperforms Protocols 1 and 2 in terms of percent Hgb within target range. As expected, increasing intra-patient variability decreases percent Hgb within target. Anemia management with SAM results in a 36% relative decrease in ESA dose compared to Protocol 1 and 2. Intra-patient variability does not significantly affect ESA utilization.

Performance comparison between SAM and standard protocols

	Protocol 1		Protocol 2		SAM	
Intrapatient variability (g/dL)	% Hgb 10-12	Mean Epo per Pt-wk	% Hgb 10-12	Mean Epo per Pt-wk	% Hgb 10-12	Mean Epo per Pt-wk
0.0	68	11,200	50	11,500	74	7,300
0.5	54	10,400	44	12,000	67	7,300
1.0	40	9,800	33	13,000	48	7,100

Conclusions: In-silico validation supports that Smart Anemia Manager™ improves cost-effectiveness of anemia management compared to standard ESA dosing protocols.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1584

Red Blood Cell Life Span and Erythropoietin Resistance Index in Hemodialysis Patients Yanna Dou,[1,2] Anja Kruse,[1,2,3] Georges Ouellet,[1,2,4] Stephan Thijssen,[1,2] Nathan W. Levin,[1] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York; [3]Bern University Hospital, Switzerland; [4]Maisonneuve-Rosemont Hospital, Canada.*

Background: Anemia is a common complication in hemodialysis (HD) patients. Poor response to erythropoiesis-stimulating agent therapy is an adverse prognostic factor with inflammation and reduced iron availability as leading causes. This study investigated the relationship between red blood cell life span (RBCLS) and the clinically used erythropoietin resistance index (ERI).

Methods: In chronic HD patients, hemoglobin (Hgb), high-sensitivity CRP (hsCRP), RBCLS, serum iron, ferritin and TSAT were measured. RBCLS was estimated from endogenous alveolar carbon monoxide concentration (determined by gas chromatography) and hemoglobin concentration (Strocchi, 1992). Demographics and weekly Epoetin alfa (EPO) dose were recorded. ERI was defined as weekly EPO dose per kilogram of body weight divided by Hgb level in g/dL. We performed logistic regression of ERI (categorized as above or below the median) on age, diabetes status, hsCRP (>5 or ≤5 mg/L), RBCLS (<60 or ≥ 60 days), TSAT, ferritin, and serum iron.

Results: Thirty-six HD patients (24 males, age 59±15 years, dialysis vintage 59±51 months, 17 patients with diabetes) were studied. Bivariate correlation analysis showed borderline significance for patients with lower RBCLS having higher ERI (Spearman rho = –0.32; P=0.057). Median ERI was 10.5 (U*dL)/(week*kg*g). Among all variables analyzed, elevated ERI was associated only with RBCLS below 60 days (OR 5.2; 95% CI 1.3 to 21.6, P<0.05), but not with age, diabetes, hsCRP>5 mg/dL, TSAT, ferritin, and serum iron.

Conclusions: RBCLS is inversely related to ERI, a parameter commonly used to assess HD patients' responsiveness to EPO. Both EPO dose and Hgb level enter the calculation of the ERI, and both are closely intertwined with RBCLS, a fact that is often neglected or underappreciated in clinical practice. A consideration of RBCLS is vital for any interpretation of ERI, particularly in populations with notably variable RBCLS, such as dialysis patients. Research to reveal the causes of reduced RBCLS in HD patients is needed.

FR-PO1585

Smart Anemia Manager Results in Better Hemoglobin Control and Cost Savings over a Traditional Algorithmic Approach Michael E. Brier,[1,2,3] George R. Aronoff,[2,3] Alfred A. Jacobs,[2] Adam E. Gaweda.[2,3] *[1]Robley Rex Veterans Medical Center, Louisville, KY; [2]University of Louisville, KY; [3]Pharos Medicine, Louisville, KY.*

Background: Erythropoietin (EPO) is dosed by protocols derived from NKF/KDOQI guidelines and EPO package information. We developed an intelligent decision support system for individualized EPO dosing based on model predictive control called Smart Anemia Manager (SAM). We tested the hypothesis that SAM will better control Hb response to EPO and use less EPO than a traditional algorithmic approach (TAP).

Methods: In a retrospective controlled clinical trial of SAM (n=68) vs. TAP (n=66) we compared anemia management in our dialysis facility for 6 months prior to the introduction of SAM to the 6 months after SAM and between TAP and SAM. The SAM program is implemented as a stand alone program that reads EPO dose and hemoglobin data from an electronic medical record. It then determines the best patient-specific future dose of EPO based on a model predictive control algorithm. The TAP is developed as an expert system based on the EPO package insert and KDOQI guidelines. The target Hb was 11.0 g/dl in both groups. We measured the mean Hb and weekly EPO dose.

Results: Shown below are the mean and SD for Hb and mean weekly EPO dose for the period 6 months prior to and 6 months after the implementation of SAM.

	Hb Before	Hb After	EPO Before	EPO After
TAP	11.5±0.93	11.3±1.00	10,777	8,734
SAM	11.3±0.9	11.0±0.9	9,029	5,902

SAM was able to achieve the target Hb within the time course of the study while TAP was not. Decreasing the population mean Hb resulted in EPO sparing in both groups but SAM resulted in an additional EPO sparing of about 2,800 units per week. SAM also resulted in a 10% decrease in Hb variability as measured by Hb standard deviation.

Conclusions: Prospective prediction of Hb response to EPO dosing with SAM is superior to an expert rule based technique used in the management of the anemia of end-stage renal disease. SAM exposes patients to less EPO, better achieves the target Hb, and results in cost savings to dialysis units in the range of $1,100-$3,800/patient/year, depending on current EPO usage.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1586

Red Blood Cell Life Span Is Associated with Hemoglobin Variability in Chronic Hemodialysis Patients Yanna Dou,[1,2] Anja Kruse,[1,2,3] Georges Ouellet,[1,2,4] Stephan Thijssen,[1,2] Nathan W. Levin,[1] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York; [3]Bern University Hospital, Switzerland; [4]Maisonneuve-Rosemont Hospital, Canada.*

Background: Increased hemoglobin (Hgb) variability is associated with higher mortality in chronic hemodialysis (HD) patients. Erythropoiesis-stimulating agent dose, iron deficiency, infections and intercurrent events can influence Hgb variability. The relationship between red blood cell life span (RBCLS) and Hgb variability has not previously been investigated.

Methods: Chronic HD patients from a single center were enrolled. Hgb, high-sensitivity CRP (hsCRP), iron status, reticulocyte production index (RPI) and RBCLS were assessed. RBCLS was estimated from endogenous alveolar carbon monoxide level (determined by gas chromatography) and Hgb concentration (Strocchi, 1992). Intra-individual standard deviation (SD) of subsequent Hgb levels indicated Hgb variability. Patients were stratified into high (SD>1 g/dL) or low (SD≤1 g/dL) Hgb variability groups. We performed logistic regression of Hgb variability on RBCLS (<40 or ≥40 days), mean weekly Epoetin alfa (EPO) dose, and mean RPI.

Results: 33 HD patients (22 males, age 59±16 years, dialysis vintage 52±53 months, 15 diabetics) were enrolled. Patients with high Hgb variability (N=12) showed a shorter RBCLS (56±16 vs. 73±23 days, P<0.05), a higher hospitalization rate (3.03 vs. 0.18 hospitalizations per patient-year, P<0.05), higher EPO doses, and higher variability of RPI; indicators of iron status and inflammation did not differ between the two groups. In logistic regression, RBCLS<40 days was associated with increased risk of high Hgb variability (OR 6.0; 95%CI 1.2-29.0; P<0.05).

Conclusions: This study reveals short RBCLS is a novel risk factor for Hgb variability. Short RBCLS in this context is presumably not causally linked to higher Hgb variability *per se*. Rather, the association may be a reflection of intercurrent events that effect both shorter RBCLS and reduced Hgb levels (e.g., via oxidative stress or other mechanisms). The fluctuating nature of such states may explain higher Hgb variability and shorter average RBCLS in the affected patients.

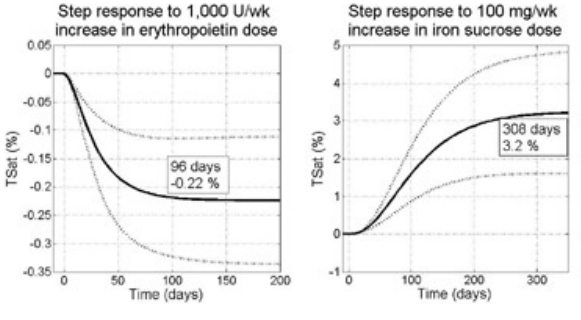
FR-PO1587

Mathematical Modeling of Erythropoietin and Iron Dosing in Anemia Management Adam E. Gaweda,[1] Yossi Chait,[2] Michael E. Brier,[1,3] George R. Aronoff.[1] *[1]University of Louisville, KY; [2]University of Massachussets, Amherst, MA; [3]Robley Rex VA Medical Center, Louisville, KY.*

Background: Sufficient iron is required for the optimum response to erythropoietin. To help guide parenteral iron supplementation, we derived a mathematical model which quantifies the effect of parenteral iron and erythropoietin on transferrin saturation (TSat), a frequently used marker of iron stores in anemia management.

Methods: We prospectively collected Hemoglobin (Hgb), TSat, erythropoietin and iron sucrose dose weekly for one year in a cohort of 56 hemodialysis patients at the University of Louisville dialysis facility. Using these data, we estimated the response of TSat (output) to changes in erythropoietin and iron sucrose dose (inputs) using a population-based additive second-order model. This model has two physiologic parameters for each input: steady-state gain (sensitivity) and time constant (dynamics). Model estimation was performed in Matlab®.

Results: The left hand side plot below shows the predicted response to a 1,000 U increase in weekly erythropoietin dose with constant iron sucrose dose. TSat decreases by about 0.22% at a steady-state achieved after approximately 96 days. The right hand side plot below shows the predicted response to a 100 mg iron sucrose dose increase with constant erythropoietin dose. A 100 mg increase in weekly iron sucrose dose increases TSat by 3.2% at a steady-state achieved after about 308 days.



Conclusions: Our proposed mathematical model quantitatively describes the interaction of erythropoietin and iron during anemia management in dialysis patients. Using this model, we can estimate how much iron supplementation is required to maintain iron repletion that maximizes the response to erythropoietin.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1588

Defining Erythropoiesis Stimulating Agent (ESA) Hyporesponsiveness David T. Gilbertson, Yi Peng, Tom Arneson, Stephan C. Dunning, Allan J. Collins. *CDRG, Minneapolis, MN.*

Background: 'ESA hyporesponsiveness' in hemodialysis (HD) patients refers to high doses of ESAs to treat anemia. It is generally the result of functional iron deficiency, bone marrow fibrosis and/or inflammation. There does not appear to be a standardized definition of ESA hyporesponsiveness, and dose cutoffs used as a definition have changed over time as ESAs have been used at higher doses. We investigated 3 definitions of hyporesponsiveness in 2008 prevalent Medicare HD patients.

Methods: 3 definitions of hyporesponsiveness (90th percentile of each: EPO Total Dose, EPO Total Dose/kg, and EPO Total Dose/Hb) were applied to the cohort of HD patients. Each of five observed months was defined as hyporesponsive or not, for each patient. Patients with ≥1 month of hyporesponsiveness were classified as such. Further classifications included chronic (hyporesponsive 4+ consecutive months) and acute hyporesponsiveness (≤3 consecutive months of hyporesponsiveness with at least one month not hyporesponsive before and after the hyporesponsive month(s).

Results: The 3 definitions produced similar proportions in hyporesponsiveness categories. 20-21% were hyporesponsive: 5-6% acute, 4-5% chronic, and 10-11% meeting neither the acute nor the chronic definition. Factors associated with all hyporesponsive patients (any, acute, or chronic) were similar across the 3 definitions. The strongest factors associated with any hyporesponsiveness included: Low Hb, decreasing trend of Hb, younger age, non-diabetic cause of renal failure, increased dialysis vintage, cancer, GI bleeding, congestive heart failure, IV antibiotic use, increased months with iron use, hospital admissions, for-profit dialysis provider, and large dialysis organizations.

Conclusions: The three definitions of hyporesponsiveness produced similar estimates of prevalence and associations with patient characteristics. Thus for the sake of clinical simplicity, Total ESA Dose may be most appropriate measurement of hyporesponsiveness. With the bundled reimbursement system implemented in Jan 2011, ESA dosing patterns may change significantly, and these associations should be reassessed.

Funding: Pharmaceutical Company Support

FR-PO1589

Intra-Individual Variability of Serum Hepcidin-25 in Hemodialysis Patients Hilde P. Peters,[1] Coby M.M. Laarakkers,[2] Jan A.J.G. van den Brand,[1] Dorine W. Swinkels,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Gelderland, Netherlands; [2]Laboratory of Genetic, Endocrine and Metabolic Diseases, Radboud University Nijmegen Medical Center, Nijmegen, Gelderland, Netherlands.*

Background: Hepcidin-25 regulates iron metabolism by integrating input from erythropoietic, inflammatory and iron signaling pathways. Serum hepcidin levels are increased in patients with CKD and may contribute to deficient erythropoiesis. Thus, (changes in) hepcidin-25 levels could become a tool to guide treatment with iron and erythropoiesis stimulating agents. For meaningful interpretation of data, it is essential to know the intra-individual variability of serum hepcidin levels. We aimed to assess the intra-individual variability of serum hepcidin-25 in hemodialysis patients and to identify significant determinants.

Methods: We included hemodialysis patients (n=43, 56% male, age 62±15 yrs) who attended our hospital for regular dialysis. Blood samples were drawn prior to dialysis once weekly during 6 weeks. The majority was treated with iron and epoetin beta. Hepcidin-25 was determined by mass spectrometry and the mean coefficient of variance (CV_1; standard deviation/mean) was calculated for each patient.

Results: At baseline, median hemoglobin was 11.4 (IQR 10.5-11.9) g/dl and serum hepcidin-25 was 46.7 (26.2-73.4) ng/ml. Median CV1 of hepcidin-25 was 26% (IQR 17-48). CV1 was higher in patients with low hepcidin and ferritin. CV1s of CRP and ferritin were 41% (IQR 8-63) and 12% (IQR 11-26), respectively. By multivariate regression analysis we found baseline ferritin and CV1 of transferrin saturation (TSAT) , but not CRP, to be independent predictors of intra-individual hepcidin-25 variability. In the majority of patients there was no correlation between hepcidin-25 and ferritin, iron, TSAT, CRP, or hemoglobin. Changes in serum hepcidin-25 could not be predicted from changes in ferritin or CRP.

Conclusions: Our results indicate that there is significant intra-individual variability of serum hepcidin-25 levels in hemodialysis patients. We found no correlation between variability of hepcidin-25 and CRP levels, thus excluding the inflammatory status as an important determinant of hepcidin variability.

FR-PO1590

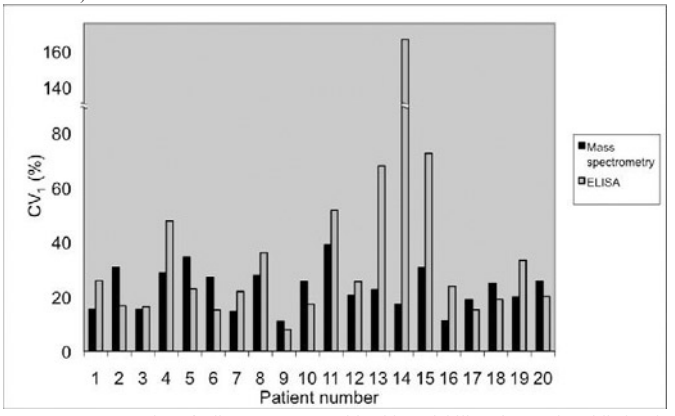
Intra-Individual Variability of Hepcidin in Haemodialysis Patients Using Mass Spectrometry and ELISA Adam Rumjon,[1] Sukhvinder Singh Bansal,[2] Jolanta Malyszko,[3] Iain C. Macdougall.[1] *[1]King's College Hospital; [2]King's College London; [3]Medical Academy, Bialystok.*

Background: Measurement of serum hepcidin levels may provide a useful alternative to the current methods of determining iron status in chronic hemodialysis patients. However, the biological variability of this pivotal regulator of iron homeostasis is unclear, and the impact of dialysis clearance and iron therapy on hepcidin variability has not been established.

Methods: Serum hepcidin levels were measured in 20 stable, chronic hemodialysis patients at the start of 9 consecutive dialysis sessions. Liquid chromatography mass spectrometry (employing hepcidin-25 as an internal standard) and a competitive ELISA

were both used to calculate the coefficient of variance (CV_1) in this population. Potential factors affecting CV_1 were also examined.

Results: The median CV1 was 23.7% (16.8, 27.9) and 23.3% (17.1, 38.9), with the MS and cELISA assays respectively. The median CV1 was similar in patients receiving and not receiving regular IV iron (p=0.77). High sensitivity CRP levels were also determined at each timepoint in all patients, and its correlation with serum hepcidin levels was weak (r=0.154, p=0.043). Hepcidin levels appeared to be higher following an inter-dialytic period of 3 days versus 2 days (p=0.02). No relationship was found between serum hepcidin and dialysis quantity, hemoglobin levels, erythropoietin dosage, or serum ferritin levels (data not shown).



Conclusions: These findings suggest considerable variability of serum hepcidin levels in hemodialysis patients. Inflammation and the use of IV iron therapy did not impact on the degree of variability. Hepcidin levels were higher after an inter-dialytic period of 3 days versus 2 days. These findings need to be taken into account in any future studies assessing the utility of serum hepcidin as a guide to the use of IV iron or ESA therapy.

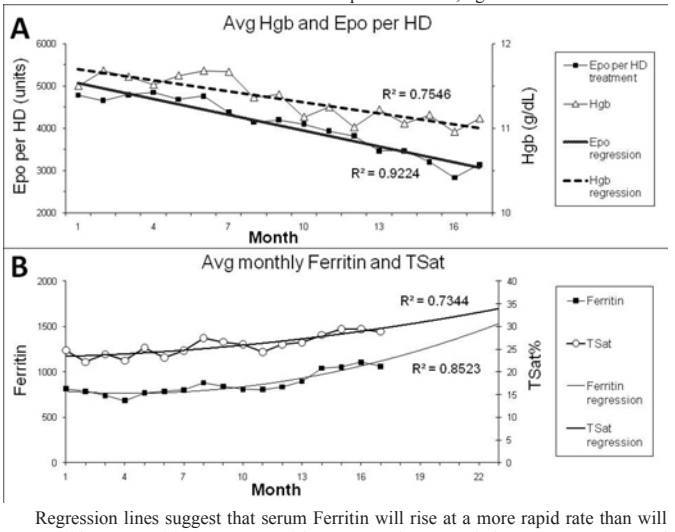
FR-PO1591

Increasing Iron and Decreasing Erythropoietin Use in Hemodialysis Patients: How High Will Serum Ferritins Become? Preeti R. Nargund, Andrew I. Chin. *Division of Nephrology, University of California, Davis School of Medicine, Sacramento, CA.*

Background: With curtailed use of erythropoietin (EPO) in anemia management of patients on HD, many centers are utilizing automated iron protocols. We describe the changes in mean serum Ferritin and Transferrin saturation (TSat) in HD patients with these new protocols in effect. By extrapolation of these data, we provide a glimpse of how high Ferritin levels may become with common iron targets.

Methods: Retrospective analysis of mean monthly serum Ferritin, TSat, Hgb, Albumin, EPO dose per HD, and administered iron from a 16 month period in which iron protocols were implemented and EPO protocols were adjusted. CQI data from 4 hemodialysis clinics affilitated with an urban academic medical center. Best fit regression was performed to provide trend lines for the parameters of interest.

Results: Mean Hgb and units of EPO per HD treatment are shown in figure A. There is a clear reduction in the amount of EPO being given, driven not only by a change in the target Hgb levels (from 11-12 g/dL to 10-12 g/dL), but also by an increased use of intravenous iron replacement for deficiency based on new protocols. As a result, mean serum Ferritin and TSat levels have increased over this same period of time, figure B.



Regression lines suggest that serum Ferritin will rise at a more rapid rate than will TSat within a TSat-driven, automated iron protocol. Extrapolating to a mean TSat of 35% (a common lower-limit TSat target range), mean serum Ferritins may go well over 1600 µg/L.

Conclusions: There has been a significant reduction in EPO use and an increase in iron use in anemia management of HD patients. With iron protocols targeting a higher range of TSat, serum Ferritins will necessarily rise. Our data suggests that Ferritin will rise at a more rapid rate than will TSat, perhaps up to levels that may cause concern for many nephrologists.

Funding: Clinical Revenue Support

FR-PO1592

Dose of Erythropoiesis-Stimulating Agents (ESAs) and Adverse Outcomes in Chronic Kidney Disease (CKD): A Meta-Regression Ioannis Koulouridis,[1,2] Mansour Alfayez,[1] Thomas Trikalinos,[3] Ethan M. Balk,[3] Bertrand L. Jaber.[1,2] *[1]Department of Medicine, Division of Nephrology, St. Elizabeth's Medical Center, Boston, MA; [2]Department of Medicine, Tufts University School of Medicine, Boston, MA; [3]Center for Clinical Evidence Synthesis, Tufts Clinical and Translational Science Institute, Boston, MA.*

Background: Higher target hemoglobin (Hb) during ESA therapy for anemia of CKD is associated with increased cardiovascular morbidity. We conducted a meta-regression to examine whether the ESA dose is associated with adverse outcomes independent of Hb.

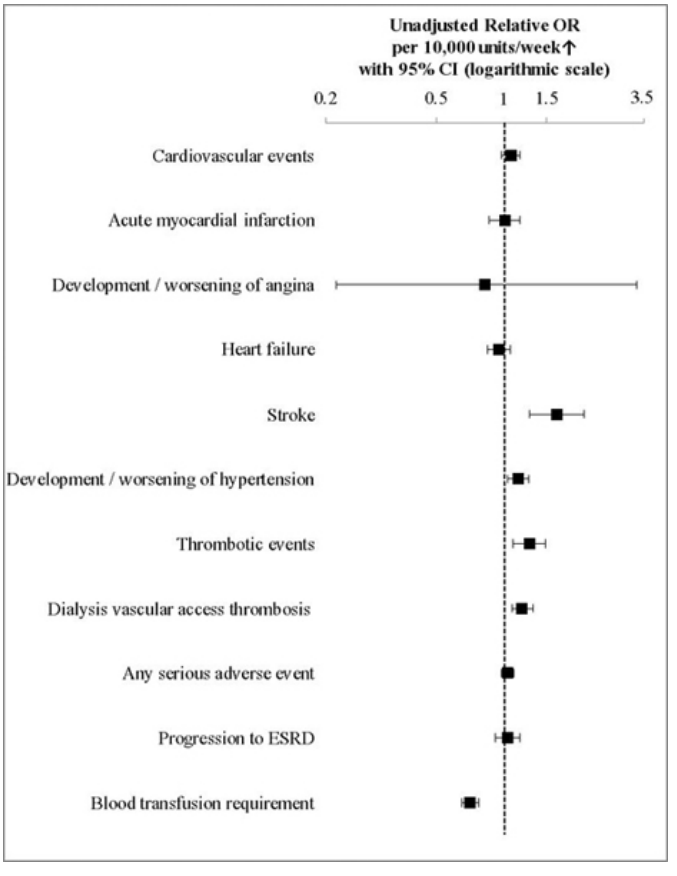
Methods: We searched MEDLINE and prior meta-analyses for randomized controlled trials of ESAs for anemia of CKD. We extracted data on ESA dose, Hb level, and outcomes. Using study arm (or cohort) as the unit of analysis, mixed effects generalized linear meta-regression was performed to examine the association between ESA dose and all-cause and cardiovascular mortality.

Results: We identified 31 trials (12,956 patients). Higher first 3-month and total study-period mean ESA dose was associated with higher unadjusted odds for all-cause (but not cardiovascular) mortality, which remained significant after adjustment for first 3-month mean Hb and target Hb, respectively (Table). Total study-period mean ESA dose was also associated with higher unadjusted odds for several secondary outcomes (Figure).

Conclusions: Higher ESA dose for anemia of CKD may be associated with higher all-cause mortality.

Predictor	No. cohorts	Relative OR (95% CI)	P value
First 3-month mean ESA dose*			
Unadjusted	11	1.42 (1.10, 1.83)	0.007
Adjusted for target Hb	10	1.71 (0.90, 3.24)	0.1
Adjusted for first 3-month mean Hb	11	1.48 (1.02, 2.14)	0.04
Total study-period mean ESA dose*			
Unadjusted	21	1.09 (1.02, 1.18)	0.02
Adjusted for target Hb	21	1.40 (1.08, 1.82)	0.01
Adjusted for total study-period mean Hb	21	1.27 (0.97, 1.65)	0.08

*per 10,000 units/week↑



FR-PO1593

Use of Crit-Line Hematocrit Monitoring To Assist with Anemia Management in Chronic Hemodialysis Frank Jiann-Gang Luo,[1,2] Steven R. Fast,[1] Amul K. Jobalia.[1,2] *[1]Division of Nephrology, Santa Clara Valley Medical Center, San Jose, CA; [2]Stanford University School of Medicine, Stanford, CA.*

Background: Hemodialysis patients receiving erythropoiesis-stimulating agent (ESA) require frequent monitoring to keep hemoglobin (Hb) levels within target. Frequent blood draws are impractical, costly, and do not provide point-of-care decision making on ESA administration. Crit-Line (Hema Metrics, Utah, USA) is a non invasive, photometric device which measures the percent change in blood volume during dialysis based on changes in the hematocrit (Hct). We present our experience of incorporating the Hct reading from Crit-Line in an anemia protocol to aid ESA administration.

Methods: We amended our existing anemia protocol with a 'hold' parameter - the prescribed dose of ESA was held for the dialysis session if the Crit-Line Hct at the start of treatment was greater than 36%. No other aspect of the protocol was changed. We analyzed Hb/Hct levels, ESA dose, ferritin/transferrin saturation and intravenous iron usage for 6 months before and after protocol amendment.

Results: Data from 136 patients receiving dialysis during the trial period was analyzed. Crit-Line Hct at the start of dialysis correlated well with laboratory measured predialysis Hct (r2=0.85). Mean Hb, Hct and Epoetin alfa (Epo) dose for two 3-month periods before (Q2-Pre, Q1-Pre) and after (Q1-Post, Q2-Post) protocol amendment were:

	Q2-Pre	Q1-Pre	Q1-Post	Q2-Post
Hb (g/dl)	12.0±1.1	12.1±1.1	11.7±1.0 *†	11.8±1.1 *†
Hct (%)	36±4	36±3	35±3 *†	35±3 *†
Epo (units/month/patient)	63434±51421	59269±44573	47924±38746 *†	44069±37110 *†

mean±SD

Quarter (Q)

∗ p<0.05 vs Q1-Pre

† p<0.05 vs Q2-Pre

Mean Hb and Hct improved to within target range (Hb 10-12g/dl; Hct 30-36%). Epo usage decreased 19% in the first quarter after protocol amendment. No difference was seen in intravenous iron usage. On average, 2.32 million units of Epo per month were held because of Crit-Line Hct > 36%.

Conclusions: Crit-Line Hct readings can be used to guide ESA administration at each dialysis treatment. This may result in improved anemia management without the need for more frequent blood draws and prevent ESA overuse which could translate to significant cost savings.

FR-PO1594

Clinical and Laboratory Features of Hemodialysis Patients with Adequate Control of Renal Anemia without Erythropoietin Stimulating Agents Remus Aurel Orasan,[1] Stefan H. Jacobson,[2] Bjorn Englund,[3] Ljubisa M. Veljancic,[4] Andrzej Jan Swiderski,[5] Andre L. Weigert.[6] *[1]Nefromed Dialysis Centers, Cluj-Napoca, Romania; [2]Dept Nephrol., Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden; [3]Euromedic International; [4]Clinic of Nephrology MMA, Belgrade, Serbia; [5]IDC Leszno, Poland; [6]Eurodial Obidos, Portugal.*

Background: There is little data in literature comparing clinical and laboratory features of hemodialysis (HD) patients who were prescribed erythropoietin stimulating agents (ESA) with patients who, for medical and/or socio-economical reasons, were not.

Methods: We followed 12,873 HD patients from 7 countries (Bosnia Herzegovina, Croatia, Poland, Portugal, Romania, Russia and Turkey) for 3 years, divided in 2 subgroups: subgroup A – patients without ESA for at least 6 consecutive months (n=1,314;10.2%) and subgroup B – patients receiving ESA (n=11,559;89.8%). In both subgroups we assessed body mass index (BMI), dialysis vintage (DV), Charlson comorbidity index (CI)(range 0-6), hemoglobin (Hb), ferritin (Fer), transferrin saturation (TSAT), albumin, Kt/V, blood flow rate (QB), calcium (Ca), phosphorus (P), intact PTH and systolic blood pressure (SBP).

Results: In subgroup A, age (56.3±13.2 vs 60±15 years, p<0.0001), CI (3.5±1.3 vs 3.8±1.5, p<0.0001), Fer (486.5±574.7 vs 735.9±622.7 ng/mL, p<0.0001), Kt/V (1.5±0.3 vs 1.5±0.9, p<0.0001), SBP (128.3±21.8 vs 134.5±21.9 mmHg, p<0.0001) and TSAT (32.8±22.6 vs 34.8±32.2 %, p<0.0001) were lower than in subgroup B, while Hb (12.7±1.6 vs 10.7±1.3 g/dL, p<0.0001), albumin (40.9±4 vs 39.6±4.8 g/L, p<0.0001), BMI (25.5±4.7 vs 25.1±5.1 kg/m2, p<0.0001), Ca (9±1 vs 8.8±1.4 mg/dL, p<0.0001), PTH (521.4±633.5 vs 398.7±568.8 pg/mL, p<0.0001), P (5.4±1.7 vs 5.2±1.7 mg/dL, p<0.0001), QB (345.4±42.3 vs 327.4±44.7 mL/min, p<0.0001) and DV (93.7±68 vs 65.3±56.6 months, p<0.0001) were higher.

Conclusions: HD patients not receiving ESA for at least 6 consecutive months maintained a higher Hb, they were younger, better nourished, longer DV, and they had a lower CI, SBP and Fer than patients treated with ESA. HD patients with no ESA treatment had adequate dialysis, fairly well controlled mineral bone disease markers and TSAT>30%.

FR-PO1595

Standardized vs. Patient-Specific Computer-Assisted Erythropoietin Dosing: A Randomized Controlled Trial Kevin Ho,[1] John McMichael,[2] Christos Argyropoulos,[1] Vladimir Ladik,[3] Laurie Zickgraf,[3] Alice A. Martin,[4] Klemens B. Meyer,[4] Dana C. Miskulin.[4] *[1]Univ of Pittsburgh, Pittsburgh, PA; [2]Dimensional Dosing Sys Inc, Wexford, PA; [3]Dialysis Clinic Inc, Nashville, TN; [4]Tufts Medical Center, Boston, MA.*

Background: EPO (epoetin) is the largest modifiable factor determining incenter hemodialysis (HD) treatment cost highlighted by the CMS Bundled Payment/QI programs.

Methods: We performed a double blind randomized control trial to compare two computer-assisted algorithms in dosing iv EPO 3x weekly: patient-specific (Intelligent Dosing System, IDS) vs. non-individualized (DCI). Incenter HD patients (n=48) were randomized to either algorithm for 6 months (intervention). Biweekly Hb values electronically triggered dose adjustments. EPO usage, Hb, EPO Resistance Index (ERI=EPO/weight/Hb) data were aggregated monthly on a patient basis. Observed Hb (g/dL) was classified as: at target 10-12, >12, or <10. Run-in and intervention measurements were analyzed with mixed linear models (continuous outcomes) or mixed logistic regression (discrete outcomes) to account for repeated assessments of the same individuals.

Results: From intervention mos. 1 to 6, the % patients at target increased 52.1% to 61.9%; 59.4% of IDS vs. 54.6% of DCI patients were at target Hb on average. During run-in, IDS patients had more variable Hb levels (variance ratio 1.23, 95%CI 1-1.52), higher EPO use (p=0.004), and higher ERI (p=0.028) than DCI patients. However with intervention, IDS patients were more likely to achieve target Hb each mo. (OR=2.1, 95% CI 1.02-4.28, p=0.043), less likely to overshoot (Hb>12: OR 0.35, 95% CI: 0.17-0.73, p=0.005), and no more likely to undershoot (Hb<12: OR 2.6, 95% CI 0.69-11.3, p=0.21). The no. of dose adjustments by IDS vs. DCI algorithms leading to Hb values at target were 107 vs. 111; for Hb<10, 20 vs. 9; for Hb>12, 125 vs. 152. Average EPO use declined in both arms, but the decline was larger in the IDS arm (p=0.0006).

Conclusions: Unlike 'one-size fits all' EPO protocols, a patient-specific algorithm may account for non-linear Hb-EPO dose relationships and yield comparable anemia outcomes without increasing dose requirements. Further testing in a larger population is indicated.

Funding: Clinical Revenue Support

FR-PO1596

Anemia Day: Simultaneous, Once Every Two Weeks (Q2W) Administrations of Darbepoetin Alfa (DA) and Iron (V), Improve Anemia Management in Hemodialysis (HD) Patients Jacques B. Rottembourg, Alain P. Guerin, Diaconita Mirela. *Hemodialysis Unit, Diaverum Group, Paris, France.*

Background: Most HD patients (Pts) require erythropoietin-stimulating-agents (ESA) and iron to control anemia in the long run. In HD Pts, several studies show that IV iron reduces ESA doses, Q2W DA IV dose requirements are similar to every week (QW) DA IV one's and less-frequent ESA administration might result in decreased in-centre nursing time and potentially reduce costs. The aim of the "Anemia Day" was to explore the potential add-on benefit of a simultaneous Q2W injection of both ESA (DA) and iron sucrose (V) during the same dialysis session.

Methods: By September 2010, all stable HD Pts treated in the unit by IV DA Q2W and IV V QW were prospectively switched to simultaneous Q2W IV DA and V dose regimen. Hb level (target 11.5-12 g/dl), ESA and iron parameters were assessed for an 8 months period. Hb was measured every two weeks and iron parameters (ferritin [F]and TSAT) every 2 months. Data at Baseline (BL) defined by the month before inclusion, month 4 (M4) and month 8 (M8) were analyzed and paired Student's t-tests were performed.

Results: 110 HD Pts were included : male 57%, mean age (SD) 60.5 (15.7) years, mean time on dialysis of 52.7(45.7) months, with 30% of Pts with diabetes as primary renal disease.

	BL	M4	M8	p M8 vs BL
Hb g/dl: mean (SD)	11.43(1.08)	11.61(1.33)	11.73(0.96)	0.02
Ferritin µg/l: mean (SD)	608 (375)	684 (349)	911 (509)	0.001
TSAT %: mean (SD)	37.0 (16.3)	36.0 (14.6)	48.7 (20.1)	0.001
DA µg/kg/week: mean (SD)	0.58 (0.42)	0.56 (0.46)	0.48 (0.44)	0.005
V mg/Q2W: mean (SD)	124 (59)	111 (61)	100 (68)	0.001

Hb level was maintained within the targets, with a significant dose decrease in ESA (17.3%) and V (18.5%). Iron parameters should be more carefully monitored in order to decrease V use, and avoid any iron overload. Such a strategy is advantageous for the entire anemia management team, with additional benefits for nurses in terms of convenience, reduced risk of omission and traceability.

Conclusions: Synchronized administration of DA and iron sucrose may result in erythropoïesis optimisation in HD Pts. These findings, which may have important implications for economic reasons, need to be confirmed by further controlled trials.

FR-PO1597

Metoxipolietilenglicol-Epoetina Beta (MIRCERA): An Efficient Treatment for Anaemia in Peritoneal Dialysis Patients. Final Results. CAPRI STUDY

Rosa Ramos,[1] Teresa Maria Gonzalez,[2] Manuel Vera,[3] Isabel García,[4] Francesc Barbosa,[5] Josep Teixido,[6] Carmen García,[7] Marc Cuxart,[8] Carlota Gonzalez,[2] Juan José De la Cruz.[9] *[1]Nephrology, Vall d'Hebron Hospital, Barcelona, Spain; [2]Nephrology, Bellvitge Hospital, Hospitalet de Llobregat, Barcelona, Spain; [3]Nephrology, Clinic Hospital, Barcelona, Spain; [4]Nephrology, Josep Trueta Hospital, Girona, Spain; [5]Nephrology, del Mar Hospital, Barcelona, Spain; [6]Nephrology, Germans Trias i Pujol Hospital, Badalona, Barcelona, Spain; [7]Nephrology, Joan XXIII Hospital, Tarragona, Spain; [8]Nephrology, Figueres Hospital, Figueres, Girona, Spain; [9]Statistical, Autonoma University, Madrid, Spain.*

Background: MIRCERA is a new eritrophoietic stimulating agent (ESA) with the longest half life. It is a good condition to indicate Mircera for treatment of anaemia in predialysis and peritoneal dialysis (PD). The aim is to follow the evolution of hematological parameters in PD patients in Catalonia. This is the first study on PD patients.

Methods: We included 113 PD patients that initiated MIRCERA as the first treatment of anaemia or as a change from previous ESAs.

Results: 83 patients completed follow-up at 12 months. Mean age was 57,8±16y. 59% were men. 71% of patients began PD as first treatment of CRF, 12(14,5%) were transferred from HD and 12(14,5%) returned to PD after kidney transplant failure. 10(12%) patients were naïve, 52(62%) previously treated with darbepoetin-alpha, 19(22,9%) with Epo-beta and 2(2,4%) with Epo-alpha. Mean dose of Mircera was 115,4±56,2µg/month at the beginning, 117,2±58,5µg/month at 6 months, 126±65,9µg/month at 12 months. Hb levels remained stable all through the measurements(11,9±1,4g/dl;11,8±1,4g/dl;11,8±1,5g/dl). No relation was observed between dose of dialysis administered(weekly Kt/V) and Mircera dose or Hb levels. At baseline, 36.7% of patients showed good blood pressure control(SBP/DBP <140/90 mmHg) and 51.0% at 6 months($p<0.05$). Mild adverse events were registered not directly related to Mircera.

Conclusions: Mircera once a month is safe and effective in correcting and maintaining the Hb levels. This pivotal study suggests that MIRCERA is an efficient treatment for PD patients.

FR-PO1598

Once Monthly C.E.R.A. Therapy Stabilizes Hemoglobin Levels in Peritoneal-Dialysis-Patients: Results from the BEAM-Study

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Background: The continuous erythropoietin receptor agonist (C.E.R.A.) has been approved for once monthly (QM) treatment of renal anemia. Therefore, it might be useful in peritoneal dialysis (PD) patients (pts). The BEAM study was the first study in Germany which was designed to investigate the efficacy and safety of C.E.R.A. in this pts.

Methods: In this non-interventional study 223 PD pts fulfilled the inclusion criteria and had been enrolled in the study. On average, the pts were 3.0 years exposed to PD. 61.4% of pts underwent CAPD (continuous ambulatory PD), 29,6% received APD (apparative PD) and 8,6% were treated in-centre at least three times a week with apparative PD. 48,2% of pts started with QM C.E.R.A. coming from shorter acting ESA, 27,7% were already on C.E.R.A. and 24,1% started de novo with C.E.R.A..Data about C.E.R.A treatment were analysed about a time period of up to 9 months (mt) in a descriptive way.

Results: 220 pts (98,7%) formed the safety population (SP). For 80.5% of pts Hb measurements were available over a time period of 2 mt beyond 6 mt of treatment (modified efficacy set, MES). Mean age of the study population was 59,6 years (48,6% female). 40,9% of pts exhibited cardiac disorders like coronary artery disease and 18,2% type II diabetes mellitus. C.E.R.A. administration modus was as follows: 38.6% of pts at the study centers, 35.5% of pts excl. at home and 25.9% of pts at both locations. In the MES-population the Hb increased from 11.1 to 11.4 g/dL at the end of the study. During the evaluation period (mt 7-9) 14.9, 37.9, and 39.8% of pts displayed Hb values in the target range of 11-12, 10-12, and 11-13 g/dL, respectively. The average dose of C.E.R.A.changed from 110 to 103 µg at the end of the study. In total, 37,3% of the SP population didn´t need any C.E.R.A.dose modifications during the 9-mt. Overall there were no specific drug related adverse events during the study.

Conclusions: QM treatment with C.E.R.A. is associated with a high degree of Hb-stability and tolerability within PD pts.

Funding: Pharmaceutical Company Support

FR-PO1599

Secular Trends in Anemia Management in Hemodialysis 2004-2010

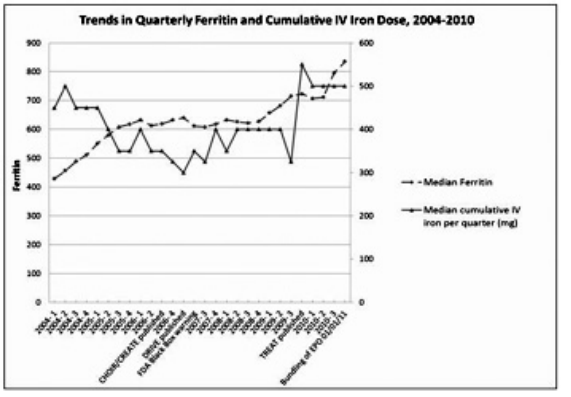
Navdeep Tangri,[1] Dana C. Miskulin,[1] Jing Zhou,[2] Courtney Cook,[2] Karen J. Bandeen-Roche,[2] L. Ebony Boulware.[2] *[1]Tufts Medical Center; [2]Johns Hopkins University; [3]DEcIDE Network Patient Outcomes in ESRD Study Investigators.*

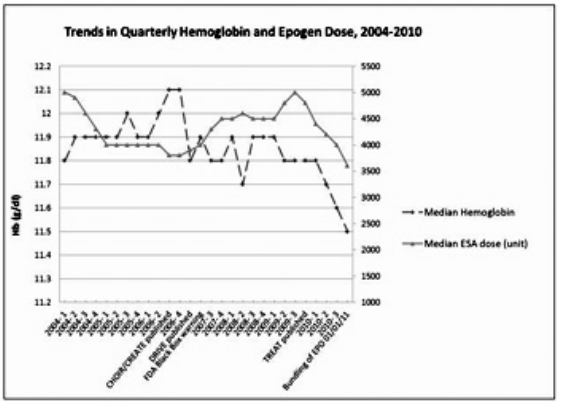
Background: Several factors may have influenced anemia management in US hemodialysis patients, including: clinical trials showing increased adverse events and mortality with targeting higher hemoglobin (Hb), a FDA black box warning advising clinicians to avoid Hb >13 g/dl, a trial showing Hb responds to IV iron with serum ferritin up to 1000 ng/ml, and Medicare payment bundling of erythropoietin stimulating agents in Jan 2011.

Methods: We describe quarterly changes in median Hb, serum ferritin, transferrin saturation (TSat), and erythropoietin and IV iron use from 2004-2010 among patients treated with HD for ≥3 months in Dialysis Clinic Inc.

Results: As shown in the figures below, among 27798 patients, the median Hb increased between 2004 and 2006, peaking at 12.1 g/dl in 2006, declined in 2007, and gradually declined to its lowest point of 11.5 g/dLin 2010. Iron use declined from 2004-2005 and increased sharply in 2010. The median erythropoietin dose per treatment decreased steadily from 2004 (4200 units) to 2007 (3000 units), and slowly declined through 2010, at 2300 units by year end. The median serum ferritin was 429 ng/ml in early 2004 and steadily increased to 835 ng/ml in late 2010. Transferrin saturation (TSat) started to increase only in late 2010.

Conclusions: Hb and Epogen use declined and IV iron use increased from 2004-2010, with the largest changes occurring in 2010. Concurrently, serum ferritin increased from 2004 but median TSat and IV iron use per quarter increased only since 2010. Trends in utilization may reflect the influence of recent research findings and policy changes. Further investigation is warranted of reasons for stable TSat in the setting of rising ferritins, as well as of the safety of IV iron administration in the setting of higher ferritin levels.





Funding: Other U.S. Government Support

FR-PO1600

Hyporesponsiveness to Erythropoiesis Stimulating Agents Is Associated with Increased Mortality among Hemodialysis Patients

Bed P. Chhatkuli, Kambiz Kalantarinia. *Division of Nephrology, University of Virginia Health System, Charlottesville, VA.*

Background: Higher hemoglobin concentrations are associated with higher mortality in CKD. We investigated if this increased mortality is related to higher degree of hyporesponsiveness to erythropoiesis stimulating agents (ESA).

Methods: Retrospective cohort study of all prevalent UVA ESRD patients on chronic hemodialysis for at least 3 months in 2008. Data on patient demographics, comorbidities and 3 consecutive monthly values for body weight, hemoglobin, ESA dose and other lab values was collected. Responsiveness to ESA was determined by dividing the ESA dose per kilogram of body weight by the Hb concentration. The association between tertiles of ESA responsiveness (EResp) and mortality was investigated in a multivariate model including patient demographics, diabetes status, albumin, Kt/V, PTH, iron status and use of renin angiotensin blockers.

Results:

Patient Characteristics

	All (n = 702)	Tertile 1 (n = 234)	Tertile 2 (n= 234)	Tertile 3 (n = 234)	p value
Age (SD)	62.5 (13.9)	61.9 (12.7)	63.6 (14.0)	62 (15)	0.98
Gender (%) F	45.2	39.7	46.2	49.6	0.09
Race (%) AA/C/O	(57/41/2)	(54/44/2)	(55/42/3)	(62/37/1)	0.25
RAAS blocker (%)	42	58	62	54	0.24
Diabetes (%)	57.8	55.1	59.8	58.5	0.57
Albumin (g/dL)	3.9 (0.5)	4.0 (0.4)	4.0 (0.5)	3.8 (0.6)	<0.0001
PTH (pg/mL)	336 (302)	297 (309)	341 (241)	372 (343)	0.008
Ferritin (ng/mL)	608 (634)	572 (367)	598(378)	654 (959)	0.18
Transferrin sat (%)	24.1 (9.5)	25.8 (8.9)	24.1 (8.1)	22.4 (11)	0.0001
Hemoglobin (g/dL)	11.5 (1.1)	12.0 (0.8)	11.6 (0.8)	11 (1.3)	<0.0001
Aranesp dose (mcg)	174.2 (105 – 327)	83.3 (58.3 – 108.3)	174.2 (141.7 – 226.7)	402.1 (300.0 – 620.0)	<0.0001
EResp (mcg/ kg/g Hb)	0.32 (0.32)	0.09 (0.03)	0.22 (0.05)	0.65 (0.38)	<0.0001
Alive (%)	64.4	73.9	62.8	56.4	0.0003

In multivariate analysis, age (p<0.0001), race (p =0.047) and EResp (p <0.001) were associated with mortality. In comparison to the lowest quartile of EResp, tertiles 2 and 3 had OR of 1.80 and 2.7 for mortality. In a different model where Hb and ESA dose were entered separately, ESA dose and not Hb was associated with mortaliy.

Conclusions: Hyporesonsiveness to ESA and higher doses of ESA are associated with higher all cause mortality among HD patients.

FR-PO1601

IV Versus SC ESA Dosing Requirements in US and Non-US Hemodialysis (HD) Patients Brigitte Schiller,[1] Iain C. Macdougall,[1] Francesco Locatelli,[1] Andrzej Wiecek,[1] Adrian Constantin Covic,[1] Carol Francisco,[2] Helen Tang,[2] Sandra Tong,[2] Minjia Chen,[2] Anne-Marie Duliege,[2] Krishna R. Polu,[2] Martha Mayo.[2] *[1]AFX-01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc, Palo Alto, CA.*

Background: Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that acts via stimulation of the erythropoietin receptor.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials assessing the safety and efficacy of peginesatide (once monthly) compared with epoetin alfa/beta (epoetin; 1-3 times weekly) in HD patients previously on stable epoetin doses (EMERALD 1 and 2). Patients received study drug via the same administration route used at the time of screening. This analysis compares hemoglobin (Hb) levels and intravenous (IV) versus subcutaneous (SC) dosing requirements in US and non-US HD patients.

Results: The median epoetin dose during the evaluation period (weeks 29-36) tended to be lower for the SC than IV route across regions, whereas this was not observed for peginesatide (Table). For both routes, ESA doses were higher in US than non-US patients. Mean Hb levels were similar for US and non-US patients regardless of administration route (Table). Mean total IV iron administered was 2246 mg for US and 2613 mg for non-US patients during weeks 0-52.

	SC		IV	
Evaluation Period	**Peginesatide**	**Epoetin**	**Peginesatide**	**Epoetin**
US Patients				
Mean Hb level (SE), g/dL	11.3 (0.1) [n = 34]	11.2 (0.2) [n = 15]	11.1 (0.04) [n = 698]	11.2 (0.05) [n = 380]
Median dose (Q1-Q3), mg/mo or U/wk	6.8 (3.5-11.8) [n = 33]	7,200 (3,800-13,800) [n = 15]	5.6 (3.4-10.2) [n = 683]	9,800 (5,400-18,700) [n = 374]
Non-US Patients				
Mean Hb level (SE), g/dL	10.9 (0.1) [n = 62]	10.8 (0.1) [n = 27)	11.2 (0.09) [n = 139]	10.9 (0.1) [n = 63]
Median dose (Q1-Q3), mg/mo or U/wk	3.9 (2.5-6.8) [n = 62]	4,100 (2,300-6,100) [n = 27]	3.5 (2.2-5.7) [n = 138]	5,000 (2,700-8,000) [n = 61]

Conclusions: For peginesatide, the SC route was not associated with reduced doses compared with IV. Despite similar Hb values in the range of 10-12 g/dL, ESA doses in US patients were higher than in non-US patients.

Funding: Pharmaceutical Company Support

FR-PO1602

Nutrition and Erythropoietin Resistance Index in Hemodialysis Patients. CARNIDIAL Trial [NCT 00322322] Lucile Mercadal,[1] Mathieu Coudert,[2] Anne Vassault,[3] Laurence Pieroni,[3] Messaoud Ouziala,[4] Hélène De Préneuf,[5] Aude Servais,[6] Nader Bassilios,[7] Ubald Assogba,[8] Gilbert Deray.[1] *[1]Nephrology, AP-HP, Pitie Salpetriere Hospital, Paris, France; [2]Biostatistics, AP-HP; [3]Biology, AP-HP; [4]CMC Pantin; [5]AURA; [6]AP-HP, Necker hospital; [7]Clinique de Turin; [8]Institut Mutualiste Montsouris.*

Background: Nutrition influences anaemia correction in hemodialysis by several pathways, such as nutrient deficiencies and a decrease of anti-oxidative capacity leading to a pro-inflammatory effect. We described herein the relation between nutrition markers and rHuEPO responsiveness.

Methods: The data of 92 incident hemodialysis patients randomized to receive in double blind placebo or intravenous L-carnitine for a 1 year period were considered for analysis. Primary outcome was presented elsewhere. Determinant factors of rHuEPO resistance index (RI) defined as weekly rHuEPO (UI/kg body weight) / hemoglobin level (g/dL) were studied on 1144 observations taking into account patient effect and using a Box-Cox transformation. Albuminemia dosage was centralized and done by a turbidimetry method. Relations between RI and nutritional markers and supply were studied in multivariate analysis adjusted for iron status, CRP, dialysis vintage, renin angiotensin system inhibitors, dietary supplement, and carnitine group.

Results: Mean caloric supply was 1691 ± 460 kcal/day. Albuminemia increased during the study period from 34.0 ± 6.4 to 36.8 ± 5.2 g/L (p=0.0045). Albuminemia, lymphocytes count were negatively associated with RI whereas serum bicarbonates were positively associated with it. After adjustment,the nutrition marker being either albuminemia or lymphocytes count or serum bicarbonates remained strongly associated with RI (β -0.12 ± 0.02 per g/L albuminemia, p<0.0001; β -0.03±0.01 per % increase in lymphocytes, p=0.01; β 0.08 ± 0.03 per mmol/L bicarbonates, p=0.005). Oral dietary supplement remained associated with a higher RI (β 2.9±0.7 for patients taking supplement, p=0.0003).

Conclusions: Nutrition status independently of iron and inflammation status and carnitine supply is a potent determinant factor of rHuEPO resistance index.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1603

The Effects of Feraheme® (ferumoxytol) Administration on Target Hemoglobin Levels in Hemodialysis Patients across Three Dialysis Chains Amit Sharma,[1] Premila Bhat,[2] Brigitte Schiller,[3] Justin Mclaughlin,[4] Zhu Li,[4] Annamaria T. Kausz,[4] William Strauss.[4] *[1]Boise Kidney and Hypertension Institute, Boise, ID; [2]Atlantic Dialysis Management Services, Ridgewood, NY; [3]Satellite Healthcare, San Jose, CA; [4]AMAG Pharmaceuticals, Lexington, MA.*

Background: Dialysis clinics typically aim to maintain their IDA patients' hemoglobin (Hgb) level between 10-12 g/dL. The IV iron ferumoxytol (Feraheme®) was approved by the FDA in 2009, with a recommended dose of 2x510 mg 3 to 8 days apart. The intent of this study was to examine the success of maintaining Hgb levels within the target range using ferumoxytol across a range of real-world administration practices and patient characteristics.

Methods: Three separate US-based dialysis chains provided laboratory and dosing data from all patients treated with ferumoxytol throughout 2010. Throughout this period, all available values for Hgb, TSAT, and ferritin were obtained for each patient from electronic laboratory databases, while all ferumoxytol doses were obtained from billing records. Monthly Hgb values were based on the last available measurement within the calendar month. Across the three chains, different IV iron treatment paradigms were employed: Chains A and B primarily dosed ferumoxytol in courses of 2 x 510 mg administered within 3 to 8 days, while Chain C primarily gave single 510 mg doses monthly as needed. All 3 chains targeted Hgb between 10 to 12 g/dL.

Results: Over the one-year period across the three chains, 8,666 patients were treated with 33,358 doses of ferumoxytol. Following conversion to ferumoxytol, all three chains were able to maintain a majority of their patients within the target Hgb range over the following 12 months despite variations in IV iron administration practices and patient characteristics (Chain A = 55 to 66%; Chain B = 63 to 78%; and Chain C = 52 to 72%).

Conclusions: Across the 3 dialysis chains, the use of ferumoxytol either by period course or by monthly dosing allowed for the majority of patients to be maintained within the target Hgb range of 10-12 g/dL.

FR-PO1604

Reduction in Erythropoeitin Usage Following Change in Iron Therapy to Ferumoxytol Roger J. Haley. *KDHCD, Visalia, CA.*

Background: Based upon a favorable reimbursement model, the two hemodialysis facilities operated by Kaweah Delta Health Care District, a California Hospital District, changed parenteral iron preparation from ferrous sucrose [V] to ferumoxytol [F].

Due to looming "bundling" of dialysis related charges, which would nullify the favorable reimbursement, a retrospective study was carried out to ascertain overall financial performance of continued use of [F] versus [V]. The change-over for all patients occurred in the same calendar month.

Methods: The patient cohort consisted of all patients who underwent hemodialysis in one of the facilities in the six months prior to the change over and the 7 months following the changeover. Patients were censored if: no [V] was given in the 6 month [V]-phase, were hospitalized at any point in the 13 months, received a blood transfusion as any point in the 13 months, or missed more than 2 dialysis treatments during any calendar month. 92 patients met these criteria. Data was analyzed for this study group (n=92).

Administration of EPO and Iron was determined by a renal pharmacist acting under a protocol for Epo and Iron administration, that remained unchanged during the study time frame, other than iron preparation.

The "change-over month" was excluded from analysis due to the fact both EPO and F were administered based upon laboratory determinations at the beginning of the changeover month, which reflected response to [V].

Results: Values in table are means ± SD. The 6 months preceding change-over was compared to the 6 months following the change-over month in this stable patient group.

Results

Group	Hgb	Ferritin	Iron Sat	Epo u/dialysis	Iron mg/month
[V]	11.7±0.8	670±427	28±12	3679±3255	172±275
[F]	11.7±0.8	782±352	31±13	2846±3762	137±265
p value	0.74	<0.0001	<0.05	<0.0001	<0.01

The data shows controlled Hgb, improved iron studies, and lower iron and EPO usage.

Conclusions: Based upon current population and acquisition and administration costs, progressive bundling of reimbursement (to 100% cost borne by the facilities for all EPO and Iron) the use of [F] should result in $986 savings per patient annually, with overall savings exceeding $300,000 annually.

FR-PO1605

C.E.R.A. Once-Monthly (QM) Maintains Stable Hemoglobin (Hb) Values in Patients (pts) with Chronic Kidney Disease (CKD) on Dialysis: A Pooled Analysis of Nine Trials Conducted in a Real-World Setting Frank Dellanna,[1] Michael Dickenmann,[2] Ricardo Correa-Rotter,[3] Christos E. Iatrou,[4] Valeriy Y. Shilo,[5] Sylvie Sulkova,[6] Joan Fort,[7] Neval Duman,[8] Francesco Locatelli.[9] *[1]Dialysezentrum Karlstrasse, Düsseldorf, Germany; [2]University Hospital, Basel, Switzerland; [3]Instituto Nacional de la Nutrición, Salvador Zubirán, Mexico; [4]General Hospital of Nikea Piraeus, Athens, Greece; [5]Moscow University of Medicine and Dentistry, Moscow, Russian Federation; [6]Institute of Clinical and Experimental Medicine, Prague, Czech Republic; [7]University Hospital Vall d'Hebron, Barcelona, Spain; [8]Ankara University School of Medicine, Ankara, Turkey; [9]Alessandro Manzoni Hospital, Lecco, Italy.*

Background: To investigate the impact of different Hb targets on Hb levels, Hb stability and C.E.R.A. dose in pts with CKD on dialysis switched from maintenance therapy with shorter-acting erythropoiesis-stimulating agents (ESAs) to C.E.R.A. QM.

Methods: Data were pooled from nine 24-week trials in adult pts with CKD on dialysis converted to C.E.R.A. QM. Clinical endpoints during the final 8-week period (evaluation period [EP]) were analysed according to the target Hb range: 10-12 g/dL (4 trials), 10.5-12.5 g/dL (3 trials), or 11-13 g/dL (2 trials).

Results: Pts (n=1473) had a median age of 63 (range 19-93) years. The table shows: mean Hb; mean Hb fluctuation; the percentage of pts with stable Hb (average Hb within the target range or a change from baseline ≤1 g/dL); and the mean dose of C.E.R.A. QM for the 3 groups during the EP.

Target range (n)	Mean Hb (g/dL)	Mean Hb fluctuation (g/dL)	Pts maintaining stable Hb levels (%)	Mean C.E.R.A. dose (µg)
Overall (n=1473)	11.4±1.02	0.48±0.30	77	134±86
10-12 g/dL (n=603)	11.2±0.94	0.47±0.28	82	128±92
10.5-12 5g/dL(n=459)	11.6±1.11	0.53±0.34	74	136±91
11-13 g/dL (n=411)	11.5±0.96	0.44±0.28	75	143±62

On average there were 2.0 dose changes per pt over 24 weeks of treatment. Tolerability of C.E.R.A. QM was similar to that of prior ESA therapy.

Conclusions: These pooled data in pt on dialysis show homogenous results with C.E.R.A. QM across all study endpoints, irrespective of the target Hb.

Funding: Pharmaceutical Company Support

FR-PO1606

Characteristics of Patients with Acute vs. Chronic Erythropoiesis Stimulating Agent (ESA) Hyporesponsiveness David T. Gilbertson, Yi Peng, Tom Arneson, Stephan C. Dunning, Allan J. Collins. *CDRG, Minneapolis, MN.*

Background: 'ESA hyporesponsiveness' in hemodialysis (HD) patients refers to high doses of ESAs to treat anemia. A presumed need for high-dose ESA is often the result of functional iron deficiency, bone marrow fibrosis and inflammation. Episodes of hyporesponsiveness may resolve quickly, or may persist. We assessed unadjusted associations of patient characteristics with acute vs. chronic hyporesponsiveness.

Methods: We used point prevalent HD patients on May 1, 2008 with Medicare coverage, surviving through Dec 2008. The 90th percentile of total EPO dose each of 5 months (Aug-Dec) was used to define monthly hyporesponsiveness for each patient. Further classifications included chronic (hyporesponsive in 4+ consecutive months) and acute hyporesponsiveness (≤3 consecutive months of hyporesponsiveness with at least one month not hyporesponsive before and after the hyporesponsive month(s)). Comorbidity was defined using Medicare Part A & B claims during May-Jun (antecedent), and separately, Aug-Dec (concurrent).

Results: 4.5%, and 5.2% of patients were classified as chronic, and acute, respectively. There were no differences by gender or race between patients with acute vs. chronic hyporesponsiveness. However, patients who were younger, with diabetes as cause of renal failure, lower BMI, and of shorter dialysis vintage had proportionally greater acute hyporesponsiveness. Most comorbidities, whether antecedent to the study period or concurrent, were more prevalent in patients who had episodes of acute versus chronic hyporesponsiveness. The largest differences between the two groups were for cerebrovascular accident/transient ischemic attack, ASHD, and infectious and all-cause hospitalizations.

Conclusions: Episodes of acute and chronic hyporesponsiveness are not uncommon, and early identification of patients at risk for such episodes may lead to improved anemia management in these patients. The impact of the bundled reimbursement system, implemented in Jan 2011, on the management of anemia in these patients is unknown, and should be assessed.

FR-PO1607

Hemoglobin (Hb) Stability during Peginesatide Versus Epoetin Treatment in Hemodialysis (HD) Patients Robert Provenzano,[1] Brigitte Schiller,[1] Mark Kaplan,[1] Bruce S. Spinowitz,[1] Carol Francisco,[2] Anne-Marie Duliege,[2] Alex Yang,[2] Krishna R. Polu,[2] Martha Mayo.[2] *[1]AFX-01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc., Palo Alto, CA.*

Background: Hb variability is associated with an increased risk of mortality in HD patients (Pisoni 2011). Limiting it is desirable and important for reimbursement metrics like the quality incentive program (QIP). Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that is designed to specifically stimulate the erythropoietin receptor.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label studies assessing safety and efficacy of peginesatide (once monthly; N = 1066) compared with epoetin alfa/beta (epoetin; 1-3 times wkly; N = 542) in HD patients (EMERALD 1 and 2). Hb variability during the evaluation period (EP; wks 29-36) was measured using the standard deviation (SD) of Hb levels within patients and median of the absolute deviation (MAD) from the within-patient Hb level. An Hb analysis similar to the QIP determined the proportion of patients with average monthly Hb levels over 1 yr within the 10-12 g/dL target range (wks 29-80). A composite safety endpoint (CSE) was evaluated that consisted of 6 events: all causes of death, stroke, myocardial infarction, and serious adverse events (AEs) of congestive heart failure, unstable angina, and arrhythmia.

Results: Hb variability was similar for the peginesatide and epoetin groups using the SD (median = 0.51 vs 0.48) and MAD (median = 0.44 for both) methods. In the QIP analysis, most patients in the peginesatide and epoetin groups had average monthly Hb levels within the target range (91% vs 95%). Fewer patients in the peginesatide than epoetin group had dose adjustments (>20% change from previous dose) during the EP (47% vs 68%). The frequency of CSE events during the studies was similar for both groups (23% vs 24%).

Conclusions: Hb stability and the proportion of patients within the target range were similar for peginesatide and epoetin; fewer patients in the peginesatide group had dose adjustments. Similar rates of cardiovascular AEs were reported for peginesatide and epoetin.

Funding: Pharmaceutical Company Support

FR-PO1608

Prescribing EPO to Chronic HD Patients: A Physiologic Approach Implemented with Continuous Quality Improvement Leads to Decreased EPO Dose Changes and Decreased Hemoglobin Variability Jonathan Lorch,[1] Victor E. Pollak.[2] *[1]New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY; [2]University of Colorado HSC, Denver, CO; [3].*

Background: Prescribing EPO has been influenced by widely used guidelines whose validity in practice has never been tested rigorously. Physicians have been urged to maintain patient Hb in the 100-120 g/L range, and to adjust EPO dosage to meet this target range. Allowing Hb to exceed 120 g/L has been thought to have an adverse effect on survival despite compelling contrary evidence.

Methods: Starting in February 2010, we tested an approach using patient-specific data collected during daily care and stored in a commercially available electronic medical record (EMR) in one dialysis unit (the protocol unit, 260 patients). Repleting iron insufficiency, ensuring iron sufficiency, and reducing EPO stepwise were key elements, while changing dose every 6-8 weeks rather than every 2-4 weeks.

Year	2004-2005	2006-2007	2008	2009	2010-1	2010-2	2011
Protocol Unit	12.6	14.9	10.6	8.4	4.8	3.8	2.1
Comparator Unit 1	18.9	20.1	20.0	20.5	21.2	17.0	8.5
Comparator Unit 2	8.8	19.8	19.3	15.2	17.6	14.3	8.5

Results: This was associated with an increase in Hb to 123.6 g/L and a low mortality (11.7 deaths per 100 patient years). Two other units (210, 186 patients respectively) served as comparators. In each unit, using data stored in the EMR, we counted and report in the Table new and discontinued orders for EPO and analyzed duration of treatment by HD per patient year from 2004 to May 2011. Data for 2010 are shown in two 6-month periods because the protocol, with its EPO prescribing changes, was instituted in 2010.

Conclusions: In the protocol unit. changes for new and discontinued EPO orders decreased by 78% and 87% per patient year respectively. In 2004-2009 there was an average of 15.8 EPO dose changes per year in the 3 units, a rate 4.2 and 7.5 times that in the protocol unit in the last two time periods. Also, the median coefficient of variation (CV) of individual patient Hb decreased 49% from 11.3% to 5.7% in the protocol unit. Hb CV was 49%, 30%, and 29% greater in the 3 units in 2004-2009 when KDOQI prescribing guidelines were in use.

FR-PO1609

Prescribing EPO to Chronic Maintenance HD Patients: A Physiologic Approach Implemented with Continuous Quality Improvement Leads to Decreased EPO Use and Increased Hemoglobin Jonathan Lorch,[1] Victor E. Pollak.[2] *[1]Medicine, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY; [2]Medicine, University of Colorado HSC, Denver, CO; [3].*

Background: EPO mitigates severity of anemia in chronic HD patients. Data from the US, Australia/New Zealand, and 8 European countries found that the per country weekly EPO dose varied from 9,500 to >21,300 units, with no relationship between dose and achieved Hb which ranged from 116 to 121 g/L (McFarlane PA, et al. Kidney Int 78:215, 2010). The therapeutic approach has been influenced by widely used guidelines whose validity in practice has never been tested rigorously.

Methods: With the objectives of achieving optimal Hb with minimum required EPO and maintaining stable Hb and EPO, we designed and tested an approach using patient-specific data collected during daily care and stored in a commercially available electronic medical record (EMR). Patient specific data collected over 9.5 years informed the study which was implemented in 250 HD patients in a single dialysis unit receiving EP0 prior to February 1, 2010, and followed to April 30, 2011. Repleting iron insufficiency, ensuring iron sufficiency, and reducing EPO stepwise were key elements. Decision support tools were used that enabled relevant data display over prolonged periods in patient-centered reports. EPO dose, adjusted at 6-8 week intervals, was based on current clinical condition and past responses.

Results: In the study patients, IV iron administration and TSAT increased after protocol start. Hb increased by months 1-2; EPO decrease began from month 4 onward. By months 11-15, EPO had decreased 32% from 15,488 to 10,580 units/week while patient median Hb increased 8% from 114 to 123.6 g/L. Both Hb and EPO administered changed little from month 7 onward, and were stable in months 11-15. Comparable results were not observed in two comparator units that reduced EPO administration, but with neither a defined plan nor a CQI approach.

Conclusions: It is possible to reduce EPO administration and its cost while maintaining or improving patient Hb and maintaing a low patient mortality (11.7 deaths per 100 patient years).

FR-PO1610

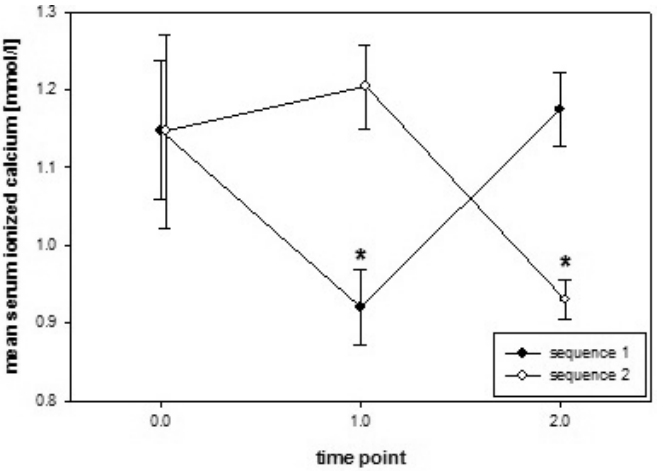
Serum Ionized Calcium Levels Determine Arterial Stiffness in Dialysis with Regional Citrate Anticoagulation Matthias B. Moor, Anja Kruse, Dominik E. Uehlinger, Ute Eisenberger. *Nephrology and Hypertension, University Hospital Bern, Bern, Switzerland.*

Background: Hemodynamic effects of changes in serum ionized calcium (iS_{Ca}) are difficult to determine during conventional hemodialysis (HD) using a fixed dialysate concentration of calcium. The model of regional citrate anticoagulation (RCA) using continuous calcium infusion allows to study the effects of predefined iS_{Ca} changes on arterial stiffness and blood pressure during HD.

Methods: In a cross-over study, 15 patients with chronic kidney failure underwent two HD sessions with RCA. Each session was divided into 2 study phases in which iS_{Ca} was titrated either to 0.8-1.0 mmol/l or to 1.1-1.4 mmol/l. Sequence of phases was randomly chosen and alternated for the second session. 30 minutes after reaching a stable iS_{Ca} level, pulse wave velocity (Pulse Trace PWV, Micro Medical Ltd, UK), arterial blood pressure and heart rate were measured. Statistical analysis was performed with SAS 9.2 for Windows on an X64_VSPRO platform.

Results: ISCa levels were modified during sequence 1 (iSCalow-high) from a predialysis baseline value of 1.15 ± 0.09 mmol/L, first to 0.92 ± 0.05 mmol/L (time point 1; *p< 0.001 vs baseline) and then to 1.18 ± 0.05 (time point 2; ns). During during sequence 2 (iSCahigh-low), iSCa levels were modified from 1.15 ± 0.12 mmol/L first to 1.20 ± 0.05 mmol/L (time point 1; ns vs baseline) and then to 0.93 ± 0.03 (time point 2; *p < 0.001), see figure.

Assuming a basic linear repeated measures model, PWV was positively related to iS_{Ca} levels ($p < 0.03$) independent of systolic or diastolic blood pressure, heart rate or ultrafiltration rate.



Conclusions: PWV, an indirect measure of arterial stiffness known to impact on long-term survival in chronic hemodialysis patients, is closely related to serum ionized calcium levels in HD patients using RCA as a study model.

Funding: Clinical Revenue Support

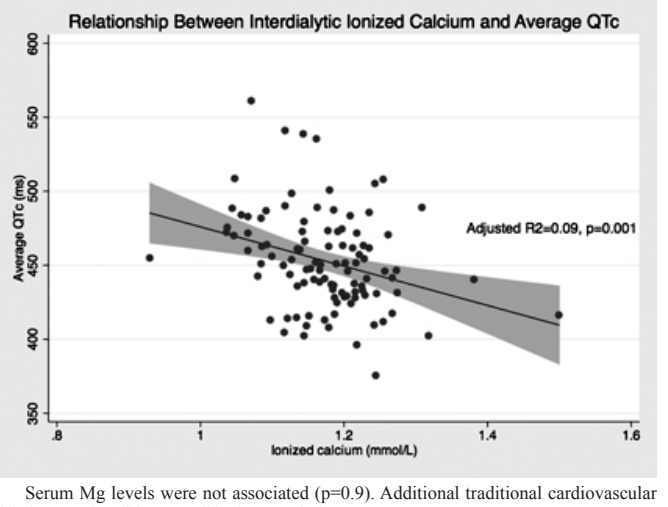
FR-PO1611

Interdialytic Ionized Calcium Is Associated with a Pro-Arrhythmic Phenotype in Incident Dialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in ESRD (PACE) Study Danica Lam,[1] Wen Hong Linda Kao,[2] Stephen M. Sozio,[2] Lucy A. Meoni,[2] Tariq Shafi,[2] Bernard G. Jaar,[2] Julia J. Scialla,[2] Larisa Tereshchenko,[2] Rulan S. Parekh.[1,2] *[1]University of Toronto; [2]Johns Hopkins University.*

Background: Patients on hemodialysis are at high risk of malignant arrhythmias and sudden cardiac death (SCD). We hypothesize that low levels of interdialytic ionized calcium (iCa) and magnesium (Mg) are associated with arrhythmias.

Methods: We investigated the cross-sectional association of prolonged QT interval, a pro-arrhythmic electrocardiogram (ECG) metric, with levels of Mg and iCa in an incident hemodialysis cohort of 114 PACE participants. At the baseline visit on a non-dialysis day, iCa corrected for pH and Mg levels were measured. A 5-minute signal-averaged ECG was recorded in a quiet room. Corrected QT (QTc) intervals were calculated and prolonged QTc was defined as >460 ms in women and >440 ms in men.

Results: In the 114 participants studied, mean age was 55±15 years. 54% were male, and 74% African-American. 32% had self-reported coronary artery disease, 54% diabetes, 25% atrial fibrillation, and 22% congestive heart failure. Prevalence of a prolonged QTc was 58% in men and 46% in women. The mean iCa levels were 1.2±0.1 mmol/L and mean Mg levels were 1.8±0.2 meq/L. Linear regression showed that iCa has a significant effect on QTc (Figure).



Serum Mg levels were not associated (p=0.9). Additional traditional cardiovascular risk factors also did not modify the association.

Conclusions: Prevalence of prolonged QTc on non-dialysis days was very high among incident dialysis patients. Interdialytic iCa but not Mg is significantly inversely associated with length of the QTc. Additional work is needed to determine if clinical dialysis parameters lower iCa and increase the risk of long QTc, which ultimately may lead to SCD.

Funding: NIDDK Support, Private Foundation Support

FR-PO1612

Association of Cardiac Valve Calcification and C-Reactive Protein (CRP) with Cardiovascular and All-Cause Mortality in End-Stage Renal Disease Patients – 10-Year Follow-Up Study from Induction of Hemodialysis Therapy

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Background: Cardiac valve calcification is frequently seen in patients with end-stage renal disease (ESRD), and may potentially reflect systemic atherosclerosis. Serum CRP is also reported to predict future cardiovascular (CV) events. We investigated the association of cardiac valve calcification, CRP and their joint role with prediction of mortality in patients on hemodialysis (HD).

Methods: A total of 1,290 consecutive patients who started HD therapy were screened by echocardiography. Patients were divided into 3 groups; those without valve calcification (NC group, n=548), those with calcification in a single (aortic or mitral) valve (SC group, n=487) and those with calcification in both valves (BC group, n=255). They were also divided into tertiles according to serum CRP levels; T1: <0.9, T2: ≥0.9 to >4.9 and T3: ≥4.9 mg/l, respectively. They were followed up for up to 10 years.

Results: Serum CRP levels were 4.0±6.7, 6.8±9.9 and 7.2±9.8mg/l in the NC, SC and BC group, respectively (p<0.0001), and were independently associated to valve calcification (Odds ratio 1.13, 95%CI 1.06-1.21, p=0.0003). Adjusted hazard ratio (HR) of valve calcification was 2.64 (95%CI 1.53-4.56, p=0.0023 for group BC vs. NC) for CV mortality and 1.86 (95%CI 1.30-2.67, p<0.0001 for group BC vs. NC) for all-cause mortality, respectively. Similarly, adjusted HR of elevated CRP levels was 3.09 (95%CI 1.54-6.16, p=0.0050 for T3 vs. T1) for CV mortality and 2.44 (95%CI 1.64-3.64, p<0.0001 for T3 vs. T1) for all-cause mortality, respectively. In the joint setting of valve calcification and CRP, the risk of CV and all-cause mortality was 6.4-fold (p=0.0073) and 3.6-fold (p<0.0001) in the BC group with T3 of CRP compared with the NC group with T1 of CRP even after adjustment, respectively.

Conclusions: Cardiac valve calcification and elevated CRP levels were closely linked, and interactively increased risk of mortality in ESRD patients who started HD therapy.

FR-PO1613

Phosphate Plasma Level as Risk Factor for Cardiovascular Morbidity and Mortality and Association of Sevelamer with Outcomes in Hemodialysis Patients – Posthoc Analysis of the AURORA Trial

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Background: AURORA was a randomized trial in 2776 hemodialysis patients, studying the effect of rosuvastatin10 mg on major CV events. There was no effect on any CV endpoint, but the database provides an opportunity to explore risk factors for CV events and mortality.

Methods: Baseline plasma phosphate as risk factor for CV disease (CVD) in HD patients was examined for CV endpoints used in the study, including mortality. Cox proportional analysis was used for risk factor assessment, after adjustment for covariates and presented as hazard ratio (HR) per unit phosphate increase. Ranking of risk factors at baseline (age, diabetes, high-sensitivity C-reactive protein [hsCRP], albumin, phosphate, low-density lipoprotein cholesterol (LDL-C), previous CVD, smoking, medication, etc) was performed. Concomitant medication was analysed as as well. Sevelamer was used in 508 patients (18%) , and its association with outcome was analysed.

Results: Plasma phosphate at baseline was an independent and strong risk factor for Major CV events (HR=1.49; p<0.0001; Rank 3), death from any cause (HR=1.34; p<0.0001; Rank 5), CV death (HR=1.58; p<0.0001; Rank 3), Atherosclerotic event (HR=1.46; p<0.0001; Rank 3), Major CV event or death (HR=1.35; p<0.0001; Rank 5), Nonfatal MI (HR= 1.51; p<0.0013; Rank 4) but not for Non-CV death (HR=1.11; p= 0.24; Rank 11) . Sevelamer use at baseline was associated with reduced mortality (HR= 0.84 ; P<0.029). Thus, plasma phosphate was one of the strongest and highest ranking risk factors for CV events and mortality and the use of sevelamer was associated with a reduced mortality, basically because of influence on CV death.

Conclusions: Baseline plasma phosphate levels is one of the most important risk factor for CV events and mortality in hemodialysis patients in the AURORA trial. Sevelamer treatment seems to be associated with a better patient survival.

Funding: Pharmaceutical Company Support

FR-PO1614

The German Calciphylaxis (Calcific Uremic Arteriolopathy) Registry

Vincent Brandenburg,[1] Paula Specht,[1] Jurgen Floege,[2] Markus Ketteler.[3] *[1]Cardiology, University Hospital Aachen, Aachen, Germany; [2]Nephrology, University Hospital Aachen, Aachen, Germany; [3]Nephrology, Klinikum Coburg, Coburg, Germany.*

Background: Calcific uremic arteriolopathy (CUA, calciphylaxis) is a rare condition associated with high morbidity and mortality. CUA is clinically characterised by painful, ischemic, skin ulcerations. Pathomorphologically, media calcification of cutaneous arterioles is the hallmark of the disease.

Methods: We established an internet-based registry (www.calciphylaxie.de) to allow online notification for all cases of established or suspected CUA. The registry is a comprehensive data base including various parameters (patient characteristics, laboratory data, clinical background and presentation as well as therapeutic strategies). Follow-up of the patients is planned by systematic queries of long-term outcome. We try to gain overview about current treatment strategies and link them to the clinical course.

Results: Altogether 127 CUA patients from 105 centers have been documented during 54 months: n = 76 (60 %) female; median age 69 yrs (range 21 - 89); 80% Caucasians; n = 99 (78%) dialysis patients; n= 57 (45%) diabetic patients.

Lab data from dialysis patients (median, interquartilen-range): Serum total calcium: 2.23 (2.08 - 2.39) mmol/L; serum phosphorus: 1.7 (1.3 - 2.1) mmol/L; PTH 156 (61 - 357) pg/mL. Oral anticoagulation with coumadins was common (46%). Cutaneous lesions were localized in more than 80% at the lower extremities or gluteal region. Among the most frequently recorded therapeutic procedures were: surgical necrosectomy, intensifying dialysis modality, i.v. sodium-thiosulfat application, lowering dialysate and oral calcium burden, systemic antibiotics, and stopping coumadins

Conclusions: CUA is associated with end-stage renal disease, female gender, diabetes and oral anticoagulation. PTH levels do not exceed current KDIGO target levels for ESRD in most cases. Decisions on therapeutic strategies vary significantly. The present internet based CUA registry is a valuable tool to collect data upon CUA cases and will serve as a basis for prospective studies.

Funding: Pharmaceutical Company Support

FR-PO1615

The Calcium Content of Hard Coronary Plaques Is a Predictor of Mortality in Maintenance Hemodialysis Patients

Antonio Bellasi,[1] Emiliana Ferramosca,[2] Geoffrey A. Block,[3] Paolo Raggi.[4] *[1]Azienda Ospedaliera S.Anna, Italy; [2]Azienda Ospedaliera S.Orsola-Malpighi, Italy; [3]Denver Nephrology; [4]Emory University.*

Background: The prognostic value of coronary artery calcification (CAC) has been documented in CKD patients. Nonetheless, several investigators maintained in the past that calcified plaques are stable and not prone to cause events

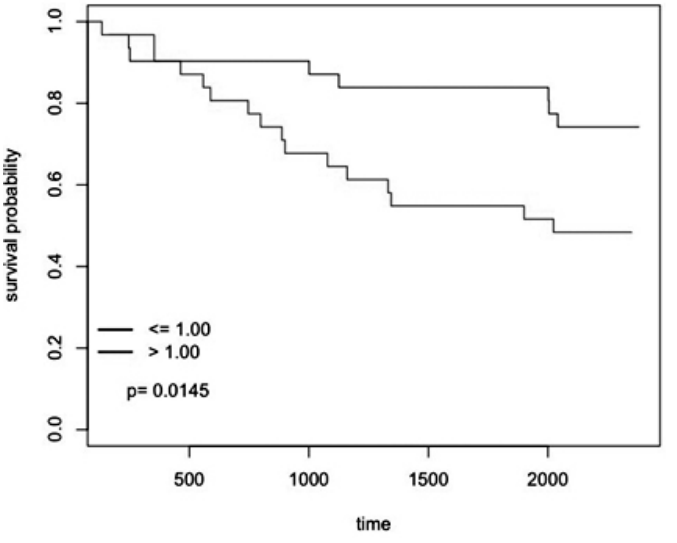
Methods: Between 2004 and 2005, 130 individuals underwent cardiac CT imaging for quantification of CAC via the Agatston and the Volume score. Since the Agatston score is derived by multiplying the density and the volume of calcified lesions, the ratio of the Agatston and the Volume score (AVR) standardizes the overall calcium content per plaque volume unit. Patients were classified as high AVR (>1) or low (≤1) AVR. Survival analyses tested the association between AVR and all-cause mortality during a median follow-up of 5 years

Results: Overall 63% of patients had a high AVR. The study cohort characteristics are summarized in the table.

Table 1

	Overall (n=130)	Low ratio (n=48)	High ratio (n=82)	p
Age (SD)	55(14)	50(13)	59(13)	0.005
%Men	52	64	49	0.29
Dialysis Vintage (SD)	4.2(4.2)	4.1(3.8)	4.4(2.9)	0.72
Hypertension (%)	95	96	96	0.66
Diabetes (%)	52	44	58	0.32
ASCVD (%)	38	28	48	0.11
Framingham (% risk)	12(10)	11(11)	14(11)	0.25
Congestive heart failure (%)	22	12	28	0.17

The mortality rate of patients with high AVR was higher than in those with low AVR calcification.



After adjustment for age, sex, race, diabetes and history of atherosclerotic disease (ASCVD) high AVR remained independently associated with all-cause mortality (HR: 2.24; 95% CI: 1.02 – 4.88, p=0.042)

Conclusions: Increased AVR is an independent predictor of all-cause mortality in hemodialysis patients. These results suggest that presence of calcium content in the coronary arteries in not an index of stability but rather a harbinger of adverse future events

Funding: Pharmaceutical Company Support

FR-PO1616

Study on the Relationship of Serum 25-hydroxyvitamin D Levels with Vascular Calcification in Hemodialysis Patients Jae Hyun Chang, Sejoong Kim, Hyun Hee Lee, Wookyung Chung, Ji Yong Jung. *Department of Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea.*

Background: Cardiovascular disease (CVD) is the main cause of mortality in chronic kidney disease patients. Vascular calcification is highly prevalent in this population and is an independent predictor of cardiovascular mortality. The role of vitamin D in this process remains controversial. The aim of this study was to determine the prevalence of vitamin D deficiency (25-hydroxyvitamin D [25D] ≤ 15 ng/ml), insufficiency (25D levels between 16 and 30 ng/ml), and moreover the relationship between vitamin D levels and vascular calcificationin hemodialysis patients.

Methods: We performed a cross-sectional study with 289 hemodialysis patients.Patients were 56.9 ± 14 years of age, 49.5% males,46.4% diabetics and 34.9% with a history of CVD. PlainX-ray images of lateral lumbar spinefrom all subjects were studied for calculation of semiquantitativevascular calcification scores as described by Kauppila.

Results: Only 3.1% of patients had adequate levels of25D (>30 ng/mL), 10.7% of them had insufficientlevels and 86.2% had deficient levels. Female gender and diabetes were associated with vitamin deficiency. We also found a high prevalence of vascular calcificationin this population. Kauppila scores revealed 180 patients (62.3%) with vascular calcification. In univariate analysis, 25D levels were inversely related to vascular calcification (r = – 0.170, P = 0.004). However, after correction for confounding factors, this relation lost statistical significance. Multivariate analysis showed that age, systolic blood pressure, andLDL-cholesterol were directly associated with higher vascular calcification scores (>7).

Multiple logistic regression analysis of factors associated with higher vascular calcification scores

Parameters	OR	95% CI	P
Dependent variable:Kaupilla> 7			
Age	1.068	1.041-1.095	< 0.001
SBP	1.014	1.002-1.026	0.021
LDL-C	1.012	1.001-1.023	0.031
25D	0.974	0.920-1.031	0.370

Conclusions: Vitamin D deficiency and insufficiency were highly prevalent in hemodialysis patients. However, low 25D levels could not be identified as an independent predictor of vascular calcification in thesepatients.

FR-PO1617

Septadian Variation in Cardiac Mortality in Haemodialysis (HD) but Not Peritoneal Dialysis (PD) Patients Rathika Krishnasamy,[1,2] Carmel M. Hawley,[1,2] Sunil V. Badve,[1,2] Brian E.R. Livingston,[1] Stephen P. McDonald,[1] Philip A. Clayton,[1] Fiona Brown,[1] Kevan R. Polkinghorne,[1] Kym M. Bannister,[1] Neil Boudville,[1] Kathryn J. Wiggins,[1] David W. Johnson.[1,2] *[1]Australia and New Zealand Dialysis and Transplant Registry, Adelaide, Australia; [2]Department of Nephrology, University of Queensland at Princess Alexandra Hospital, Brisbane, Australia.*

Background: Cardiovascular disease (CVD) represents the leading cause of death in dialysis patients. However there is limited evidence that dialysis modality may also influence mortality related to CVD. The aim of this study was to evaluate the effects of dialysis modality and HD frequency on the septadian pattern of cardiac and non-cardiac mortality in the Australian and New Zealand (ANZ) end-stage kidney failure (ESKF) cohorts, using data from the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry.

Methods: We analysed all adult ESKF patients (n=14594, HD n= 10338, PD n = 4256) receiving maintenance dialysis in ANZ who died between 1 January 1999 and 31 December 2008. The independent predictors of cardiac and non-cardiac death were determined by multivariable logistic regression.

Results: Cardiac deaths accounted for 40% of deaths. Cardiac death was significantly more likely to occur on Mondays and Tuesdays for HD patients receiving 3 or fewer dialysis sessions per week (n=9967), [Monday adjusted odds ratio(OR)1.42(95% CI 1.22-1.66), Tuesday OR 1.29(95% CI 1.10-1.51)].This pattern of increased deaths on Mondays and Tuesdays was not seen among PD patients, HD patients receiving more than 3 sessions per week (n =371) or home HD patients (n=573). Subgroup analyses showed sudden cardiac deaths also had a septadian pattern among HD patients.This pattern was not seen for non-cardiac deaths.

Conclusions: Our novel finding is that patients on PD, home HD and HD patients receiving more than 3 sessions per week did not demonstrate the usual septadian pattern of CV deaths on Mondays and Tuesdays. This data may provide insights into mechanisms of CV deaths in the dialysis cohort.

FR-PO1618

Carotid Artery Calcification at the Initiation of Hemodialysis Is a Risk Factor for Cardiovascular Events in Patients with End-Stage Renal Disease Yoriko Ura,[1] Hirofumi Ikeda,[1] Masaharu Nagata,[2] Masaru Nakayama.[1] *[1]Division of Nephrology and ClinicalResearch Institute, Department of Internal Medicine, National Kyushu Medical Center Hospital, Fukuoka, Japan; [2]Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: Vascular calcification has been recognized as a risk factor for cardiovascular (CV) events in patients with end-stage renal disease (ESRD). However, the association of carotid artery calcification (CAAC) with CV events remains unknown. The aim of this study was to elucidate whether CAAC is associated with composite CV events in ESRD patients.

Methods: One-hundred thirty-three patients who had been started on hemodialysis between 2004 and 2008 were included in this retrospective cohort study. These patients received multi-detector computed tomography to assess CAAC at the initiation of hemodialysis. Composite CV events, including ischemic heart disease, heart failure, cerebrovascular diseases, and CV deaths after the initiation of hemodialysis, were examined in each patient.

Results: CAAC was found in 94 patients (71%). At the end of follow-up, composite CV events were seen in 47 patients: ischemic heart disease in 20, heart failure in 8, cerebrovascular disease in 12, and CV deaths in 7. The incidence of CAAC was 87% in patients with CV events, which was significantly higher than the rate (62%) in those without. Kaplan-Meier analysis showed a significant increase in composite CV events in patients with CAAC compared with those without CAAC (p=0.001, log-rank test). Univariate analysis using a Cox hazard model showed that age, smoking, common carotid artery intima-media thickness and CAAC were risk factors for composite CV events. In multivariate analysis, only CAAC was a significant risk factor for composite CV events (hazard ratio, 2.85; 95% confidence interval, 1.18-8.00; p=0.02).

Conclusions: CAAC is an independent risk factor for CV events in ESRD patients. The assessment of CAAC at the initiation of hemodialysis is useful for predicting the prognosis.

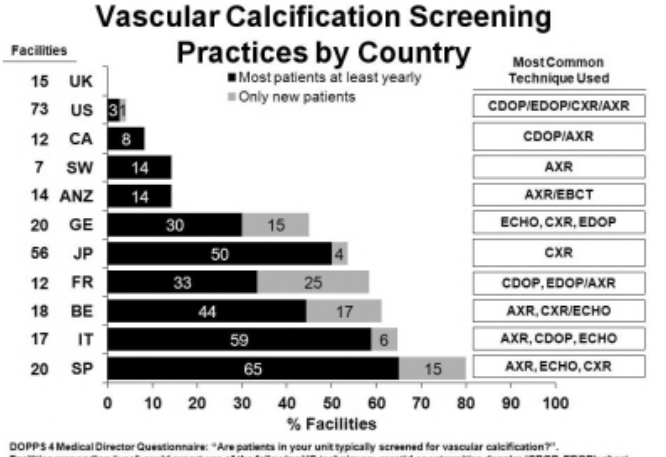
FR-PO1619

Dramatic International Variation in Vascular Calcification Screening Practices: Results from the DOPPS Bruce M. Robinson,[1,2] Douglas S. Fuller,[1] Brian Bieber,[1] Christian Combe,[3] Yun Li,[1,2] David C. Mendelssohn,[4] Francesca Tentori,[1] Shunichi Fukuhara.[5] *[1]Arbor Res Collab for Hlth, Ann Arbor; [2]Univ of MI, Ann Arbor; [3]CHU Bordeaux, France; [4]Humber River Reg Hosp, Canada; [5]Kyoto Univ, Japan.*

Background: The 2009 KDIGO MBD guidelines indicate CKD stage 3-5D patients with vascular/valvular calcification (VC) are at highest cardiovascular (CV) risk, but do not recommend for or against VC screening. We describe facility VC screening practices internationally and associations with clinical outcomes.

Methods: Data were from 7,703 chronic in-center hemodialysis (HD) patients in 266 DOPPS 4 facilities (2010). Medical directors were asked if they typically screen for VC. Among 6 countries with >45% of units screening for VC, clinical practices and laboratory measures were compared with generalized estimating equations adjusted for demographics, comorbidity, and intra-facility correlation. Rates of CV hospitalizations and mortality were compared with adjusted Cox models.

Results: Routine VC screening was reported by <5% of US and UK units but was common (45-80%) in Japan and most European DOPPS countries. Somewhat lower Ca-based phosphate binder (51.5% vs 58.5%, p=0.16) and dialysate Ca (<3 mEq/L; 25.5% vs 36.7%, p=0.14) use was seen in VC screening units. No notable differences in vitamin D use or serum calcium, phosphorus, and PTH levels were seen. VC screening units had similar CV hospitalizations (hazard ratio [HR]=0.86; 95% CI=0.61, 1.21) and all-cause mortality (HR=0.99; 95% CI=0.79, 1.25) as non VC-screening units.



DOPPS 4 Medical Director Questionnaire: "Are patients in your unit typically screened for vascular calcification?". Facilities responding "yes" could report use of the following VC techniques: carotid or extremities doppler (CDOP, EDOP); chest, abdominal, or hand X-ray (CXR, AXR, HXR); echocardiogram (ECHO); or electron-beam computed tomography (EBCT). Most common techniques listed in order of frequency.

Conclusions: Use of VC screening and preferred screening tests vary dramatically across DOPPS countries. VC screening units may choose practices that limit calcium exposure to a greater extent than other units. Whether tailoring therapy based on VC screening limits VC progression or improves clinical outcomes needs additional study.

Funding: Pharmaceutical Company Support

FR-PO1620

Association of Omega-3 Polyunsaturated Fatty Acids with Carotid Arteriosclerosis in Patients on Chronic Hemodialysis Hirotake Kasuga,[1] Ryo Takahashi,[1] Keiko Kimura,[1] Chieko Matsubara,[1] Rei Okada,[1] Seiichi Matsuo.[2] *[1]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Nephrology, Nagoya University Hospital, Nagoya, Japan.*

Background: Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are widely recognized to have beneficial effects on cardiovascular disease. In hemodialysis (HD) patients, blood levels of n-3 PUFAs have been reported to be more suboptimal compared to general population. However, the association between n-3 PUFAs levels and cardiovascular risk is little known in this population. We investigated the association of n-3 PUFAs levels with carotid arteriosclerosis in HD patients.

Methods: Carotid ultra-sound was performed in a total of 461 patients (male 67%, age 67±12years, diabetes 46%) stably undergoing HD. Intima-media thickness (IMT) and plaque score (PS) in common carotid artery were measured. Carotid arteriosclerosis was defined as IMT >1.2mm and/or PS >5.0mm. The levels of n-6 PUFAs [dihomo-gamma-linolenic acid (DHLA) and arachidonic acid (AA)] and n-3 PUFAs [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] were also measured prior to carotid ultra-sound.

Results: Carotid arteriosclerosis was seen in 94 patients (20.4%). Individual PUFAs were comparable between patients with and without carotid arteriosclerosis, however, the ratio of EPA / AA and the ratio of n-3 / n-6 PUFAs were significantly lower in patients with carotid arteriosclerosis than in those without (0.43±0.29 vs. 0.53±0.44, p = 0.027 and 0.94±0.43 vs. 1.07±0.55, p = 0.036, respectively). On multivariate logistic regression analysis, the ratio of EPA / AA [odds ratio (OR) 0.45, 95% confidential interval (CI) 0.22-0.93, p = 0.029] and the ratio of n-3 / n-6 PUFAs (OR 0.60, 95%CI 0.36-0.99, p = 0.046) were independently associated with carotid arteriosclerosis, respectively. Based on the cut-off level of 0.27 determined by ROC analysis (AUC = 0.63), the incidence rate of carotid arteriosclerosis was significantly higher in patients with low EPA / AA ratio than those with high EPA / AA ratio (27.9% vs. 17.5%, p = 0.012).

Conclusions: These data suggest that low levels of both EPA / AA ratio and n-3 / n-6 PUFAs ratio were closely associated with incidence of carotid arteriosclerosis in patients on HD.

FR-PO1621

Long-Term Outcomes of Cardiac Risk Stratification Using Gated Single Photon Emission Computed Tomography (SPECT) in Asymptomatic End-Stage Renal Disease Patients at the Start of Dialysis Jwa-Kyung Kim,[1] Ja-Ryong Koo,[2] Young Rim Song.[1] *[1]Department of Internal Medicine & Kidney Research Institute, Hallym University Medical Center, Anyang, Gyunggi-do, Korea; [2]Department of Internal Medicine & Kidney Research Institute, Hallym University Medical Center, Chuncheon, Gangwon-do, Korea; [3]Korea.*

Background: Screening for occult coronary artery disease (CAD) might permit early identification of subjects at increased risk of an adverse cardiac event. The aim of this study was to investigate the long-term effects of cardiac risk stratification using gated single photon emission computed tomography (SPECT) in ESRD patients at the start of dialysis.

Methods: This is an observational cohort study performed in Hallym University Hospital between January 2005 and April 2009. Baseline echocardiography were performed in all patients. For high risk patients who presented at least one cardiovascular risk factor, or decreased ejection fraction(<50%) or regional wall motion abnormality (RWMA) on echocardiography, SPECT was recommended. Cardiac events were defined as cardiac death and non-fatal acute coronary syndrome.

Results: Among 303 patients, 254 were high-risk patients. SPECT was performed in 143 of the patients and 66 showed reversible perfusion defects. During the mean follow-up of 59.0 months, overall cardiac event rate per person-year of follow-up was 6.0%; it was significantly higher in high-risk group compared to that of low-risk group (6.6 vs. 1.6%, HR3.43, 95% CI 1.61-7.24). Multivariate Cox analysis showed that old age, diabetes and RWMA were independent predictors of adverse cardiac events in total patients. Among high-risk patients who underwent SPECT, summed stress score ≥9 (HR 2.60 95%CI 1.32-5.13) and reversible perfusion defect (HR3.37 95% CI 1.86-6.10) was additionally associated with the increased risk of cardiac event. The subgroup analysis in patients with reversible perfusion defects showed that intensive revascularization therapies decreased the risk of cardiac events by 50% compared to patients who treated only medically.

Conclusions: In conclusion, intensive cardiac work-up with SPECT may provide important prognostic information, particularly in high-risk dialysis patients.

FR-PO1622

Temporal Evolution of Systolic and Diastolic Blood Pressure in the Frequent Hemodialysis Network (FHN) Trials Peter Kotanko,[1] John B. Stokes,[2] Amit X. Garg,[3] Thomas A. Depner,[4] Christopher T. Chan,[6] Andreas Pierratos,[7] Brett Larive,[5] Gerald J. Beck,[5] Tom H. Greene,[5] Nathan W. Levin,[1] Alan S. Kliger,[8] The FHN Trial Group.[5] *[1]Renal Research Institute, New York, NY; [2]Iowa U and VA Med. Center, Iowa City, IA; [3]Div of Nephrology, U Western Ontario, London, Canada; [4]UC Davis, Davis, CA; [5]NIDDK, NIH, Bethesda, MD; [6]Univ. Health Network, Toronto, Canada; [7]U Toronto, Toronto, Canada; [8]Yale U, Princeton, NJ.*

Background: As part of the FHN Trials we investigated the impact of 6x weekly in-center hemodialysis (HD; Daily Trial) and 6x weekly nocturnal home HD (Nocturnal Trial) over 12 months on systolic and diastolic blood pressure (SBP; DBP).

Methods: In the Daily Trial 245 patients (pts) were randomized to 6x or 3x weekly HD, in the Nocturnal Trial 87 pts were randomized to 6x weekly nocturnal HD or 3x weekly HD. Pre-HD SBP and DBP were measured at baseline and then monthly. Intradialytic weight loss (IWL) was used as a proxy of interdialytic weight gain.

Results: In the Daily Trial, compared to 3x weekly HD, 6x HD resulted in lower blood pressure at 1 month (SBP [mean±SE; mm Hg]: -5.9±1.8, P<0.001; DBP: -3.4±1.1, P<0.01) and at 12 months (SBP: -10.3±2.0; DBP: -5.5±1.2; all P<0.001). 6x HD led to a 0.4±0.2 kg (P=0.06) lower post-HD weight and a lower IWL of 1.0± 0.1 (P<0.001) at month 1; this difference was maintained through month 12.

In the Nocturnal Trial SBP and DBP did not differ between the 2 groups after 1 month; at 12 months both were lower in 6x HD pts (SBP: -8.3±3.3; DBP: -4.9±1.9; all P<0.05). Post-HD weight did not differ throughout the trial. IWL at month 1 was similar in the 2 arms; by 12 months 6x HD pts had a lower IWL by 0.5±0.2 (P<0.01).

Conclusions: Compared to 3x weekly HD, 6x HD produced a comparable fall in SBP and DBP in both the Daily and Nocturnal Trials; the difference became evident earlier in the Daily Trial. This indicates that frequent HD reduces blood pressure whether HD is given during the day or in longer nocturnal sessions. Potential mechanisms may be reduction of extracellular volume, as evidenced by reduced post-HD weight in the Daily Trial, and reduced IWL in both trials.

Funding: NIDDK Support

FR-PO1623

Absolute Values of Systolic Blood Pressure (SBP) Do Not Predict Second Year Survival in Incident Hemodialysis Patients with Stable SBP Jochen G. Raimann,[1,2] Len A. Usvyat,[1] Stephan Thijssen,[1,2] Peter Kotanko,[1,2] John Rogus,[3] Eduardo K. Lacson,[3] Nathan W. Levin.[1,2] *[1]RRI, NY; [2]BIMC, NY; [3]FMC NA.*

Background: The U-shaped relationship between systolic blood pressure (SBP) and mortality is recognized in incident and prevalent hemodialysis (HD) patients (Li 2006, Zager 1998). Incident HD patients whose pre-HD SBP increased or decreased by more than 1 mmHg per month over their 1st year had greater mortal risk compared to those with "stable" SBP (Usvyat, WCN 2011; ASN 2011). This analysis investigates the influence of absolute values of SBP on second year survival among patients with stable SBP (i.e. lacking linear trend of increase or decrease).

Methods: Patients who started HD in between Jan 1, 2001 to Feb 28, 2010 with at least 13 HD treatments in their second year of HD were stratified into 4 groups according to the SBP during the first month in Year 1: <120, 120-150, 151-180 and above 180 mmHg. Changes were quantified as the slope of a linear regression of SBP values per patient in the first year. Patients selected for survival studies showed SBP between -0.5 and +0.5 mmHg change per month. Cox Regression was used to analyze the hazard ratio (HR) of death by SBP group.

Results: Of 10245 eligible, incident HD patients (57% male, 38% black, 54% white, 54% diabetic and 62.1±15.6 years old), 1385 patients with stable SBP in Year 1 were included. HR for mortality adjusted for age, gender, black race, BMI, cardioprotective drugs (CPD), diabetes and various other co-morbidities did not differ between the SBP groups. Only age (HR 1.02; P<0.001) and race (HR 1.83, P<0.05) remained significant predictors. Use of CPD showed a trend of improved survival (HR 0.7, P=0.10).

Conclusions: The risk of death did not vary in different SBP groups, supporting prior findings that implicate change in SBP (compared to absolute levels) as the more significant factor impacting mortality risk in chronic HD patients. It cautions against the current practice whereby treatment targets are derived from epidemiologic studies, specifically that absolute thresholds should not be used in isolation for the development of guidelines and treatment strategies, because they do not reflect SBP changes.

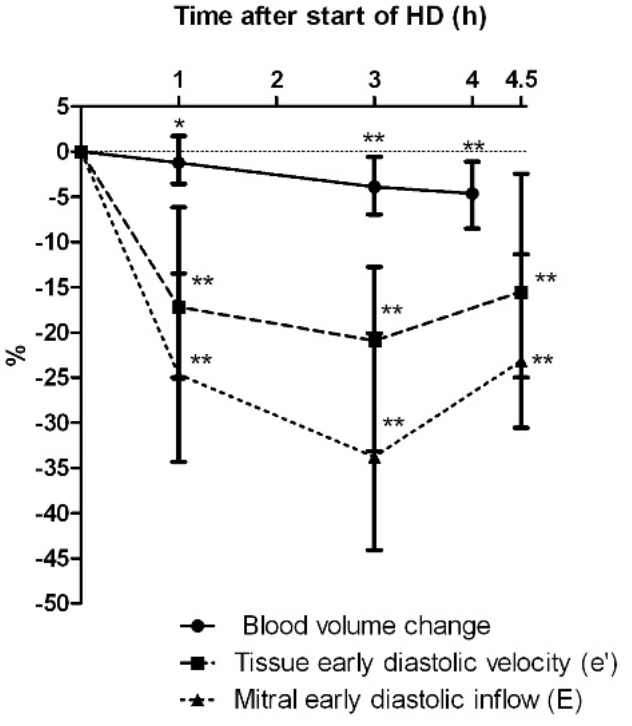
FR-PO1624

Hemodialysis-Induced Diastolic Dysfunction: It Is Not Only Volume! Solmaz Assa,[1] Yoran M. Hummel,[2] Adriaan A. Voors,[2] Johanna J. Kuipers,[3] Ralf Westerhuis,[3] Paul E. de Jong,[1] Casper F. Franssen.[1] *[1]Nephrology; [2]Cardiology, University Medical Center Groningen; [3]Dialysis Center Groningen, Netherlands.*

Background: Left ventricular (LV) diastolic dysfunction is common in hemodialysis (HD) patients. However, acute changes in diastolic function have been thus far studied before and after HD but not during HD. We evaluated in detail changes in LV diastolic function in relation to volume parameters during a single HD session.

Methods: Hundred and nine adult HD patients with a mean (±SD) age of 62.5 ± 15.6 year and median dialysis vintage of 2 (1-4) years participated in this study. Echocardiographic examination was performed 4 times: before HD, 60 min and 180 min after the start of HD and 30 min after the end of HD. Diastolic function was evaluated using mitral early inflow (E) and tissue-Doppler derived early diastolic velocity (e'). The change in blood volume (BV) during HD was calculated from the changes in hematocrit.

Results: Pre-HD median (IQR) E and mean (±SD) e' were 0.9 (0.8-1.1) m/s and 6.6 ± 2.1 cm/s, respectively. The figure describes the changes in LV diastolic function parameters during HD. E and e' decreased significantly at 60 min of HD by 24.7% (13.5-34.3%) and 17.2% (6.1-25.0%), respectively (both p<0.001), whereas BV had decreased by only 1.3% (-3.4-1.7%) (p<0.05, compared with pre-HD). There was no correlation between the change in BV or ultrafiltration (UF) volume and changes in E and e' at 60 min of HD, indicating that these changes were unrelated to changes in volume. At 180 min HD onwards the change in E correlates significantly with BV and UF volume. Such a correlation was not seen for the change in e'.



Conclusions: Diastolic function acutely deteriorates early during HD. This is not related to changes in BV or UF volume. Therefore, other factors than hypovolemia seem to cause an early impairment in diastolic function.

Funding: Government Support - Non-U.S.

FR-PO1625

Predictors of Blood Pressure Variability Change over Time in Incident Hemodialysis Patients

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Background: Blood pressure variability (BPV) increases the risk of death in hemodialysis (HD) patients but the factors associated with BPV are not well described.

Methods: We assessed factors influencing within-patient variability in predialysis systolic BP (SBP) among 25,031 incident HD patients treated at Dialysis Clinic, Inc. (DCI). We assessed comorbidity using a previously validated index and fluid removal using change in weight during dialysis. We examined BPV in 3-month windows over the first year of HD using the residual-intercept ratio obtained from mixed-effects linear regression models estimating changes in SBP over time. This ratio reflects each individual's BPV over time with positive values reflecting greater BPV.

Results: Patients' mean age was 62 years; 35% were black and 44% were female. Patients who were older, female, black and had more comorbidity had consistently higher BPV during the first 6 months of dialysis. Greater fluid removal was associated with lower BPV in the first 6 months and calcium-phosphate product was associated with higher BPV after 6 months.

Predictors of Systolic BP Variability[1] in Incident Hemodialysis Patients

	0-3 months	4-6 month	7-9 months	10-12 months
N	25,031	21,190	18,729	16,665
Mean SBP±SD, mmHg	148±27	149±26	149±26	149±26
Predictors				
Age, years (Ref:18-44)				
45-64	**4.4**‡	**2.9**‡	**2.5**‡	**2.4**‡
>64	**3.6**‡	**1.7**†	1.1	**1.6**†
Female vs. Male	**4.6**‡	**4.9**‡	**5.0**‡	**5.2**‡
Black vs. White	**4.9**‡	**5.4**‡	**4.1**‡	**4.3**‡
Comorbidity (Ref:0-3)				
4-6	**1.2**†	**1.1**∗	**1.7**‡	**1.1**∗
7-9	**1.9**†	**1.5**∗	1.3	1.3
>9	**2.0**∗	1.6	0.02	0.7
Fluid Removal, per 10% weight	**-8.7**‡	**-4.3**‡	-2.4	-2.2
S. Albumin, per 1 g/dL increase	**-6.4**‡	**-7.3**‡	**-8.5**‡	**-9.3**‡
S.CaXPhos, per 10 higher	0.1	0.3	**0.7**‡	**0.7**‡

[1]Coefficients represent individual BPV in a 3 month period. Positive values represent higher BP variability. Coefficients are scaled by 10^{-1}. *p<0.05; †p<0.01; ‡p<0.001

Conclusions: In the first of year of HD, factors influencing within-patient BP variability can change significantly over time. Studies are needed to better understand these dynamic changes and their influence on clinical outcomes in HD patients.

Funding: NIDDK Support

FR-PO1626

Randomized Cross-Over Study of Daily Versus Conventional Hemodialysis To Explore Mechanisms of Blood Pressure Improvement

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Background: Hypertension (HTN) is poorly controlled in many end stage renal disease (ESRD) patients treated with conventional hemodialysis (CHD). Our study had 2 objectives: 1) To determine if short daily hemodialysis (SHD) is associated with improved systolic blood pressure (SBP) compared to CHD in hypertensive ESRD patients, and 2) To explore the potential mechanisms of BP improvement.

Methods: Randomized cross-over study of prevalent HD patients with a history of HTN (pre-dialysis SBP >140 and/or on 2 or more antihypertensive medications). After informed consent, patients underwent a 3 month run-in phase in which the dialysate Na was reduced to 138 mM, dry weight and antihypertensives were optimized to achieve a pre-dialysis SBP of <140mmHg. At the end of the run in phase, patients were then randomized to a further 3 months of CHD or SHD and then crossed over to the other treatment arm. SBP, ECFV via bioimpedance and catecholamines were measured after each phase, and intensity of antihypertensive therapy was estimated.

Results: 22 patients consented to participate in the study; 3 patient withdrew prior to randomization, 2 patients did not complete the SHD arm (included in the intent to treat analysis). There was a statistically significant decrease in SBP from study entry to the end of the run in phase (151 vs 138 mm Hg, p=0.004) without a change in dry weight (77.4 vs 77.1 kgs, p=0.63) or intensity of antihypertensive medications (p=0.57). There was no difference in SBP between CHD versus SHD (142 vs 139 mmHg, p=0.39) although more medication was required to control SBP in CHD (5.0 vs 3.7, p=0.02). These differences were not explained by plasma catecholamine levels (norepinephrine, p=0.33; epinephrine, p = 0.48), ECFV (p=0.77) or changes in dry weight (p=0.70).

Conclusions: A protocol-based approach to HTN management is associated with a significant reduction in SBP in ESRD patients treated with HD. Once target SBP has been achieved, both CHD and SHD are associated with maintenance of SBP but more antihypertensive medications are required in CHD. The mechanism(s) by which SHD improves SBP such that the number of medications can be reduced remains unclear.

Funding: Private Foundation Support

FR-PO1627

Incidence, Prevalence and the Risks of Atrial Fibrillation in Dialysis Patients

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Background: ESRD patients appear to be at high risk for atrial fibrillation (AF) but the risks and benefits of anticoagualtion for stroke prevention remain unclear. We undertook a systematic review to clarify the risks of mortality and stroke in patients with ESRD and AF.

Methods: A literature search using Medline and embase from 1990 to November 2010 was conducted that included ESRD patients treated with dialysis. Studies described incidence or prevalence and/or complications of AF with or without anticoagulation. Abstracts were reviewed and data abstracted by two investigators with conflicts resolved by a third investigator. Observational study quality was judged using the NewCastle Ottawa Scale. Event rates were calculated in patient-years and were combined using a random effects model.

Results: The average Ottawa New Castle Score was 6.1 (range 3-9) in the 21 studies that met our eligibilty criteria. The majority of patients were male (56%) with an mean age of 61 years. The overall prevalence of AF was 12.3% (range 4.5%-27%) and the overall incidence was 2.4/100 patient-years (range 0.97-5.9 events/100 patient-years). The risk of mortality was increased in ESRD patients with AF compared to ESRD patients without AF, 21.6/100 patient-years and 14.2/100 patient-years respectively. The risk of stroke was increased in patients with AF at 5.2/100 patient-years compared with 2.5/100 patient-years. The effects of anticoagulation on reducing the risk of stroke were heterogeneous.

Conclusions: The incidence and prevalence of AF in ESRD patients is high and is associated with an increased risk of mortality and stroke. Study variability was observed in design, population characteristics and method of AF documention. Given the limitations of the study designs and the equipoise about the risks and benefits of anticoagulation in patients with ESRD, a randomized controlled trial is required to clarify the optimal anticoagulation strategy in the ESRD population.

Funding: Clinical Revenue Support

FR-PO1628

Determinants of Prolonged QTc Interval over 5 Years in Patients Undergoing Regular Hemodialysis Shigeru Oikawa,[1] Akihiko Kato,[2] Fumio Takayama,[1] Hisanori Azekura,[1] Narikazu Iijima,[1] Akira Shimomura.[1] *[1]Sanaru Sun Clinic, Hamamatsu, Shizuoka, Japan; [2]Blood Purification Unit, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

Background: Prolongation of the corrected QT interval (QTc) on the surface electrocardiogram (ECG) is a predictor of total death and cardiac event in general population and patients with end-stage kidney disease. QTc prolongation is often observed in patients on chronic hemodialysis (HD), while it remains to be determined which factors are more associated with QTc prolongation over time.

Methods: In this study, we longitudinally measured QTc interval over 5 years, and examined the determinants of progression of QTc prolongation in stable 76 HD patients who had not taken any medication to prolong QTc interval (age: 63±12 [35-89] years, time on HD: 148±66 [63-387] months, male/female = 45/31, diabetes; n=12).

Results: Basal QTc was significantly and inversely correlated with albumin-corrected serum calcium (r=-0.36, p<0.01) and creatinine (r=-0.28, p=0.01). A longer QTc was also observed in female HD patients. During the 5-year observation, mean QTc was significantly increased from 421±18 to 428±21ms (p<0.01). The prevalence of borderline/abnormal QTc was increased from 18.4 to 36.8% (p<0.01). There was a significant and positive relationship between absolute changes in QTc and HD period (r=0.25, p<0.03). Older patients (≥65 years, n=34) disclosed a significant increase in QTc from 422±18 to 433±22ms during the follow-up (p<0.01). QTc interval was also prolonged more markedly in patients with serum albumin lower than 4.0 g/dL (420±20 vs. 434±22ms, p<0.05) (n=32) than those without (421±1 vs. 425±21ms). In contrast, diabetes and other co-morbid factors did not affect QTc prolongation.

Conclusions: These findings suggest that HD therapy increases QTc interval over time. In addition, prolongation of QTc is associated with ageing and hypoalbuminemia in stable patients on long-term HD.

FR-PO1629

Pulmonary Hypertension the Predictor of Mortality in HD and PD Patients: A Prospective Chinese Study Limeng Chen, Xuemei Li, Xiaohong Fan, Hong Xu, Jianling Tao, Xuewang Li. *Department of Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: Pulmonary hypertension (PH), a disease which carries substantial morbidity and mortality has been reported. No prospective evaluation of the prevalence or clinical significance of PH in ESRD patients in China has been undertaken. The objective of this study was to evaluate the impact of PHT and mortality among ESRD patients receiving chronic HD and PD therapy.

Methods: Echocardiograms were performed prospectively in chronic PD (n=73) and HD (n=89) patients at a single dialysis center. The patient's general clinical data were collected. The arterial stiffness was prospectively estimated by measure the Ankle-Brachial Index and brachial-ankle pulse wave velocity (baPWV). ABI and baPWV were remeasured , clinical outcome (death) were recovered at 36months.

Results: In these cohort patients, 44.9% of patients receiving HD, and in 34.2% of the patients receiving PD met the definition of PH (sPAP ≥ 35 mm Hg). Of those 30.3% HD patients and 16.4% PD patients met the definition of more severe PH (sPAP>45 mmHg). At 36 months, mortality was significantly higher in patients with PH (26.2%) compared with patients without PH(8.2%, p=0.002). And the PH was the independent risk factor of mortality in HD patients. Echocardiographic findings showing impaired left ventricular(LV) function and elevated diameter of inferior vena cava and left atrial were significantly associated with more severe PH. Anemia and lower body weight was also connected with PH. The other clinical features, such as dialysis time, nPCR, hypertension, CRP, P, PTH, Alb, TG, TC, HDL, Pwv and ABI had no significant difference between patients with or without PHT.

Conclusions: This prospective study of a single dialysis center suggests that PH may be present in nearly half of Chinese HD patients and 1/3 PD patients. When PH was present is associated with increased mortality. The Echocardiographic findings suggested that the PH may be secondary to diastolic dysfunction and compounded by volume overload.

Funding: Government Support - Non-U.S.

FR-PO1630

Echocardiographic Determinants of an Abnormal Spatial QRS-T Angle in Chronic Dialysis Patients Mihaly K. De Bie,[1] Nina Ajmone Marsan,[1] Arien Gaasbeek,[2] Victoria Delgado,[1] Ton J. Rabelink,[2] Jeroen J. Bax,[1] Martin J. Schalij,[1] J. Wouter Jukema.[1] *[1]Cardiology, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, Leiden University Medical Center, Leiden, Netherlands.*

Background: The spatial QRS-T angle, the angle between the mean QRS- and T-vector, describes the relation between ventricular depolarization and repolarization. Having a wide (abnormal) angle is considered a predictor of arrhythmic events in various patient groups, including dialyis patients. Given the high incidence of sudden cardiac death in dialysis patients, this parameter is of particular interest in this patient group. The objective of this study was to assess the association of (modifiable) echocardiographic parameters and an abnormal spatial QRS-T angle in dialysis patients.

Methods: A total of 93 consecutive dialysis patients (67.5 ± 7.5 yrs, 76% male) were included. In all patients a 12-lead electrocardiogram, a 2-dimensional echocardiogram and routine blood samples were obtained. Using a previously validated computer algorithm, the spatial QRS-T angle was then calculated from the 12-lead ECG. An abnormal spatial QRS-T angle was defined as ≥130° in males and ≥116° in females.

Results: An abnormal spatial QRS-T angle was present in 27 (29%) patients. Patients with an abnormal spatial angle had a higher serum phosphate (1.65 ± 0.43 mmol/L vs. 1.45 ± 0.32 mmol/L, p = 0.027). Furthermore, these patients had a lower left ventricular ejection fraction (LVEF) of 47 ± 7% vs. 55 ± 6% (p < 0.001) and had higher LV dyssynchrony as measured by tissue Doppler imaging, with a septal to lateral (S-L) delay of peak systolic velocity of 70 ± 42 ms vs. 40 ± 38 ms (p=0.001) respectively. Multivariate logistic regression analysis controlling for possible confounders demonstrated that LVEF (OR 0.82; 95%CI 0.73 -0.92, p=0.001) and S-L delay (OR 6.5; 95%CI 1.53 – 27.8, p=0.01) were independent determinants of an abnormal spatial QRS-T angle in this patient group.

Conclusions: Left ventricular ejection fraction and dyssynchrony are echocardiographic determinants of an abnormal spatial QRS-T angle in dialysis patients and might therefore represent a potential target for the prevention of sudden cardiac death in these patients.

Funding: Pharmaceutical Company Support

FR-PO1631

Effect of Hemodialysis and Trimetazidine on QTc Prolongation in Patients with End-Stage Chronic Kidney Disease Ivica Premuzic Mestrovic,[1] Boris Kudumija,[2] Damir R. Rosic,[3] Mladen Knotek.[1] *[1]Department of Medicine, University of Zagreb Medical School, Clinical Hospital Merkur, Zagreb, Croatia; [2]Dialysis, Avitum, Zagreb, Croatia; [3]Department of Anatomy, University of Zagreb Medical School, Zagreb, Croatia.*

Background: Long QTc is an independent risk factor for sudden cardiac death in patients with end-stage renal disease. Patients on chronic hemodialysis (HD) have prolonged baseline QTc that is further extended by the HD.

This study examined whether the length of the baseline QTc and its changes in response to HD procedure depended on the HD days of the week and whether QTc duration may be reduced by trimetazidine, an antiishemia drug.

Methods: In a prospective, sequential trial 31 patients (age 57 ± 10 years, 23 men), treated by chronic HD, without any significant ischemic heart disease, were included. The study was conducted in two phases: a 14-day control phase (without trimetazidine) and the 28-day implementation phase of trimetazidine (at a dose of 35 mg bid). In the first week of the control phase and in the last week of the study, immediately before and after each HD session a 12-canal ECG was done and serum K, Na, Ca^{++} and troponin I were determined. The length of QT interval was determined manually, and was corrected for heart rate by the Bazzet formula.

Results: The pre-HD QTc during both phases was similar (p = n.s.). A significant prolongation of QTc occurred after the second (443.3±25.8 vs. 429.7±20.3 ms, p<0.01) and third HD session (441.6±25.9 vs. 430.1±25.3 ms, p<0.05) in the control phase. In the last week of the treatment phase this prolongation of the QTc after the first (437.2±27.5 vs. 430.4±22.4 ms, p= n.s.), the second (433.3±25.8 vs. 430.1±25.6 ms, p= n.s.) and the third (431.5±25.6 vs. 428.5±22.6 ms, p= n.s.) HD session disappeared. Serum K, Na, Ca++ and troponin I were not significantly associated with variability of the QTc. Trimetazidine was well tolerated, with no observed side effects.

Conclusions: The QTc after the HD procedure may depend on the day of the week, with the second and third HD day of the week being associated with prolongation of the QTc in response to HD. Prolongation of the QTc in response to HD can be significantly reduced by trimetazidine.

FR-PO1632

Ventricular Repolarization, Total Mortality and Sudden Death in a Population of Hemodialysis Patients Paolo Fabbrini,[1,2] Simonetta Genovesi,[1,2] Emanuela Rossi,[1] Andrea Stella.[1,2] *[1]Università degli Studi di Milano Bicocca, Monza, Italy; [2]Clinica Nefrologica AO S Gerardo, Monza, Italy.*

Background: A prolonged ventricular repolarization time (QT interval) is associated to an increased risk of total mortality and sudden death in the general population.

Among hemodialysis (HD) patients the prevalence of QT interval prolongation is high and the HD session can induce a further QT increase, but no data are available on QT length and mortality relationship in this population.

Methods: We studied 122 patients, recruited between 2005 and 2010 in a single HD center [age 71.3 years (38.5-89.2), HD duration 3.0 years (0.0-37.9), 64.8% male]. 37.7% of patients had ischemic cardiac disease, 42.6% dilated cardiomyopathy, 84.4% were hypertensive and 27.1% were diabetic. Ejection fraction (EF) was 60.0% (22-72) and cardiac mass index (CMI) was 147.3 gr/m2 (54.0-311.2). By ECG Holter and a dedicated algorhythm 24h QT length corrected for heart rate (QTc) was calculated. QTc length was considered prolonged when longer than 450 msec in men and 460 msec in women.

Results: 43 patients out of 122 (35.3%) had a prolonged QTc. Female gender (p<0.001), dilated cardiomyopathy (p=0.004) and amiodarone therapy (p=0.037) were significantly associated to a QTc prolongation, while EF% (p=0.004) was inversely correlated to the length of ventricular repolarization. Up today 37 deaths (10 sudden death) were observed. After stratification for age, QTc prolungation (p<0.001, HR=1.27 for 10 msec of increment) and the presence of dilated cardiomyopathy (p=0.052, HR=2.15) were independent predictors of mortality, while beta-blockers therapy was weakly protective (p=0.10, HR=0.53). Sudden death was associated to a prolonged QTc (p=0.010, HR=1.40 per 10 msec of increment), digoxin therapy (p=0.014, HR=22.44) and a greater CMI (p=0.037, HR=1.19 per 10 gr/m2 of increment).

Conclusions: In a population of HD patients, a prolongation of QTc interval is an independent predictor of total and sudden mortality

FR-PO1633

Prevalent and Incident Rates of Asymptomatic Electrocardiographic Abnormalities in Hemodialysis Patients Darren Green, Paul Dunne, David I. New, Philip A. Kalra. *Vascular Research Group, Manchester Academic Health Sciences Centre, University of Manchester, Salford Royal Hospital, United Kingdom.*

Background: Dialysis patients have a high rate of cardiovascular disease. Dialysis is itself associated with arrhythmia and myocardial ischemia. Routine ECG would be of potential benefit in detecting early signs of new cardiovascular changes as it is low cost, non-invasive, and repeatable.

Methods: We performed a pilot study of incidental ECG abnormalities in a cross-section of dialysis patients from one centre. Patients were selected who had an elective ECG as part of transplant, pre-operative, or routine out-patient cardiology assessment. In patients with 2 such ECGs, comparison was made between tracings. Patients were excluded who had suffered acute troponin rises between ECGs, so as to assess changes that occur independent of distinct acute cardiac events.

Results: 176 patients were included in the cross-sectional study. The mean age was 68 years, with mean time on dialysis 4.7 months. 29% of patients had 1st degree heart block, 12% had prolonged QRS (>100ms), 28% Sokolow-Lyon indexed LVH, 26% T-wave inversion, and 3% atrial fibrillation. 89 patients had follow up assessment with mean time between ECGs 18 months. New onset QRS ischemic changes in the absence of acute coronary events occurred at a rate of 50 per 1000 patient years. The rate of new T-wave abnormalities was 48 per 1000 patient years. QTc became prolonged only in female patients (female baseline mean 431ms vs. follow up 458ms p=0.000, male baseline 434ms vs. follow up 438ms p=0.486). The rates of new conduction defects were 50 and 36 per 1000 patient years for atrial fibrillation and 1st degree heart block, respectively.

Conclusions: The prevalent rate of conduction defects and rate of new onset changes was high. This may give insight into the source of the high rate of arrhythmic death suffered by dialysis patients. The rate of onset of new ischemic changes fits with previous reports of dialysis being associated with myocardial injury. This pilot study supports the need for future work to determine whether routine ECG can improve cardiovascular risk stratification for these patients.

FR-PO1634

Pre-Dialysis Factors and the Timing of Dialysis Initiation among Older Adults Deidra C. Crews,[1,3] Julia J. Scialla,[1,3] Haifeng Guo,[2,3] Jiannong Liu,[2,3] Bernard G. Jaar,[1,3] L. Ebony Boulware.[1,3] *[1]Johns Hopkins Medical Institutions, Baltimore, MD; [2]Chronic Disease Research Group, Minneapolis, MN; [3]DEcIDE Network Patient Outcomes in ESRD Study Investigators.*

Background: In recent years patients have been initiating dialysis at increasingly higher estimated glomerular filtration rates (eGFR), despite a lack of evidence of benefit and suggestions of harm in some studies. We examined pre-dialysis factors that might influence earlier initiation of dialysis by nephrologists treating older adults.

Methods: Using USRDS data, we identified patients initiating dialysis at age 67+ years from 2006-2008, with 2 years Medicare coverage prior to initiation, and at least one outpatient nephrology visit in the 6 months preceding initiation. Medicare claims and the Medical Evidence form were used to ascertain comorbidities, and 6 months of Medicare claims were reviewed for the frequency of nephrology visits and number of congestive heart failure (CHF) admissions immediately preceding dialysis initiation. Logistic regression assessed the relation between pre-dialysis patient characteristics (comorbidities and health care utilization patterns) and early (eGFR ≥10 ml/min/1.73m^2) versus later (eGFR <10) dialysis initiation. We adjusted for demographics, year of dialysis initiation, ESRD cause, albumin, hemoglobin, and erythropoiesis stimulating agents and/or intravenous iron use.

Results: Among 70,662 patients, median age was 77 [interquartile range (IQR) 72-82] years and median eGFR at dialysis initiation was 11.0 (IQR 8.2-14.4) ml/min/1.73m2. Early initiators comprised 58%. Greater burden of comorbidities, frequent nephrology visits and CHF admissions were associated with early dialysis initiation.

Adjusted Odds Ratios Comparing Early versus Later Dialysis Initiation

Pre-Dialysis Factor	N (%)	Categories	Odds Ratios (95% Confidence Interval)
Comorbidity score (greater=more comorbidities)	18,252 (26)	≤ 5	1.00 (referent)
	15,446 (22)	>5 to ≤ 8	1.38 (1.32-1.44)
	20,232 (29)	>8 to ≤ 11	1.67 (1.59-1.75)
	16,732 (24)	>11	2.12 (2.02-2.24)
Nephrology visits in the 6 months prior to dialysis initiation	29,066 (41)	>0 to ≤ 5 visits	1.00 (referent)
	19,412 (28)	>5 to ≤ 10 visits	1.12 (1.08-1.17)
	22,184 (31)	>10 visits	1.22 (1.17-1.26)
CHF admissions in the 6 months prior to dialysis initiation	36,699 (52)	None	1.00 (referent)
	18,489 (26)	>0 to ≤ 1 admissions	1.15 (1.11-1.20)
	8,413 (12)	>1 to ≤ 2 admissions	1.54 (1.46-1.63)
	7,061 (10)	>2 admissions	2.12 (1.99-2.27)

Conclusions: Multiple comorbid illnesses and intensive pre-dialysis health care utilization may influence nephrologists to initiate dialysis early among older adults.

Funding: Other U.S. Government Support

FR-PO1635

Prospective Study on Clinical Effects of Dialysis in Treatment-Resistant Congestive Heart Failure Frank Van der Sande,[1] Trijntje T. Cnossen,[1] Jeroen Kooman,[1] Harmen P. Krepel,[2] Nicole Uszko-Lencer,[4] Karel M.L. Leunissen.[1] *[1]Nephrology, University Hospital Maastricht, Maastricht, Netherlands; [2]Nephrology, Franciscus Hospital, Roosendaal, Netherlands; [3]Nephrology, Catharina Hospital, Eindhoven, Netherlands; [4]Cardiology, University Hospital Maastricht, Maastricht, Netherlands.*

Background: The incidence of congestive heart failure (CHF) is still increasing and treatment of hypervolemic patients with treatment-resistant CHF complicated by progressive and permanent chronic renal insufficiency, known as cardiorenal syndrome (CRS) type 2, is notoriously difficult. Aim of the study was to investigate clinical outcome in CRS type 2 treated with dialysis.

Methods: Prospective observational non-randomized study. At start, after 4 and 8 months of dialysis, functional status, quality of life (QoL) using Minnesota Living with Heart failure Questionnaire and left ventricular ejection fraction (LVEF) were evaluated. Survival and hospitalization time were registered till April 1, 2011.

Results: Twenty-three patients with CRS type 2 (mean age 66±21 years, mean glomerular filtration rate 14.6±12.1 ml/min, 95% CI 0-28.5 ml/min, mean Charlson's comorbidity index 4.9±1.2) started with dialysis (12 (52%) patients started peritoneal dialysis (PD), 11 (48%) intermittent hemodialysis (IHD)). Seven patients (30%) died during the study period. Cumulative survival is shown in a Kaplan-Meier curve (figure 1). Median estimated survival time was 16 months. At the end of the follow-up period 5 patients were still treated for >20 months. Hospitalizations for cardiovascular causes were reduced (2.1±2.9 vs. 0.4±0.6 days/patient/month, p=0.000). NYHA class improved (3.8±0.4 at start vs. 2.4±0.7 after 4 months vs. 2.7±0.9 after 8 months; p=0.001) and QoL tended to improve (63±21 at start vs. 41±20 after 4 months vs. 51±25 after 8 months; p=0.056)). Left ventricular ejection fraction (LVEF) did not change.

Conclusions: After starting dialysis for CRS type 2, survival is highly variable, hospitalizations for cardiovascular causes were reduced, functional status improved and QoL tended to improve, but LVEF was not different. No differences were detected between IHD and PD.

FR-PO1636

Impact of Baseline Levels and Trimestral Variation of Triiodothyronine and Thyroxine on Mortality in Maintenance Hemodialysis Patients Christian L. Meuwese,[1] Friedo W. Dekker,[2] Bengt Lindholm,[1] Abdul Rashid Tony Qureshi,[1] Olof Heimburger,[1] Peter F. Barany,[1] Peter Stenvinkel,[1] Juan J. Carrero.[1] *[1]Renal Medicine and Baxter Novum, Karolinska Institutet, Sweden; [2]Clinical Epidemiology, Leiden University Medical Center, Netherlands.*

Background: Conflicting evidence exists with regards to the association of thyroid hormone levels and mortality risk in patients with end-stage renal disease (ESRD), which is limited to studies comprising single thyroid-hormone measurements. This study assesses the impact of basal and trimestral variation of thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) on cause-specific mortality in dialysis patients.

Methods: In 210 prevalent hemodialysis patients serum T3, T4, TSH and Interleukin-6 were measured three months apart. Cardiovascular and non-cardiovascular deaths were registered during follow-up. Based on fluctuations along tertiles of distribution, four trimestral patterns were defined for each thyroid hormone: persistently low, decrease, increase and persistently high. By means of Kaplan Meier survival curves and Cox proportional hazard models, the impact of baseline levels and trimestral variation on mortality was investigated.

Results: TSH levels did not associate with mortality. At baseline, patients with low T3 or T4 (≤66th percentile) had higher hazards of dying than patients with high levels. Longitudinally, patients with persistently low levels of T3 or T4 during the 3-month observational period had higher mortality hazards than those having persistently high levels. These associations were mainly attributable to cardiovascular-related mortality. The association between T4 and mortality was not altered after adjustment for T3.

Conclusions: Hemodialysis patients with reduced T3 or T4 levels bear an increased mortality risk, especially due to cardiovascular causes. This was true when considering both baseline measurements and trimestral variation patterns. This longitudinal design adds important observational evidence -although non-decisive- that the link may underlie a causal effect. Thus, this study supports the hypothesis that restoration of thyroid hormone alterations in ESRD may improve patient´s outcome.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1637

Biofeedback Dialysis for Hypotension and Extracellular Volume Excess: A Systematic Review and Meta-Analysis Gihad E. Nesrallah,[1,2] Elie Akl,[2] Reem Mustafa,[2] Robert M. Lindsay,[1] Rita Suri.[1] *[1]Nephrology, University of Western Ontario, Canada; [2]Clinical Epidemiology and Biostatistics, McMaster University, Canada.*

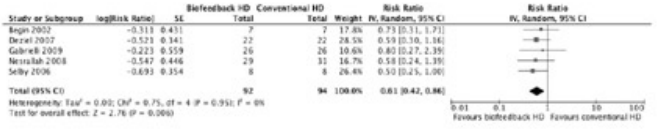
Background: Intradialytic hypotension (IDH) is associated with morbidity and mortality. Biofeedback (BF) devices which automate ultrafiltration and conductivity in response to changes in blood volume may reduce IDH and related complications. We conducted a systematic review to assess the benefits and harms of BF dialysis.

Methods: We adhered to a pre-specified protocol (PROSPERO ID: CRD42011001133). Data sources included CENTRAL (Issue 1,2011), MEDLINE (1966-2011), EMBASE (1980-2011), and ISI Web of Science (1976-2011). We included randomized parallel arm and crossover trials that randomized adults(>18 years) with symtomatic IDH or extracellular fluid volume expansion to receive HD with a BF device or usual care. All patients received 3 times weekly HD. Two authors assessed trial quality and independently extracted data in duplicate. We used a random effects model and expressed results as a risk ratio (RR) for dichotomous outcomes or mean difference (MD) for continuous data. We measured heterogeneity using the I^2 statistic.

Results: Seven studies met inclusion criteria. Two were parallel RCT's and 5 were cross-over studies. All studies were open-label. Studies were generally small (median N = 20), and not powered to assess survival or hospitalization. BF devices significantly reduced IDH (RR 0.61, 95% CI 0.42 to 0.86; I2=0%), but not predialysis systolic BP (MD 3, 95% CI -2 to 8 mmHg; I2=0%). Quality of life (QoL) data was reported in two studies, but the results could not be pooled; neither study demonstrated improved QoL. Potential harms were not assessed in any study.

Conclusions: BF dialysis reduces the frequency of IDH. Whether BF devices improve survival, hospitalization or QoL requires further study in adequately powered randomized trials.

Figure 1. Biofeedback HD vs. conventional HD with constant dialysate conductivity and ultrafiltration rate; outcome: intradialytic hypotension.



FR-PO1638

Role of Statins on C-Reactive Protein and the Kinetics of Erythrocyte Sodium Lithium Countertransport in Hemodialysis Patients Kriengsak Vareesangthip. *Renal Division, Department of Medicine, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand.*

Background: Cardiovascular disease as a result of accelerated atherogenesis is common in hemodialysis patients. Dyslipidemia may be a major contributor in this process and can be influenced by statins. Statins may exhibit additional inhibitory effects on the atherogenesis by a reduction of the inflammatory marker C-reactive protein (CRP). Erythrocyte sodium-lithium countertransport (Na/LiCT) is a sensitive membrane protein and has been reported to be abnormal (low Km) in hemodialysis patients. The activity of Na/LiCT has a positive correlation with the level of CRP in high cardiovascular risk patients. There is evidence suggesting that simvastatin can reduce the level of CRP and the other acute phase reactants in hemodialysis patients. We have hypothesized that simvastatin may decrease the CRP level and would improve the kinetics of Na/LiCT.

Methods: Twenty hemodialysed patients were divided into 2 groups. The study group 1 (n=10) has received simvastatin 10 mg daily for 4 months and the placebo group (n=10) has received placebo tablets for the same period as in the study group. We have measured serum CRP level, ESR, lipid profiles and the kinetics of Na/LiCT in all 20 patients before and after taking simvastatin.

Results: After 4 months, the CRP level was decreased in the study group more than in the placebo group (18.6% reduction in the study group vs 1.0% reduction in the placebo group). Before the treatment period, the Km for external sodium of erythrocyte of Na/LiCT was lower than that of normal controls in both groups of patients (56±3 vs 76±2 in group 1, P<0.01 and 50±2 vs 76±2 in group 2, P<0.01). After 4 months of simvastatin, the Km for external sodium was significantly improved (56±3 vs 69±5, P<0.01).

Conclusions: These results show those simvastatin exhibit comparable favorable effects on lipid profiles and the reduction of CRP level in hemodialysis patients. Moreover, the improvement of the kinetics of erythrocyte Na/LiCT as shown in this ESRD population, may indicate that these statins exhibit favorable effects on oxidative stress.

Funding: Government Support - Non-U.S.

FR-PO1639

Predictors of Antihypertensive Medication Exposure over Time for Dually Eligible Dialysis Patients Theresa I. Shireman,[1] James B. Wetmore,[1] Jonathan D. Mahnken,[1] Qingjiang Hou,[1] Purna Mukhopadhyay,[1] Sally K. Rigler,[1] Edward F. Ellerbeck.[1] *[1]University of Kansas School of Medicine, Kansas City, KS; [2]St. Luke's Mid-America Heart Instiute/UMKC, Kansas City, MO.*

Background: Renin angiotensin system antagonists (RASAs), beta-blockers (β-blockers), and calcium channel blockers (CCBs) are widely prescribed for their antihypertensive and cardioprotective benefits in patients on chronic dialysis, yet we do not know about chronic dialysis patients' degree of exposure to these classes while on dialysis.

Methods: We examined exposure patterns for a retrospective cohort (2000-2005) to determine factors associated with varying levels of use. We created a Medicare-Medicaid eligible cohort of new dialysis patients and tracked their medication exposure until death, transplant, or end of observation. The proportion of days covered (PDC), adjusted for institutional stays, was computed for each drug class from Medicaid drug claims and USRDS core data. PDC was computed across the entire window of observation for each cohort member without regard to when treatment began, reflecting medication exposure rather than adherence.

Results: Of 45,127 subjects in the cohort, 61.6% used a RASA, 64.2% a CCB, and 33.6% a β-blocker. Among users in each class, PDCs were highest for CCBs (mean 0.55, SD 0.31) followed by RASAs (mean 0.52, SD 0.31) and β-blockers (mean 0.46, SD 0.32). Advancing age was associated with higher PDCs for all classes, and Caucasians had higher PDCs than other racial groups ($p < 0.0001$). Diabetes, hypertension, and CVA were associated with higher RASA PDC ($p < 0.0001$). β-blocker PDCs were higher in the presence of HF or CAD ($p < 0.01$). The absence of HF and CAD was associated with higher CCB PDCs ($p < 0.01$). The presence of hypertension and CVA was associated with higher CCB PDC ($p < 0.0001$).

Conclusions: Despite substantial cardiac comorbidity, just under 2/3 used a CCB or a RASA and 1/3 used a β-blocker. Their respective PDCs indicate that use was limited to approximately 50% of dialysis tenure time. While exposure levels were somewhat consistent with clinical indications, there was less use among non-Caucasians, a finding that deserves further investigation.

Funding: NIDDK Support

FR-PO1640

Predicting Mortality in Incident Hemodialysis Patients: Validation of a European Model in an International Cohort Martin Wagner, Christoph Wanner, Francesca Tentori, Brian Bieber, Ronald L. Pisoni, Damian G. Fogarty, Bruce M. Robinson, Navdeep Tangri. *Medicine, University of Wuerzberg, Wuerzberg, Germany.*

Background: The risk of death varies among patients on hemodialysis. We previously developed and internally validated a model to predict mortality in incident patients from the UK Renal Registry (UKRR). The model included a number of routinely collected variables and achieved adequate performance. Here, we present the external validation of the prediction model in an international cohort of incident patients from the Dialysis Outcomes and Practice Patterns Study (DOPPS)

Methods: We included all patients initiating hemodialysis treatment in 2002-2004 (DOPPS), who survived the first 90 days on treatment. The variables in the prediction model comprised age, gender, race, primary renal disease; diabetes, CVD, smoking, albumin, hemoglobin, calcium, and creatinine. Discrimination was evaluated with a time-dependent c-statistic. Calibration was evaluated using the Nam and D'Agostino chi square statistic.

Results: The DOPPS dataset consisted of 3612 patients, of whom 355 died. The prediction model achieved adequate discrimination (c-statistic 0.74) and good calibration (observed vs. predicted risk, p-value 0.24).

	Discrimination C-statistic	Calibration observed/predicted risk across quatiles				
		low	intermediate	high	very high	p-value
UKRR	0.80 ± 0.01	0.1/1.4%	3.6/4.1%	5.3/8.2%	21.1/17.2%	<0.05
DOPPS	0.74 ± 0.02	1.2/2.1%	6.6/5.9%	10.6/11.4%	22.1/23.0%	0.24
North America	0.69 ± 0.03	4.5/2.7%	8.0/7.1%	15.0/13.1%	28.6/24.3%	0.10
Europe	0.74 ± 0.02	0.1/1.8%	7.8/5.5%	11.6/9.0%	23.8/20.0%	0.08
Asia/Pacific Rim	0.85 ± 0.04	0.1/0.2%	2.2/0.5%	2.4/1.0%	15.8%/2.5%	<0.05

While discrimination was adequate in the UKRR and the European DOPPS cohort (c statistic 0.80 and 0.74), the model was less accurate in North American patients (c-statistic 0.69) and calibration was limited in Asian patients.

Conclusions: Basic patient characteristics and laboratory variables are sufficient to accurately predict one-year mortality in patients incepting hemodialysis. Our model, developed in the UKRR, is now externally validated in an international cohort. Further research is needed to establish an easy-to-use clinical score that may provide useful information at the bedside.

FR-PO1641

Factors Predicting Mortality of New Patients Commencing Dialysis Therapy after 5 and 10 Years Follow-Up Sunil Bhandari,[1] Oliver T. Browne.[2] *[1]Renal Medicine, Hull & East Yorkshire Hopsitals NHS Trust and Hull York Medical School, Hull, East Yorkshire, United Kingdom; [2]Medical School, Hull York Medical School, Hull, United Kingdom.*

Background: The natural history of patients commencing dialysis is not well characterised and there is little evidence regarding the impact of potential pre-dialysis factors predicting mortality.This study examined pre-dialysis and co-morbid risk factors for 10y mortality post start of dialysis therapy.

Methods: A prospective study: all new subjects commencing dialysis in 2001/02 in East Yorkshire were followed up for a mean of 11.8 years. Predictors of mortality (e.g predialysis factors - creatinine, albumin, haemoglobin etc) were determined by uni-variate, multi-variate analysis and survival via Kaplin-Meier analysis.

Results: 94 patients, mean age of 63±1y were analysised. Mortality rate at 106 months was 60%. 30% (29) of patients had been transplanted during the follow-up period. 20 transplant patients were still alive with a functioning transplant (67%), 7 experienced transplant failure and returned to dialysis. 2 transplants died (myocardial infarction and calciphylaxis). Low-eGFR and haemoglobin at dialysis commencement had a significant impact on early mortality on univariate analysis. At 5y vascular disease and sepsis accounted for 71% of mortality. This decreased to 46% by 10y for these causes. Cardiac disease was the commonest cause of death. In 17% of patients death was related to dialysis or its withdrawal.

From Kaplan-Meier survival, patients with vascular disease had a cumulative survival of 14% vs. 33% of those without vascular disease (p=0.05). Diabetic patients had a cumulative survival of 18% vs. 27% without diabetes (p=0.02). Contrary to the 5 year data, calcium phosphate was no longer predictive of mortality.

Conclusions: Diabetes and vascular disease remain strong predictors of mortality. Calcium-phosphate levels are more specific predictors of early cardiac mortality. Low-eGFR and low haemoglobin concentration at dialysis commencement had a significant impact on early mortality but were not predictive of mortality on survival analysis. Aggressive management of cardiac risk factors in addition to early transplantation is key to survival.

FR-PO1642

Clinical Characteristics and Coronary Plaque Morphology in Chronic Kidney Disease (CKD) Patients Keiji Kono,[1] Hideki Fujii,[1] Kentaro Nakai,[1] Shunsuke Goto,[1] Masafumi Fukagawa,[2] Shinichi Nishi.[1] *[1]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan; [2]Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan.*

Background: Coronary artery disease (CAD) is a major cause of death in patients with chronic kidney disease (CKD). However, the pathophysiology of CAD remains unclear. Virtual Histology- Intravascular Ultrasound (VH-IVUS) can provide four major plaque components (fibrous, fibro-fatty, dense calcium and necrotic core) in vivo with a high accuracy. The aim of this study is to evaluate the clinical characteristics and coronary plaque morphology by VH-IVUS analysis in CKD patients.

Methods: Seventy-eight patients (CKD stage 1-2, n=31; CKD stage 3, n=24; CKD stage 4-5, n=11; Hemodialysis (HD), n=12) with CAD, who underwent VH-IVUS between May 2005 and December 2009 at our institute, were included in this study. They were divided into 2 groups, ACS group and non-ACS group, according to the admission diagnosis. VH-IVUS analysis on culprit segments was performed for all the study patients. From these profiles, we calculated Necrotic core/Dense calcium (NC/DC) ratio, and compared the ratio in patients with or without ACS in all the study patients.

Results: The result of VH-IVUS analysis showed that the relative volume of dense calcium (% DC) and necrotic core (% NC) gradually increased with decreasing renal function. On the other hand, NC/DC ratio gradually decreased with the development of CKD (CKD stage1-2: 2.5±1.4, CKD stage3: 2.2±1.6, CKD stage4-5: 1.6±0.9, HD: 1.4±0.7, p=0.06). Moreover, NC/DC ratio was significantly higher in patients with ACS compared to those without ACS (ACS: 2.3±1.5, non-ACS: 1.5±0.8, p<0.05). In multiple variate analysis, % DC was significantly correlated with diabetes mellitus (β=0.214, p<0.05) and estimated glomerular filtration rate (GFR) (β=-0.313, p<0.05).

Conclusions: Our findings suggested that the compositional pattern of coronary plaque was transformed from necrotic core -rich plaque into calcium-rich plaque with the development of CKD, and NC/DC ratio was associated with incidence of ACS in CKD.

FR-PO1643

Hemodialysis-Induced Regional Left Ventricular Systolic Dysfunction Is Independently Associated with Increased Mortality Solmaz Assa,[1] Yoran M. Hummel,[2] Adriaan A. Voors,[2] Ralf Westerhuis,[3] Paul E. de Jong,[1] Casper F. Franssen,[1] Johanna J. Kuipers.[1] *[1]Nephrology; [2]Cardiology, University Medical Center Groningen; [3]Dialysis Center Groningen, Netherlands.*

Background: The hemodialysis (HD) procedure may acutely induce regional left ventricular (LV) systolic dysfunction. The time course of this entity, its relation with intra-HD volume changes and long-term consequences are unknown. Therefore, we studied the occurrence of regional LV dysfunction at different times during HD in relation to volume parameters and the impact on outcome.

Methods: In 109 HD patients with a mean (±SD) age of 62.5±15.6 and median (IQR) dialysis vintage of 2 (1-4) years, echocardiography was performed pre-HD, at 60 and 180 min intra-HD and 30 min post-HD. LV global and regional systolic function was evaluated by ejection fraction (EF) and wall motion score index (WMSI). HD-induced regional LV dysfunction was defined as an increase in WMSI in ≥2 segments at either 60 or 180 min intra-HD or 30 min post-HD compared with pre-HD. Mean follow-up was 1.5 years.

Results: HD-induced regional LV dysfunction was found in 29 (27%) patients. These patients had worse pre-HD WMSI and EF, more previous cardiovascular events and higher pre-HD CRP levels (all p <0.05). In 17 (59%) of these patients, HD-induced regional LV dysfunction was manifest already at 60 min intra-HD when blood volume was only -1.3% (-3.4-1.7) lower than pre-HD. The course of blood volume, ultrafiltration (UF) volume, blood pressure and heart rate did not differ between patients with and without HD-induced regional LV dysfunction. Patients with HD-induced regional LV dysfunction had a significantly higher mortality, also after correction for age, sex, dialysis vintage, diabetes, cardiovascular history, UF volume, baseline WMSI & EF, and CRP (HR: 5.4; CI: 1.3-23.4; P=0.024).

Conclusions: HD regularly induces significant regional wall motion abnormalities, and these changes are related to a strongly increased mortality. HD-induced regional LV dysfunction occurs independently of changes in BV, suggesting that other mechanisms than hypovolemia must be involved.

Funding: Government Support - Non-U.S.

FR-PO1644

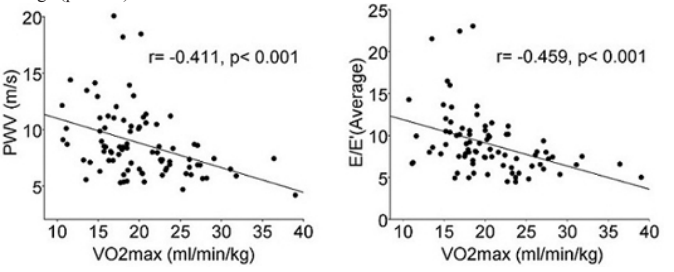
Increased Arterial and Left Ventricular Diastolic Stiffness Are Associated with Poor Cardiovascular Exercise Capacity in Advanced CKD Stephen M. Ting,[1,2] Hasan Iqbal,[1] Sudheer Koganti,[1] Prithwish Banerjee,[1] Robert Higgins,[1] Nicolas Aldridge,[1] Rosemary Bland,[2] Daniel Zehnder.[1,2] *[1]Univ. Hosp. Coventry & Warwickshire NHS Trust; [2]Warwick Medical School, United Kingdom.*

Background: Loss of large artery elasticity and LV diastolic dysfunction are notably prevalent in CKD patients. We sought to determine if LV diastolic compliance and aortic stiffness were associated with objective measure of cardiovascular functional reserve in CKD.

Methods: Patients waitlisted for kidney transplantation were prospectively assessed. Cycle ergometric exercise testing, TD echocardiography and carotid-femoral pulse wave velocity (PWV) were performed.

Results: 87 patients [age, 47 (37,59) years] were evaluated. Increased aortic stiffness (PWV: ≤8.15 vs. >8.15 m/s) was associated with reduced maximal exercise oxygen consumption [VO2max: 22.6 (18.1,26.2) vs. 18 (15.4,20.2) ml/min/kg, p< 0.001] and anaerobic threshold [VO2AT: 12.8 (10.9,14.1) vs. 11.6 (10.3,12.3) ml/min/kg, p= 0.03]. Patients with lower VO2max had a higher measure of LV filling pressure (E/E', p< 0.001). E/E' was calculated using the averaged of septal and lateral mitral annular lengthening velocities.

In univariate analysis, VO2max was inversely correlated with PWV (r= -0.411, p< 0.001) and E/E' (r= -0.459, p< 0.001). Similar correlations were observed for VO2AT with PWV (r= -0.295, p< 0.01) and E/E' (r= -0.258, p= 0.02). There were no clear associations between VO2max with LV ejection fraction (p= 0.07), LVMI (p= 0.33) and dialysis vintage (p= 0.39).



Conclusions: Arterial stiffness and abnormal LV diastolic filling pressure were strongly associated with poor cardiovascular functional reserve. This highlights the significance of ventriculo-arterial coupling in the complex mechanism of CKD mediated CHF. Further study with assessment following kidney transplantation may clarify the relationship between these prognostic factors.

Funding: Government Support - Non-U.S.

FR-PO1645

The Effect of Hemodialysis Ultrafiltration on Hemoconcentration and Changes in Whole Blood Viscosity Shayan Shirazian,[1] James Drakakis,[1] Sudharani Dikkala,[1] Paula J. Dutka,[1] Marc E. Duey,[2] Daniel J. Cho,[3] Steven Fishbane.[1] *[1]Nephrology and Hypertension, Winthrop University Hospital, Mineola, NY; [2]ProMetrics Inc., King of Prussia, PA; [3]Rheovector LLC, Pennsauken, NJ.*

Background: Whole blood viscosity (WBV) is an important predictor of cardiovascular outcomes. Hemodialysis patients may experience hemoconcentration and increases in WBV from fluid ultrafiltration (UF). The objectives of this study were to determine 1) the effect of hemodialysis on hemoconcentration/WBV and 2) whether UF volume requirements influence hemoconcentration and increases in WBV.

Methods: This was an observational analysis of 59 hemodialysis patients maintained at target Hgb 11-12 g/dL using darbepoetin alfa. Demographic and clinical characteristics were recorded. Blood samples were analyzed at initiation, midway and at the end of

dialysis. A scanning capillary viscometer (Prometrics Inc.) was used to measure WBV at multiple shear rates.

Results: For analysis, patients were divided into low-normal vs. high UF groups (cutoff 2700 ml). At baseline, patients in the high UF group were younger and had a greater proportion of diabetics. Mean hematocrit increased during dialysis in both groups. The intradialytic increase in hematocrit was significantly greater in the high versus the low UF group (3.2% vs. 1.28%, p=0.01), with a significantly higher end-dialysis hematocrit in the high UF group (40.5% vs 38%, p=0.02). At the end of dialysis both systolic (low shear rate) WBV ($p<0.01$) and diastolic (high shear rate) WBV ($p<0.01$) were significantly higher in the high UF compared to the low UF group. There was an approximately two-fold increase in systolic ($p<0.01$) and diastolic (p=0.01) WBV during dialysis in high versus low UF groups. The increase in systolic blood viscosity during dialysis was significantly correlated with an increase in Hct ($R2=0.6526$, $p<0.01$).

Conclusions: Hemodialysis results in hemoconcentration and increased WBV. Among patients requiring greater UF volumes, there are greater increases in Hct, systolic and diastolic WBV. Because of the potential harmful cardiovascular effects of increased WBV, patients with greater UF requirements may need lower Hgb targets during erythropoietin treatment.

Funding: Private Foundation Support

FR-PO1646

Serum Cystatin C as a Predictor for Cardiovascular Events in End-Stage Renal Disease Patients at the Initiation of Dialysis Sang Heon Song,[1,2] Ihm Soo Kwak,[1,2] Soo Bong Lee,[1] Eun Young Seong,[1,2] Il Young Kim,[1] Harin Rhee.[1,2] *[1]Department of Internal Medicine, Pusan National University School of Medicine, Busan, Korea; [2]Biomedical Research Institute, Pusan National University Hospital, Busan, Korea.*

Background: Cystatin C has been known to predict cardiovascular outcomes in elderly persons and stage 3 or 4 CKD patients. However, there has been no study to investigate whether cystatin C could predict the cardiovascular events in ESRD patients. Furthermore, recent studies argue that cystatin C-based eGFR level ($eGFR_{cysC}$) is a better predictor of cardiovascular disease than $eGFR_{Cr}$ because the non-GFR determinants of cystatin C also reflect cardiovascular risk. Current study was performed to delineate the role of serum cystatin C and $eGFR_{cysC}$ for prediction of the cardiovascular events and compare the other traditional variables with serum cystatin C in incident dialysis patients.

Methods: This study included 66 ESRD patients [mean age, 52.7±16.3 years; hemodialysis (HD), 46 pts; peritoneal dialysis (PD), 20 pts] who survived for more than 3 months after the start of dialysis, and serum cystain C levels were measured at the point of dialysis initiation. We conducted a retrospective charts review and median follow-up period was 14.9 months.

Results: Serum cystatin C was correlated with BUN ($r=0.537$, $p<0.001$), serum creatinine ($r=0.480$, $p<0.001$) and smoking ($r=0.284$, p=0.021). Cystatin C was inversely correlated with age ($r=-0.316$, p=0.01) and eGFRCr by MDRD ($r=-0.533$, $p<0.001$). The incidence of cardiovascular events was 16.7% (11/66). Kaplan-Meier analysis for cardiovascular events revealed that patients with lower cystatin C level (< 4.14 mg/L) had a better event-free survival rate compared with higher cystatin C group (≥4.14 mg/L) (p=0.039). In univariate analysis, serum cystatin C (HR, 2.62; 95% CI, 1.24-5.53; p=0.011), eGFRcysC (HR, 0.64; 95% CI, 0.47-0.87; p=0.004) were significant factors for the prediction of cardiovascular events. After multivariate adjustment, eGFRcysC was only an independent determinant of cardiovascular events (HR, 0.60; 95% CI, 0.39-0.95; p=0.029).

Conclusions: Our study suggested that $eGFR_{cysC}$ independently predicted cardiovascular events in incident dialysis patients

FR-PO1647

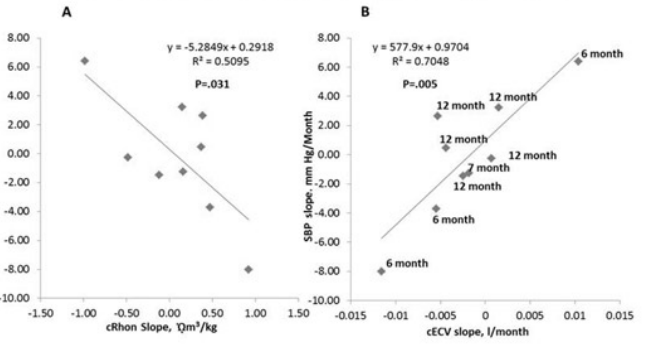
Correlation between Extracellular Volume as Assessed by Calf Bioimpedance Spectroscopy (cBIS) and Blood Pressure Changes in Hemodialysis (HD) Patients Rakesh Malhotra,[1] Olynka Vega,[1] Erik L. Penne,[2] Penny Faith Palmiero,[1] Samer Rateb Abbas,[1] Stephan Thijssen,[1] Nathan W. Levin,[1] Peter Kotanko,[1] Fansan Zhu.[1] *[1]RRI, NYC; [2]VUMC, Amsterdam.*

Background: Chronic fluid overload is associated with hypertension in HD patients. cBIS is a noninvasive technique to determine volume and distribution of body fluids (F Zhu;2008). The aim of the study is to investigate whether changes in fluid volume as assessed by cBIS correlate with changes in systolic blood pressure (SBP).

Methods: Chronic HD patients who were enrolled as part of a sodium intervention trial were followed for one year. In each subject, cBIS was done monthly pre-HD and post-HD to assess normalized calf resistivity ($\rho N,5, 10^{-2} W\ m^3/kg$) and extracellular volume (cECV) using a Hydra 4200 device (Xitron technologies, San Diego, CA). SBP and diastolic blood pressure (DBP) were recorded pre-HD and post-HD. Only patients with at least 6 month of follow-up were included. Temporal changes of pre-HD SBP, ncRho, and cECV were computed by simple linear regression.

Results: We studied 9 HD patients (6 men; age 45.8 ± 16.8 yrs). Temporal changes of SBP were significantly correlated with those of ncRho ($R2=0.50$, P=0.031; Fig.1A) and cECV ($R2 = 0.70$; P=0.005; Fig.1B)

Figure1: Correlation between SBP and ncRhon (A) and cECV (B) in HD patients



Conclusions: cBIS is a simple and non-invasive low cost means to objectively measure body fluid content in HD patients. Our study shows that cBIS derived measures (ncRho; cECV) are clinically meaningful. The use of cBIS for diagnosis and treatment guidance of fluid overload can facilitate blood pressure control.

Results of Follow-up Measurements

	Baseline	Month 3	Month 6	Month 9	Month 12
# patients	9	9	9	5	5
cECV	0.20±0.04	0.19±0.03	0.20±0.03	0.18±0.01	0.18±0.01
ρN,5,10-2Ω m3/kg	13.5±3.0	13.6±6.5	13.7±3.0	14.1±2.2	14.9±1.46
SBP, mmHg	141.9±19.7	139.9±17.2	140.6±11.7	138.8±24.9	135.5±5.4
DBP, mmHg	74.7±13.8	76.8±8.2	79.2±10.3	78.0±23.2	74.5±25.3

FR-PO1648

Mortality Due to Pulmonary Embolism, Myocardial Infarction, and Stroke among Patients Starting Dialysis Gurbey Ocak,[1] Karlijn J. Van Stralen,[2] Marion Verduijn,[1] Friedo W. Dekker,[1] Kitty J. Jager.[2] *[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden; [2]ERA-EDTA Registry, Department of Medical Informatics, Academic Medical Center, Amsterdam, Netherlands.*

Background: Dialysis patients have an increased cardiovascular mortality risk as compared to the general population. However, there is limited information on how specific cardiovascular causes contribute to this increased risk.

Methods: Age-and sex-standardized mortality rate ratios (SMRs) for cardiovascular causes were calculated for 130 439 adults starting dialysis and registered in the ERA-EDTA Registry as compared with the European general population. Furthermore, we calculated hazard ratios (HRs) with 95% confidence intervals (CIs) to investigate the association between potential risk factors and specific causes of cardiovascular death.

Results: The overall age- and sex-standardized mortality rate of cardiovascular causes was 8.9 (95% CI 8.7-9.1) times higher in dialysis patients than in the general population. The SMRs in dialysis patients as compared to the general population were 12.2 (95% CI 10.2-14.6) for pulmonary embolism, 11.0 (95% CI 10.6-11.4) for myocardial infarction, 8.4 (95% CI 8.0-8.8) for stroke, and 8.3 (95%CI 8.0-8.5) for other cardiovascular diseases. Primary kidney diseases due to diabetes and multi-system disease were associated with an increased mortality risk due to pulmonary embolism (HR 1.9; 95% CI 1.0-3.8 and HR 3.2; 95% CI 1.6-6.4, respectively), myocardial infarction (HR 4.1; 95% CI 3.4-4.9 and HR 2.2; 95% CI 1.7-2.7, respectively), stroke (HR 3.5; 95% CI 2.8-4.4 and HR 2.8; 95% CI 2.1-3.6, respectively), and other cardiovascular causes of death (HR 3.4; 95% CI 2.9-3.9 and HR 3.4; 95% CI 2.9-4.0, respectively) as compared to patients with polycystic kidney disease after adjustment for age, sex, calendar year, and country.

Conclusions: Compared to the general population, dialysis patients have an highly increased mortality risk due to myocardial infarction, stroke, and pulmonary embolism.

Funding: Government Support - Non-U.S.

FR-PO1649

Obesity Paradox in Japanese Hemodialysis (HD) Patients Jyunichiro Hashiguchi,[1] Satoshi Funakoshi,[1] Takashi Harada,[1] Junko Kubo,[1] Rica Etoh,[1] Yoshiaki Lee,[1] Kazunori Utsunomiya,[3] Mineaki Kitamura,[2] Tomoya Nishino,[2] Shigeru Kohno.[2] *[1]Divisionof Blood Purification, Nagasaki Renal Center, Nagasaki, Japan; [2]Department of Internal Medicine, Nagasaki University Graduate School of Medicine, Nagasaki, Japan; [3]Department of Diabetes, Jikei University, Tokyo, Japan.*

Background: Dialysis Outcome and Practice Patterns Study (DOPPS) had suggested that nutritional indicator, including higher body mass index (BMI), has an important factor on the survival of HD patients. On the other hand, obesity is considered to be independent risk factors for the development of cardiac risks in the general population (obesity paradox). We hereby assess the impact of BMI on cardiac function of Japanese HD patients in long term.

Methods: From April of 2005 to March of 2010, 94 HD patients in our facility with stable BMI and normal protein catabolic rate (nPCR) over 5 years were enrolled in this study after appropriate informed consent. Ultrasound cardiography including ejection fraction (EF) or left ventricle diameter (LVDd) were evaluated before and after the period of > 5 years. The objects were divided into three groups, one for the patients with BMI

< 20 (underweight), one for BMI 20-25 (optimal) and BMI > 20 (overweight), and then analyzed the correlation between BMI and cardiac function.

Results: As shown in the table, EF in underweight group did not change after 5 years, whereas EF had significantly decreased in overweight group from 0.658±0.33 to 0.617±0.25 (p<0.05).

Change in Cardiac Parameters during 5 years

	underweight	optimal	overweight
	before / after	before / after	before / after
No. of patients	44	34	19
DM / non-DM	12 / 32	10 / 24	7 / 12
average EF	0.688 / 0.707	0.679 / 0.699	0.658 / 0.617*
average LVDd (mm)	51.3 / 55.2*	54.5 / 55.8	52.5 / 50.8

* p<0.05

The increase in LVDd was observed in underweight group from 51.3±18.6 to 55.6±10.9, wheras there were no change in optimal or overweight group. Nutritional factors including nPCR stayed the same in all groups.

Conclusions: In Japanese HD patients population where the average body weight is 53-56 Kg in adults, higher BMI indicating obesity may increase cardiac risks presumably associated with various baseline health status including cardiac load.

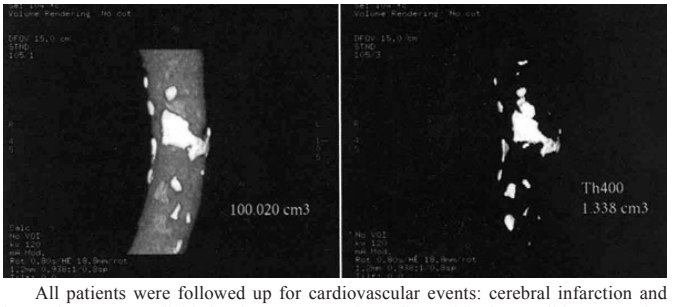
Funding: Private Foundation Support

FR-PO1650

Calcification of the Thoracic Aorta Determined by Three-Dimensional Computed Tomography Predicts Cardiovascular Complications in Hemodialysis Patients Nozomu Kamiura,[1] Kiyoko Yamamoto,[2] Shioko Okada,[2] Makoto Sakai,[1] Akira Fujimori.[2] *[1]Department of Internal Medicine, Konan Hospital; [2]Blood Purification and Kidney Center, Konan Hospital.*

Background: The most common cause of death in dialysis patients is cardiovascular disease, and this may be due in part to the presence of excessive vascular calcification.

Methods: Computed tomography using contrast medium was performed in 49 hemodialysis patients (29 males and 20 females; 17 diabetics, 32 nondiabetics; average age 68.9 ± 11.0 years). Calcification score (CS) was defined as the ratio of the volume of the vascular calcification to the volume of the thoracic aorta, 10 cm caudal part from the bifurcation of the trachea.



All patients were followed up for cardiovascular events: cerebral infarction and hemorrhage, myocardial infarction, ECG or echocardiographic abnormalities suggestive of myocardial ischemia, cardiac surgery, leg amputation, and death and hospitalization due to heart failure.

Results: After 3 years of follow-up, 12 patients reached the end point. Mann-Whitney U test showed that both high CS (P = 0.007) and male gender (P = 0.009) were significantly associated with cardiovascular outcome which did not correlate with age, dialysis duration, diabetes mellitus, smoking status, LDL cholesterol, pulse-wave velocity, maximum intima-media thickness of the carotid artery wall, systolic blood pressure, left ventricular hypertrophy. Multiple logistic regression analysis revealed that high baseline CS was a singificant predictor of the cardiovascular events (P < 0.05), independent of age, gender, dialysis duration, diabetes, smoking status, and LDL cholesterol.

Conclusions: Calcification of the thoracic aorta determined by three-dimensional computed tomography predicts cardiovascular events in hemodialysis population.

FR-PO1651

C-Reactive Protein Is a Strong and Independent Risk Factor for Cardiovascular Morbidity and Mortality in Hemodialysis Patients – Post Hoc Results from the AURORA Trial Inga Soveri,[1] Eva Carlsson,[1] Ingar Holme,[2] Hallvard Holdaas,[3] Alan G. Jardine,[4, 5] Roland E. Schmieder,[6] Eva K.A. Johnsson,[7] Faiez Zannad,[8] Bengt C. Fellstrom.[1] *[1]Nephrology, Uppsala University, Sweden; [2]Preventive Medicine, Clinical Research, Oslo University Hospital, Ullevål, Norway; [3]Nephrology, Oslo University Hospital, Rikshospitalet, Norway; [4]Renal Unit, British Heart Foundation Glasgow Cardiovascular Research Centre, United Kingdom; [5]Medicine and Therapeutics, Western Infirmary, Glasgow, United Kingdom; [6]University Hospital Erlangen-Nürnberg, Erlangen, Germany; [7]AstraZeneca, Mölndal, Sweden; [8]Inserm CIC9501 and U961, CHU Nancy, Nancy Université, France.*

Background: The AURORA trial was a controlled, randomized trial in 2,776 hemodialysis patients, studying the effect of rosuvastatin 10 mg on major cardiovascular (CV) events. There was no significant effect on any CV endpoint in the overall study; however, the data provide an opportunity to explore risk factors for CV events and mortality in hemodialysis patients.

Methods: High-sensitivity C-reactive protein (hsCRP) as a risk factor for CV disease (CVD) in hemodialysis patients was examined for the CV endpoints used in the study, including mortality. Cox proportional analysis was used for risk factor assessment, presented as hazard ratio (HR) per unit hsCRP increase. Ranking of risk factors at baseline (age, diabetes, hsCRP, albumin, phosphate, low-density lipoprotein cholesterol, previous CVD, smoking, medication, etc) was performed using a Random Forest Model.

Results: The model showed that baseline hsCRP was an independent and strong risk factor for all endpoints.

hsCRP at baseline as risk factor for endpoints in AURORA

	HR	p-value
Major CV events	1.112	0.0011
Death from any cause	1.167	<0.0001
CV death	1.151	<0.0001
Atherosclerotic event	1.128	0.0026
Major CV event or death	1.148	<0.0001
Non-fatal myocardial infarction	1.14	0.041
Non-CV death	1.207	<0.0001

Ranking of risk factors captured in the study showed that hsCRP as a risk factor was surpassed only by age and diabetes for all endpoints.

Conclusions: In this post-hoc analysis, baseline hsCRP was the 3rd-6th strongest risk factor for CV events and patient mortality among hemodialysis patients in the AURORA trial.

Funding: Pharmaceutical Company Support

FR-PO1652

The Survival Impact of Cardiac Output on Access Blood Flow in Chronic Hemodialysis Patients --A Nine-Year Cohort Study Cheng-Kai Tsai. *Division of Nephrology, St. Martin De Porres Hospital, Chia-yi, Taiwan.*

Background: Access surveillance program becomes clinical routine to maintain the adequate performance of dialysis. The previous study postulated that the Qa may serve as a maker to predict clinical outcomes of dialysis patients. We know the Qa is potentially affected by many factors, including systemic hemodynamics, the size and endothelial function of vessels supplying and draining the access, and the presence of intravascular lesions, but the role of the performance of cardiac contractivity in access blood flow has not been discussed.

Methods: The purpose of our study was to evaluate the impact of cardiac performance on access blood flow. Thus, we had conducted a nine-year prospective cohort study of 434 patients, aged from 18 to 80, who received hemodialysis treatment in our medical center from January 2002 to December 2010.The surveillance program initiated the evaluation of all eligible patients, including cardiac function assessment and the Qa measurement by the saline dilution method using Transonic HD01/02 devices twice yearly.

Results: The cardiac index (CI) was classified into three categories (<2.5, 2.5 to 4.5, >4.5), and access blood flow (Qa) into five categories (<500, 501 to 999, 1000 to 1499, 1500 to 1999, >2000 ml/min).

A big difference of access blood flow (788±396 ml/min vs 1062±524 ml/min, p<0.001) and cardiac index (3.18±0.97 vs 3.65±1.01, p<0.001) was found between diabetic and non-diabetic patients.

Patient's cardiac index between 2.5 to 4.5 and the Qa between 1500 to 2000ml/min acquired the best survival rate, and the hazard ratio after adjustment for demographic characteristics, comorbidity, and the access type was still high in patients with lowest cardiac index and Qa.

Conclusions: The patients with diabetes whose access blood flow and the cardiac index are lower lead to greater risk of death and their vulnerability.

Funding: Private Foundation Support

FR-PO1653

Outcomes of Cardiovascular Implantable Electronic Device Infections in Patients on Chronic Hemodialysis Therapy LaTonya J. Hickson,[1] Katherine Y. Le,[2] Larry M. Baddour,[3] David L. Hayes,[4] Walter R. Wilson,[3] James Steckelberg,[3] Muhammad Rizwan Sohail.[3] *[1]Nephrology and Hypertension, Mayo Clinic; [2]Internal Medicine, Mayo Clinic; [3]Infectious Diseases, Mayo Clinic; [4]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

Background: Infection is a serious complication of cardiovascular implantable electronic device (CIED) implantation. Hemodialysis (HD) dependent patients have multiple co-morbid conditions with an impaired immune system. Thus, they may be at higher risk of complicated CIED infection with poorer outcomes than non-HD patients.

Objective: To compare clinical presentations and outcomes in patients with CIED infection based on whether they are HD-dependent or not.

Methods: We reviewed all cases of CIED infections treated at our center between 1991 and 2008 and analyzed their pertinent clinical features.

Results: Among the 415 patients with CIED infection, mean age was 69±15 years and 75% were male. Seventeen (4%) had received HD therapy prior to CIED infection. HD patients were more likely to be female (59% vs 24%, p=0.001). Both localized signs at the generator pocket and systemic manifestations of infection were less frequent in HD as compared to the non-HD group. Although HD patients were more likely to be bacteremic (100% vs. 44%, p<.0001), endocarditis rates were similar (50% vs 34%, p=0.2). There were no differences in device removal complication rates between the two groups. However, HD patients were relatively less likely to undergo device removal (82% vs. 95%, p=0.02) or undergo implantation of a new device (43% vs 67%, p=0.04). Although mortality rates at 30- and 60-day were similar between HD and non-HD groups, 90-day mortality was significantly higher (24% vs 8%, p=0.02) among HD patients.

Conclusions: HD-dependent patients are more likely to present with bacteremia complicating CIED infection and have a higher 90-day mortality rate as compared to non-HD dependent patients.

FR-PO1654

Efficacy of Low-Dose Bisoprolol in Maintenance Hemodialysis Patients with Asymptomatic Left Ventricular Remolding and Diastolic Dysfunction
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Background: Left ventricular hypertrophy and diastolic dysfunction are the most frequent cardiac alteration in ESRD. The aim of this study was to determine whether β-blockers, bisoprolol, had beneficial effects in maintenance hemodialysis patients with asymptomatic left ventricular diastolic dysfunction.

Methods: In this study we enrolled 30 patients with chronic kidney disease (CKD) undergoing maintenance hemodialysis accompanying left ventricular diastolic dysfunction more than six months. Bisoprolol was started with 1.25 mg once daily orally 30 minutes after breakfast and increased every 1 week by 1.25 mg increments up to the maximum tolerated dose. Echocardiographic examination was used to measurement of left ventricular diastolic function.

Results: 27 patients finished 6 months study. After 6 months treatment, compared with the baseline, the cardiothoracic ratio was significant decreased. Echocardiographic examination showed that there was no significant change in EF, but LVDd , LVDs, PWT were significant decreased . E/A ratio significantly increased.

Conclusions: Our study demonstrate bisoprolol efficacy in improving left ventricular remolding and diastolic dysfunction in HD patient with normal blood pressure.

Funding: Government Support - Non-U.S.

FR-PO1655

Potassium-Binding Sodium-Based Resins: Associations with Serum Chemistries and Interdialytic Weight Gain
Michel Y. Jadoul,[1] Angelo Karaboyas,[2] Francesca Tentori,[2] David A. Goodkin,[2] Yun Li,[2,3] Laura Labriola,[1] Bruce M. Robinson.[2,3] *[1]Cliniques univ. St Luc, Université Catholique de Louvain, Brussels, Belgium; [2]Arbor Research Collaborative for Health, Ann Arbor, MI; [3]University of Michigan, Ann Arbor.*

Background: Na-based resins increase phosphaturia and plasma bicarbonate levels in non-uremic dogs, due to calcium binding in the gut, favoring phosphorus and bicarbonate absorption. Relevance to hemodialysis (HD) patients is unknown.

Methods: Data were from 10,487 HD patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) phases 2-4 (2002-2011) in countries with >5% Na-based K resin use: Belgium (12%), Canada (7%), France (49%), Italy (19%), and Sweden (25%). Linear mixed models were used to determine associations at baseline between K resin use and interdialytic weight gain (IDWG), serum concentrations of bicarb, P, K, Ca, and Na. Instrumental variable (IV) analyses were conducted because they can limit treatment-by-indication bias due to unmeasured confounders.

Results: Overall K resin use was 20%, with notable facility variation. The 95th percentile of facility % K resin use was 58%; 22% of facilities did not prescribe any K resins. Patients prescribed a K resin had a greater IDWG (in line with the Na load of K resins), and higher serum bicarb, P, and Na (but not Ca) than patients without a K resin Rx (Table 1). While the magnitudes of the bicarb and Na effects are small, the P effect could impact P-binder dose. Findings were directionally consistent in the IV analysis for bicarb, P, Na, and IDWG (i.e., possibly causal) but not K (treatment-by-indication bias).

Conclusions: As hypothesized, Na-based K resin use in HD patients is associated with higher serum bicarbonate, phosphorus, and sodium concentrations and greater IDWG. Additional studies are warranted to assess the impact of K resins on vascular calcification, CV events, and mortality.

Table 1: Effect of Na-based K Resin Prescription (Estimate*, 95% CI)

Outcome	Unadjusted	Adjusted	IV approach (adjusted)
Serum Bicarbonate (mEq/L)	0.09 (-0.10,0.28)	0.32 (0.14,0.51)	0.10 (-1.26,1.46)
Serum Phosphorus (mg/dL)	0.37 (0.28,0.46)	0.20 (0.11,0.28)	0.32 (-0.05,0.69)
Serum Potassium (mEq/L)	0.34 (0.30,0.38)	0.20 (0.16,0.24)	-0.16 (-0.35,0.03)
Serum Sodium (mEq/L)	0.38 (0.20,0.56)	0.35 (0.16,0.53)	0.96 (-0.02,1.93)
IDWG (kg)	0.44 (0.36,0.51)	0.26 (0.19,0.33)	0.24 (-0.07,0.55)

*Estimate is the difference in the outcome for patients with baseline K resin use compared to non-use

Adjustments: age, gender, black race, vintage, BMI, residual kidney function, vascular access, ferritin, hemoglobin, white blood cell count, serum albumin, serum creatinine, and 14 comorbid conditions
Note: All models adjusted for DOPPS phase and country and accounted for facility clustering effects

Funding: Pharmaceutical Company Support

FR-PO1656

Multiple Biomarkers Improve the Prediction of Cardiovascular Mortality in Patients on Chronic Hemodialysis
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Background: Patients on chronic hemodialysis (HD) are widely recognized to be at high risk for cardiovascular (CV) mortality, thus the more accurate prediction of CV mortality is clinically important. Three biomarkers,Serum brain natriuretic peptide (BNP), Troponin T (TnT) and C-reactive protein (CRP) ,are individually established to be predictive biomarkers for CV mortality in this population.

Methods: A total of 500 consecutive HD patients were examined by the measurement of three biomarkers for 10 years. The cut-off values were based on the tertiles of the individual biomarkers, and a multivariate Cox analysis including three biomarkers for CV mortality was performed. From the analyzed model, a simplified score was obtained by putting weight to individual biomarkers based on the adjusted hazard ratio (HR). Finally, the multi-marker score(MMS) was defined as the sum of these points, with higher points indicating a higher mortality risk.

Results: During follow-up period (80±41 months), 204 patients died including 95 CV deaths. Three biomarkers were individually independent predictors for CV mortality ($p<0.01$ for all). However, by receiver operating characteristic (ROC) analysis, area under curve (AUC) for CV mortality was larger in the multi-marker score (0.76) compared to BNP (0.64), TnT (0.70) and CRP (0.65) alone. Similarly, AUC for all-cause mortality was 0.80, 0.68, 0.74 and 0.73 in MMS. Among low-risk group (MMS< 3), middle-risk group (MMS=4-6) and high-risk group (MMS> 7), 10-year survival rate was 93.5%, 73.3% and 54.0% for CV mortality and 83.1%, 52.6% and 26.6% for all-cause mortality ($p<0.0001$ for both), respectively. Even after adjustment, MMS had strongly predictive power.

	Cardiovascular mortality		All-cause mortality	
Multi-marker score	HR (95%CI)	P value	HR (95%CI)	P value
<3	Reference		Reference	
4-6	4.00 (1.70-9.40)	0.0015	2.88 (1.68-4.94)	0.0001
>7	7.80 (3.25-18.73)	<0.0001	5.93 (3.39-10.37)	<0.0001

Conclusions: Multiple biomarkers could more accurately predict the mortality than these biomarkers alone in patients on HD.

FR-PO1657

NT-ProBNP Has No Diagnostic Value in End Stage Renal Disease Patients Presenting with Dyspnea
Jose Albert Avila, Richard K. Kasama, Shaffer R.S. Mok. *Medicine, Nephrology, Cooper University Hospital, Camden, NJ.*

Background: NT-ProBNP/BNP although used to differentiate acute dyspnea states, has not been validated in End Stage Renal Disease(ESRD) patients. We examine hemodialysis(HD) patients presenting with dyspnea.

Methods: Retrospectively, 250 HD subjects admitted to Cooper Hospital from 07/2010 to 03/2011, with acute dyspnea were broken into a high group (NT-ProBNP >=70,000) and a low group (NT-ProBNP <2,600) based on a rough cut off of 2,500 reported for chronic kidney disease patients. Analysis used Chi-square to assess differences between low and high NT-ProBNP levels in relation to performing hemodialysis, number of cardiology consults and echocardiograms ordered corrected for the continuous data set. Standard t-test analysis evaluated the difference in creatinine, volume removed, weights, weight change, and ejection fraction(EF) between groups.

Results: Out of 250 subjects, 235 had NT-ProBNP levels performed. No statistically significant difference was found in the frequency of hemodialysis, obtaining cardiology consults and echocardiograms between ESRD patients with high and low NT-ProBNP.
Chi-square analysis

Procedure	Chi-Square	P-value	Continuity correction	P-value
Hemodialysis	4.581	0.032	2.633	0.105
Cardiology consult	1.904	0.168	1.233	0.267
Echocardiogram	0.807	0.369	0.413	0.521

No statistically significant difference in creatinine, volume removed, weight change, and EF between groups with low and high NT-ProBNP was noted. There was a statistically significant difference in weight pre and post HD between the groups.

	Group (Low vs High NT-ProBNP)	Mean (N, SD)	t-test	P-value
Creatinine (mg/dL)	Low	7.24 (30, 5.25)	-0.585	0.561
	High	7.92 (31, 3.75)		
Volume removed (liters)	Low	2.14 (29, 1.19)	-0.455	0.651
	High	2.28 (31, 1.13)		
Weight pre-HD (kg)	Low	89.76 (29, 28.53)	2.180	0.033
	High	77.50 (31, 12.50)		
Weight post-HD (kg)	Low	87.53 (29, 28.86)	2.201	0.032
	High	74.97 (31, 12.85)		
Weight change (kg)	Low	2.23 (29, 2.08)	0.642	0.523
	High	2.53 (31, 1.49)		
EF (%)	Low	57.20 (30, 16.13)	1.540	0.129

Conclusions: There is no difference in the initial management strategies of HD patients presenting with NT-ProBNP levels in both extremes. In ESRD, the NT-ProBNP has no clinical value for discriminating between primary pulmonary processes and volume overload states and, therefore, should not be ordered as part of an emergency room evaluation.

FR-PO1658

Threshold Value of Serum b-Type Natriuretic Peptide (BNP) Level in Predicting Cardiovascular Disease (CVD) Risk in Hemodialyzed Patients Yusuke Tsukamoto,[1] Michio Kuwahara,[2] Sei Sasaki.[3] *[1]Department of Nephrology, Itabashi Chuo Medical Center, Itabashi-ku, Tokyo, Japan; [2]Department of Nephrology, Shuwa General Hospital, Kasukabe-shi, Saitama, Japan; [3]Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

Background: BNP has been reported to be a useful marker for CVD risk both in non-CKD and CKD patients. However, peptides accumulate in patients with decreased kidney function and threshold value in non-CKD cannot be applied in dialyzed patients.

Methods: Total of 463 patients (median HD vintage=87 months, age=60 yrs/o, male 68%, diabetic 39%) were studied from Jan. 2009 to Dec. 2010. Serum BNP level and ultrasonic cardiography was measured at the end of the first dialysis session of the week. Other serum laboratory tests were done at the beginning of dialysis.

Results: One hundred and seven CVD events (include 44 CVD deaths) were recorded during this study period. Cox-hazard revealed that BNP had a significant power to predict CVD events (hazard ratio=1.0002, p=0.0026 adjusted by age, sex, diabetes, dialysis vintage). ROC analysis demonstrated that threshold BNP level was higher than 503 pg/ml (specificity 81.2%, sensitivity 37.7%, P=0.0008) for CVD events and 708 pg/ml (specificity 87.4%, sensitivity 34.1%, P<0.05) for CVD death. Multiple regression analysis showed that BNP was correlated with ejection fraction (negatively, P=0.0002), left atrial diameter (P=0.019) but not with left ventricular mass index or average dialysis ultrafiltrate volume (n=287). In this cohort, higher ultrafiltrate volume was also associated with high risk of CVD event (hazard ratio=1.62, P<0.05 adjusted by demographics, n=362).

Conclusions: 1. Serum BNP level reflected a volume status of hemodialyzed patients and suggested to be a good marker to decide an adequate body weight. 2. Serum BNP level is a good marker to predict CVD risk not only in non-CKD but also in hemodialyzed patients. However, the threshold value for risk should be set much higher than one for non-CKD.

Funding: Clinical Revenue Support

FR-PO1659

High Cardiac Troponin T Levels Predicts High Mortality in Hemodialysis Patients with Preserved Left Ventricular Ejection Fraction Chi-Ting Su,[1,3] Kuan-Yu Hung,[2] Jenq-Wen Huang.[2] *[1]Nephrology, Internal Medecine, Nation Taiwan University Hospital, Yun-Lin Branch, Douliou, Taiwan; [2]Nephrology, Internal Medecine, Nation Taiwan University Hospital, Taipei, Taiwan; [3]Human Genetic, Graduate School of Public Health, University of Pittsburgh, PA.*

Background: The implication of cardiac troponin-T (cTnT) to evaluate of cardiac functions in heomdialysis patients with preserved left ventricular ejection fraction (LVEF) remains unclear. We conduct this study to illustrate the potential value of cTnT to predict systolic function and patient survival.

Methods: There were one hundred and nine hemodialysis patients enrolled. Patients with severe valvular heart disease, atrial fibrillation, pulmonary edema or acute coronary syndrome were excluded. Study participants received echocardiography with tissue Doppler imaging analysis, and 2-dimensional speckle-tracking echocardiography with strain analysis. Patients were stratified by level of cTnT ≥0.05 ng/ml and cTnT < 0.05 ng/ml.

Results: Between two groups, there was no significant difference of gender, age, LVEF, systolic myocardial velocity, and the level of high sensitivity C-reactive protein (hsCRP). Patients, with high level of cTnT (≥0.05 ng/ml), presented with lower serum albumin level (3.13 ± 0.33 g/dL vs. 3.38 ± 0.41 g/dL, p=0.008) than those in the other group. By strain analysis, among patients with high cTnT level, reduced global LV peak systolic longitudinal strain (GSl; high level cTnT vs. low level cTnT group:-16.8 ± 3.3% vs. -19.0 ± 3.6%, p=0.02) developed. There was a correlation between GSl and level of cTnT (r=0.36, p < 0.001). One and half year mortality rate was lower in the group with low cTnT level (high level cTnT group vs. low level cTnT group: 40% v.s. 9.8%, p= 0.003).

Conclusions: Deterioration of LV systolic function, GSl, developed along with the increasing level of cTnT. In maintenance hemodialysis patients with level of cTnT ≥0.05 ng/ml presented with worse LV systolic function and lower albumin level than others with level of cTnT<0.05 ng/ml. The lower survival rate was associated with the higher level of cTnT.

FR-PO1660

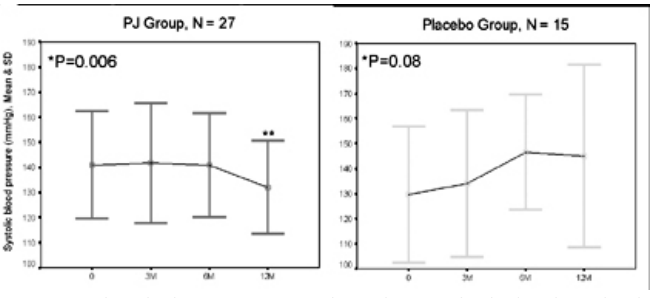
Pomegranate Juice Intake Attenuates Traditional Cardiovascular Risk Factors in Hemodialysis Patients: A Randomized Placebo Controlled Trial Lilach Shema,[1] Liora Ore,[2] Shifra Sela,[3] Ronit Geron,[1] Galina Shapiro,[3] Batya Kristal.[1] *[1]Nephrology Department, Western Galilee Hospital, Nahariya, Israel; [2]School of Public Health, University of Haifa, Israel; [3]Clinical Microbiology Lab and Eliachar Research Lab, Western Galilee Hospital, Nahariya, Israel.*

Background: The aim of the present study was to investigate the long term effects of Pomegranate juice (PJ) consumption, rich in polyphenols, on traditional cardiovascular (CV) risk factors: lipid profile, hypertension and on the progression of the atherosclerotic process.

Methods: 101 HD patients were randomized to receive 100 cc of PJ (0.7mM polyphenols) or matching placebo, three times a week for one year. The primary endpoints were systolic and diastolic blood pressure, number of antihypertensive drugs, level of triglycerides (TG), total cholesterol, HDL and LDL. Secondary endpoint was a composite of variables: Intima Media Thickness (IMT), number and structure of atherosclerotic plaques of the Carotid arteries.

Results: After one year of intervention the change in the number of antihypertensive drugs was significantly different between the two groups (P.V = 0.05). The number of antihypertensive drugs decreased in 22% of patients in the PJ group compared to 7.7% in the placebo group, while an increase was documented in 12.2% of patients in the PJ compared to 34.6% in the placebo group. Furthermore, a significant time response improvement in systolic blood pressure, triglycerides and HDL was observed in the PJ group.

Conclusions:



Among patients in the PJ group attenuation and aggravation in the atherosclerotic process was detected in 25% and 5% respectively, while more than 50% of patients in the Placebo showed progression and none showed improvement.

Conclusions: PJ consumption attenuates traditional CV risk factors. Hence, it may favor the high incidence of morbidity and mortality in HD patients.

Funding: Government Support - Non-U.S.

FR-PO1661

Treatment of Chronic Low Back Pain in Hemodialysis Patients: A Randomized Controlled Trial Ricardo Sesso, Tatiana Cristofolini, Jamil Natour. *Nephrology, Federal University of São Paulo, Brazil.*

Background: Low back pain is a very common and disabling symptom that has not been properly studied in hemodialysis patients. The aim of this study was to evaluate the effectiveness of a physiotherapy approach to chronic low back pain.

Methods: 104 patients with chronic low back pain (lasting more than 3 months) not related to infection, tumor or fractures, and undergoing chronic hemodialysis in two hemodialysis units were prospectively randomized to one of two intervention groups: 1. Physiotherapy (N=53) with the McKenzie method consisting of repetitive and sustained flexion/extension exercises of the lumbar spine. Four movements were selected: flexion in standing, extension in standing, flexion in lying and extension in lying position; repeated twenty times per session (30 min. total) three times a week for eight weeks. Group 2 (N=51): received transcutaneous electrical nerve stimulation (TENS) for 20 min at 50-100 Hz frequencies for the same time frame. Outcome measures: lumbar pain visual analogue scale (VAS, ranging from 0-10[worse]), and the functional lumbar spine status measured by the Roland Morris (RM) disability questionnaire (with 24 items and scores ranging from 0 to 24[worse]) reported by the patients 1 week after the end of treatment.

Results: Mean patients' age was 58 yr.; median time on dialysis was 3.9 yr. Baseline sociodemographic, laboratory and clinical patients' characteristics were similar in both groups. Mean±SD scores of the VAS pre- and post-treatment were 6.9±1.6 and 1.4±1.4, respectively, in group 1, and 7.1±1.6 and 6.4±1.6, respectively in group 2. Mean change from baseline to after treatment was 5.5±1.8 and 0.8±0.6 for groups 1 and 2, respectively, P<0.001. Baseline RM scores were 20.0±2.6 and 20.8±2.4, for groups 1 and 2, respectively. Mean change from baseline to after treatment was 16.0±2.6 and 1.1±1.0 for groups 1 and 2, respectively, P<0.001. The effectiveness of the physiotherapy intervention was observed in all strata of the sociodemographic, laboratory and clinical variables.

Conclusions: Physiotherapy with the McKenzie method is effective in decreasing chronic low back pain and improving disability in chronic hemodialysis patients.

Funding: Government Support - Non-U.S.

FR-PO1662

Hemodialysis Time of Day and Restless Legs Complaint: USRDS Special Study Data

Nancy G. Kutner,[1] Donald L. Bliwise,[1] Rebecca H. Zhang,[1] Kirsten L. Johansen,[1,2] Lynda A. Szczech.[3] *[1]USRDS Rehabilitation/QoL Special Studies Center, Emory University, Atlanta, GA; [2]Nephrology Section, San Francisco VA Medical Center, San Francisco, CA; [3]Nephrology, Duke University Medical Center, Durham, NC.*

Background: Restless legs is a prevalent complaint among patients on dialysis, but contributors are not well specified. Worsening of symptoms later in the day is a defining characteristic of restless legs syndrome (RLS). Data from the Comprehensive Dialysis Study (CDS) allowed us to explore variation in patients' restless legs complaint by HD treatment time, a factor that has received little study.

Methods: The CDS surveyed incident dialysis patients aged >18 from 296 randomly selected clinics throughout the US. Participants included 1,174 patients whose HD started before 2 pm (early shift) and 270 patients whose HD started 2 pm or later (late shift). RLS was defined by positive response to the three NIH workshop criteria: unpleasant sensations plus urge to move legs, sensations occur mainly at rest and improve with movement, and symptoms worse in evening/night than in morning. Patient characteristics potentially associated with RLS were compared by HD shift using t-test and chi-square analysis, and logistic regression was used to predict RLS.

Results: RLS was reported more often by late shift patients than by early shift patients (35% vs 28%; p = 0.008). Race/ethnicity, gender, educational level, and mean hemoglobin level were similar for early and late shift patients, while early shift patients were more likely to have diabetes and to be older. With adjustment for diabetes and age, the odds ratio for RLS among late shift patients compared to early shift patients was 1.37 (CI 1.05-1.79); p = 0.02.

Conclusions: RLS is time-of-day and activity dependent. For patients at risk for RLS, undergoing HD later in the day may increase distress and could contribute to patients' shortening treatment; it has been shown that RLS has negative implications for patient survival. CDS data suggest the value in clinical practice of early screening for restless legs complaint and avoiding late shift HD in at-risk patients.

Funding: NIDDK Support

FR-PO1663

A Prospective Trial Assessing the Longitudinal Stability of CKD-MBD Parameters in Hemodialysis Patients

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Background: PTH is known to exhibit significant intraindividual variability in hemodialysis (HD) patients. Fibroblast growth factor 23 (FGF23) is an independent risk factor of mortality in dialysis. The variability of FGF23 in serial measurements has not yet been determined.

Methods: In this prospective, monocentric study over 32 weeks 56 chronic HD pts. (63% male, mean age 65±13 yrs, 35% diabetics) were investigated . 49 pts. underwent three to six serial measurements of FGF23 and other routine CKD-MBD parameters. We calculated the intraclass correlation (ICC) from estimates of between-subject variance ($\sigma 2b$) and within-subject variance ($\sigma 2w$) derived from mixed linear models. We applied the following formula: $\sigma 2b / (\sigma 2b + \sigma 2w)$. FGF23 was measured by c-terminal assay.

Results: During the study period the within-subject variability of FGF23 accounted for 15 % of the total variability compared to 30% in PTH, 50% in Ca, 27% in PO4, 30% in CaxPO4 and for 5.5% in bone alkaline phosphatase (BAP).

According to serial serum FGF23 measurements HD pts could be grouped into categories: group 1 (n=13): constantly low values <2000 RU/ml, group 2 (n=14): medium values 2000-10.000 RU/ml, group 3 (n=10): constantly high values >10.000 RU/ml and group 4 (n=12): changing values. These groups were sign. different in classical CKD-MBD parameters. Pts. with high FGF23 (group 3) had sign. higher time averaged values for Ca [2.53±0.12 vs 2.36±0.08 mmol/l], ion.Ca [1.32±0.07 vs 1.25±0.04 mmol/l], PO4 [2.41±0.38 vs 1.65±0.27 mmol/l] and CaxPO4- [6.08±0.85 vs 3.89±0.6 $mmol^2/l^2$] then pts. in group 1 (all $p< 0.01$).

Conclusions: FGF23 and BAP were characterized by lower within-subject variability compared to PTH, Ca and PO4 in HD pts.. About 75% of the pts. showed stable values in different ranges in this cohort. These data may help establishing FGF23 as diagnostic marker in HD pts.

Funding: Pharmaceutical Company Support

FR-PO1664

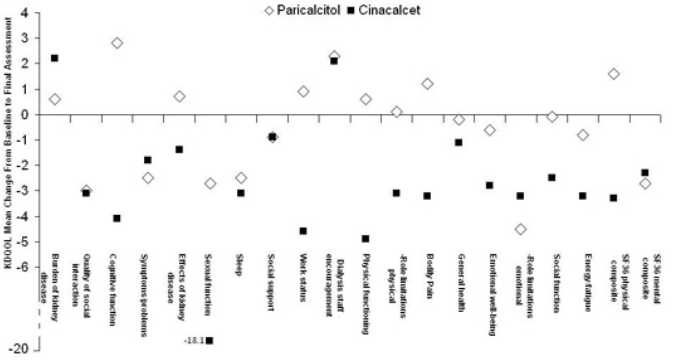
IMPACT-SHPT Study: Comparative Quality of Life Analysis of the Treatment of Secondary Hyperparathyroidism

David J. Goldsmith,[1] Markus Ketteler,[2] Kevin J. Martin,[3] Mario Cozzolino,[4] Myles S. Wolf,[5] Amit Sharma,[6] Michael Amdahl,[7] Samina Khan,[7] Steven E. Marx.[7] *[1]Guy's Hosp; [2]Klinikum Coburg; [3]Saint Louis U; [4]University of Milan; [5]U of Miami; [6]Boise Kidney and Hypertension Inst; [7]Abbott.*

Background: The IMPACT-SHPT is a randomized, open-label, 28-week, multicenter trial to compare paricalcitol and cinacalcet to determine the most effective therapy for the treatment of SHPT in subjects undergoing hemodialysis. The objective of this analysis is to assess patient reported outcomes using the KDQOL-SF in subjects receiving paricalcitol (IV Stratum-US) compared with cinacalcet plus low dose vitamin D.

Methods: 268 Subjects were randomly assigned to IV paricalcitol or cinacalcet treatment groups and received at least one dose for up to 28 weeks. The cinacalcet group received a fixed dose of doxercalciferol. If serum calcium was >10.5 mg/dL on two consecutive levels cinacalcet was administered in the paricalcitol arm. The treatment goal was to achieve a iPTH between150 to 300 pg/mL during weeks 21–28. KDQOL-SF questionnaire was self administered. The baseline and final KDQOL-SF measurement were compared between US treatment arms.

Results: KDQOL Mean Change From Baseline to Final Measurement



Conclusions: Paricalcitol treated patients experienced a 5 point mean difference in change from baseline to final measurement in cognitive function, sexual function, work status, and physical function compared with cinacalcet plus low dose vitamin D. Paricalcitol treated patient's mean difference in change from baseline to final measurement were higher in15 out 20 domains compared with cinacalcet plus low dose vitamin D. Higher scores represent a better health state. Clinicians and healthcare decision makers may consider these findings when evaluating treatment options for secondary hyperparathyroidism.

Funding: Pharmaceutical Company Support

FR-PO1665

Achievement of KDOQI Guidelines for Bone Metabolism and Mortality in Incident Hemodialysis Patients in Relationship to Age

Olynka Vega,[1, 2] Len A. Usvyat,[1] Rakesh Malhotra,[1, 2] Stephan Thijssen,[1, 2] Nathan W. Levin,[1, 2] Peter Kotanko.[1, 2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York.*

Background: The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines for bone and mineral metabolism (BMM) do not take patient's age into account. This study aims to investigate the effect of age on the achievement of BMM NKF-KDOQI goals during the first three months in HD and the impact of this achievement on all-cause mortality at 24 moths.

Methods: The study included all incident patients aged >18 years that received HD at the facilities of the Renal Research Institute from 2001 to 2008. Patients were divided into four age groups at start of dialysis: 1: 18-60, 2: 61-70, 3: 71- 80, and 4: >80 years old. Demographic data, etiology of ESRD, the average levels during the first 3 months on HD of total calcium (Ca), phosphorus (P) and parathyroid hormone (PTH) were collected by chart review. Multivariate Cox regression models were used to calculate HR and 95% CI.

Results: We included 3456 patients, 56.6% male, 51% Diabetics and 48.1% whites.

HR for all cause Mortality. At target groups were used as reference.

	18- 60 years, N= 1612	61-70 years, N= 808	71-80 years, N= 697	>80 years, N= 339
	HR (CI)	HR (CI)	HR (CI)	HR (CI)
Calcium				
< target	1.5 (1.1-2.3)*	1.7 (1.1-2.7)*	2.2 (1.4-3.4)‡	1.1 (0.7-1.8)
>target	0.6 (0.3-1.1)	0.9 (0.4-1.8)	0.4 (0.1-1.1)	0.6 (0.2-1.4)
Phosphorus				
< target	1.7 (0.8-3.4)	2.45 (1.1-5.0)†	1.2 (0.6-2.5)	0.9 (0.5-1.7)
> target	0.6 (0.4-0.9)	0.9 (0.5-1.5)	0.9 (0.5-1.1)	1.2 (0.6-2.5)

*P=0.02, † P=0.01, ‡P= 0.002

All-cause mortality risk increased with hypocalcemia in all age groups bellow 80 years. Hypophosphatemia increased mortality in age group 61-70. PTH levels did not impact survival.

Conclusions: Hypocalcemia increased all cause mortality risk in patients bellow 80 year old. The reasons for this association are unclear, but may be related to arrhythmias. Hypophosphatemia, a likely reflection of poor nutrition, was associated with increased all cause mortality risk in the age group 61-70 years.

Funding: Private Foundation Support

FR-PO1666

Influence of Age on Bone Mineral Metabolism Parameters: Differences and Commonalities between Two Countries Olynka Vega,[1, 2] Gero D. von Gersdorff,[3] Mathias Schaller,[3] Len A. Usvyat,[1] Nathan W. Levin,[1, 2] Peter Kotanko,[1, 2] Claudia Barth.[4] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York; [3]Nephrology, University of Cologne Medical Center, Germany; [4]Kuratorium fur Heimdialyse, Neu-Isenburg, Germany.*

Background: The KDOQI guidelines for bone and mineral metabolism (BMM) do not take patient's age into account. This study aims to investigate the effect of age on the achievement of BMM KDOQI goals using two cohorts of HD patients from the US and Germany.

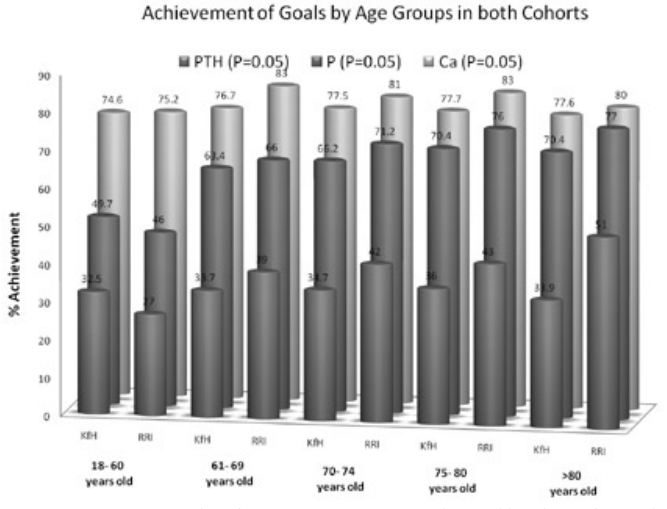
Methods: We included all patients aged >18 years who received in 2009 HD at the facilities of the German Kuratorium für Heimdialyse (KfH), and the Renal Research Institute (RRI), USA. Patients were stratified by age: 18-59; 60-69; 70-75; 75-80, and >80 years. Etiology of ESRD, levels of total calcium (Ca), phosphorus (P), and parathyroid hormone (PTH) were collected from the databases. ANOVA was used.

Results: There were 8638 patients in the KfH-cohort and 2446 in the RRI-Cohort. Diabetes was the most common cause of ESRD in both cohorts (KfH=26% and RRI 33%). HR for all cause Mortality. At target groups were used as reference.

Table 1. Ca, P and PTH levels by age groups. Values are in mean ± SD

	18-60 years		75-80 years		>80 years		
	Kfh	RRI	Kfh	RRI	Kfh	RRI	P
No.	3520	787	1106	283	389	421	
P (mg/dL)	5.5±1.3	5.6±1.2	4.7±1	4.7±0.9	4.7±1	4.5±0.8	<0.001
Ca (mg/dL)	8.9 ± 0.6	8.9±0.7	8.9±0.5	9±0.5	8.9±0.5	8.9±0.5	<0.001
PTH (ng/mL)	278±258	536±563	211±183	356±257	205±191	304±221	<0.001
% Patients with PTH <150 ng/mL	32.5	9	44	12	46	16	<0.001

Ca, P, CaxP product levels, and the % of patients achieving BMM KDOQI goals did not differ between the countries. The % of patients achieving BMM KDOQI goals increased with the age in both cohorts. Noteworthy, PTH <150 ng/mL was >3 times more frequent in the KfH cohort.



Conclusions: Irrespective of country, BMM KDOQI goals are achieved more frequently with increasing age. Older patients may increase susceptibility to low PTH levels

FR-PO1667

Long-Term Treatment of Bixalomer in Chronic Kidney Disease Patients on Hemodialysis with Hyperphosphatemia Tadao Akizawa,[1] Chisato Kameoka.[2] *[1]Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; [2]Astellas Pharma Inc., Tokyo, Japan.*

Background: Bixalomer is a new phosphate-binding polymer in development for the treatment of hyperphosphatemia. In this study, the long-term safety and efficacy of bixalomer in Japanese Chronic Kidney Disease (CKD)-Hemodialysis (HD) patients were assessed.

Methods: This was a multicenter, open-label study in CKD-HD patients with hyperphosphatemia. Main efficacy endpoints were time course of serum phosphorus (Pi) level and achievement rate of the target Pi range in Japanese guideline (3.5 to 6.0mg/dL). For safety assessment, the incidence of adverse drug reactions (ADRs) was tabulated. The starting dose was 1.5g/day and then the dose was titrated up to 7.5g/day depending on the serum Pi levels. Treatment period was up to 48 weeks.

Results: A total of 248 subjects were investigated and 179 subjects completed the study. The mean serum Pi level decreased over time and remained around 5.5 mg/dL until Week 48. The results of the main efficacy endpoints are summarized in the following table.

	Week 0	Week 48	End of Treatment
Serum Pi level (mg/dL)	7.71	5.47	5.77
The achievement rate of the target Pi range (%)	7.0	70.8	62.6

The incidence of AEs and ADRs was 94.4% and 29.4%, respectively. Serious adverse events which were assessed by investigators as related to the study drug were colitis ischaemic, diverticulum intestinal haemorrhagic, oesophageal ulcer, and cholecystitis acute (n=1 each). The most common ADR was constipation (21.0%). The majority of ADRs occurred in the early treatment period up to Week 12 and the incidence did not tend to increase with long-term treatment. Furthermore, bixalomer did not decrease HCO_3 levels.

Conclusions: Bixalomer was able to control serum Pi levels for a long period and would not raise any major safety concerns when administered for a long period. Therefore, bixalomer could be a clinically effective drug that can be administered for a long period to treat hyperphosphatemia.

Funding: Pharmaceutical Company Support

FR-PO1668

IMPACT-SHPT Study: Comparative Economic Analysis of the Treatment of Secondary Hyperparathyroidism Amit Sharma,[1] Markus Ketteler,[2] Kevin J. Martin,[3] Myles S. Wolf,[4] Mario Cozzolino,[5] David J. Goldsmith,[6] Michael Amdahl,[7] Samina Khan,[7] Steven E. Marx.[7] *[1]Boise Kidney and Hypertension Inst; [2]Klinikum Coburg; [3]Saint Louis U; [4]U of Miami; [5]University of Milan; [6]Guy's Hosp; [7]Abbott.*

Background: The IMPACT-SHPT is a randomized, open-label, 28-week, multicenter trial that compared paricalcitol and cinacalcet to determine the most effective therapy for the treatment of SHPT in subjects undergoing hemodialysis. The objective of this analysis is to assess the cumulative dose and cost of paricalcitol and cinacalcet treatment during the study period.

Methods: Subjects were randomly assigned to receive paricalcitol or cinacalcet and fixed IV doxercalciferol or oral alfacalcidol for 28 weeks. Cinacalcet was administered if serum calcium is ≥ 10.5 mg/dL on two consecutive levels in the paricalcitol arm. The treatment goal was to achieve a mean iPTH value between150 to 300 pg/mL during weeks 21–28. Data were collected from 268 subjects who received at least one dose of randomized study drug. Using a US perspective, costs were estimated by utilizing average whole price minus fifteen percent.

Results: The 28 week treatment dose in the paricalcitol arm was: paricalcitol 56,668 mcg, and cinacalcet 20,850 mg, compared to the cinacalcet arm: cinacalcet 895,200 mg, doxercalciferol 4,087 mcg, and alfacalcidol 2,780 mcg. The treatment cost was $184,279 in the paricalcitol arm compared to $490,611 in the cinacalcet arm over 28 weeks.

Conclusions: These results suggest the annual treatment cost of targeting the same iPTH control is 62% ($612,663) lower with paricalcitol compared with cinacalcet plus low-dose vitamin D in this study. There is an estimated savings of about $229,462 per 100 patients per year from a US perspective. Paricalcitol therapy costs are much lower than cinacalcet plus low dose vitamin D for the same target iPTH control. This is a conservative estimate since oral doses were not included if subjects did not return their study drug bottles for the determination of doses taken, as instructed per protocol. Healthcare decision makers should consider these findings when assessing therapies for the treatment for secondary hyperparathyroidism.

Funding: Pharmaceutical Company Support

FR-PO1669

A Phase III, Sevelamer HCl-Controlled Study of Bixalomer in Chronic Kidney Disease Patients on Hemodialysis with Hyperphosphatemia Tadao Akizawa,[1] Hideki Origasa,[2] Chisato Kameoka.[3] *[1]Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; [2]Division of Biostatistics and Clinical Epidemiology, University of Toyama School of Medicine, Japan; [3]Astellas Pharma Inc., Tokyo, Japan.*

Background: Bixalomer is a new phosphate-binding polymer in development for the treatment of hyperphosphatemia in Chronic Kidney Disease(CKD)-Hemodialysis(HD) patients in Japan. In this study, the non-inferiority of bixalomer to Sevelamer HCl(SH) in efficacy was assessed and the safety of bixalomer was also compared with that of SH.

Methods: This was a multicenter, randomized, SH-controlled, open-label study in CKD-HD patients with hyperphosphatemia. Primary endpoint was serum phosphorus(Pi) levels at the end of treatment. The starting dose was 1.5g/day(bixalomer) and 3g/day or 6g/day(SH, based on the serum Pi level before treatment period). Depending on serum Pi levels, the dose of bixalomer or SH was titrated up to 7.5g/day and 9g/day, respectively. Subjects received the study drug for 12 weeks.

Results: Overall 110 subjects were randomized to either bixalomer group or SH group(n=55 each). The adjusted mean serum Pi level at the end of treatment was 5.87mg/dL and 5.55mg/dL, respectively. The upper limit of the 95%CI for the difference between the adjusted mean for bixalomer group and SH group (bixalomer-SH) was below 1.0mg/dL(non-inferiority margin in this study). Thus the non-inferiority of bixalomer to SH was confirmed. The incidence of common gastrointestinal adverse drug reactions(GI ADRs) which were assessed by investigators as related to the study drug is summarized in the following table with the final mean daily dose. No serious or severe GI AEs were reported. In addition, bixalomer did not decrease HCO3 levels, which were measured to assess the metabolic acidosis risk.

	Bixalomer	SH
GI-ADRs	29.1%	47.3%
-Constipation	18.2%	29.1%
-Abdominal distension	1.8%	12.7%
Mean Daily Dose (Week11 to 12)	4.78g/day	5.00g/day

Conclusions: Bixalomer was as effective as SH in decreasing serum Pi level. Bixalomer caused fewer GI ADRs and showed no deterioration of metabolic acidosis. These results indicated the clinical benefits of bixalomer as a treatment for hyperphosphatemia.

Funding: Pharmaceutical Company Support

FR-PO1670

N-DEPTH – Nephrology DVT and Pulmonary Embolism Prophylaxis STudy in Hospitalized Patients Christine M. Ribic, Andrew M. Burke, Sarah Karkhanechi, Catherine M. Clase, Azim S. Gangji. *Medicine, McMaster University, Hamilton, ON, Canada.*

Background: In hospitalized patients, the risk of venous thromboembolism (VTE) has been reported to be higher in patients with chronic kidney disease. Prophylaxis prescription practices, and the risk of VTE and bleeding in hospitalized patients treated with dialysis are unknown.

Methods: This single centre, retrospective chart review was designed to determine the rate of VTE pharmacological prophylaxis in adult patients treated with chronic dialysis (≥ 3 months) on index admission during Sept 2008 to Sept 2009. Exclusion criteria included admission ≤ 24 hours, for suspected VTE, bleed or renal transplant. We defined VTE prophylaxis as administration of prophylactic doses of heparin within 48 hours of admission. We collected data on VTE risk factors as per the ACCP guidelines, VTE events defined objectively with imaging and major bleeding events.

Results: We screened 329 patient admissions: 143 met eligibility criteria. Of these, 23(16.1%) patients were on full dose anticoagulation (coumadin with INR > 2.0, or heparin) and were ineligible for VTE prophylaxis. Of the 120 eligible patients, 31(25.8%) received VTE prophylaxis. The number of baseline VTE risk factors (range 1 to 7) and anticoagulant or antiplatelet use did not predict the use of VTE prophylaxis (p=0.86). There was a trend towards less use of prophylaxis in patients receiving clopidogrel at baseline (p=0.05). VTE events (1 pulmonary embolism; 1 deep vein thrombosis) occurred in 2(2.2%) eligible patients not receiving prophylaxis and no events occurred in those receiving prophylaxis (p=0.55). There was no difference in minor or major bleeds between those that did (n=3) and did not receive prophylaxis (n=6; p=0.66). The majority of bleeding events were gastrointestinal (n=7).

Conclusions: To our knowledge this is the first account of use of prophylaxis in this population. Three-quarters of apparently eligible patients on dialysis admitted to a tertiary care centre under 13 different nephrologists were not prescribed prophylaxis, and baseline VTE risk factors did not predict for whom it was prescribed. Bleeding risk was not increased in patients receiving prophylaxis. This issue deserves wider study.

FR-PO1671

Retrospective Analysis of Etiology and Management of Pneumonia in End Stage Renal Disease Rupam Ruchi,[1] Matthew Whitbeck,[1] Sanjeev Kumar,[2] Milagros Zegarra.[1] *[1]Internal Medicine, Wayne State University; [2]Anesthesiology, Wayne State University, Detroit.*

Background: The American Thoracic Society guidelines for management of pneumonia recommends that pneumonia in ESRD should empirically be treated as health care associated pneumonia(HCAP). We conducted this study to investigate if patients who are initially treated as HCAP do better vs patients who are empirically treated as community acquired pneumonia(CAP).

Methods: It is a retrospective cohort study including patients 18-89 yrs with ESRD on hemodialysis for ≥3 months, admitted with pneumonia. According to initial antibiotic used, patients were divided into group A (treated empirically as HCAP) and group B (treated empirically as CAP). Data was collected regarding demographic profile, severity of pneumonia (as assessed by PSI and CURB-65), co-morbidities, microbiology, length of hospital stay, in-hospital mortality. The chi-square statistics & multiple linear regression were used.

Results: 103 patients were included. Group A (n=51) and group B (n=52) were comparable with respect to all parameters except number of patients with COPD (p=0.03), nursing home residents (p=0.02), PSI (96.1 ± 22.4 in group A vs in 116.5 ± 34.8 in group B, p≤0.01), CURB-65 (45.1% patients in group A had CURB-65 of 2 and above, vs 57.6% in group B, p≤0.01). Length of hospital stay was significantly lower in group A, 6.4 ± 6.9 days as compared to 9.5 ± 6.9 days in group B, p=0.02. This difference persisted even after adjusting for age, race, PSI, hypertension, COPD, and being nursing home residents. No patient died in group A whereas 13.5% patients died in group B, p≤0.01. However, after adjusting for PSI, this difference was not found to be significant (p=0.09). A microbiological diagnosis was established in 31 patients, identifying a total of 44 organisms. In group B, 27.1% patients grew MRSA and 11.5% grew Pseudomonas, vs none of those in group A.

Conclusions: Empiric treatment of pneumonia in ESRD patients on hemodialysis as CAP reduces the length of hospital stay without any significant difference in mortality. We suggest stratification of these patients according to the severity of their pneumonia and treating less severe pneumonia as CAP, rather than HCAP.

FR-PO1672

High Prevalence of Incidental Findings in Chest Computed Tomography in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Events in End-Stage Renal Disease (PACE) Study Bernard G. Jaar,[1] Svetlana V. Chembrovich,[1] Stephen M. Sozio,[1] Julia J. Scialla,[1] Tariq Shafi,[1] Joao A.C. Lima,[1] Wen Hong Linda Kao,[1] Rulan S. Parekh,[1 and 2] Lucy A. Meoni.[1] *[1]Johns Hopkins University; [2]University of Toronto.*

Background: Routine evaluation of research images by radiologists can result in identification of incidental findings. To our knowledge no studies have reported on the prevalence of these findings in research chest computed tomography (CT) of incident hemodialysis (HD) patients (pts) and its association with clinical characteristics.

Methods: We performed a cross-sectional analysis of the first 205 pts who underwent a research cardiac imaging protocol with a 320 slice multidector CT scan (Toshiba Aqulion) with under 5 msv of radiation.

Results: Of the 205 pts, 25% were ≥ 65 years, 57% male, 28% white race, 61% smoker, 33% had body mass index (BMI) of ≥ 30 and 45% had existing cardiovascular disease (CVD). Overall, 44% had at least one and 15% had 2 or more incidental findings with 11% of the pts requiring follow-up to rule out malignancy. Among the 205 pts, lung nodules represented the most frequent findings (25%). The likelihood of any incidental findings was 3 times higher in pts ≥ 65 years, Odds Ratio (OR) [95% Confidence Interval] 2.96 [1.41-6.20] and 1.8 times higher in pts with existing CVD (OR 1.79 [1.01-3.19]); whereas a BMI of ≥ 30 was associated with lower odds (OR 0.49 [0.27-0.89]). Both older age and existing CVD, but not lower BMI, were also associated with higher odds of lung nodule. After adjustment, age ≥ 65 years remain associated with a higher likelihood of any incidental findings and pulmonary nodule; (OR 2.58 [1.20-5.55]) and (OR 3.94 [1.93-8.01]) respectively; while a BMI of ≥ 30 was associated with lower odds of any incidental findings (OR 0.46 [0.25-0.86]). Neither pulmonary nodules nor any incidental findings were associated with smoking, chronic lung disease or history of cancer.

Conclusions: The high occurrence of these incidental findings in incident HD pts requiring further investigations raises important practical, ethical and medico-legal issues that need to be carefully considered in research projects using chest CT scanning.

Funding: NIDDK Support

FR-PO1673

Prevalence of Oral Lesions in Hemodialysis Patients: A Multinational Cohort Study Giovanni F.M. Strippoli. *Diaverum Medical Scientific Office, Lund, Sweden.*

Background: Oral diseases are common in the general population and particularly in underprivileged portions of society. It is plausible that prevalence would be high in people with end stage kidney disease receiving hemodialysis but this has not been formally established. We globally surveyed the prevalence of any oral lesion in people on hemodialysis.

Methods: In this ongoing multinational cross-sectional and prospective cohort study, we enrolled people receiving hemodialysis in 30 outpatient clinics selected randomly from a collaborative network. Prevalence of dental, periodontal, mucosal and salivary lesions was assessed based upon standard dental practice methodology. Analysis was with descriptive statistics.

Results: Of 1744 hemodialysis patients in the participating clinics, 1308 (75%) received a complete oral visit (mean age 66.81 (13.85) years). 323 (27%) were edentulous, 371 (40%) reported attrition and dental erosion (bruxism), 21 (2%) had enamel hypoplasia. DMFT score was 23.94 (8.58). Salivary pH was 7.39 (0.85). Buffer capacity was high in most patients (868, 69% high capacity; 96 patients (8%) low capacity). The salivary flow rate before dialysis was 0.69 (0.64), versus 0.76 (0.74) post dialysis. 469 patients (36%) had mucosal lesions (any), 172 candidiasis (15%), 46 unrecognized neoformations (4%), 17 gingival overgrowth (2%), among others. 538 patients (45%) reported dryness of mouth, 72 (6%) burning and 57 (5%) pain in their mouth. Finally, periodontitis was present in 496 (58%) of 873 dentate patients undergoing periodontal evaluation.

Conclusions: In conclusion, we found oral lesions to be highly prevalent in people receiving hemodialysis. This ongoing study will be completed in 2012 and prospectively analyzed the relationship between exposure to any oral lesions and the risk of major patient level endpoints including mortality and cardiovascular events. Focus on oral health could be an essential component of managing people with end stage kidney disease.

ORAL-D steering committee/participants: Stroumza P, Frantzen L, Leal M, Torok M, Bednarek A, Dulawa J, Gelfman R, Celia E, Craig JC, Johnsohn D, Ruospo M, Palmer S, Hegbrant J, Wollheim C, Strippoli GF.

FR-PO1674

Thirst and Oral Symptoms in Hemodialysis Patients: A Multinational Cohort Study Giovanni F.M. Strippoli. *Diaverum Medical Scientific Office, Lund, Sweden.*

Background: Thirst and xerostomia, the subjective complaint of dry mouth due to a lack of saliva, are common side-effects of various medications, and it is plausible that their prevalence would be high in people with end stage kidney disease receiving hemodialysis as well as dialysis treatment itself being a major determinant. In this cohort study, we globally survey the prevalence of any oral symptoms in hemodialysis.

Methods: In this ongoing multinational cross-sectional and prospective cohort study, we enrolled consenting people receiving hemodialysis in 30 outpatient clinics selected

randomly from a collaborative dialysis network. Xerostomia inventory and dialysis thirst inventory were assessed based upon validated methodology. Analysis was with descriptive statistics.

Results: Of 1733 hemodialysis patients in the participating clinics, 1308 (75%) received a self administered questionnaire on oral symptoms. 557 patients (43%) reported occasional use of candies for dry mouth sensation, 313 (24%) had difficulties to swallow and 635 (49%) needed to sip to swallow, 693 (54%) wake up during the night to drink, 479 (37%) had dry mouth and 642 (50%) had dry lips, for a total xerostomia inventory score of 21.14 (5.47). Thirst as a symptom was problem for 823 patients (64%), 1028 (79%) were thirsty during the day and 667 (51%) during the night, 425 (33%) patients felt that thirst influenced their social life. The final dialysis thirst inventory score is 18.42 (5.61).

Conclusions: In conclusion, we found oral symptoms to be highly prevalent and heavily affecting people receiving hemodialysis. This ongoing study will be completed in 2012 and prospectively evaluate the relationship between these symptoms and major patient level endpoints including mortality and cardiovascular events. ORAL-D steering committee/participants: Stroumza P, Frantzen L, Leal M, Torok M, Bednarek A, Dulawa J, Gelfman R, Celia E, Craig JC, Johnsohn D, Ruospo M, Palmer S, Hegbrant J, Wollheim C, Strippoli GF.

FR-PO1675

Oral Hygiene Habits in Hemodialysis Patients: A Multinational Cohort Study Giovanni F.M. Strippoli. *Diaverum Medical Scientific Office, Lund, Sweden.*

Background: Oral hygiene habits in people with end stage renal disease receiving hemodialysis has never been formally studied. In this study we globally survey oral hygiene habits in a large hemodialysis population.

Methods: ORAL-D is an ongoing multinational cross-sectional and prospective cohort study of oral diseases in people on hemodialysis. We enrolled consenting people receiving hemodialysis in 30 outpatient clinics selected randomly from a collaborative dialysis network. Oral hygiene habits were assessed based upon standard patient questionnaire methodology. Analysis was with descriptive statistics.

Results: Of 1733 hemodialysis patients in the participating clinics, 1308 (75%) responded to a self administered oral hygiene questionnaire. Of these, 723 (56%) did not remember when they had the latest visit, or never had one, 372 (29%) had the first dental visit after they were 30 years old, 259 (19%) never brushed their teeth, 426 (33%) used mouthwash and only 67 (5%) used dental floss, 524 (44%) changed the toothbrush as needed, and only 378 (30%) spent more than 2 minutes for the daily oral hygiene.

Conclusions: In conclusion, according to existing validated instruments for evaluation of oral hygiene, people receiving hemodialysis display very poor oral hygiene patterns. This ongoing study will be completed in 2012 and explore the association of such patterns with major patient level endpoints including mortality and cardiovascular events.

ORAL-D steering committee/participants: Stroumza P, Frantzen L, Leal M, Torok M, Bednarek A, Dulawa J, Gelfman R, Celia E, Craig JC, Johnsohn D, Ruospo M, Palmer S, Hegbrant J, Wollheim C, Strippoli GF.

FR-PO1676

The Use of External Carotene Cream (CC) Can Inhibit Hypertrophic Scars Caused by Needle Injury in Hemodialysis (HD) Patients Satoshi Funakoshi,[1] Jyunichiro Hashiguchi,[1] Rica Etoh,[1] Junko Kubo,[1] Yoshiaki Lee,[1] Takashi Harada,[1] Kazunori Utsunomiya,[3] Mineaki Kitamura,[2] Tomoya Nishino,[2] Shigeru Kohno.[2] *[1]Division of Blood Purification, Nagasaki Renal Center, Nagasaki, Japan; [2]Department of Internal Medicine, Nagasaki University Graduate School of Medicine, Nagasaki, Japan; [3]Department of Diabetes, Jikei University, Tokyo, Japan.*

Background: Hypertrophic scars and keloids both represent fibrotic skin conditions which is difficult to treat with a high recurrence rate. Since upper arms are most susceptible to developing these types of conditions, HD patients are under high risks as they receive needle injuries repeatedly in HD session on the brachial arteriovenous fistula.

Carotene, an antioxidant, is also known to reduce squamous metaplasia in skin tissues. We herein evaluate the effects of CC on prevention of developing keloids and hypertrophic scars on the brachial skin in HD patients.

Methods: Fourteen HD outpatients with moderate – severe scars and keloids on their forearms were enrolled in this study after appropriate IC. After every HD session CC or control creams was put on the needle injuries of their skin, and the skin color and thickness of skin in scar area were monitored using photometer (Spectrophotometer NF333, SOUKEN, Tokyo) and superficial ultrasound study (LOGIQ E9, GE Yokokawa, Tokyo) for 4 weeks (the period of skin turn over).

Results: As shown in the table, there was significant difference between CC and control creams in both the skin color and the thickness of skin after 4 weeks.

Effects of carotene cream (CC) on skin color and thickness in arteriovenous fistula area

		pre	4 weeks	p-value
Skin color	control	55.81±0.99	57.38±2.9	0.23
	CC	56.78±2.06	59.92±4.38	0.044*
Skin thickness (mm)	control	0.69±0.06	0.56±0.05	0.085
	CC	0.79±0.11	0.53±0.05	0.0038*

Adverse effects including skin rash or erosion were not observed.

Conclusions: Our results strongly indicate the potential effects of CC on protecting the formation of keloids and hypertrophic scars, and eventually improving QOL of HD patients.

Funding: Private Foundation Support

FR-PO1677

Prevalence of Nephrogenic Systemic Fibrosis (NSF) in Dialysis Patients: The Pro-FINEST Study Sabine Amet,[1] Vincent Launay-Vacher,[1] Benedicte Stengel,[2] Anne Castot,[3] Camille Frances,[4] Nicolas Grenier,[5] Jean-Yves Gauvrit,[6] Genevieve M. Reinhardt,[7] Olivier Clement,[8] Nicolas Janus,[1] Carmen Kreft-Jais,[3] Gabriel Choukroun,[9] Maurice Laville,[10] Gilbert Deray.[11] *[1]ICAR Department, Nephrology, Pitie-Salpetriere Hospital, Paris, France; [2]INSERM U1018, Paul Brousse Hospital, Villejuif, France; [3]Drug Monitoring, Afssaps, Saint-Denis, France; [4]Dermatology, Tenon Hospital, Paris, France; [5]Radiology, Pellegrin Hospital, Bordeaux, France; [6]Radiology, Pontchaillou Hospital, Rennes, France; [7]Radiology, Haguenau Hospital, Haguenau, France; [8]Radiology, G. Pompidou Hospital, Paris, France; [9]Nephrology, South Hospital, Amiens, France; [10]Nephrology, Edouard Herriot Hospital, Lyon, France; [11]Nephrology, Pitie-Salpetriere Hospital, Paris, France.*

Background: NSF is a cutaneous and systemic disorder characterized by widespread tissue fibrosis. It has been linked with gadolinium-based contrast agents (GBCA), especially in dialysis patients. The Pro-FINEST study is a national prospective study endorsed by the French Drug Agency (Afssaps), and the French Societies of Nephrology, Dermatology, and Radiology. It aims at determining the prevalence of NSF after a Magnetic Resonance Imaging (MRI) examination, +/- GBCA, in dialysis patients.

Methods: The study is based on a 3-section patient form. Section 1: demographics and dialysis; Section 2: MRI examination; Section 3: any dermatological event (DE). Further investigations are planned in case of DE. When a NSF diagnosis is confirmed, an ancillary study is scheduled, with random selection of 4 patients (same gender, dialysis technique, centre, GBCA and without any DE after MRI).

Results: Since 01/2009, 571 patients have been included (247 centres): mean age 63.3 years, 58.5% males, 368 forms received: 58.7% received GBCA, 90.7% Gadoterate. 14 patients reported a DE. Dermatological diagnoses did not report any evidence of NSF.

Conclusions: So far thus, no case of NSF has been reported in 571 dialysis patients among whom the majority received a GBCA. Most patients received a macrocyclic gadolinium chelate for which no unconfounded case of NSF has been observed yet worldwide (Gadoterate).Final results will be available at the time of the congress.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1678

Skin Pathologies and Associated Mortality in a Cohort of End-Stage Renal Disease Undergoing Chronic Hemodialysis Therapy Stefan Walter, Andreas Kribben, Oliver Witzke, Thorsten Feldkamp, Stefan Becker. *Department of Nephrology, University Hospital Essen, Essen, NRW, Germany.*

Background: Pathologies and symptoms of the skin are frequently encountered in end-stage renal disease patients undergoing chronic hemodialysis therapy. Uremic pruritus, pigmentary disorders or further skin-diseases like calciphylaxia are often described in the literature.

Aim of the study was to detect skin pathologies of such patients, to identify risk factors and to test for associated mortality.

Methods: 508 patients undergoing chronic hemodialysis therapy (8 centres in North Rhine-Westphalia, Germany) were included into the study. Patients were interviewed and further data was taken from their medical records. Physical examination included upper and lower extremities and focused on xerosis, edema, papules, pustula, hardening, hyperpigmentation, and tethering of the skin.

To identify risk factors, we performed a multivariate analysis. 6 month after the visit the mortality-status was checked.

Results: 86.8 % (441/508) of the patients presented at least one of the respected skin pathologies. Frequently were found: xeroderma (71.9%, 365/508), itching (39.0%, 198/508) and edematous (17.5%, 89/508) skin, as well as hyperpigmentation (14.8%, 73/508), induration (1.6%, 8/508) and tethering of the skin (0.4%, 2/508).

Risk factors for hyperpigmentation, induration or tethering of the skin were simultaneous presence of coronary heart disease ($p<0.005$), warfarin therapy ($p<0.001$), peripheral arterial disease ($p<0.002$) or a previous thrombosis ($p<0.003$).

Patients with this skin lesions had a significantly higher mortality ($p<0.002$, OR=2.6) than patients without these lesions.

Conclusions: Skin lesions are a frequent finding in end-stage renal disease patients undergoing chronic hemodialysis therapy. One may assume that certain findings are associated with certain comorbidities and hence increased mortality.

FR-PO1679

Efficacy of Glycerol-Paraffin in Uremic Xerosis: A Randomized, Double-Blind, Comparative Study Jacek Szepietowski,[1] Elias V. Balaskas,[2] Didier Bessis,[3] Dimitrios Ioannides,[2] Claudio Ponticelli,[4] Corinne Ghienne,[5] Patrick Dupuy.[5] *[1]Department of Dermatology, Venereology and Allergology, University of Medicine, Wroclaw, Poland; [2]Department of Nephrology, Ahepa University Hospital, Thessaloniki, Greece; [3]Department of Dermatology, Hospital Saint Eloi, Montpellier, France; [4]Department of Nephrology, Scientific Institute Humanitas, Rozzano, Italy; [5]Division of Research and Development, Orfagen, Toulouse, France.*

Background: Uremic xerosis is a bothersome condition that is poorly responsive to moisturizing and emollient therapy. It is associated with cutaneous deficiency in glycerol and exacerbated pruritus (uremic pruritus).

Methods: A randomized, double-blind, intra-individual (left vs right comparison), multicentric clinical study was performed on 100 patients with moderate to severe uremic xerosis for 7 days, during which the patients applied twice daily an emulsion combining glycerol and paraffin (test product) on one allocated lower leg, and the emulsion alone (comparator) on the other lower leg. This was followed by an open-labeled use of the test product on all the xerotic areas for 49 days. The main efficacy parameter was treatment response on each lower leg, as defined by a reduction from baseline of at least 2 grades in a pre-defined 5-point clinical score, on day 7.

Results: Among the ninety-nine (99) patients analyzed, the test product was highly effective with a treatment response in 72 patients (73%), whereas 44 patients (44%) responded to the comparator ($p<0.0001$, inter-group analysis). This was associated with an objective reduction in the density and thickness of the scales using D-squames on day 7 ($p<0.0001$ compared to the comparator), and a substantial improvement of the uremic pruritus (75%) and quality of life at study end ($p<0.001$, intra-group analysis). The test product was very well tolerated, product-related local intolerance (exacerbated pruritus, local burning, erythema) occurring in only 5 patients (5%).

Conclusions: In conclusion, uremic xerosis and pruritus can be managed successfully when an appropriate skin protectant is used.

Funding: Pharmaceutical Company Support

FR-PO1680

Quality of Life in Patients with Uremic Xerosis Patrick Dupuy,[1] Jacek Szepietowski,[2] Elias V. Balaskas,[3] K.M. Taube.[4] *[1]Division of Research and Development, Orfagen, Toulouse, France; [2]Department of Dermatology, Venereology and Allergology, University of Medicine, Wroclaw, Poland; [3]Department of Nephrology, Ahepa University Hospital, Thessaloniki, Greece; [4]Department of Dermatology, Martin Luther University, Halle, Germany.*

Background: Although uremic xerosis (rough and scaly skin affecting patients with end-stage renal disease, ESRD) is a neglected disease, it is a prominent feature that aggravates uremic pruritus and may have some psychological and social consequences.

Methods: Three hundred and thirty four (334) ESRD patients with moderate-to-severe uremic xerosis were surveyed for quality of life (QoL) assessment, using the generic Short-Form (SF-12) scale and the Dermatology Life Quality Index (DLQI). In parallel, the intensity of xerosis on 4 sites (the two lower legs, chest, forearm without arterio-venous shunt) was assessed, using a 5-point lesional intensity score. Pruritus was auto-assessed by the patients, using a 100-mm visual analogue scale (VAS).

Results: Uremic xerosis patients had a marked deterioration of the Physical Component Summary (PCS) of SF-12 (mean ± SD: 34.92 ± 9.98) and DLQI (5.06 ± 4.73). Younger age ($r = - 0.20$), xerosis intensity ($r = 0.14$), and the presence of pruritus ($p < 0.0001$) and its intensity ($r = 0.50$) were shown to be significant worsening factors of DLQI. Because a weak but significant correlation between the intensity of xerosis and pruritus was also demonstrated ($r = 0.18$, $p = 0.001$), the direct contribution of age, xerosis and pruritus on DLQI was analysed in a multiple linear regression model. Age and pruritus intensity, but not xerosis intensity, were found to be independent contributors to DLQI deterioration ($p < 0.0005$). On the other hand, uremic xerosis without associated pruritus still resulted in DLQI alteration (3.24 ± 3.99).

Conclusions: It was concluded that young age and intensity of uremic pruritus compromise QoL in uremic xerosis patients. Some characteristics of uremic xerosis other than xerosis intensity may also be involved in QoL alteration.

Funding: Pharmaceutical Company Support

FR-PO1681

Sexual Dysfunction in Women Requiring Hemodialysis: A Multinational Cross-Sectional Study Suetonia Palmer,[1] Giovanni F.M. Strippoli.[2,3] *[1]University of Otago, Christchurch, New Zealand; [2]Diaverum Medical Scientific Office, Lund, Sweden; [3]Mario Negri Sud Consortium, S Maria Imbaro, CH, Italy.*

Background: Existing data for the prevalence and correlates of sexual dysfunction, including depression, in women on hemodialysis are limited by suboptimal study design.

Methods: Using a multinational cross-sectional study, we studied women receiving hemodialysis between January and June 2008 within a collaborative network in Europe and South America. Sexual dysfunction (SD) was identified using the 19-item Female Sexual Function index questionnaire based on self-reported sexual experiences in the four weeks before questionnaire. Depression was measured using the Center for Epidemiological Studies-Depression (CES-D) questionnaire. Correlates of self-reported SD were identified by multiple regression analyses. We used recursive partitioning and amalgamation analysis to group clinical characteristics associated with SD.

Results: 659 of the 1472 eligible women (45%) completed the questionnaires. Over half (386 [56%]) lived with a partner and 232 (35%) were sexually active. Overall, 555 (84%) of respondents reported SD. Women with a partner were less likely to experience SD (78% versus 92% without a partner). In multivariate analysis, age was a strong correlate of SD; for each 1 year increase in age, sexual dysfunction increased by 8% (adjusted odds ratio [AOR] 1.08 [95% CI, 1.06-1.11]). SD was also independently associated by depressive symptoms, lower educational attainment, menopause, diabetes, and diuretic therapy. Nearly all women who were not wait-listed for a kidney transplant and not living with a partner (249/260 [96%]) reported SD. Over half (55%) of sexually active women reported SD which was associated with age, depressive symptoms, menopause, low serum albumin, and diuretic therapy. Analyses were limited by the response rate and residual confounding.

Conclusions: This descriptive study suggests most women on hemodialysis may experience sexual problems. Additional research on the relevance of SD to the quality of life, well-being, and outcomes in these women is now required.

Steering Commitee/ Participants: Mariacristina Vecchio, Jonathan C Craig, Giorgia De Berardis

FR-PO1682

Effect of Hypnosis on Anxiety, Depression, Fatigue and Sleepiness in People Undergoing Hemodialysis Philippe Chauveau,[1] Aurelie Untas,[2] Catherine Dupre,[4] Anne Kolko-Labadens,[3] Nicolas Cazenave.[4] *[1]Aurad-Aquitaine, Bordeaux, France; [2]Universite Paris Descartes, Paris, France; [3]Hopital Foch, Paris, France; [4]CHU de Toulouse, Toulouse, France.*

Background: Hypnosis has shown positive effects in stress related disorders (e.g., cancer, surgery, burns), but has not been studied in chronic renal disease. We investigated the effects of a hypnosis session on anxiety, depression, fatigue and sleepiness in patients undergoing hemodialysis.

Methods: The sample consisted in 29 patients (mean age 62.6 years, SD=16.8, 52% of men). Patients assigned to take part to a hypnosis session during a hemodialysis session. Anxiety, depression, fatigue and sleepiness were measured weekly with validated scales (Hospital Anxiety and Depression Scale, Multidimensional Fatigue Inventory, Epworth Sleepiness Scale). Fatigue was also measured daily using a numeric scale. Study participation lasted fifteen days. Hypnosis session took place on the eighth day.

Results: Depression was significantly associated to anxiety, fatigue and sleepiness. Fatigue was strongly correlated to sleepiness. Anxiety, depression and sleepiness significantly decreased after hypnosis, whereas fatigue remained constant.

Conclusions: This preliminary study shows encouraging results which suggest that hypnosis is an effective intervention to help hemodialysis patients face treatment consequences, such as feelings of negative emotions. Future studies should investigate the effect of a longer intervention getting people to do self-hypnosis.

FR-PO1683

Subjective Sleep Quality in Kidney Disease Patients Sameer Shakir, Gabrielle R. Paoletti, Elizabeth K. Lee, Dahlia Raymond, Brett A. Tomlin, Sarah Ramer, Mark L. Unruh. *University of Pittsburgh School of Medicine, PA.*

Background: Sleep affects one's quality of life. Patients' subjective ratings of their sleep, however, do not correlate well with objective measures. This study examined predictors of subjective sleep quality in patients with CKD and ESRD compared to controls.

Methods: The cross-sectional sample consisted of 75 CKD and 77 ESRD patients as well as 224 controls from the Sleep-Strategies Concentrating on Risk Evaluations study. The outcome measure, the Pittsburgh Sleep Quality Index (PSQI), is a 19-item scale with a total score of 0-21. Lower scores indicate higher sleep quality. Patients underwent home polysomnography (PSG), filled out the Perceived Stress Scale-4 (PSS-4), a measure of life stress, and the Life Orientation Test-Revised (LOT-R), a scale of optimism. Other variables included demographics; anthropometrics; hypertension, diabetes, depression status; and medications.

Results: Mean age was 56.8±11.5 years. Males made up 56.3% and non-African-Americans 62.5% of the sample. The mean PSQI score for CKD patients was 6.5±3.7; for ESRD patients, 8.5±4.6; and for controls, 6.4±3.4 ($p=0.002$). CKD patients, ESRD patients, and controls differed significantly in age, sex, race, PSS-4 score, sleep apnea status, sleep efficiency, sleep latency, and total sleep time. In a multivariate linear regression controlling for age and race, female sex (1.40, 95% CI 0.62, 2.17), ESRD (vs. control) (1.81, 95% CI 0.82, 2.81), and sleep efficiency (-0.03, 95% CI -0.06, -0.005) significantly predicted higher PSQI score. In another multivariate linear regression controlling for the above covariates and adding PSS-4 and LOT-R scores, PSS-4 score became a significant predictor (0.26, 95% CI 0.12, 0.41) in addition to female sex and ESRD (vs. control). Sleep efficiency became non-significant.

Conclusions: ESRD patients experience significantly worse subjective sleep quality, as measured by the PSQI, than controls, but female sex and higher stress level are stronger predictors of worse sleep quality than any objective measure obtained from PSG. A full assessment of sleep quality must therefore include subjective and objective ratings.

Funding: NIDDK Support

FR-PO1684

The Prevalence of Sleep Disturbances (SD) in Pediatric Chronic Kidney Disease (CKD): A Report of the Midwest Pediatric Nephrology Consortium

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Background: Although SD are common in adults with CKD, little is known about the prevalence of SD in pediatric CKD.

Methods: Between May 1, 2006 and July 1, 2008, a clinic-based survey of sleep habits and common symptoms (sx) of SD was conducted in 159 school-aged CKD patients (pts) and/or parent-proxy. Three pt study groups of CKD were assessed: 1) not on dialysis, not transplanted (n=68); 2) dialysis (n=30); 3) functioning transplant (n=61). Four sx domains for SD were assessed: 1) Excessive daytime sleepiness (EDS) using pediatric modification of the Epworth Sleepiness Scale; 2) Sleep disordered breathing (SDB) sx using Pediatric Sleep Questionnaire; 3) Restless Legs Syndrome sx using a standard questionnaire; and 4) Insufficient Sleep duration based on normative data for age, gender. Pts and parent-proxy completed the Pediatric Quality of Life Inventory Version 4.0 Generic Core Scales questionnaire (PedsQL) to assess health-related quality of life (HRQOL).

Results: The overall characteristics of these pts were: mean (SD) age=13.8 (3.6) years, 50.9% male, 30.2% non-Caucasian, 50.9% congenital CKD; no significant difference between CKD study groups were noted ($p > 0.05$). Overall, 93 pts (58.5%) had sx of a SD in at least one sleep domain, the most common being EDS (n=73) and SDB (n=37). Multivariate-adjusted logistic regression of associations (age, gender, CKD study group, CKD diagnosis, BMI z-score, PedsQL Total Score, and steroid use) with SD revealed that lower PedsQL Total Scores were associated with a higher likelihood of any SD ($p < 0.001$), SDB ($p< 0.001$), or EDS ($p = 0.003$). The association of a SD and a decrease in HRQOL scores was independent of the CKD pt study group.

Conclusions: SD are common throughout the spectrum of pediatric CKD and are associated with diminished HRQOL scores independent of the CKD pt study group.

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FR-PO1685

Sudden Discontinuance of On-Line HDF Clearly Proved the Clinical Advantage of Itself in Relieving Dialysis Related Symptoms

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Background: On 11th march 2011, a terrible disaster smashed the north east part of Japan. Our facility is just next to the smashed area and we experienced 3 days of electric power failure. We had been treated many dialysis patients by on-line HDF; however, just after the disaster we were not able to continue on-line HDF for 18 days because we could not validate the quality of dialysis fluid as safe as enough for on-line HDF. In the current study we reviewed the changes in dialysis related symptoms through the discontinuance of on-line HDF.

Methods: In our facility 94 out of 159 chronic dialysis patients (59%) had been treated by on-line HDF for relieving their itchiness, restless leg syndrome and insomnia and so on. On-line HDF was stopped on 11th March 2011 and restarted on 28th March. The changes in their subjective symptoms were retrospectively monitored 2 or 3 weeks after the disaster and followed up 1 and 2 months later. Ninety one out of 94 patients admitted to be monitored about their symptoms.

Results: Forty-three out of 91 patients (47%) recognized recurrence of their symptoms or new onset dialysis related symptoms. The recurrence of the same symptom for that on-line HDF was introduced was observed in 37% of the patients. The frequent symptoms were itchiness (29%), irritable state (15%), fatigue (15%) and insomnia (7%), and its degree was different in each patient. These symptoms gradually disappeared as at 51% by 2 weeks later the restart of on-line HDF, at 68% by 1 month, and at 99% by 2months.

Conclusions: We have proposed that on-line pre-dilution HDF is effective to relieve the dialysis patients of their dialysis related symptoms such as itchiness, irritability, insomnia and skin pigmentation. (NDT plus 3 (suppl)28-35, 2010) However, this proposal is not based on any prospective controlled studies, and actually it is hard to build randomized control study to prove the benefit of on-line HDF. Our patients don't accept for the enrollment to this kind of study because they don't want to be treated by any other than on-line HDF. The disaster gave us the opportunity to reconsider what is a good dialysis modality and a natural evidence for the clinical advantage of on-line HDF.

FR-PO1686

Trends in the Occurrence and Outcomes of Acute Non-Variceal Upper Gastrointestinal Bleeding in U.S. Patients with End-Stage Renal Disease (1998-2007)

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Background: Renal failure is a risk factor for acute nonvariceal upper gastrointestinal bleeding (ANVUGIB) and associated with poor outcomes. We examed the burden of ANVUGIB and its outcomes on dialysis patients.

Methods: Using the United States Renal Data System, we quantified the occurrence rate and 30-day mortality of ANVUGIB (2 criteria) in dialysis patients. We used Poisson and logistic regression to estimate occurrence rates of ANVUGIB and 30-day mortality from 1998-2007, respectively.

Results: 958533 patients contributed 2403545 patient-years for study. The occurrence rates for ANVUGIB were 56 and 318 episodes per 1000 person-years for stringent and lenient criterion respectively. Crude occurrence rates remained flat (stringent) or increased (lenient) over the decade; only adjustment for socio-demographics and comorbidities resulted in significant declining trends. Patients had higher hematocrits prior to and were more likely to receive blood transfusions during their ANVUGIB episodes in later years. Overall 30-day mortality was 11.7% and declined over time.

Annual changes in the occurrence of and 30-day mortality rate after ANVUGIB

		change of an increment of one year (%)	95% Confidence interval (%)	
Stringent algorithm				
Occurrence rate ratio	Unadjusted	-0.2	-0.4	0.04
	Adjusted	-2.7	-2.9	-2.5
Mortality odds ratio	Uadjusted	-2.3	-2.7	-1.8
	Adjusted	-3.3	-3.8	-2.8
Lenient algorithm				
Occurrence rate ratio	Unadjusted	3.2	3.1	3.3
	Adjusted	-1.5	-1.6	-1.4
Mortality odds ratio	Unadjusted	-2.8	-3.0	-2.5
	Adjusted	-3.6	-3.8	-3.4

Conclusions: Despite declining trends worldwide, crude rates of ANVUGIB among dialysis patients did not decrease over the decade. Though 30-day mortality from ANVUGIB declined, ANVUGIB remains a significant burden in dialysis patients.

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FR-PO1687

Exercise Interventions in Chronic Kidney Disease: A Systemic Review and Meta-Analysis

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Background: Exercise interventions improve physical performance and cardiovascular risk factors in the general population. Their beneficial effects are unclear in various stages of chronic kidney disease (CKD). We systematically reviewed the effects of exercise interventions in CKD.

Methods: We searched MEDLINE (1966- September 2010) and SCOPUS (September 2010) for relevant randomized trials comparing exercise interventions (aerobic, resistance or combination of aerobic and resistance regimen) in non-dialysis dependent CKD, dialysis and renal transplant recipients. Two reviewers independently extracted data on relevant outcomes from included studies. Results were summarized as mean difference (MD) with 95% confidence intervals (CI) using a random effects model.

Results: Thirty-three trials (3 non-dialysis dependent CKD, 25 dialysis and 5 renal transplant studies) were included; most were small and of short duration. When compared with control group, exercise interventions significantly improved peak oxygen consumption (11 trials, 467 patients; MD 4.79 ml/kg/min; 95% CI 3.31 to 6.26) and lowered systolic blood pressure (MD -4.76 mmHg; 95% CI -8.89 to -0.62), and diastolic blood pressure (MD -3.43 mmHg; 95% CI -5.76 to -1.10) at the end of treatment period among dialysis patients. There were no significant differences in the 6 minute walk time between exercise and control groups in the dialysis population. No significant heterogeneity was noted in these analyses. Few studies reported better health related quality of life with exercise using different scales. Studies that included renal transplant recipients and non-dialysis dependent CKD patients did not report outcomes consistently and was not pooled.

Conclusions: Short-term exercise interventions improve physical performance and blood pressure and with possible beneficial effects on health-related quality of life in dialysis patients. Data among non-dialysis dependent CKD and renal transplant recipients are sparse. Larger, long-term and better quality studies exploring various exercise interventions are warranted.

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FR-PO1688

Open Dissection Versus Laparoscopic Peritoneal Dialysis Catheter insertion: A Randomized Prospective Comparison on Outcome and Economical Evaluation

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Background: Peritoneal dialysis (PD) catheter malfunction owing to displacement is another important complication lead to technique failure besides peritonitis. Previous study indicated the success of PD depended more on placement technique than on catheter design. Currently available methods for catheter placement are principally including open dissection and laparoscopic insertion. The best method is still controversial while the laparoscopy is much expensive. In this study, we compared the outcome and economical expense between the two methods.

Methods: We conducted a prospective randomized study in which patients underwent PD catheter placement by either the open dissection or the laparoscopic technique . Open dissection was performed under local anesthesia and laparoscopic insertion was performed by one general surgeon under general anesthesia. Tenckhoff direct PD catheters were placed

in all patients and continuous ambulatory peritoneal dialysis was started at the third day of the procedure.

Results: Seventy-four patients were enrolled from January 2011 to April 2011. The mean hospital stay time was 13.2 days and 8.66 days in open dissection group and laparoscopic group respectively ($P < 0.05$). The mean operative cost was CNY 400.00 and CNY 5000.00 ($P < 0.001$) and the mean hospital expense was CNY 15356.25 and CNY 15768.70 in open dissection group and laparoscopic group respectively ($P > 0.05$). Fluid leakage was observed in 1 patient in open dissection group, but in no patients in laparoscopic group ($P > 0.05$). Bleeding occurred in 2 patients in open dissection group and in 1 patient in laparoscopic group ($P > 0.05$). Catheter displacement occurred in 5 patients in open dissection group and in 3 patients in laparoscopic group ($P > 0.05$). Neither group had exit site infection, peritonitis or death during the follow-up.

Conclusions: Compared to open dissection, although the operative cost is higher, laparoscopic placement does not increase the total hospital expense maybe due to the shortened hospital stay. Current study did not find which method could lower the perioperative complications and catheter displacement.

FR-PO1689

Sarpogrelate Hydrochloride Improves Skin Perfusion Pressure of Lower-Extremity in the Hemodialysis Patients with Peripheral Arterial Disease

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Background: Peripheral arterial disease (PAD) is more prevalent in patients on hemodialysis (HD), however, little is known about the effectiveness of drugs in HD patients. This study aimed to clarify the effects of sarpogrelate hydrochloride, a selective 5-HT2A antagonist,on HD patients with PAD comparing with cilostazol.

Methods: We conducted prospective, randomized, open-label, multicenter trial for 6 months on HD patients with PAD. PAD was diagnosed by the clinical symptoms (Fontaine classification: I or II) and positive sign of the following test; 1) ultrasonography, multidetector-row CT or angiography for arteries of lower legs, 2) ankle-brachial pressure index of either foot below 1.0 or skin perfusion pressure (SPP) below 50mmHg. All eligible patients (n=35) were divided into age-, and HD duration-matched two groups, with medication given sarpogrelate of 300mg/day (n=17) or cilostazol of 200mg/day (n=18) followed by 6 months. We measured SPP before and 6 months after with serum levels of hsCRP, MDA-LDL, plasma levels of fibrinogen and pentosidine.

Results: There was no significant difference in the patient's characteristics. At 6 months, SPP was increased in both groups (sarpogrelate: 43±17 to 55±15 mmHg, cilostazol: 49±21 to 66±29 mmHg, $p<0.05$) and there was no difference in both groups before and after 6 months. The levels of hsCRP and fibrinogen showed no differences between both groups before and after 6 months. Plasma pentosidine levels were decreased in both groups (0.65±0.24 to 0.48±0.12 μg/ml, 0.58±0.22 to 0.47±0.17 μg/ml, respectively: $p<0.05$) and there was no difference in both groups. Serum MDA-LDL level was not increased in sarpogrelate group, while significantly increased from 60.5±25.5 to 87.0±56.5 U/l in cilostazol group ($p<0.05$) . Heart rate was significantly increased in cilostazol group from 77±13 to 83±16 bpm ($p<0.05$), but not in sarpogrelate group.

Conclusions: Sarpogrelate clearly improved SPP without increases in heart rate and serum MDA-LDL level in HD patients with PAD.

FR-PO1690

Predictors of Intradialytic Blood Pressure Drops in Hospitalized Hemodialysis Patients

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Background: Estimation of the hydration status of hospitalized and multimorbid hemodialysis (HD) patients by clinical examination only is often imprecise and can result in severe drops in blood pressure (BP). We analyzed whether a combination of bioimpedance spectroscopy (BIS), blood gas analysis (BGA), electrocardiogram (ECG) and clinical evaluation could predict BP instability during the course of HD treatments.

Methods: Prospective, non-interventional study in 72 hospitalized HD patients (38% female, age 65±15 yrs, BMI 25.5 ± 5.2; 38% diabetics), examined by BIS (hand-to-foot), ECG, BGA, BP and clinical evaluation every 30 min during a 4 hrs HD; 3 groups were separated: A: n=30, stable BP, no hypotensive symptoms (symptoms defined as dizziness, vomiting, nausea, numbness or an intervention like: Trendelenburg maneuver, stop ultrafiltration, fluid infusion); B: n= 25, drop in BP > 25 mmHg, no symptoms; C: n=17, drop in BP > 25mmHg plus symptoms.

Results: Based on the clinical evaluation 40% of group C patients were considered to be hypervolemic and 50% to be euvolemic prior to the dialysis session. Clinical evaluation did not correlate with BIS measurements. The three groups differed significantly in the measured parameters:

groups	A	B	C
Extracellular resistance (Re[Ω])	534±79 [1]	535±101 [2]	625±95
Extracellular water (ECW [ml])	19.4±2.6 [1]	19.5±2.8 [2]	6.5±3.3
Intracellular water (ICW [ml])	25.4± 4.5 [1]	26.6±4.8 [2]	21.7±6.7
Potassium [mmol/l]	3.93±0.04 [1]	4.07±0.05	4.16± 0.06
Hematocrit [%]	31±0.5 [1]	33±0.5	34±0.7
pH	7.43±0.05 [1]	7.42±0.05	7.40±0.07

[1] $p<0.05$ between group A and C; [2] $p<0.05$ between group B and C

A measured ECW <18ml early in the course of the HD session identified patients with subsequent symptomatic BP drops (and so belonging to group C) with a sensitivity of 67% and a specificity of 66%.

Conclusions: HD patients who develop symptomatic intradialytic BP drops reveal different BIS and BGA profiles prior to the BP drops. BIS and BGA monitoring might help preventing BP drops in hospitalized HD patients.

FR-PO1691

Comparison of the Efficacy of Senna Glycoside Versus Lactulose in the Treatment of Chronic Constipation in Maintenance Hemodialysis Patients

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Background: Chronic constipation is one of the frequent gastrointestinal symptoms in patients with maintenance hemodialysis (MHD). There are several inevitable factors contributed to the symptom and often the patients are more likely to use laxatives. We aimed to compare the effecacy of senna glycoside and lactulose in treating chronic constipation in MHD patients and to demonstrate the colonic transit time among those.

Methods: The randomized, double blind, cross-over study in MHD patients at Phramongkutklao Hospital was conducted during July to December 2010. The MHD patients with chronic constipation matched to ROME III criteria were eligible and randomized to 2 arms; the senna glycoside (S) and the lactulose (L). The colonic transit time was performed by using radio-opaque marker before and after the study periods. The stool daily charts defined by ROME III criteria were evaluated as the tool during the study.

Results: Twenty-nine of patients per protocol were studied. Sixteen were male (55%) with average age 58.17±12.26 years. Median colonic transit time was 38.4(16.8-52.8) hours. Only 3 patients were reported having prolonged colonic transit time over 72 hours. There was no difference between the cathartic effect of senna glycoside and lactulose (58.6% vs. 75.9%, p=0.403). The S-group had higher abdominal cramp than the L-group (65.5% vs. 13.8%, $p<0.001$), while the L- group had higher abdominal bloating than the S- group (93.1% vs. 6.9%, $p<0.001$). The average doses related with adverse effect was 4.00±1.76 tablets for senna glycoside and 22.8±12 mL for lactulose. No serious drug adverse events were reported.

Conclusions: The present study shows that both laxative agents are effective in treatment of constipation in MHD patient. However, each of them may have different gastrointestinal adverse effects.

FR-PO1692

Dialyzability and Pharmacokinetics of Oral Levofloxacin in Infected Hemodialysis Patients

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Background: Pharmacokinetic parameters of the drugs are altered by infection, because increase of systemic cytokines during the infection changes metabolism and excretion of the drug in the body. This study was performed to evaluate the dialyzability and pharmacokinetics of oral levofloxacin simultaneously in infected hemodialysis patients, which has not been reported previously.

Methods: Seven infected maintenance hemodialysis patients due to pyelonephritis lacking residual renal function were enrolled. Levofloxacin (500 mg after hemodialysis session for the first day and 250 mg for the 4 hours before scheduled hemodialysis session on the 3rd day) was orally administered. On the 3rd day, blood was taken from arterial and venous sides before and 2 and 4 hours after session initiation. Another sampling was performed on the 5th day of the study. Drug concentration, hematocrit, and urea nitrogen concentration were measured. Dialyzability and pharmacokinetic parameters of levofloxacin were evaluated by arterio-venous difference method.

Results: All patients exhibited improved symptoms without major problems. Drug concentrations in all arterial samples were above MIC of targeted bacteria. Dialyzer clearance and elimination fraction were 57.8±3.9 ml/min/m2 and 64.1±1.6%, respectively. Apparent half-lives during and after dialysis session were 4.6±0.5 and 27.1±3.8 hours, respectively. Dialyzer clearance was positively correlated with urea reduction ratio and negatively correlated with serum albumin concentration. About 1/3 of the drug was removed by dialysis when administered 4 hours before the session.

Conclusions: Oral dosing of this drug at 500 mg on the 1st day, followed by 250 mg on the 3rd day, in infected maintenance hemodialysis patients provides safe drug concentration compatible with that of healthy subjects orally receiving 500 mg daily. Because a significant amount of the drug was removed, administration might be undertaken after the dialysis session.

Funding: Government Support - Non-U.S.

FR-PO1693

An Efficacy Study of Combined Hepatitis A and B Vaccine(TWINRIX®) in Non-Responders to Double Dose Hepatitis B Vaccination in Dialysis Patients

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Background: Dialysis is an established route of transmission for Hepatitis B virus(HBV).The Centers for Disease Control and Prevention recommends routine immunization in dialysis patients against HBV.An average of 64% of dialysis patients develops seroprotective antibodies with immunization when compared to 90-95% of healthy, immunocompetent adults.Recent reports indicate that combined hepatitis B and hepatitis A vaccine(Twinrix®,GSK,UK) may improve immunogenicity in healthy non-responders to hepatitis B vaccine.The purpose of our study is to determine whether Twinrix® results in increased hepatitis B surface antibody titers(anti-HBs) in dialysis patients who are non-responders to a primary course of double dose monovalent hepatitis-B vaccine.

Methods: This pilot study was designed as a randomized controlled trial.Twenty three subjects (15 male) who were non-responders to an initial double dose monovalent hepatitis-B vaccine were recruited.Twelve patients were randomized to receive a double dose of Twinrix®at 0,1 and 6 months(treatment arm) and 11 patients received repeat double dose monovalent hepatitis B vaccine at 0,1 and 6 months(control arm).The anti-HBs titers were determined before vaccination and 4 weeks after each dose.

Results: Of the 18 patients who completed the study,7 of 9 (77.8%) in treatment arm and 5 of 9 (55.5%) in control arm had antibody titers >10 mIU/ml (Relative Risk[RR]=1.4 (95%CI 0.71-2.77).There was no difference in adverse events in either group.

Conclusions: Vaccination of non-responders to monovalent hepatitis B vaccine with combined hepatitis B and hepatitis A vaccine produced improved hepatitis B seroconversion rates in dialysis patients compared to monovalent hepatitis B vaccine.This is the first evaluation in a US population and merits larger studies to better evaluate this potential benefit.

FR-PO1694

Proteinase-Activated Receptor-2, a Novel Mechanism of Uremic Pruritus: A Pilot Study

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Background: Pruritus is a common problem in ESRD patients. The underlying mechanisms are not yet fully understood. We examined the relationship of the proteolytic activity, expression of PAR2, and pruritus in the skin of ESRD patients.

Methods: The Skin of ESRD patients with pruritus (n=12) and without pruritus (n=4) were compared about activity of serine protease and expression of PAR2. The skin biopsy was conducted on the abdomen. In addition, the effect of soybean extracts treatment for 4 weeks, containing protease inhibitor was examined in ESRD patients with pruritus (n=6). The status of pruritus was examined by pruritus score and VAS scale.

Results: Proteolytic activity and PAR2 expression was increased in ESRD patients with pruritus compared with those without pruritus. Soybean extracts treatment decreased the activity of proteolytic activity and expression of PAR2, and the severity of pruritus.

Conclusions: We suggested that the soybean extracts containing protease inhibitor is a new candidate for the treatment of uremic pruritus and the new pathogenesis based on the relationship of PAR2 and uremic pruritus.

FR-PO1695

Clinicopathological Study on 11 Patients with End-Stage Renal Disease Showing Intestinal Perforation Due to Cation-Exchange Resin Against Hyperkalemia

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Background: The administration of cation-exchange resin (CER) of potassium absorbing agent such as sodium polystyrene sulfonate (Kayexalate) or calcium polystyrene sulfonate (Kalimate) is used extensively for treatment against hyperkalemia, especially in the patients with end-stage renal disease (ESRD). The purpose of this study to survey a rare side effect of CER.

Methods: The 11 patients with ESRD, who experienced intestinal perforation after oral administration of CER, were selected . Evidence of CER administration was documented by identifying basophilic polygonal crystals, which are characteristic of both Kayexalate and Kalimate.

Results: Median age of the patients (male 7, female 4) was 72.5 years old (43-90 years). The site of perforation was sigmoid colon in 9 patients and rectum in 2 patients. Underlining diseases consisted of CGN in 8 patients and diabetic glomerulosclerosis in 3 patients. Regardless of underlining diseases, all patients showed the event of intestinal perforation during CKD stage 4 to 5D. The 8 patients and 3 patients were orally administrated with Kayexalate (without sorbitol) and with Kalimate, respectively. Total dose of both CER was 30g/day. After emergent events, all patients were treated by Hartmann's operation. Resected intestine was examined. Microscopically, all patients revealed scattered basophilic and polygonal crystals at necrotic perforating site. Diverticulitis was confirmed in all patients. Besides necrotic lesion, remaining intact mucosa penetrated between discontinuous muscularis propria suggesting underlying diverticulum.

Conclusions: Patients with ESRD have a common physiological background to generate a diverticulum. Even though the patients were treated only with CER without sorbitol, which can be one of the causes of colonic necrosis, an intestinal perforation occurred on the basis of diverticulum. The present study proposes that the clinicians can decrease the risk of intestinal perforation by avoiding administration of CER in the ESRD patients with diverticulum.

Funding: Government Support - Non-U.S.

FR-PO1696

The Incidence of Intestinal Perforation in U.S. Dialysis Patients Remained Unchanged after Approval and Subsequent Widespread Adoption of Sevelamer Hydrochloride

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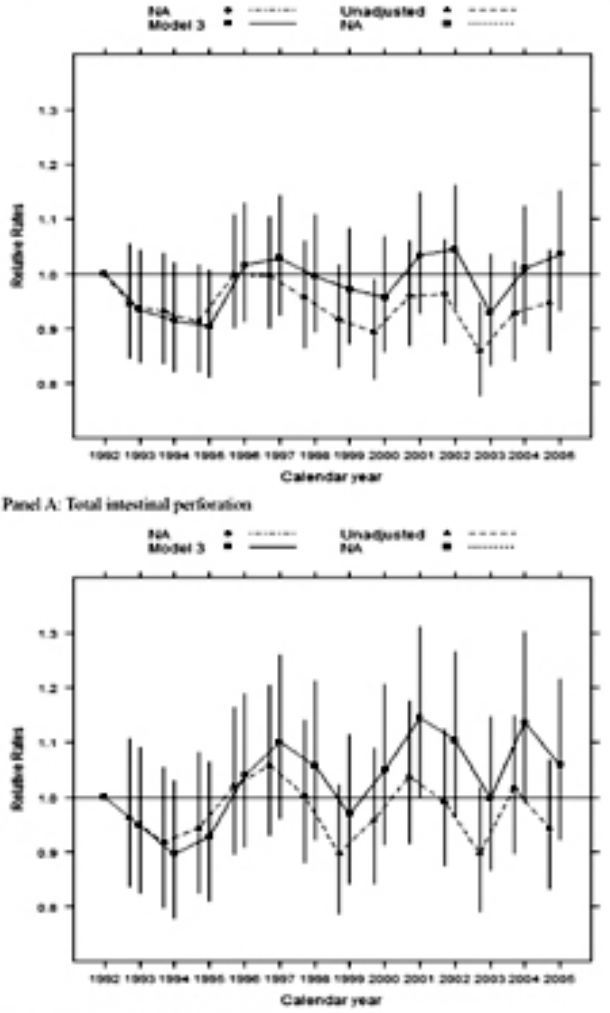
Background: Concerns have been raised that sevelamer hydrochloride may increase the risk of intestinal perforation. We examined temporal trends of the incidenc of intestinal perforation among U.S. dialysis patients between 1992 and 2005.

Methods: In the United States Renal Data System database, we studied dialysis patients between 1992 and 2005. We used ICD-9 diagnosis code 569.83 to ascertain events of intestinal perforation. We defined as spontaneous perforations those events that had no identifiable leading disease or no potential iatrogenic procedures. We used Poisson regression to model the annual number of intestinal perforations, and interrupted time-series analysis was used to test for any changes of incidence rates before versus after 1999.

Results: Overall, 1068594 patients contributed 2.85 Million patient-years. We observed 12518 events of intestinal perforation of which 7928 were considered spontaneous perforations. Annual incidence rates before and after adjustment for demographic and comorbid factors over time are shown in the figure.

Figure 1 Relative incidence rates of intestinal perforation (1992-2005)



Legend: Rate ratios and corresponding 95% confidence intervals
Model 1: adjusted for age, gender and race
Model 2: additionally adjusted for Medicaid coverage, dialysis vintage and modality
Model 3: additionally adjusted for history of past kidney transplantation and 11 comorbidities.

Formal tests for any changes in the level or slope of incidence comparing time periods before and after 1999 indicated no evidence for any changes. (p-values for changes in level/trend: 0.35/0.63 and 0.39/0.22, for total and spontaneous episodes).

Conclusions: In this population-based study, we did not find any significant changes in the incidence of intestinal perforation before versus after approval of Sevelamer in 1998.

Funding: Private Foundation Support

FR-PO1697

Venous Needle Dislodgement Prevention in Hospital Based Hemodialysis

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Background: Venous needle dislodgement (VND) is a serious complication of hemodialysis (HD) and, if undetected, can cause serious morbimortality. VND is not a reportable statistic so precise incidence is unavailable. Prior reports of ~414 episodes/year in USA are likely an underestimate.

Our hospital HD unit cares for high-acuity patients, frequently with altered mental status, with a higher than average risk for VND.

In February 2010, we introduced a quality improvement project aiming to reduce VND rates, with a goal of zero undetected VND episodes.

Internal data showed 3 undetected VND episodes during the prior 3 months (2 class IV hemorrhages), incidence of 1 VND per 538 HD treatments.

Methods: Components of the project were: 1) standardizing cleaning and disinfection procedure of the arteriovenous (AV) access and surrounding skin; 2) implementing a unique anchoring needle taping (silk or plastic) technique, and a standard protocol for anchoring the blood lines to the patient; 3) setting the lower limit of the venous pressure alarm as close as possible to the current venous pressure; 4) ensuring that AV access and needles were visible at all times; 5) using a fiber optic blood detection device for all patients with AV access. The device has a single-use sensor patch placed over the venous needle site where it will absorb blood if VND occurs and emitting an audible alarm; 6) modifying staffing levels with the addition of a quality control nurse, and ensuring adequate staff-to-patient ratios to allow routine AV access monitoring during HD.

Results: All staff were trained in a 1-month time frame and required to demonstrate proficiency in the above. These included physicians, nurses, HD technicians, risk management personnel, development of educational materials and technical support.

Between 2/15 and 12/31/2010 there have been zero undetected VND episodes and the overall occurrence dropped from 13 in 2009 to 4 in 2010 (incidence 1 VND per 1750 treatments).

Conclusions: In conclusion, prevention of VND in high-risk hospitalized HD patients is achievable with effective education, protocol standardization and ongoing monitoring. Minimization of undetected VND episodes can be aided by blood loss detection devices use.

FR-PO1698

Lymphangiogenesis Develops during Peritoneal Fibrosis in Peritoneal Dialysis Patients and Rat Peritonitis Model

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Background: Peritoneal fibrosis/sclerosis (PF) causes ultrafiltration failure (UFF) and is an important complication in long-term peritoneal dialysis (PD). We studied the roles of lymphangiogenesis and vascular endothelial growth factor-C (VEGF-C), which is a potentially important mediator of lymphangiogenesis, in the relationship between PF and UFF.

Methods: VEGF-C contents in human dialysate effluent (n=124) and VEGF-C tissue expression (n=69) were investigated by ELISA, real-time PCR and immunohistochemistry. VEGF-C production with TGF-β1 in Met-5A mesothelial cells and human mesothelial cells (HPMC) from the spent patient peritoneal dialysate (n=28) were studied. Expression of lymphatic vessels was examined by immunohistochemistry. We developed a rat model of PF induced by intraperitoneal injection of chlorhexidine gluconate (CG) every other day. Rats were treated with TGF-β Type I receptor inhibitor (TGFβRI). Lymphatic vessels and VEGF-C were evaluated by immunohistochemistry and real-time PCR.

Results: The dialysate-to-plasma ratio for creatinine (D/P Cr) was positively correlated with dialysate VEGF-C concentration. VEGF-C mRNA expression was 4.3-fold higher in peritoneal membranes with UFF than in pre-PD renal failure peritoneum. Lymphatic vessels and VEGF-C, which was detected in the mesothelial cells and some macrophages, were higher in the advanced fibrotic peritoneum. VEGF-C expression was upregulated by TGF-β1 in cultured Met-5A cells, which was specifically suppressed by TGFβRI. In cultured HPMC, TGF-β1 upregulated VEGF-C mRNA expression at 12 hours, and was correlated with D/P Cr ($R=0.64$, $p<0.001$). In the rat CG model, lymphatic vessels and VEGF-C expression were high, and significantly suppressed by TGFβRI.

Conclusions: Our results suggest that high peritoneal transport is associated with lymphangiogenesis and fibrosis via the TGF-β-VEGF-C pathway.

FR-PO1699

Effects of Icodextrin on Inter-Cellular Adhesion Molecule-1 Expression Via Protein Kinase C in Human Endothelial Cells

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Background: Intracellular adhesion molecule (ICAM)-1, which is one of the adhesion molecules, plays an important role in inflammatory processes of peritoneal damages in patients on peritoneal dialysis (PD) by mediating leukocyte-endothelial cell adhesion, leukocyte migration, and T cell-antigen-presenting cell interactions. We compared the effects of glucose and icodextrin on ICAM-1 expression in human endothelial cells.

Methods: We used icodextrin powder-dissolved culture medium to exclude the effects of ingredients of the PD fluid. Quiescent cultured human umbilical vein endothelial cells (HUVECs) were exposed to either glucose or icodextrin, and the expression levels of ICAM-1 were analyzed by flow cytometry using FACScan. Phosphorylation of protein kinase C (PKC) was analyzed by western blotting using antibodies against phosphorylated forms of PKC.

Results: High glucose levels induced ICAM-1 expression on cell surface of HUVEC in time- and concentration-dependent manners, while icodextrin did not influence the expression levels of ICAM-1. Glucose increased phosphorylation levels of PKC, and calphostin C, which is a specific inhibitor of PKC, completely suppressed glucose-induced PKC phosphorylation and ICAM-1 expression. Icodextrin had no effects on phosphorylation levels of PKC.

Conclusions: Our results indicate that glucose induces ICAM-1 by activating PKC, while icodextrin has no effects on PKC activation and ICAM-1 expression; hence, icodextrin-containing PDF has superior biocompatibility and does not influence leukocyte-endothelial cell adhesion in the peritoneum of patients on PD.

FR-PO1700

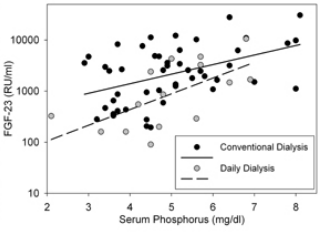
Effects of Short Daily Versus Conventional Hemodialysis on Serum FGF-23 Levels Joshua Zaritsky,[1] Anjay Rastogi,[2] George E. Fischmann,[2] Kenneth S. Kleinman,[2] Georgina Chow,[1] Barbara Gales,[1] Isidro B. Salusky,[1] Katherine Wesseling-Perry.[1] *[1]Department of Pediatrics, UCLA, Los Angeles, CA; [2]Department of Medicine, UCLA, Los Angeles, CA.*

Background: Increased FGF-23 levels have been associated with cardiac abnormalities, vascular calcifications and mortality in hemodialysis (HD) patients. However, it remains unknown whether the frequency and type of HD influence FGF-23 levels.

Methods: We therefore compared FGF-23 levels as well as other biochemical variables between 15 patients (pts) undergoing short daily home HD (DHD) using the NxStage System® and 48 pts undergoing conventional in-center HD (CHD) (table). FGF-23 levels were measured using the 2nd generation Immutopics® C-terminal assay.

Results: DHD pts were younger but there were no differences in the duration of end stage renal disease (ESRD). A greater number of DHD patients received vitamin D sterol therapy than CHD patients while there was no difference in calcimimetic therapy between groups. Overall serum calcium, phosphorus and intact parathyroid hormone (iPTH) levels were similar and phosphorus levels correlated with FGF-23 in both groups as expected; r=0.67 (p<0.005) and r=0.42 (p<0.003) in DHD and CHD respectively (figure). However, FGF-23 levels were lower in the DHD group despite greater use of vitamin D sterols.

	Daily Dialysis Mean±SD or Median (25-75%)	Conventional Dialysis Mean±SD or Median (25-75%)	p
Age (yrs)	45 ± 18	62 ± 19	<0.05
ESRD duration (yrs)	1.8 (1-18)	2.5 (1-5)	NS
Male/Female (N)	11/4	30/18	NS
Vitamin D use (%)	47 (25-70)	10 (5-20)	<0.05
Calcimimetic use (%)	33 (15-59)	40 (29-52)	NS
Hgb (mg/dl)	12 ± 1	12.1± 1.5	NS
Calcium (mg/dl)	8.8 ± 0.6	8.7 ± 0.7	NS
Phosphorus (mg/dl)	4.9 ± 1.3	5 ± 1.4	NS
iPTH (pg/ml)	198 (110-376)	237 (130-320)	NS
FGF-23 (RU/ml)	860 (222-3037)	2520 (909-5556)	<0.03



Conclusions: These findings suggest that FGF-23 levels may be a more sensitive biomarker of cumulative phosphate burden than single phosphate determinations. Future trials are needed to assess whether targeted reduction of FGF-23 by DHD will result in improved cardiovascular outcomes.

Funding: NIDDK Support

FR-PO1701

Effects of Toll-Like Receptor 2 on the Inflammatory Response and Cytokine Production from Intraperitoneal Polymer Catheters Michael F. Flessner,[1] Elise Peery Gomez-Sanchez,[2] Xiaorong Li.[2] *[1]KUH, NIDDK, Bethesda, MD; [2]Medicine, GV Montgomery VA Medical Center, Jackson, MS.*

Background: To test the hypothesis that inflammation from a sterile intraperitoneal (ip) foreign body is mediated by Toll-Like Receptor 2 (TLR2), we compared the responses of normal C57Bl mice (C, n=12) and TLR2-Knock-out mice (TKO, n=20) to polyethylene catheters.

Methods: Five 5Fr catheter rings were placed ip in each treated animal, while sham animals underwent a laparotomy only. After 1 or 2 weeks, the rings were aseptically recovered, and cells adhering to the catheters were separated using ultrasound and stained for immunochemical cell markers (ICC). Cells, catheter, and a swab from the abdomen were cultured for 96 hours to insure sterility. Abdominal wall tissues were collected after sacrifice and processed for CD31 (angiogenesis marker), Trichrome (peritoneal thickness), and immunohistochemistry for cytokines (IHC, 1=no stain to 4=heavy stain).

Results: While week number was not a significant factor, marked differences were noted between animals with catheters and those without for: peritoneal thickness (μm±SE): 74.4±3.5 vs 16.1±4.8; angiogenesis (vessels/mm±SE): 41.5±3.6 vs 6.5±5.0; IHC for: αSMA: 3.1±.1 vs 1.4±.2 and TGFβ: 2.7±.1 vs 1.3±.1 (p<10-5, ANOVA). Adherent cells showed ICC staining for macrophages, mesothelial cells, myofibroblasts and lymphocytes. Staining for macrophages increased from 1 to 2 weeks, but all other cells maintained the same degree of ICC stain. Comparison of TKO animals to the C demonstrated that deletion of TLR2 decreased inflammatory angiogenesis (vessels/mm±SE, p<.01): TKO, 31.7±3.1 vs C, 61.3±4.4, but increased peritoneal staining for αSmooth Muscle Actin (p<.05): 3.4±.1 vs 2.6±.2. No significant differences were noted in fibrotic thickness or in Transforming Growth Factor β staining.

Conclusions: From these observations, we conclude that deletion of TLR2 decreases angiogenesis in the inflammatory response to biomaterials. The fibrotic response appears to be more complex and likely involves other pathways.

Funding: Private Foundation Support

FR-PO1702

Fibrinogen Enhances Inflammatory Cell Adherence to Intraperitoneal Polymer Catheters and Decreases Cytokine Production Michael F. Flessner,[1] Xiaorong Li,[2] Elise Peery Gomez-Sanchez.[2] *[1]KUH, NIDDK, NIH, Bethesda, MD; [2]Medicine, GV Montgomery VA Hospital, Jackson, MS.*

Background: Binding of fibrinogen has been shown to be a potential step of inflammatory cell adherence to foreign materials in the peritoneal cavity (Tang, 1993).

Methods: To test the hypothesis that pretreatment with fibrinogen alters cell adherence and cytokine appearance after peritoneal implantation, we incubated sterile 5Fr polyethylene catheter rings in solutions of saline (S), saline + 1.5 mg/ml fibrinogen (F), or saline + 5 mg/ml albumin (A) for 4 hours and placed 5 rings each into the peritoneal cavity of 18 C57Bl mice. After 1 week, the rings were aseptically recovered, and cells adhering to the catheters were separated using ultrasound and stained for immunochemical cell markers (ICC). Cells, catheter, and a swab from the abdomen were cultured for 96 hours to insure sterility. Abdominal wall tissues were collected after sacrifice and processed for CD31 (angiogenesis marker), Trichrome (peritoneal thickness), and immunochemistry for cytokines (IHC, 1=no stain to 4=heavy stain).

Results: Cell densities on the catheter material (10^3#cells/cm^2, mean±SE) were: (A), 138.2±21.0; (S), 177.2±23.0; (F), 273.3±21.0 (p<.002, 1-way ANOVA). ICC for macrophages (MAb F4/80) was lowest with (F), 2.0±.3; (S), 2.7±.3, and highest for (A), 3.6±0.3. Other ICC (CD3, CD8, cytokeratin, vimentin) were similar for adherent cells. Tissue CD31 staining, peritoneal thickness, and IHC for Fibroblast Growth Factor and Transforming Growth Factor β were not different among the treatments (1-way ANOVA). IHC for Vascular Endothelial Growth Factor (VEGF:A, 2.5±.2; S, 2.6±.2; F, 1.9±.2; p<0.004) and for α-Smooth Muscle Actin (αSMA: A, 3.4±.2; S, 3.3±.2; F, 2.8±.2; p<0.05) were significantly less for F than the other treatments.

Conclusions: We conclude that pretreatment of catheters with fibrinogen enhances overall cell adhesion, while decreasing the relative macrophage number when compared to saline or albumin solutions. F also leads to decreased appearance of VEGF and αSMA in the peritoneal response at 7 days. We conclude that the addition of fibrinogen affects the foreign body response to polyethylene in mice.

Funding: Private Foundation Support

FR-PO1703

Interleukin-10 Prevents the Progression of Peritoneal Fibrosis Akira Onishi,[1/2] Yoshiyuki Morishita,[1] Masashi Urabe,[2] Ichiro Hirahara,[1] Shigeaki Muto,[1] Keiya Ozawa,[2] Eiji Kusano.[1] *[1]Division of Nephrology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan; [2]Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

Background: Peritoneal fibrosis (PF) is an intractable complication of peritoneal dialysis (PD). Interleukin-10 (IL-10) was reported to have anti-fibrotic effects; however, little is known about its effects on PF. In the present study, we examined the effects of IL-10 on PF.

Methods: *In vitro* study: Rat peritoneal mesothelial cells (RPMCs) purified from Sprague-Dawley (SD) rats were incubated with IL-10 for 48 h. Then, 50 mM methylglyoxal (MGO); an inciting agent of PF was added. After 24 h, the phenotype and signal transduction in RPMCs were analyzed by qRT-PCR. *In vivo* study: Male SD rats aged 6 weeks were injected intramuscularly with an AAV type 1-based vector encoding IL-10 (AAV-IL-10). The rats injected with the vector encoding GFP (AAV-GFP) or PBS served as controls. From the 28th day after the injection, PD fluid containing 10 mM MGO was injected intraperitoneally every day for 3 weeks. Then, the parietal peritoneum was sampled for analyses.

Results: *In vitro study:* IL-10 mitigated a decrease in epithelial markers (E-cadherin and occludin), an increase in mesenchymal markers (fibronectin and vimentin) and up-regulation of TGF-β1 and Snail induced by MGO in RPMCs. *In vivo* study: Histological analysis revealed marked fibrous thickening of the peritoneum in AAV-GFP and PBS groups. In AAV-IL-10 group, the thickness of the peritoneum was reduced to less than one-half of those in the other groups. Immunohistochemistry showed that decreased epithelial markers and increased mesenchymal markers of the peritoneal cells were mitigated in AAV-IL-10 group compared to AAV-GFP or PBS group. qRT-PCR analysis revealed that TGF-β1 and Snail expression levels in the peritoneum in AAV-IL-10 group was less than 0.3-fold compared to those in AAV-GFP or PBS group.

Conclusions: The results of present study suggested that IL-10 inhibited the progression of PF through the suppression of TGF-β1 signaling. AAV vector-mediated systemic IL-10 delivery would be a potentially powerful option for treatment of PF.

FR-PO1704

Protective Effect of Icodextrin on Mesothelia-to-Mesenchymal Transition (MMT) of Mesothelial Cells (MCs) Induced by Peritoneal Dialysis (PD) Liquids Abelardo I. Aguilera,[2] Pilar Sandoval-Correa,[1] Rafael Selgas,[3] Maria Luisa Perez Lozano,[1] Manuel Lopez-Cabrera.[1] *[1]Biologia Molecular, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain; [2]Servicio de Nefrología, Hospital Universitario de la Princesa, Madrid, Spain; [3]Servicio de Nefrología, Hospital Universitario de la Princesa, Madrid, Spain.*

Background: MMT of MCs is a key process in the initiation of peritoneal membrana (PM) damage in PD. MCs exposed to glucose degradation products (GDPs), low pH and pro-inflammatory cytokines undergo on MMT. Some clinical studies suggest that Icodextrine (Ico) can show better biocompatibility. This study analyzes the effects of Ico on MMT of CMs as triggering of PM damage.

Methods: On the last 10 years we periodically cultured MCs from PD effluents. In MCs lysate we determined E and N-cadherin, Snail and α-SMA as MMT markers. Fibronectin and collagen-I as extrecellular matrix-component (ECM) and VEGF as pro-angiogenic factor. These values were compared between the different PD liquids and clinical events. MCs from omentum (HPMO) were used to *in-vitro* experiments. In our PD mice model we are studying the effects of Ico on PM

Results: We included 157 PD patients of whom 137 were extracted. 63 cultures were from 23 patients using Ico, 23 were from 12 using Dianeal. 26 and 35 cultures

were from 9 and 13 patients using Physioneal 35 and 40, respectively. 66 cultures were classified as fibroblastoid phenotype, 59 as cobblestone and 12 as mixed or not growth. Transdifferentiated MCs showed higher levels of snail (PCR), fibronectin, VEGF, TGF-β and IL-8. High Cr-MTC and lower UF capacity were also found in patients who drained these cells. Although there were 2 more cases of peritonitis in the Ico group, the frequency of MMT was 50% compared to 75% of Dianeal. As expected, Ico group showed lower values of EMC, VEGF and TGF-β and similar frequency of MMT than physioneal. In HPMO, Ico partially inhibited the MMT induced by TGF-β and PD liquids but decreased ECM. Animal experiments are underway.

Conclusions: In PD Ico showed a protective effect on PM through a partial inhibition of MMT of MCs.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1705

Are Matrix Metalloproteinase-2 and Plasminogen Activator Inhibitor-1 Determined by Peritoneal Transport or Local Production?

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Background: Solute transport in peritoneal dialysis occurs from the peritoneal microcirculation to the peritoneal cavity, and visa versa by diffusion and by hydrostatic, and osmotic pressure gradients. Peritoneal effluent contains clinical relevant substances derived from intraperitoneal production or transperitoneal transport or both. Matrix metalloproteinase-2 (MMP-2) is a glycoproteinase that cleaves denatured collagen, and complement other collagenases in the degradation of fibrillair collagens. Elevated intraperitoneal levels of plasminogen activator inhibitor-1 (PAI-1) have been demonstrated to be present in patients with intra-abdominal adhesions. Therefore, the aim of this study was to investigate the potential use of MMP-2 and PAI-1 in effluent as markers in the development of peritoneal alterations.

Methods: For this purpose the roles of peritoneal transport and local peritoneal production of these parameters was studied. This single centre cohort study included 86 incident PD patients. All patients were treated with biocompatible dialysis solutions and underwent a standard peritoneal permeability analysis (SPA). The presence of local production as well as correlations between MMP-2, PAI-1 and peritoneal transport parameters were studied.

Results: Median effluent levels of 22.1ng/mL for MMP-2 and 0.95ng/mL for PAI-1 were found. Local peritoneal production averaged 93% of effluent MMP-2 concentration and 77% for PAI-1. Also, when expressed as ratio, D/P_{MMP-2} or D/P_{PAI-1} over $D/P_{Albumin}$ exceeded 1 and therefore local production could be established on top of transport. Furthermore, correlations between $MTAC_{creatinine}$ and MMP-2 ($r=0.38$, $p<0.001$) or PAI-1 ($r=0.42$, $p<0.001$) were present.

Conclusions: In conclusion this study demonstrates that MMP-2 and PAI-1 pass the peritoneal membrane via peritoneal transport. However, the presence of local production was much more important. This data illustrates the potential of MMP-2 and PAI-1 as biomarkers of peritoneal modifications, but constituents of peritoneal transport and local production should be separated in every patient.

FR-PO1706

Indoxyl Sulfate and *p*-cresyl Sulfate Concentrations Increase in Incident Peritoneal Dialysis Patients along the Loss of Residual Renal Function

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Background: Residual renal function (RRF) is of critical importance for the clearances of p-cresyl sulfate (PCS) and indoxyl sulfate (Inds) in peritoneal dialysis (PD) patients. Besides other mechanisms, high serum PCS and IndS concentrations may contribute to the association between poor RRF and increased cardiovascular (CV) morbidity in PD patients. Studies evaluating the impact of RRF on serum levels of PCS and IndS have so far yielded conflicting results, probably related to small sample size and retrospective design.

Methods: We performed a prospective, observational cohort study in incident PD patients. Serum concentrations, mass removal and total, renal and dialytic clearances of creatinine, urea nitrogen, PCS and Inds were assessed 1, 6, 12 and 24 months after start of PD. Data from 35 patients (19 male, age 55±17 year) with a technique survival exceeding 2 years were analyzed.

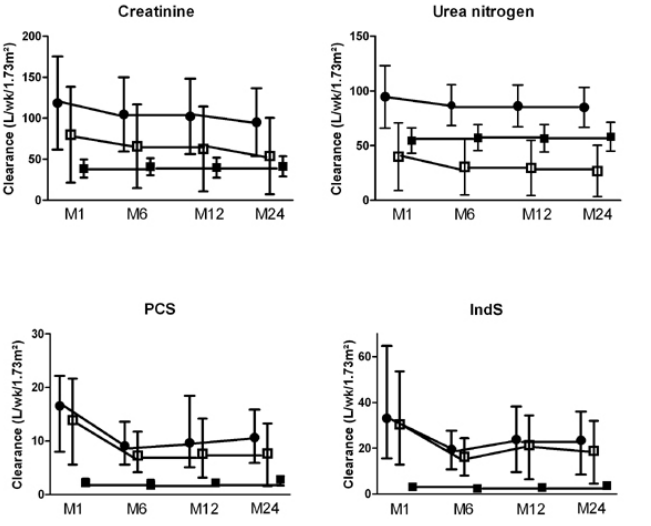
Results: Serum concentrations of PCS and IndS increased with dialysis vintage, along the decline in RRF. Conversely, mass removal of both toxins remained stable.

Evolution of serum concentrations and mass removal (MR) of PCS and IndS

	M1	M6	M12	M24	P
nPNA	1.8	1.8	1.9	1.9	0.9
PCS (μM)	84	99	107	122	0.01
IndS (μM)	43	57	60	69	0.0009
Urea nitrogen (mg/dL)	105	108	109	110	0.3
Total MR PCS (mg/week)	235	209	215	195	0.7
Total MR IndS (mg/week)	260	210	237	266)	0.6

Median values are shown.

Compared to urea nitrogen, total clearances of PCS and IndS showed a more prominent decline along the loss of RRF.



Time course of renal (open squares), peritoneal (closed squares) and total (closed circles) clearances of creatinine, urea nitrogen, PCS and IndS.

Conclusions: Contrary to previous reports, we demonstrate that serum PCS and IndS concentrations in incident PD patients increase along the loss of residual renal function.

FR-PO1707

Baroreceptor Function Is Similar in Chronic Peritoneal Dialysis and Hemodialysis Patients

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Background: The baroreflex function (BRS), a measure of the autonomic nervous system activity was not well characterized in patients with end-stage renal disease on chronic peritoneal dialysis treatment (CCPD/CAPD).

Methods: To assess BRS indices, continuous beat-to-beat intervals (IBI) and systolic blood pressure (SBP) were monitored using the Finometer equipment in a group of patients on chronic peritoneal dialysis treatment (CCPD/CAPD, n=10) as compared with age matched patients on chronic hemodialysis (HD, n=91), and control subjects (C, n=27).

Results: Mean SBP and IBI variability , BRS indices and BEI (baroreflex effectiveness index) were (median and interquartile range):Table 1.

	C	HD	p vs.C	CCPD/CAPD	p vs.C	p vs. HD
Age* (years)	56±7	56±12	NS	56±15	NS	NS
SBP (mm Hg)	126 (24)	137 (32)	NS	127 (43)	NS	NS
BEI (%)	11.8 (12.2)	3.0 (7.3)	0.005	2.3 (7.3)	0.001	NS
Slope (msec/mmHg)	6.34 (2.47)	4.88 (3.32)	0.005	4.35 (4.77)	0.001	NS
LF IBI ($msec^2$/Hz)	2285 (2166)	743 (1570)	0.001	876 (1633)	0.008	NS
HF IBI $msec^2$/Hz)	250 (222)	125 (157)	0.008	94 (78)	0.003	NS
LFα(msec/mmHg)	4.99 (2.66)	3.23 (2.51)	0.002	3.32 (1.73)	0.015	NS
HFα(msec/mmHg)	6.19 (5.17)	3.90 (2.47)	0.002	3.38 (1.90)	0.004	NS

* mean±SD; Slope: the slope of the regression line between IBI and SBP for at least 3 beats; % BEI: % of sequences out of the number of ramps; LF IBI and HF IBI: power spectrum of IBI in the low frequency (0.04-0.15 HZ, LF) and the high frequency (0.15-0.40 Hz, HF) bands.α: the square root of the ratio of average power spectral density of IBI and SBP in the low (LF) or high (HF) frequency bands.

Mean IBI, mean diastolic blood pressure (DBP) and the variablity of the blood pressure were in the same range in all groups.The proportion of diabetic patients was similar in HD and CCPD/CAPD groups.

Conclusions: These results show that heart rate variability and baroreceptor indices are markedly reduced in patients with end-stage renal disease on both maintenance hemodialysis and CCPD/CAPD, as compared with normal individuals. The dialysis modality, however, has no effect on the degree of suppression of the autonomic function. The prognostic significance of these findings remains to be defined.

FR-PO1708

Is the Sodium Restriction Harmful or Beneficial in the Long-Term Peritoneal Dialysis?

Jie Dong, Yanjun Li, Rong Xu. *Institute of Nephrology, Peking University, Beijing, China.*

Background: Sodium restriction is routinely recommended for patients on peritoneal dialysis (PD). However, a very low sodium intake was shown to be associated with protein-energy wasting and high mortality in patients on PD most recently. We aimed to determine whether a reduction in sodium intake in the early stage of PD is associated with a decline in dietary and nutritional parameters, and with a high risk for mortality.

Methods: A total of 305 incident patients were enrolled in our single-center cohort study. All patients were followed until death or censored. Demographic data was collected at baseline. Biochemical, dietary and nutrition data were examined at baseline and thereafter at regular intervals. Three groups of patients were defined according to the change of sodium intake over the first two quarters: one in which sodium intake decreased, one in which sodium intake remained stable, and a third group in which sodium intake increased.

Results: Participants with decreased sodium intake tended to be young, non-diabetic and less inflammatory status. There were no significant differences in the longitudinal change of dietary protein and energy intake, serum albumin and lean body mass during the long-term follow-up between groups. The decreased sodium intake significantly predicted a lower risk for cardiovascular mortality with HR of 0.57 (0.38~0.86) and for first cardiovascular event with HR of 0.68 (0.52~0.88) after adjusting for recognized confounders.

Conclusions: Our study revealed that decreased sodium intake in the early stage of PD was not associated with declined dietary and nutritional status in the long term. The decreased sodium intake independently predicts a lower risk for cardiovascular death and first cardiovascular event.

FR-PO1709

Sleep-Disordered Breathing, Restless Legs Syndrome and Daytime Sleepiness in Automated Peritoneal Dialysis

Maria-Eleni Roumelioti, Christos Argyropoulos, Filitsa H. Bender, Beth M. Piraino, Mark L. Unruh. *Renal and Electrolyte, University of Pittsburgh.*

Background: Sleep-Disordered Breathing (SDB), Restless Legs Syndrome (RLS), and excessive daytime sleepiness (EDS) are highly prevalent among hemodialysis (HD) patients (pts). The burden of these conditions remains poorly defined among Automated Peritoneal Dialysis (APD) pts.

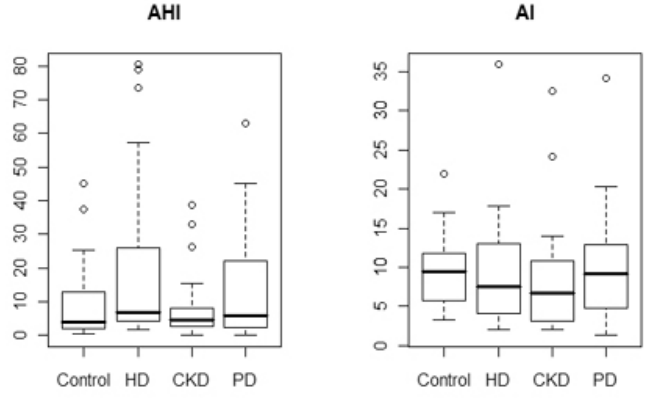
Methods: APD pts were matched to pts with CKD (MDRD eGFR<40 ml/min),on HD and healthy control participants from the Sleep-SCORE Study of sleep and cardiovascular risk with respect to age, gender and BMI. We used in-home unattended polysomnography to measure total sleep time (TST), sleep efficiency (SE) (TST as a proportion of the time spent in bed); Apnea/Hypopnea Index (AHI,apneas and hypopneas/hour)and Arousal Index (AI, microarousals/hour). EDS was defined by a score≥10 on the Epworth Sleepiness Scale (ESS). Presence of RLS was examined with the Hopkins RLS Diagnostics Questionnaire.

Results: A total of 88 pts were studied (22 in each group):

Patient Characteristics

	APD	CKD	HD	Controls
Age	42±14	47±12	50±6	52±6
Women	16(73%)	15(68%)	16(73%)	16(73%)
African Americans	11(50%)	9(40%)	11(50%)	11(50%)
BMI	26.2±6	30±7	28.8±6	31±7
Waist Circumference (cm)	98±17	107±21	104±18	96±18
Neck Circumference (cm)	36±4	37±6	40±6	37±3
SBP (mmHg)	134±15	147±22	148±32	127±15
DBP (mmHg)	87±28	85±16	82±14	80±11

HD pts had the lowest TST (302±114 min) and SE (69±17%) among the 4 groups. APD pts were similar to controls(TST: 355±113 min vs 366±102 min p=0.78 , SE: 72±19% vs 74±13% p=1). The prevalence of EDS was 45% and did not differ among the 3 groups $\chi 2$=0.32). AHI and AI (SBD) scores were not different among the groups.



RLS was present in 35% of the 3 groups, with no difference among them ($\chi 2$=0.93)

Conclusions: The burden of SBD, RLS and EDS is similar among pts on APD, HD and advanced CKD and should be discussed when opting for a dialysis modality.

FR-PO1710

Effluent Markers and Epithelial Mesenchymal Transition in CAPD

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Background: Epithelial mesenchymal transion (EMT) of peritoneal mesothelial cells is important for peritoneal deterioration in CAPD. It is reported that hepatocyte growth factor (HGF) and bone morphogenic protein-7 (BMP-7) ameliorate EMT, but the clinical significance of effuluent HGF and BMP-7 levels remain unclear. It has also been reported that dialysate growth factor levels should be measured relative to the level of cancer antigen 125 (CA125).

Methods: We evaluated the association between peritoneal solute transport rate (PSTR) and effluent markers related to EMT with adjusted values for effluent cancer antigen 125 (CA125). One hundred five incident peritoneal dialysis (PD) patients on PD for 25 (12-68) months with biocomapatible solutions were included in the study. Fast peritoneal equilibration test was used to evaluate PSTR. Effluent hepatocyte growth factor (HGF), bone morphogenic protein-7 (BMP-7), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and CA125 at 4h were measured.

Results: Clinical data are shown in the table.

Clinical data

Group	Patients with high transport rate	Others
n	14	91
Age	58 (44-68)	55 (46-64)
D/P creatine	0.90 (0.87-0.96)*	0.71 (0.60-0.75)
Diabetics	8/14	22/91
Duration of PD	10 (7-11)*	32 (15-75)
Urine volume	400 (176-624)	100 (0-875)
Serum albumin	2.70 (2.69-2.98)*	3.40 (3.03-3.78)

*P<0.001 compared with others

Patients with dialysate/plasma creatinine ≥0.82 showed significantly higher effluent HGF [240 (198-319) vs. 133 (107-216) pg/ml, P<0.001], VEGF [33 (29-34) vs 25 (20-31) pg/ml, p<0.01] and IL-6 [24 (14-35) vs 12 (7-24) pg/ml, p<0.05] levels than the others. Dialysate/plasma creatinine levels were significantly correlated with HGF (r=0.53, p<0.001), VEGF (r=0.35, p<0.001), IL-6 (r=0.35, p<0.001), IL6/CA125 (r=0.25, p<0.05) but not with BMP7, BMP7/CA125, HGF/CA125, VEGF/CA125.

Conclusions: Effluent HGF levels as a compensatory mechanism is a marker of peritoneal deterioration, but controversy remains regarding the adjustment of markers for CA125.

Funding: Private Foundation Support

FR-PO1711

Biological Effects Research of Maltose Peritoneal Dialysis Solution

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Background: Over the past 15 years, peritoneal dialysis (PD) has undergone considerable development from a technological point of view, and osmotic agent has played the essential role in peritoneal dialysis fluid.

Methods: We set up a method to quantitative the maltose by HPLC. Rabbits were enrolled as acute renal failure model to study transport capability in small molecule solute. Anew non-uremic rat model of long-term peritoneal dialysis were enrolled to research the influence of peritoneum structure in long-term peritoneal dialysis.

Results: The glucose and maltose concentrations can be determined with a recently established HPLC methods . The elution peak retention time of glucose and maltose are 5.485min and 4.112min respectively. In our study we select 2.5%and 4.25%maltose peritoneal dialysis solution as subject investigated. The osmotic pressure of maltose is lower than that of glucose but higher than that of Icodextrin. The PH of maltose is higher than that of glucose and icodextrin. The non-uremic rat model of long-term the peritoneal dialysis and the acute renal failure rabbit model have the certain usability. The net ultrafiltration of maltose peritoneal dialysis solution excelled equal concentration glucose peritoneal dialysis solution in 4h.Meanwhile the net ultra filtration of 4.25% maltose peritoneal dialysis solution excelled 7.5% Icodextrin. In 8h the net ultra filtration of maltose peritoneal dialysis solution still excelled equal concentration glucose peritoneal dialysis solution, but lower than that of 7.5% Icodextrin.

Conclusions: The net ultrafiltration of maltose peritoneal dialysis solution excelled equal concentration glucose peritoneal dialysis solution in 4h. The transport capability in small molecule solute of maltose peritoneal dialysis solution overmatches that of equal concentration glucose peritoneal dialysis solution.The biocompatibility of maltose peritoneal dialysis solution overmatches traditional glucose peritoneal dialysis solution.

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FR-PO1712

The Definition of Histological Criteria for Encapsulating Peritoneal Sclerosis – A Standardized Approach Niko Braun,[1] Peter Fritz,[2] Christoph Ulmer,[3] Martin Kimmel,[1] Dagmar Biegger,[2] German Ott,[4] Fabian R. Reimold,[1] Klaus-Peter Thon,[3] Juergen Dippon,[5] Stephan Segerer,[6] Mark Dominik Alscher.[1] *[1]Department of Internal Medicine, Division of Nephrology, Robert-Bosch Hospital, Stuttgart, Germany; [2]Margarete-Fischer–Bosch Institute of Clinical Pharmacology, University of Tuebingen, Stuttgart, Germany; [3]Department of Surgery, Robert-Bosch Hospital, Stuttgart, Germany; [4]Department of Diagnostic Medicine, Division of Pathology, Robert-Bosch Hospital, Stuttgart, Germany; [5]Department of Mathematics, University of Stuttgart, Stuttgart, Germany; [6]Division of Nephrology, University Hospital Zurich, Switzerland.*

Background: The two most relevant pathologies of long-term peritoneal dialysis (PD) are simple sclerosis and encapsulating peritoneal sclerosis (EPS). Aim of the study was to define relevant and reproducible histological parameters in patients with EPS compared to patients on PD without EPS.

Methods: 31 EPS patients and 27 PD patients were analyzed. Two blinded investigators recorded the following histological characteristics: mesothelial denudation, fibrosis, acute and chronic inflammation, hemorrhage, cellularity, number of vessels, vasculopathy, iron content, number of fibroblast-like-cells (FLC), calcification, ossification and fibrin deposits and podoplanin.

Results: The following findings were significantly more common in EPS: FLC ($p<0.001$), meothelial denudation ($p<0.001$), decreased cellularity ($p=0.008$), fibrin deposits ($p<0.03$), positive iron staining ($p=0.05$) and IHC podoplanin vascular ($p<0.001$), podoplanin avascular ($p<0.001$). Multivariate analysis yielded mesothelial denudation ($p=0.04$), FLC ($p=0.04$), acute and chronic inflammation ($p=0.03$), fibrin deposits (0.04) and cellularity ($p=0.02$) as significant histological parameters. Inclusion of podoplanin resulted in different parameters according to binary logistic regression: podoplanin vascular ($p=0.008$), calcium deposits ($p=0.02$), cellularity ($p=0.02$) and chronic inflammation ($p=0.006$).

Conclusions: We demonstrated that a binary logistic regression model differentiates between histological findings in PD compared to EPS. Our next goal is to establish a scoring system with a larger cohort of patients.

FR-PO1713

Visceral Fat Thickness Is Associated with Carotid Atherosclerosis in Peritoneal Dialysis Patients Jisun Paeng,[1] Mi Jung Lee,[1] Dong Ho Shin,[1] Tae Ik Chang,[2] Seung Jun Kim,[1] Dong Eun Yoo,[1] Hyung Jung Oh,[1] Seung Hyeok Han,[1] Tae-Hyun Yoo,[1] Shin-Wook Kang.[1] *[1]Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea; [2]Dept. of Int. Medicine, NHIC Ilsan Hospital, Goyang, Korea.*

Background: Visceral fat is known to be more metabolically active and to be associated with atherosclerosis, inflammation, and insulin resistance. However, the impact of visceral fat on cardiovascular disease in patients on peritoneal dialysis(PD) has never been elucidated. This study was conducted to investigate whether visceral fat thickness(VFT) has a predictive role in carotid atherosclerosis(CA) determined by carotid intima-media thickness(cIMT) in PD patients.

Methods: A cross-sectional study was undertaken in 88 prevalent PD patients. Body mass index(BMI) and waist circumference(WC) were measured as anthropometric indices of obesity. VFT and subcutaneous fat thickness(SFT) were determined by sonographic measurement of abdominal fat. CA was defined as increased cIMT(>1.0 mm) or presence of plaque. Logistic regression analysis(age and sex-adjusted, and multivariate-adjusted) was performed to confirm the independent association of obesity indices with CA. In addition, various biochemical markers for inflammation and insulin resistance according to VFT category were compared by ANOVA.

Results: Thirty-two patients(36.3%) had CA. Patients with CA had significantly higher VFT, BMI, and WC compared to patients without CA. In univariate logistic analysis, BMI, WC, and VFT, but not SFT, were significant risk factors for CA. However, multivariate analysis revealed VFT was an independent factor associated with CA after adjusting for demographic, biochemical parameters, and anthropometric indices(per 1mm increase; OR, 2.294; 95% CI, 1.048-5.021; P=0.038). When the patients were divided into three groups according to VFT, log high-sensitivity C-reactive protein levels, fibrinogen concentrations, and HOMA-IR were significantly higher in the 3rd tertile compared to the other tertiles.

Conclusions: VFT, not SFT, is independently associated with CA in PD patients. Therefore, sonographic measurement of VFT could be useful to stratify the risk of cardiovascular disease in these patients.

FR-PO1714

Oral Active Vitamin D and the Risk of Peritonitis: The Benefit of an Early Start Ana Pinho,[1] Helena Carreira,[2] Ana Cabrita,[1] André Fragoso,[1] Anabela Malho,[1] Isabel Pinto,[1] Idalecio Bernardo,[1] Pedro Neves.[1] *[1]Nephrology Department, Faro Hospital, Faro; [2]Public Health Institute of Oporto Universtity, Oporto.*

Background: Peritonitis is a major complication of peritoneal dialysis (PD), being associated with high hospitalization rate and increased mortality. Data on the relationship between medication and the incidence of peritonitis is scarce and inconsistent. It has been suggested that baseline treatment with vitamin D could be associated with a lower risk of peritonitis. The aim of our study was to clarify the role of active vitamin D exposure in our PD patients.

Methods: We included and followed prospectively all patients who were on PD for at least 3 months, since 2000. Comorbidities and laboratory parameters were assessed at baseline. Data regarding oral active vitamin D treatment (Alfacalcidol and Calcitriol) was recorded throughout the follow-up and in a low clearance clinic period (pre-PD).

Results: We evaluated 105 incident patients, 43% female, with a mean age of 53 years and a mean follow-up time of 31months. The incidence rate of peritonitis was 0.25episodes/patient-year. Peritonitis was the major cause both for hospitalization (36.1%) and death (22.4%), being associated with 2.8 increased risk for mortality ($p=0.029$). Of our population, 17 patients were treated with vitamin D (0.69±0.54mcg/week) for 115±46 weeks during the pre-PD. At the start of PD therapy, 11 patients began vitamin D and during follow-up period another 44 patients also initiated vitamin D treatment.

In the univariate analysis, the treatment with vitamin D in pre-PD was associated with a significantly lower risk of peritonitis ($p=0.012$). After adjustment for time on PD, age, diabetes, baseline serum albumin and CRP, pre-PD vitamin D therapy was associated with an 72% of reduction of peritonitis risk [OR: 0.28; IC95%: 0.08-0.93). The therapy with vitamin D after the beginning of PD wasn't associated with reduction of peritonitis risk.

Conclusions: Treatment with oral vitamin D in a pre-PD period is associated with a lower risk of peritonitis. Further studies are still needed to corroborate our results and to get a better understanding of how vitamin D exerts this protection capacity peritonitis.

FR-PO1715

Automated Cell Analysis Improves Diagnostic Accuracy in Peritoneal Dialysis Associated Peritonitis A.M. Van Alphen, Robert Jonge, Marjen W. Fieren. *Erasmus University Medical Center, Rotterdam, Netherlands.*

Background: According to current guidelines PD associated infectious peritonitis is recognised by elevated white blood cell (WBC) counts ($>100*10^6$ /L) in the effluent. Of these leukocytes >50% are required to be polymorphonuclear cells (PMN's). Microscopic analysis has been gold standard to determine differential WBC count. However, microscopic analysis is time consuming, requires skilled personnel, suffers from substantial variability and is seldom available 24 hours per day. Since immediate antibiotic treatment of peritonitis is warranted time to diagnosis should be as short as possible. Automated hemocytometric analysis may overcome these problems.

Methods: We report on our three year experience with automated hemocytometric analysis using the Sysmex® XE-5000 body fluids mode. Previously we showed that this method enables highly accurate WBC differential counting in body fluids including PD effluent, even at low WBC numbers. The system has been implemented in March 2008 in our hospital. Since implementation data on differential WBC is available within one hour after sampling. We evaluated 61 episodes of symptomatic effluent leukocytosis occurring from March 2008-March 2011. We excluded the first week after start training PD from our analysis.

Results: Six episodes were characterised by predominant monocytosis. All 6 episodes recovered without antibiotic treatment and cultures, evaluated using Bactec® technique, remained sterile. In 55 cases, patients presented with predominant neutrophilic leucocytosis in the effluent (1 episode/23 patient months). These patients were all treated with antibiotics. The average percentage of PMN's in the effluent was 79%. 39 (71%) episodes resolved, 6 (11%) episodes resulted in relapse peritonitis and 10 (18%) proved refractory to treatment. In 5 cases (9%) no pathogen could be identified on culture. Because PMN ratio was >50% in these 5 patients, they were treated with antibiotics. All five recovered readily.

Conclusions: Immediate availability of differential WBC counts in PD effluents, using automated hemocytometric analysis, prevents unnecessary treatment of PD associated peritonitis with antibiotics in 10% of presenting cases.

FR-PO1716

Low Calcium Dialysate as a Risk Factor for Decline in Bone Mineral Density in Female Patients on Peritoneal Dialysis: A Single-Centre Retrospective Observational Study Seokhui Kang, Jun-Young Do, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. *Division of Nephrology, Department of Internal Medicine, Yeungnam University Hospital, Daegu, Korea.*

Background: Previous studies have showed that low calcium dialysate (LCD) induce an increase of serum intact-parathyroid hormone (i-PTH) in PD patients. There are few reports on the effects of LCD for decline in bone mineral density (BMD) in PD patients.

Methods: We reviewed the medical records at Yeungnam University Hospital in Korea and identified all the female patients who received PD between 2001 April and 2009 March. Among them, patients with < 2 years of follow-up were excluded. BMD measurement was performed yearly by the Hologic (Discovery Wi). One hundred ten patients were enrolled. The following data was documented from the patients' record: age at the initiation of PD, underlying disease, the time averaged laboratory findings, types of dialysate and changes in total body BMD during 2 years.

Results: Twenty four underwent LCD and 86 patients underwent standard calcium dialysate (SCD). The mean age was 49.8 ± 11.4 years old in low calcium dialysate group and 49.5 ± 13.7 years old in standard calcium dialysate group. Total BMD (g/cm^2) was 1.02 ± 0.13, 1.02 ± 0.13 and 1.00 ± 0.12 at baseline, 1 year and 2 years after the initiation of PD. There was a significant decrease in the BMD between 1 and 2 years after the initiation of PD. Time averaged intact-PTH was 249.4 ± 161.3 in LCD group and 141.3 ± 1189 in SCD group ($p = 0.000$). Time averaged alkaline phosphatase (ALP) was 215.4 ± 87.6 in LCD group and 186.4 ± 74.6 in SCD group ($p = 0.023$). On the univariate analysis, LCD, residual renal function, normalized protein equivalent of nitrogen appearance, weekly Kt/V, i-PTH, ALP and initial total body BMD were associated with decline of total body BMD. On the multivariate analysis, LCD, ALP and initial total body BMD were proved to be the independent risk factors for decline in total body BMD.

Conclusions: Low calcium dialysate is a risk factor for decline in BMD. LCD may be associated with increment of ALP and intact-PTH. Therefore, LCD should be carefully used for female PD patients with risk of decline in BMD.

FR-PO1717

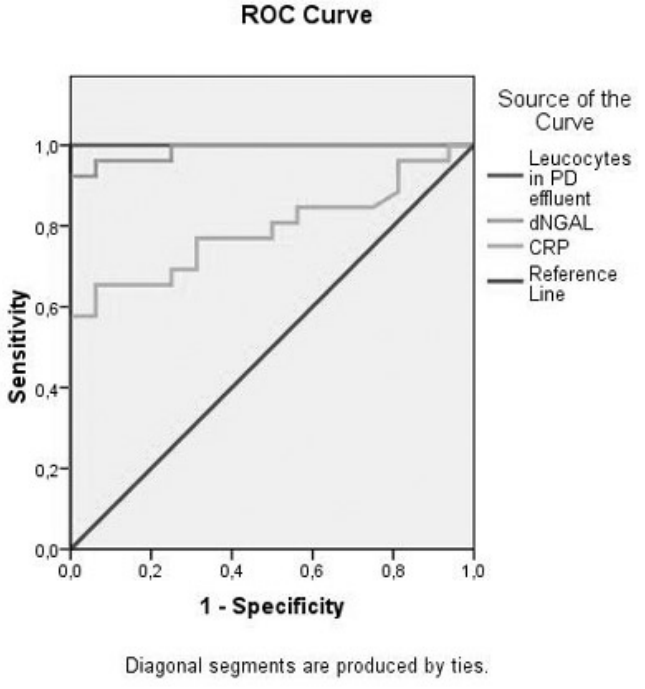
Neutrophil Gelatinase-Associated Lipocalin in Peritoneal Dialysate Effluent as a Marker of Acute Episode of Peritonitis Francesca K. Martino,[1] Pierluigi Di Loreto,[1] Ilenia Filippi,[2] Maria Pia Rodighiero,[1] Claudio Ronco.[1-2] *[1]Nephology, Dialysis and Kidney Transplant, San Bortolo Hospital, Vicenza, Italy; [2]IRRIV, Vicenza, Italy.*

Background: The major infective complication of peritoneal dialysis (PD) is the development of peritonitis. Serum neutrophil gelatinase-associated lipocalin (NGAL) was reported as biomarker of bacterial infection and a previous study of Leung showed its increase during the first days of peritonitis. The aim of study is to access the utility of NGAL in peritoneal dialysate effluent (dNGAL) for the diagnosis of peritonitis in PD patients.

Methods: In this cohort study dNGAL concentration (evaluated by Archictet NGAL assay Abbott) was measured in heterogeneous group of PD patients (n=57) with or without any suspect of peritonitis. Moreover we evaluated CRP, Procalcitonin in the blood and leucocytes in the blood and in PD effluent. The episodes of peritonitis are defined in agreement with the guidelines of the International Peritoneal Dialysis Society.

Continuous variables were presented as the median values and interquartile range (IQR). The Mann-Whitney U tests was used to compare continuous variables. Binary regression analysis was performed to study the ability of variables to predict peritonitis and ROC analysis was used to calculate area under curve (AUC) for biomarkers. All statistical analysis were performed with SPSS version 17.0.

Results: During 8 months of observation, we had 38 peritonitis. In univariable analysis CRP and dNGAL were significantly associated with peritonitis, with OR of 1,5 (p=0,02) and 1,008 (p<0,01), respectively. In multivariable analysis, only NGAL (p=0,042) in PD effluent was independent predictor of peritonitis (H.R. 1,007). AUC for dNGAL was 0,988 while AUC for CRP was 0,792.



Conclusions: In our analysis, dNGAL could be used as a potential biomarker of peritonitis in PD patients.

FR-PO1718

Non-Infectious Complications of Fistulas in Patients Receiving Frequent Hemodialysis Deborah Lynn Zimmerman,[1] Sarah Daisy Kosa,[2] Christopher T. Chan,[2] Charmaine E. Lok.[2] *[1]Ottawa Hospital, Ottawa, ON, Canada; [2]Toronto General Hospital, Toronto, ON, Canada.*

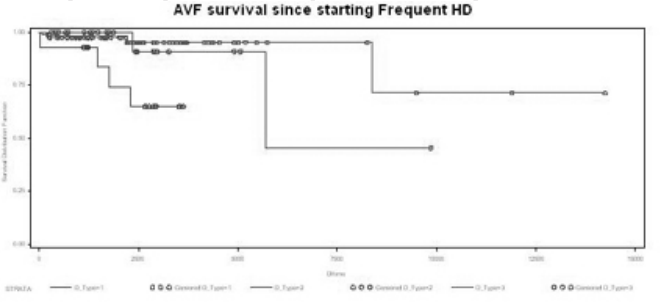
Background: Frequent hemodialysis includes short daily dialysis (SDH), frequent conventional hemodialysis (FHD) and nocturnal dialysis (NHD). FHD is associated with many beneficial clinical outcomes. There is a paucity of data directly comparing these modalities in terms of vascular access complications, interventions, and fistulas (AVF) survival.

Methods: Patients who received SDH (>5x/wk), FHD (4 days/wk; <4 hrs/session) and NHD (>3 x/week, >5 hrs/session) who were dialyzed with a catheter (CVC), AVF, or graft (AVG) were prospectively followed between Jan 2001-Dec 2010. The intervention rate for each type of vascular access between FHD, SD, and NHD were compared. AVF survival in SDH vs. NHD were compared by Kaplan Meier analysis (log rank test) using SAS, v 9.2.

Results: 193 accesses were studied.

	FHD (n=19)	SDH (n=46)	NHD (n=128)
Fistula	10 (53%)	32(70%)	68 (53%)
Graft	2 (11%)	1 (2%)	6 (5%)
Catheter	7 (36%)	13 (28%)	54 (42%)
AVF interventions (avg/pt)	1.28	0.93	1.45

On average, more AVF are attempted and used in NHD (0.51/pt) compared with SDH (0.20/pt); p<0.0001. Patients cannulating AVF with buttonhole technique needed more retraining than rotating site cannulation. On average there were more AVG interventions (for stenosis and thrombosis) in NHD than in SDH (p=0.04). However, the overall intervention rates and complication rates did not differ between NHD and SDH. There was longer AVF survival for NHD patients compared with both frequent FHD and SDH (p=0.004)



(d_type=3 is NHD; D_type=2 is SDH)

Conclusions: Patients on frequent dialysis have similar vascular access interventions. AVF in patients on NHD have greater patency compared with SDH and FHD. Further evaluation of the impact of patient characteristics and prior dialysis history on AVF outcomes in frequent dialysis is required.

Funding: Clinical Revenue Support

FR-PO1719

Impact of Early Decline in Residual Renal Function on the Body Composition Changes and Clinical Outcomes during the First Year in the Continuous Ambulatory Peritoneal Dialysis Patients Kyu-Hyang Cho,[1] Jun-Young Do,[1] Seokhui Kang,[1] Jong-Won Park,[1] Kyung-Woo Yoon,[1] Tae Woo Kim.[2] *[1]Internal Medicine, Yeungnam University Hospital, Daegu, Republic of Korea; [2]Internal Medicine, Soonchunhyang University Gumi Hospital, Gumi, Republic of Korea.*

Background: Preservation of residual renal function in peritoneal dialysis patient is essential to improve clinical outcomes. Therefore, the authors investigated the effect of rapid decline of residual renal function in early period on clinical outcomes and body composition changes in CAPD patients.

Methods: Among new incident CAPD patients from May 2001 to December 2009 in our hospital, 200 patients who finished 12 month protocol (male: 103, mean age: 50.0 ± 13.2 years, DM: 94) were analyzed. Patients were assigned to high GDP group (n=103, Dianeal® and Stay-safe®) and low GDP group (n=97, Physioneal® and Balance®). We defined early RRF decline group (n=68) as more than 5 ml/min decline in RRF at the first month after initiation of PD from GFR just before initiation of PD. Clinical indices and UFV during the PET were measured at the first , 6th and 12th month. Body composition including LBM and fat mass were measured using BIA at the first and 12th month.

Results: 1) Baseline characteristics between early RRF decline group and non-early RRF decline group were not significantly different. 2) Incidence of early RRF decline group was 34%. There is significant positive correlation between RRF at the first month and RRF at the 12th month . 3) Early RRF decline group showed significant lower RRF at the 6th and 12th month, and also significant higher CRP and lower serum albumin at the 12th month than non-early RRF decline group. Early RRF decline group showed significant lower BW, TBW and LBM at the 12th month than non-early RRF decline group. There were no significant differences in RRF at the 12th month between the high GDP and the low GDP group.

Conclusions: Early decline of RRF in the first month could be associated with body composition changes and clinical outcomes during the first year in CAPD patients. It is suggested that preservation of RRF in early period of peritoneal dialysis is important to improve clinical outcomes and nutritional status.

FR-PO1720

Adequate Kt/V and Its Practical Data in Infants Receiving Peritoneal Dialysis Shojiro Okamoto, Tomoyuki Sakai, Riku Hamada, Yuko Hamasaki, Kenji Ishikura, Hiroshi Hataya, Masataka Honda. *Department of Nephrology, Tokyo Metropolitan Children's Medical Center.*

Background: In children who are receiving peritoneal dialysis (PD), the adequate Kt/V urea/week remains controversial. The K/DOQI Guidelines recommendation that an adequate Kt/V urea/week is above 1.8/week. The Japanese guidelines recommend a value above 2.5/week for children and above 3.0/week for infants.

Methods: In our institution, the target BUN level is below 70 mg/dl for children who are receiving PD and adequate nutrition. We studied infants who were receiving ambulatory PD treatment during the 1-year period from January 2009. In these patients, we analyzed the Kt/V urea/week and n-PNA (neutralized-protein equivalent of nitrogen appearance). Residual kidney function (RKF) was also evaluated.

Results: Nine patients were studied, including 4 with RKF. The median age was 5.0 years. Average Kt/V urea/week was 2.83 (2.66 in patients with RKF, 2.97 in anuric patients). Average n-PNA was 1.31 g/kg/day (1.34 g/kg/day in patients with RKF, 1.29 g/kg/day in anuric patients). Five patients received nightly intermittent PD (4 had RKF), 2 received continuous cyclic PD (both were anuric), and 2 received continuous cyclic PD + continuous ambulatory PD (both were anuric). The average BUN level in patients with RKF was 64.05 mg/dl, which was higher than that in anuric patients (61.4 mg/dl). The average ultrafiltration volume of PD was higher in anuric patients. The average rate of increase in the height standard deviation was 0.11. The average intraperitoneal volume was 895 ml/m^2. Only 1 patient had a complication of inguinal hernia.

Conclusions: In our series, the Kt/V urea/week of infants who were receiving PD was higher than the value recommended by the K/DOQI guidelines. Growth was satisfactory under appropriate nutrition, without complications. In anuric patients, the elevated level of Kt/V was in contrast to the lower level of BUN, suggesting that the Kt/V resulted from other determinants of the PD prescription, such as ultrafiltration volume. To perform PD appropriately in infants, not only the Kt/V, but also growth, nutrition, membrane permeability, and ultrafiltration volume should be comprehensively considered.

FR-PO1721

The Effect of Frequent Hemodialysis (HD) Therapies on Serum Beta2-Microglobulin (B2M) Levels in Patients Having Residual Renal Function (RRF) Alp Akonur, Baris U. Agar, Angelito A. Bernardo, J. Ken Leypoldt. *Renal, Baxter Healthcare Corporation, McGaw Park, IL.*

Background: Extracorporeal removal of B2M using high-flux hemodialyzers is largely hindered by the compartmentalization of its distribution volume. While the CONTRAST study has recently demonstrated that therapies using enhanced convective transport can reduce serum B2M levels in patients with and without RRF (Penne et al, CJASN 2010), an equivalent analysis is not yet available for frequent HD therapies.

Methods: We used a variable-volume, two-compartment kinetic model (Ward et al, KI 2006) to calculate weekly mean pretreatment serum B2M concentrations (MPC) with in-center (ICHD), short-daily (SDHD), and nocturnal (NHD) HD therapies in patients with increasing degrees of residual renal B2M clearance (RRC-B2M). The modeled B2M parameters were the intercompartmental mass transfer coefficient (40 ml/min), generation rate (0.17 mg/min), non-renal clearance (3 ml/min), and distribution volume (13.3 L) (Clark et al, JASN 1999). The RRC-B2M considered ranged from 0 to 4 ml/min. Dialysis frequency and duration were: 3 times/week and 4 hours/treatment (3X/4hr) (ICHD), 6X/3hr (SDHD), and 6X/8hr (NHD). A dialyzer clearance rate of 50 ml/min was assumed.

Results: Considerable reductions in MPC were achieved with both frequent therapies compared with ICHD. Similar, less pronounced, reductions were also found when RRC-B2M was gradually increased from 0 to 4 ml/min. NHD in patients without RRF and ICHD in patients with RRC-B2M of 4 ml/min resulted in comparable reductions.

	RRC-B2M (ml/min)	0	1	2	3	4
ICHD	MPC (mg/L) / Reduction (% a)	35.8 / NA	30.0 / 16.1	25.8 / 28.0	22.6 / 37.0	20.1 / 44.0
SDHD	MPC (mg/L) / Reduction (% b)	28.9 / 19.3	25.1 / 16.6	22.1 / 14.4	19.8 / 12.6	17.8 / 11.1
NHD	MPC (mg/L) / Reduction (% b)	19.6 / 45.3	17.9 / 40.4	16.5 / 36.2	15.2 / 32.6	14.2 / 29.4
a	Reduction in MPC calculated with respect to ICHD when RRC-B2M=0					
b	Reduction in MPC calculated with respect to ICHD at the corresponding value of RRC-B2M					

Conclusions: These simulations show that frequent HD therapies may help achieve considerable reductions in serum B2M levels in patients with and without RRF.

Funding: Pharmaceutical Company Support

FR-PO1722

PD-Associated Peritonitis as a Risk Factor for the Development of Encapsulating Peritoneal Sclerosis (EPS): The Historical Cohort Study of Fifty Patients with EPS in Japanese Single Center Masatsugu Nakao, Keitaro Yokoyama, Yudo Tanno, Ichiro Ohkido, Hiroshi Hayakawa, Masato Ikeda, Hiroyasu Yamamoto, Tatsuo Hosoya. *Division of Nephrology and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.*

Background: Encapsulating peritoneal sclerosis (EPS) is a serious complication that occurs in peritoneal dialysis (PD) patients. PD-associated peritonitis is speculated to be involved in the pathogenesis of EPS, however there have been very few statistical reports.

Methods: To evaluate PD-associated peritonitis as a risk factor for EPS, we compared a group of 50 PD patients who developed EPS with a group of 57 PD patients who did not develop EPS in the number of PD-associated peritonitis episodes, the causative organisms, and the duration of treatment.

Results: In this study, as we previous reported, a high permeability on peritoneal function testing and the β2MG level were examined as predictors of EPS. Comparison between the EPS and non-EPS groups yielded the following serum levels: 33.8 ± 8.54 vs. 29.2 ± 8.18 for β2 MG (P = 0.0062), 3.82 ± 5.38 vs. 0.20 ± 0.28 for CRP (P = 0.0000017), and 0.82 ± 0.10 vs. 0.67± 0.12 for D/PCr (P = 0.0000032). In addition, a past history of peritonitis was significantly more common (P = 0.0073) and the number of peritonitis episodes was significantly higher (1.80 ± 2.19 vs. 0.75 ± 1.07, P = 0.0019) in the EPS group. Moreover, the duration of treatment of peritonitis was significantly longer in the EPS group than in the non-EPS group (18.11 ± 15.3 vs. 10.2 ± 4.90, P = 0.002).On the other hand, *Streptococcus sp.* was more common causative organisms in the non-EPS group than in the EPS group. Oppositely *Staphylococcus aureus* was more common causative organisms in the EPS group. These results might indicate that the prevalence of antibiotic-resistant peritonitis is higher in the EPS group.

Conclusions: The number of peritonitis episodes and the prevalence of antibiotic-resistant peritonitis might be associated with the development of EPS.

FR-PO1723

Macrophage Infiltration and Factor V Expression in the Peritoneum through Peritoneal Dialysis Hideki Nosaka,[1] Michiko Bessho,[2] Toshiya Takeda,[3] Fumiaki Nogaki,[1] Yoshiaki Isogawa,[3] Tomomi Takeda,[3] Keiko Nomura,[3] Yoko Matsuo,[4] Noriko Mori,[4] Takahiko Ono.[1] *[1]Shimada Municipal Hospital, Shimada, Japan; [2]University of Shizuoka, Japan; [3]Kyoto Takeda Hospital, Kyoto, Japan; [4]Shizuoka General Hospital, Shizuoka, Japan.*

Background: Fibrin deposition was frequently observed in peritoneal fibrosis induced with long-term peritoneal dialysis (PD). Factor V in its active form (Va) serves as a membrane-bound cofactor to factor Xa, which facilitates activation of prothrombin to thrombin. Previously we have reported that expression of factor V in infiltrated macrophages, together with factor X deposition, progressed fibrosis in an experimental model (Perit Dial Int 29: 340-351, 2009). In the present study, we have conducted a clinicopathological study to examine factor V expression in the peritoneum through PD.

Methods: Peritoneal specimens were obtained at the opportunity of PD tube exchange, removal, or reinsertion in ten cases. Those included one case of encapsulating peritoneal sclerosis (EPS), one case of peritoneal fibrosis, three cases of peritonitis, three cases of reintroduction after each pause of PD, and two cases of tunnel infection. Specimens from ten cases at initiation of PD were evaluated as pre-PD controls, and compared with PD cases. Factor V was detected with a specific rabbit antibody, and macrophages were stained with a monoclonal anti-CD 68 antibody.

Results: In all pre-PD specimens, CD-68-positive cells infiltration was negatively or scarcely observed. CD-68-positive cells were moderate- or abundantly observed in the peritoneal specimens with EPS, peritoneal fibrosis, or peritonitis: spindle-shaped morphology in the EPS case, and rounded in the peritonitis cases. Accordingly, factor V-positive cells were also observed in the same manner as CD-68-positive cells. The frequency of CD-68-positive cells infiltration was significantly correlated with that of factor V-positive cells ($p < 0.01$).

Conclusions: Present data suggest that the factor V expression in macrophages may be induced in the peritoneum through inflammation during PD procedures, and that macrophages may change morphology from round to spindle-shape in the advanced phase.

Funding: Government Support - Non-U.S.

FR-PO1724

Reduced Sodium Sieving on Chronic Peritoneal Dialysis: Clinical and Physiologic Correlates Javier De Arteaga,[1,2] Carlos R. Chiurchiu,[1,2] Walter Guillermo Douthat,[1,2] Jorge Luis De la Fuente.[1,2] *[1]Nephrology, Hospital Privado, Cordoba, Argentina; [2]Postgraduate Nephrology, Catholic University, Cordoba, Argentina.*

Background: A reduced sodium sieving (RNAS) during a hypertonic glucose dwell can be the consequence of aquaporin dysfunction or a reduced osmotic conductance to glucose. Also has been claimed that a high transport status associates to RNAS. Other clinical correlates that could be associated to this situation like pts age, gender , previous transplants or total dialysis time have not been extensively evaluated.**Objective**: to study these clinical and physiologic variables that could be linked to RNAS in our chronic PD pts.

Methods: Since 2001, modified PET (4.25%) tests have been done yearly with ultrafiltration measured every hour mannualy in 46 chronic PD pts. In PET, initial plasmatic and hourly dialysate NA were analysed by ISE (indirect ion selective electrode). A High transport state was defined as a D/P creat > 0.81 at 4hs and RNAS was defined as a delta NA (dialysate NA 60 min- dialysate NA time 0) < 4.5. In 3 RNAS pts osmotic conductance to glucose was evaluated by a UNIPET.(Lamilia ,2010). None of these patients has an ultrafiltration failure (UF < 400cc at 4 hs)

Results:

Gr 1: (Reduced NA sieving): vs Gr 2: (normal)

	Group1.	Group2:	P value
N	11	35	
OCG (n=3)*	4,45 ± 0,64	_	_
D/P Cr 4hs	0,87 ± 0,056	0,76 ± 0,10	0,0006
Age (ys)	52,81 ± 17,16	51,2 ± 13,43	0,82
Total DP (ms)	23,64 ± 17,16	29,77 ± 30,94	0,75
Total dialysis(ms)	31,27 ± 26,72	43,72 ± 37,14	0,26
Gender (Fem)	18%	54,28%	0,10
Transport High	90,99%	31,43%	0,0006
Transplant (yes)	36%	25,71%	0,37

* osmotic conductance for glucose (UNIPET)

Conclusions: 11 patients (23.9%) have a reduced sodium sieving (RNAS). There is a strong association with a high transport state (p <.0.0006). A reduced osmotic conductance for glucose was not associated to RNAS in the only 3 patients evaluated (unipet).Although not significant (p< 0.10), there seems to be a trend towards a male gender in the RNAS group of our population.

FR-PO1725

Risk Factors for Mortality in Stable Peritoneal Dialysis Patients Seokhui Kang, Jun-Young Do, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. *Division of Nephrology, Departement of Internal Medicine, Yeungnam University Hospital, Daegu, Korea.*

Background: Peritoneal dialysis (PD) is an established treatment modality for patients with end stage renal disease (ESRD). The mortality rates of ESRD patients have significantly declined over the past decade. However, there are few reports on the risk factor for mortality in stable PD patients who survive for a considerable time.

Methods: We reviewed the medical records and we identified all the adult patients who received PD. Among these patients, those with <2 years of follow-up were excluded. Two hundred thirty-six patients were enrolled. The following data was documented from the patients' record: age at the initiation of PD, gender, the laboratory findings, the comorbidities (Davies index) and survival.

Results: The mean follow-up was 62.4 ± 24.2 months. The cumulative survival was 95.5% at 3 years and 80.3% at 5 years. On the univariate regression analysis, old age (>60-years-of-age), hypoalbuminemia, low residual renal function (RRF) (≤4 ml/min) and a high Davies index were associated with increased mortality for the stable PD patients. On the multivariate analysis, old age, low RRF and a high Davies index were proved to be the independent risk factors for mortality.

Table 1. Predictive factors affecting the mortality of long-term survivors on PD

Variables	Univariate analysis		Multivariate analysis	
	Odd ratio	p-value	Odd ratio	p-value
Age at the initiation of PD (≥60-years)	4.169 (2.350-7.395)	0.000	4.542 (2.446-8.436)	0.000
Gender (Female)	0.850 (0.505-1.429)	0.540		
TA-albumin (≤35g/L)	2.015 (1.198-3.391)	0.008	1.123 (0.627-2.010)	0.697
TA-CRP (≥5mg/L)	1.672 (0.975-2.868)	0.062	1.296 (0.725-2.318)	0.381
RRF (≤4ml/min) at 24 months	2.380 (1.021-5.548)	0.045	2.876 (1.178-7.021)	0.020
Davies index				
Low risk (reference)	1		1	
Intermediate risk	3.410 (1.703-6.828)	0.001	3.552 (1.727-7.307)	0.001
High risk	8.348 (3.147-22.143)	0.000	6.112 (2.132-17.516)	0.001

Abbreviations: CI, confidence interval; PD, peritoneal dialysis; TA, time-average; CRP, C-reactive protein; BMI, body mass index; RAS, rennin-angiotensin system; RRF, residual renal function.

Conclusions: The preservation of the RRF and proper management of the comorbidities may help to improve the survival of stable PD patients.

FR-PO1726

Efficacy of Lanthanum Carbonate in Patients on Peritoneal Dialysis: An Opportunity To Improve Nutrition? Rosamund Wilson,[1] Maggie Gill,[2] John Brian Copley.[3] *[1]Spica Consultants, Marlborough, United Kingdom; [2]Shire Pharmaceuticals, Basingstoke, United Kingdom; [3]Shire Pharmaceuticals, Wayne.*

Background: There may be an increase in the use of peritoneal dialysis (PD) in patients with chronic kidney disease (CKD) in the USA as a result of recent changes in dialysis reimbursement. Both PD and dietary phosphate restriction may have a negative effect on nutritional status so the use of an effective phosphate binder to control hyperphosphatemia may allow higher protein intake. This analysis assesses the efficacy of the non-calcium, non-resin phosphate binder lanthanum carbonate (LC) in reducing serum phosphorus (P) in patients on continuous ambulatory PD (CAPD).

Methods: This was a double-blind, placebo-controlled, parallel-group study, conducted in two parts. Part 1 involved dose titration up to 2250mg/day of LC over a 4-week period to achieve P<5.57mg/dL. In part 2 (a double-blind period), patients were randomized to receive their maintenance dose of LC or matching placebo for 4 weeks.

Results: Patients enrolled in this study had albumin levels of approximately 3.8g/dL (normal range=3.4–5.4g/dL) and these levels were maintained during LC treatment. Twenty-one patients receiving CAPD entered the double-blind phase; 10 were randomized to LC and 11 to placebo. At the end of treatment, 60% of patients treated with LC had controlled P (4.03–5.57mg/dL) vs. 10.0% in the placebo group. There was no difference in P levels between treatment groups at the start of the double blind phase (LC=4.87mg/dL, placebo=4.89mg/dL; *P*=0.96), but there was a significant difference at last visit (LC=4.83 mg/dL, placebo=6.96 mg/dL; *P*=0.0015). The most common adverse events during LC treatment were vomiting (26%) and nausea (23%) in part 1 and localized infection (20%, not considered related to LC) in part 2.

Conclusions: Patients on PD may require higher protein intake than those on hemodialysis, so it is important not to impair nutritional status while controlling serum P. Treatment with LC resulted in significantly reduced P levels in patients receiving CAPD at doses up to 2250mg. The more commonly used dose of 3000mg may be expected to give further reductions, possibly allowing greater protein intake.

Funding: Pharmaceutical Company Support

FR-PO1727

Baseline Residual Renal Function and Female Sex Are Associated with Technique Survival in Incident Peritoneal Dialysis Patients Kai Lu,[1] Jason Jones,[2] David C. Selevan,[3] Peggy Balcius,[2] Victoria A. Kumar.[1] *[1]Nephrology, SCPMG, Los Angeles, CA; [2]Research and Evaluation, SCPMG, Pasadena, CA; [3]Renal Business Group, SCPMG, Pasadena, CA.*

Background: Previous studies have reported that technique survival for peritoneal dialysis (PD) patients is highest in our region (Network 18) compared to the rest of the nation. We therefore sought to examine predictors of technique survival in a cohort of PD patients at a large healthcare maintenance organization.

Methods: We identified all adult patients in our database who initiated PD at our institution between January 1, 2001 and December 31, 2010. We included only patients with a renal creatinine clearance ($rCrCl_0$) and a total creatinine clearance ($totCrCl_0$) at initiation of PD. Baseline peritoneal creatinine clearance ($pCrCl_0$) was obtained by subtraction of $rCrCl_0$ from $totCrCl_0$ Patients who received a renal transplant during the study period were censored from the analysis. A Cox proportional hazards model was used to individually evaluate the effect of $rCrCl_0$, $pCrCl_0$ and $totCrCl_0$ on technique survival after adjusting for age, sex and Charleson Comorbidity Index (CCI).

Results: Baseline patient demographics are shown in Table.

Patient demographics

Number of patients	647
Median age in years	56.8 (47.5-65.6*)
Number of females (%)	296 (45.7)
CCI	6 (3-7*)
Number of diabetics (%)	399 (61.7)
PD as first modality (%)	402 (62.1)

* 95% Confidence Interval (CI)

Median technique survival for our patients was 4.64 years (CI 3.57-5.68). For each 10L/week increment in $rCrCl_0$ and $totCrCl_0$, there was a 4% increase in technique survival (Hazard ratio [HR] 0.996 for both, CI 0.996-0.999, p=0.006 and 0.0996-0.999, p=0.007, respectively). The HR for $pCrCl_0$ was 1.0 (CI 0.995-1.003, p=0.947). Female sex predicted technique survival in all 3 models, most strongly in the $rCrCl_0$ model (HR 0.582, CI 0.441-0.769, p<0.001).

Conclusions: $rCrCl_0$ and $totCrCl_0$ are associated with PD technique survival, but $pCrCl_0$ is not. Baseline renal function and female sex appear to be the strongest predictors of technique survival in our patients.

Funding: Pharmaceutical Company Support

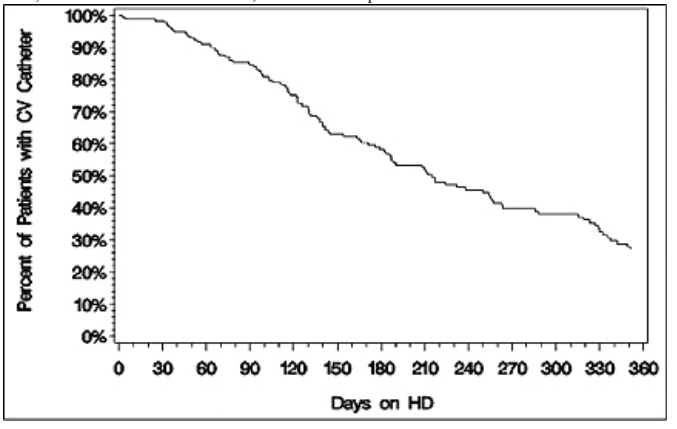
FR-PO1728

Vascular Access Use in Patients Who Transfer from Peritoneal Dialysis to Hemodialysis Leslie P. Wong, Sumi J. Sun, David Francis Nash. *Medical and Clinical Affairs, Satellite Healthcare, San Jose, CA.*

Background: PD dropout is common, with 45% of incident patients leaving PD by 2 years. Many patients transfer to HD and require vascular access. Unfortunately, there is little data describing access use and outcomes in these patients.

Methods: We analyzed the Satellite Healthcare database between January 1993 and May 2010 for patients who transferred from PD to HD. After excluding 432 records with insufficient data or multiple transfers, 439 patients were studied. One-year outcomes included probability of central venous catheter (CVC) use over time, rate of access or bacteremia-related hospitalization and subsequent modality status.

Results: Demographics were: mean age 63, 56% white, 55% male, 49% DM, 13% CHF, 95% Kt/V > 1.7, 85% high transporters, 31% with residual kidney function and 75% with albumin < 3.5. Median duration on PD was 358 days and 48% were incident PD patients. Leading reasons for transfer were peritonitis 15% and psychosocial 11% but only recorded for 42% of patients. Sixty-six percent of patients transferred to HD with a CVC. More incident PD patients started with a CVC (73%) compared to patients with prior HD (59%) p=0.003. Median number of CVC days was 215, with 84% of patients still using a CVC after 90 days (see figure). Forty percent of CVC patients switched to an AVF/AVG within 1 year. Access or bacteremia-related hospitalization rate was 49 admissions/1000 patient years and did not differ between groups. At 1 year 53% of patients remained on HD, 15% died, 21% transferred back to PD, 3% were transplanted and 8% moved.



Conclusions: CVC use predominates in patients who transferred from PD to HD. Though most patients stayed on HD, conversion to AVF/AVG was suboptimal. Hospitalization rate was not higher for CVC patients but may reflect underreporting. Vascular access planning for PD patients warrants further study.

FR-PO1729

Modeling Intraperitoneal Cefazolin and Cefepime for Nocturnal Intermittent Peritoneal Dialysis Pisut Katavetin, Talerngsak Kanjanabuch, Kriang Tungsanga, Somchai Eiam-Ong. *Divsion of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand.*

Background: Nocturnal intermittent peritoneal dialysis (NIPD) has been increasingly. Cefazolin and cefepime are the principal drugs for treatment of peritoneal dialysis (PD)-related peritonitis. Current guideline suggested daily intraperitoneal (IP) administration of drugs in the additional long day dwell which is not always practical. We, therefore, developed a mathematical model to test whether intermittent IP drug delivery into the first bag (5 liter) of NIPD could yield an optimal antibiotics level both in serum and in peritoneal fluid in patients undergoing 10 liters (L) NIPD in 5 cycles over 12 hours.

Methods: Two-compartment, fixed volume model for pharmacokinetics of cefazolin and cefepime in PD patients were developed. Level of cefazolin and cefepime in serum and in dialysate were then calculated according to the model using the pharmacokinetic parameters derived from the literature. The concentration of antibiotics in the PD fluid of the second to fifth cycle were assume to be about 0.6 time of that in the previous cycle due to the dilution of antibiotics by adding 2 L of fresh PD fluid from the second bag into the remaining 3 L first antibiotics containing bag.

Results: In a 70 kilogram (kg) NIPD patient, adding 1.5 gram (g) of cefazolin in the first 5 L bag of NIPD could yield optimal serum and peritoneal antibiotics concentration during the on-cycler period but may yield marginal concentration in peritoneal fluid (6-7 µg/ml) despite adequate serum concentration during the off-cycler period. For cefepime, 1 g of cefepime in 70 kg patient could yield optimal serum and peritoneal antibiotics concentration during both on-cycler and off-cylcer period although there was a considerably delay in time to reach optimal serum concentration in the first day. Increasing dose of cefepime to 1.5 g could ameliorate this problem.

Conclusions: Intermittent intraperitoneal administration of cefazolin and cefepime in the first bag of NIPD is a reasonable option for treatment of NIPD-related peritonitis with favorably increasing dose by 1.5 time.

FR-PO1730

Periostin- A Matricellular Protein Involved in Peritoneal Injury during Peritoneal Dialysis Niko Braun,[1] Kontheari Sen,[2] Mark Dominik Alscher,[1] Peter Fritz,[3] Martin Kimmel,[1] Achim Joerres,[4] Clemens D. Cohen,[2] Stephan Segerer.[2] *[1]Department of Internal Medicine, Division of Nephrology, Robert-Bosch Hospital, Stuttgart, Germany; [2]Division of Nephrology, University Hospital Zurich, Switzerland; [3]Department of Diagnostic Medicine, Division of Pathology, Stuttgart, Germany; [4]Department of Nephrology and Medical Intensive Care, Charité-Universitätsmedizin, Campus Virchow-Klinikum, Berlin, Germany.*

Background: Periostin is a matricellular protein involved in tissue remodelling. We hypothesized that this protein might be expressed in the peritoneal cavity of patients on peritoneal dialysis (PD) and patients with signs of encapsulating peritoneal sclerosis (EPS).

Methods: We localized periostin in peritoneal biopsies from patients on PD with EPS (n=7), on PD without signs of EPS (n=10), and compared it with biopsies taken during hernia repair as controls (n=11).

Results: Periostin was found in the wall of larger arteries and focally in the submesothelial zone in control biopsies. Patients on PD demonstrated interstitial periostin in variable amounts depending on the severity of submesothelial fibrosis. In EPS there was a very prominent expression in the sclerosis layer. Commonly the superficial layer was periostin negative. A semiquantitative score was most prominently associated with the diagnosis of EPS, as well as with the thickness of the submesothelial fibrosis zone.

Conclusions: Periostin is expressed in EPS and to a lesser extend in simple peritoneal sclerosis. It might play a role in the progression of peritoneal injury.

FR-PO1731

Bone Mineral Density and Abdominal Aortic Calcifications Detected by Computed Tomography in Peritoneal Dialysis Patients Pierluigi Di Loreto, Francesca K. Martino, Claudio Ronco. *Nephrology Dialysis Transplantation, San Bortolo Hospital, Vicenza, Italy.*

Background: Bone mineral density (BMD) is negatively correlated with vascular calcification and cardiovascular risk in CKD pts. Aim of this study is to confirm these observations in Peritoneal Dialysis (PD) patients.

Methods: We studied 45 PD patients (mean age 61,9 ±14,5 years, 41,3% Women and 58,7% Man, mean age Man 65,7±11 Y, Women 56,2±17 Y p=0,031 for sex) mean duration of PD was 21,9±25,4 months. These patients underwent an abdominal computerized tomography scan. The severity score for Abdominal aortic Calcifications (AOC) was: 1=none, 2=mild, 3=moderate, 4=severe. For each patient we collected the following laboratory data: calcium corrected for albumin, PTH, phosphorus, alkaline phosphatase, haemoglobin, BMI, diuresis, KT/V, use of Vitamin D, Cinacalcet, phosphate binders, ESA, warfarin. Quantitative CT measurements of BMD (mg/ml) using fully automated software were obtained at the first, second, third and fourth lumbar vertebrae. Statistical analysis was performed with SPSS.

Results: We found a negative correlation between BMD and age (M p=<0,006, r =-0,516, W p=<0,001, r= -0,763) and between BMD and calcium in women (p=0,037, r = 0,481). 25 pts (55,6% Man and 42,1% Women with no statistically significant difference between sexes) were osteoporotic (BMD <160 mg/ml), and respectively 14 and 11 of them showed severe and mild AOC. After 2 years the overall prevalence of fracture was 15,5% (7 pts). 12 pts (26%) died. All the pts who died and those with fracture showed a low BMD and a severe AOC. No correlations was found between laboratory data, BMI, diuresis, KT/V, use of medications, lenght of dialisys and BMD-AOC.

Conclusions: Our data confirm that low BMD is correlated with age, vascular calcification and cardiovascular risk as in CKD pts. We could not confirm the findings of other Authors who suggested that low body weight and low KT/V were the most important risk factors for low BMD in PD pts

FR-PO1732

Effects of Spironolactone on Residual Renal Function in Patients Receiving Peritoneal Dialysis Berna Yelken,[1] Numan Gorgulu,[2] Meltem Gursu,[3] Yasar Caliskan,[4] Halil Yazici,[4] Ay?egül Telci,[5] Rumeyza Kazancioglu,[6] Tevfik Ecder,[4] Semra Bozfakioglu.[4] *[1]Division of Nephrology, Department of Internal Medicine, Gaziosmanpasa Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey; [2]Division of Nephrology, Memorial Hospital, Istanbul, Turkey; [3]Division of Nephrology, Department of Internal Medicine, Haseki Education and Research Hospital, Istanbul, Turkey; [4]Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [5]Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Turkey; [6]Faculty of Medicine, Bezmialem vakif university, Istanbul, Turkey.*

Background: There is increasing evidence that long-term peritoneal dialysis (PD) is associated with structural changes in the peritoneal membrane. It is unknown whether spironolactone affects RRF in addition to the structural changes in the peritoneal membrane.

Methods: 23 (13 female) patients with RRF (>400 ml/day) receiving PD were evaluated.After measuring baseline serum high sensitive C-reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), connective tissue growth factor (CTGF) and N- terminal pro-B-type natriuretic peptide (NT-proBNP) level, ultrafiltration (ml/day), RRF, creatinine clearance (CrCL), Kt/V, normalized protein catabolic rate (nPCR), peritoneal transport status and dialysate CA125 level and VEGF, spironolactone (25 mg daily) therapy was given for six months. At the end of six months all measurements were repeated.

Results: The mean age of the patients was 46±13 years. Duration of PD was 15±21 months (range; 2-88). After spironolactone therapy, the mean dialysate CA125 level significantly increased as compared to baseline (20.52±12.06 U/ml vs. 24.44±13.97 U/ml; p=0.028).Serum hs-CRP, VEGF, TGF-β, CTGF and NT-proBNP levels, dialysate VEGF levels, daily ultrafiltration, Kt/V, nPCR and peritoneal transport status of patients were similar between two study periods. There was no decrease in RRF and CrCL at the end of six months.

Conclusions: Spironolactone therapy for six months appears to preserve RRF and peritoneal structure in patients with PD.

FR-PO1733

Agreement between Peripheral Venous and Arterial Blood Gas Measurements in the Intensive Care Unit Ho Sik Shin, Jin Hee Park, Sung Bin Kim, Yeon Soon Jung, Hark Rim. *Internal Medicine, Kosin University College of Medicine, Busan, Korea.*

Background: Venous blood gas (VBG) analysis is safer than arterial blood gas (ABG) analysis and may be a suitable alternative for determining acid-base status. The objective of this study was to examine the agreement between ABG and peripheral VBG samples for all commonly used parameters in medical intensive care unit (ICU) patients.

Methods: We performed a single-center, prospective trial to assess the agreement between arterial and peripheral VBG measurements in a medical ICU. When an ABG was deemed necessary as part of ICU management, a peripheral venous sample was also obtained within 2 minutes to examine the agreements among the pH, PCO_2, bicarbonate and total CO_2 measurements. All of the samples were immediately analyzed using the same arterial blood gas analyzer. A maximum of 5 paired ABG-VBG samples were obtained per patient to prevent any single patient from dominating the data set.

Results: Regression equations were derived to predict arterial values from venous values as follows: Arterial pH = 0.763 X venous pH + 1.786, arterial bicarbonate = 0.822 X Venous bicarbonate + 2.815 and arterial HCO_3 = 0.639 X Venous total CO_2 + 5.360. The mean ABG minus peripheral VBG differences for pH, PCO_2, and bicarbonate were not clinically important.

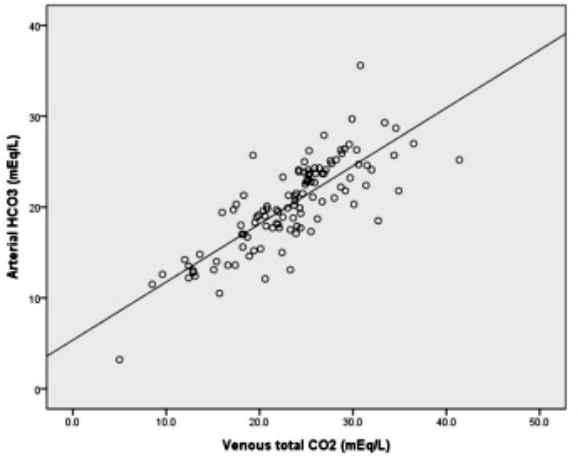


Figure 4. Correlation between peripheral VBG value for total CO_2 and ABG values for HCO_3 R = 0.802, Arterial HCO_3 = 0.639 X Venous total CO_2 + 5.360

Table 1. Patient characteristics

Characteristic	Value
Age (years; mean±SD)	65.5 ± 12.4
Gender (male/female; n %)	20 (58.8) / 14 (41.2)
Intubated (n/N %)	8/34 (23.5)
Hypotensive (n/N %)	30/34 (88.2)
Inotropic agent use (n/N %)	30/34 (88.2)
Primary diagnosis (%)	
Sepsis	14.7
Upper GI bleeding	2.9
Renal failure	67.6
Pneumonia	2.9
Pancreatitis	5.9
Respiratory failure of unknown cause	5.9

Conclusions: Peripheral venous pH, PCO_2, bicarbonate and total CO_2 can replace their arterial equivalents in many clinical contexts encountered in the ICU.

FR-PO1734

A Phase 1, Open-Label Study of Intravenous Conivaptan in Subjects with Renal Impairment Keith Erdman,[1] Michael Roy,[1] Anura Abeyratne,[1] Lisa Plumb,[1] Dennis Riff,[2] Jim Keirns.[1] *[1]Astellas Pharma Global Development, Inc., Deerfield, IL; [2]Advanced Clinical Research Institute, Anaheim, CA.*

Background: Conivaptan is a vasopressin $V_{1A/2}$ receptor antagonist approved for intravenous treatment of euvolemic and hypervolemic hyponatremia in hospitalized patients. This study assessed the pharmacokinetics, protein binding, and safety of conivaptan in subjects with normal renal function or with mild or moderate renal impairment.

Methods: Subjects with normal renal function (Cockroft-Gault formula, eGFR >80mL/min), or mild (50–80 mL/min) or moderate (30-49 mL/min) renal impairment were administered conivaptan as a loading dose of 20 mg intravenously over 30 minutes, followed by a 20 mg/23.5 hour continuous infusion on day 1 and a 20 mg/24 hour continuous infusion on day 2. Serial blood sampling for calculation of conivaptan pharmacokinetic parameters C_{LD}, C_{48}, and $AUC_{0-\infty}$ and urine collection to assess urinary conivaptan excretion began immediately before the start of the loading dose and continued for 96 hours.

Results: Mean pharmacokinetic parameters tended to be higher in the mild renal impairment group due to one subject with a low free fraction and one subject with a spurious conivaptan concentration at hour 24.

Conclusions: Renal Impairment

	Normal (n=8)	Mild (n=8)	Moderate (n=8)
C_{LD} (ng/mL)	548 (151)	794 (251)	592 (185)
C_{48} (ng/mL)	52 (15)	93 (66)	55 (38)
$AUC_{0-\infty}$ (hr•ng/mL)	2857(638)	5053 (2815)	3267(1649)
Fraction unbound	0.0073 (0.0015)	0.0067 (0.0028)	0.0077 (0.0028)
Ae_{last} %	1.5 (0.4)	1.5 (0.2)	1.0 (0.4)
CL_R (L/hr)	0.33 (0.10)	0.22 (0.09)	0.20 (0.08)

Data are mean (SD)

Conivaptan was minimally excreted in the urine and >99% protein bound. Ae_{last}%, renal clearance, and protein binding values were similar across groups. The most frequently reported AEs were associated with infusion site reactions. All events were mild in intensity, there were no serious AEs, and no subjects discontinued from the study.

Conclusions: Conivaptan was well tolerated in all subjects with no indication of safety issues. There were no significant differences in exposure between the 3 groups.

Funding: Pharmaceutical Company Support

FR-PO1735

Aggressive and Unnecessary Treatment of Mild Hyperkalemia: Inappropriate Use of Kayexalate Gabriel El-kass, Farhanah Yousaf, Emily Yan, Bruce S. Spinowitz, Chaim Charytan. *Department of Nephrology, New York Hospital Queens, Flushing, NY.*

Background: Although hyperkalemia is potentially a lethal metabolic complication which may require emergent treatment, there is no consensus defining clinically significant hyperkalemia, nor the settings which require K^+ lowering therapy. The literature suggests that clinically significant hyperkalemia occurs at K^+ levels >;6.0 mEq/L with EKG changes or at K^+ >;6.5 mEq/L [Levinsky, 1966; Greenberg, 1998; AHA 2005]. A recent study suggested that even more severe hyperkalemia may not require aggressive therapy nor hospitalization [Charytan D, 2000]. By extrapolation, it may be assumed that lower levels of hyperkalemia might not require aggressive intervention. This becomes particularly important in view of concerns raised about the efficacy and safety of Kayexalate (SPS) [Sterns, 2010]. During an investigation on the efficacy and safety of SPS at our institution, we decided to explore how frequently clinically mild hyperkalemia, a serum K^+ ≤5.6 mEq/L, was treated with SPS with or without additional interventions.

Methods: Medical records of patients ≥18 years receiving SPS in emergency department or as in-patients between June 2010 and August 2010 were reviewed.

Results: 154 patients were prescribed SPS 249 times. Of these, 92 patients, 45 males and 47 females, aged 76.0±13.3 yrs, received 106 doses of SPS for a serum K^+ ≤5.6 mEq/L. SPS was administered as a 30 g dose in 99 cases and as a 15 g dose in 7 cases. 103 doses were given orally, 2 doses rectally, and 1 dose via PEG.

	Pre SPS K+	Post SPS K+	Delta Δ	p Value
Mean±SD	5.37±0.26	4.84±0.66	0.53±0.07	<0.001

31 available EKGs were unremarkable for hyperkalemia-associated changes. 7 patients required K^+ supplements due to subsequent development of hypokalemia.

Conclusions: The above experience suggests that mild clinically insignificant hyperkalemia is often treated aggressively and unnecessarily. Considering the infrequent but potentially serious adverse events associated with SPS, intestinal necrosis and perforation, cost of therapy, and possible unnecessary hospitalization or delayed discharge, it may be appropriate to redefine significant hyperkalemia and develop new therapeutic guidelines.

FR-PO1736

Hospital Pharmacy Guidelines for the Administration of 3% Sodium Chloride in Children Siddharth Shah,[3] Juan Carlos Ayus,[2] Michael L. Moritz.[1] *[1]Pediatrics, Chidlren's Hosptial of Pittsburgh of UPMC; [2]Renal Consultants of Houston; [3]Chidlren's Hospital of Pittsburgh of UPMC.*

Background: Hyponatremic encephalopathy is a medical emergency with the treatment of choice being 3% sodium chloride (NaCl). A 100 cc or 2 cc/kg bolus of 3% NaCl is the preferred therapy (NEJM 2005:353) that has now been accepted as the standard of care (NephSAP 2011:10). We suspect that children's hospital pharmacies may have policies that would restrict the administration of a 3% NaCl bolus due to concerns for overcorrection of hyponatremia.

Methods: An internet survey was distributed to the pharmacy directors of 43 children's hospitals participating in the Child Health Care Corporation of America (CHCA) network in order to assess their policies for administering 3% NaCl.

Results: The response rate was 65%. 71.4% of respondents had policies for the administration of 3% NaCl. The majority of respondents had policies restricting the volume dispensed (53.6%) and rate of administration allowed (67.9%). The majority of pharmacies (57.1%) also would not allow 3% NaCl to be used in a non-ICU setting or via a peripheral IV. Of those who allowed 3% NaCl through a peripheral IV, 57.1% had restrictions on its use. Only 24% of respondents had similar policies for the administration of hypertonic sodium bicarbonate or mannitol.

Conclusions: The majority of children's hospital pharmacies have restrictive policies for the rate, volume, route and setting of administration of 3% NaCl. These restrictive policies could prevent the timely use of a 3% NaCl bolus for the treatment hyponatremic encephalopathy in children.

FR-PO1737

Urine of Patients with Cerebral/Renal Salt-Wasting Syndrome Contains a Substance That Inhibits Reabsorptive Sodium Flux in LLC-PK1 Cells Steven J. Youmans,[1] John K. Maesaka.[2] *[1]Dept. of Biomedical Sciences, NY College of Osteopathic Medicine, NYIT, Old Westbury, NY; [2]Dept. of Medicine, Winthrop University Hospital, Mineola, NY.*

Background: We have proposed that the persistent elevation of fractional excretion (FE) of urate after correction of hyponatremia identifies patients with cerebral/renal salt-wasting syndrome (RSW), being most common in patients with neurosurgical (NS) diseases. Our study sought to demonstrate whether natriuretic activity, previously found in the plasma of NS patients with increased FEurate >10% and normonatremia, is also present in urine. The control population was made up of normonatremic NS patients with normal FEurate and SIADH.

Methods: Ammonium precipitates (ppts) of urine were dialyzed (10 kDa), lyophilized, and quantitated by protein content. Ppts were applied to monolayers of a pig proximal tubule cell line (LLC-PK1) cultured on commercial transwells and the transcellular movement of 22Na was determined over time.

Results: Ppts from 5 of 6 RSW patients inhibited the absorptive sodium flux by -30.5 ±2.9% at 5 µg protein/ml and -31.9±3.3% at 10 µg protein/ml versus 4 Controls

at the same concentrations. (Mean ± SEM, P=.002 and P=.003 at 5 and 10 µg protein/ml respectively; 3-5 repetitions per RSW and 2-4 per Control patient.) It was noted that means for Controls showed some stimulation of Na absorption versus vehicle, which did not reach significance (P=.18 and P=.14). In paired experiments in which different concentrations of RSW ppt were tested in the same run, a dose-response was seen, inhibition being greater for a change from 10 to 20 µg/ml than for a change from 5 to 10 µg/ml (-23.0±3.8% vs. -11.6±4.4% respectively; P=.045).

Conclusions: The results suggest that ammonium precipitable substance(s) in urine from NS patients with increased FEurate and normonatremia inhibits transcellular transport of Na as compared to ppts from normonatremic NS patients with normal FEurate and SIADH. The natriuretic substance may contribute to the renal Na loss seen in RSW. These studies support our proposal that normonatremia with increased FEurate might identify patients with RSW and that RSW is common in NS patients.

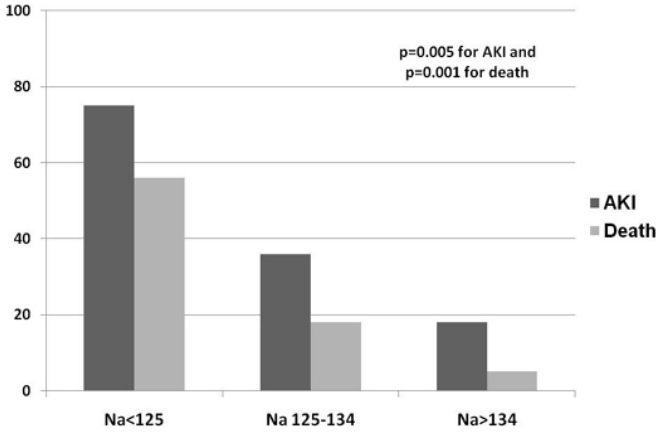
FR-PO1738

Hyponatremia and Acute Kidney Injury (AKI) Are Associated with Mortality in HIV Patients with Neurotoxoplasmosis Alexandre Braga Liborio,[1] Geraldo B. Silva,[1,2] Carolina Gaspar Cavalho Heil Silva,[2] Adalberto Studart Neto,[2] Francisco José Correia Lima Filho,[2] Elizabeth De Francesco Daher.[2] *[1]School of Medicine, University of Fortaleza, Fortaleza, Ceara, Brazil; [2]Department of Internal Medicine, School of Medicine, Federal University of Ceara, Fortaleza, Ceara, Brazil.*

Background: Toxoplasmosis a main cause of neurologic symptoms among HIV-infected patients. AKI has not been studied in this population. Hyponatremia is a potential complication of neurologic-related disorders. The objective of this study is to describe the occurrence of hyponatremia and its relationship with AKI and mortality in HIV-associated neurotoxoplasmosis.

Methods: Retrospective cohort of patients with HIV-related neurotoxoplasmosis. AKI was considered only if RIFLE criteria were achieved after hospital admission. Serum creatinine (Scr) at admission was considered baeline Scr.

Results: A total of 92 patients were included, with an average age of 36 +/- 9 years old; 73.9% were male. Hyponatremia at admission was observed in 40 (43.4%) patients and AKI developed in 25(27.1%) during hospital-stay. Sulfadiazine was the choice treatment in 81% of cases and SMZ-TMT in the remaining, with no difference in AKI-occurrence. Death occurred in 13 cases (14.1%). Sodium level was associated with AKI and mortality.



After adjusting for other clinical and laboratory variables, only male gender (OR 7.89, p=0.03) and hyponatremia at admission (OR 4.73, p=0.02) were predictors for AKI. Independent risk factors for death were AKI (OR 8.3, p<0.0001), hyponatremia (OR 9.9, p<0.0001) and diarrhea (OR 5.86, p<0.053).

Conclusions: Hyponatremia and AKI are common events in neurotoxoplasmosis and are associated with mortality. As patients presented hyponatremia at admisson and developed AKI during hospital-stay, we suggest hyponatremia is a primary event in this population and may have a causative role in AKI-development.

Funding: Government Support - Non-U.S.

FR-PO1739

Association of Hyponatremia and Hypernatremia with Mortality in Burn Patients Molly A. Tilley,[1] Ian J. Stewart,[1] Benjamin D. Morrow,[1] Keith W. Kramer,[1] Chris A. Gisler,[2] James K. Aden,[3] Evan Renz,[3] Kevin Chung.[3] *[1]San Antonio Military Medical Center; [2]University Texas Health Sciences Center San Antonio; [3]Burn Center, US Army Institute of Surgical Research.*

Background: Dysnatremias are associated with mortality in many groups. However, this relationship has not been well described in burn patients. Our study seeks to determine if moderate to severe hyponatremia or hypernatremia are independently associated with mortality in this population.

Methods: We examined all admissions to our institution's burn center from January 2003 to December 2008. Patients were included if they were at least 18 years old and had a serum sodium obtained during their hospitalization. Exclusion criteria included end stage renal disease and death within 24 hours of admission. Independent variables included age, gender, percentage total body surface area burned (%TBSA), percentage of third degree burn, inhalation injury, injury severity score (ISS), Acute Kidney Injury Network (AKIN) stage, hypernatremia, and hyponatremia. These variables were examined via multiple logistic regression analysis against death. Moderate to severe hyponatremia and hypernatremia were defined as serum sodium less than 130mmol/l and greater than 150mmol/l, respectively.

Results: In 1973 subjects with a mean age of 36±16, average %TBSA of 16±18, and average ISS of 10±12, hypernatremia occurred in 9.8% (n=194) while hyponatremia occurred in 7.0 % (n=138) during their admission. Overall in-hospital mortality was 7.6% (n=150). Among those with hypernatremia and hyponatremia, mortality was 33.5% and 13.5% respectively which were both significantly higher than the mortality rate (4.5% and 6.8%) among those without these abnormalities (p<0.0001 and p=0.0027, respectively). On multiple logistic regression, however, only age, %TBSA, ISS, and AKIN stage were found to be significant predictors of mortality. Hypernatremia (OR 0.66, 95% CI 0.35-1.25, p=0.20) and hyponatremia (OR 0.46, 95% CI 0.21-1.03, p=0.06) were not.

Conclusions: In contradistinction to other groups, neither hyponatremia nor hypernatremia are independent predictors of mortality in the burn population.

Funding: Other U.S. Government Support

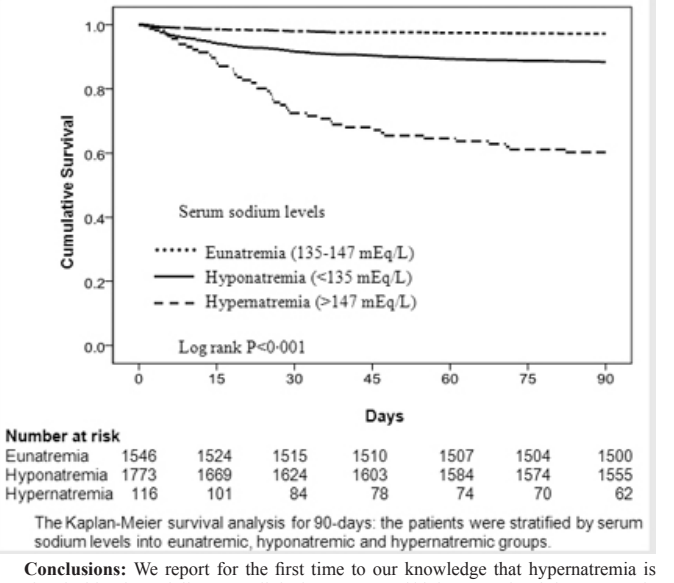
FR-PO1740

The Cost and Clinical Outcomes of Hypernatremia in Hospitalized Cancer Patients Elson Thomas,[1,2] Simit Doshi,[2] Amit Lahoti,[2] Abdulla K. Salahudeen.[2] *[1]Department of Internal Medicine, Division of Renal Disease & Hypertension, University of Texas Medical School at Houston, TX; [2]Division of Internal Medicine, UT MD Anderson Cancer Center, Houston, TX.*

Background: Disorders of sodium balance are the most common of the electrolyte abnormalities seen in clinical practice. However, unlike hyponatremia, little is known about hypernatremia, especially related to outcomes. The objective was to evaluate the frequency and severity of hypernatremia in patients admitted to the hospital and its associations with clinical outcomes and hospital costs.

Methods: We analyzed prospectively collected data on patients with cancer admitted to the UT MD Anderson Cancer Center over a 3-month period in 2006. Clinical outcomes and costs were compared among hypernatremics, eunatremics and hyponatremics (serum Na >147 mEq/L, 135-147 mEq/L and <135 mEq/L, respectively).

Results: Of 3886 patients admitted, 3%, 46% and 51% were respectively hypernatremic, eunatremic and hyponatremic. The length of hospital stay in hypernatremics was 4-fold higher than eunatremics (e.g., 27 ± 22 days vs. 6 ± 5 days, p < 0.001) and 2-fold higher than hyponatremics. Moreover, the multivariate hazard ratios (HR) for mortality of hypernatremics were significantly higher than eunatremics (in-hospital HR: 3.83, 95% CI: 2.10 - 6.95; p<0·001 and 90-day HR: 10.20, 95% CI: 6.50-15.99; p<0·001) or hyponatremics (in-hospital HR: 1.73, 95% CI: 1.20-2.50; p<0.005 and 90-day HR: 2.99, 95% CI: 2.14-4.20; p<0.001) (see figure: survival curve). The cost was higher for hypernatremics compared to rest of the groups (46% higher than eunatremics, 37% higher than hyponatremics; p<0.001 for both).



The Kaplan-Meier survival analysis for 90-days: the patients were stratified by serum sodium levels into eunatremic, hyponatremic and hypernatremic groups.

Conclusions: We report for the first time to our knowledge that hypernatremia is associated with substantially worse clinical outcomes and higher costs than is hyponatremia and eunatremia. These findings warrant similar studies in non-cancer patients.

FR-PO1741

Inverse Correlation of Serum Sodium Level with Body Temperature in Children with Common Febrile Diseases Hideki Matsumura,[#1] Akira Ashida,[#2] Akihiko Shirasu,[#2] Hyogo Nkakura,[#2] Motoshi Hattori,[#3] Hiroshi Tamai.[#2] *[1]Pediatrics, Hirakata City Hosptital, Osaka, Japan; [2]Pediatrics, Osaka Medical College, Osaka, Japan; [3]Pediatric Nephrology, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Hyponatremia is a common electrolyte abnormality in hospitalized patients. Routine use of hypotonic maintenance fluids can lead to potentially fatal hyponatremia in cases of excess antidiuretic hormone (ADH) production. Several studies have demonstrated that non-osmotic ADH activity contributes to the development or persistence of hyponatremia in children with common febrile diseases. However, as the relationship between hyponatremia and body temperature has remained unclear, we examined this relationship in children with common diseases.

Methods: In this retrospective case study based on chart review, 2,027 children (1,150 males and 877 females, 1,418 febrile and 609 non-febrile patients) presenting with acute illnesses at Hirakata City Hospital, between November 2008 and October 2009 and for whom blood test data were available, were enrolled. The median age of this cohort was 2.7 years and the mean serum sodium concentration was 136.5 mEq/L: 454 of the 2,027 patients showed hyponatremia, with a serum sodium level of <135 mEq/L. The patients were classified into four groups on the basis of body temperature: <37°C, 37°C (37.0 - 37.9), 38°C (38.0 - 38.9) and ≥39°C, and serum sodium concentration was compared among them.

Results: The mean sodium level was significantly lower in febrile than in non-febrile patients: 135.7 mEq/L (95%CI, 135.5 - 135.8) versus 138.4 mEq/L (138.2 - 138.6), respectively. The serum sodium levels in the temperature groups were in ascending order, 138.6mEq/L (138.4 - 138.8), 137.3 mEq/L (137.1 - 137.6), 136.1 mEq/L (135.8 - 136.3) and 134.6 mEq/L (134.4 - 134.9), respectively. The serum sodium level in each individual temperature range was significantly different from that in the others, and became lower as the body temperature increased.

Conclusions: Fever may play an important role in the development of hyponatremia in children with common febrile diseases. Care is necessary when administering intravenous fluid to febrile pediatric patients.

FR-PO1742

Severe Hyperkalemia Requiring Hospitalization; Predictors of Mortality and Improvement Jung Nam An,[1] Jung Pyo Lee,[1,2] Yun Kyu Oh,[1,2] Chun Soo Lim.[1,2] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea.*

Background: Severe hyperkalemia (K≥6.5mEq/L) is a potentially life-threatening electrolyte disorder. To treat it promptly and effectively, it is essential to know its causes, risk factors, clinical manifestations, and predictors of mortality and improvement.

Methods: A total of 610 Korean patients over age 18 who were hospitalized with a diagnosis of severe hyperkalemia from August 2007 to July 2010 became the subject of this retrospective study. The medical records were reviewed.

Results: Hypertension was the most common underlying medical condition, followed by diabetes and chronic kidney disease (CKD). The most common precipitating factor was metabolic acidosis, followed by acute kidney injury (AKI), drugs such as angiotensin receptor blocker, K-sparing diuretics, beta blocker, and non-steroidal anti-inflammatory drugs (NSAIDs), and infection. The higher serum potassium levels were significantly associated with the use of NSAIDs, metabolic acidosis, and the history of recurrent severe hyperkalemia. Also, mortality was strongly correlated with the severity of hyperkalemia. Changes in electrocardiogram findings were associated with higher potassium levels, but didn't increase the mortality rate themselves. The mortality rate was higher in patients with underlying disease such as malignancy and severe medical conditions including infection and bleeding. In addition, AKI in patients with normal baseline renal function and metabolic acidosis were definite predictors of mortality. Whereas, mortality rate had a lowering tendency in patients with underlying disease such as hypertension, AKI on underlying CKD, and drug-induced hyperkalemia.

Conclusions: Severe hyperkalemia requiring hospitalization and prompt treatment occurred in various medical conditions. The precipitating factors were also diverse. The mortality rate was especially higher in patients with severe underlying diseases and with new-onset AKI rather than AKI on underlying CKD.

FR-PO1743

Role of Kidney-Specific WNK1 in the Regulation of NCC and ROMK by High Dietary Potassium Intake Zhen Liu, Chou-Long Huang. *Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: Upregulation of secretory K+ channels including ROMK in the distal nephron is an important adaptive response to high dietary K+ loading. Earlier studies in adrenalectomized animals suggest that the regulation is independent of aldosterone. Recent studies in rodents show that K+ loading also downregulates NCC, which would lead to increased Na+ reabsorption via ENaC and enhance K+ secretion via ROMK. The mechanism for inhibition of NCC by K+ loading remains elusive. An increase in the aldosterone by K+ loading should stimulate NCC. WNK1 is a ubiquitous protein kinase of which increased expression causes hypertension and hyperkalemia. KS-WNK1 is a kidney-specific splice variant of WNK1 and reportedly activates NCC and inhibits ROMK, respectively. High dietary K+ intake stimulates KS-WNK1, and in this study we examine the role of KS-WNK1 in the regulation of NCC and ROMK by K+ loading.

Methods: KS-WNK1-null mice were generated by homologous recombination to delete exon 4A, the unique coding exon of KS-WNK1.

Results: Previously, we reported that KS-WNK1-null mice have Na+ retention, increased surface expression of NCC and elevated blood pressure on a high-Na+ diet compared to WT littermates. Here, we reported that serum K+ levels were not significantly different between null and WT mice fed a normal K (1%) diet (4.4 ± 0.2 vs 4.2 ± 0.2 mM), but were significantly higher in KS-WNK1-null mice fed a high K (10%) diet for 10 days (5.0 ± 0.2 vs 4.2 ± 0.2 mM, $p<0.05$). Immunofluorescent staining using antibodies against NCC and p-T58-NCC showed that dietary K+ loading decreased the abundance of total NCC and phospho-NCC in WT but had lesser effect in KS-WNK1-null mice. Western blot analysis confirmed the differential effect of K+ loading on NCC in WT vs KS-WNK1-null mice. Immunofluorescent staining revealed that dietary K+ loading increased the apical abundance of ROMK in AQP2-positive tubules in WT mice, but had a much less effect on ROMK in KS-WNK1 mice.

Conclusions: These results support the hypothesis that increased expression of KS-WNK1 contributes to the downregulation of NCC and upregulation of ROMK by dietary K+ loading.

Funding: NIDDK Support

FR-PO1744

Characterisation of Mouse Models of Specific Inactivation of WNK1 in the Distal Nephron Cara J. Busst,[1] Xavier Jeunemaitre,[1,2] Juliette Hadchouel.[1] *[1]Paris Centre for Cardiovascular Research, INSERM U970 - Université Paris Descartes, Paris, France; [2]Genetic Department, Hopital Européen Georges Pompidou, Paris, France.*

Background: Identification of mutations in the WNK1 (With No Lysine (K) kinase 1) gene as responsible for the Mendelian disease Familial Hyperkalemic Hypertension (FHHt) led to the discovery of a new pathway for blood pressure regulation. To date, most studies have been conducted in vitro and have focused on the role played by the ubiquitously expressed long isoform and the kidney-specific isoform of WNK1 (L- and KS-WNK1 respectively) in the distal convoluted tubule (DCT). Recent research on a mouse model of KS-WNK1 inactivation suggests that this segment is not solely responsible for the development of hypertension observed in FHHt patients and that there are compensatory mechanisms acting in the connecting tubule (CNT) and/or collecting duct (CD).

Methods: To characterise the role of L-WNK1 in the late distal tubule, we generated a mouse model with specific inactivation of L-WNK1 in the collecting duct by crossing mice carrying a conditional allele of WNK1, containing LoxP sites encasing exons 4 and 4a, with mice expressing Cre-recombinase under the control of the Hoxb7 promoter. Control and mutant males were maintained in metabolic cages to measure blood and urine electrolytes.

Results: There were no differences in blood or urinary electrolytes when comparing mutant and control animals, nor was there a difference in urinary aldosterone levels. Western blot analysis of cortical membrane protein extracts did not reveal a difference in expression of the Na-Cl cotransporter, the epithelial sodium channel or pendrin between the two groups.

Conclusions: In summary, inactivation of WNK1 in the collecting duct does not appear to have a strong phenotype in terms of electrolyte transport in the kidney, likely due to compensatory mechanisms acting in other kidney segments, similar to what was observed in the mouse model of CD-specific inactivation of ENaC. To gain further insights in the role played by L-WNK1 in nephron segments downstream of the DCT, we are currently characterising a mouse model of L-WNK1 inactivation in Principal cells of the CNT and CD.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1745

Alternatively Spliced PY Cassette Exons in WNK1 Decrease Stability by Enhancing Sensitivity to the E3 Ubiquitin Ligase Nedd4-2 Ankita Roy,[1] Avin C. Snyder,[1] Shaheen Khadem,[1,2] Rodrigo Alzamora,[1] Yen-Pei Christy Chang,[3] Arohan R. Subramanya.[1,2] *[1]Department of Medicine, University of Pittsburgh; [2]VA Pittsburgh Healthcare System, Pittsburgh, PA; [3]Department of Medicine, University of Maryland, Baltimore, MD.*

Background: With-No-Lysine kinase 1 (WNK1) is a large serine-threonine kinase that regulates Na+ and K+ handling in the aldosterone-sensitive distal nephron (ASDN). Two major functional isoforms of WNK1 are expressed in the kidney: full-length WNK1 (L-WNK1), and a truncated kidney-specific kinase-defective product (KS-WNK1). Alternative splicing of exons 11 and 12 of WNK1 has also been reported in kidney, but the significance of these events is unknown.

Methods: To clarify the role of exons 11 and 12 in WNK1 function, we assessed WNK1 mRNA expression in human kidney by RT-PCR. Exons 11 and 12 were cloned from rat kidney cDNA and integrated in-frame to a rat L-WNK1 splice variant lacking the two exons (Xu et al, JBC 2001). WNK1 cDNAs were then expressed in HEK293 cells for biochemical assessments of protein abundance, interaction, and stability.

Results: We detected a contingent of 16 renal L-WNK1 and KS-WNK1 mRNAs containing a complete assortment of exon 11 and 12 combinations. Bioinformatic analysis revealed that exons 11 and 12 each harbor a canonical PY motif ([L/P]PXY), which binds to WW domain proteins such as the E3 ligase Nedd4-2; remarkably, no other PY motifs are present in the WNK1 sequence. When expressed in cells bearing endogenous Nedd4-2, exons 11 and 12 decreased steady state L-WNK1 protein abundance by enhancing its proteasomal degradation. Conversely, ablation of both PY motifs by site-directed mutagenesis increased L-WNK1 abundance relative to wild type, suggesting that the exons decrease protein stability by promoting Nedd4-2 interaction.

Conclusions: These observations indicate that exons 11 and 12 function as alternatively spliced "PY cassettes" that enhance WNK1 sensitivity to Nedd4-2 mediated degradation. Since Nedd4-2 is inhibited by signaling intermediates downstream of aldosterone and vasopressin, we propose that hormonal stimuli for NaCl reabsorption in the ASDN augment WNK-dependent signaling through Nedd4-2 dependent control of WNK1 protein abundance.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-PO1746

Renal Phenotype of WNK3 Knockout Mouse Katsuyuki Oi,[1] Eisei Sohara,[1] Motoko Chiga,[1] Dario Alessi,[2] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan; [2]MRC Protein Phosphorylation Unit, University of Dundee, United Kingdom.*

Background: Mutations of WNK1 and WNK4 genes cause the human hereditary hypertensive disease, pseudohypoaldsteronism type II (PHAII). By analyzing the PHAII model mouse, we discovered the novel kinase cascade (WNK4-OSR1/SPAK) in kidney that regulates Na-Cl cotransporter (NCC). WNK3, another member of WNK kinase family, reportedly regulates Na-K-Cl cotransporter (NKCC) and NCC when co-expressed in Xenopus oocytes. However, it remains to be determined whether WNK3 is in fact involved in the regulation of these transporters in vivo. To investigate this issue, we generated and analyzed WNK3 knockout mice.

Methods: The construct was designed to result in the insertion of loxP sequences flanking exon 2 of Wnk3. Then, we mated WNK3 (flox/+) offspring with CAG Cre transgenic mouse and successfully obtained WNK3 knockout mouse. The knockout of WNK3 gene was confirmed by RT-PCR and immunoblot in various organs. To determine the transporters potentially regulated by WNK3 in kidney, we first determined WNK3 expression along nephron by laser capture microdissection and RT-PCR.

Results: We found that WNK3 is expressed in proximal tubules, thick ascending limb of Henle's loop, distal tubules and collecting ducts, where NHE3, NKCC2, NCC and ENaC are expressed, respectively. We then performed immunoblot of these transporters. Under normal diet and even under low salt diet, we could not see any significant difference in the protein abundance and the magnitude of phosphorylation of NKCC2 and NCC between WNK3 knockout and wild-type mice. There was also no remarkable difference in ENaC and NHE3 protein levels between the two genotypes.

Conclusions: Thus, it is unlikely that WNK3 has a major role in WNK-OSR1/SPAK kinase cascade in kidney.

FR-PO1747

NCC Modulation by WNK3 Requires WNK3-SPAK Interaction Diana Pacheco-Alvarez,[1] Norma Hilda Vázquez,[2] Norma Bobadilla,[2] Maria Castañeda-Bueno,[2] Gerardo Gamba.[2] *[1]Escuela de Medicina, Universidad Panamericana, Mexico City, Mexico; [2]INNSZ-IIB-UNAM, Mexico City, Mexico.*

Background: With-no-lysine kinase 3 (WNK3) is a powerful activator of NKCCs and NCC while it is an inhibitor of KCCs. It has been shown that WNKs lie upstream of the STE-20 kinases SPAK/OSR1 and that interaction with SPAK occurs through the SPAK-binding motif, RFx(V/I), three of which are present in WNK3. In a previous study we observed that NKCC2 activation by WNK3 is prevented by elimination of the WNK3 SPAK binding site F1337 (PNAS, 2008). Here we define the role of SPAK/OSR1 in the modulation of NCC and other members of the SLC12 family by WNK3.

Methods: The SPAK binding sites of WNK3 (F242A, F873A, or F1337A; one at a time), NCC (F19A), and NKCC2 (F17A) were eliminated. Basal activity, the effect of WNK3 or low chloride hypotonic stress was assessed by measuring the tracer $^{22}Na^+$ or $^{86}Rb^+$ uptake in *Xenopus* oocytes injected with combinations of transporters and kinases wild type or mutant cRNAs. Proteins extracted from oocytes were used to define the interaction between WNK3-SPAK-NCC by immunoprecipitation and NCC-T58 phosphorylation by using phosphoantibodies.

Results: Basal activity of NCC-F19A and NKCC2-F17A was reduced by 50%. However, the response to WNK3 and to low chloride hypotonic stress was still present. WNK3-F1337A no longer activates NKCC2, but the effect towards NCC, NKCC1 and KCC4 is preserved. In contrast, the effect of WNK3 towards these cotransporters, but not NKCC2, was prevented in WNK3-F242A. Elimination of F873 had no consequences in WNK3 effects. Catalytic activity is preserved in WNK3-F242A. Immunoprecipitation revealed that WNK3-SPAK-NCC forms a protein complex that is still present with NCC-F19A, but not with WNK3-F242A. WNK3 promoted NCC or NCC-F19A phosphorylation at T58. This effect was not observed with WNK3-F242A.

Conclusions: Our observations suggest that basal activity of NCC, but not activation by WNK3, requires the presence of the SPAK binding site on the cotransporter. However, modulation of NCC (and NKCC1 or KCC4) requires interaction between WNK3 and SPAK through the binding site F242 at WNK3. Interestingly, a different SPAK binding site on WNK3 is required for NKCC2 activation.

Funding: Government Support - Non-U.S.

FR-PO1748

WNK4 Is a Novel Regulator of Thiazide Sensitive NaCl Transport in the Collecting Duct Cara J. Busst,[1] Dominique Eladari,[2] Francoise Leviel,[2] Christelle Soukaseum,[1] Richard P. Lifton,[4] Xavier Jeunemaitre,[1,3] Juliette Hadchouel,[1] Régine Chambrey.[2] *[1]Paris Centre for Cardiovascular Research (INSERM U970), Paris, France; [2]Cordeliers Research Centre (INSERM U872), Paris, France; [3]Hôpital Européen Georges Pompidou, Paris, France; [4]Yale University School of Medicine, New Haven, CT.*

Background: Mutations in the WNK1 and WNK4 (With no lysine (K)) kinases were found to cause Pseudohypoaldosteronism type II (PHAII), an autosomal dominant hypertension with hyperkalemia and hyperchloremic metabolic acidosis. Transgenic mice expressing WNK4 containing a PHAII mutation (TgWNK4PHAII) display all features of PHAII, thought to be due solely to increased Na-Cl cotransporter (NCC) activity in the distal convoluted tubule (DCT). However, recent research on mice lacking the kidney-specific isoform of WNK1 (expressed only in the DCT) showed that NCC overactivity is not sufficient to generate PHAII due to compensatory mechanisms in the cortical collecting duct (CCD).

Methods: To investigate CCD function, we performed isolated, perfused tubule studies on CCDs dissected from WT and TgWNK4PHAII mice.

Results: We observed increased Na+ and Cl- transport in TgWNK4PHAII mice while K+ and epithelial voltage were unchanged, suggesting that increased Na+ reabsorption in these mice is not mediated by the epithelial sodium channel (ENaC). We then examined ENaC activity in vivo by subjecting mice to amiloride injections while housed in metabolic cages and found that ENaC activity was indeed decreased in TgWNK4PHAII mice, as the natriuretic and anti-kaliuretic response was abolished. We recently showed that parallel action of the Na+-dependent Cl-/HCO3- exchanger NDCBE and the Cl-/HCO3- exchanger pendrin drives electroneutral thiazide-sensitive and amiloride-insensitive Na+ transport in the CCD. We thus repeated microperfusion experiments in the presence of hydrochlorothiazide and observed inhibited Na+ and Cl- transport in TgWNK4PHAII mice.

Conclusions: In conclusion, WNK4 appears to be an important regulator of NaCl transport in the CCD, modifying activity of the electroneutral NaCl transport system mediated by NDCBE and pendrin, and indicates that this system may play a key role in PHAII.

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FR-PO1749

WNK4 Suppresses WNK3-Driven Phosphorylation of NCC in Mammalian Kidney Cells Chao-Ling Yang,[1,2] Shaunessy L. Rogers,[1,2] James A. McCormick,[1] David H. Ellison.[1,2] *[1]Nephrology & Hypertension, Oregon Health & Science University, Portland, OR; [2]Renal Section, VA Medical Center, Portland, OR.*

Background: WNK kinases regulate renal salt transport via the thiazide-sensitive Na-Cl cotransporter, NCC. Previous work has shown that WNK4 and KS-WNK1 inhibit NCC activity, acting in opposition to WNK1 and WNK3, both in vitro and in vivo. It has also shown that WNK3 is more potent as a kinase than WNK4. We reported that WNK3 is highly expressed along the distal nephron, where it is expressed with NCC. Based on their ability to form protein-protein complexes and on the functional attenuation data, we proposed that WNK kinases form a signaling complex to regulate NCC activity to maintain salt homeostasis (JCI 2007). NCC must traffic to the apical membrane of distal convoluted tubule cells, and be phosphorylated along its amino terminus, to be active.

Methods: Here, we compared effects of WNK3 and WNK4 in HEK293 cells, which express SPAK endogenously. We transfected the cells with NCC, WNK3, and WNK4, and performed immunoblotting and immunocytochemistry.

Results: We first showed that these cells glycosylate and phosphorylate NCC normally. Transfected WNK3 strongly promoted NCC phosphorylation at T53, one of the sites essential for NCC activation. This effect required SPAK, because knockdown of endogenous SPAK with siRNA, or co-expression of kinase-inactive SPAK T243A, prevented the increased phosphorylation. Unlike WNK3, WNK4 suppressed NCC protein expression, as well as NCC T53 phosphorylation. Furthermore, WNK4 could block WNK3-mediated NCC phosphorylation. This inhibition was independent of WNK4 kinase activity, because a WNK4 C-terminal fragment, without a kinase domain, had a similar effect as full-length WNK4.

Conclusions: In conclusion, WNK3 and WNK4 exert opposite effects on NCC phosphorylation in mammalian cells, and WNK4 inhibits the effect of WNK3 to activate SPAK and phosphorylate NCC. These effects appear to be additive to those of WNK4 on NCC protein trafficking to the plasma membrane, suggesting WNK kinases regulate transporter activity at several steps.

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FR-PO1750

Upregulation of Thiazide-Sensitive and Furosemide-Sensitive Sodium Absorption, NCC, and NKCC2 Expression in PHAII Mutant Mice Qingshang Yan, Laxiang Wan, Junhui Zhang, Nanami Gotoh, Jesse Rinehart, Richard P. Lifton, Gerhard H. Giebisch, Tong Wang. *C. & M. Physiology, Yale University, School of Medicine, New Haven, CT.*

Background: WNK4 mutations cause pseudohypoaldosteronism type II (PHAII), a disease featuring increased renal NaCl reabsorption and impaired K^+ secretion. PHAII mutant mice exhibited phenotypes of higher blood pressure, hyperkalemia and hypercalciuria. We investigate the mechanism of increased NaCl absorption in PHAII mice.

Methods: The renal expression levels of NKCC2 (Na/2Cl/K-cotransporter), thiazide-sensitive Na^+-Cl^- cotransporter (NCC) and the ENaC (epithelia Na^+ channel) were examined by Q-PCR and their activities by measuring the effect of furosemide (Furo), hydrochlorothiazide (HCTZ) and benzamil (BEZ) on Na^+ and K^+ excretion by renal clearance. The urine volume (UV), GFR and absolute (ENa, EK) and fractional (FENa, FEK) Na^+ and K^+ excretion were measured before and after bolus iv of diuretics in wild-type (WT) and WNK4 mutant (PHAII) mice.

Results: All three diuretics produced significant diuretic and natriuretic effects in WT, but Furo and HCTZ produced stronger and benzamil produced smaller diuretic and natriuretic effects significantly in PHAII mice compared to the WT. The fractional increase of UV was 8.6- vs. 6.5-fold, 5.6- vs. 1.9-fold and 0.30- vs. 1.2-fold; ENa was 17.8- vs. 4.9-fold, 17.6- vs. 4.4-fold and 3.9- vs. 5.6-fold; and FENa was 34.6- vs. 10.1-fold, 19.4-vs. 5.4-fold and 2.85- vs. 4.4-fold with Furo, HCTZ and BEZ treatment in PHAII mice vs. WT, respectively. Furo and HCTZ increase and BEZ reduced both EK and FEK but there was no significant difference in the fractional changes in EK and FEK caused by the diuretics between WT and PHAII mice. Q-PCR data show NKCC2 and NCC expression levels were 25% and 61% higher in PHAII than in WT mice. NHE3, α, β and γ ENaC expression were not significantly changed but ROMK expression was reduced by 46% in PHAII vs. WT.

Conclusions: We conclude that 1) elevated NKCC2 and NCC activities contributes to increased NaCl absorption; 2) reduced ROMK expression limits ENaC activity; and 3) other K^+ secretion mechanisms may be upregulated when the ROMK channel is downregulated in PHAII mice.

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FR-PO1751

Epithelial Ion Flux in the Fly Renal Tubule Occurs through NKCC, and Is Regulated by WNK and SPAK Aylin R. Rodan,[1] Michel G. Baum,[1,2] Chou-Long Huang.[1] *[1]Medicine, UTSW; [2]Pediatrics, UTSW, Dallas, TX.*

Background: NCC and NKCC play key roles in renal epithelial ion transport. Recent evidence suggests that the WNK and SPAK/OSR1 kinases regulate these transporters, but many questions remain. Analysis in mammalian systems is complicated by multiple NKCC, WNK, and SPAK/OSR1 family members.

Methods: We are using *Drosophila melanogaster*, which has sophisticated genetics, a rapid life cycle, and well-characterized renal tubular epithelial transport, to better understand these pathways. The fly homologs of NKCC, SPAK/OSR1 and WNK are *Ncc69*, *fray* and *wnk*, respectively. In the fly tubule, an apical H^+-ATPase generates a lumen-positive transepithelial potential difference (TEP), which drives K^+ secretion by K^+/H^+ exchange. To investigate the roles of *Ncc69* in basolateral K^+ uptake, and *wnk* and *fray* in K^+ transport regulation, we have measured TEP and K^+ flux in isolated fly renal tubules.

Results: 100 μM bumetanide, an NKCC inhibitor, reversibly decreased TEP from 42 mV to 21 mV when added basolaterally to perfused tubules. In *Ncc69* mutants, transepithelial K^+ flux was slightly decreased, from 59 pmol/min in wild-type to 48 pmol/min in mutants. Loss of *Ncc69* is compensated by other mechanisms, including upregulation of a cAMP-sensitive pathway: in mutant tubules, 1 mM cAMP increased K^+ flux 2.7-fold, compared to only 1.4-fold in wild-type. To isolate the role of *Ncc69*, K^+ flux through the Na-K-ATPase was inhibited with a submaximal dose of ouabain (100 μM). Under these conditions, K^+ flux decreased from 92 pmol/min in wild-type to 52 pmol/min in *Ncc69* mutants, and addition of 100 μM bumetanide decreased K^+ flux in wild-type to 52 pmol/min, but did not significantly decrease *Ncc69* mutant K^+ flux (45 pmol/min). Thus, *Ncc69* is the bumetanide-sensitive transporter in the fly tubule. Tubule knockdown of *wnk* decreased K^+ flux to 40 pmol/min, compared to 75 pmol/min in the control. Similarly, tubule knockdown of *fray* decreased K^+ flux to 58 pmol/min, compared to 76 pmol/min in the control.

Conclusions: WNK and SPAK play an evolutionarily conserved role in the regulation of renal epithelial ion flux, likely through the regulation of NKCC. Future studies will examine the mechanisms by which this occurs.

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FR-PO1752

SPAK and OSR1 Act as Interdependent Kinases That Control Renal Salt Excretion and Blood Pressure Via Regulation of NCC Phosphorylation and Distal Convoluted Tubule Cell Mass Paul R. Grimm,[1] Tarvinder K. Taneja,[1] Jie Liu,[1] Richard A. Coleman,[1] Eric J. Delpire,[2] James B. Wade,[1] Paul A. Welling.[1] *[1]Department of Physiology, University of Maryland School of Medicine, Baltimore, MD; [2]Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN.*

Background: The STE20/SPS-1-related proline-alanine-rich protein kinase (SPAK) and its homolog, Oxidative Stress Related Kinase (OSR1), co-localize at the apical membrane of the TAL and DCT and have the capacity to regulate NKCC2 and NCC, yet genetic ablation of SPAK in mice causes a salt-wasting nephropathy that is restricted to the DCT, reminiscent of Gitelman's syndrome. Here, we explore why proper DCT function is especially dependent on SPAK.

Results: In SPAK-/- mice, NKCC2 becomes hyper-phosphorylated, and this is paralleled by a concomitant increase in OSR1 expression, consistent with a compensatory mechanism in the TAL. By contrast, OSR1 is not augmented in the DCT. In fact, OSR1 becomes largely displaced from NCC and the apical membrane, redistributing to dense punctate structures within the cytoplasm. These changes are paralleled by a dramatic decrease in NCC abundance and phosphorylation; without SPAK and proper localization of OSR1, phosphorylation-dependent regulation of NCC by dietary sodium restriction is lost. As assessed by immunohistochemical and transcript analysis, SPAK-/- also exhibit a remarkable decrease in the distal convoluted tubule, exclusive to DCT1. As a result, SPAK-/- mice are highly sensitive to dietary salt-restriction, displaying prolonged negative sodium balance and hypotension.

Conclusions: In conclusion: 1) OSR1is compensatory to SPAK removal in the TAL; 2) In the DCT, SPAK and OSR1 act as interdependent kinases that are necessary for phospho-regulation of NCC and the control of DCT1 cell mass.

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FR-PO1753

SPAK and OSR1 Kinases Modulate Luminal Trafficking and Cellular Abundance of NKCC2 Aljona Borschewski,[1] Kamel Laghmani,[2] Kerim Mutig,[1] Sylvie Demaretz,[2] Christin Dathe,[1] Alexander Paliege,[1] Nicholas R. Ferreri,[3] Eric J. Delpire,[4] Sebastian C. Bachmann.[1] *[1]Institut für Vegetative Anatomie, Charité Universitätsmedizin, Berlin, Germany; [2]INSERM, Centre de Recherche des Cordeliers, Paris, France; [3]Dept. of Pharmacology, New York Medical College, Valhalla, NY; [4]Dept. of Anesthesiology, Vanderbilt University School of Medicine, Nashville.*

Background: Na^+,K^+,$2Cl^-$-cotransporter (NKCC2) of the thick ascending limb (TAL) is essential for urinary concentration and volume regulation. Sterile 20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative-stress responsive kinase 1 (OSR1) regulate NKCC2 activity by phosphorylation of conserved N-terminal threonines. The aim of the present study was to evaluate the effects of SPAK and OSR1 on total abundance and surface expression of NKCC2.

Methods: Localization of SPAK and OSR1 in TAL was verified immunohistochemically. Effects of SPAK and OSR1 on NKCC2 phosphorylation (pT96 and pT101), surface expression, and total abundance were studied in cultured rat TAL cells and in opossum kidney (OPK) cells by means of knockdown or transient overexpression of the kinases and NKCC2.

Results: Confocal evaluation revealed co-localization of SPAK and OSR1 with NKCC2 in subapical compartment of mouse and rat TAL. The interactions between the kinases and NKCC2 were established by co-immunoprecipitation experiments using rat medullary kidney homogenates. In cultured TAL cells, the endogenous abundance of SPAK and NKCC2 was verified by immunoblotting. Knockdown of SPAK in cultured TAL cells resulted in significantly decreased total abundance (-75%) and phosphorylation of NKCC2 (-82%). In OPK cells, co-transfections of NKCC2 with SPAK or OSR1 produced significant increases of NKCC2 surface expression (+71% and +74%, respectively) and total abundance (+22% and +26%, respectively) as revealed by immunoblotting from total cell lysates and biotynylated fractions.

Conclusions: This study provides evidence that SPAK and OSR1 may regulate the activity of NKCC2 not only by phosphorylation of the conserved N-terminal threonines but also by modulation of the total abundance and surface expression of the transporter.

FR-PO1754

Cytoplasmic Hsp70/Hsp90 Multichaperone Complexes Regulate Thiazide-Sensitive NaCl Cotransporter Biogenesis and Degradation Arohan R. Subramanya,[1,3] Patrick G. Needham,[2] Shaheen Khadem,[1,3] Avin C. Snyder,[1] Jeffrey L. Brodsky.[2] *[1]Department of Medicine, University of Pittsburgh; [2]Department of Biological Sciences, University of Pittsburgh; [3]VA Pittsburgh Health Care System, Pittsburgh, PA.*

Background: The thiazide-sensitive NaCl cotransporter (NCC) is the primary mediator of sodium reabsorption in the distal convoluted tubule. Loss-of-function mutations of NCC cause Gitelman's syndrome, a hypotensive salt-wasting disorder. NCC is a complex polytopic membrane glycoprotein that is susceptible to misfolding and endoplasmic reticulum-associated degradation (ERAD). Most Gitelman's mutants are unable to escape ER quality control, but the molecular details of this process remain unknown.

Methods: To begin to define these mechanisms, we utilized yeast as a model system to compare mouse NCC processing in wild type strains and in strains with targeted mutations in individual chaperones. Findings were subsequently verified in MDCK epithelia. We employed a variety of methods to study NCC ERAD, including cell fractionation and pulse-chase assays, co-IPs, and MALDI-TOF Mass Spectrometry.

Results: NCC behaves as a bona fide ERAD substrate in yeast, as it is membrane-integrated, is predominantly ER localized, and undergoes ubiquitylation and proteasomal degradation. While the ER luminal chaperone Kar2/BiP is dispensible for NCC ERAD, the cytoplasmic Hsp70 Ssa1 is required. In MDCK cells, cytoplasmic mammalian Hsp70 (Hsp72) coexpression enhanced NCC ERAD, decreasing its half-life by ~50%. Proteomic analyses of NCC immunoprecipitates confirmed that the cotransporter was associated with a mammalian cytoplasmic chaperone complex consisting of Hsp70, Hsp90, and Hsp40. Modulation of the activity of this complex by pharmacological Hsp90 inhibition, or by the distal nephron-expressed Hsp70/Hsp90 co-chaperone CHIP, altered the rate of NCC turnover.

Conclusions: These findings demonstrate that cytoplasmic chaperones play a critical role in NCC ER quality control. We propose that cytoplasmic multichaperone complexes sense NCC folding status, and that disease-causing conformational variants of NCC may be predisposed to enhanced ERAD through altered interactions with this quality control machinery.

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FR-PO1755

Acute Insulin Stimulation Induces Phosphorylation of the Na-Cl Cotransporter in Cultured Distal mpkDCT Cells and Mouse Kidney Eisei Sohara,[1] Tatemitsu Rai,[1] Sung-Sen Yang,[2] Akihito Ohta,[1] Shih-Hua P. Lin,[2] Alain Vandewalle,[3] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan; [2]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan; [3]INSERM U773, Université Paris 7 -Deni Diderot, Paris, France.*

Background: The NaCl cotransporter (NCC) is essential for sodium reabsorption at the distal convoluted tubules (DCT), and its phosphorylation increases its transport activity and apical membrane localization. Although insulin has been reported to increase sodium reabsorption in the kidney, the linkage between insulin and NCC phosphorylation has not yet been investigated.

Methods: This study examined[ensp]whether insulin[ensp]regulates NCC phosphorylation in cultured cells and mouse.

Results: In cultured mpkDCT cells, insulin increased phosphorylation of STE20/SPS1-related proline-alanine-rich kinase (SPAK) and NCC in a dose-dependent manner. Ly294002, a PI3K inhibitor, decreased the insulin effect on SPAK and NCC phosphorylation, indicating that insulin phosphorylates SPAK and NCC through PI3K in mpkDCT cells. Moreover, acute insulin administration to mice increased phosphorylation of oxidative stress-responsive kinase-1 (OSR1)/SPAK and NCC in the kidney. Time-course experiments in mpkDCT cells and mice suggested that SPAK is upstream of NCC in this insulin-induced NCC phosphorylation mechanism, which was confirmed by the lack of insulin-induced NCC phosphorylation in SPAK knockout mice. Moreover, insulin administration to WNK4 hypomorphic mice did not increase OSR1/SPAK phosphorylation in the kidney, suggesting that WNK4 is also involved in the insulin-induced OSR1/SPAK phosphorylation mechanism.

Conclusions: The present results demonstrated that insulin is a potent regulator of NCC phosphorylation in the kidney, and that WNK4 and SPAK are involved in this mechanism of NCC phosphorylation by insulin.

FR-PO1756

Aldosterone Affects NCC Phosphorylation Involving MAPK ERK1/2 Signaling Pathway Xiuyan Feng,[1] Yanhui Wang,[1,4] Ping Wu,[1] Eric J. Delpire,[3] Hui Cai.[1,5] *[1]Renal Division, Emory University School of Medicine, Atlanta, GA; [2]Department of Nephrology, Second Affiliated Hospital, Wenzhou Medical College, Wenzhou, Zhejiang, China; [3]Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN; [4]Department of Nephrology, First Affiliated Hospital, Wenzhou Medical College, Wenzhou, Zhejiang, China; [5]Renal Section, Atlanta VA Medical Center, Atlanta, GA.*

Background: The thiazide-sensitive Na^+-Cl^- cotransporter (NCC) is one of the key regulators in renal sodium handling. Chronic treatment with aldosterone increased NCC abundance *in vivo*. We have previously shown that knock-down of ERK 1/2 expression increased NCC protein expression in mouse distal convoluted tubule (mDCT) cells.

Methods: We used cell cutlure, western blot analysis and siRNA knock down technique to carry out the experiments.

Results: To determine whether acute aldosterone treatment affects NCC regulation involving MAPK ERK1/2 signal pathway, we treated the mDCT cells with aldosterone 1uM at different time point and found that the ERK1/2 phosphorylation increased at 15 minute of aldosterone treatment but deceased after 1 hour and lasted at least 24 hour. However, SPAK phosphorylation at S373 was not significantly altered. We then determined whether reduction of SPAK expression by siRNA affects ERK1/2 phosphorylation and NCC phosphorylation. Under knock-down of SPAK expression, we still observed the dynamic change of ERK1/2 phosphrylation in a similar fashion as before. We found that aldosterone increased NCC phosphoralation at T60 at 6 hour of aldosterone treatment. Knock-down of SPAK expression did not alter aldosterone-mediated increased NCC phosphorylation at T60.

Conclusions: These results suggest that ERK1/2 signaling pathway is involved in the acute aldosterone-mediated NCC phosphorylation and potential regulation of NCC function and protein expression.

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FR-PO1757

Development of New Systems To Measure total and Phosphorylated Na-Cl Cotransporter(NCC) Protein in Human Urine Kiyoshi Isobe, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

Background: Thiazide-sensitive sodium chloride cotransporter (NCC) localizes to the apical membrane of the distal convoluted tubule (DCT) in kidney, and was responsible for reabsorbing 5-10% of the filtered load of sodium chloride. By analyzing the pathogenesis of pseudohypoaldosteronism type II (PHAII), we discovered that WNK-OSR1/SPAK kinase cascade is a powerful regulator of NCC. NCC phosphorylated by OSR1/SPAK kinases was shown to be functionally active and concentrated on the plasma membranes of DCT. Thiazide, an inhibitor of NCC, is one of the first-line drugs for treating hypertension. However, it is difficult to predict the thiazide sensitivity in each patient prior to the administration. Since NCC protein is known to be excreted in urine, we hypothesized that NCC excretion in urine could be a biomarker for predicting the thiazide sensitivity in hypertensive patients. In order to investigate this possibility, sensitive, efficient and reproducible methods measuring urine NCC are necessary.

Methods: In this study, we developed a sandwich ELISA method to measure NCC in human urine along with a conventional but improved Western blotting method.

Results: Sandwich ELISA and Western blotting are able to detect urinary NCC as low as 2.5 pmol/ml and 5.0 pmol/ml, respectively. Using these methods, we found that NCC concentration in spot urine samples remained constant within a day when they were corrected by respective creatinine concentration. This result suggests that single spot urine can be used to estimate total excretion of NCC for 24 hours. We also confirmed that urine NCC excretion varied according to different salt intake. Furthermore, we for the first time succeeded to detect phosphorylated NCC (pNCC) in human urine by using phospho-specific anti-human NCC antibodies. Since pNCC is an active form of NCC, pNCC in urine could be more sensitive marker for predicting in vivo activity of NCC than total NCC.

Conclusions: Thus, we established methods to measure NCC and pNCC in human urine samples. The relationship of thiazide sensitivity and urine NCC and pNCC will be efficiently investigated by these systems.

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FR-PO1758

Urinary Excretion of Na-Cl Cotransporter in Exosomes Is Increased by High Salt Diet as Well as Low Salt Diet Muhammad Zakir Hossain Khan, Eisei Sohara, Akihito Ohta, Shotaro Naito, Motoko Chiga, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Na-Cl co-transporter, the key regulator of sodium reabsorption in the distal convoluted tubule (DCT) is responsible for reabsorption of 5-10% of filtered sodium in the kidney. We recently demonstrated that constitutive activation of the WNK kinase-OSR1/SPAK kinase-NaCl cotransporter (NCC) phosphorylation cascade is the molecular pathogenesis of salt-sensitive hypertension in pseudohypoaldosteronism type II. We also have reported that dietary salt intake regulates expression and phosphorylation of NCC.In order to establish a method to monitor the activity of NCC in kidney by urine examination; we sought to determine the correlation between NCC in kidney and NCC in urine exosomes.

Methods: After 6 days feeding with three different diets (normal, low and high salt), we collected urinary exosomes from wild-type C57/BL6 mice.

Results: Western blotting of these samples revealed total and phosphorylated NCC (S71 and T53) were increased in mice fed low salt diet than in mice fed normal diet, which is consistent with previous reports, and also well correlated with the increases of total and phosphorylated NCC in the kidney. However, we found that total and phosphorylated NCC in urine exosomes from mice fed high salt diet was also increased compared with those in mice fed normal diet.

Conclusions: Since urinary exosomal proteins are derived from multi-vesicular bodies, formed by endocytosis of plasma membranes, we speculate that, the increased excretion of NCC in exsosomes may be caused by the increased endocytosis of NCC from the apical plasma membranes in DCT. This result suggests that urinary NCC excretion in exosomes may not necessarily reflect NCC abundance in the apical plasma membranes of DCT, at least in mice.

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FR-PO1759

Genotype and Phenotype Analysis in Taiwanese Patients with Gitelman's Syndrome Min-Hua Tseng,[1,2] Sung-Sen Yang,[2,3] Yu-Wei Fang,[2] Shih-Hua P. Lin.[2,3] *[1]Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan; [2]Graduate Institute of Medical Sciences, National Defense Medical Center., Taipei; [3]Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan.*

Background: Phenotype and genotype analysis is rarely performed in a large number of patients with Gitelman's syndrome (GS) caused by mutations in *SLC12A3* encoding the thiazide-sensitive NaCl cotransporter of distal convoluted tubules .

Methods: 106 Taiwanese GS patients (M:F = 64:42, age 24 ± 10) with *SLC12A3* mutations belonging to 85 unrelated families were investigated. All had chronic hypokalemia with renal salt and potassium wasting, metabolic alkalosis, and normotension. Genomic DNA and/or cDNA from blood leukocytes were done for the detection of *SLC12A3* mutations and haplotype analysis with intra- and extra-genic markers for recurrent *SLC12A3* mutations. Clinical symptoms and biochemical studies on the first presentation and follow-up studies were recorded.

Results: 38 different *SLC12A3* mutations including 25 missense, 3 nonsense, 3 deletion, 3 splice site, 3 deep intronic, 1 insertion were identified with compound heterozygosity being the most common. Approximately 10 % and 15% of patients had triple and heterozygous *SLC12A3* mutations, respectively. 14 mutations including 8 missense, 2 nonsene, 2 deep intronic, 1 deletion and 1 insertion were recurrent with only one founder mutation (S710X). Typical hypocalciuria and hypomagnesemia were not found in 7 and 9 patients, respectively. In addition to male patients having an earlier age of onset, more severe hypokalemia and neuromuscular symptoms, patients with homozygous and deep intronic mutation in the intron 13 (c.1670-191C>T) also had severe hypokalemia. Seven and four patients had chronic kidney disease (CKD, stage III and IV) and type 2 diabetes mellitus at follow up, respectively.

Conclusions: GS patients with *SLC12A3* mutations may not always have hypocalciuria and hypomagnesemia. Screening of recurrent "hot spot" *SLC12A3* mutations may provide an early molecular diagnosis. Besides gender effect, the nature of homozygous and deep intronic mutations may influence the phenotype. GS patients have a significant risk for the development of CKD and abnormal glucose metabolism.

FR-PO1760

Diagnostic Value of Diuretics Loading Test in Gitelman Syndrome
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Background: We investigated the diagnostic significance of diuretics test in Gitelman syndrome (GS) and evaluated the superiority among various indices of renal clearance test.

Methods: Sixteen patients with clinically typical Gitelman syndrome were enrolled. One patient with factitious vomiting due to bulimia nervosa and 1 normal volunteer were also studied as controls. Mutation analysis of *SLC12A3* and *CLCNKB* gene was done in all the subjects. Diuretics tests were performed with thiazide (HCT) and furosemide (FUR). Clearance indices (CI) such as FE_{Na}, FE_{Cl}, C_{Cl} and DFCR (distal fraction of chloride reabsoprtion) were calculated. The ratio of clearance index (CI(HCT/FUR) which stands for CI(HCT)/CI(FUR)) and the ratio of delta CI (ΔCI(HCT/ FUR) which stands for ΔCI(HCT)/ΔCI(FUR)) were also calculated.

Results: All subjects had normotensive hypokalemic metabolic alkalosis, hyperreninemia and increased aldosterone level. *SLC12A3* mutation was detected in 11 patients (8 compound heterozygous, 3 homozygous mutations), but not in 5 patients. No *CLCNKB* mutation was detected in all the subjects. GS patients showed blunted response to thiazide administration (the range of FE_{Na}(HCT/Basal) was 0.75 ~ 5.74). The ranges of FE_{Na}(HCT/FUR), FE_{Cl}(HCT/FUR) and ΔFE_{Na}(HCT/FUR) of GS patients were 0.05 ~ 0.24, 0.05 ~ 0.30 and -0.05 ~ 0.14, and those of control group were 0.38 ~ 0.39, 0.31 ~ 0.38 and 0.34 ~ 0.36, which clearly discriminated patients from controls. Indices calculated from FE_{Cl}, C_{Cl}, and DFCR except FE_{Cl}(HCT/FUR) and ΔCCl(HCT/FUR) overlapped between GS patients and controls.

Conclusions: We suggest that the diagnosis of GS can be done with clinical characteristics and renal clearance test with diuretics instead of *SLC12A3* mutation analysis. FE_{Na} associated indices rather than absolute values were more reliable among various clearance indices in the diagnosis of GS.

FR-PO1761

Activation of the Bumetanide-Sensitive Na^+,K^+,2Cl^--Cotransporter NKCC2 Is Facilitated by Tamm-Horsfall Protein in a Chloride-Sensitive Manner
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Background: Active transport of NaCl in the thick ascending limb (TAL) is accomplished by Na^+,K^+,2Cl^--cotransporter (NKCC2). The activation of NKCC2 depends on intracellular chloride concentration ($C_{[i]}$) and includes its amino-terminal phosphorylation. We hypothesized that co-expressed Tamm Horsfall protein (THP) modulates NKCC2 activity in TAL.

Methods: Effects of THP on NKCC2 phosphorylation (T96/T101) and transport activity were studied in THP-deficient (THP-/-) and wild type (WT) mice, cultured TAL cells, and frog oocytes.

Results: THP-/- mice displayed decreased phosphorylation of NKCC2 (-49%, $p<0.05$) compared to WT mice. Cultured TAL cells with low endogenous THP levels displayed sharp increases in NKCC2 phosphorylation (+38%, $p<0.05$) along with a pronounced decrease of $C_{[i]}$ (-40.4%, $p<0.05$) upon transfection with THP. In NKCC2-expressing frog oocytes, co-injection with THP cRNA significantly enhanced the activation of NKCC2 under low chloride hypotonic stress (+112% vs. +235%, $p<0.05$). Stimulation of the vasopressin V2 receptor pathway by V2R agonist (dDAVP; 30 min) resulted in enhanced NKCC2 phosphorylation in WT mice and cultured TAL cells transfected with THP whereas in the absence of THP, NKCC2 phosphorylation upon dDAVP was blunted in both systems. Attenuated effects of furosemide along with functional and structural adaptation of the distal convoluted tubule in THP-/- mice further supported the notion that NaCl reabsorption was impaired in TAL lacking THP.

Conclusions: In summary, these results are compatible with a permissive role for THP in the modulation of NKCC2-dependent TAL salt reabsorptive function.

FR-PO1762

cAMP Stimulates NKCC2 Recycling in Thick Ascending Limbs (TALs) Via PKA: Role of Ser126 Phosphorylation
Gustavo R. Ares, Pablo A. Ortiz. *Hypertension & Vascular Research Division, Henry Ford Hospital, Detroit, MI.*

Background: The apical cotransporter NKCC2 mediates NaCl reabsorption by the thick ascending limb (TAL). Surface NKCC2 is maintained by constitutive trafficking into and out of the apical membrane that involves recycling of internalized transporters. cAMP, the second messenger of AVP and β-adrenergic agonists, stimulates apical membrane NKCC2 and NaCl reabsorption by increasing the rate of NKCC2 exocytosis via protein kinase A (PKA). However, it is not known whether cAMP stimulates NKCC2 exocytosis from a recycling compartment or the biosynthetic pool. cAMP also enhances NKCC2 phosphorylation at Ser126, Ser874, and Thr96, and 101. We hypothesized that cAMP stimulates NKCC2 recycling in TALs *via* PKA, and Ser126 phosphorylation is involved in this trafficking event.

Methods: We measured NKCC2 recycling and phosphorylation in rat TAL suspensions by a modified surface biotinylation protocol and Western blot.

Results: TALs were incubated in the absence or presence of forskolin/IBMX (F/I) to stimulate cAMP and recycling of internalized NKCC2 measured and expressed as a percent of the internalized pool. We found that internalized NKCC2 recycles back to the surface in a constitutive manner (7 min = 11.4±2.1%, 15 min = 17.2±3.0%, 30 min = 25.1±3.3%, n=6). cAMP enhanced the rate of NKCC2 recycling by up to 160% by 15 min (7 min = 22.8±3.6%, 15 min = 44.6±4.8 %, 30 min = 47.3±1.6%, n=6, $p<0.05$). The internalized pool did not loose the biotin label nor was degraded over 30 min. We found that the PKA inhibitor H-89 (10 μM) completely blocked cAMP-stimulated NKCC2 recycling by 30 min (Basal = 39.5±1.9, cAMP = 59.8±1.7, cAMP+H-89 = 27.3±4.3, n=5, $p<0.05$). cAMP enhanced phospho-Ser126 by 33±6-fold and H-89 blocked this effect by 82% ($p<0.05$), we did not detect Ser874 phosphorylation. cAMP also enhanced Thr96 and Thr101 phosphorylation by 1.7±0.4-fold ($p<0.05$), however this was not blocked by H-89 (1.8±0.4-fold).

Conclusions: We conclude that cAMP stimulates NKCC2 recycling in TALs *via* PKA. PKA inhibition blocked NKCC2 recycling and Ser126 phosphorylation. Our data suggest that Ser126 rather than Thr96,101 is involved in cAMP stimulated trafficking in TALs.

Funding: Other NIH Support - NHLBI

FR-PO1763

Renal Na^+-K^+-Cl^--Cotransporter Is Structurally and Functionally Associated with Lipid Rafts
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Background: Apical Na^+-K^+-2Cl^- cotransporter (NKCC2) is an integral membrane protein responsible for the reabsorption of NaCl in the thick ascending limb (TAL). NKCC2 activity is dependent on the phosphorylation of conserved N-terminal threonine residues. 40-70% of NKCC2 was identified in cholesterol-enriched lipid rafts (LR). This study addresses the distribution of NKCC2 in different types of LR and evaluates their role for NKCC2 function.

Methods: Distribution of NKCC2 was evaluated by colocalization of the transporter with the LR markers, ganglioside GM1 and GPI-anchored Tamm-Horsfall protein (THP) on native membrane sheets from cultured rat TAL cells. Short term effects of methyl-β-cyclodextrin (MβCD)- or sphingomyelinase (SM)-induced disruption of LR on steady state and vasopressin (AVP)-stimulated phosphorylation and transport activity of NKCC2 were evaluated.

Results: Immunogold labeling of luminal membrane sheets from rat TAL cells revealed an association of NKCC2 and phospho (p)-NKCC2 (pT96/pT101) with electron-dense membrane regions. There was little or no colocalization of NKCC2 with GM1 but significant colocalization of the transporter with THP (57% of NKCC2 signal) indicating that NKCC2 is participating in LR containing GPI-anchored proteins. SM-induced disruption of LR markedly decreased the amount of pNKCC2 in the membrane sheets (-70%, $p<0.05$) which was accompanied by redistribution of NKCC2 to membrane regions with lower electron density. SM-treatment also decreased pNKCC2 levels in isolated mouse TALs (-66%, $p<0.05$). AVP increased the phosphorylation of NKCC2 in TAL cells (+33%, $p<0.05$) and its transport activity in isolated perfused mouse TALs (+44%, $p<0.05$). The effects of AVP were significantly blunted after MβCD-induced LR disruption.

Conclusions: We conclude that LR play an important role for NKCC2 phosphorylation and activity.

FR-PO1764

Heterogeneity in the Processing of NKCC2 Mutants Related to Bartter Syndrome Type 1
Yingying Zhu, Sylvie Demaretz, Nadia Defontaine, Nancy Zaarour, Kamel Laghmani. *INSERM, UMRS 872 - Equipe 3- ERL7226, Universités Paris V, Paris VI, Paris, France.*

Background: Mutations in the apically located Na-K-2Cl co-transporter, NKCC2, lead to type I Bartter syndrome (BS1), a life-threatening kidney disease. Regulatory characterizations of NKCC2 mutants were essentially limited to Xenopus laevis oocytes. Consequently, our knowledge of the molecular mechanisms underlying membrane trafficking of NKCC2 mutants in mammalian cells is poor. Undeniably, only analysis of the expression of such NKCC2 mutants in renal cells would definitively establish their cellular fate. Here, we investigated the consequences of previously reported, pathogenic, missense mutations of NKCC2 in renal cells.

Methods: The effect of NKCC2 mutations on protein processing was examined in OKP and HEK cells, using immunoblot analysis and confocal imaging. NKCC2 transport activity was measured as bumetanide-sensitive NH4 influx.

Results: WT NKCC2 was detected as two protein bands representing the core glycosylated (120 kDa) and the complex glycosylated (160 kDa) forms of the protein, located at the endoplasmic reticulum (ER) and the plasma membrane, respectively. Immunocytochemistry analysis showed that WT NKCC2 staining co-localized with biotinylated cell-surface proteins indicating correct targeting of NKCC2 to the cell surface. Immunoblot analysis of three out of six NKCC2 mutants showed only the core glycosylated form of NKCC2. Co-immunolocalization assay revealed that these mutants were not detected at the cell surface due to retention in the ER. Accordingly, they were not functional. One NKCC2 mutant displayed partial loss of complex glycosylation at the

cell surface, which was associated with reduced transport activity. Finally, two NKCC2 mutants were correctly glycosylated and trafficked normally to the cell surface, but exhibited a significantly decreased transport activity.

Conclusions: In summary, our study revealed distinct cellular mechanisms accounting for NKCC2 loss of function in BS1. They are consistent with a model whereby the processing of NKCC2 mutant is determined by mutant specific mechanisms, suggesting that a mutant specific method would be required to rescue the functional expression of each NKCC2 mutant.

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FR-PO1765

A Primary Culture System of Mouse Thick Ascending Limb Cells Established on Collagen-Coated Membranes Bob Glaudemans,[1] Olivier Devuyst,[1,2] Sara Terryn.[1,2] *[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Nephrology, UCL Medical School, Brussels, Belgium.*

Background: The epithelial cells lining the thick ascending limb (TAL) of the loop of Henle play essential roles in the normal and diseased kidney.

Methods: We report a new method to develop primary cultures from TAL cells, obtained from microdissected tubules from the outer medulla of 1-month-old collagenase-treated mouse kidneys.

Results: The selected tubules specifically express uromodulin (Tamm-Horsfall protein) and the Na+,K+,2Cl- cotransporter NKCC2, whereas markers such as podocin (glomerulus), aquaporin-1 (proximal tubule) and aquaporin-2 (collecting duct) are negative. The TAL tubules were then cultured on permeable filter supports for 7-10 days, allowing the formation of well-polarized confluent monolayers with apical and basolateral domains. The TAL cells maintain a differentiated state, with expression of functional markers such as uromodulin, NKCC2 and the chloride channel ClC-Kb. Confocal microscopy confirmed the apical staining of both NKCC2 and uromodulin. The staining partly overlapped, as NKCC2 was located within the apical membrane, while GPI-anchor protein uromodulin showed additional staining at the extracellular compartment. Further analyses evidenced the typical filaments made of uromodulin in the apical extracellular compartment. In line with in vivo measurements, electrophysiological recordings of primary cultured TAL cells grown on filters show a high transepithelial resistance (1200 ± 175 ohm/cm2) and a lumen-positive transepithelial potential (Vte) (9.3 ± 1.2 mV/cm2) (n=9). The activity of NKCC2 and its role in maintaining the TAL lumen-positive Vte was confirmed by addition of apical bumetanide (100 µmol/L), which caused a sudden drop in Vte, with recovery after washing.

Conclusions: The establishment of this primary culture system will allow to investigate the role of TAL cells obtained from transgenic mouse models, providing insights for understanding the role of that segment in health and disease.

FR-PO1766

Regulation of the Epithelial Na+ Channel by Intracellular Na+ – Mechanism and Time Course Ankit B. Patel,[1,2] Lawrence G. Palmer.[1] *[1]Physiology and Biophysics, Weill Cornell Medical College, New York, NY; [2]Tri-Institutional MD-PhD Program, Weill Cornell/Rockefeller University/Sloan-Kettering Institute, New York, NY.*

Background: Feedback inhibition of the epithelial Na+ channel (ENaC) by intracellular Na+ is a homeostatic mechanism to regulate epithelial sodium transport. Decreases in cell surface expression and open probability of ENaC are thought to be responsible for this regulation.

Methods: We investigated the time course and mechanism of feedback inhibition using two-electrode voltage clamp (TEVC) to measure ENaC activity and biotin labeling to examine cell surface expression. Brefeldin A was used to inhibit insertion of newly ENaC subunits in the plasma membrane.

Results: Incubation of rENaC-expressing Xenopus oocytes in high-Na+ buffer decreased ENaC currents by 70% over 80 minutes but had little effect on oocytes expressing Liddle's mutant (βR564X). However, western blots showed little to no change in cell-surface γ ENaC of WT or βR564X with 1-1.5 hour high-Na+ incubation. Overnight incubation in high-Na+ caused a 92% and 75% decrease in ENaC currents in WT and βR564X-expressing oocytes, respectively with a concomitant decrease in cell-surface γ ENaC in both groups. There was no change in cell surface expression of β ENaC during 1-1.5 hour or overnight high Na+ incubation. In the presence of Brefeldin A, high-Na+ incubation compared to low-Na+ incubation decreased ENaC currents by 82% over 8 hours. We also observed decreased surface expression of cleaved γ ENaC but not total γ or β ENaC.

Conclusions: We show here that increases in intracellular Na+ can decrease ENaC activity independent of changes in cell surface γ ENaC. Retrieval of Liddle's mutant (βR564X) with overnight high-Na+ incubation suggests a Nedd-4 independent retrieval of cell surface γ ENaC. The lack of change in cell surface β ENaC with high Na+ incubation suggests that cell surface expression of the β and γ subunits of ENaC is differentially regulated. Lastly, we show with Brefeldin A that cleaved γ ENaC is preferentially retrieved with high-Na+ incubation suggesting a key role for the regulation of cleaved γ ENaC in the feedback inhibition of the channel.

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FR-PO1767

Epithelial Na^+ Transport Regulation by AMP-Activated Protein Kinase in Kidney Collecting Duct and Its Role in Ischemia Hui Li, J. Darwin King, Rodrigo Alzamora, Jeffrey Lee, Nuria M. Pastor-Soler, Kenneth R. Hallows. *Medicine, University of Pittsburgh, PA.*

Background: The epithelial Na^+ channel (ENaC) in the kidney collecting duct regulates total body volume and blood pressure and is regulated by hormones and cellular signals, including metabolic stress induced by ischemia. The metabolic sensor AMP-activated protein kinase (AMPK) inhibits ENaC in kidney and lung epithelia, but the potential role of AMPK activation in the acute inhibition of ENaC following metabolic stress is unknown.

Methods: We used mouse polarized kidney cortical collecting duct ($mpkCCD_{c14}$) cells cultured on Transwells for surface biotinylation and Ussing chamber short-circuit current (I_{sc}) measurements of ENaC following AMPK activity modulation and chemical ischemia. We also used the ex vivo kidney slices method to examine treatment-dependent changes in ENaC immunolocalization by confocal microscopy.

Results: AMPK activation by 5-aminoimidazole-4-carboxamide riboside (AICAR) treatment (1 mM) inhibited both baseline and 10 nM desmopressin (dDAVP)-induced β-ENaC apical membrane expression in $mpkCCD_{c14}$ cells. Similarly, ex vivo AICAR treatment of kidney slices disrupted dDAVP-induced ENaC apical pole accumulation as assessed by immunolocalization in the CD. Treatment of $mpkCCD_{c14}$ cells with chemical ischemic agents, either an inhibitor of glycolytic metabolism (10 mM 2-deoxyglucose) or inhibitors of oxidative metabolism (0.1 µM antimycin A or 2-5 µM CCCP), elicited rapid reductions in amiloride-sensitive ENaC-dependent I_{sc}. These ENaC inhibition responses were blunted in the presence of the AMPK inhibitor Compound C (50 µM) or with inducible RNAi-mediated AMPK-α1 knockdown in $mpkCCD_{c14}$ cells, suggesting that AMPK activation plays a significant role in the ENaC inhibition response to ischemia.

Conclusions: AMPK inhibits ENaC activity and apical surface expression in kidney CCD cells and participates in the ischemia-induced down-regulation of ENaC activity. Inhibition of ENaC and other transport proteins by AMPK under conditions of metabolic stress may play an adaptive role by preventing the dissipation of ionic gradients generated by cellular pumps, thereby limiting cellular ATP consumption.

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FR-PO1768

ENaC-Mediated Sodium Reabsorption in $P2X_4$ Null Mice Eilidh Craigie,[1] Robert J. Unwin,[1] Scott S.P. Wildman,[2] David G. Shirley.[1] *[1]Centre for Nephrology, University College London Medical School, London, United Kingdom; [2]Department of Veterinary Basic Sciences, Royal Veterinary College, London, United Kingdom.*

Background: There is now overwhelming evidence that extracellular nucleotides, acting via P2 receptors, can modify ENaC-mediated sodium reabsorption in collecting ducts (Bailey & Shirley 2009, Purinergic Signal. 5:473). However, the P2 receptor subtype(s) involved have not been clearly defined. Pharmacological profiling and the use of genetically engineered mice point to an important role for $P2Y_2$ receptors (Pochynyuk *et al* 2010,FASEB J 24: 2056), but a patch-clamp investigation of rat cortical collecting duct principal cells has provided evidence that apical $P2X_4$ and/or $P2X_{4/6}$ receptors may also modulate ENaC activity (Wildman *et al* 2008, JASN 19: 731).

Methods: The present study was designed to assess, in vivo, the possible role of the $P2X_4$ receptor subunit in the regulation of ENaC activity. $P2X_4$ null mice ($P2X_4^{-/-}$), and their wild-type littermate controls ($P2X_4^{+/+}$), were anaesthetised and surgically prepared for renal clearance studies. After control collections of urine and plasma, mice were given the ENaC inhibitor benzamil (1 mg/kg bolus, IV), and further collections were made. Data are presented as mean±SEM; statistical comparisons were made using ANOVA.

Results: In animals used as drug-vehicle controls, GFR and fractional sodium excretion (FE_{Na}) remained stable. Benzamil treatment did not affect GFR significantly in either $P2X_4^{+/+}$ or $P2X_4^{-/-}$ mice. In $P2X_4^{+/+}$ mice, benzamil caused an increase in FE_{Na} from 0.6 ± 0.2 % to 1.9 ± 0.3% (n = 8, P<0.001; ΔFE_{Na} = 1.3 ± 0.2 %). In $P2X_4^{-/-}$ mice, benzamil increased FE_{Na} from 0.8 ± 0.2 % to 1.8 ± 0.2 % (n = 8, P<0.001; ΔFE_{Na} = 1.0 ± 0.3 %). The benzamil-induced increase in FE_{Na} was not significantly different between the two groups.

Conclusions: These data provide no solid evidence for a role for $P2X_4$ receptors in the regulation of ENaC-mediated sodium reabsorption in mice on a normal sodium intake. It remains to be seen whether this is the case when ENaC expression and activity is up regulated under sodium-restricted conditions.

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FR-PO1769

Extracellular ATP Directly Activates Ion Channels in a Mouse Collecting Duct Cell Line Toby S. Scott-Ward, Rebecca Birch, Claire M. Peppiatt-Wildman, Scott S.P. Wildman. *Urinary System Physiology Unit, Royal Veterinary College, London, United Kingdom.*

Background: Extracellular nucleotides have emerged as potent modulators of various renal functions. They exert their effects by binding to and activating cell-surface P2 receptors. These are subdivided into ionotropic P2X receptors (14 subtypes) and metabotropic P2Y receptors (8 subtypes). In the collecting duct, ENaC, AQP2, and ROMK activity and/or expression is altered by extracellular nucleotides. Due to a lack of selective agonists and antagonists, and notoriously unreliable antibodies, controversy exists over the

P2 receptor subtype(s) responsible. Arguably, P2X subtypes are largely overlooked when considering the receptor(s) responsible for extracellular nucleotide-evoked modulation of these key solute and water transport mechanisms.

Methods: Using a cell line (M1) derived from principal cells of the CCD, and perforated whole-cell and excised outside-out configurations of the patch-clamp technique, we have sought functional evidence for extracellular ATP-gated P2X receptor ion channel expression in the CD. The external (bath) solution contained 145 mM NaCl and the internal (pipette) solution contained 145 mM K^+ (70 mM Cl^-).

Results: Upon application of ATP (100 μM) at -60 mV, a large inward whole-cell current activated rapidly and then desensitised in approximately 50% of cells. Interestingly, whole-cell currents appeared to be comprised of several components including an initial rapidly desensitizing current. Furthermore, in outside-out patches, application of external ATP (100 μM) transiently activated flickery single-channel currents (t_{act} ~2 s, t_{des} ~30 s). At -100 mV, the mean single-channel current amplitude (i) and open probability (P_o)* of these channels were -0.59 ± 0.03 pA and 0.63 ± 0.02 (n = 3).

Conclusions: Our result demonstrate that M1 cells express functional ATP-sensitive P2 receptors and, moreover that ATP is able to directly activate channels with properties similar to those of P2X subtypes. Based on these data, we suggest P2X receptors may be key in the regulation of CD solute and water transport.

* from start of first open event to end of last open event.

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FR-PO1770

Functional Coupling between Antinatriuretic Ang II and Natriuretic Bradykinin Signaling Cascades Is Critical for Aldosterone-Independent Regulation of ENaC by Dietary Sodium Intake Oleh Pochynyuk, Mykola Mamenko, Oleg L. Zaika. *Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, TX.*

Background: It is generally accepted that aldosterone affects activity of the epithelial Na^+ channel (ENaC) in the distal nephron to regulate circulating volume and, consequently blood pressure. However, growing experimental evidence suggest that complementary to aldosterone mechanisms might have a role in regulation of ENaC activity in response to variations in dietary salt intake.

Methods: We used patch clamp electrophysiology in freshly-isolated split-opened murine distal nephrons to test if ENaC can be regulated by systemic salt intake independently of aldosterone.

Results: Inhibition of MR receptors with spironolactone, while decreasing ENaC membrane levels, did not affect regulation of ENaC open probability by dietary sodium. We hypothesized that activation Ang II signaling may contribute to regulation of ENaC by salt intake. Indeed, we found that Ang II in the range from 5 to 500 nM acutely and reversibly increases ENaC Po. Activation of AT1 receptors with subsequent stimulation of NADH oxydase mediated Ang II actions on ENaC. Importantly, saturation of MR status with DOCA treatment did not perturb Ang II regulation of ENaC suggesting that the effect of Ang II is non-redundant. In addition, we found that activation of Ang II cascade further increases ENaC activity via Angiotensin Converting Enzyme (ACE)-dependent mechanism by limiting the inhibitory actions of locally produced Bradykinin (BK). ACE inhibition augmented the inhibitory action of BK on ENaC and caused marked natriuresis in wild type but not in mice lacking BK receptors.

Conclusions: We concluded that the balance between stimulatory Ang II and inhibitory BK cascades allows fine-tuning of ENaC activity during variations in dietary salt independently of aldosterone.

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FR-PO1771

Aldosterone Induces Epigenetic Reprogramming of the αENaC Gene in mIMCD3 Cells Zhiyuan Yu, Qun Kong, Bruce C. Kone. *University of Texas-Houston Medical School.*

Background: Aldosterone increases renal tubular Na^+ reabsorption in part by enhancing α*ENaC* transcription. In addition to known effects of aldo to *trans*-activate α*ENaC* via the mineralocorticoid receptor (MR), we previously reported that α*ENaC* transcription is also governed by an aldosterone-sensitive epigenetic pathway involving histone H3K79 methyltransferase Dot1 and DNA binding protein Af9. The Dot1-Af9 complex associates with and represses basal α*ENaC* transcription in mIMCD3 cells, and aldo releases this repression, increasing αENaC promoter activity and endogenous mRNA levels beginning at 3 h of treatment (J Biol. Chem 284:20917-26, 2009). Determining how genes move through repressed, primed, and active chromatin states is central to understanding transcriptional induction. To understand aldo-induced activation of α*ENaC* transcription, a high-resolution kinetic analysis of aldo-induced changes in the histone code, the recruitment and action of enzymes mediating these changes, and the recruitment and action of MR is needed.

Methods: Time-course ChIP/qPCR assays of the R0 (-988/-713), R1 (-735/-415), R2 (-414/+80), and R3 (-57/+494) subregions of the α*ENaC* promoter from chromatin harvested from mIMCD3 cells in response to 1 μM aldo or vehicle (as a time control) were performed. A common internal genomic fragment was used for normalization.

Results: Under basal conditions, the epigenetic signature of the α*ENaC* promoter included repressive chromatin marks H3K27me3 at R1 and R2, H4K20me3 at R2, and H3K9me3 at R3 (as well as the previously described H3K79 methylation at R0-R3), and occupancy of Dot1, Af9, Sirt1, and Suv39H1. With aldo treatment, Af9 occupancy and H3K79 methylation diminished within 30 min, whereas MR occupancy was first evident at 1 h. Maximal Pol II occupancy, indicative of transcriptional activation, occurred at 1.5 h. In addition, H4K20me3 at R2 and Suv39H1 at R3 decreased while the active chromatin marks H3K36me3, H3K9me1, and H3K4me3 were increased at R1 and R3 during this time frame.

Conclusions: Aldo induces α*ENaC* transcription through an initial, complex reprogramming of the histone code that dismisses the Dot1-Af9 complex to effect de-repression, followed by MR-mediated *trans*-activation of the gene.

Funding: NIDDK Support

FR-PO1772

An Aldosterone-Regulated NH_2-Terminal Sgk1 Variant with Enhanced Function Is Expressed in the Collecting Duct Christie P. Thomas,[1,2] Nandita S. Raikwar.[1] [1]*Internal Medicine, University of Iowa, Iowa City, IA;* [2]*VAMC, Iowa City, IA.*

Background: Serum and glucocorticoid-regulated kinase 1 (Sgk1), regulates ENaC-mediated Na^+ transport in the distal nephron.

Methods: Sgk1 transcripts were examined in mouse nephron segments and in the collecting duct cell line, $mpkCCD_{c14}$, by qRT-PCR. Sgk1 isoforms were expressed with or without ENaC subunits in $mpkCCD_{c14}$ and FRT epithelia and Na^+ transport measured in Ussing chambers. ENaC surface expression and cleavage were studied in HEK293 cells. Ubiquitination and half-life measurements of Sgk1 isoforms were also studied in HEK293.

Results: Previously, we identified a cell-surface expressed Sgk1 isoform (Sgk1_i2) that stimulates Na^+ transport (AJP 2008, 295: 321-326). We have now identified an aldosterone and insulin-regulated alternate transcript of Sgk1 that is expressed in the native mouse DCT, CNT and CCD and in $mpkCCD_{c14}$. The encoded protein, Sgk_i3 has a variant NH_2-terminus that results in a Sgk1 isoform that is less susceptible to ubiquitination, is more stable and significantly increases Na^+ transport when overexpressed in $mpkCCD_{c14}$ and when co-expressed with ENaC in FRT cells. The increase in Na^+ transport requires an intact kinase domain. Sgk1_i3 increases ENaC cleavage at the cell surface and inhibits the effect of Nedd4-2 to reduce ENaC cleavage, similar to the prototypic Sgk1. Mutation of a conserved polybasic amino acid motif (KKR) in its variant NH_2-terminus converts Sgk1_i3 from a combined nuclear and cytosolic protein to exclusively cytosolic suggesting that the KKR motif may be part of a nuclear localization signal. This KKR motif is also a destabilizing motif; possibly a target for ubiquitination, since mutation of KKR increases the abundance of Sgk1_i3 which correlates with reduced ubiquitination and an increase in the half-life of Sgk1_i3.

Conclusions: In conclusion, we have identified a regulated NH_2-terminal variant of Sgk1 that is expressed in the distal nephron. The encoded protein isoform inhibits Nedd4-2, increases ENaC cleavage and stimulates epithelial Na^+ transport and probably contributes to aldosterone and insulin-stimulated epithelial Na^+ transport in the CNT and CCD.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support

FR-PO1773

Mechanistic Basis for Specific Activation of SGK1 by mTOR Ming Lu, Jian Wang, Harlan Ives, David Pearce. *Medicine, University of California, San Francisco, CA.*

Background: The serum- and glucocorticoid-induced kinase 1 (SGK1) plays an important role in hormone regulation of ENaC-dependent Na^+ transport. We have previously reported that the mTOR complex-2 (mTORC2) activates ENaC by phosphorylating SGK1. It is, however, unknown which mTORC2 component mediates this interaction, or whether this interaction plays a physiologically relevant role in specific activation of SGK1

Methods: We used the yeast two-hybrid system coupled with random mutagenesis to identify a mutant mSIN1 that does not interact with SGK1.

Results: Expression of the mSIN1 mutant does not restore SGK1 phosphorylation to wild-type levels in mSIN1-deficient murine embryo fibroblasts. Furthermore, in kidney epithelial cells, the mSIN1 mutant has a dominant-negative effect on SGK1 phosphorylation and on SGK1-dependent ENaC-mediated Na^+ transport. Interestingly, the role of mSIN1 to recruit SGK1 to mTOR appears to be specific for SGK1: although mSIN1 is essential for phosphorylation of another mTORC2 substrate, Akt, it does not interact with Akt and its ability to phosphorylate and activate Akt is unaffected by the point mutation that abrogates interaction with SGK1.

Conclusions: These data support the conclusion that mTOR, which regulates a wide array of cellular processes, uses distinct strategies to phosphorylate its various substrates, and suggest a mechanism for specific regulation of ENaC-mediated Na^+ transport without inadvertent effects on unrelated cellular processes.

Funding: NIDDK Support

FR-PO1774

Role of Sgk2 in Mediating the Regulation of ENaC by Oxygen Russell F. Husted,[1] Rita D. Sigmund,[2] John B. Stokes.[1,2] [1]*Internal Medicine, University of Iowa, Iowa City, IA;* [2]*Internal Medicine, VA Medical Center, Iowa City, IA.*

Background: We have previously demonstrated that changing the ambient oxygen concentration between 1% and 40% has a potent effect on ENaC-mediated Na transport independent of the actions of corticosteroids. This oxygen effect requires hours to become evident, exhibits a clear dose response, and is completely reversible. The purpose of the present experiments was to determine possible molecular mediators of this oxygen effect.

Methods: We used the mouse collecting duct cell line mpkCCD-c14 grown on filters where corticosteroids had been withdrawn for 24 h and measured Na transport by short-circuit current (Isc).

Results: We first investigated the possible role of candidate kinases. At normal oxygen levels inhibitors of ERK1-2 and p38 had minimal effect while inhibitor of JNK reduced Isc by ~50%. However, none of these inhibitors altered the relative effects of 8% or 40% oxygen on Isc. Thus, while JNK may regulate baseline Na transport, it appears not to be involved in the effects of oxygen to regulate ENaC activity. Microarray analysis identified Sgk2 as a possible candidate. Real time PCR demonstrated an increase in Sgk2 mRNA with higher oxygen but no effect of oxygen on Sgk1 or Sgk3 mRNA. Immunoblot demonstrated a similar effect on Sgk2 protein abundance. We constructed Sgk1 and Sgk2 in a tetracycline regulated adenovirus expression system and found that increased expression of Sgk2 had a more potent stimulatory effect on Isc than did Sgk1 at normal oxygen content. Overexpression of Sgk1 did not alter the relative response to changing oxygen. In contrast, overexpression of Sgk2 produced a greater effect at low oxygen and a blunted effect at high oxygen content. Mutation of the analogous phosphorylation site in Sgk2 required for activation by PDK2 (probably mTORC2) greatly blunted the regulatory effect of oxygen.

Conclusions: Regulation of Sgk2 activity has not previously been reported to our knowledge. The results of these studies raise the possibility that Sgk2 plays a role in the regulation of ENaC activity by oxygen.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1775

ENaC Inhibition Stimulates Conductive Cl^- Secretion in the Cortical Collecting Duct Vladimir Pech, Susan M. Wall. *Deparment of Medicine, Emory University School of Medicine, Atlanta, GA.*

Background: In cortical collecting ducts perfused in vitro, Cl^- absorption is reduced when epithelial Na^+ channel inhibitors, such as benzamil, are applied to the luminal fluid. Since ENaC does not transport Cl^-, this study explored the mechanism of benzamil-sensitive Cl^- absorption.

Methods: To do so, Cl^- absorption was measured in CCDs perfused in vitro, taken from mice given aldosterone for 7 days.

Results: Luminal hydrochlorothiazide had no effect on either Cl^- absorption or transepithelial voltage. However, application of benzamil (3 mM) to the luminal fluid reduced Cl^- absorption and transepithelial voltage by ~75% in wild type mice, but had no effect in CCDs from collecting duct-specific ENaC null mice ($Scnn1a^{loxloxcre}$). Eliminating the transepithelial voltage with application of a Na,K-ATPase inhibitor (ouabain) to the bath, also abolished Cl^- absorption, suggesting that benzamil reduces Cl^- absorption by reducing the lumen-negative transepithelial voltage. Benzamil-sensitive Cl^- absorption did not depend on the apical H^+-ATPase or maxi-K^+ channels. In the absence of benzamil, addition of the Cl^- channel inhibitor, DIDS (100 mM), to the luminal fluid had no effect. However, application of DIDS increased Cl^- absorption from 4.3 ± 3.3 to 19.5 ± 4.4 pmol/mm/min ($n=6$, $P < 0.05$) when benzamil was present in the lumen. In addition, with benzamil in the lumen, application of the Na-K-2Cl cotransporter, bumetanide(100 mM) to the bath increased Cl^- absorption from 7.4 ± 3.3 to 16.7 ± 4.9 pmol/mm/min ($n=6$, $P < 0.05$).

Conclusions: 1) in the CCD of aldosterone-treated mice, most Cl^- absorption is benzamil-sensitive, while thiazide-sensitive Cl^- absorption is undetectable and 2) Benzamil applied to the perfusate reduces Cl^- absorption most likely by eliminating the transepithelial voltage, thereby eliminating the driving force for paracellular Cl^- absorption and stimulating conductive Cl^- secretion mediated by an apical Cl^- channel that acts in series with Cl^- uptake across the basolateral membrane mediated by NKCC1.

Funding: NIDDK Support, Private Foundation Support

FR-PO1776

Preeclampsia Is Associated with Significant Urinary Excretion of Plasmin(ogen) and the Ability of Urine To Activate ENaC In Vitro Kristian B. Buhl,[1] Ulla G. Friis,[1] Bente Jespersen,[2] Per Ovesen,[2] Per Svenningsen,[1] Claus Bistrup,[3] Britta Frederiksen-Møller,[3] Boye Jensen.[1] *[1]University of Southern Denmark, Denmark; [2]Aarhus University Hospital, Denmark; [3]Odense University Hospital, Denmark.*

Background: In patients with nephrotic syndrome, plasminogen is filtered from plasma to pre-urine and activated. Plasmin can proteolytically activate ENaC by cleavage of the γ-subunit, leading to pathophysiological sodium reabsorption.

It was hypothesized that excretion of plasmin(ogen) and the ability of urine to stimulate ENaC activity is a feature of preeclampsia.

Methods: In a cross-sectional study, urine samples were obtained from 16 preeclamptic patients (PE) and 17 normotensive, non-proteinuric pregnant women matched on age and gestational age (Ctrl). Urine was analysed for plasminogen, creatinin, albumin and proteolytic activity. ENaC current after exposure to urine was monitored in mouse cortical collecting duct cells (M1) by whole cell patch clamp.

Results: Urine plasmin(ogen)-creatinine ratio was elevated significantly in PE compared to Ctrl. (geometric mean: 1.7×10^{-4} vs. 3.3×10^{-6}, $p<0.0001$). A significant positive correlation was found in the PE group between urinary plasmin(ogen) and diastolic blood pressure ($p= 0.05$). In the PE-group, proteolytic activity was detected at 75 kD by gelatin zymography in 9 of 13 compared to 1 of 13 in Ctrl. Western blotting of urine yielded results compatible with presence of both plasminogen and plasmin in PE samples. Whole cell patch clamp on M1 cells showed significant increases in current when M1 cells were exposed to urine from PE ($172.6\% \pm 51.4\%$, $n=6$, $p = 0.0002$). The ability of the PE samples to evoke current was abolished by amiloride (2 μM), α_2-antiplasmin (1 μM) and by boiling of the urine samples prior to cell exposure.

Conclusions: Preelampsia is associated with significant urinary excretion of plasmin(ogen) and activation of ENaC by urine. Urinary plasminogen is significantly correlated to blood pressure. We speculate that pathophysiological activation of ENaC by urinary plasmin may contribute to hypertension and edema in preeclampsia.

Funding: Pharmaceutical Company Support, Private Foundation Support

FR-PO1777

Urinary Content of Plasmin(ogen) and Activation of ENaC Current by Urine Resides during Remission of idiopathic Nephrotic Syndrome Kristian B. Buhl,[1] Rene Frydensbjerg Andersen,[2] Ulla G. Friis,[1] Per Svenningsen,[1] Boye Jensen,[1] Soren Rittig.[2] *[1]University of Southern Denmark, Denmark; [2]Aarhus University Hospital, Denmark.*

Background: In nephrotic syndrome (NS), data show glomerular filtration of plasminogen to pre-urine and activation to plasmin. Urine plasmin activates the epithelial Na^+ channel (ENaC) in vitro. It was hypothesized that this mechanism is causal for NaCl retention and therefore that plasmin and the ability of urine to activate ENaC disappears in the remission phase of nephrotic syndrome.

Methods: Spot urine samples from 20 children with active idiopathic NS were collected and compared to urine samples obtained after remission in the same patients. Urine samples were analyzed for plasmin and plasminogen concentration (ELISA) and urinary protease activity (zymography). Ability of urine to evoke ENaC currents were assessed by whole cell patch clamp using a murine cortical collecting duct cell line (M1).

Results: 20 patients (7 girls) mean age 9.1 ± 3.2 yrs were included. Urine plasmin(ogen) concentration normalized to urine creatinin concentration was found significantly differently in the active phase of NS in comparison to remission by paired t-test ($p<0.0001$, geometric mean: 2244 μg/g vs. 83 μg/g). Gelatin Zymography showed protease activity in urine in 10 of 17 patients in active phase compared to 3 of 17 patients at remission. Western blotting analysis of urine from active phase of NS showed results compatible with the presence of both plasmin and plasminogen whereas at remission, 10 out of 10 tested urine samples were negative. Urine from the active phase of NS evoked a significant increase in ENaC current in M1-cells ($201\% \pm 31\%$, $p<0.006$, $n=6$) that was significantly larger than current evoked by a urine sample from the same individual in the remission phase ($p<0.0006$). Addition of amiloride (2 μmol/L) to urine samples from patients in active phase abolished inward currents.

Conclusions: The parallel observation of remission and normalisation of urine plasmin excretion and proteolytic activity is compatible with a plasma origin of plasmin in urine and a causal role in edema. Further studies should test this by intervention.

Funding: Private Foundation Support

FR-PO1778

Proteinuria Induces Increased Insulin- and IGF1-Receptor Signalling, a Mechanism of ENaC-Mediated Volume Retention Franziska Theilig,[1] Christoph Geers,[1] Daniela Corinne Spohr,[1] Anne Enke,[1] Harm Peters.[2] *[1]Department of Medicine, Institute of Anatomy, Fribourg, Switzerland; [2]Institute of Nephrology, Berlin, Germany.*

Background: Proteinuria is a symptom of many renal glomerular diseases. It is associated with signs of volume retention such as edema formation or hypertension. In the collecting duct a dysregulation of ENaC is assumed to be causative and hormonal activation of aldosterone and vasopressin was excluded.

Therefore, we hypothesized an activation of the insulin- and IGF-receptor signalling to account for the volume retention in proteinuric kidney diseases.

Methods: For the induction of an experimental glomerulonephritis (GN; type Thy1) and puromycin-induced nephritic syndrome (PAN) Wistar rats were injected either with OX-7, puromycin or vehicle. After 6 days, kidneys were prepared for histochemical or biochemical analysis.

Results: Urinary excretion of insulin (control 2.19 ± 0.5; GN 25.1 ± 9.9 und PAN 18.6 ± 6.4 ng/ 24h) and IGF-1 (control 11.9 ± 1.7; GN 264.6 ± 65.5 und PAN 431.9 ± 58.24 ng/ 24h) were strongly increased. Insulin- and IGF-1-R were localized to the apical and basolateral membrane of the collecting duct. Proteinuria induced an increased phosphorylation of the apical insulin/IGF-1 receptor. Insulin and IGF-1 induced a "priming effect" of their respective receptors as determined by cell surface biotinylation experiments and confocal microscopy of mpkCCD cells. An activation of the insulin/ IGF-1R induced signalling cascade with phosphorylation of PDK1, Akt, WNK1, sGK1, Nedd4-2 and consecutive increased ENaC expression was observed upon insulin/ IGF-1 mediated apical stimulation of mpkCCD cells as well as in GN and PAN.

Conclusions: In summary, our results show that proteinuria activated the apical insulin/ IGF-1R pathway and may therefore be an additional mechanism for ENaC mediated volume retention in proteinuric kidney diseases.

FR-PO1779

Nephron Expression and Distribution of the Plasminogen Receptor, PLG-R_{KT}, and Colocalization with ENaC and uPAR, in Murine Kidney Samir Nangia,[1] Hongdong Bai,[1] William B. Kiosses,[2] Kevin W. Chen,[1] Volker Vallon,[1] Lindsey A. Miles,[2] Robert J. Parmer.[1] *[1]University of California, San Diego, and VA San Diego Healthcare System, San Diego, CA; [2]Scripps Research Institute, La Jolla, CA.*

Background: Recent studies suggest a key role for the plasminogen (PLG) activation system in the proteolytic processing and activation of ENaC, providing an important mechanism for the Na^+ retention associated with nephrotic syndrome, in which increased PLG concentrations are present in urine. We recently identified a novel transmembrane PLG receptor, PLG-R_{KT}, which markedly enhances cell surface activation of PLG to the active protease plasmin. Here, we investigated the expression, distribution, and cellular localization of PLG-R_{KT} in murine kidney, and performed quantitative colocalization studies of PLG-R_{KT} and the urokinase receptor (uPAR, another key component of the PLG activation system), with ENaC.

Methods: C57Bl6 mice were perfused *in situ* with 4% paraformaldehyde. Kidneys were fixed, placed through sucrose gradients and frozen in OCT. Sections were immunostained with antisera specific for PLG-R_{KT}, uPAR, and γENaC, followed by fluorescent secondary antibody, and examined using high resolution laser scanning confocal microscopy.

Results: PLG-R_{KT} was prominently expressed in proximal and distal nephron segments, particularly in the distal tubule and collecting duct (as revealed by co-staining with antisera to the sodium chloride co-transporter and aquaporin 2). PLG-R_{KT} was observed primarily on the apical surface, with some labeling also in a punctate distribution in the cytoplasm, in a pattern similar to that observed for γENaC. uPAR (a GPI-linked membrane protein) was primarily observed in the distal nephron, and was almost exclusively on the apical surface. Quantitative analyses of merged images showed substantial apical colocalization of PLG-R_{KT} with ENaC (70.1±1%, n=507 cells), and uPAR with ENaC (62±1%, n=314 cells).

Conclusions: These results demonstrate that PLG-R_{KT}, uPAR, and ENaC are co-localized on the apical surface in the distal nephron, and are present in an orientation to promote PLG activation and ENaC processing.

Funding: Other NIH Support - NHLBI, Veterans Administration Support

FR-PO1780

PAR2 Controls the Activity and Expression of Electroneutral Thiazide-Sensitive Sodium Transport in Collecting Duct and Blood Pressure Luciana Morla, Gaëlle Brideau, Lydie Cheval, Gilles Crambert, Suresh Krishna Ramakrishnan, Alain Doucet. *UMRS 872 team 3, UPMC Univ Paris 06 and INSERM and CNRS, Paris, France.*

Background: We previously showed that PAR2 activation increases sodium absorption in cortical collecting ducts (CCD). Therefore, we evaluated whether it participates in the maintenance of blood pressure.

Methods: For this purpose, we compared blood pressure and sodium handling in PAR2$^{-/-}$ and wild type (WT) mice under basal state and sodium depletion *in vivo* and *in vitro* microperfused CCDs.

Results: Under basal state, PAR2$^{-/-}$ mice displayed normal sodium excretion and blood pressure. During the first five days of sodium depletion, PAR2$^{-/-}$ mice showed a blunted ability to maximally decrease their urinary excretion of sodium, as compared to WT mice. This inappropriate ability to conserve sodium was associated with decreased systolic blood pressure (15-20 mmHg) in PAR2$^{-/-}$ mice whereas WT mice maintained a normal blood pressure.

CCDs from WT and PAR2$^{-/-}$ mice fed a normal diet displayed no sodium transport when perfused *in vitro*. However, activation of PAR2 by trypsin or a specific agonist peptide increased electroneutral, thiazide-sensitive sodium reabsorption in WT but not PAR2$^{-/-}$ mice. Sodium depletion induced both electrogenic amiloride-sensitive and electroneutral thiazide-sensitive sodium transport in CCDs of WT mice, but exclusively the electrogenic component in PAR2$^{-/-}$ mice. Thus, PAR2 not only stimulates electroneutral reabsorption of sodium in the CCD, but it is also responsible for the induction of this pathway observed in response to sodium depletion.

Conclusions: In conclusion, through its actions on electroneutral sodium transport, PAR2 participates to the maintenance of blood pressure during sodium depletion.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1781

Reduced Anti-Natriuretic Response to Insulin and Benzamil in Mice Lacking Insulin Receptors in the Collecting Duct Lijun Li, Radha Mayuri Garikepati, Carolyn M. Ecelbarger. *Department of Medicine, Georgetown University, Washington, DC.*

Background: The epithelial sodium channel (ENaC) has been shown to be upregulated by insulin in a variety of cell systems and in perfused tubules, but whether circulating insulin plays a role in day-to-day sodium balance is unclear.

Methods: To address this, the IR was selectively knocked out from the collecting duct using mice with Cre recombinase driven by the aquaporin-2 promoter. Natriuretic responses to insulin, dextrose in saline, and select sodium transporter and channel antagonists were studied in both male (M) and female (F) knockout (KO) and wild-type (WT) littermates (3-4 months old).

Results: There were no differences in body weight (bw), basic renal function, or kidney size due to genotype in either sex, with M mice about 5 grams heavier than F. Insulin (0.5 U/kg bw) or vehicle was administered intraperitoneally, and urine collected in metabolic cages for 4 hours. The anti-natriuretic response to insulin was significantly blunted in the KO mice: (μmol Na^+/25g bw/4hr): 5.3 ± 1.6, 13.6 ± 2.7, 8.7 ± 2.5, and 12.4 ± 2.7 for MWT, MKO, FWT, and FKO, respectively ($p = 0.02$ for genotype). Sex differences in this response were not observed. To determine whether reduced basal ENaC activity might play a role in this response, mice were injected with benzamil, ENaC antagonist (1.4 mg/kg bw). KO mice had a significantly blunted diuretic response suggesting reduced basal activity of ENaC in these mice (μl/25g bw/4hr): 399 ± 59, 341 ± 78, 445 ± 75, and 225 ± 30 for MWT, MKO, FWT, and FKO, respectively ($p = 0.04$ for genotype). Sodium excretion trended toward being lower as well in this test in KO, but wasn't quite significant ($p = 0.13$). In contrast, diuretic and natriuretic responses to hydrochlorothiazide (NaCl-cotransporter antagonist) were not affected by genotype, but significantly higher in females.

Conclusions: Our findings suggest that insulin, via its own receptor, is important in renal sodium handling by the collecting duct via activation of ENaC. Fluctuations in circulating insulin levels, therefore, due to diet, disease, or therapy, may be expected to alter sodium handling independently of the renin-angiotensin-aldosterone system.

Funding: NIDDK Support, Private Foundation Support

FR-PO1782

Mechanism of the Epithelial Na+ Channel (ENaC) Regulation by Insulin Tengis S. Pavlov,[1] Vladislav Levchenko,[1] Carolyn M. Ecelbarger,[2] Alexander Staruschenko.[1] *[1]Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Medicine, Georgetown University, Washington, DC.*

Background: Sodium reabsorption via the epithelial Na^+ channel (ENaC) in the aldosterone-sensitive distal nephron (ASDN) plays a central role in body fluid volume regulation. Insulin is recognized as a powerful regulator of ENaC in the collecting duct.

Methods: To study mechanisms of ENaC regulation by insulin, we generated insulin receptor knockout (IR-KO) mice targeted specifically to the collecting duct principal cells using Cre-lox mediated recombination. Mice with loxP sites flanking the IR gene were crossed with mice possessing Cre-recombinase driven by the AQP2-promoter.

Results: After one week of sodium-deficient diet the IR-KO mice demonstrated significantly lower ENaC activity compared to their wild type littermates as was demonstrated by cell attached patch clamp measurements in freshly isolated split opened collecting duct. Acute insulin application in such experiments revealed that loss of insulin reception prevented increase of ENaC activity which was observed in wild type mice. Immunohistochemical and western-blot assays demonstrated that total abundance of all three ENaC subunits in the kidney cortex were not different between WT and IR-KO mice. Thus, these results suggest that insulin via IR increases ENaC activity affecting the channel open probability (Po). To further determine mechanism of insulin's action on ENaC, we used immortalized mpkCCD$_{c14}$ principal cells. Insulin rapidly increased amiloride-sensitive transepithelial flux in mpkCCD$_{c14}$ cells with the EC_{50}=12.2±1.7nM. Pretreatment of the mpkCCD$_{c14}$ cells with PI3-kinase or mTOR inhibitors LY294002 or PP242, respectively precluded the effect of insulin.

Conclusions: Thus, we propose that insulin is a key regulator of ENaC activity and its effects are mediated via PI3-kinase and mTOR signaling.

Funding: NIDDK Support, Private Foundation Support

FR-PO1783

Effect of Formate on Flow-Dependent Transport of Sodium, Bicarbonate and Chloride in Proximal Tubule Zhaopeng Du,[1] Nanami Gotoh,[1] Sheldon Weinbaum,[2] Alan Mark Weinstein,[3] Tong Wang.[1] *[1]C. & M. Physiology, Yale University, School of Medicine, New Haven, CT; [2]Biomedical Engineering, City College of New York, New York, NY; [3]Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY.*

Background: Glomerulotubular balance (GTB) refers to the nearly proportional change in salt and water reabsorption in proximal tubules with variation in GFR. We have previously demonstrated that axial flow impacts proximal reabsorption of $Na^+$and HCO_3^- by modulating both NHE3 and H-ATPase activity. In this study we investigated whether Cl^- absorption is also impacted by axial flow in the proximal tubule.

Methods: Mouse proximal tubules were perfused in vitro at low (5nl/min) and high (20 nl/min) perfusion rates in the absence and presence of 0.5mM of formate to activate the Cl^-/formate-exchanger. The fluid (J_v) and HCO_3^- (J_{HCO3}) absorption were measured by the changes of ^{3}H-Inulin and total CO_2 concentrations in the original and collected fluid, and the J_{Na} was estimated from the change of J_v and the assumption of isotonic transport. The change in J_{Cl} was estimated as the difference between J_{Na} and J_{HCO3}.

Results: Similar to our previous report, a 53% increase of fluid and Na^+ and doubling of HCO_3^- absorption were observed when the flow rate was increased from 5 to 20 nl/min. Flow did not affect Cl^- absorption, as J_{Cl} was 47.8 and 40.5 pmole/min/mm at low and high flow rates, respectively. Addition of formate significantly increased both Na^+ and HCO_3^- absorption with a stronger increment in Cl^-, but the percentage of increment of transport activity by formate was similar at both low and high flow rates. J_{Na}, J_{HCO3} and J_{Cl} increased by 43%, 39% and 50% and by 42%, 39% and 51% at the low and high flow rate, respectively. Specifically, in the presence of formate, J_{Cl} was 71.4 and 61.3 pmol/min/mm ($P>0.05$) at low and high flow rates.

Conclusions: These results indicate that Cl^- absorption is not impacted by axial flow, and that the absence of a flow effect persists in the presence of formate. They suggest that there is unlikely to be a flow-dependent change in luminal membrane density of the Cl^-/formate-exchanger.

Funding: NIDDK Support

FR-PO1784

Adenosine Receptors Modulate Sodium Uptake in Human Renal Proximal Tubule Cells Earl H. Rudolph, Christopher S. Wilcox, William J. Welch. *Medicine/Nephrology, Georgetown University, Washington, DC.*

Background: Adenosine (Ado) generated in renal proximal tubule cells (RPTCs) acts on local receptors to mediate sodium (Na^+) reabsorption. Inhibition of Ado type 1 receptors (A_1-AR) leads to natriuresis and diuresis, primarily due to its effects in the PT, whereas the effects of inhibition of type 2A receptors (A_{2A}-AR) are unknown.

Methods: To explore the role of Ado in RPTCs, we tested the effects of A_1-AR and A_{2A}-AR inhibition and stimulation on $^{22}Na^+$ uptake in cultured human RPTCs (HK-2 cells). We hypothesized that Ado signals through A_1-AR and A_{2A}-AR to modulate the activity of the Na^+-hydrogen exchanger 3 (NHE3), the major Na^+ reabsorption pathway in RPTCs.

Results: As expected, inhibition of NHE3 with S1611 (10^{-5}M) reduced ^{22}Na uptake by ~60-65% vs. control (Control: 4678±358 vs S-1611: 1772±448 cpm, p<0.001). The A_1-AR antagonist PSB-36 (10^{-8} M) reduced $^{22}Na^+$ uptake by ~20% vs. control (Control: 4678±358 vs PSB: 3707±302 cpm, p<0.001). PSB had no additional effect on $^{22}Na^+$ uptake in the presence of S-1661 vs. NHE3 inhibition alone. Ado deaminase (AD, 5U/mL), which metabolizes endogenous Ado and thereby attenuates AR signaling, inhibited $^{22}Na^+$ uptake by ~20-25% vs. control (Control: 4678±358 vs AD: 3601±424 cpm, p<0.005). Addition of the A_1-AR agonist CHA (10^{-6}M) in the presence of AD restored $^{22}Na^+$ uptake vs. AD alone (CHA+AD: 4689±279 vs. AD: 3601±424 cpm, p<0.005). However, CHA had no additional effect on $^{22}Na^+$ uptake in the presence of S-1611. The A_{2A}-AR antagonist ZM241385 (10^{-8}M) had no effect on $^{22}Na^+$ uptake, with or without inhibition of NHE3. Similarly, the A_{2A}-AR agonist CGS (10^{-8}M) in the presence of AD had no effect on $^{22}Na^+$ uptake, with or without NHE3 inhibition.

Conclusions: In summary, inhibition of A_1-AR decreased $^{22}Na^+$ uptake and stimulation of A_1-AR reduced $^{22}Na^+$ uptake in RPTCs and both actions were attenuated by simultaneous NHE3 blockade. These results suggest that Ado acts on A_1-ARs through signaling pathways that inhibit or promote NHE3 activity. However, neither inhibition nor stimulation of A_{2A}-AR had an effect on $^{22}Na^+$ uptake, either in the presence or absence of NHE3 inhibition, suggesting A_{2A}-AR does not modulate Na^+ uptake in this model.

Funding: NIDDK Support, Other NIH Support - NHLBI

FR-PO1785

MPGES-1-Derived PGE2 Mediates Dehydration Natriuresis Zhanjun Jia,[1,2] Gang Liu,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah, Salt Lake City, UT; [2]Internal Medicine, Veterans Affairs Medical Center, Salt Lake City, UT.*

Background: Dehydration natriuresis is an important physiological response, aiming to reduce the plasma Na^+ and maintain normal plasma osmolality. PGE2 is a natriuretic factor whose production is elevated after water deprivation.The goal of this study was to investigate the role of mPGES-1 in dehydration natriuresis.

Methods: mPGES-1 WT and KO mice were subjected to the 24-h water deprivation (WD).

Results: After 24-h WD, WT mice exhibited a significant increase in 24-h urinary Na^+ excretion (159.2 ± 21.1 vs. 231.2 ± 24.9 μmol/24hl, p<0.01), accompanied with normal plasma Na^+ concentration and osmolality. In contrast, WD-induced elevation of urinary Na^+ excretion was completely abolished in KO mice (152.6 ± 17.3 vs. 128.6 ± 25.9 μmol/24h p>0.05), in parallel with trend increases in plasma Na+ concentration (139.2±1.5 vs. 142.2±1.4 mmol/L, p=0.06) and osmolality (278.4±2.26 vs. 284.0±2.34 mOsm/kgH2O, p=0.051). By qRT-PCR, renal medullary COX-2 mRNA in dehydrated WT mice was upregulated by 2.5 folds, contrasting to unaltered renal mRNA expression of COX-1 and mPGES-1. WD induced 1.8-fold increase in urinary PGE2 output and 1.6-fold increase in PGE2 content in the renal medulla but not the renal cortex of WT mice, both of which were completely abolished in Ko mice. In response to WD, WT mice exhibited a 20% increase in urinary nitrate/nitrite output (p<0.05) and a 2.6-fold increase of urinary cGMP (p<0.01), both of which was completely blocked in the KO mice. By qRT-PCR, the natriuretic EP1 and EP3 subtypes in the renal medulla but not renal cortex were upregulated by 1.5-fold and 1.7-fold, respectively, following WD. In primary IMCD cells EP3 mRNA was elevated by hypertonicity at 540 mOsm/kg H20, irrespective of the type of solutes, whereas the elevation of EP1 mRNA was dependent on the solutes with the stimulation by NaCl, Na-gluconate, but not Cl^- or mannitol.

Conclusions: We conclude: 1) mPGES-1-derived PGE2 contributes to dehydration natriuresis likely via NO/cGMP pathway; 2) EP1 and EP3 in renal collecting cells may distinctively involved in natriuretic effect of PGE2 during water deprivation.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1786

EP1 Receptors Mediate Renal Compensatory Response to Thiazolidinedione-Induced Fluid Retention Via NO/cGMP Zhanjun Jia,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah; [2]Internal Medicine, VA Medical Center.*

Background: In vitro microperfusion demonstrated the natriuretic property of EP1 receptors but its in vivo action is poorly characterized. This study was to examine the role of EP1 in natriuretic response to rosiglitazone (Rosi)-induced fluid retention.

Methods: WT mice were treated with vehicle or EP1 antagonist SC51322 by minipump and fed a diet incorporated with or without Rosi (320 mg/kg diet).

Results: A 2-week Rosi treatment induced 2-fold increase of urinary PGE2 output, accompanied by 4.2-fold and 2.5-fold increases in renal cortical but not medullary mRNA expression of EP1 and EP2, respectively, contrasting to unaltered expression of EP3 and EP4 in either kidney regions. Rosi treated mice exhibited increased urinary Na^+ excretion (Rosi:1.2±0.06 mmol/24h vs Cont: 0.78±0.14 mmol/24h, p<0.05) and reduced net Na accumulation (intake-excretion: Rosi 0.248±0.043 mmol/24h vs Cont 0.433±0.09mmol/24h, p<0.05), both of which were completely blocked by EP1 antagonism (Na^+excretion:0.756±0.075 mmol/24h, p<0.05 vs Rosi group; Na^+ intake-excretion: 0.399±0.014, p<0.05 vs Rosi group). The changes in 24-h urine volume and water balance followed the same pattern as Na^+. Rosi treatment significantly decreased Hct (Rosi: 47.2±1.4% vs Cont 51.8±0.9%, p<0.05) and this decrease was greater in Rosi+SC51322 group(42.2±1.4%, p<0.05 vs Rosi group), indicating accelerated plasma volume expansion. qRT-PCR showed widespread downregulation of all Na^+ transporters examined, including NHE3, NKCC2, NCC, α-Na-K-ATPase and ENaC subunits in Rosi mice. Interestingly, EP1 antagonism restored the levels of NHE3 and NCC but not other Na^+ transporters. Rosi treatment markedly elevated the urinary output of nitrate/nitrite (Rosi: 323.5±24.5 nmol/24h vs Cont: 94.4±23.2 nmol/24h, p<0.05) and cGMP (Rosi: 10.2±3.1 nmol/24h vs Cont: 3.1±0.77 nmol/24h, p<0.05), both of which were significantly blocked by EP1 antagonism (nitrate/nitrite:163.3±51.2 nmol/24h, p<0.05 vs. Rosi group; cGMP: 4.0±0.54 nmol/24h, p<0.05 vs Rosi group).

Conclusions: Taken together, these data suggest renal PGE2/EP1 pathway play an important role in mitigating Rosi-induced plasma volume expansion via NO/cGMP.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1787

Sodium Delivery and ENaC Regulate Collecting Duct Endothelin Production Donald E. Kohan, Meghana Pandit, Brianna Lyon-Roberts. *Division of Nephrology, University of Utah Health Sciences Center, Salt Lake City, UT.*

Background: Collecting duct (CD) endothelin-1 (ET-1) is an autocrine inhibitor of CD Na reabsorption. Salt loading increases CD ET-1; the mechanisms transducing this effect are poorly understood. Tubule fluid flow increases in response to Na loading, hence we studied flow modulation of CD ET-1. We have previously reported that flow increases mpkCCD cell ET-1 mRNA content via intracellular Ca, PLC and PKC.

Methods: We now extend these studies in MPK cells to examine how flow modulates CD ET-1. MPK cell ET-1 mRNA was assessed at 2 dyne/cm2 for 2 hr using various agents.

Results: Increasing perfusate osmolality to 450 mOsm/L (from 300 mOsm/L) with NaCl, but not mannitol, increased ET-1 mRNA response to flow by 75%. Increasing osmolality with Na acetate, but not choline chloride, increased ET-1 mRNA by 85%, indicating Na-, but not Cl-, dependence. Amiloride (1 μM) or benzamil (0.2 μM) markedly reduced flow-stimulated ET-1 mRNA, indicating ENaC-dependence. Two days of aldosterone increased the flow response by 95%; amiloride reduced the aldosterone response by 75%. Aprotonin reduced flow-stimulated ET-1 mRNA content by 80%. We then examined Ca entry pathways (primarily apical) known to exist in CD. Removal of primary cilia (chloral hydrate or NH4SO4) did not alter the flow response. Blockade (RN1734) or stimulation (La or Gd) of TRPV4 did not affect the flow response, nor did nifedipine. Inhibition of TRPC3/6 channels with SKF96365, BTP2 or Pyr3 modestly reduced the flow response. Blockade of Na/Ca exchange with SEA0400 had no effect on the flow response.

Conclusions: In summary, flow-stimulated CD ET-1 production appears to be mediated in large part by Na delivery whose detection is critically dependent upon ENaC. How this leads to alterations in intracellular Ca signaling remains to be fully determined. These data describe a novel pathway wherein CD Na delivery per se increases CD ET-1 production, thereby potentially down-regulating Na-stimulated ENaC-mediated Na reabsorption. Such a system may be important in natriuretic states wherein diminished CD Na reabsorption is desirable.

Funding: Other NIH Support - NHLBI

FR-PO1788

Mice with the XX Sex Chromosomal Complement Have a Differential Natriuretic Response to Aldosterone Plus High-NaCl Diet as Compared to XY Lijun Li, Radha Mayuri Garikepati, Carolyn M. Ecelbarger. *Department of Medicine, Georgetown University, Washington, DC.*

Background: Young females (F), on average, have lower blood pressure (BP) than young males (M), in a variety of species. The mechanisms underlying these sex differences are not fully understood but may involve sex steroid and sex chromosomal complement (SCC) influences on sodium handling.

Methods: Utilizing the unique mouse model in which Sry (male sex-determining gene) was translocated from the Y chromosome to an autosome, we evaluated the independent influences of sex (M vs. F) and sex chromosomal complement (XX vs. XY) on natriuretic responses to aldosterone plus high-NaCl diet. Mice of 4 genotypes: 1) XX-F, 2) XY-F 3) XX-M, and 4) XY-M were gonadectomized to remove masking effects of sex steroids. After 2 weeks, they were placed on a low-NaCl diet (0.085%) for 2 days, and then osmotic minipumps were implanted to infuse aldosterone (40 mg/40 g•bw/d). After 4 days, all mice were switched to a high-NaCl diet (3%) for 3-additional days. 24-hr urine was collected. Mice consumed diet and drank water *ad libitum*.

Results: No significant differences in body weight or weight change were observed. By day 2 of high-NaCl diet, mice of the XX SCC demonstrated a significantly more robust aldosterone escape, as evident by higher urine sodium excretion (mmol Na^+/40 g•bw/d): 3.5 ± 0.9 (XX-F), 1.5 ± 0.2 (XY-F), 2.1 ± 0.3 (XX-M), and 1.5 ± 0.4 (XY-M), p = 0.028 for SCC. This significantly increased excretion of sodium in the XX SCC was maintained on day 3 of high-NaCl diet (p = 0.031 for genotype). Interestingly, potassium excretion was also significantly increased in the XX SCC on day 3 (p = 0.045).

Conclusions: These results suggest either: 1) XX SCC are more aldosterone sensitive, leading to early sodium retention necessitating greater pressure natriuresis or, 2) XX SCC have a greater efficiency of escape mechanisms relative to XY. Studies to evaluate BP in this model are currently ongoing. Overall, these studies highlight important differences due to SCC in renal sodium handling in response to high-NaCl diet with aldosterone infusion. This findings may be particularly relevant in the absence of sex steroids, e.g., post-menopausally.

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FR-PO1789

Crosstalk between Kidney and Heart Angiotensin II Contributes to Diabetic Cardiomyopathy through Volume Expansion and Overload Takahiro Masuda, Shigeaki Muto, Eiji Kusano. *Division of Nephrology, Department of Medicine, Jichi Medical University, Tochigi, Japan.*

Background: Chronic kidney disease, including diabetic nephropathy, is a risk factor for cardiovascular disease. In diabetes, kidney and heart angiotensin II (Ang II) play a critical role in each organ damage; however, it is unclear how crosstalk between them is involved in the pathogenesis of diabetic cardiomyopathy (DCM).

Methods: We used male Spontaneously Diabetic Torii (SDT) rats (a novel non-obese human type 2 diabetes model) treated without and with either olmesartan medoxomil (Olm) (an Ang II receptor blocker) or insulin for 16 weeks after diabetes onset. Systolic blood pressure, echocardiographic findings, blood and urinary biochemical findings, and histological findings in left ventricular (LV) and kidney tissues were compared among the groups.

Results: In SDT rats, kidney and heart Ang II, but not circulating Ang II, increased and colocalized with aquaporin 1 (a proximal tubule marker) and cardiac myosin (a cardiomyocyte marker), respectively. SDT rats showed LV chamber dilatation, LV hypertrophy and increases in plasma atrial natriuretic peptide (a plasma volume marker) and tubular Na^+ reabsorption without hypertension. The expression of aquaporin 1 and Na^+-H^+ exchanger 3, both of which contribute to proximal tubule fluid reabsorption, was upregulated in the SDT rat kidney cortex. In SDT rats, cardiomyocyte hypertrophy, interstitial fibrosis and overexpression of Ang II and atrial natriuretic peptide were restricted to the LV subendocardium, which is more susceptible to local environmental changes than is the subepicardium. These events (except the hyperglycemia) were reversed by Olm.; insulin abolished them all.

Conclusions: We conclude that volume expansion via the stimulatory effect of Ang II overproduced in proximal tubules on the tubules' Na^+ reabsorption induces Ang II upregulation in LV subendocardial cardiomyocytes as volume overload, leading to DCM. Therefore, crosstalk between kidney and heart Ang II, which involves the downstream processes of persistent hyperglycemia, contributes to DCM through volume expansion and overload.

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FR-PO1790

Decreased Na-K-ATPase Maximal Activity and Expression in Angiotensin II-Induced Hypertension Agustin Gonzalez-Vicente, Jeffrey L. Garvin. *Hypertension and Vascular Research Division, Henry Ford Health System, Detroit, MI.*

Background: Thick ascending limbs (TALs) reabsorb 25% to 30% of the total filtered NaCl load. NaCl enters TAL cells via NKCC2 and exits via basolateral Na-K-ATPase. We have shown an increase in TAL NaCl reabsorption in Angiotensin II (AngII) induced hypertension. However the transporters affected in this model are not known. Thus we hypothesize that in AngII-induced hypertension Na-K-ATPase activity is enhanced.

Methods: we infused rats with 200ng/kg/min AngII or vehicle for 7 days. Direct femoral mean arterial blood pressure (MABP) was measured. TAL suspensions were obtained and Na-K-ATPase activity measured in permeabilized tubule fragments. Na-K-ATPase expression was analyzed in TAL lysates by Western blots using an anti α1-subunit antibody. We used ^{3}H-Ouabain binding to measure the number of Na-K-ATPases in the plasma membrane.

Results: AngII increased MABP by 20 ± 5 mmHg, (116 ± 4 mmHg, n=10 vs 137 ± 3 mmHg, n=11, $p < 0.001$). Contrary to our hypothesis in AngII-induced hypertension TAL total Na-K-ATPase activity (membrane + intracellular) was decreased by 13% (1.55 ± 0.05 vs 1.35 ± 0.04 mmol PO4/μg protein/min, $p < 0.005$, n=12). We also found a 16 ± 4 % ($p < 0.01$, n=4) decrease in Na-K-ATPase expression. There were no significant differences in the k½ for Na (7.5 ± 0.2 vs 7.4 ± 0.4 mM n=5) or K (1.9 ± 0.1 vs 1.8 ± 0.1 mM, n=7). Despite decreased total activity and expression, we found no differences in the number of pumps located in the membrane (6.5 ± 0.7 x109 vs 6.1 ± 0.8 x109 units/μg protein, n=6).

Conclusions: 1) in contrast to the increase in Na transport, total Na-K-ATPase activity is decreased in AngII-induced hypertension possibly due to decreased expression; 2) changes in enzymatic parameters can not explain the decrease in activity; 3) the increase in NaCl reabsorption that occurs in AngII-induced hypertension must be due to increases in apical Na entry; 4) since total expression is decreased without changes in the number of Na-K-ATPases in the membrane, AngII-induced hypertension may enhance trafficking.

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FR-PO1791

Effect of Chronic Tempol on Sodium Transporter Regulation in Sprague Dawley Rats Fed Control Diet Mien T.X. Nguyen, Donna Lee, Nicholas K. Fletcher, Muhammad Madkour, Alicia A. McDonough. *Keck School of Medicine of USC, Los Angeles, CA.*

Background: Both Ang II, which is anti-natriuretic, and high salt diet, which is natriuretic, increase reactive oxygen species (ROS) generation. ROS increases Na+ reabsorption in perfused TALH. Scavenging ROS with Tempol, a superoxide dismutase mimetic, reverses the effects of ROS in the TALH (Ortiz,2002). In contrast, scavenging ROS with Tempol doubles Na+ reabsorption in proximal tubule of Spontaneously Hypertensive Rats (SHR)(Panico,2009). This study aimed to examine effects of Tempol on urinary electrolytes and renal Na transporter abundance and phosphorylation in Sprague Dawley rats.

Methods: Rats fed a normal diet (0.74%NaCl, 2%KCl) were given drinking water without (C, n=5) or with 4mM Tempol (T, n=4) for 2 weeks. Urine was collected overnight in metabolic cages. NHE3, NKCC and NCC abundance and phosphorylation were analyzed in homogenates of renal cortex by immunoblot.

Results: Compared to C, T group had: 38% lower rate of weight gain/wk, 25% lower urine volume (UV), 15% lower UosmV, 30% lower UNa+V, unchanged UK+V and decreased UNa/K ratio (all $p<0.03$). Proximal tubule (PT) NHE3 abundance did not change, NHE3pS552 increased 55% and NHE3p/NHE3total ratio increased 45%, a molecular marker for depressed NHE3 activity (both $p <0.03$). Cortical NKCC and NKCCpT96,T101 both tended to increase (ns) and DCT NCC increased 30% ($p=0.01$) in T treated vs. C.

Conclusions: These results are consistent with effects of Tempol in normal rats to: 1) decrease food and water intake (based on decreased output), 2) decrease PT Na+ reabsorption (in contrast to reports in SHR), 3) increase DCT Na+ reabsorption. We conclude that scavenging basal levels of ROS in control animals significantly alters renal Na+ transporter regulation in a reciprocal manner in PT and DCT.

Funding: NIDDK Support

FR-PO1792

Cellular and Subcellular Aspects of Renal Vasopressin V2 Receptor Distribution Adelina Stoessel,[1] Turgay Saritas,[1] Luca Rampoldi,[2] Sebastian C. Bachmann,[1] Kerim Mutig.[1] *[1]Anatomy, Charite Universitätsmedizin, Berlin, Germany; [2]Molecular Genetics of Renal Disorders Unit Dibit, San Raffaele Scientific Institute, Mailand, Italy.*

Background: Vasopressin (AVP) regulates salt and water transport in renal epithelia, chiefly via vasopressin V2 receptors (V2R). Previous studies of the segment- and cell-type-related aspects of V2R abundance and subcellular distribution produced in part controversial results which may be due to restricted affinities and limited availability of specific antibodies. This study aimed to present a high-resolution analysis of cellular and subcellular V2R distribution in mammalian kidney epithelia.

Methods: A specific anti-V2R antibody was generated and characterized. Renal distribution of V2R was studied using confocal microscopy. Basolateral and luminal subcellular aspects were resolved using immunogold labeling of V2R on kidney sections and confocal evaluation of cultured thick ascending limb (TAL) cells transfected with GFP-V2R.

Results: Application of our anti-V2R antibody on rat, mouse, and human kidney sections produced comparably strong signals in TAL, distal convoluted tubule, and in the principal cells of the connecting tubule and collecting duct as identified using specific markers for the indicated renal tubules. Abundance of the receptor in macula densa (MD) cells was low to absent. No signals were detected in glomeruli, proximal tubules or vascular elements. Confocal evaluation of kidney sections and cultured TAL cells transfected with GFP-V2R revealed no significant co-localization of V2R with luminal proteins, such as Na^+,K^+,$2Cl^-$- cotransporter type 2, Tamm-Horsfall protein, or aquaporin 2, suggesting basolateral distribution of the receptor. Electron microscopic analysis confirmed the predominant basolateral distribution of V2R.

Conclusions: This study provides an extensive analysis of cellular and subcellular V2R distribution in rat, mouse, and human kidney epithelia. Low abundance of the receptor in MD cells may be related to their specific role for TGF.

FR-PO1793

Rho-Kinase Pathway Activated in HIV-Associated Nephropathy Jin Judy Song,[1] Rungwasee Rattanavich,[2] Mohammad Husain,[2] Ashwani Malhotra,[2] Pravin C. Singhal.[2] *[1]Department of Medicine, St. Luke's Roosevelt Hospital Center, New York City, NY; [2]Feinstein Institute for Medical Research, North Shore LIJ Health System, Great Neck, NY.*

Background: Epithelial mesenchymal transition (EMT) plays an important role in the progression of renal interstitial tubular fibrosis. EMT has been shown to contribute to the manifestation of the proliferative phenotype in HIV-associated nephropathy (HIVAN) (Am J Physiol 2010). Activation of Rho A/Rho kinase is one of the major signaling pathways involved in EMT. The inhibition of Rho kinase has been shown to reduce the EMT and renal fibrosis in different animal models. We hypothesized that Rho kinase signaling pathway is contributing to occurrence of EMT in HIVAN. In the present study, we examined the role of Rho kinase in HIVAN.

Methods: Kidneys were harvested from age (4 weeks old) and sex matched control and Tg26 mice. Renal cortical sections were immunolabled for Rho kinase and alpha-SMA (a marker of EMT). To establish a temporal and spatial relationship between Rho kinase and alpha-SMA, serial sections of renal cortical sections of Tg26 mice were labeled either

for Rho kinase or alpha-SMA. Immunoblots were prepared from renal tissues of control and Tg26 mices and probed for Rho kinase. In *in vitro* studies, human tubular cells (HK2) were transduced with either empty vector (EV/HK2) or NL4-3 (HIV/HK2). Immunoblots of EV/HK2s and HIV/HK2 were probed for Rho-Kinase and associated down stream signal- phos-MYPT1.

Results: Tubular cells in Tg26 mice displayed enhanced expression of both Rho kinase and alpha-SMA when compared with control mice. Immunolabling studies revealed spatial relationship between the expression of Rho kinase and alpha-SMA in tubular cells. In *in vitro* studies, HIV/HK2 also showed enhanced expression of Rho kinase. Moreover, HIV/HK2s displayed enhanced expression of phos-MYPT1.

Conclusions: •These findings indicate that Rho kinase is activated in tubular cells in HIVAN.

Funding: NIDDK Support

FR-PO1794

SIRT1/PGC-1α Activation Protects Against Aldosterone-Induced Podocyte Injury Via the Amelioration of Mitochondrial Dysfunction Aihua Zhang, Songming Huang, Guixia Ding. *Department of Nephrology, Nanjing Children's Hospital Affiliated to Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Podocyte injury causes proteinuria and is found in many glomerular diseases. Mitochondria maintain podocyte energy homeostasis, and mitochondrial dysfunction (MtD) is an early event in podocyte injury. This study investigated whether the transcriptional coactivator, peroxisome proliferator activated receptor-g coactivator 1α (PGC-1α), a major regulator of oxidative metabolism and mitochondrial function, prevented podocyte damage by improving MtD.

Methods: MPC5 conditionally immortalized mouse podocyte clonal cells (kindly provided by Peter Mundel at Mount Sinai School of Medicine) were cultured. C57BL/6J mice had osmotic minipumps implanted subcutaneously. Pumps delivered a continuous infusion of aldosterone (0.15 μg/h). MtD was assessed by mitochondrial membrane potential (MMP), mtDNA copy number, ATP content, and ROS production. Podocyte damage was assessed by apoptosis, nephrin and podocin expression.

Results: Aldosterone (Aldo) decreased PGC-1α expression and induced MtD and podocyte injury in dose- and time-dependent manners. Endogenous PGC-1α suppression by RNAi induced podocyte MtD and apoptosis. Increased PGC-1α levels in podocytes by transfection with a PGC-1α vector prevented Aldo-induced MtD and inhibited injury. The protective effects of PGC-1α overexpression were not observed when a PGC-1α T177A S538A mutant vector was used. *SIRT1* (silent mating type information regulation 2 homolog 1), a gene upstream of PGC-1α, was also investigated. We confirmed that SIRT1 overexpression restored Aldo-induced MtD and podocyte injury by upregulating PGC-1α at both the transcriptional and posttranslational levels. Finally, we found that resveratrol (RSV), a SIRT1 activator, attenuated Aldo-induced MtD and podocyte injury *in vitro* and in Aldo-infused mice *in vivo*.

Conclusions: PGC-1α is important in maintaining normal mitochondrial function, and SIRT1/PGC-1α activation protected podocytes from Aldo-induced MtD and injury. SIRT1 activators, such as RSV, may be useful therapeutically for glomerular diseases to promote and maintain PGC-1α expression and mitochondrial function.

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FR-PO1795

Disparate Effects of Ang II during the Initiation and Progression of HIV-Associated Nephropathy (HIVAN) Divya Salhan,[1] Dileep Kumar,[1] Guohua Ding,[2] Praveen N. Chander,[3] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [3]Pathology, New York Medical College, Valhalla, NY, China.*

Background: Ang II may contribute to the progression of HIVAN through its hemodynamic effects or its direct effects. We evaluated the role of Ang II in the initiation and progression of HIVAN in genetically engineered HIVAN mice (Tg26) with variable copies of angiotensinogen (agt).

Methods: Tg26 mice with 2, 3, and 4 copies of Agt were evaluated for expression of AT1 and AT2 receptors during embryogenesis (E13, E15, and E18) and after birth, on day 1 and day 10 by immunohistochemical, in situ hybridization, and confocal microscopy. In addition, aging Tg 26 mice with various copy numbers of Agt were evaluated for severity of proteinuria, BUN, renal lesions, collagen deposition, blood pressure and arteriosclerosis, renal tissue mRNA for PAI-1 at 4, 9, 12 and 16 weeks (n=5).

Results: During embryogenesis and on days 1 and 10, renal cells showed greater expression of AT2 receptors when compared to AT1 receptors. Both tubular cells and podocytes showed temporal and spatial relationship between AT1 and AT2 receptors. Mice with 4 Agt copies showed lower blood pressure (mean 110/80 mm Hg) at 4 and 8 wks when compared to mice with two Agt copies (mean 140/90 mm Hg). Mice with 4 Agt copies showed higher blood pressure at 16 wks. Four wks old mice with 4 Agt copies displayed attenuated expression of PAI-1 when compared to age-matched mice with 2 Agt copies; whereas, 16 wks old mice with 4 Agt copies showed 3-fold greater PAI-1 expression than age-matched mice with 2 Agt copies. Tg26 mice (2 Agt copies) aged to nine weeks developed renal lesions that were more severe than those seen in age-matched Tg26 mice with 3 or 4 Agt copies. However, 16 wks old mice with 4 copies of Agt, displayed more advanced renal lesions when compared to wild type Tg26 mice with 2 Agt copies.

Conclusions: We conclude that higher Agt copies-induced protective effect during the initiation of HIVAN may be mediated through the temporo-spatial expression of AT1 and AT2 receptors during embryogenesis and post-natal period.

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FR-PO1796

Telmisartan Improves Spatial Memory Dysfunction through Reduction of Cerebral Oxidative Stress in Experimental Uremic Mice Kiichiro Fujisaki, Toshiaki Nakano, Masatomo Taniguchi, Kazuhiko Tsuruya. *Department of Medical and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: Advanced chronic renal failure (CRF) is associated with the brain dysfunction generally referred to as uremic encephalopathy. Although we previously showed an increased oxidative stress in the brain of uremic rodents, a detailed association of oxidative stress with cognitive impairment remains to be elucidated. Recently, antioxidants have been shown to play a neuroprotective role and blockade of renin angiotensin system has been reported to ameliorate brain infarction through reduction of oxidative stress in the brain.

Methods: We investigated the effect of telmisartan (Tel), an angiotensin II type-1 receptor blocker (ARB), against uremia-induced cognitive dysfunction and oxidative stress in mice brain. CRF was induced in male C57BL/6 mice using unilateral nephrectomy and 2/3 electrocoagulation of renal cortex. 8 weeks old mice were divided into four groups: control mice orally administered normal water (CON-NW, n=8), control mice treated with Tel (CON-Tel, n=8), CRF mice orally administered normal water (CRF-NW, n=8), CRF mice treated with Tel (CRF-Tel, n=8). In the radial arm water maze (RAWM) test performed after 8 weeks, we trained all groups for initial four days to memorize water maze and examined on the fifth day. We sacrificed the mice and examined the content of malondialdehyde (MDA), a product of lipid peroxidation, in the brain specimens.

Results: The errors in RAWM test were more frequent in CRF-NW mice than in the control mice, whereas those in CRF-Tel mice were comparable to the controls. The accumulation of MDA was increased in CRF-NW mice, but not in CRF-Tel mice.

	CON-NW	CON-Tel	CRF-NW	CRF-Tel
Blood urea nitrogen (mg/dl)	25 ± 1.8	29 ± 1.8	69 ± 3.9*	68 ± 4.6*
The number of error in RAWM test	1.8 ± 0.64	1.9 ± 0.44	3.9 ± 0.57*♦	1.6 ± 0.50

*p < 0.05 vs. CON-NW and CON-Tel, ♦p < 0.05 vs. CRF-Tel, mean ± SEM.

Conclusions: The present study is the first report that Tel inhibited cerebral oxidative stress and improved memory dysfunction in the uremic mice. It is concluded that Tel has the potential to protect against neuronal cell damage by uremia.

FR-PO1797

Effects of Combined ARB and PPARγ Agonist To Treat Established Glomerulosclerosis in 5/6 Nephrectomized Rats Keizo Matsushita, Li-Jun Ma, Haichun Yang, Agnes B. Fogo. *Department of Pathology, Vanderbilt University Medical Center, Nashville, TN.*

Background: We previously observed that PPARγ agonist can attenuate glomerulosclerosis in rats, with specific protection of podocytes. Azilsartan medoxomil (AZL) is a potent new ARB. We now assessed effects of combination therapy with AZL and pioglitazone on established glomerulosclerosis.

Methods: Sprague Dawley male rats underwent 5/6 nephrectomy (5/6Nx). Body weight (BW), systolic blood pressure (SBP) and urinary protein (UP) were measured at intervals. In pilot studies, AZL in varying doses was given to 5/6Nx rats starting at time of surgery. AZL 3 mg/kg q.d. was effective in controlling SBP (147.3±2.7 vs VEH 199.7±6.1 mmHg at wk12), decreasing UP (309±35 vs VEH 594±92 mg/d at wk12) and sclerosis (GS, 0-4 scale, 0.29±0.06 with preemptive AZL vs 0.96±0.21 in VEH at wk12). Additional 5/6Nx rats were untreated until wk8, then underwent renal biopsy and were randomized to groups with equal starting moderate GS, and treated with AZL (3 mg/kg q.d., n=6), pioglitazone (2.5 mg/kg q.d.; PIO; n=7), combination (AZL+PIO; n=7), or vehicle (VEH; n=7) for 4 wks.

Results: In rats with established sclerosis, SBP, UP and GS were equal in all groups at wk8 by study design (194.4±5.0 mmHg, 254.6±14.9 mg/24hr and 0.84±0.10, respectively). AZL alone from wk8 to 12 prevented increased SBP (195.5±9.1 at wk12), in contrast to further hypertension in VEH (222.0±11.6 at wk12). AZL+PIO significantly decreased SBP (173.4±7.6 at wk12, p<0.05 vs VEH). There was one death in VEH from wk8 to 12, and none in treatment groups. UP at wk12 was not different amongst groups. Sclerosis progressed in VEH from biopsy to autopsy (0.82±0.28 to 1.67±0.35). AZL alone or PIO alone both ameliorated GS at wk12 (1.47±0.42 and 1.36±0.30, respectively). Combination AZL+PIO GS markedly reduced sclerosis at wk12 to 0.70±0.23 (p<0.05 vs VEH). Podocyte number (WT-1/glomerulus) in VEH was 9.6±0.9, and AZL+PIO increased podocyte number to 12.1±0.7 (p<0.05 vs VEH).

Conclusions: Azilsartan is highly effective in primary prevention of hypertension and sclerosis. In combination with pioglitazone, there is enhanced benefit in established sclerosis, linked to podocyte preservation.

Funding: Pharmaceutical Company Support

FR-PO1798

Immune Complexes from Patients with IgA Nephropathy Containing Galactose-Deficient IgA1 and Anti-glycan Antibodies Induce Protein-kinase Signaling and Proliferation in Cultured Human Mesangial Cells Zhi Qiang Huang,[1] Joshua Anderson,[1] Stacy D. Hall,[1] Timothy D. Rohrbach,[1] Rhubell T. Brown,[1] Bruce A. Julian,[1] Christopher D. Willey,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, AL; [2].*

Background: Circulating immune complexes (CIC) in patients with IgA nephropathy (IgAN) consist of galactose-deficient IgA1 (Gd-IgA1) and anti-glycan antibodies. Several *in vitro* studies have shown that these CIC are pathogenic in that they activate mesangial cells (MC) and induce cellular proliferation.

Methods: Using sera of patients with IgAN, we fractionated CIC by size-exclusion chromatography. Cultured primary human MC were stimulated with CIC, samples collected at various time points, and analyzed by SDS-PAGE/Western blotting with anti-phospho-tyrosine (P-Y) antibody. Sera depleted of IgA-containing CIC served as negative control. Maximal changes in P-Y phosphorylation in MC were detected 5 min after stimulation with CIC. These samples were further analyzed using PamStation® 12 high-content peptide substrate microarray to profile global tyrosine kinase (TK) activity (kinomic profiling) to identify CIC-stimulated pathways.

Results: Results showed that CIC increased P-Y of multiple proteins in MC by 3-42%. Kinomic profiling showed that CIC activated multiple TK-mediated signaling pathways, including PDGF signaling and anti-apoptosis processes. To validate these results, similar experiments were performed with engineered immune complexes (EIC) formed *in vitro* from Gd-IgA1 myeloma protein and a recombinant human IgG specific for Gd-IgA1. The EIC increased phosphorylation of proteins in MC by 5-41%. PamStation® kinomic profiling indicated that EIC activated TK in a fashion similar to that for native CIC.

Conclusions: In summary, Gd-IgA1-containing complexes, CIC and EIC, activated multiple signaling pathways in MC and led to cellular proliferation. Importantly, EIC may provide an excellent substitute for native CIC in the future IgAN studies and may be used to develop animal models more closely reflecting human disease.

Funding: NIDDK Support

FR-PO1799

Protein-kinase Inhibitors Can Block Cellular Proliferation and Signaling Induced in Cultured Human Mesangial Cells by Immune Complexes from Patients with IgA Nephropathy Zhi Qiang Huang, Joshua Anderson, Timothy D. Rohrbach, Stacy D. Hall, Rhubell T. Brown, Bruce A. Julian, Christopher D. Willey, Jan Novak. *University of Alabama at Birmingham, AL.*

Background: IgA1-containing circulating immune complexes (CIC) isolated from sera of patients with IgA nephropathy (IgAN) stimulate proliferation of mesangial cells (MC) *in vitro*. Here, we studied cellular proliferation and signal transduction induced by CIC in cultured primary human MC and the potential blocking effects of tyrosine-kinase inhibitors (TKI).

Methods: CIC were isolated from sera of patients with IgAN by size-exclusion chromatography. Sera depleted of IgA-containing CIC served as negative control. MC stimulated with CIC were analyzed by Western blotting with anti-phospho-tyrosine (P-Y) antibody and by PamStation®12 high-content peptide substrate microarray to profile global tyrosine kinase activity (kinomic profiling) to identify the pertinent signaling pathways. TKIs, dasatinib and sorafenib (50 nM), were tested in assays of cell proliferation and signaling. Cell proliferation was measured as ^{3}H-thymidine incorporation; phosphorylation was assessed by Western blotting with anti-P-Y antibody after MC were incubated with CIC with or without inhibitors.

Results: CIC increased MC proliferation 7-fold compared to the baseline. Dasatinib and sorafenib inhibited the induced MC proliferation by 86% and 19%, respectively. The inhibition of proliferation was reflected by decreased P-Y signaling on Western blots. CIC enhanced P-Y of proteins from MC by 19-67%, based on densitometric analysis. Dasatinib and sorafenib inhibited phosphorylation induced by CIC by 26-64% and by 0-20%, respectively. Kinomic profiling using PamStation®12 showed that CIC increased P-Y of multiple proteins, including those involved in PDGF signaling and in anti-apoptotic processes. Dasatinib significantly blocked CIC-induced P-Y of these proteins, whereas sorafenib was ineffective.

Conclusions: In summary, CIC-induced MC proliferation was mediated by tyrosine-kinase signaling and a TKI can block MC proliferation and signaling, thus raising novel possibilities for potential new options for therapy of IgAN.

Funding: NIDDK Support

FR-PO1800

Quantitative Analysis of O-Glycosylation of IgA Hinge Portion (HP) in IgA Nephropathy (IgAN) Patients and Its Responsiveness to the Therapy of Tonsillectomy Combined with Corticosteroid IV (TLX+S) Hirotsugu Iwatani,[1] Yasuyuki Nagasawa,[1] Takahiro Inoue,[2] Yoshiyasu Ueda,[1] Ryohei Yamamoto,[1] Maki Shinzawa,[1] Yoshitsugu Obi,[1] Junya Teranishi,[1] Toshihiro Ishigami,[1] Hideki Iijima,[2] Yoshinao Wada,[3] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Gastroenterology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Osaka MCHRI, Izumi, Osaka, Japan.*

Background: Several patterns of sugar chain attached to Ser/Thr residue of IgA1 HP constitute O-glycosylation. The component sugars are N-acetylgalactosamine (GalNAc), galactose (Gal) and sialic acid. Although its qualitative analyses in IgAN patients have been performed and aberrant glycosylation has been already reported, neither its quantitative analysis itself nor its clinical significance has been reported.

Methods: The fully glycosylated form of human IgA1 is as follows; the sugar adjacent to the Ser/Thr residue is GalNAc which is connected to Gal, and sialic acids are attached to GalNAc and Gal. But there are a variety of glycosylated forms of IgA in one individual. We analyzed the serum IgA glycosylation from IgAN patients and control using MALDI-TOF-MS. We calculated the number of GalNAc and Gal at HP and the ratio of Gal to GalNAc. We also analyzed the serum IgA glycosylation of the IgAN patients before and after the therapy of TLX+S.

Results: The ratio of IgA with 5 GalNAc to that with 4 GalNAc in HP and the average number of Gal joined to one GalNAc in HP (Gal/GalNAc) are significantly decreased in patients with IgAN (n=9) compared to healthy control (n=30). In cases of patients with IgAN undergoing TLX+S, the number of GalNAc per HP significantly increased from 4.2 ± 0.085 at pretreatment to 4.4 ± 0.11 at 46 ± 9.5 months post tonsillectomy (p= 0.02). The number of Gal per HP or the ratio of Gal/GalNAc did not change significantly. In remission cases, all patients exhibited the increase of the number of GalNAc per HP.

Conclusions: The O-glycosylation of IgA in patients with IgAN seems to be deeply involved with the pathogenesis of the disease. It can be recovered by the therapy of TLX+S.

FR-PO1801

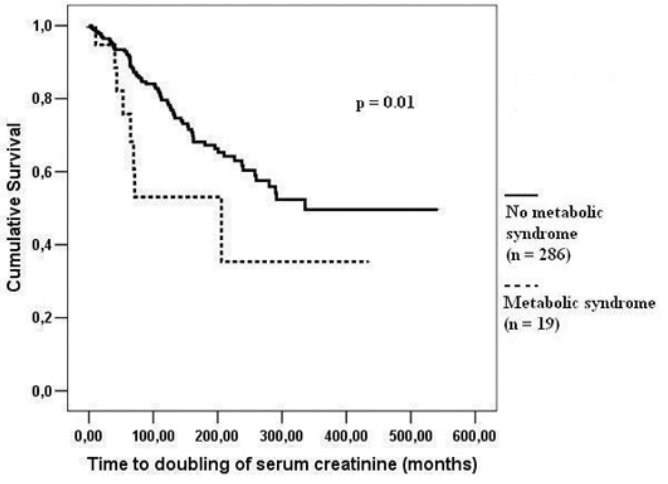
Metabolic Syndrome and Progression of IgA Nephropathy Tibor Vas,[1] Csaba P. Kovesdy,[2] Istvan Kesoi,[1] Balazs Sagi,[1] Istvan Wittmann,[1] Tibor Kovacs,[1] Judit Nagy.[1] *[1]Nephrology Center and 2nd Department of Internal Medicine, Faculty of Medicine, University of Pécs, Hungary; [2]Division of Nephrology, University of Virginia, Charlottesville, VA.*

Background: The components of the metabolic syndrome are major cardiovascular risk factors and the metabolic syndrome itself is associated with a modest but independent and additive risk of new-onset chronic kidney disease (CKD). The purpose of this study is to determine whether there are differences in the progression of IgA nephropathy (IgAN) according to the presence of metabolic syndrome at the diagnosis of IgAN.

Methods: Three hundred and five IgAN patients (19 with metabolic syndrome and 286 without at the diagnosis of the CKD, mean follow-up 142.3 months) were analyzed. Metabolic syndrome was defined on the basis of the modified WHO criteria. End points for renal outcome were doubling of serum creatinine or reaching of end-stage renal disease (serum creatinine >500 umol/l or initiation of dialysis treatment or transplantation).

Results: IgAN patients with metabolic syndrome were significantly older (46.9±15.2 yr), had significantly lower eGFR (54±21 ml/min/1.73m2) than IgAN patients without metabolic syndrome (34.8±13.1 yr, 81±30 ml/min/1,73m2, p<0.001 each). Patients with metabolic syndrome had a significantly lower mean survival time to reach the doubling of serum creatinine than patients without metabolic syndrome (215 months vs 346 months, p=0.01) (Figure).

Conclusions: Metabolic syndrome is associated with faster deterioration of renal function in patients with IgAN. Therefore, the early diagnosis and appropriate treatment of metabolic syndrome may be beneficial in the prevention of progression of IgAN.



FR-PO1802

Metformin Increases Renal Medullary Interstitial Cell Apoptosis In Vitro and In Vivo Youfei Guan,[1,2] Qifei Han,[1] Xiaoyan Zhang.[1] *[1]Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China; [2]Diabetes Center, Peking University, Beijing, China.*

Background: Metformin is the most commonly used pharmacological therapy for type 2 diabetes worldwide. It improves glycemic control in type 2 diabetic patients mainly via the activation of AMP-activated protein kinase (AMPK). AMPK is a Ser/Thr protein kinase acting as a sensor of cellular energy status and is abundantly expressed in the kidney, where it plays an important role in regulating a variety of physiological and pathological processes including ion transport, glomerular and medullary cell function and diabetic nephropathy. We aimed to examine the effect of metformin on the survival of renal medullary interstitial cells (RMICs) under hyperosmotic conditions both in vitro and in vivo. AMPK activity was decreased in RMICs under the hypertonic condition within 12 hr and then gradually returned to the baseline level. Metformin activated AMPK and markedly increased hypertonicity-induced RMIC apoptosis. Similarly, AICAR and A-769662, two selective AMPK activators, or a constitutively active AMPKα construct resulted in a significant increase in apoptosis of RMICs. AMPK activation was associated with the suppression of hypertonicity-induced NFκB nuclear translocation and activation of the cytoprotective cyclooxygenase-2 (COX-2). AMPK activation also resulted in a marked reduction in ROS generation and nuclear expression of tonicity-responsive enhancer binding protein (TonEBP), which prevented up-regulation of osmoprotective genes in hypertonicity-treated RMICs. In vivo study using normal C57Bl/6 mice with water deprivation further demonstrated massive apoptosis of RMICs after treatment with metformin and two other AMPK activators (AICAR and A-769662). Furthermore, treatment of type 2 diabetic db/db mice with AMPK activators including metformin caused a marked increase in RMICs' apoptosis under both normal and dehydration conditions. Taken together, these results identify AMPK as a critical factor involved in the maintenance of RMIC viability in type 2 diabetes and raise safety concerns for metformin in diabetic patients with dehydration.

Funding: Government Support - Non-U.S.

FR-PO1803

Decreased Expression of PGC-1α in Skeletal Muscle May Contribute to Protein-Energy Wasting in CKD: Role of Glucocorticoids Russ Price,[1] Bin Zheng,[1] Jennifer L. Gooch,[1,2] Xiaonan H. Wang.[1] *[1]Renal Division, Emory University, Atlanta, GA; [2]Atlanta VA Medical Center, Decatur, GA.*

Background: Accelerated muscle proteolysis contributes to the protein-energy wasting that occurs during CKD and diabetes. PGC-1α is a transcription coactivator that integrates energy metabolism. In muscle, PGC-1α antagonizes the FOXO transcription factors which induce components of proteolytic systems. Previously, we demonstrated that diabetes decreases PGC-1α mRNA as well as calcineurin (Cn)/MEF2/NFAT signaling in rodent muscle; these pathways are implicated in the regulation of PGC-1α transcription. Presently, we tested whether similar changes occur in muscle of CKD mice.

Methods: CKD was induced in mice by partial nephrectomy; controls underwent sham operations. The mice were pair-fed and sacrificed approximately 3 weeks later. mRNAs were measured by qRT-PCR. PGC-1α transcription was evaluated in L6 muscle cells transfected with a PGC-1α-promoter luciferase reporter gene (PGC-1α-Luc).

Results: PGC-1α mRNA was decreased 88±2% (P<0.05) in gastrocnemius muscle of CKD vs controls. The transcriptional activities of MEF2 and NFAT were evaluated by measuring the mRNA levels of their respective gene targets, MRF4 and MCIP1.4 in muscle. CKD decreased MRF4 mRNA by 57±8% and MCIP1.4 mRNA by 43±12% (P<0.05). Since glucocorticoids are necessary for protein wasting in CKD, we tested whether treatment of L6 muscle cells with dexamethasone (DEX; 100 nm, 48 h) affected PGC-1α expression and Cn/MEF2/NFAT signaling. DEX decreased PGC-1α, MRF4 and MCIP1.4 mRNAs by 61±8%, 46±4% and 40±11%, respectively (P<0.05). The decrease in PGC-1α mRNA was due, at least in part, to suppression of transcription because DEX reduced luciferase activity by 30±4% (P<0.05) in muscle cells transfected with PGC-1α-Luc. Importantly, co-expression of constitutively active Cn with PGC-1α-Luc increased luciferase activity 94±20% (P<0.05).

Conclusions: These data indicate that glucocorticoids reduce Cn/MEF2/NFAT signaling and PGC-1α expression in muscle which could lead to higher FOXO activity and accelerated proteolysis. If unchecked, the responses would contribute to the protein-energy wasting associated with CKD.

Support: NIH DK50740.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1804

Dysfunction in Renal Protein Handling in Rats Fed a High Fat Diet Deanne H. Hryciw, Esther Grinfeld, Adam M. Miller, Kayte A. Jenkin, Michael L. Mathai, Andrew J. McAinch. *School of Biomedical and Health Sciences, Victoria University, Melbourne, Victoria, Australia.*

Background: Obesity is frequently associated with chronic kidney disease. The earliest marker of chronic kidney disease is microalbuminuria, with an association between the severity of obesity and the magnitude of microalbuminuria. Albumin uptake is processed by a macromolecular complex in the proximal tubules, via interactions with megalin. The molecular mechanism linking obesity and albuminuria is yet to be elucidated, however reduced processing of albumin by the macromolecular complex contributes to the excessive excretion of albumin in the urine. We investigated changes to the mRNA and protein of the macromolecular complex in rats fed a high fat diet, compared to control.

Methods: 18 male Sprague-Dawley rats were fed either a control chow diet (4%) or a high fat diet (22%) for 12 weeks. At weeks 3, 6 and 10, 24 hour urine samples were collected and analysed for sodium, albumin and protein content. Kidneys were excised from animals at week 12. mRNA and protein was extracted, and the levels of megalin, NHE3, ClC-5, NHERF1 and NHERF2 were assessed by real time PCR and Western blot analysis, respectively.

Results: Compared to control, rats fed a high fat diet had a significant increase in body weight. At weeks 3, 6, and 10, there was a significant increase in urinary albumin excretion in rats fed a high fat diet, however the protein in the urine was only significantly increased at week 10. Sodium secretion in rats fed a high fat diet was significantly decreased at 10 weeks. Analysis of the expression of megalin, NHE3, ClC-5, NHERF1 and NHERF2 mRNA indicated no significant difference between control and high fat fed animals. Importantly, the level of NHE3 protein was altered in rats fed a high fat diet which may account for the changes in sodium excretion.

Conclusions: Therefore, in rats fed a high fat diet, there is an increase in renal albumin and protein secretion, and a reduction in sodium secretion. Significantly, albuminuria was shown to precede proteinuria in this model of obesity. This may be due to the altered expression of components of the megalin macromolecular complex observed in these rats.

Funding: Private Foundation Support

FR-PO1805

Endogenous PPAR Agonist Nitro-Oleic Acid Protects Against Adriamycin-Induced Nephropathy in Mice Shanshan Liu,[1,2] Zhanjun Jia,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah; [2]VA Medical Center.*

Background: Adriamycin (ADR) is an anthracycline antibiotic utilized in antitumor therapy but its clinical use is frequently impeded by renal toxic effects. Nitroalkene derivatives of nitro-oleic acid (OA-NO2) are endogenous lipid products with novel signaling properties, particularly the activation of PPARγ. Our previous studies demonstrated renoprotective action of OA-NO2 in various mouse models of acute and chronic renal injuries. The present study was undertaken to examine the possible role of OA-NO2 in a mouse model of ADR nephropathy.

Methods: BABL/c mice were pretreated for 2 days with OA-NO2 at 5mg/kg/day via osmotic mini-pump, followed by a single injection of vehicle or adriamycin (10mg/kg) via tail vein. Albuminuria and renal function were analyzed at 1 wk of ADR treatment.

Results: ADR mice developed prominent albuminuria (ADR: 508.9±48.5ug/24h vs. Cont: 42.4±7.1ug/24h, p<0.01), hypoalbuminemia (ADR: 0.28±0.08g/dl vs. Cont: 1.01±0.15g/dl, p<0.01), and hyperlipidemia (triglyceride, ADR: 396.2±70.9mg/dl vs. Cont: 61.41±2.7mg/dl, p<0.01), with 90% of the mice in this group having severe ascites. In contrast, the ADR+OA-NO2 group showed less levels of albuminuria (342.4±33.3ug/24h, p<0.01 vs. ADR), hypoalbuminuria (0.58±0.13g/dl, p<0.01 vs. ADR) and hyperlipidemia (212.7±39.2mg/dl, p<0.05 vs. ADR) than ADR group; only 20% of ADR+OA-NO2 mice had mild ascites. The urine thiobarbituric acid-reactive substances (TBARS) significantly increased in ADR group mice (ADR: 0.98±0.1pmol/24h vs. Cont: 0.5±0.07pmol/24h, P<0.05), and this increase was attenuated in the ADR+OA-NO2 group (0.7±0.07pmol/24h, P<0.05 vs. ADR). Kidney histology by PAS staining revealed increased mesangial matrix and mesangial cells in glomeruli, narrow Bowman's capsule, and a large number of protein casts in the tubules. In contrast, these pathological changes were significantly improved in ADR + OA-NO2 group.

Conclusions: Together, these findings suggest a novel therapeutic potential of OA-NO2 in adriamycin nephropathy.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1806

Urinary α-1 Microglobulin Is a Stronger Predictor of Glomerular Filtration Rate Than Urinary Albumin in Patients with Diabetic Nephropathy

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Background: Urinary albumin excretion is a strong predictor of diabetic nephropathy progression, which presumably reflects abnormal glomerular filtration. In the current study, we evaluated whether measurement of a low molecular weight protein (LMWP, α-1 microglobulin, α1m) or a high MWP IgG is a stronger predictor of GFR.

Methods: A cross-sectional cohort of 114 diabetic patients evaluated in the Mayo Nephrology Clinic were recruited for the study. Random urine samples were obtained for measurement of albumin (Roche immunoturbidometric assay), creatinine, α1m (Siemens nephelometric assay), IgG (ALPCO ELISA), and total protein. Recent serum creatinine, 24-hour albumin and total protein, and other demographic data were abstracted from the chart.

Results: Urinary albumin correlated strongly with urinary total protein (r^2=0.89). Urinary α1m also correlated with urinary total protein (r^2=0.75) and urinary albumin (r^2=0.73). IgG correlated weakly with urinary total protein (r^2 = 0.19). Urinary albumin correlated weakly with GFR (r^2= -0.04). However, urinary α1m had a much stronger correlation with GFR (r^2 = -0.34), while urinary IgG correlated only weakly with GFR (r^2= -0.23). In multivariate analysis, adjusting for comorbidities (hypertension, hyperlipidemia, CAD) and medication use (ACE inhibitors and ARBs), only urinary α1m remained a predictor of GFR (p= 0.018). In a similar model, both urinary urinary α1m (p= 0.0003) and urinary albumin (p= 0.0001) were significant predictors of urinary total protein.

Conclusions: Urinary excretion of α1m is a good indicator of GFR in patients with established diabetic nephropathy, perhaps due to progressive tubulointerstitial disease and proximal tubular dysfunction. Further investigation in longitudinal or prospective cohorts will be necessary to determine if increases in urinary α1m or IgG predict future decline in GFR or carry other prognostic value.

FR-PO1807

The Role of Salt Inducible Kinase in Sodium Retention in the Proximal Tubule

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Background: Renal angiotensin II (Ang II) expression is increased in diabetic nephropathy leading to salt retention. Similarly, the peroxisome proliferator activated receptor (PPARγ) pioglitazone, used therapeutically in the treatment of patients with Type 2 diabetes to achieve glyaemic control, also results in excessive sodium reabsorption with salt and water rentention being a limiting side effect to clinical use. We have previously demonstrated that both AngII and pioglitazone increase sodium reabsorption in renal proximal tubular cells (PTC) through increased expression of the sodium hydrogen exchanger 3 (NHE3). Salt inducible kinases (SIK) are members of the AMP-activated protein kinase (AMPK) family. Their levels are regulated by an increase in salt intake and increased SIK1 expression is linked to high blood pressure. The role of SIK1 and 2 in the proximal tube is not known.

Methods: PTCs were exposed to pioglitazone (3μM) or Ang II (100 nM) for 24, 48 or 72 hrs, SIK 1 and 2 mRNA levels were determined by real time PCR. SIK1 and 2 localisation, in the presence and absence of either pioglitazone or AngII, were determined by confocal microscopy. The effect of increasing PPARγ on SIK 1 and 2 expression was determined by Western blot following PPARγ over-expression. The levels of SIK1 and 2 in hypertensive mRen2 rat kidney was determined using immunohistochemistry.

Results: Exposure of PTCs to either AngII or pioglitazone not only increased SIK 1 and 2 in a time dependent matter (P<0.05) but also increased shuffling of SIK1 and 2 from the nucleus to the cytoplasm, reflecting activation. Over-expression of PPARγ increased SIK1 and SIK2 protein levels. Both SIK 1 and 2 were abundantly expressed in hypertensive Ren-2 rats compared to control.

Conclusions: Our data suggest that SIK 1 and 2 are expressed in the proximal tubule and their levels are increased and activated in the presence of both AngII and Pioglitazone. We have additionally demonstrated that SIK1 and 2 are over-expressed in the renal tubules of hypertensive Ren-2 rats. This finding suggests a role of SIK in sodium retention which may be exacerbated in diabetes and further increased by PPARγ agonists.

Funding: Government Support - Non-U.S.

FR-PO1808

Glomerulosclerosis (GS) in the Rat Diet Induced Obesity (DIO) Model Correlates with Endothelial Dysfunction but Not Renal Hyperperfusion/ Hypertrophy

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Background: Hemodynamic and metabolic pathways have been incriminated in the pathogenesis of GS in obesity models. Yet, minimal GS is seen after 12 wks of DIO. The present studies investigated if significant GS develops after a longer follow-up in the DIO model and the role of hemodynamic mechanisms in its pathogenesis.

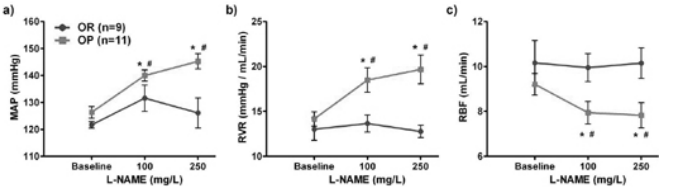
Methods: Obesity prone (OP) and obesity resistant (OR) rats (6-8 wks, Charles River) were placed on a moderately high fat (MHF) diet and BP was monitored radiotelemetrically until ~36 wks of age before sacrifice for histopathology. Additional OP and OR rats were instrumented with a BP radiotransmitter and renal blood flow (RBF) probe (Transonic) at ~20 wks of age before the development of renal injury. After 5-7 days, BP & RBF were recorded (2-4h) in conscious rats at baseline and during escalating doses of L-NAME (100 and 250 mg/L; drinking water) to assess transfer functions and endothelial reserve.

Results: OP but not OR developed modest hypertension, significant proteinuria and GS but not renal hypertrophy.

	BW (grams)	KW (grams)	SBP (mmHg)	Proteinuria (mg/day)	GS (%)
OR (n=8)	588.4 ± 17.7	2.4 ± 0.1	127.5 ± 1.7	12.0 ± 1.4	0.4 ± 0.2
OP (n=10)	699.1 ± 18.9 *	2.4 ± 0.1	142.2 ± 3.0 *	62.4 ± 12.2 *	7.7 ± 1.4 *

mean±SE, * p < 0.05

Fig. 1a-c: MAP, renal vascular resistance (RVR) and RBF were not significantly different at ~20 wks in OP vs. OR rats. L-NAME increased BP & RVR and reduced RBF only in OP rats (*p<0.05 vs baseline; #p<0.05 vs OR)



Conclusions: The mechanism(s) by which endothelial dysfunction leads to GS in DIO models remain to be defined.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1809

CKD-Induced Glucocorticoid Production Suppresses Muscle Satellite Cell Function and Stimulates Myostatin Expression Contributing to Atrophy

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Background: We find that chronic kidney disease (CKD) impairs muscle stem or satellite cell function (JASN, 2010). The mechanism for this finding is unclear. We examined if glucocorticoids (GC) suppress satellite cell function because CKD induces GC production and GC are required to stimulate muscle wasting.

Methods: C57/BL6 mice were given physiologic levels of GC (dexamethasone or Dex: 2 μg/100 g/day for 10 days). We measured body and muscle weights and the expression of myogenic genes by satellite cells. Satellite cell function was also analyzed using the standard technique, cardiotoxin (CTX) injection into muscle.

Results: Dex reduced both body and muscle weights vs control values (mice injected with PBS). In gastrocnemius muscles of Dex-treated mice, there was decreased expression of myogenic genes, MyoD, myogenin and Myf-5, indicating impaired satellite cell function. We examined how Dex influences satellite cell activation *in vivo* using the standard CTX method and monitoring muscle regeneration. At 3 days after injury, Dex suppressed Brdu incorporation and myogenin expression indicating impaired proliferation and delayed satellite cell differentiation. At day 5, the newly formed myofibers (detected by their central nuclei) were fewer; after one month, new myofibers in Dex-treated mice were smaller vs those in control mice. Dex also impaired satellite cell function directly: isolated myofibers from gastrocnemius muscles that were cultured on matrigel plates with/without Dex, more satellite cells migrated away from myofibers and proliferated compared to results with Dex. Dex also increased myostatin expression *in vivo* and *in vitro* (cultured C2C12 muscle cells), indicating that the mechanism impairing satellite cell function involves stimulation of myostatin by GC. Indeed, inhibition of the function of myostatin with a myostatin peptibody in Dex-treated mice improved satellite cell proliferation and differentiation and muscle regeneration.

Conclusions: CKD induced GC production upregulates myostatin in satellite cells, suppressing their function, contributing to muscle atrophy.

Funding: NIDDK Support, Private Foundation Support

FR-PO1810

Activated Prorenin and (Pro)renin Receptor Are Up-Regukated in Glomeruli of Diabetic Nephropathy and IgA Nephropathy Using Renal Biopsy Specimen

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Background: Activated prorenin plays a key role in the regulation of the tissue renin-angiotensin system (RAS), and a direct renin inhibitor has been reported to reduce proteinuria in diabetic nephropathy through inhibiting the tissue RAS. However, little information is available regarding the localization of activated prorenin and (pro)renin receptor (P)RR in human kidney under pathophysiological conditions.We examined the localization of activated prorenin and (P)RR in kidney biopsy specimens of the patients with IgA nephropathy, diabetic nephropathy, and minimal change nephropathy using parts of renal biopsy specimens.

Methods: This study was approved by Kochi Medical School review boards. The antiserum against activated prorenin was raised in a rabbit by injecting the peptide fragment corresponding to the gate region of human prorenin. Immunohistochemical analyses were performed by confocal microscopy.

Results: We analyzed 21 renal biopsy specimens (n=7 IgA nephropathy, n=6 diabetic nephropathy, n=8 mininal change). Immunohistochemistry with the anti-(P)RR antibody showed positive immunostaining in mesangial area in 14 patients (n=5/7 IgA nephropathy, n=6/6 diabetic nephropathy, n=3/8 mininal change). We also observed positive immunostaining for activated prorenin in podocyte area in 12 patients (n=4/7 IgA nephropathy, n=6/6 diabetic nephropathy, n=2/8 mininal change). These were no significant staining for activated prorenin in endothelial cell in glomeruli. These findings indicate that activated prorenin and (P)RR are up-regulated in mesangial area in diabetic nephropathy and IgA nephropathy, suggesting that activated prorenin and (P)RR may contribute to the pathophysiology of glomerulonephritis.

Conclusions: Activated prorenin and (P)RR are up-regulated in mesangial area and podocyte in diabetic nephropathy and IgA nephropathy, and may contribute to the pathophysiology of these nephropathy.

FR-PO1811

The VEGF Receptor Blocker, Sunitinib (SU), Promotes Glomerular Microthrombosis and Aggravates Segmental Sclerosis in the Remnant Kidney (Nx) Model Flavia G. Machado, Patricia Semedo Kuriki, Clarice K. Fujihara, Camilla Fanelli, Simone R. Costa, Cristiene Okabe, Claudia R. Sena, Grasiela P. Barlette, Vivian L. Viana, Denise M. Malheiros, Niels O.S. Camara, Roberto Zatz. *Univ Sao Paulo, Brazil.*

Background: We showed previously that treatment with SU, used as an antiangiogenic agent, did not influence interstitial capillary density (ICD) in Nx, suggesting that the rate of new vessel formation is not increased in this model. However, SU treatment aggravated glomerulosclerosis (GS). We now investigated whether the latter effect might involve podocyte (POD) injury and/or formation of microthrombi (MT).

Methods: Adult male Munich-Wistar rats underwent Nx and were immediately assigned to Groups Nx+V (vehicle) or Nx+SU (SU, 4 mg/Kg/d). Forty five days later, % GS, % glomerular MT(%GMT), % cortical interstitium (%INT), % glomerular endothelial area (%GE), ICD (capillary profiles/mm^2), glomerular volume (V_G, x 10^{6}μm^3), % glomerular zonula occludens 1 area (%ZO1) and the number of POD per glomerular tuft (POD/G) were assessed in 10 NX+V and 10 Nx+SU rats. Fourteen sham-operated rats receiving vehicle (S+V) or SU (S+SU) were also studied. Results (Mean±SE, ^{a}p<0.05 vs. respective S, ^{b}p<0.05 vs. respective untreated):

Results:

	%GS	%GE	%GMT	%ZO1	POD/G	%INT	ICD
S+V	0±0	53±3	0±0	84±3	16±1	0.1±0.1	609±42
S+SU	0±0	55±3	1±1	85±1	16±1	0.1±0.1	609±30
Nx+V	7±2^a	40±3^a	2±1^a	68±3^a	13±1	2.5±0.4^a	356±39^a
Nx+SU	22±4^{ab}	38±2^a	7±3^{ab}	61±5^a	13±1	3.1±0.4^a	298±33^a

In addition, SU treatment significantly raised serum creatinine in Nx (1.5±0.1 in Nx+SU vs. 1.2±0.1 in Nx+V). SU promoted no glomerular or interstitial change in S, but markedly exacerbated GS in Nx. This effect could not be explained by a reduction in the number of POD or endothelial cells, or by a functional change of POD. However, SU-treated Nx exhibited a marked increase in the frequency of MT, the organization of which may have been the basis for the observed worsening of GS.

Conclusions: Chronic VEGF inhibition can promote glomerular endothelial injury in previously compromised kidneys. However, the antiangiogenic effect of VEGF inhibition may have little influence on glomerular and interstitial injury in the Nx model.

Funding: Government Support - Non-U.S.

FR-PO1812

Curcumin Prevents CKD in 5/6 Nephrectomized Rats by Blocking LPS Secretion Siddhartha S. Ghosh, George Bassam Saffouri, Domenic A. Sica, Shobha Ghosh, Todd W. Gehr. *Int Medicine/Nephrology, VCU, Richmond, VA.*

Background: Bacterial lipopolysaccharide (LPS), a known mediator of inflammation is elevated in the circulation of inflammatory disorders such as chronic kidney disease (CKD). This increase in circulatory LPS has been suggested to be due to paracellular transport of bacterial LPS from the gut. Orally acting antibacterial agents by changing gut microbiota can cause decrease LPS translocation. Curcumin which has anti-inflammatory and antibacterial properties ameliorates inflammatory disorders such as CKD. We speculated that one of the mechanisms by which curcumin can decrease inflammation and slow renal failure progression is by decreasing the circulatory content of LPS.

Methods: CKD was induced in Sprague-Dawley rats by 5/6 nephrectomy. 18 Nx animals were divided into untreated (Nx), and curcumin-treated (Cur) groups. The Cur treatment (75 mg/kg) was carried out for 10-weeks and results were compared with Nx and sham-operated animals (SH). Before sacrificing, 4 animals from each group were given thioglycollate (ip) and macrophages (MO) harvested 72 hours later. Proteinuria and creatinine was used as measures of renal function. Lipooxygenase (LO) and cyclooxygenase (COX-2) were measured by western Blot.

Results: As previously observed there was significant improvement in proteinuria and creatinine in Cur treated animals. Plasma LPS concentration in the Nx cohort was (3.3±0.72 EU/ml) significantly higher than in SH (1.1±0.14 EU/ml; p<0.001) and was reduced by Cur treatment (2.1±0.6 EU/ml; p<0.05). Macrophage infiltration is a common phenomenon in CKD and LPS is known to stimulate inflammatory biomolecules such as TNFα, COX-2 and 5 (LO) in macrophages. Compared to SH; macrophages from Nx cohort had a 4 fold higher TNFα mRNA,; 2.3 fold higher LO (p<0.05) and 4 fold higher COX-2 (p<0.01). Compared to the Nx cohort, TNF, LO, and COX-2 were significantly lower in curcumin treated animals (p<0.05).

Conclusions: We reason that the antibacterial aspects of curcumin reduces parcellular transport of LPS and lowers inflammatory marker burden. These findings suggest that paracellular intestinal transport of LPS may play an important role in inflammatory disease states, such as CKD.

FR-PO1813

Adenine-Induced Chronic Kidney Disease: Less Is Best for Equivalence to Human Disease Vishal Diwan,[1] Glenda C. Gobe,[2] Lindsay Brown.[3] *[1]Sch of Biomedical Sciences, Univ of Queensland, Brisbane, Queensland, Australia; [2]Sch of Medicine, Univ of Queensland, Brisbane, Queensland, Australia; [3]Dept of Biological and Physical Sciences, Univ of Southern Queensland, Toowoomba, Queensland, Australia.*

Background: Animal models of chronic kidney disease (CKD), which mimic the insidious development of CKD in humans, are limited. Adenine diet offers an accepted model of CKD pathogenesis but the rapid development from 0.75% adenine can detract from applicability to human CKD. This project aimed to investigate low dose, prolonged, adenine diet in rats as a model of human CKD.

Methods: Male Wistar rats were given 0.25% or 0.75% adenine in comparison with a normal powdered chow (PC) as control (N=12 per group; 300±30g initial body weight), over 16 weeks. We studied: rate of development of CKD; morphometry for glomerular and tubular damage, fibrosis and infiltration/activation of macrophages and myofibroblasts; Western blots for oxidative stress (HO-1), inflammation (TNF-α) and fibrosis (TGF-β); and renal function as blood urea nitrogen (BUN), plasma creatinine (PCr), and their clearances.

Results: 0.75% adenine diet induced renal failure quickly over 4 weeks with approximately 10-fold increase in PCr, marked renal edema, loss of body mass, and the animals were moribund at 4 weeks. In comparison, 0.25% adenine diet induced CKD more slowly, similar to prolonged development in humans. At 16 weeks, in comparison with PC diet, the 0.25% diet induced only moderate loss of body weight, significantly increased % renal fibrosis (35.6±2.8 vs 4.8±0.48), marked tubular atrophy, >10-fold increased macrophage and activated myofibroblast numbers and increased expression of HO-1, TNF-α and TGF-β (all p<0.05). Functionally, 0.25% vs PC diet caused increased BUN (56.5±5.4 vs 6.2±0.6mmol/L) and PCr (267.9±22.9 vs 41.9±2.8μg/L), and decreased BUN clearance (526.7±22.9 vs 753.9±71.2mg/dL/hr) and PCr clearance (380±33 vs 2220±57mg/dL/hr) (both p<0.05).

Conclusions: Development of CKD occurred successfully over 16 weeks with 0.25% adenine diet, with many of the characteristics of human CKD replicated. The slower development of CKD using this model will better allow analysis of modulation of the CKD characteristics with new therapies.

Funding: Government Support - Non-U.S.

FR-PO1814

Salutary Effect of a Novel Glutathione Precursor on Chronic Progressive Tubulointerstitial Nephropath Nosratola D. Vaziri,[1] Jun Yuan,[1] Susanne B. Nicholas,[2] Albert B. Crum,[3] Keith C. Norris.[2] *[1]Medicine/Nephrology, University of Califronia, Irviine, CA; [2]Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]ProImmune Company, L.L.C., Rhinebeck, NY.*

Background: Oxidative stress and inflammation play a critical part in the development and progression of CKD. Oxidative stress in CKD is associated with depletion of glutathione (GSH) which is the most abundant and potent intracellular antioxidant. Degradation of GSH by digestive enzymes precludes its use by oral supplementation. Oral administration of F1, a GSH precursor containing cystine as a cysteine carrier has been shown to restore tissue GHS. We tested the hypothesis that GSH repletion with F1 may attenuate oxidative stress, inflammation and severity of CKD in rats with adenine-induced tubulointerstitial nephropathy.

Methods: Male SD rats were randomized to the control (fed regular rat chow), CKD (fed chow containing 0.7% adenine for 2 weeks), and F1-treated CKD (fed chow containing 0.7% adenine for 2 wks during which they received F1, 0.5 g/Kg/day) groups. They were then placed on regular diet and monitored for 2 weeks, after which they were euthanized.

Results: Consumption of 0.7% adenine-containing diet resulted in severe swelling, discoloration and deformation of the kidney, massive interstitial inflammation, heavy tubular and glomerular damage, impaired urinary concentrating capacity, marked elevation of plasma urea and creatinine and severe anemia. Concurrent treatment with F1 significantly attenuated interstitial inflammation, tubular and glomerular injury, renal dysfunction, and anemia in this model.

Conclusions: Administration of the novel glutathione precursor, F1, results in marked attenuation of kidney tissue damage and inflammation in the animal model of severe progressive chronic interstitial nephropathy. Further studies are planned to determine the effect of this intervention in other models of acute and chronic kidney diseases.

FR-PO1815

Indoxyl Sulfate Causes Accumulation of Uremic Toxins through Down-Regulation of SLCO4C1 Transporter Yasutoshi Akiyama,[1] Yoichi Takeuchi,[1] Eikan Mishima,[1] Takehiro Suzuki,[1] Sadayoshi Ito,[1] Takaaki Abe.[2,3] *[1]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Division of Medical Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan; [3]Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Japan.*

Background: With the progression of CKD, various uremic toxins accumulate, subsequently causing renal damage and hypertension. Recently, we have revealed that human kidney-specific organic anion transporter SLCO4C1 excretes uremic toxins, resulting in the reduction of blood pressure and renal inflammation (PNAS 2004, JASN2009). However, in the renal failure, the SLCO4C1 expression level is decreased. So far, the down-regulation mechanism of renal transporters in the renal failure has not been clarified.

Methods: Toxic potentials of various uremic toxins identified by our capillary electrophoresis-based MS analysis (HTN Res 2010) were examined at the translational and transcriptional levels in vitro and in vivo.

Results: Among the compounds tested (18 compounds), indoxyl sulfate (IS) decreased the human SLCO4C1 mRNA level in a dose-dependent manner. Because of the existence of GATA sequence at the 5' UTR of human SLCO4C1, we examined the correlation of SLCO4C1 with GATA transcriptional factors. The mRNA expression level of GATA3 was increased by IS in contrast to down-regulation of SLCO4C1. In the human kidney cells, over-expression of GATA3 significantly inhibited the expression level of SLCO4C1 and conversely, knockdown of GATA3 increased SLCO4C1 expression. GATA-inhibitor K-7174 completely canceled the effects of IS. In 5/6 nephrectomized rats, treatment with oral absorbent AST-120 significantly decreased plasma IS level and conversely increased the renal SLCO4C1.

Conclusions: The accumulation of IS in the renal failure decreases human SLCO4C1 through GATA transcriptional pathway. This down-regulation of SLCO4C1 further causes the rise of uremic toxin levels and subsequently, exacerbates the renal function. Thus, the usage of AST-120 to reduce IS level is a new aspect for treating CKD patients.

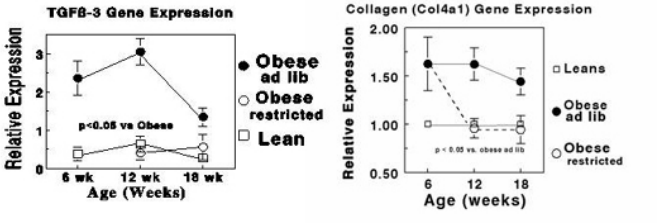
FR-PO1816

Food Limitation Reverses Increased Collagen Type 4 α1 Gene Expression (Col4a1) in the Obese Zucker Rat Bayard C. Carlson,[1] Leah B. Callahan,[1,2] David A. Maddox.[1] *[1]Sioux Falls VA Healthcare System, Sioux Falls, SD; [2]Avera Research Institute.*

Background: Obesity is closely linked to hypertension, Type II diabetes, and hypercholesterolemia, which are among the leading causes of kidney failure. Obese Zucker rats at 6, 12, and 18 weeks of age over-express transforming growth factor beta-3 (TGF- β3) compared to their lean littermates. This effect is prevented by food restriction which also prevents kidney damage. TGF- β3 belongs to a pro-collagen generation pathway leading to kidney damage. We examined the effects of food restriction on Col4a1 gene expression in the obese rat.

Methods: Six week-old obese Zucker rats were fed *ad libitum* or restricted in food intake to that consumed by lean Zucker rats, then tested at 6, 12, and 18 weeks of age. The animals were anesthetized, and their kidneys perfusion-fixed (RNA*Later*™). Tissue mRNA was extracted and quantified from the kidney cortex, converted to cDNA, then run on custom RT-PCR arrays (SABiosciences). Values are mean ± SE.

Results: Previously we found that TGF-β3 gene expression was increased in 6, 12, and 18 week-old obese animals compared to lean. Food restriction reduced TGF-β3 expression to control at 12 and 18 weeks (left panel). Col4a1 gene expression was also significantly increased at 6, 12, and 18 weeks of age in obese animals compared to controls. Food limitation prevented these changes (right panel).



Conclusions: Similar to that seen for TGF-β3, gene expression of Col4a1 in the obese rat was elevated at 6 weeks of age. Food restriction in obese rats beginning at 6 weeks of age led to a significant drop in gene expression levels of Col4a1 by 12 weeks. These data suggest that elevations in the expression of TGF-β3 and Col4a1 are important initiating events leading to kidney damage in these animals. The contents do not present the views of the Department of Veterans Affairs or the United States Government.

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FR-PO1817

Renoprotective Effects of Astragaloside IV Synergizes with Ferulic Acid in Rats with 5/6 Nephrectomy Liqiang Meng, Lei Qu, Jiawei Tang, Xiaomei Li. *Renal Division, Department of Medicine, Peking University First Hospital.*

Background: Astragaloside IV (AS-IV) and Ferulic acid (FA) are two major active constituents of Chinese herbs *Astragali* and *Angelicae*, which could alleviate renal tubulointerstitial fibrosis. This study was to investigate whether AS-IV and FA could retard the progression of chronic renal failure in rats with 5/6 Nephrectomy (Nx).

Methods: The Sprague-Dawley rats were randomly divided into sham, Nx, Nx+FA (FA 12mg/kg/d), Nx+AS-IV (AS-IV 20.4mg/kg/d) and Nx+AF (FA 12mg/kg/d, AS-IV 20.4mg/kg/d). After therapy for 2, 4, 8 and 12 weeks, the tail-cuff blood pressure, serum creatinine (Scr), 24 hour protein excretion rate, semiquantitatively evaluated for glomerulosclerosis, tubulointerstitial lesion and vascular damage in PAS stained tissue sections were evaluated.

Results: The blood pressure was significantly increased in Nx group compared with sham group at 4th,8th and 12th week. Compared with the Nx group, blood pressure was reduced in AF-treated group at 4th week (113±4/84±2mmHg vs 139±9/104±19mmHg, P<0.01), and in the three groups with therapies at 8th week (FA: 127±9/96±8mmHg, AS: 115±7/83±7mmHg, AF: 111±12/77±10mmHg, vs Nx:152±9/100±15mmHg, P<0.001). But there was no statistical difference in groups with therapies at 12th week. Scr and proteinuria were gradually increased in the Nx group compared with sham group (P<0.05). After treatment with AF, Scr was decreased at 4th week (42.3±4.5μmol/L vs 55.4±4.7μmol/L, P<0.05), 8th week (47.3±1.2μmol/L vs 58.0±0.0μmol/L, P<0.05) and 12th week (60.3±4.6μmol/L vs 80.2±9.5μmol/L, P<0.05). The similar effect on Scr was shown in the groups treated with AS-IV or FA. The proteinuria was decreased after AS-IV and/or FA at 4th, 8th and 12th week. The pathological injury in kidney was significantly exacerbated from 4th week in the Nx group, and alleviated after treated by AS-IV, FA and AF characterized by total injury index reduced to 83%, 85% and 79% at 12th week, respectively.

Conclusions: AS-IV and/or FA therapy retarded the progression of renal failure in rats with 5/6 Nephrectomy via effective control of hypertension and proteinuria, and the synergistic effect of AS-IV and FA was more effective than AS-IV or FA alone.

Funding: Government Support - Non-U.S.

FR-PO1818

Urinary Levels of Angiopoietin-Like 4 in Pediatric Steroid Sensitive Nephrotic Syndrome Support a Possible Role in Proteinuria Michael R. Bennett, Nuntawan Piyaphanee, Prasad Devarajan. *Cincinnati Children's Hospital Medical Center.*

Background: Idiopathic nephrotic syndrome (NS) is the most common glomerular disorder of childhood. Roughly 90% of children under 10 years of age with nephrotic syndrome have minimal change disease (MCD), which is typically responsive to steroid treatment (SSNS). While some structural proteins in the glomerulus have been found that may contribute filtration problems in MCD, many of the disease mechanisms remain unknown. Recent studies in animal models of SSNS suggest a role for podocyte excreted angiopoietin-like 4 protein (ANGPTNL4)in modulating proteinuria in this disease. We set out to determine if urinary levels of ANGPTNL4 were elevated in pediatric patients with SSNS with active proteinuria.

Methods: Urine and clinical data were collected from patients at Cincinnati Children's recently diagnosed with active nephrotic syndrome and healthy controls. Patients with a history of gross hematuria, active/recurrent UTI or nephrotic syndrome secondary to systemic disease were excluded. ANGPTNL4 was measured using a commercially available ELISA. Median ANGPTNL4 levels were calculated and subjected to Kruskal-Wallis One Way Analysis of Variance on Ranks and Dunn's multiple comparison test.

Results: This study included three groups: biopsy-proven FSGS and steroid resistant clinical course-SRNS (n=14), steroid sensitive clinical course-SSNS (n=14), and normal controls (n=10). SSNS was divided into relapse (active nephrotic grade proteinuria; n=7) and remission (n=7). Median ANGPTNL4 levels were significantly (p=0.001) higher in SSNS-relapse (22.2 ng/ml; IQR 1.9-69) vs SSNS-remission (0.0 ng/ml; IQR 0.0-0.6). While median ANGPTNL4 levels were 16 fold higher in SSNS-relapse than SRNS (1.4 ng/ml; IQR 0.4-11.7), it failed to reach significance (p=0.06). ANGPTNL4 was undetectable in healthy control urine.

Conclusions: Significantly increased ANGPTNL4 in urine from pediatric patients with active SSNS vs those in remission is consistent with the animal findings that this protein may be involved in the genesis of proteinuria in this disease. Urinary ANGPTNL4 may serve as a non-invasive biomarker to differentiate between SSNS-relapse and SRNS.

FR-PO1819

Knockout of α2A-Adrenoceptors Delay Progression of Chronic Kidney Disease Henning Hoch, Johannes Stegbauer, Sebastian Alexander Potthoff, Eva Koenigshausen, Lars C. Rump, Oliver Vonend. *Department of Nephrology, Medical Faculty, Heinrich Heine University, Duesseldorf, Germany.*

Background: Chronic kidney disease (CKD) is a major health issue. Investigations were carried out to analyse the function of α2A-adrenergic receptor (AR) for progression of CKD. The α2A-AR is known as main regulator of presynaptic noradrenaline release.

Methods: A murine knockout model (KO) with deletion of α2A-AR was used and compared to its wild-type (WT). Experimental renal failure was induced by subtotal nephrectomy (SNX). Kidneys of WT and KO mice were isolated perfused for evaluation of presynpatic noradrenaline release and angiotensin II pressor response.

Results: In kidneys of KO mice presynaptic noradrenaline release after renal nerve stimulation was significantly higher than in WT mice. After SNX WT and KO mice developed albuminuria which was surprisingly significant higher in WT mice. Kaplan-Meier survival analysis revealed a diminished mortality of KO mice. In isolated perfused kidneys the α2-agonist UK14,304 showed a facilitatory effect on angiotensin II-induced vasoconstriction. In addition, UK14.304 induced a concentration- and time-dependent phosphorylation of extracellular signal-regulated-kinases ERK1/2 in α2A-AR transfected HEK 293T-cell.

Conclusions: The presented data confirms the noradrenaline release regulating effect of presynaptic α2A-adrenergic receptors. Moreover, our data reveal a major role of postsynaptic α2A-adrenergic receptors regulating vascular tone for progression of CKD. The cell culture experiments might hint to an ERK1/2 dependent pathway which could explain the effect of catecholamines modulating fibrotic or inflammatory processes in CKD.

FR-PO1820

Renoprotective Effects of Nicorandil in the Rat Remnant Kidney Model Yoshifuru Tamura, Carlos Alberto Roncal-Jimenez, Christopher J. Rivard, Katsuyuki Tanabe, Wataru Kitagawa, Richard J. Johnson, Takahiko Nakagawa. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: Nicorandil has been used to treat patients with ischemic heart disease. Interestingly recent literatures have documented that nicorandil exhibits the protective effects in the acute renal disease in rodents, including acute kidney injury and glomerulonephritis. We hypothesized that nicorandil also exhibits beneficial effects in the chronic renal disease. In order to test our hypothesis, nicorandil was administered in the rat remnant kidney (RK) model. We also examined if nicorandil could be more potent with angiotensin converting enzyme inhibitor (ACEI) in chronic renal disease.

Methods: RK rats were divided into five groups: Sham operated rats (SHAM, n = 7), Untreated remnant kidney rats (RK, n = 7), RK rats treated with Enalapril 5 mg/kg/day (ENAL, n = 7), RK rats treated with low dose Nicorandil 3 mg/kg/day (L-NICO, n = 7), RK rats treated with high dose Nicorandil 30 mg/kg/day (H-NICO, n = 7). Twelve weeks later, the rats were sacrificed.

Results: Enalapril significantly reduced blood pressure (108 ± 2 vs. 147 ± 2 mmHg, $p<0.05$). Nicorandil at high dose tended to reduce blood pressure (136 ± 6 mmHg, $p = 0.11$), while low dose had no effect on it (153 ± 0 mmHg) at 12 weeks. Urine albumin excretion was significantly ameliorated by nicorandil as well as enalapril compared with that in RK (1.66 ± 0.5 in H-NICO, 7.98 ± 3.0 in L-NICO, 0.28 ± 0.2 in ENAL vs. 14.5 ± 5.4 mg/day in RK) at 9 weeks. Interestingly, the preventive effect of L-NICO on albuminuria could be independent of blood pressure. In histology, the development of glomerular sclerosis was significantly attenuated in the group H-NICO and ENAL compared with non-treated RK.

Conclusions: High dose of nicorandil prevents the progression of chronic renal disease as potently as enalapril. Low dose nicorandil may also reduce proteinuria independently of blood pressure. These findings suggest that nicorandil could be a therapeutic option for chronic renal disease.

FR-PO1821

Prolactin Receptor was Up-Regulated in the Proximal Tubules of the Kidney in the Cardio-Renal Syndrome Model Mice Yohei Tsuchida, Yoshikatsu Kaneko, Akihiko Saito, Tadashi Yamamoto, Ichiei Narita. *Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata City, Niigata Prefecture, Japan.*

Background: Many epidemiological investigations revealed the interaction between dysfunctions of kidney and cardiac. These pathological states were recently recognized as cardio-renal syndrome (CRS). However, the precise pathogenesis and the physiological mechanisms of CRS are to yet be elucidated. Hence the aim of our study was to investigate the molecular mechanisms of the development of CRS by generating the CRS model mice.

Methods: We generated CRS model mice by the combination of uninephrectomy (Nx) and abdominal aorta banding (Ab), which caused mild decrease of cardiac function through the continuous pressure load. To evaluate cardiac and renal function, we measured ejection fraction (EF) by echocardiogram and serum cystatin C level by ELISA, respectively. Comprehensive gene expression analysis in the kidney of the CRS model mice, comparing those in the kidney of the mice treated with Nx alone, was conducted using DNA array.

Results: In CRS mice, serum cystatin C level as significantly higher and EF was lower compared with those in the control mice with Nx alone 6 weeks after Nx. DNA array analysis revealed that prolactin receptor (Prlr) gene expression was enhanced in CRS mice kidney compared with that in Nx mice. Real-time RT PCR using mRNA purified from the kidney showed that Prlr gene expression was significantly increased in both Ab and CRS mice. Gene expression levels of several alternative Na^+ channels were not affected by Ab and Nx. *In situ* hybridization study in the fixed kidney tissue demonstrated that the gene expression of prlr was distributed in the proximal tubules along the border between the cortex and the medulla. Serum prolactin (Prl) level was not affected by Ab or Nx.

Conclusions: We newly generated CRS model mice and discovered that prlr expression was up-regulated in the proximal tubules of the kidney in these model mice. Prl may act as a natriuretic hormone by inhibiting proximal tubular Na^+ / K^+-ATPase activity, and the diuresis might be accelerated by up-regulation of the prlr in CRS.

FR-PO1822

Hypoalbuminemia and microRNAs in Chronic Kidney Disease Carmen Josefina Mora,[1] Diego Julio Arenas,[2] Carmen María del Prado,[1] Teresa Renata Romero,[1] Virgilia Soto,[3] Marcela Avila.[1] *[1]UIMEN, CMN SXXI IMSS, Mexico, DF, Mexico; [2]UIMGH, CMN SXXI IMSS, Mexico, DF, Mexico; [3]S. Patologia, H Cardiol, Mexico, DF, Mexico.*

Background: AIbumin synthesis is controled by positive and negative transcription factors. Its transcript translation regulation by microRNAs (miRNAs) is unknown.

The aim was to investigate the relationship between albumin synthesis and a miRNAs profile in a CKD mice model.

Methods: Four mice groups were formed: CKD group, induced with Nx 5/6, Inflammation group (I) induced with E. coli, LPS, food restriction group 50%, (FR), and control group (C). The animals were sacrificed after eight weeks of treatment; total-blood samples and liver were extracted, and plasma creatinine, albumin, and total proteins were measured. Total RNA was extracted from liver for Albumin mRNA measurement in RT-PCR. miRNAs were obtained for miRNAs microarrays. Gene target prediction was made with "miRANDA" database. Liver histological analysis was made to corroborate fibrosis development.

Results: Plasma albumin was different in CKD (1.59 g/dL, $p<0.016$) and I groups (1.68 g/dL, $p<0.009$) from C group (1.80 g/dL). The albumin transcript level was diminished in the CKD (12.30 UR $p<0.009$) and I (11.99 UR, $p<0.005$) groups regarding C group (17.51 UR); FR group (11.66 UR $p<0.006$) had the lowest albumin mRNA level. Liver fibrosis in pixeles was: in I group (58.63 ± 6.04, $p<0.001$), followed by CKD group (55.56 ± 4.03, $p<0.001$) and FR group (24.38 ± 5.61, $p<0.001$), and C group (1.78 ± 0.75). The obtained miRNAs profile, showed 74 miRNAs differentially expressed (-2< Z Score >2).Four sub-expressed miRNAs (miR21, miR30b, miR350 and 376A), had CEBP β as gene target predicted. Other miRNAs target genes predicted, involve structural, and inflammation genes

Conclusions: We found miRNAs related with extracellular matrix genes, and with CEBP β which is a negative transcription factor in albumin transcription. It might be that CEBP is up-regulated by down-expressed miRNAs, promoting transcription suppression of albumin. The heterogeneity of miRNAs differential expression and the gene targets diversity, show that the albumin synthesis regulation in CKD involves several mechanisms

Funding: Government Support - Non-U.S.

FR-PO1823

Globotriaoxylceramide (GB3)-Induced Endothelial-to-Mesenchymal Transition as a New Mechanism of Renal Progression in Fabry Disease Mina Yu,[1] Yang-Hee Jang,[1] Jin Ok Choi,[2] Sung Cheol Jung,[2] Duk-Hee Kang.[1] *[1]Nephrology, Ewha Womans University School of Medicine, Seoul, Korea; [2]Biochemistry, Ewha Womans University School of Medicine, Seoul, Korea.*

Background: The lysosomal storage disorder Fabry disease is characterized by excessive globotriaosylceramide (Gb3) accumulation in major organs such as kidney. Defective lysosomal alpha-galactosidase A (Gla) is responsible for excessive Gb3 accumulation, and vascular endothelium is one of the sensitive cells to the effects of Gb3 accumulation. Although renal fibrosis is known to be associated with Fabry disease, it is not known whether it plays any roles in the development of organ damage in Fabry disease or whether Gb3 per se is related to renal progression. Recent data suggest that endothelial-to-mesenchymal transition (endo-MT), which is characterized by the loss of endothelial cell markers and an acquisition of mesenchymal cell markers, is a potential mechanism of renal fibrosis.

Methods: We investigated whether Fabry kidney showed an evidence of endo-MT and whether Gb3 induced endo-MT in cultured human endothelial cells.

Results: Double immunofluorescence staining of CD31 (FITC) and α-SMA (cy3) in the kidney of animal model of Fabry disease, Gla deficient mice, showed a decreased microvascular endothelial staining both in glomerular and peritubular capillaries compared to wild type mice with an appearance of α-SMA (+) and CD31 (+) endothelial cells. Treatment of Fabry mice with of recombinant adeno-associated virus (rAAV) vector encoding alpha-Gal A cDNA (rAAV2/8-hAGA) resulted in the clearance of accumulated Gb3 in kidney with concomitant elevation of alpha-Gal A enzyme activity. rAAV2/8-hAGA therapy also ameliorated endo-MT of glomerular and peritubular capillary endothelial cells. Stimulation of HUVEC with Gb3 (0.1-10 uM) and lyso-Gb3 (10uM) down-regulated the expression of CD31 with an up-regulation of α-SMA from 48 hours in a dose-dependent and time-dependent manner. Blocking of Gla using siRNA and Gb3 after siRNA decreased the expression of CD31 and increased α-SMA expression.

Conclusions: These finding suggest that Gb3-induced endo-MT is one of the mechanisms of nephropathy in Fabry disease.

FR-PO1824

Correlation of HIF-1 α and Twist in the Proximal Tubular Epithelial Cells of Hypoxia Kidney Diseases Shiren Sun, Rui Du. *Deparment of Nephrology, Xijing Hospital, FMMU, Xi'an, China.*

Background: Our previous studies indicate that the Twist plays an important role in the development of hypoxia-induced tubular epithelial-mesenchymal transition and kidney fibrosis. However, the expression of Twist in the human kidney and its mechanisms are not well known.

Methods: Twist expression was examined by Immunohistochemistry (IHC) study, and consecutive slides were stained for HIF-1α and E-cadhrein. Immunostaining was

evaluated in a blinded manner. A 0–3 relative scale was used to grade the amount of immunostaining: 0, <5% staining; 1+, 5–25% staining; 2+, 25–50% immunostaining; 3+, >50% immunostaining.

Results: Twist was predominantly located in the nuclear of renal tubular epithelial cells from CKD patients including diabetic nephropathy (DN), FSGS, immunoglobulin (Ig) A nephropathy, hypertensive nephrosclerosis, tubulointerstitial nephritis that had different degrees of chronic hypoxia. While little positive staining for Twist was found in the renal tubules of normal kidneys (Twist score of <2), prominent Twist staining (>25% of all cells positive) was found in 48 of 74 patients with CKD (Twist score of ≥2+, P = 0.018 compared with normal controls, when grouped by score of ≥2+ or <2+; Fisher's exact test;). Positive HIF-1α was observed in nuclear in 62.2% (46/74) of CKD kidney tissues, which was closely associated with Twist (P= 0.000). Loss or reduced expression of E-cadherin was observed in 70.83% (34/48) Twist-positive tubular epithelial cells, but only in 26.9% (7/26) of Twist-negative tumors (P =0.000), which was significantly associated with higher levels of Twist in all kidney tissues from CKD patients. Using linear regression, we found a positive linear correlation between active Twist and tubulointerstitial fibrosis in the biopsy samples (r=0.57, P= 0.000).

Conclusions: We found that in the human kidney activated Twist is expressed in a wide range of renal diseases, which was correlated with HIF-1α activation, E-cadherin suppression and tubulointerstitial fibrosis. Our findings could indicate that Twist activation might represent a common downstream pathway for hypoxia-induced tubulointerstitial fibrosis.

FR-PO1825

Assessment of Erythropoietin Effects in the Progression of Experimental Chronic Kidney Disease Fernando Felippe Carvalho, Vicente de Paulo Castro Teixeira, Waldemar S. Almeida, Nestor Schor. *Nephrology, Federal University of São Paulo, São Paulo, Brazil.*

Background: Erythropoietin (EPO) has been used primarily to treat anemia caused by chronic kidney disease. Recent studies have shown a renoprotective EPO effect in ischemic kidney diseases. The mechanisms of renoprotection include antiapoptotic effects and stimulation of endothelial progenitor cells. Thus, the aim of this study is to evaluate the influence of EPO on progression of kidney disease in experimental chronic kidney disease

Methods: Male Wistar rats weighing 280-300g underwent 5/6 nephrectomy and were divided into two groups: (NX) only nephrectomized(n=6) and (NX-EPO) nephrectomized(n=6) and treated with a weekly dose of erythropoietin(250UI/kg/ip).

All animals were sacrificed 8 weeks after surgery. Hematocrit, serum creatinine, proteinuria, indirect blood pressure measurement, glomerular score and tubular lesion were assessed.

Results: The NX-EPO group showed significant improvement in serum creatinine (NX 1.6 ± 0.4 versus NX-EPO 0.8 ± 0.1, P ≤ 0.001) and protein/urine creatinine ratio (NX 11.2 ± 6.0 versus NX-EPO 4.1 ± 2.2, P = 0.021). Preliminary results suggest an improvement in glomerular score and tubular lesion in animals that received EPO.

There were no significant differences in hematocrit and blood pressure between the two groups

Conclusions: Our study suggests a beneficial effect of EPO in the model of progression of experimental chronic renal disease reflected by the improvement of serum creatinine, attenuation of proteinuria and a lower glomerular lesion score, regardless of its effect on the hematocrit and blood pressure.

FR-PO1826

IL-6 Alters Glomerular Structure and Function and Influences Renal Development Mukut Sharma,[1] Jianping Zhou,[1] Madhulika Sharma,[2] Ram Sharma,[1] Ellen T. McCarthy,[2] Jean-Francois Gauchat.[3] *[1]Nephrology Research, KC VA Medical Ctr, Kansas City, MO; [2]Kidney Institute, KUMC, Kansas City, KS; [3]Pharmacology, University of Montreal, QC, Canada.*

Background: Systemic inflammation is a characteristic of obesity, diabetes and hypertension (the metabolic syndrome). Increased levels of the inflammatory cytokine IL-6 in obese mothers may influence renal development, function and susceptibility to disease in the offspring. The role of IL-6 family cytokines in glomerular function and pathophysiology is not known. We hypothesize that IL-6 alters glomerular filtration barrier structure and function and influences renal development.

Methods: 1. Isolated rat glomeruli were incubated for 15 min with IL-6 (0.01-10 ng/mL) and glomerular albumin permeability (P_{alb}) was determined using an *in vitro* assay .

2. Immortalized murine podocytes were incubated with IL-6 (1 ng/mL) for 15 minutes and total and phosphorylated Akt and ERK1/2 were determined by Western blotting.

3. Metanephroi from embryonic mouse kidneys were incubated with IL-6 (10 pg/mL) for 3 days starting at ED13.5. Tissue growth was measured daily using Image J software (NIH) and branching morphogenesis was studied using immunostaining for cytokeratin.

Results: 1. IL-6 increased P_{alb} within 15 min in a dose-dependent manner (10 pg/mL, $P<0.05$ vs. control; 1 ng/mL, $P<0.001$).

2. IL-6 decreased phosphorylation of ERK at 10 pg/mL (<50%) and 1 ng/mL (>50%) within 15 minutes. IL-6 did not alter phosphorylation of Akt.

3. IL-6 (10 pg/mL) resulted in 20% less growth of metanephroi and altered immunostaining for cytokeratin compared to the untreated control.

Conclusions: Physiologically relevant concentrations of IL-6: 1) alter glomerular filtration barrier function as evidenced by increased P_{alb}, 2) alter podocyte signaling via the MAPK/ERK pathway as evidenced by decreased phospho-ERK, and 3) impacts kidney growth and development as evidenced by growth restriction and altered cytokeratin distribution. We conclude that IL-6 may play multiple important roles in glomerular structure and function. Further studies will determine the role of IL-6 in the onset and progression of glomerular dysfunction in CKD.

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FR-PO1827

Albumin Endocytosis Inhibits Autophagy in Proximal Tubular Epithelial Cells Andrea Havasi, Jonathan M. Gall, Zhiyong Wang, Steven C. Borkan, John H. Schwartz. *Department of Medicine, Boston University Medical Center, Boston, MA.*

Background: Proteinuria is associated with progressive chronic kidney disease. It is well known that exposure of proximal tubular epithelial cells (PTEC) to large amounts of albumin leads to the development of tubular atrophy and fibrosis. However, the possible pathogenic role of albumin in this process has not been fully elucidated. To address this issue, we examined the effect of albumin exposure on autophagy and lysosomal function in cultured primary PTEC.

We show that albumin exposure, mimicking nephrotic glomerular filtrate, causes both lysosomal dysfunction and decreased autophagy. In primary mouse tubular cells, albumin exposure decreased LC3-I to LC3-II conversion in a concentration-dependent manner. Similar results were obtained using either recombinant human albumin or fatty acid free bovine albumin. In the presence of bafilomycin, an H+-ATPase inhibitor, autophagic flux was shown to be inversely related to albumin concentration. In addition, albumin treatment decreased the number of autophagosomes (AP) both in normal media (basal autophagy) and in starved cells (induced autophagy). Albumin exposure over 3-5 days markedly reduced the number of AP, suggesting that prolonged albumin exposure blocks AP formation. After a brief exposure of up to 48 hr, albumin caused marked AP enlargement, suggesting that albumin inhibits fusion of AP with lysosomes without impairing the maturation of small, punctate autophagosomes into larger ones. Normal lysosomal function is required for clearance of AP, hence lysosomal pH and enzyme activity were examined. Albumin exposure increased lysosomal pH and decreased lysosomal enzyme activity in PTEC, suggesting that autophagy inhibition might be the result of lysosomal dysfunction.

Taken together, these data show that albumin overload compromises autophagic and lysosomal function that could lead to cell toxicity. Therapy directed at rescuing autophagy during proteinuric states might be a rational approach for preventing or slowing the progression of chronic kidney disease.

Funding: Private Foundation Support

FR-PO1828

Uremia-Induced Cardiac Activity Modifications: A Uremic Rabbit Model Emiliana Ferramosca,[1] Cristiana Corsi,[2] Eleonora Grandi,[2] Luciano Pisoni,[3] Ilaria Rivolta,[4] Boris Dalpozzo,[3] Stefano Severi,[2] Antonio Santoro.[1] *[1]Malpighi Nephrology, S.Orsola-Malpighi Hosp, Bologna, Italy; [2]DEIS, Bologna Univ, BO, Italy; [3]Veterinary Med., Bologna Univ, BO, Italy; [4]Experimental Med., Bicocca Univ, Milano, Italy.*

Background: Cardiovascular disease is the main cause of death in ESRD patients. Besides the increased arrhythmic events, a larger dispersion in the QT interval duration has also been described. The reasons explaining these alterations in the ventricular repolarization are still poorly understood.

So, the cellular mechanisms underlying electrocardiographic and hemodynamic alterations is of great interest.

Methods: We present a set up of an animal model of uremic cardiomiopathy, the cardiac morphological and functional evaluation, and the electrophysiological properties at the single cell level. We set up a rabbit uremic model obtained by partial resection of renal parenchima. Echocardiographic evaluation of the morphology and hemodynamic parameters revealed the onset of left ventricular (LV) hypertrophy. The action potential of ventricular cardiomyocytes was recorded through whole-cell patch clamp technique in current clamp mode and its duration at the 90% of repolarization (APD90) was assessed.

Results: Plasmatic levels of urea, creatinine and K+ increased 2.6 fold, 5 fold and 20%, respectively.

Ecocardiographically, the thickness of posterior wall increased from 3.3 cm up to 6 cm at the end of the diastolic phase and from 4 cm up to 7.2 cm at the end of the systolic phase, while the thickening of the intraventricular septum was 0.07 cm before the surgery and became 0.17 cm after the development of the uremic state. The LV mass grew from 3.9 g to 7.7 g.

Cardiomyocytes isolated form the LV were paced at 2Hz while perfused with a solution with typical post-dialisys plasma electrolyte concentrations. APD90 decreased by 26% compared to that measured on the same cardiomyocytes perfused with a solution mimicking the electrolyte concentrations in the uremic plasma.

Conclusions: Uremic rabbit may represent a useful model for a deeper understanding of the cellular ionic mechanisms underlying uremia-associated cardiac function alterations and for the development of potential therapeutical strategies.

FR-PO1829

Genetic Analysis of Mesangial Matrix Expansion in Aging Mice and Identification of Far2 as a Candidate Gene Gerda A. Noordmans,[1] Yuan Huang,[1] Kenneth A. Walsh,[2] Susan Marie Sheehan,[2] Jan-Luuk Hillebrands,[1] Peter Heeringa,[1] Harry Van Goor,[1] Ron Korstanje.[2] *[1]Pathology and Medical Biology, University Medical Center Groningen, Netherlands; [2]The Jackson Laboratory, Bar Harbor, ME.*

Background: Aging of the kidney is associated with glomerular mesangial matrix expansion (MME). To unravel the mechanism of aging it is important to identify genes involved in this process. Identifying aging-associated genes might help design novel therapeutic modalities in order to prevent histological changes like MME and to reduce the decline of renal function.

Methods: Glomerular structural changes in the aged kidney of 26 mouse inbred strains (www.jax.org/phenome) were characterized in male mice at 20 months of age in PAS stained sections. Haplotype Association Mapping (HAM) was used to determine genetic loci associated with the presence of MME.

Results: Thirteen out of 26 strains showed MME. HAM showed a peak on chromosome 6 and the only gene found within this 200Kb haplotype block is *Far2*. The strains with MME revealed to have a 9bp indel in the 5'UTR of *Far2*, which is absent in most of the strains without MME. RT-PCR showed a 2-fold increase in the expression of *Far2* in the strains with MME compared to strains without.

Conclusions: Our study found an association between *Far2* expression and glomerular mesangial matrix expansion. *Far2* catalyzes the reduction of fatty acyl-CoA to fatty alcohols, the precursors of platelet activating factor (PAF). PAF is shown in vitro in human and rat mesangial cells to increase the expression of the mesangial matrix. Our data suggests that *Far2* plays an important role in renal aging.

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FR-PO1830

Biomarker of Irreversible Glomerular Injury – A Metabolomic Analysis Experimental Glomerulonephritis Kyoung Hee Han,[1] Bora Kim,[2] Se Eun Lee,[1] Seong Heon Kim,[1] Yo Han Ahn,[3] Hee Gyung Kang,[1] Hae Il Cheong,[1] Joo-Youn Cho,[2] IL-Soo Ha.[1] *[1]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Department of Clinical Pharmacology, Seoul National University College of Medicine, Seoul, Republic of Korea; [3]Department of Pediatrics, Center for Pediatric Oncology, National Cancer Center, Goyang, Republic of Korea.*

Background: Some injuries of the kidney recover completely but many other diseases are not completely reversible and progress to chronic kidney diseases(CKD). While complex network of cellular and molecular events have been known to be involved in the pathophysiological mechanism of CKD, the diverging point between the reversals versus progression and the triggering event of the progression are still unknown. An indicator that predicts progression or regression of injuries is not yet available. To understand the different mechanisms between the reversible and irreversible kidney diseases and to search for biomarkers predicting the prognosis, a metabolomic analysis was applied in comparison of acute and chronic experimental nephritis model.

Methods: Urine samples were collected on day 0, 7, and 14 from rats that were treated with anti-Thy 1 MoAb(OX-7) with or without heminephrectomy. Samples were analyzed by ultra-performance liquid chromatography coupled with Synapt Q-TOF mass spectrometry(Waters, USA). Multivariate analysis was performed on 2597 positive ion markers and 7537 negative ion markers using SIMCA-P(ver. 12.0).

Results: A total of 4 peaks in Electrospray Ionization(ESI)+ and 5 peaks in ESI- were detected after peak alignment. Principal components analysis(PCA) revealed that the chronic and acute nephritis models had two discrete phenotypes and orthogonal partial least squares discriminate analysis(OPLS-DA) showed direction of the time trends was different between these two models. Candidates of biomarkers for chronic nephritis were searched from the human metabolome database(HMDB) and confirmed by tandem mass spectrometry of authentic compounds.

Conclusions: These results suggested that metabolomic analysis could provide insights into mechanism of disease progression and biomarkers for early diagnosis of chronic nephritis.

FR-PO1831

PDGF-C Neutralization Protects Collagen 4A3 Deficient Alport Mice from the Development of Renal Fibrosis Claudia R.C. van Roeyen,[1] Ana Kaitovic,[1] Peter Boor,[1] Ina V. Martin,[1] Stephanie Zok,[1] Ulf P.E. Eriksson,[2] Jurgen Floege,[1] Oliver Gross,[3] Tammo Ostendorf,[1] Frank Eitner.[1] *[1]Nephrology, RWTH Aachen, Aachen, Germany; [2]Karolinska Institute, Stockholm, Sweden; [3]Nephrology&Rheumatology, University Medicine Göttingen, Göttingen, Germany.*

Background: PDGF-C is upregulated at sites of renal fibrosis. Reduced renal fibrosis has been demonstrated following neutralization of PDGF-C in mice with ureteral obstruction. We now studied the role of PDGF-C antagonism or deficiency in collagen 4A3 deficient ("Alport") mice, which serve as a model for progressive renal fibrosis.

Methods: Alport mice were crossbred with PDGF-C deficient mice (n=15) and analyzed at 10 weeks of age. In a second experiment, Alport mice (n=24) were treated with neutralizing anti-PDGF-C antibody or control IgG from week 6 until sacrifice at week 9. We analyzed renal function, histological damage and renal inflammation.

Results: PDGF-C deficient Alport mice developed less renal injury: significantly less glomerular injury (76% reduction of glomeruli containing extracapillary proliferates; 65% reduction of glomerular fibrin deposition) and significantly less cortical inflammation (90% reduction of CCL2 mRNA, 80% reduction of CCL5 mRNA). Treatment with neutralizing anti-PDGF-C antibody resulted in significantly less renal injury in 9 week old mice: better renal function (58% reduction of serum urea), less glomerular injury (87% reduction of glomeruli containing extracapillary proliferates), less cortical matrix accumulation (63% reduction of fibronectin, 62% reduction of collagen type I), and less cortical inflammation (73% reduction of CCL2 mRNA, 63% reduction of CCL5 mRNA, 94% reduction of infiltrating macrophages).

Conclusions: In conclusion, both PDGF-C deficiency as well as PDGF-C antagonism significantly reduced the development of progressive renal fibrosis in collagen 4A3 deficient mice. These potent antifibrotic effects of PDGF-C neutralization in an animal model that closely resembles all features of progressive human glomerular diseases make PDGF-C a prime target for the treatment of progressive human renal fibrosis.

Funding: Government Support - Non-U.S.

FR-PO1832

Gadolinium-Based Magnetic Resonance Imaging (MRI) Contrast Leads to Organ-Specific Fibrosis Associated with Fibrocyte Accumulation in a Rodent Model of Nephrogenic Systemic Fibrosis Brent Wagner,[1,2] Seema S. Ahuja,[1,2] Jeffrey L. Barnes.[1,2] *[1]South Texas Veterans Health Care System, San Antonio, TX; [2]Department of Medicine, University of Texas Health Science Center at San Antonio, TX.*

Background: Nephrogenic systemic fibrosis (NSF) is associated with gadolinium-based MRI contrast exposure in the setting of acute or chronic renal compromise. Given its systemic nature, it has been proposed that circulating fibrocytes mediate the disease. A study was conducted to determine if bone marrow-derived cells are involved in mediating dermal fibrosis in a rodent model of NSF.

Methods: Lethally-irradiated rats s/p 5/6 nephrectomy were used, salvage bone marrow transplant from human placental alkaline phosphatase- (hPAP-) expressing donors. Animals were treated with gadodiamide contrast (Omniscan, 2.5 mmol/kg IP) for 4 weeks or an equivalent volume of normal saline.

Results: Within the 4th week of treatment, contrast-treated animals demonstrated subtle signs of stress (e.g., perioribital porphyrin staining and muzzle swelling). Dermal cellularity in the contrast-treated group was greater than control. Skin from the contrast-treated group demonstrated greater hPAP expression, which co-localized with α-smooth muscle actin-positive stress fibers. These cells were also pro-collagen I+. The donor cells were also CD34+. Heart and liver tissues demonstrated subtle histological differences, yet the latter was characterized by increased fibronectin on immunoblot. Contrast-treated animals demonstrated greater dihydroethidium in the dermis. Skin demonstrated greater NADPH oxidase 4 (Nox4) by immunoblot.

Conclusions: The animal model demonstrates that a radio-sensitive bone marrow-derived cell population is increased in the dermis of MRI contrast-treated rodents. The expression of α-smooth muscle actin, pro-collagen I, and CD34 are consistent with fibrocytes mediating the disease. Further elucidation of the mechanisms of MRI contrast-induced fibrosis may aid in discovering therapies to this devastating disease.

Funding: Veterans Administration Support

FR-PO1833

Bone Marrow-Derived Cells Play a Major Role in Unilateral Ureteral Obstruction-Induced Kidney Fibrosis Hee-Seong Jang, Jee In Kim, Kwon Moo Park. *Department of Anatomy, Kyungpook National University School of Medicine, Daegu, Korea.*

Background: Interstitial fibrosis is a hallmark of chronic renal failure. Increased fibroblast induces the accumulation of extracellular matrix proteins in the interstitium leading to kidney fibrosis. However, the origin of these cells contributing to the fibrosis has not been defined yet. In the present study we investigated the role of bone marrow-derived cells in the fibrosis induced by ureteral obstruction in mice.

Methods: Bone marrow cells collected from eGFP transgenic mice were transplanted into lethally irradiated mice which are produced by same strain with eGFP mice. 8 weeks after bone marrow transplantation the chimeric mice were subjected to unilateral ureteral obstruction (UUO). 1, 3 and 12 days after UUO kidneys were harvested and used for further experiments including immunostaining against GFP, fibroblast specific protein-1 (FSP-1), collagen III, and proliferating cell nuclear antigen (PCNA).

Results: UUO resulted in gradual increases of GFP-positive cell number in the interstitium and expansion of interstitial area overtime. Over 80% of interstitial cells were GFP-positive cells at 12 days after UUO. Over 90% of GFP-positive cells in the interstitium expressed FSP-1 at 12 days after UUO. Collagen III also expressed in the GFP-positive cells. 49% and 55% of interstitial PCNA-positive cells were GFP-positive cells 3 and 12 days after UUO, respectively. Some of GFP-positive cells were dividing. Cells presenting weaker GFP signal expressed stronger FSP-1 and collagen III. It suggests that infiltrated cells into the injured kidney may differentiate into fibrogenic cells.

Conclusions: Bone marrow-derived cells play as major contributor in UUO-induced kidney fibrosis via infiltration, accumulation into injured site, and subsequent proliferation and differentiation into fibroblasts.

Funding: Government Support - Non-U.S.

FR-PO1834

Bone Marrow-Derived Macrophage Myofibroblast Transition (MMT) Is a Previously Unrecognized Major Pathway in Renal Fibrosis Shuang Wang,[1] Yee-Yung Ng,[2] Xiao Ru Huang,[1] Hui Y. Lan.[1] *[1]Departments Of Chemical Pathology, and Medicine & Therapeutics, and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Shatin, NT, Hong Kong; [2]Division of Nephrology, Veterans General Hospital-Taipei, National Yang-Ming University, Taipei, Taiwan.*

Background: Myofibroblast is a key cell type in renal fibrosis. However, the origin of myofibroblasts during renal fibrosis remain largely debated. The present study tested the hypothesis that bone marrow (BM)-derived macrophage myofibroblast transition (MMT) may be a key pathway leading to renal fibrosis in patients with CKD and in a mouse model of UUO.

Methods: We first determined MMT in renal biopsies from patients with CKD. Then the critical role of BM-derived cells and MMT in renal fibrosis were examined in three mouse models of UUO: 1) BM deletion followed with/without GFP^+BM, GFP^+Smad3^+, GFP^+Smad3^- BM transplantation or; 2) GFP-BM chimeric mice; 3) mice with inducible macrophage deletion (lysM-Cre/DTR). MMT was determined by confocal microscopy and flow cytometry with α-$SMA^+CD68^+/CD11b^+$.

Results: Surprisingly, in CKD patients, upto 70% of α-SMA^+ myofibroblasts were of macrophage phenotype (α-SMA^+CD68^+), contributing to renal fibrosis and fibro-cellular crescents. In mouse models of UUO, irradiative deletion of BM prevented tubulointerstitial fibrosis including α-SMA^+ cells and collagen I/III accumulation, which was restored by GFP-BM transplantation. By confocal microscope and flow cytometry, 70-80% of α-SMA^+ myofibroblasts were $GFP^+F4/80^+$, indicating that BM-derived MMT ($GFP^+\alpha$-$SMA^+F4/80^+$) is a major pathway of myofibroblast origin during renal fibrosis. This was confirmed by the finding that conditional deletion of macrophages largely reduced MMT (60-70%↓) and renal fibrosis after UUO. Moreover, MMT was TGF-β/Smad3-dependent because BM transfusion with GFP^+Smad^+, but not GFP^+Smad3^-, restored MMT and renal fibrosis.

Conclusions: BM-derived macrophage myofibroblast transition (MMT) is a major pathway of myofibroblast origin during renal fibrosis. TGFβ/Smad3 may play an important role in MMT.

Funding: Government Support - Non-U.S.

FR-PO1835

Smad3 Regulates Bone Marrow-Derived Fibroblast Precursors in Renal Fibrosis Jiyuan Chen, Song-Chang Lin, William E. Mitch, Yanlin Wang. *Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Progressive renal fibrosis is the final common manifestation of chronic kidney disease resulting in irreversible loss of kidney parenchyma and renal function. Activated fibroblasts play a critical role in the pathogenesis of renal fibrosis. Their origin remains controversial. We and others have recently shown that bone marrow-derived fibroblast precursors termed fibrocytes migrate into the kidney and contribute to renal fibrosis. However, the molecular mechanisms that are responsible for the recruitment and maturation of bone marrow-derived fibroblast precursors in the kidney are not fully understood. Since TGF-beat1/Smad3 signaling has been shown to play an important role in the pathogenesis of kidney fibrosis, we investigated the role of Smad3 in the recruitment and maturation of bone marrow-derived fibroblast precursors in the kidney in a murine model of renal fibrosis.

Methods: Wild type (WT) and Smad3 knockout (Smad3-KO) mice were subjected to unilateral ureteral obstruction and maintained for up to 2 weeks.

Results: Bone marrow-derived fibroblast precursors, identified as cells positive for both CD45 and vimentin were accumulated in obstructed kidneys of WT mice in response to obstructive injury, which were significantly reduced in obstructed kidneys of Smad3-KO mice. Furthermore, the number of bone marrow-derived myofibroblasts that are positive for both CD45 and α-SMA were significantly decreased in obstructed kidneys of Smad3-KO mice compared with WT mice. Immunohistochemical studies and Western blot analysis revealed that the levels of type I collage, fibronectin, and α-SMA were significantly increased in obstructed kidneys of WT mice . In contrast, all of these parameters were dramatically attenuated in obstructed kidneys of Smad3-KO mice.

Conclusions: These data indicate that Smad3 plays a significant role in the recruitment and maturation of bone marrow-derived fibroblast precursors during the pathogenesis of renal fibrosis.

Funding: Other NIH Support - NHLBI
AHA

FR-PO1836

CTP-499, a Novel Drug for the Potential Treatment of Chronic Kidney Disease, Has Anti-Fibrotic, Anti-Inflammatory, and Anti-Oxidative Activities with *In Vivo* Efficacy Ara Aslanian, Kristine Hogan, Shixin Qin, Julie Fields Liu, Changfu Cheng, Gary W. Bridson, Philip B. Graham, Roger Tung, Lijun Wu. *Concert Pharmaceuticals, Lexington, MA.*

Background: Chronic Kidney Disease (CKD) is a complex, multifactorial disease in which renal function is chronically compromised. Decreased glomerular filtration due to dysregulated extracellular matrix (ECM) deposition is a hallmark of progressive CKD. However, oxidative imbalance and inflammation are now increasingly recognized as major pathogenic mechanisms in CKD. Here, we show that CTP-499, a novel, deuterated analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine (HDX), the active M1 metabolite of pentoxifylline, exhibits efficacy in key cellular pathological mechanisms involved in CKD and in a rat model of diabetic nephropathy.

Methods: To assess the efficacy of CTP-499 *in vitro*, cells were challenged with various stimuli to induce pro-fibrotic gene expression, inflammatory response, and oxidative stress. To establish the efficacy of CTP-499 in an *in vivo* model of diabetic nephropathy, STZ-treated rats were dosed with CTP-499 for seven weeks and markers of disease progression were measured.

Results: CTP-499 demonstrated anti-fibrogenic, anti-inflammatory and anti-oxidative activities in a series of *in vitro* experiments. In a rat model of diabetic nephropathy, CTP-499 treatment resulted in significantly decreased kidney weights, a trend towards lower albuminuria, and significantly reduced cytokine levels compared to vehicle controls.

Conclusions: In summary, CTP-499 exhibits significant biological activities in multiple inter-related pathological mechanisms that contribute to kidney dysfunction. These data support our continued interest in CTP-499 as a novel compound for the potential treatment of CKD.

Funding: Pharmaceutical Company Support

FR-PO1837

Thymosin β4 Augmentation of Fibrosis Is Dependent on PAI-1 Yiqin Zuo,[1] Sebastian Alexander Potthoff,[2] Haichun Yang,[1] Li-Jun Ma,[1] Agnes B. Fogo.[1] *[1]Pathology, Vanderbilt University, Nashville, TN; [2]Nephrology, University of Duesseldorf, Germany.*

Background: Thymosin β4 (Tβ4), a G-actin sequestering protein, is degraded by prolyl oligopeptidase (POP) to Ac-SDKP. Our previous study shows that inhibition of POP shifted the balance of Tβ4 and Ac-SDKP and exacerbated tubulointerstitial fibrosis induced by unilateral ureteral obstruction (UUO) in wild type (WT) mice. Both transforming growth factor β (TGF-β) and plasminogen activator inhibitor 1 (PAI-1) pathways promote fibrosis. We now investigated whether PAI-1 deficiency affects fibrosis. We further examined the effects of augmenting Tβ4 in PAI-1-/- mice.

Methods: WT or PAI-1-/- C57BL6 male mice underwent UUO and were sacrificed 5 days and 14 days after UUO. At day 5, there were two groups: WT with or without Tβ4+POP inhibitor. At day 14, there were three groups: WT without treatment, PAI-1-/- with and without Tβ4+POP inhibitor.

Results: Tubulointerstitial fibrosis, assessed by polarized Sirius red morphometry, was increased similarly in WT vs. PAI-1-/- after 14 days of UUO (3.01±0.13 vs. 3.02±0.45%) compared to non-obstructed kidneys (0.33±0.02%). Tβ4+POP inhibitor treatment resulted in enhanced fibrosis in WT mice at day 5 after UUO (WT with treatment 1.75±0.06% vs WT no treatment 1.50±0.04%, $p<0.05$) with more TGF-β1 and fewer infiltrating macrophages. In contrast, PAI-1-/- mice even at day 14 after UUO showed no increase in fibrosis in response to Tβ4+POP inhibitor administration (2.74±0.09 vs. 3.02±0.45%, p NS). Phosphorylated Smad2, an indicator of TGF-β1 activation, was similarly increased in all UUO kidneys at day 14 compared to non-obstructed kidneys. Infiltrating macrophages were similar in WT vs. PAI-1-/- (3.05±0.64 vs.2.54±0.38% F4/80 positive area), and were not increased in PAI-1-/- by adding Tβ4+POP inhibitor (2.85±0.18%).

Conclusions: We conclude that PAI-1-/- mice are not protected from UUO-induced tubulointerstitial fibrosis, and unlike WT mice, do not show enhanced fibrosis when Tβ4 is increased. Thus, Tβ4 in combination with POP inhibitor enhances fibrosis in WT mice, and this effect is dependent on the presence of PAI-1.

Funding: NIDDK Support

FR-PO1838

Long-Term Administration of a Novel Glutathione Precursor Attenuates Age-Related Oxidative Stress and Kidney Damage Xin J. Zhou,[1] Indrani Sinha-Hikim,[3] Ting Ye,[1] Dinesh Rakheja,[1] Albert B. Crum,[2] Keith C. Norris,[3] Nosratola D. Vaziri.[4] *[1]pathology, UT Southwestern Medical Center, Dallas, TX; [2]Proimmune, Rhinebeck, NY; [3]Medicine, Charles Drew University, Los Angeles, CA; [4]Medicine, UC Irvine, Irvine, CA.*

Background: Oxidative stress (OS) plays a key role in the pathogenesis of renal senescence. Glutathione (GSH) is the most abundant and potent endogenous antioxidant whose tissue contents can be exhausted by OS and its deficiency can cause/intensify OS. The present study was undertaken to examine the effect of long-term oral administration of a novel GSH precursor, F1 (a compound containing cystine as the carrier of cysteine), on the aging kidney.

Methods: Eighteen-month old male B6 mice were randomized to groups fed regular diet or diet containing F1 (0.5 g/Kg/day) for six months. Young adult (2 month old) mice consuming regular diet served as controls. Animals were then euthanized and kidneys were harvested and processed for histological examination, measurement of of markers of OS, and expression of key molecules involved in the redox and inflammatory pathways.

Results: Compared to the young mice, kidneys in the untreated aged mice showed marked GSH depletion, mesangial matrix expansion, focal tubular atrophy, interstitial fibrosis and patchy interstitial inflammation. This was associated with accumulation of TBARs, upregulation of MPO, and down-regulation of GSH peroxidase (GPX), PPARγ, SIRT1, and catalytic and modifier subunits of glutamate cysteine ligase (GCL). Treatment with F1 restored renal tissue GSH content and attenuated age-related histological abnormalities. This was accompanied by amelioration of OS, reduction in MPO abundance and partial or complete restoration of GPX, PPARγ, SIRT1, and GCL expression. Despite OS, which should have evoked activation of Nrf2, its nuclear content was unchanged reflecting impaired response to oxidative stress in the aged mice. Amelioration of OS with F1 administration was associated with the maintenance of Nrf2 activity in the kidneys of the aged mice.

Conclusions: These findings illustrate the efficacy of F1 in mitigating age-associated oxidative stress and renal injury in this model.

Funding: Private Foundation Support

FR-PO1839

Disrupted TGF-β Receptor II Abrogates Smad3-Mediated Renal Fibrosis but Exaggerates Inflammatory Response In Vivo and In Vitro Xiaoming Meng, Xiao Ru Huang, Jun Xiao, Haiyong Chen, Hui Y. Lan. *Department of Medicine and Therapeutics and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Shatin, NT, Hong Kong, China.*

Background: It is well known that TGF-β1 plays distinct roles in fibrosis and inflammation. The present study tested the hypothesis that TGF-β1 may signal through its receptor II to exert the diverse effects on renal fibrosis and inflammation in vivo and in vitro.

Methods: The hypothesis was examined in vivo in a mouse model of unilateral ureteral obstructive (UUO) nephropathy induced in TGFRII (RII) conditional knockout mice (KspCre/RII$^{f/f}$) in which RII was deleted specifically from the kidney tubular epithelial cells and in vitro in primary kidney fibroblasts and NRK52E cell line where the RII was deleted by adenovirus Cre or by overexpressing dominant negative RII (DNRII).

Results: Disrupted RII from the kidney as well as from kidney fibroblasts substantially inhibited fibrogenesis including collagen and fibronectin expression (50% ↓) and α-SMA+ myofibroblasts accumulation (approximately 20% ↓) in the UUO kidney and in TGF-β1-stimulated kidney fibroblasts. These changes were associated with significant reduction of TGF-β1($p<0.05$) and CTGF ($p<0.01$) and deactivation of Smad3 signaling (80%↓). In contrast, deletion of RII enhanced inflammatory response as demonstrated by up-regulation of IL-1β, TNFα (20%↑) in the UUO kidney and in IL-1β-stimulated DNRII-NRK52E cells. Further studies revealed that enhanced renal inflammation in the UUO kidney and cells with conditional deletion of RII was associated with a further increase in activation of NF-κB signaling.

Conclusions: In conclusion, TGF-β1 may signal through its RII to exert its diverse role in causing renal fibrosis while inhibiting renal inflammation under pathophysiological conditions.

Funding: Government Support - Non-U.S.

FR-PO1840

Suramin: A Novel Treatment for Chronic Kidney Disease Shougang Zhuang.[1,2] [1]*Department of Medicine, Brown University School of Medicine, Providence, RI;* [2]*Department of Nephrology, Tongji University Shanghai East Hospital, Shanghai, China.*

Background: Current drug discovery efforts for fighting renal fibrosis are largely focused on compounds that are specific for a particular receptor or protein kinase. Since renal fibrogenesis is associated with increased production of multiple cytokines/growth factors, inhibitors with broad specificity might offer improved therepautic benefit in fibrotic diseases of the kidney. We here assessed the therapeutic effect of suramin, an FDA approved drug for treating selected malignancies, on the activation of renal interstitial fibroblasts and the development and progression of renal fibrosis in animal models of chronic kidney injury.

Methods: Unilateral ureteral obstruction and remnant kidney models were used.

Results: In a model of unilateral ureteral obstruction (UUO), administration of a single dose of suramin (20 mg/kg) immediately after injury prevented the onset of renal fibrosis as shown by abolishing expression of fibronectin, suppressing expression of alpha-SMA and type I collagen and reducing deposition of extracellular matrix proteins. In a rat model of remnant kidney disease, suramin also prevented progressive renal injury as demonstrated by inhibiting the rise of 24 hour-proteinuria and serum creatinine, preserving renal tissue architecture and preventing glomerular and tubulointerstitial damage. Furthermore, delayed administration of suramin starting at day 3 of obstruction completely blocked further increase in expression of type 1 collagen and fibronectin and largely suppressed expression of alpha-SMA in both treatment groups. UUO injury or renal ablation induced phosphorylation of epidermal growth factor receptor and platelet derived growth factor receptor and several signaling molecules including Smad-3, STAT3 and ERK1/2 that are associated with renal fibrogenesis. Suramin treatment completely blocked phosphorylation of all these molecules in the injured kidney and also repressed expression of multiple cytokines and decreased leukocyte infiltration to the interstitium.

Conclusions: These findings indicate that suramin is a potent anti-fibrotic agent and may have therapeutic potential in treating patients with CKD.

Funding: NIDDK Support

FR-PO1841

Lipocalin-Type PGD2 Synthase (L-PGDS) Play a Key Role in Kidney Interstitial Fibrosis Via the Activation of Th2-Dominant Inflammatory Response Hideyuki Ito,[1,2] Motoaki Sano,[2] Yasunori Utsunomiya,[1] Keiichi Fukuda,[2] Tatsuo Hosoya.[1] [1]*Division of Kidney and Hypertension, Jikei University School of Medicine, Minato-ku, Tokyo, Japan;* [2]*Division of Cardiology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan.*

Background: L-PGDS, which was identified as an enzyme responsible for PGD2 biosynthesis, is expressed in the kidney. It has reported that urinary L-PGDS excretion increased in diabetic nephropathy and hypertensive nephrosclerosis. However, the pathophysiological role of PGD2 biosynthesis in the kidney is still unknown.

Methods: We examined the interaction of kidney expression of L-PGDS and tissue injury in an animal model of unilateral ureteral obstruction (UUO).

Results: L-PGDS expression was induced in cortex after 3days of UUO and continued to increase thereafter. Tubular-epithelial de novo synthesis of L-PGDS was also demonstrated by in situ hybridization. Of note, the interstitial collagen deposition and mRNA expression of collagen-I was less in the UUO-kidneys of L-PGDS knockout (LKO) mice compared to those of wild-type (WT) mice. In addition, the number of infiltrating CD4+ T cells, not macrophages, was significantly decreased in LKO mice. Intracellular staining in the kidney revealed that the infiltration of IL-4 producing Th2 cells, but of IFN-γ producing Th1 cells, was reduced in LKO mice. CRTH2, which is one of the PGD2 receptors, is expressed on Th2 cells and mediates Th2-cytokine production. CRTH2-KO mice showed lower expression levels of IL-4 and IL-13 in the UUO-kidneys. Interestingly, UUO-induced interstitial fibrosis was markedly reduced in CRTH2-KO mice. In the further support to these notes, both IL-4-KO and IL-13-KO mice reduced the degree of UUO-induced interstitial fibrosis compared to WT mice. Furthermore, administration of CRTH2 antagonist, CAY10471, following UUO attenuated the progression of interstitial fibrosis.

Conclusions: L-PGDS is de novo synthesized in tubular epithelium of the obstructed kidneys and contributes to the progression of interstitial fibrosis via the activation of Th2 cells. Blockade of CRTH2-signalings is a promising strategy to attenuate the tubulointerstitial fibrosis via inhibition of Th2-dominant inflammatory response.

FR-PO1842

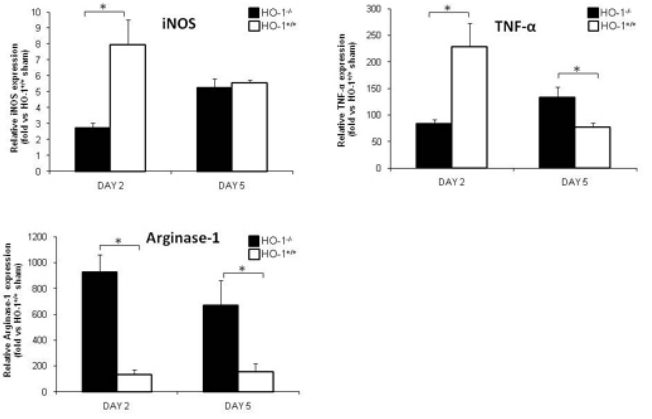
Regulation of Macrophage Polarization by Heme Oxygenase-1 in Renal Fibrosis Abolfazl Zarjou,[1] Anjana Perianayagam,[2] Subhashini Bolisetty,[1] Anupam Agarwal.[1] [1]*Medicine, Nephrology, University of Alabama at Birmingham;* [2]*Medicine, Nephrology, University of Arkansas.*

Background: Heme oxygenase-1 (HO-1) is known to modulate innate and adaptive immune responses. We previously showed that there is increased renal fibrosis following unilateral ureteral obstruction (UUO) in HO-1 deficient (HO-1$^{-/-}$) mice compared to HO-1$^{+/+}$ mice. Macrophages play an essential role in such fibrosis. The M1 (classically activated) macrophages predominate during early phases of kidney injury and inflammation, whereas M2 (alternatively activated) macrophages predominate at later time points and promote cellular repair and fibrosis. The purpose of this study was to determine the effect of HO-1 on the distribution of renal macrophage subtypes following UUO.

Methods: Renal macrophage infiltration and polarization was investigated at 2 and 5 days following UUO in HO-1$^{+/+}$ and HO-1$^{-/-}$ kidneys using flow cytometry and real time-PCR.

Results: A significantly higher number of macrophages were detected in HO-1$^{-/-}$ kidneys at 48h post UUO. Following isolation of CD11b^{+} (a macrophage marker) population from UUO kidneys, we performed real time-PCR evaluation of M1 (iNOS and TNFα) and M2 (Arginase-1) markers (Figure 1). As expected, the M1 subpopulation in HO-1$^{+/+}$ kidneys was higher at 2 days while the predominant inflammatory cells were M2 macrophages at 5 days post UUO. Although there were no significant differences in sham animals, polarization was dysregulated in HO-1$^{-/-}$ mice that underwent UUO. The M2 macrophages were the most abundant inflammatory cells at 2 days in HO-1$^{-/-}$ kidneys and M1 macrophages were significantly increased only 5 days post UUO (*$P < 0.05$).

Figure 1



Conclusions: Our findings elucidate the key role of HO-1 in macrophage polarization and suggest that modulation of HO-1 expression in inflammatory disorders has the potential as a novel therapeutic modality.

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FR-PO1843

Inflammation Exacerbates the Progression of Vascular Calcification in Hemodialysis Patients through the Disruption of LDL Receptor Pathway
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Background: Chronic microinflammation in hemodialysis patients played crucial roles in the progression of vascular calcification (VC). Our previous studies *in vivo* and *in vitro* demonstrated that inflammation accelerated the progression of atherosclerosis (AS) via the dysregulation of low density lipoprotein receptor (LDLr) pathway. This study was to investigate whether LDLr pathway is involved in the progression of VC under inflammatory stress.

Methods: Twenty-eight hemodialysis patients receiving arteriovenostomy were divided into two groups by the plasma level of C-reactive protein: Control (n=14), inflamed group (n=14). Hematoxylin-eosin staining and alizarin red S staining were respectively used to check foam cell formation and VC using tissues surgically removed from radial artery. Immunohistochemistry and immunofluorescent staining were used to check protein expressions related with intracellular cholesterol trafficking and VC.

Results: There was parallel change for increased foam cell formation and significant VC in continous cross-sections of radial arteries in inflamed group compared to control, which were correlated with the increasing protein expressions of LDLr, bone morphogenetic proteins-2 (BMP-2) and collagen I respectively. Confocal microscopy observation showed that inflammation enhanced the protein expressions of alkaline phosphatase, and reduced the protein expression of alpha-smooth muscle actin, contributing to the phenotype conversion of vascular smooth muscle cells in calcified vessels from the fibroblastic to the osteogenic, which was one of the main cell components involved in VC. Further analysis showed that the dysregulation of LDLr pathway induced by inflammation was significantly associated with the enhanced expression of BMP-2 and collagen I.

Conclusions: Our study firstly demonstrated that inflammation accelerated the progression of VC in hemodialysis patients through the disruption of LDLr pathway, suggesting a new potential mechanism involved both in the progression of VC and AS.

Funding: Government Support - Non-U.S.

FR-PO1844

M2 Macrophages Contribute to Kidney Repair Via Promoting Degradation of Extracelluar Matrix through Legumain
Xiaoyue Tan, Min Xiong. *Pathology, School of Medicine, Tianjin, China.*

Background: Studies have suggested that the switch of macrophage phenotype from M1 to M2 regulates the kidney injury and repair. In this study, we investigated that the role of Legumain in the mechanism underlying the renal beneficial effect of M2 macrophage.

Methods: Adult male C57 mice underwent Recovery unilateral ureteral obstruction (R-UUO). Liposome cholodronate (LC) was used to deplete the macrophages. LC/control liposome was administrated either before the operation or at the time when recovery. 20 mice were randomly divided into 4 groups: LC before operation; Control liposome before operation; LC recovery; Control liposome recovery (n=5). Macrophage and Legumain expression were identified by Immunofluorescence staining ; FACS analysis further evaluated the phenotype of Macrophage. Legumain expression was confirmed via Western blot. *In vitro* experiments were performed in co-culture system of M2 macrophage and HK-2 cells. HK-2 cell were treated with or without 5 ng/ml TGF-beta1. Degradation of Fibronectin and Collagen I in the condition medium were measured via western blots. Legumain inhibitor was usd to evaluate the effect of legumain. IP assay were performed to evaluate the possible interaction between legumain and extracellular matrix.

Results: Compared with control liposome recovery group, depletion of macrophage at the time of UUO recovery increased extracellular matrix deposition and the legumain expression was inhibited in LC recovery group with the depletion of M2 macrophage. Result of immunofluorescence staining and FACS analysis showed the M2 macrophages were the major source of legumain in the process of kidney repair. *In vitro* experiment showed that co-culture with M2 macrophage promoted the degradation of Fibronetin and collagen I while small inhibitor of Legumain blocked this effect. IP assay confirmed the interaction between legumain and two major extracellular matrix components, Fibronectin and Collagen I.

Conclusions: Our results suggest that M2 macrophages exhibit protective effect on the kidney recovery from fibrosis. The underlying mechanism is most likely related with inducing expression of legumain thus promoting the degradation of extracellular matrix.

Funding: Government Support - Non-U.S.

FR-PO1845

PBI-4050, a Novel Orally Active Anti-Inflammatory/Anti-Fibrotic Agent, Reduces Fibrosis and Sclerosis in 5/6 Nephrectomized Rats
Lyne Gagnon, François Sarra-Bournet, Brigitte Grouix, André Doucet, Valérie Perron, Jean-Simon Duceppe, Abdallah Ezzitouni, Boulos Zacharie, Christopher Penney, Pierre Laurin. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: PBI-4050 is a novel first-in-class, orally active low molecular weight compound which displays anti-inflammatory/anti-fibrotic activities via a novel mechanism of action. The aim of this study was to investigate the effect of PBI-4050 on 5/6 nephrectomized rats.

Methods: Wistar rats were partially nephrectomized (2/3 of the left kidney) on day 0. On day 7 the right kidney was removed. Oral treatment with PBI-4050 (200 mg/kg, once a day) or vehicle was initiated at day 21. GFR was measured at day 21 and assessed every 3 weeks up to day 190 at which time the animals were sacrificed.

Results: Treatment with PBI-4050 resulted in a significant improvement (up to three fold relative to control) in GFR as demonstrated by an increase in creatinine clearance. Histological lesion scores of kidney were also significantly ($p<0.05$) reduced in PBI-4050-treated rats (2.7 ± 1.5) compared to control (3.9 ± 1.4), as determined by HPE, PAS and Masson's trichrome staining. Tubulo-interstitial fibrosis and sclerosis were significantly ($p<0.05$) reduced by treatment with PBI-4050. Also, a reduction in blood pressure was observed in PBI-4050-treated rats. Furthermore, oral treatment with PBI-4050 induced a significant reduction of urine MCP-1 level in treated 5/6 nephrectomized rats. This reduction correlates with GFR improvement and inhibition of fibrosis observed in PBI-4050-treated rats, indicating that PBI-4050 delays disease progression.

Conclusions: Taken together, these results suggest that PBI-4050 offers the potential as a novel therapy for chronic kidney disease by reduction of fibrosis and sclerosis.

Funding: Pharmaceutical Company Support

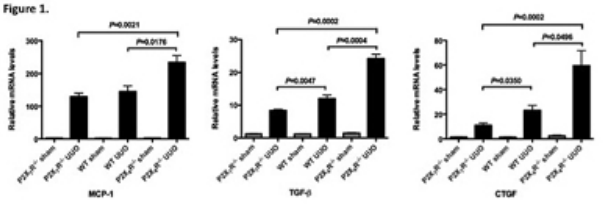
FR-PO1846

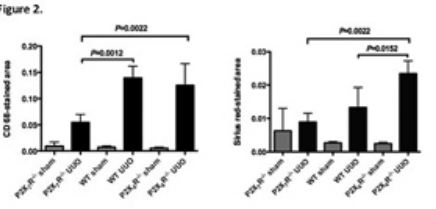
Deficiency of P2X4 Receptor May Promote Renal Fibrosis in a Mouse Model of Unilateral Ureteral Obstruction
Min Jeong Kim,[1] Reiko Hewitt,[1] Jennifer Smith,[1] Robert J. Unwin,[2] Frederick W.K. Tam.[1] *[1]Kidney and Transplant Institute, Imperial College London, United Kingdom; [2]Centre for Nephrology, University College London, United Kingdom.*

Background: Among the seven mammalian P2X receptors, P2X7 (P2X7R) has been shown to have pro-inflammatory effects in two models of renal injury: nephrotoxic nephritis and unilateral ureteral obstruction (UUO). P2X4 (P2X4R) is a related receptor with similarities to P2X7R; it is adjacent on the same chromosome, but has a wider tissue distribution. We compared the function of these receptors in the UUO model using knockout (KO) mice.

Methods: Ten to 12-week-old male P2X4R-/-, P2X7R-/- (a model expressing a novel splice variant of P2X7R) - gifts from GSK - and WT mice were subjected to either UUO or sham operation. Kidney samples taken at day 7 or day 14 were evaluated for MCP-1, TGF-β and CTGF expression by qPCR; macrophage infiltration and collagen deposition were assessed by immunostaining for CD68 and staining for picrosirius red (SR).

Results: On day 7 after UUO there were no significant differences in mRNA expression of MCP-1, TGF-β and CTGF, or CD68+ staining (P2X7R-/-, WT or P2X4R-/-); however, SR staining was significantly greater in P2X4R-/- compared with P2X7R-/-. By day 14 mRNA expression of MCP-1, TGF-β and CTGF was significantly higher in P2X4R-/- compared with P2X7R-/- and WT (Fig 1); CD68+ staining was significantly greater in both P2X4R-/- and WT compared with P2X7R-/-; SR staining was significantly greater in P2X4R-/- compared with P2X7R-/- and WT (Fig 2).





Conclusions: These findings suggest: (1) that P2X4R deficiency increases renal fibrosis in UUO; (2) that the reduced inflammation and fibrosis in another P2X7R KO model is consistent with previous findings, and that there is no functional role for the P2X7R splice variant in UUO.

FR-PO1847

Accelerated Tubulointerstitial Alterations Induced by Unilateral Ureteral Obstruction in Mice Lacking Vasohibin-1 Hiroyuki Watatani,[1] Yohei Maeshima,[1] Daisuke Saito,[1] Hiroko Yamasaki,[1] Masaru Kinomura,[1] Norikazu Hinamoto,[1] Haruyo Ujike,[1] Hitoshi Sugiyama,[1] Hikaru Sonoda,[2] Yasufumi Sato,[3] Hirofumi Makino.[1] *[1]Dept. of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Discovery Research Laboratories, Shionogi & Co., Ltd., Settsu, Osaka, Japan; [3]Dept. of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku Univ, Sendai, Miyagi, Japan.*

Background: Tubulointerstitial injuries are crucial histological alterations predicting deterioration of renal function in chronic kidney disease. Vasohibin-1(VASH-1), serves as a negative feedback regulator of angiogenesis as well as a maturation factor of neovessels by inducing pericyte attachment. We previously reported the protective role of Vasohibin-1 in diabetic nephropathy, but its role on tubulointerstitial injuries remains to be elucidated. In the present study, we aimed to evaluate the potential role of endogenous VASH-1 to regulate tubulointerstitial alterations in a mouse unilateral ureteral obstruction (UUO) model.

Methods: UUO was induced in female VASH-1 heterozygous knockout mice (VASH1+/-) or wild-type (VASH1+/+) littermates (C57/BL6J background). Mice were sacrificed on Day 3 or 7 after inducing UUO and the obstructed kidneys (OBK) were obtained.

Results: Glomerular or tubulointerstitial alterations were not observed in the kidneys of sham-operated VASH1+/- mice. Interstitial fibrosis, accumulation of type I and III collagen and F4/80+ monocytes/ macrophages in the OBK on Day 7 after inducing UUO were significantly accelerated in VASH1+/- mice compared with VASH1+/+ mice. Increase in the levels of transforming growth factor (TGF)-beta1 (immunoblot) and the number of interstitial fibroblast specific protein (FSP)1+ cells in the OBK were significantly aggravated in VASH1+/- mice compared with the wild-type mice.

Conclusions: These results suggest that endogenous VASH-1 may play a role in suppressing tubulointerstitial alterations induced by UUO via regulating inflammation and fibrosis, thus implicating its potential use to serve as a novel therapeutic reagent for renal disorders.

FR-PO1848

S1PR3 Is Pivotal Factor in Fibrosis in the Kidney Shunji Shiohira,[1] Takumi Yoshida,[1, 2] Junko Kohei,[1] Hidekazu Sugiura,[1] Michihiro Mitobe,[1] Kosaku Nitta,[1] Ken Tsuchiya.[1] *[1]Department of Medicine IV, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan; [2]Internal Medicine, Yoshida Medical Clinic, Suginami, Tokyo, Japan.*

Background: The major sphingolipid metabolite, sphingosine-1-phosphate (S1P), is now attractive mediator of renal fibrosis. In our past report, S1P induced fibrosis *in vitro* in normal rat kidney interstitial fibroblast cells (NRK-49F). S1P is likely to have potentiality to mediate directly renal fibrotic processs in addition to via inflammaroy pathway. On the other hand, S1P is known to have unique tissue distribution of the receptor subtypes and the differing signaling pathways resulting from S1P receptor (S1PR) subtype activation. In our past study, NRK-49F showed mRNA expressions of S1PR 1, 2, 3 and 5, but not 4.And FTY720 inhibited fibrosis induced by S1P. FTY720 is S1PR 1,3,4,5 antagonist. S1PR 1 is mostly related with inflammatory pathway and S1PR 5 is involved in the regulation of nervous system. Thus, these results suggest that S1PR 3 is likely to play main role in renal fibrosis.

In this study, the role of S1P as a migratory mediator and which subtype of S1PR was related to fibrosis in the kidney were investigated in NRK-49F.

Methods: NRK-49F were stimulated with S1P and the expressions (mRNA/western blotting) of a-SMA, E-cadherin, collagen type 1 (COL1), collagen type 4 (COL4), TIMP1 and PAI1 were examined. The morphological changes of the NRK-49F after stimulation by S1P were examined. Increased migration of the cells was evaluated by the cell count within a field of view. And these examinations were adapted to siRNA (S1PR3) model.

Results: S1P stimulated fibrosis of NRK-49F in a dose- and time-dependent manner, and induced fibrotic transformation of the NRK-49F. Increase in a-SMA, COL1, COL4, TIMP1 and PAI1 expressions and decrease in E-cadherin expression were observed in the S1P-stimulated cells. Treatment of S1P-induced morphological changes (elongation of the cell shape with spindle-like extension, increased migration) in NRK-49F. In the presence of siRNA, fibrotic changes induced by S1P were suppressed.

Conclusions: In conclusion, S1P is a novel fibrotic mediator in the kidney, and S1PR3 is pivotal factor in fibrosis.

FR-PO1849

BMP2 Induces a Pro-Fibrotic Phenotype in Adult Renal Progenitor Cells (ARPCs) through Nox4 Activation in Transplant Recipients with Delayed Graft Function (DGF) S. Simone,[1] M. Cariello,[1] C. Cosola,[1] Antonia Loverre,[1] F. Rascio,[1] Fabio Sallustio,[1] Loreto Gesualdo,[1] Francesco Paolo Schena,[1] G. Grandaliano,[2] G. Pertosa.[1] *[1]Dept. of Emergency and Organ Transplantation, University Aldo Moro, Bari, Italy; [2]Dept. of Biomedical Sciences, University of Foggia, Italy.*

Background: ARPCs contribute to repair featuring acute kidney injury (AKI). Bone morphogenetic proteins (BMPs) regulate differentiation, modeling and regeneration in several tissues. Aim of the study was to evaluate the biological actions of BMP2 in ARPCs.

Methods: BMP2/BMP2 Receptors (BMP-R) gene (RT-PCR) and protein (ELISA/ immunoblotting) expression was evaluated in ARPCs isolated from adult human kidney (magnetic cell sorting). Intracellular reactive oxygen species (ROS) generation was measured by 2',7'DCF. Nox4 protein expression was studied by immunoblotting. BMP2, CD133, a-SMA and Nox4 protein expression was evaluated in renal biopsies of patients (pts, n=10) with DGF by confocal microscopy.

Results: BMP2 was expressed by ARPCs in adult human kidney and was upregulated in vivo after DGF (p=.02). ARPCs expressed type I and II BMP-R. ARPCs treated with BPM2 induced ROS production (basal 36.2±15.3 Arbitrary Unit (AU); BMP2 15' 65.5±17.8 AU, p=.03), NADPH oxidase activity (basal 86.5±38.2 AU; BMP2 15' 212±75.4 AU, p=.01) and Nox4 protein expression (p=.03). In vivo, Nox4 was localized in BMP2+CD133+cells at the tubular level after DGF. BMP2 incubation induced a-SMA (basal .3±.1 AU; BMP2 .8±.2 AU, p=.02), collagen-I (p=.03) and fibronectin (p=.04) protein expression in ARPCs. a-SMA colocalized with CD133 in vivo after DGF. H2O2 induced a-SMA expression in ARPCs (basal 1.0±.2 AU; H2O2 2.6±.8 AU, p=.03), while N-acetyl-cysteine inhibited BMP2-induced a-SMA expression (BMP-2 2.2±.6; BMP2+NAC 1.3±.4 fold change, p=.04). Nox4 silencing (siRNA) abolished BMP2-induced NADPH oxidase activation and myofibroblastic induction (p=.01).

Conclusions: In conclusion: a) ARPCs express BMP2; b) this expression is increased in pts with DGF; c) BMP2 may induce the commitment of ARPCs towards a myofibroblast phenotype; d) this profibrotic effect is mediated by Nox4 activation suggesting a novel mechanism linking DGF with progressive graft damage

FR-PO1850

RGS2, a Regulator of G Protein Signaling 2, Is Essential for the Prevention of Renal Fibrosis Induced by Unilateral Ureteral Obstruction Hee-Seong Jang, Jee In Kim, Kwon Moo Park. *Department of Anatomy, Kyungpook National University, Daegu, Korea.*

Background: A regulator of G protein signaling 2 (RGS2) is the most potent negative regulator of angiotensin II (AngII) type 1 receptor (AT1R), which is well-known contributor in the progression of chronic kidney disease. However, the role of RGS2 in kidney fibrosis remains to be defined. Here, we investigated the role of RGS2 in renal interstitial fibrosis following unilateral ureteral obstruction (UUO).

Methods: RGS2 deficient (RGS2-/-) and wild type (RGS2+/+) mice were subjected to UUO or sham-operation and kidneys were harvested at 3 and 5 days after operation. Expressions of RGS2 mRNA and protein were determined by RT-PCR and Western blot analysis, respectively. AT1R, AngII type 2 receptor (AT2R) and α- smooth muscle actin (α-SMA) expressions, and collagen deposition were evaluated by Western blot analysis and trichrome staining.

Results: UUO elevated the levels of RGS2 mRNA and protein along with increased expression of α-SMA and collagen deposition in the kidney. AT1R and AT2R expressions also significantly increased in the kidney after UUO. RGS2 gene deletion accelerated the kidney fibrosis with increase in the expression of α-SMA and collagen deposition. UUO-induced increase of AT1R expression was greater in the kidney of RGS2-/- mice than in those of RGS2+/+ mice and AT2R was vice versa. Well known downstream effector of AT1R such as PAI-1 and phosphorylated-ERK (p-ERK) were also greater in the kidney of RGS2-/- than in those of RGS2+/+ mice.

Conclusions: Our findings suggest that RGS2 prevents the progression of UUO-induced renal fibrosis through inhibition of AT1R.

Funding: Government Support - Non-U.S.

FR-PO1851

A Synthetic Serine Protease Inhibitor Camostat Mesilate Attenuates Renal Interstitial Fibrosis in Rats Jun Morinaga,[1] Yutaka Kakizoe,[1] Taku Miyoshi,[1] Tomoaki Onoue,[1] Teruhiko Mizumoto,[1] Manabu Hayata,[1] Kohei Uchimura,[1] Naoki Shiraishi,[1] Masataka Adachi,[1] Sakai Yoshiki,[2] Kimio Tomita,[1] Kenichiro Kitamura.[1] *[1]Department of Nephrology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan; [2]Ono Pharmaceutical Co., Ltd, Osaka, Japan.*

Background: Interstitial fibrosis is an important common pathway to end stage renal disease. It has been shown that serine proteases such as plasmin and thrombin have important roles in the pathogenesis of fibrosis. Therefore, we hypothesized that orally active serine protease inhibitor camostat mesilate (CM; FOY 305) can improve renal fibrosis.

Methods: In vivo, we investigated the effect of CM on unilateral ureteral obstruction (UUO) model. Sprague-Dawly rats were divided into three groups: Sham, UUO placebo, UUO CM (7mg/day). Rats were sacrificed after 2 weeks from UUO treatment. In vitro, we investigated the effect of CM on TGF-β1 signaling. Rat kidney fibroblasts (NRK cells) were divided into three groups: Control, TGF-β1+vehicle, TGF-β1+CM. After incubation in serum free medium for overnight, NRK cells were premedicated by CM or vehicle. Then, active TGF-β1 was administered to the NRK cells. Cells were harvested with time.

Results: In vivo, renal collagen I and III mRNA expression, percentage of sirius red positive area, hydroxyproline content, αSMA mRNA expression and its protein quantity was increased by UUO treatment. Administration of CM attenuated these fibrotic changes. Active TGF-β1 concentration of the kidney, phosphorylation of smad2 and ERK were increased by UUO, and significantly ameliorated by CM induction. Matrix metalloproteinase (MMP) -2 activity was increased by UUO, and ameliorated by CM administration. In vitro, mRNA expression of PAI-I, αSMA, CTGF, and protein quantity of αSMA, collagen I were increased by TGF-β1 treatment. Phosphorylation of ERK and smad2 were increased by TGF-β1 treatment. Administration of CM significantly ameliorated these changes.

Conclusions: CM attenuated renal interstitial fibrosis in UUO rats, probably through the inhibition of activating process of TGF-β1 by serine proteases. And more, in vitro study suggested that CM directly inhibited TGF-β1 signaling.

FR-PO1852

Silencing of Pericyte MicroRNA-132 Reduces Renal Fibrosis and Is Associated with Altered Sirt1 Expression Roel Bijkerk,[1,2] Ton J. Rabelink,[1] Benjamin D. Humphreys,[2] Anton Jan Van Zonneveld.[1] *[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Renal Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Background: Lineage analysis has shown that during nephrogenesis, FoxD1-positive mesenchymal cells give rise to adult interstitial pericytes. These FoxD1-derivative interstitial cells expand and differentiate into smooth muscle actin (α-SMA) positive myofibroblasts during renal fibrosis, accounting for a large majority of myofibroblasts, which are responsible for scar formation in fibrotic kidney disease. MicroRNAs (miRNAs) involved in this differentiation could serve as a target to decrease myofibroblast formation in fibrotic kidney disease.

Methods: Fibrosis was induced in FoxD1-GC;Z/Red mice by unilateral ureteric obstruction (UUO) and FoxD1-derivative interstitial cells (dsRed positive) were isolated using FACS sorting. To identify differentially expressed microRNAs we profiled these specific cell populations in UUO vs. healthy controls. To investigate the role of miRNA-132 *in vivo* in renal fibrosis we injected antagomirs i.v. to silence its function in the UUO model. Mice were sacrificed both 5 and 10 days after surgery.

Results: miR-132 was amongst the most highly up regulated microRNAs in the FoxD1-derivative interstitial cells in the fibrotic kidney. Blocking the function of miR-132 with antagomir-132 in the UUO model resulted in an almost 20% decrease in collagen deposition after 10 days as compared to scramblemir controls, as determined by Sirius Red staining. In addition, immunohistochemical analyses show that the number of interstitial α-SMA positive cells, representing the myofibroblast population, is decreased by 25%. Western blot analyses showed a two fold reduction in the expression of α-SMA. This protection from a fibrotic phenotype is associated with a 3-fold reduction in the expression of the NAD-dependent deacetylase Sirt-1.

Conclusions: Silencing miR-132 protects against the development of renal fibrosis and is associated with strongly altered Sirt-1 levels.

FR-PO1853

In Tubulointerstitial Inflammation of the Kidney Notch3 Receptors Play a Non-Redundant Profibrogenic Role Sonja Djudjaj,[1,2] Christos Chatziantoniou,[3] Ute Raffetseder,[1] Dominique Guerrot,[3] Peter Boor,[1] Tammo Ostendorf,[1] Jurgen Floege,[1] Clemens D. Cohen,[4] Peter R. Mertens.[2] *[1]Department of Nephrology & Immunology, University Hospital RWTH Aachen, Germany; [2]Department of Nephrology & Hypertension, Otto-von-Guericke University, Magdeburg, Germany; [3]INSERM U702, Hospital Tenon, Paris, France; [4]Division of Nephrology & Institute of Physiology, University Zürich, Switzerland.*

Background: Tubulointerstitial fibrosis is the final common pathway in chronic kidney diseases. Recent studies link Notch receptor expression with kidney damage and scarring. We performed in vitro and in vivo experiments to analyze the participation of Notch3 receptors in the inflammatory response and tissue fibrosis.

Methods: Primary human mesangial cells were challenged with TGF-β and analyzed for Notch receptor expression. We extended our studies to an in vivo model of tubulointerstitial fibrosis: unilateral ureteral obstruction was performed with 20 week-old females. The right ureter of C57Bl6 and Notch3 -/- mice was ligated for 5 and 14 days, respectively. Kidneys were harvested for qRT-PCR and immunohistochemical analyses.

Furthermore qRT-PCR for Notch receptor and ligands was performed in human renal biopsies collected by the European Renal cDNA Bank.

Results: TGF-β challenge increased Notch1 and Notch3 receptor expression/activation at protein and mRNA levels within 30 minutes in hMC.

The expression of Notch3 mRNA and protein was significantly increased in the obstructed kidneys of C57Bl6 mice as compared with contralateral kidneys (6- and 3.8-fold at 5 and 14 days of UUO). Furthermore, analysis of renal tissue on day 5 showed that in comparison with wild type animals Notch3 -/- mice had fewer activated myofibroblasts (αSMA:-48%), markedly less matrix deposition (Sirius Red:-70%, collagen I:-73%, collagen III:-69%), tubular cell loss and infiltration of inflammatory cells (F4/80 staining:-95%). Upregulated Notch3 transcripts were also found in various inflammatory glomerular and tubulointerstitial human kidney diseases.

Conclusions: Upregulation of Notch3 receptor expression is a prerequisite for key events in fibrosing kidney diseases. The receptor may be a novel target for therapeutic intervention.

FR-PO1854

Oral Treatment with a Novel First-in-Class Anti-Fibrotic Compound, PBI-4419, Delays Tubulo-Interstitial Fibrosis and Sclerosis in 5/6 Nephrectomized Rats Lyne Gagnon, Lilianne Geerts, André Doucet, François Sarra-Bournet, Shaun Abbott, Jean-François Bienvenu, Jean-Simon Duceppe, Boulos Zacharie, Christopher Penney, Pierre Laurin, Brigitte Grouix. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: Recently, we discovered a novel, first-in-class, orally active low molecular weight compound which displays anti-inflammatory and anti-fibrotic activities via a new mechanism of action. The aim of this study was to investigate the effect of this first-in-class compound PBI-4419 on 5/6 nephrectomized rats.

Methods: Male Wistar rats were partially nephrectomized (2/3 of the left kidney) on day 0. On day 7 the right kidney was removed. Treatment with vehicle or PBI-4419 (10 and 50 mg/kg, oral once a day) was from day 1 through day 125. GFR was measured at day 21 and assessed every 3 weeks up to day 126 and animals were sacrificed at day 133. Serum urea and creatinine were also assessed every 3 weeks.

Results: Treatment with PBI-4419 increased survival. Rats treated with PBI-4419 demonstrated lower serum urea and creatinine levels than control. A significant GFR improvement (threefold increase) was also observed in PBI-4419-treated rats. Histological lesion scores of kidney were also reduced in PBI-4419-treated rats, as determined by HPE, PAS and Masson's trichrome staining. Tubulo-interstitial fibrosis and sclerosis were significantly ($p<0.05$) reduced by treatment with PBI-4419. Furthermore, oral treatment with PBI-4419 induces a significant reduction of urine MCP-1 level. This reduction correlates with GFR improvement and inhibition of fibrosis observed in PBI-4419-treated rats, indicating that PBI-4419 delays disease progression. 5/6 Nephrectomized rats showed an increase in TGFβ and CTGF mRNA expression in the kidney. Treatment with PBI-4419 gave a significant reduction of the expression of TGFβ (37%, $p=0.002$) and CTGF (30%, $p< 0.0001$) in the kidney.

Conclusions: Taken together, these results suggest that PBI-4419 offers the potential as a novel therapy for chronic kidney diseases by prevention or reduction of fibrosis and sclerosis.

Funding: Pharmaceutical Company Support

FR-PO1855

Kinin B1- and Angiotensin1-Receptor Antagonists Display Comparable Curative Antifibrotic Effects in an Accelerated Model of Renal Fibrosis Antoine Huart,[2] Julie Klein,[1,3] Julien Gonzalez,[1,3] Eric Neau,[1,3] Benedicte Buffin-Meyer,[1,3] David Ribes,[2] Joost Schanstra,[1,3] Jean-Loup Bascands.[1,3] *[1]Inserm U1048, Toulouse, France; [2]Department of Nephrology, CHU-Rangueil, Toulouse, France; [3]Université Toulouse III Paul-Sabatier, Toulouse, France.*

Background: Renal tubulointerstitial fibrosis is the pathological hallmark of chronic kidney disease. Currently, inhibitors of the renin angiotensin system (RAS) remain the sole therapy in humans displaying antifibrotic effects. Therefore new antifibrotic drugs are needed and should be evaluated in combination with RAS inhibitors. We have recently reported that delayed treatment with a kinin B1 receptor antagonist (B1Ra) reduced renal fibrosis in the unilateral ureteral obstruction (UUO) model. The usefulness of new drugs also resides in outperforming the gold standards and being potentially additive or complementary to existing therapies. For this reason we compared the efficacy of a B1Ra with that of an angiotensin type 1 receptor antagonist (AT1a) in a curative model of UUO and determined whether bi-therapy presented higher efficacy than any of the drugs alone.

Methods: Treatments with the AT1 (Valsartan-TAREG) and B1 (SSR240612) receptors antagonists were started 3 days after UUO surgery and continued throughout the time (8 days) of obstruction. A control group received only the vehicle.

Results: Delayed B1Ra treatment was as efficient as the gold-standard AT1a treatment. However bitherapy did not improve the antifibrotic effects. We sought for the reason of the absence of this additive effect by studying the modifications of a panel of genes involved in the fibrotic process. We observed that at the gene expression level the drugs exhibited clear differences in their efficacy to down regulate the different players in fibrosis that, however, in this severe model did not result in improved reduction of fibrosis at the protein level.

Conclusions: As the B1R is induced specifically in the diseased organ and thus potentially displays low side effects, it might be an interesting alternative in cases where AT1a are not effective.

Funding: Government Support - Non-U.S.

FR-PO1856

Age Affects Kidney Remodeling in Response to Ureteral Obstruction Lucas Falke,[1] Amelie Dendooven,[1] Roel Broekhuizen,[1] Reinout Stoop,[2] Jaap A. Joles,[3] Tri Q. Nguyen,[1] Roel Goldschmeding.[1] *[1]Pathology, University Medical Center, Utrecht, Netherlands; [2]Metabolic Health Research, TNO, Leiden, Netherlands; [3]Nephrology and Hypertension, University Medical Center, Utrecht, Netherlands.*

Background: Tubulointerstitial fibrosis (TI) is the final common pathway for all CKD leading to ESRD. CKD incidence increases with age in humans. However, for most experimental studies, young mice are used, and little is known about possible effects of aging on renal matrix biology in the TI compartment.

Methods: Unilateral ureteral obstruction (UUO) was performed in young (16wks; n=5) and old (48wks; n=5) C57Bl6 mice. Structural damage was scored after 14 days on PAS-stained slides. Gene expression was assessed by qPCR. Hydroxyproline and proline were measured by HPLC (hyp/proline ratio). Contralateral kidneys were used as an internal control.

Results: Old mice had 1.2-fold more tubular atrophy ($p=0.02$) and 2-fold more dilation ($p=0.02$) in the obstructed kidney than young mice. Contralateral non-obstructed kidneys in old mice tended to have lower 'baseline' hyp/proline ratio than in young mice, which, after UUO also increased 30% less in old than in young kidneys ($p=0.03$). Accordingly, COL1A2 mRNA was induced in obstructed kidneys in both young and old mice (12-fold, $p<0.0001$), but tended to be lower (1.6-fold, $p=0.07$) in obstructed kidneys of old vs. young mice, while collagenase MMP1/13 mRNA was decreased similarly in old and young obstructed kidneys (2.4-fold, $p<0.001$). In response to injury, BMP6 mRNA was decreased in young (1.9-fold, $p=0.03$), but not old mice after UUO, while decrease of BMP7 (4-fold, $p=0.01$), and increase of TGFβ (4-fold, $p=0.03$) and CTGF mRNA (1.7-fold, $p=0.04$) by UUO were not affected by age.

Conclusions: We conclude that age affects renal response to UUO. The more severe tubular atrophy and dilation upon obstruction might reflect decreased resistance to stretching due to differences in matrix remodeling in old kidneys. The latter might involve preserved BMP6 expression.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1857

Silencing Ki-Ras with Antisense Oligonucleotides Reduces Renal Fibrosis in Unilateral Ureteric Obstruction but Does Not Affect Wound Healing Lucy Jade Newbury,[1] Jia-Hui Wang,[1] A. S. Knisely,[2] Bruce M. Hendry,[1] Claire C. Sharpe.[1] *[1]Renal Medicine, King's College London, United Kingdom; [2]Institute of Liver Studies, King's College London, United Kingdom.*

Background: Pathogenic fibrosis in the kidney is thought to occur via many different signaling pathways, many of which converge on Ras GTPases. Work in our laboratory has highlighted that the Ki-Ras isoform is important in renal fibrosis. In this project we used antisense oligonucleotides (ASO) to silence Ki-Ras expression to prevent fibrosis in a unilateral ureteric obstruction (UUO) model and assessed the effects on surgical wound healing.

Methods: Male Adult Wistar rats underwent unilateral ureteric ligation. The treatment group (n=7) received 12.5mg/kg ASO in saline subcutaneously on alternate days for 16 days. The control group (n=7) received saline alone. Obstructed kidney, contralateral kidney, liver, heart, and wound tissues were fixed in formalin at sacrifice (day 16). Microscopy of sections stained with H&E and Picro-Mallory Trichrome techniques allowed assessment of fibrosis and inflammation whilst an ASO-specific antibody was used to visualize tissue ASO accumulation. Anti-aquaporin 1 immunostaining was used to identify proximal tubular cells whilst anti-aquaporin 2 marked collecting ducts.

Results: In ASO-treated rats, ASO accumulated in all examined tissues, including surgical wounds. In obstructed kidneys ASO were seen primarily in proximal tubules and the interstitium of obstructed kidneys; they was absent from collecting ducts. Silencing Ki-Ras expression markedly reduced fibrosis in the obstructed kidneys. No difference between ASO-treated and vehicle only-treated rats was identified in inflammation or fibrosis at healing wounds.

Conclusions: Subcutaneously administered ASO are deposited in multiple tissues around the body. Although Ki-Ras ASO can prevent pathological renal fibrosis it does not inhibit normal wound healing, which permits the inference that fibro-inflammatory change in UUO and in wound healing operate via different mechanisms. Ki-Ras ASO use in clinical settings may not be contraindicated by wound-healing concerns.

FR-PO1858

Hyaluronan Synthase 1 Gene Expression Modulates Fibroblast Phenotype Timothy Bowen,[1,2] Rachel D. Neville,[1,2] John Martin,[1,2] Robert Steadman,[1,2] Aled O. Phillips.[1,2] *[1]Institute of Nephrology, Cardiff University School of Medicine, Cardiff, United Kingdom; [2]Cardiff Institute of Tissue Engineering and Repair, Cardiff University, Cardiff, United Kingdom.*

Background: To identify the processes underlying scar-free healing we are using donor-matched primary human dermal (DFs) and oral mucosal fibroblasts (OFs) as models of scarring and non-scarring fibroblast phenotypes, respectively. Dermal and oral mucosal wounds heal via inflammation, proliferation and extracellular matrix (ECM) remodelling. However, OF-mediated wound healing lacks scar formation, occurring more rapidly via increased migration, experimental wound repopulation and ECM reorganization.

ECM glycosaminoglycan hyaluronan (HA) is synthesised by the HA synthase (HAS) enzymes, encoded by genes HAS1-3. Transcriptional induction of HAS1 in DF by pro-inflammatory cytokine interleukin (IL)-1β up-regulates HA synthesis and alters cell migration. Fibrotic mediator transforming growth factor (TGF)-β1 also stimulates HAS1 mRNA synthesis in DF, driving differentiation to a myofibroblastic phenotype. Neither quiescent nor cytokine-treated OFs express HAS1, and these cells do not differentiate in response to TGF-β1. We describe here studies on the effect of modulation of HAS1 expression on DF and OF phenotype.

Methods: We used 5'-RACE to identify the HAS1 transcription start site. Selected transcription factor mRNAs were then subjected to small interfering (si)RNA knockdown. siRNA knockdown of HAS1 and HAS2 open reading frames (ORFs) was also carried out, together with forced expression of these ORFs.

Results: siRNA knockdown in DFs showed that HAS1 induction by IL-1β was Sp3-dependent, while TGF-β1 up-regulated HAS1 mRNA synthesis specifically via Smad3. Forced HAS1 ORF expression in OFs drove up-regulated transcription of alpha-smooth muscle actin (α-SMA), a key marker of fibroblast-to-myofibroblast transition. Further analyses in DF, following siRNA knockdown of HAS1 and / or HAS2, showed that visible α-SMA was absent only when siRNAs to both isoforms were used.

Conclusions: Our data provide evidence of synergy in the function of HAS1 and HAS2 in fibroblast phenotypic plasticity, with HAS1 facilitating differentiation to a myofibroblastic phenotype.

FR-PO1859

Regulation and Function of Hyaluronan Synthase 2-Antisense 1 Noncoding RNA, HAS2-AS1 Timothy Bowen,[1,2] Daryn R. Michael,[1,2] James Edward Redman,[3] Abdalsamed Altaher,[1] John Martin,[1,2] Aled O. Phillips.[1,2] *[1]Institute of Nephrology, Cardiff University School of Medicine, Cardiff, United Kingdom; [2]Cardiff Institute of Tissue Engineering and Repair, Cardiff University, Cardiff, United Kingdom; [3]School of Chemistry, Cardiff University, Cardiff, United Kingdom.*

Background: Noncoding (nc)RNAs are emerging rapidly both as novel regulators of gene expression and potential therapeutic targets. Glycosaminoglycan hyaluronan (HA) is a ubiquitous extracellular matrix component that is synthesised by the HA synthase (HAS) enzymes. HA synthesis and pericellular assembly drive the differentiation of renal proximal tubular epithelial cells (PTCs) and fibroblasts to a pro-fibrotic, myofibroblastic phenotype. HAS2 gene expression is essential to this process, and HAS2-antisense 1 (HAS2-AS1) is a natural antisense for the HAS2 gene that down-regulates HAS2 transcription and HA synthesis in osteosarcoma cells. We have recently shown that HAS2-AS1 transcriptional induction facilitates HAS2 expression in PTCs. In the present investigation we investigated HAS2-AS1 and HAS2 expression in fibroblasts as well as the mechanism of HAS2-AS1:HAS2 ncRNA:mRNA interaction in PTCs.

Methods: We used RT-qPCR and siRNA knockdown to analyse HAS2-AS1 and HAS2 expression in fibroblasts. HAS2-AS1 ncRNA and HAS2 mRNA secondary structures were investigated in silico to verify the thermodynamic feasibility of heterodimer formation. Minimum free energy and partition function calculations were performed using the Vienna suite of programs. Analysis of ncRNA:mRNA duplex location in PTCs was carried out using endpoint RT-PCR.

Results: Our data showed coordinated expression of HAS2-AS1 and HAS2 in fibroblasts, supporting our previous analyses in PTCs. The thermodynamic feasibility of HAS2-AS1/HAS2 heterodimer formation was demonstrated, and locus-specific cytoplasmic double-stranded RNA was detected.

Conclusions: We provide evidence that the interaction between HAS2 mRNA and HAS2-AS1 ncRNA stabilises and/or augments HAS2 expression via cytoplasmic duplex formation. Our results emphasise the potential of modulating antisense RNA expression to regulate both HAS2-driven HA synthesis, and the downstream myofibroblastic conversion of PTCs and fibroblasts.

FR-PO1860

The Senescence of Renal Tubular Epithelial Cells Correlates with Tubulointerstitial Fibrosis in Immunoglobulin A Nephropathy Yani He, Jun Liu, Jurong Yang, Wei-Wei Zhang, Lirong Lin, BenGang Huo, Jun Zhan. *Department of Nephrology, Daping Hospital, the Third Military Medical University, Chongqing, China.*

Background: Tubulointerstitial fibrosis (TIF) is the key pathophysiology basis in the development of immunoglobulin A nephropathy (IgAN). Here we report our studies on the relationship between the senescence of RTECs and TIF in IgAN.

Methods: Patients with IgAN were initially classified according to Lee's classification method. With immunohistochemical staining, the expression of Col III and fibronectin (FN) in tubulointerstitium were examined, and expression of p16, p21, cyclin D1 in RTECs were examined, also positive staining of SA-β-gal in RTECs was evaluated. Immunohistochemical staining in serial sections was also performed to examine the expression of p16, p21, cyclin D1 and FN in IgAN with Lee's Grade V. Correlation analysis show the expression of Col III, FN and positive staining of SA-β-gal in RTECs.

Results: With the increased level of Lee's IgAN grades, positive staining of SA-β-gal and the nuclear positive expression of p16 and p21 were progressively increased ($P < 0.01$), which was consistent with the increased expression of Col III and FN in tubulointerstitium; while the expression of cyclin D1 was significantly decreased ($P < 0.01$). Immunohistochemical staining in serial sections in Lee's Grade V IgAN showed that the increased p16 and p21 in RTEC associated with elevated FN expression in tubulointerstitium, whereas the expression of cyclin D1 notably decreased. Correlation analysis suggested that positive staining of SA-β-gal in RTECs presented significant positive correlation with the expression of Col III and FN in tubulointerstitium ($r=0.665$, $r=0.682$, $P < 0.01$).

Conclusions: Our results revealed that the senescence of RTECs tightly links with TIF in IgAN, which is the possible underlying mechanism response for the progression of TIF in IgAN.

FR-PO1861

Increased Expression of Intranuclear Matrix Metalloproteinase 9 in Atrophic Renal Tubules Is Associated with Renal Fibrosis Jen-Pi Tsai,[1,2] Jong-Da Lian,[2,3] Horng-Rong Chang.[2,3] *[1]Department of Nephrology, Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan; [2]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan; [3]Division of Nephrology, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan.*

Background: Reduced turnover of extracellular matrix has a role in renal fibrosis. Matrix metalloproteinase (MMP) is associated with many glomerular diseases, but the histological association of MMP and human renal fibrosis is unclear.

Methods: We studied the role of MMP-9 in the pathogenesis of renal fibrosis in 46 patients who received nephrectomies by immunohistochemical analysis of renal expression

of MMP-9 in nephrectomized kidneys, and determination of the association of renal expression of MMP-9 and renal fibrosis. MMP-9 expression in individual renal components and fibrosis was graded as high or low based on MMP-9 staining and fibrotic scores.

Results: Patients with high interstitial fibrosis scores (IFS) and glomerular fibrosis scores (GFS) had significantly higher serum creatinine, lower estimated glomerular filtration rate (eGFR), and were more likely to have chronic kidney disease (CKD) and urothelial cell carcinoma. Univariate analysis showed that IFS and GFS were negatively associated with normal and atrophic tubular cytoplasmic MMP-9 expression and IFS was positively correlated with atrophic tubular nuclear MMP-9 expression. Multivariate stepwise regression indicated that MMP-9 expression in atrophic tubular nuclei ($r = 0.315$, $p = 0.013$) was an independent predictor of IFS, and that MMP-9 expression in normal tubular cytoplasm ($r = - 0.483$, $p < 0.001$) was an independent predictor of GFS.

Conclusions: Our results indicate that renal fibrosis is associated with a decline of MMP-9 expression in the cytoplasm of normal tubular cells and increased expression of MMP-9 in the nuclei of tubular atrophic renal tubules. We postulate that increased intranuclear MMP-9 expression may reflect intranuclear gelatinase proteolysis, play a role in oxidative DNA damage by cleaving nuclear matrix proteins (PARP-1 and/or XRCC1), and contribute to cell death and fibrosis.

Funding: Clinical Revenue Support

FR-PO1862

HIV-1 Promotes Renal Tubular Epithelial Cell Protein Synthesis: Role of mTOR Pathway Shabina Rehman,[1] Mohammad Husain,[1] B. S. Kasinath,[2] Ashwani Malhotra,[1] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Medicine, University of Texas Health Science Center, San Antonio, TX.*

Background: Tubular cell HIV-infection has been reported to manifest in the form of cellular hypertrophy. The mammalian target of rapamycin (mTOR) has been considered to be a central pathway of protein synthesis. In the present study, we evaluated the role of the mTOR pathway in the HIV-induced tubular cell protein synthesis and hypertrophy.

Methods: Mouse proximal tubular epithelial cells (MPTECs) were transduced with either gag/pol-deleted NL4-3 (HIV/MPTEC) or empty vector (Vector/MPTEC). Vector/MPTECs and HIV/MPTECs were evaluated for DNA synthesis by BrdU labeling and for protein synthesis by immunoblotting for β-laminin and fibronection. Immunoblots prepared from vector/MPTECs and HIV/MPTECs were probed for phosphorylation of mTOR, p70S6 kinase and associated down stream molecules (eEF2, eIF4B and 4EBP-1). We also evaluated the effect of rapamycin on HIV-induced tubular cell mTOR activation and associated downstream signaling. In addition, we studied the effect of mTOR inhibition on HIV-induced tubular cell protein synthesis. In vivo studies, tubular cell mTOR activation was also examined.

Results: HIV/MPTECs showed enhanced ($P<0.01$) production of β-laminin and fibronection in addition to increased ($P<0.01$) protein content per cell. Analysis of mTOR revealed increased expression of phospho (p)-mTOR in HIV/MPTECs when compared to vector/MPTECs. Further downstream analysis of mTOR pathway revealed enhanced ($P<0.01$) phosphorylation of p70S6 kinase and associated diminished phosphorylation of eEF2 in HIV/MPTECs; moreover, HIV/MPTECs displayed enhanced phosphorylation of eIF4B and 4EBP-1. Rapamycin not only attenuated ($P<0.01$) phosphorylation of p70S6 kinase and associated down stream signaling in HIV/ MPTECs but also inhibited HIV-1 induced tubular cell protein synthesis. In in vivo studies, renal cortical sections from HIVTg mice and HIVAN patients showed enhanced tubular cell phosphorylation of mTOR.

Conclusions: The mTOR pathway activation in HIV-expressing tubular cells results in increased protein synthesis and cellular hypertrophy.

Funding: NIDDK Support

FR-PO1863

Matrix Metalloproteinase-7 as a Surrogate Marker Predicts Renal Wnt/β-Catenin Signaling in Progressive Chronic Kidney Diseases Weichun He, Roderick J. Tan, Yingjian Li, Dan Wang, Youhua Liu. *Department of Pathology, University of Pittsburgh, PA.*

Background: Matrix metalloproteinase-7 (MMP-7) is a secreted, zinc and calcium dependent endopeptidase that cleaves a variety of extracellular matrix components and other substrates. In this study, we investigated MMP-7 regulation in progressive chronic kidney diseases and delineated its relation to renal Wnt/β-catenin signaling. In mouse model of obstructive nephropathy, both MMP-7 mRNA and protein were upregulated in a time-dependent manner. Induction of MMP-7 mRNA and protein was also found in adriamycin nephropathy. The pattern and extent of MMP-7 induction were closely correlated with an increased Wnt/β-catenin signaling in these models. Activation of β-catenin through ectopic expression of Wnt1 gene *in vivo* promoted MMP-7 expression, whereas delivery of endogenous Wnt antagonist Dickkopf-1 gene abolished its induction. MMP-7 protein was detectable in urine and its urinary levels correlated with renal Wnt/β-catenin activity. Pharmacologic blockade of Wnt/β-catenin signaling by paricalcitol also inhibited MMP-7 expression in diseased kidneys and its excretion to urine. *In vitro*, β-catenin activation induced MMP-7 expression and secretion, and promoted the occupancy of T cell factor to the MMP-7 promoter in kidney epithelial cells, as shown by ChIP assay. MMP-7 expression was also induced, and correlated with an increased β-catenin, in human kidney biopsies from patients with various nephropathies. These results suggest that MMP-7 level could serve as a surrogate marker for predicting renal Wnt/β-catenin activity, thereby monitoring the progression of renal fibrotic lesions.

Funding: NIDDK Support

FR-PO1864

Albumin Caused the Activation of NLRP3 in Proximal Tubule Cells Dan Liu, Linli Lv, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

Background: Proteinuria is considered to play a central role in the pathogenesis of progressive renal dysfunction, especially, in the renal tubulointerstitial inflammation and fibrosis. Recent studies have indicated that NLRP3 inflammasome is a key platform for the formation of inflammation, it is thereby interesting to understand the potential influence of albuminuria on the expression of NLRP3 inflammasome in kidney tubular cells. In this study, we firstly investigated whether albumin would induce the activation of NLRP3 inflammasome in tubular epithelial cells.

Methods: Proximal tubule cell (HK-2) was cultured and stimulated with different concentrations (2.5 mg/ml, 5 mg/ml, 10mg/ml) of Bovine Serum Albumin (BSA). The expression of mRNA for NLRP-3, caspase-1, IL-1beta and IL-18 was detected by Real time PCR. Immunohistochemistry and immunofluorescence was applied to detect the expression of NLRP3 in cytoplasm of HK-2 cells. The secreted IL-1beta and IL-18 in the supernatant from cell culture was determined by ELISA.

Results: After the 10 mg/ml BSA stimulation, real time RT-PCR analysis revealed the increasing expression of mRNA of NLRP3, caspase-1 and IL-1beta compared with control group. Study by immunohistochemistry and immumofluorescence showed that the expression of NLRP-3 was significantly increased in cytoplasm. Furthermore, it was shown that secretion of IL-1beta and IL-18 in supernatant significantly increased when HK2 cells exposed to the treatment of BSA stimulation ($P<0.05$).

Conclusions: Our study firstly demonstrated that albumin would cause the activation of NLRP-3 in proximal tubular cells, which provide a novel insight about the albuminuria in inducing tubulointerstitial inflammation and subsequent fibrosis.

Funding: Government Support - Non-U.S.

FR-PO1865

Core Fucosylaion of Megalin Is Required for Albumin Binding and Endocytosis in HK-2 Cells Hong Li Lin, Wang Da Peng, Zheng Mei Jie, Yan Ling Sun, Hua Xie. *First Affiliated Hospital of Dalian Medical University.*

Background: Proteinuria is an independent risk factor leading to end-stage renal failure. Over-reabsorption of filtered proteins has been proved to trigger interstitial inflammation and fibrosis in glomerular disease. Megalin, an endocytic receptor expressed on the renal tubular brush border, is responsible for albumin reabsorption. It has been reported that megalin was a glycoprotein and there was amount of core fucose structure in megalin. In this study, we investigated the role of core fucose of megalin which catalysed by its specific transferring enzyme FUT8 in albumin overloading injury in human renal proximal tubular epithelial cells lines (HK-2)

Methods: An albumin overload cell mode was induced by adding 10mg/mL BSA in HK-2 cells. FUT8siRNA or FUT8 full length cDNA vector was transiently transfected into HK-2 cells respectively. The spatial relationship in localization between megalin and core fucose was examined by double immunostaining. BSA binding and endocytosis was determined by flow-cytometry and laser scanning confocal microscope. Expression of megalin protein and core fucose of megalin were detected by immunoprecipitation and lectin blotting. Furthermore,we detected the expression of MCP-1 and RANTES to investigate the effect of core fucosylaion of megalin on the inflammation iuduced by BSA in HK-2 cells.

Results: BSA binding and endocytosis was in a time- and dose-dependent manner in HK-2 cells after BSA administration. Core fucosylation of megalin was inhibited by FUT8siRNA and up-regulated by FUT8 full length cDNA. Our data firstly showed that megalin was modified by core fucosylation, and furthermore the core fucosylation of megalin was essential for its binding and endocytosis of albumin in HK-2 cells .Incubation with BSA led to a significant increase of MCP-1 and RANTES in HK-2 cells. Reducing expression of core glycosylation of megalin markedly decreased expression of MCP-1 and RANTES.

Conclusions: Core fucosylaion of megalin was required for albumin binding and endocytosis. Reducing expression of core glycosylation of megalin markedly decreased expression of inflammation factors in HK-2 cells induced by albumin overload.

Funding: Government Support - Non-U.S.

FR-PO1866

Oncostatin M Is a Novel Inhibitor of TGF-β1-Induced CTGF Expression in Human Proximal Tubular Cells Markus Pirklbauer, Rita Sarkozi, Christine M. Hauser, Susie-Jane Noppert, Andreas Kronbichler, Viktoria Maria Haller, Gert J. Mayer, Herbert Schramek. *Department of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical University, Innsbruck, Austria.*

Background: Matricellular proteins (MP) such as connective tissue growth factor (CTGF), thrombospondin-1 (TSP-1), tenascin-C (TNC), and SPARC (secreted protein, acidic and rich in cystein) have been implicated in the development of tubulointerstitial lesions in human and experimental diabetic nephropathy. This study investigated potential anti-fibrotic effects of the cytokine oncostatin M (OSM) in human proximal tubular cells (hPTC), particularly with regard to inhibition of pro-fibrotic events mediated by transforming growth factor-β1 (TGF-β1).

Methods: Cell culture, Western blot, real-time PCR.

Results: In quiescent hPTC 10 ng/ml OSM diminished TGF-β1-induced expression of the transcriptional EMT mediator FoxC2. Real-time PCR analysis revealed time-dependent induction of CTGF, TSP-1, TNC, and SPARC in hPTC stimulated with TGF-β1 (10 ng/

ml). While TGF-β1-mediated induction of TNC, TSP-1, and SPARC was highest after 24 h, strongest stimulation of CTGF mRNA and protein was detected after 3 h and 6 h, respectively. Exposure to OSM attenuated basal and TGF-β1-induced expression of the four MPs regardless of the sequence of ligand administration. OSMs inhibitory effect on TGF-β1-induced CTGF mRNA expression was effective after 2 h and not affected by inhibition of DNA methylation. OSM resulted in rapid and sustained phosphorylation of Stat1 and Stat3 and a transient phosphorylation of Smad2/3 in contrast to TGF-β1, which demonstrated a gradually building phosphorylation of Smad2/3 and a brief phosphorylation of Smad1/5/8.

Conclusions: In hPTC OSM is a potent inhibitor of basal and TGF-β1-induced CTGF, TSP-1, TNC, and SPARC mRNA expression. Utilising receptor-blocking molecules we found the inhibitory effect of OSM on TGF-β1-induced CTGF mRNA expression occurs independently of Smad2/3 signaling and present evidence that this effect may be partially driven by OSM receptor-mediated Stat1 and/or Stat3 signaling pathways. Thereby providing a mechanism whereby OSM can contribute to tubulointerstitial protection.

Funding: Government Support - Non-U.S.

FR-PO1867

HSP27 Inhibits Renal Fibrogenesis Via Stabilization of Membrane-Bound E-Cadherin Aparna Vidyasagar, Shannon Reese, Lingjin Huang, Jose R. Torrealba, Lynn M. Jacobson, William F. Swain, Arjang Djamali. *Medicine and Surgery, UW-SMPH, Madison, WI.*

Background: We hypothesized that HSP27, a small stress response protein, protects the kidney from tubulointerstitial fibrosis in obstructive nephropathy.

Methods: To test this hypothesis we assessed HSP27 expression in human kidneys with ureteropelvic junction (UPJ) obstruction. Next, we generated transgenic mice that specifically overexpress human HSP27 in renal tubules under a kidney androgen protein promoter, and investigated the effects of HSP27 on epithelial-to-mesenchymal transition (EMT) and tubulointerstitial fibrosis following unilateral ureteral obstruction (UUO). We confirmed our findings in the in vitro model of TGF-β-induced EMT in NRK52E cells.

Results: There was a significant increase in tubular HSP27 in human kidneys with UPJ obstruction, supporting the clinical importance of these studies (Fig 1)

Figure 1. Tubular Hsp27 was increased in kidneys with UPJ obstruction

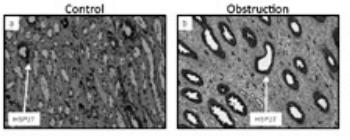


Figure 2. Hsp27 was increased after UUO in Transgenic Mice

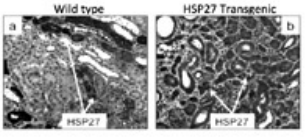
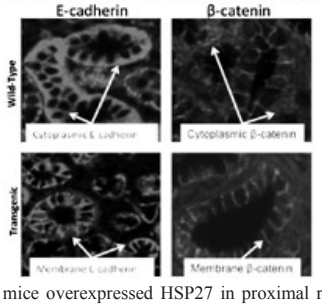


Figure 3. E-cadherin and beta-catenin remained at the cell membrane in HSP27 transgenic mice



HSP27 transgenic mice overexpressed HSP27 in proximal renal tubular epithelial cells after UUO (Fig 2). HSP27 upregulation was associated with decreased fibrosis as evidenced by significant reduction in trichrome staining and decreased oxidative stress (OS) measured by HNE and nitrotyrosine western blots. Notably, E-cadherin and β-catenin were preserved at the cell membrane of tubular cells in transgenic mice undergoing UUO (Fig 3). These studies were confirmed in vitro, where human HSP27 transfection preserved E-cadherin and β-catenin at the cell membrane of rat tubular epithelial cells during TGF-β-induced EMT.

Conclusions: Our studies demonstrate that HSP27 overexpression in renal tubular epithelial cells is associated with reduced fibrogenesis and OS after UUO. The inhibitory effects of HSP27 on fibrogenesis may be mediated via stabilization of the E-cadherin-β-catenin complex at the cell membrane. The molecular mechanisms that regulate HSP27-E-cadherin interactions need to be determined.

Funding: NIDDK Support

FR-PO1868

Matrix Metalloproteinases-2 Was Involved in the Development of Renal Interstitial Fibrosis through Tubular Epithelial-to-Mesenchymal Transition Xuanyi Du,[1] Akira Shimizu,[1] Yukinari Masuda,[1] Akiko Mii.[2] *[1]Analytic Human Pathology, Nippon Medical School, Tokyo, Japan; [2]Internal Medicine (Division of Neurology, Nephrology, and Reumatology), Nippon Medical School, Tokyo, Japan.*

Background: Renal fibrosis is common characteristic findings of the progressive renal diseases. Matrix metalloproteinases (MMPs) is involved in the epithelial-to-mesenchymal transition (EMT). We investigated the role of MMP-2 and the effect of inhibition of MMPs on the development of renal fibrosis.

Methods: Renal fibrosis was induced in MMP-2 wild type (MMP-2+/+) mice by unilateral ureteral obstruction (UUO). Renal histology, EMT-associated molecules, and activity of MMP-2 and MMP-9 were examined during the development of interstitial fibrosis. Renal fibrosis using UUO was also induced in MMP-2 deficient (MMP-2-/-) and MMP-2+/+ mice treated with inhibitor of MMPs (minocycline; 150 mg/kg body wt/day).

Results: In MMP-2+/+ mice, MMP-2 and MMP-9 expressed on the dilated or atrophic damaged tubules, and their activities increased in a time-dependent manner at days 3 to 14 after UUO. At day 14, interstitial fibrosis developed with the deposition of type I and type III collagens, new expression of mesenchymal phenotypes (S100A4, vimentin, αSMA, and HSP-47) in damaged tubular epithelial cells, and F4/80+ macrophage infiltration. In the fibrotic kidneys, EMT-associated molecules (TGF-b1, smad, ILK, and snail) were up-regulated. In contrast, the kidneys of MMP-2-/- mice and MMP-2+/+ mice treated with minocycline showed reduction of renal fibrosis with decreased expression of mesenchymal phenotypes of tubular epithelial cells, inhibition of the up-rugulated EMT-associated molecules, and suppression of macrophage infiltration.

Conclusions: We conclude that MMP-2 play a pathogenic role in the progression of renal interstitial fibrosis, possibly through inducing EMT and macrophage infiltration. Inhibition of MMPs may provide a novel therapeutic advantages aimed at attenuating progressive renal diseases.

Funding: Private Foundation Support

FR-PO1869

Targeted Inhibition of NF-κB Activation in Tubular Epithelia Protects Mice Against Renal Fibrogenesis Following Nephrotoxic Serum Nephritis (NTN) Tsutomu Inoue,[1] Matsuhiko Hayashi,[2] Hiromichi Suzuki,[1] Hirokazu Okada.[1] *[1]Nephrology, Saitama Medical University, Irumagun, Saitama, Japan; [2]Apheresis and Dialysis Center, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan.*

Background: Although it is well known that NF-κB is involved in renal fibrogenesis following NTN, the relative role of NF-κB activation in tubular epithelia is unclear.

Methods: Two lines of transgenic (tg) mice were used. IκB-dominant negative (IκBdN) tg mice that carry the IκBdN gene (IκBdN gene product inhibits NF-κB activation *in vivo*; JASN 21:2047-2052, 2010) separated from a universal CAG promoter by a floxed STOP sequence were crossed with γGT.Cre tg mice that express Cre recombinase in tubular epithelia. Male, 6-8 week-old, double-tg mice (γGT.Cre;IκBdN) and wild-type mice were then challenged with NTN. Inflammation/Fibrosis-related parameters in the kidneys were determined by real-time RT-PCR and immunohistochemistry.

Results: Either urinary protein excretion or glomerular damage such as crescent formation was significantly increased at the similar degree in the γGT.Cre;IκBdN and wild-type mice with Day14 NTN. In contrast, the number of p-p65^{+} nuclei was significantly lower in tubular epithelia in the NTN kidneys of the γGT.Cre;IκBdN mice due to tubular epithelium-specific induction of the IκBdN gene, compared to those of the wild-type mice ($p<0.05$). In contrast, F4/80^{+} monocyte infiltration and matrix deposition by Masson's trichrome staining in peritubular interstitium were significantly decreased in the NTN kidneys of the γGT.Cre;IκBdN mice, compared to those of the wild-type mice ($p<0.05$). Levels of mRNAs encoding KIM-1, MCP-1, PAI-1, and fibronectin-EIIIA were also significantly decreased in the NTN kidneys of the γGT.Cre;IκBdN mice, compared to those of the wile-type mice ($p<0.05$).

Conclusions: Targeted expression of IκBdN in tubular epithelia provides evidence of the critical role of NF-κB activation in tubular epithelia in proinflammatory cell recruitment and subsequent fibrogenesis in the NTN kidneys.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1870

Adenovirus Mediated Gene Transfer of Gremlin1 to the Peritoneal Membrane Is Associated with Epithelial Proliferation and Cellular Transition Simon P. Curran,[1] Imad U. Siddique,[2] Peter Margetts.[2] *[1]Medicine, University of Toronto, ON, Canada; [2]Medicine, McMaster University, Hamilton, ON, Canada.*

Background: Gremlin1 (grem1) is a bone morphogenetic protein (BMP) antagonist that displays increased expression in models of fibrosis, including diabetic nephropathy. Grem1 expression is associated with epithelial to mesenchymal transition (EMT) due to reduced BMP-7 signaling. Grem1 has also been identified as an agonist of vascular endothelial growth factor receptor 2. EMT and angiogenesis are important processes leading to peritoneal membrane injury and alteration in solute transport properties for patients on peritoneal dialysis (PD) therapy.

Methods: C57Bl/6 mice received an intraperitoneal injection of an adenovirus vector encoding grem1 or control adenovirus (AdDL). Grem1 was delivered at $1.5x10^{8}$ pfu (AdGrem1(low dose)) or $3x10^{8}$ pfu (AdGrem1(high dose)). As a positive control for fibrosis, we treated animals with an adenovirus expressing active transforming growth factor β (AdTGFβ). Animals were sacrificed 7, 14 and 21 days after infection.

Results: mRNA was extracted from peritoneal tissues. Gene expression for grem1 was increased in both low dose and high dose AdGrem1 animals. On histology, we observed epithelial proliferation and increased submesothelial thickening, most prominent in the high dose group. Compared with AdTGFβ1 treated mice, the thickened submesothelium zone in AdGre1 treated animals demonstrated little collagen accumulation on trichrome stain. This was confirmed by PCR. AdGrem1 did not induce significant collagen gene expression. AdGrem1 did increase α-smooth muscle actin and decreased E-Cadherin gene expression, suggestive of an EMT process.

Conclusions: Grem1, by inhibiting BMP-7, appears to cause EMT and epithelial proliferation without strong evidence of fibrosis. The clinical importance of Grem1 in peritoneal dialysis patients is unknown.

Funding: Pharmaceutical Company Support

FR-PO1871

The Spectrin-Based Cytoskeleton Organizes Differential Functional Domains along the Mouse Nephron Gilbert W. Moeckel, Michael C. Stankewich. *Pathology, Yale University School of Medicine, New Haven, CT.*

Background: The spectrin-based cytoskeleton lines the intracellular side of membranes of many cell types, forming a molecular scaffold that links the actin cytoskeleton to many membrane-spanning proteins either directly or through its partner, ankyrin. Seven spectrin genes and three ankyrin genes (spectrin αI/αII; βI-βV and ankyrins I (R), II (B) & III (G) are known in vertebrates.

Methods: Their expression profiles were investigated using RT-PCR/ q-PCR/Western analysis/immuo-fluorecence and electron microscopy in the mouse kidney.

Results: mRNA for seven of the ten-spectrin/ankyrin genes is found (all except for αI, βIV and βV spectrin). αII/βII are the most abundant spectrin transcripts and Ank3 (G) is the most abundant ankyrin. βII spectrin is predominantly expressed together with αII spectrin in the proximal tubules at the basolateral membrane and in coated vesicles near the lateral and apical membrane, while βIII spectrin is expressed in distal segments of the kidney. AnkyrinB is predominantly found in the thick loop of Henle and the distal convoluted tubule, while AnkyrinG reactivity is observed in most renal tubules. In the glomerulus, αII/βII/βIII spectrins and ankyrinB are found in podocytes and capillary endothelial cells.

Conclusions: Together, the full repertoire of spectrin and ankyrin genes provide a membrane scaffold for apical and basolateral surfaces and for vesicular structures in both the proximal and distal renal tubules. We propose that the distinct distribution of spectrin and ankyrin proteins to intracellular, lateral or apical membrane domains suggest their involvement in the cellular location and properties of receptors, ion channels and transporters in kidney cells.

Funding: NIDDK Support

FR-PO1872

Mouse Renal Fibrosis after Unilateral Ureteral Obstruction Is Reduced by Klotho Protein Overexpression through Wnt Signaling Inhibition Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Department of Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Wnt proteins play important roles in regulating cell differentiation, proliferation, and polarity. Increased Wnt signaling is implicated in many fibrotic diseases including obstructive nephropathy. Blockade of Wnt/β-catenin signaling may offer a novel therapeutic approach to renal fibrosis. Klotho, characterized as an anti-aging gene, is reported to function as a secreted Wnt antagonist. The Wnt-klotho interaction results in suppression of Wnt biological activity. Here we explored the possibility that klotho protein could improve renal fibrosis by inhibition of Wnt signaling.

Methods: Unilateral ureteral obstruction (UUO) performed in transgenic mice overexpressing the alpha-klotho gene under control of human elongation factor 1 alpha promoter (KLTG; C57BL/6 background) and in C57BL/6 mice (WT) was studied after 14 days. In some WT, plasmid encoding mouse klotho (pCAGGS-KL) was transferred into skeletal muscle by electroporation. Wnt/β-catenin signaling activation was examined by crossing with BAT-LacZ mice overexpressing the β-galactosidase gene under control of seven repeat T cell factor/lymphoid enhancer-binding factor 1 promoter.

Results: In WT, UUO led to increased extracellular matrix deposition and tubulointerstitial fibrosis, and significantly decreased wet weight of the obstructed kidney compared with sham-operated kidney. In KLTG and pCAGGS-KL, however, there was markedly less extracellular matrix deposition compared to WT mice. At 7 days post-UUO, Wnt/β-catenin signaling activation was activated in the tubulointerstitium in WT but less so in KLTG. Expression of Wnt/β-catenin target genes was increased in WT and reduced in KLTG and pCAGGS-KL. Expression of connective tissue growth factor, transforming growth factor-β1, and type III collagen post-UUO was decreased in KLTG and pCAGGS-KL compared to WT. KLTG also showed less progression of epithelial to mesenchymal transition compared to WT post-UUO, as evidenced by reduced amounts of alpha-smooth muscle actin.

Conclusions: Klotho protein is a critical negative regulator of Wnt signaling and plays negative regulating roles in renal fibrosis after UUO.

Funding: Government Support - Non-U.S.

FR-PO1873

Tubulointerstitial De Novo Expression of the α8 Integrin Chain Attenuates Tubulointerstitial Fibrosis after Unilateral Ureteral Obstruction (UUO) Andrea Hartner,[1] Nada Cordasic,[2] Carlos Menendez-Castro,[1] Bernd Klanke,[2] Wolfgang Rascher,[1] Karl F. Hilgers.[2] *[1]Department for Pediatrics and Adolescent Medicine, University Hospital of Erlangen, Erlangen, Germany; [2]Department of Nephrology and Hypertension, University Hospital of Erlangen, Erlangen, Germany.*

Background: In the normal kidney, the α8 integrin chain is expressed only on mesangial and vascular smooth muscle cells. α8 integrin ligates several matrix molecules including fibronectin (FN), osteopontin (OPN) and fibrillin-1 (FB1). Recently, we detected de novo expression of α8 integrin on epithelial cells in the wall of renal cysts. We hypothesized that the α8 integrin chain is induced in dedifferentiating tubular epithelia and that binding to its ligands adds to the fibrotic response in the tubulointerstitium after UUO.

Methods: Rats underwent right UUO or sham operation for 4, 7 or 14 days. mRNA expression of α8 integrin and its ligands was evaluated by real-time PCR. The localization of α8 integrin and its ligands was determined by immunoflourescence. UUO was also induced in α8 integrin-deficient mice. Blood pressure and renal function was measured and tubulointerstitial (TI) damage was quantified in renal sections.

Results: The α8 integrin chain was induced 10fold after 7 days of UUO and an induction of the ligands FN (4fold), OPN (20fold) and FB1 (70fold) was observed. α8 integrin was localized to some cytokeratin-positive, but also to vimentin-positive dedifferentiating epithelial cells and to TI fibroblasts; and was detected adjacent to FN, OPN and FB1 positive extracellular matrix. Lack of α8 integrin led to more severe TI damage after UUO compared to controls. Blood pressure, albumin excretion and creatinine clearance, however, were not different in α8 integrin-deficient and wildtype mice after 7 days of UUO.

Conclusions: We conclude that the expression of the α8 integrin chain and its ligands is strongly induced in UUO. Moreover, a colocalization of α8 integrin with its ligands in the tubulointerstitium was observed. Mice lacking the α8 integrin chain are more susceptible to TI damage than wildtypes. Thus, interactions of α8 integrin with its ligands seem to be relevant for the development or progression of TI fibrosis in UUO.

Funding: Government Support - Non-U.S.

FR-PO1874

Role of Heme Oxygenase-1 in Mediating Epithelial-to-Mesenchymal Transition of Peritoneal Mesothelium Jongho Shin,[1] Jinuk Jeong,[1] Kitae Bang,[1] Jo Seong-Min,[1] Chang Nam Kim.[2] *[1]Department of Internal Medicine, Eulji University of Medicine, Daejeon, Korea; [2]Department of Surgery, Eulji University of Medicine, Daejeon, Korea.*

Background: Epithelial-to-mesenchymal transition (EMT) or mesothelial-to-mesenchymal transition of peritoneal mesothelial cells has been regarded as an early mechanism of peritoneal fibrosis. The effects of HO-1 expression on Epithelial-to-mesenchymal transition , which plays a critical role in the development of peritoneal membrane fibrosis, are unknown and its roles in peritoneal fibrosis has not been studied, yet.

Methods: We treated the human peritoneal mesothelial cells(HPMCs) with high glucose solution, HO-1 inducer(hemin, 10μmol/l) and HO-1 antagonist(SnPPIX, 10 μmol/l). To further investigate the pure effect of HO-1 on EMT of mesothelium, Gene transfer of recombinant Adenovirus-harboring human HO-1(Adv-HO-1 Gene) to HPMCs was done.

Results: Exposure of HPMCs to HG solution (30, 60, and 120 mM D-glucose) for 2 to 7 d increased the expression of mesenchymal markers such as α-smooth muscle actin(α-SMA) and vimentin, associated with an decrease in the expression of epithelial markers, E-cadherin. HO-1 protein expression was decreased in the same situation. Treatment of HPMCs with HO-1 inducer, hemin (2.5, 5, 10, and 20 mM) showed a dosage-dependent amelioration of HG induced changes in markers of EMT with increase of expression of HO-1. Treatment of HPMCs with HO-1 antagonist(SnPPIX, 10 μmol/l) simultaneously with HG or after the treatment of HO-1 inducer reversed the effect of HO-1 inducer. Adenovirus-harboring human HO-1 (Adv- HO-1) gene transfer resulted in a significant increase in HO-1 expression and ameliorated HG-induced changes in expression of E-cadherin, α-SMA, vimentin. Consistent with the protein data, HO-1 treatment resulted in an amelioration of HG-induced changes in *mRNA* of those markers.

Conclusions: Taken together, our results suggest that HO-1 has a critical role in the modulation of peritoneal fibrosis, and, more important, the suppression of EMT. Modulation of EMT, a major contributor to peritoneal fibrosis, through the HO-1 pathway may provide a means for novel therapeutic interventions aimed at progressive peritoneal diseases.

Funding: Private Foundation Support

FR-PO1875

Altered Podocyte Integrins and Cytoskeleton in *Col4a3*$^{-/-}$ Alport Mice Brooke M. Steenhard,[1] Kathryn S. Isom,[1] Larysa Stroganova,[1] Adrian T. Zelenchuk,[1] Patricia St. John,[1] Billy G. Hudson,[2] Roberto M. Vanacore,[2] Dale R. Abrahamson.[1] *[1]Anatomy and Cell Biology and the Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Medicine, Vanderbilt University Medical Center, Nashville, TN.*

Background: The Alport mouse harbors a deletion of the *Col4a3* gene, resulting in an absence of the α3(IV) α4(IV) α5(IV) collagen heterotrimer in the mature glomerular basement membrane (GBM), recapitulating many aspects of human Alport disease. Glomeruli in *Col4a3* mutants retain the infantile collagen IV network of α1(IV) and α2(IV) chains, progressively deteriorate, and kidneys become massively proteinuric. Here, we studied how glomeruli of Alport mice respond to the incorrect GBM collagen IV network.

Methods: Glomeruli from Alport and wildtype littermates were purified, protein digests from each were fluorescently labeled with spectrally distinct dyes (Cy3 and Cy5), combined, and separated by two dimensional gel electrophoresis (DIGE) (n=3). Differentially expressed peptides ($p<0.05$) were robotically picked for MALDI-TOF mass spectroscopy for protein identification. We also designed PCR primers to assess mRNA changes, and carried out quantitative immunofluorescence microscopy.

Results: Among the most significantly upregulated proteins identified in Alport glomeruli by DIGE, qPCR, and quantitative immunfluorescence were the intermediate filament protein vimentin and the actin-associated protein tropomyosin. Overexpression of both occurred specifically in Alport podocytes. Reasoning that an Alport GBM lacking the α3(IV), α4(IV), α5(IV) collagen IV network affected the podocyte cytoskeleton through

integrins, we quantified integrin mRNA and protein abundance. Significant mRNA increases were seen for integrin α3 and integrin β1. In contrast, glomerular immunofluorescence signals for both integrin α1 and integrin α3 were significantly upregulated in Alport podocytes whereas integrin α2 and integrin β1 were unchanged.

Conclusions: We conclude that the Alport GBM alters the normal expression and distribution pattern of podocyte integrins, which in turn may lead to cytoskeletal reorganization and other signaling events important for pathogenesis of the disease.

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FR-PO1876

Prevention of Cyclosporin A-Induced Fibronectin Synthesis by Simvastatin in Rat Mesangial Cells Chong Myung Kang, Joon-Sung Park, Chang Hwa Lee, Gheun-Ho Kim. *Internal Medicine, Hanyang University Hospital, Seoul, Korea.*

Background: Cyclosporin A(CsA) is an effective immunosuppressant for prevention of rejection after kidney transplantation. CsA has nephrotoxicity leading to chronic allograft nephropathy and renal fibrosis. Simvastatin improves hypercholesterolemia after renal transplantation and preserves structures and functions of glomeruli. But, it is not well known how simvastatin acts on CsA induced fibronectin synthesis. We studied to investigate the effect of simvastatin(simva) on Smad pathway, which is the key pathway of CsA induced fibronectin synthesis in rat mesangial cells(RMCs).

Methods: Transforming growth factor-β1 (TGF-β1) was induced by CsA cultured RMCs. Optimal concentration and time of CsA were measured according to its concentration (0.1 ~ 10 μM) and TGF-β1 expression time (1~72 h) measured by ELISA. All RMCs were divided into six experimental goups: control, CsA (1 μM) alone, simva (1 μM) alone, simva 0.1 μM pretreatment plus CsA 1 μM, simva 1 μM pretreatment plus CsA 1 μM, and simva 10 μM pretreatment plus CsA 1 μM. The level of cytokines such as phosphorylated Smad3 (phospho-Smad3), Smad7 and fibronectin proteins were measured by semiquantitative immunoblotting.

Results: TGF-β1 expression was maximally increased at 1 μM dose of CsA, 24h after CsA treatment. After treatment with CsA alone, the level of TGF-β1 expression significantly increased (3.995 ± 1.272-fold, p=0.0035). Phospho-Smad3 expression and fibronectin production significantly increased after CsA treatment (2.900 ± 0.053-fold, p=0.006, 1.812 ± 0.223-fold, p=0.0085). When all the groups treated with CsA and simva were compared with those treated with CsA alone, phospho-Smad3 expression (p = 0.0010, 0.0001, 0.0003) and fibronectin production (p = 0.0064, 0.0005, 0.0002) significantly decreased. But, in simva 10 μM pretreatment CsA 1 μM group, the level of Smad7 expression was significantly increased compared with control (p=0.0316).

Conclusions: This study demonstrates that simvastatin may ameliorate CsA-induced fibronectin synthesis by modulating the TGF-β1/Smad pathway in RMCs. These results suggest that simvastatin may inhibit the development of nephrosclerosis and subsequent chronic CsA nephropathy.

FR-PO1877

Parathyroid Hormone Induces Endothelial-to-Mesenchymal Transition in Human Aortic Endothelial Cells Min Wu, Rining Tang, Linli Lv, Kun Ling Ma, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University Medical School, Nanjing, Jiangsu Province, China.*

Background: Cardiovascular disease (CVD) is the leading cause of death among patients with end-stage renal disease (ESRD). Recent studies have suggested that highly secreted parathyroid hormone (PTH) might be involved in the development of uremic vascular impairment. However, the underline mechanism has not been well understood. Our previous study have showed that endothelial-mesenchymal transition (EndMT) involves in the cardiac fibrosis. In this study, we addressed the question of whether PTH as an inducer of EndMT, and subsequently contributes to the vascular sclerosis in ESRD.

Methods: Primary human aortic endothelial cells (HAECs) between passage 3 to 5 were stimulated with 10^{-11} to 10^{-7} mol/L PTH. Pathological changes were examined by confocal microscopy for co-expression of endothelium marker (CD31) and fibroblast markers (FSP1 and α-SMA). The mRNA and protein expressions of CD31, FSP1 and α-SMA were detected by real-time PCR and western blot. Cellular ultrastructure was observed with electron microscopy.

Results: CD31 has been found to be expressed in HAECs and its mRNA and protein levels were inhibited by increasing concentrations of PTH (P<0.05). Besides, the expression of FSP1 and α-SMA upon the treatment with PTH were significantly increased in dose-dependent manners compared with control (P<0.05). Double staining indicated that a co-expression of CD31 and FSP1 when HAECs were exposed to PTH, and some cells acquired spindle-shaped morphologies accompanying with a loss of CD31 staining. Ultrastrctural investigation showed HAECs incubated with PTH (10^{-7} mol/L) have an increasing roughed endoplasmic reticulum.

Conclusions: We firstly demonstrated that PTH could stimulate HAECs developing phenotypic changes as EndMT, which may provide a novel insight that secondary hyperparathyroidism being involved in the vascular sclerosis in CKD.

Funding: Government Support - Non-U.S.

FR-PO1878

Serum Galectin-3 and Leucocyte-Endothelial Adherence Are Increased in a Murine Model of Chronic Uremia Andrew Duncan Stewart Findlay, Julius Edward Kieswich, Magdi Yaqoob. *William Harvey Research Institute, Barts and the London, Queen Mary's School of Medicine and Dentistry, London, United Kingdom.*

Background: Galectin -3 is a galactoside binding protein with diverse roles including leucocyte adhesion with unknown roles in chronic uremia. Intravital microscopy visualises leucocyte-endothelial interactions in post-capillary venules *in vivo*.

We measured serum Galectin-3 levels in an adenine diet model of chronic uremia. We then established the feasibility of intravital microscopy in this model and described endothelial-leucocyte interactions.

Methods: Male c57bl6 wild-type mice were fed 0.25% adenine diet for 4 weeks after which intravital microscopy was performed.

The cremaster muscle was isolated and placed over a plexi-glass stage perfused with bicarbonate buffered saline. The muscle was placed under a microscope and transilluminated. After a 30 min stablisation period leucocyte rolling velocity, flux, adhesion and transmigration was recorded in 3-5 vessels per mouse for off-line analysis.

Serum Galectin-3 was subsequently measured by ELISA in sham and adenine diet uremic mice

Results: Serum Galectin-3 was significantly raised in uremic mice compared to sham mice (60.35ng/ml vs 34.23ng/ml, p=0.0303)

The uremic mice had significantly more leucocyte adherence to endothelium but equivalent levels of leucocyte flux, rolling velocity and transmigration.

Leucocyte Interactions with Endothelium Uremic (Adenine diet) vs Sham

	Adenine Diet (n=5)	Sham Diet (n=6)	p-value
Urea mmol/l	37.4	9.8	
Leucocyte Flux (per min)	12.4	13.2	p=0.9269
Leucocyte Velocity (μm/s)	42.3	65.6	p=0.4205
Leucocyte Adherence (per100μm)	9.7	4	p=0.0043
Leucocyte Transmigration(per 100x50μm ²)	1.2	0.6	p=0.0814

Values shown Mean

Conclusions: Serum Galectin -3 is raised in the adenine diet chronic uremic mouse model. The same model also demonstrated significantly more adherent leucocytes to endothelium. This could represent a mechanism of inflammatory uremic microvascular dysfunction. We intend to persue the significance of Galectin-3 on leucocyte-endothelial interactions in uremia with Galectin-3 knockout mice.

Our results demonstrate intravital microscopy is a feasible technique to gain novel insights into uremic microvascular dysfunction.

FR-PO1879

Continuous Rituximab Therapy for Antineutrophil Cytoplasmic Antibody-Associated Vasculitis John Niles, Karen A. Laliberte. *Renal, Massachusetts General Hospital, Boston, MA.*

Background: We identified 68 patients with AAV who were initiated on continuous B-cell depletion treatment with rituximab prior to 5/6/2009.

Patients: The average age was 56.5. 39 patients had PR3 and 33 patients had MPO. Patients were followed for a total of over 2448 patient months.

Treatment: Most patients received 2 or 4 doses of rituximab to start and then one dose every 4 months. Other medications were initially continued, but then tapered slowly with the goal of coming off all other immunosuppression.

Results: All patients entered complete remission except for the patient with acute airway obstruction. Medications were slowly weaned off in most patients. 6 patients had major relapses (BVAS-WG >/= 3). All were in the setting of weaning of their other medications. All were with positive ANCA titers. In only one, did the ANCA titer rise significantly with the flare. 5 patients developed late onset neutropenia. One developed bacteremia. IgG levels gradually fell in most patients, ultimately leading to discontinuation of therapy in 2 patients. 4 patients died. One patient died of disease related sudden airway obstruction (36 yo). One died of lung ca (71 yo), and one of aortic stenosis (90 yo). An additional patient died of acute disease related lung injury (90 yo), 21 months after stopping all therapy.

Conclusion: Continuous rituximab therapy was associated with a high rate of remission, ongoing disease control and facilitated the withdrawal/weaning of other immunosuppressive medications and was associated with a low rate of relapses and complications. Routine monitoring for late onset neutropenia, on maintenance rituximab dosing, should become a standard of care. More data is needed about the delayed adverse effects of rituximab.

We declare no potential conflicts of interest.

Funding: Private Foundation Support

FR-PO1880

Soluble fms-Like Tyrosine Kinase 1 (sFLT) Correlates with Proteinuria in ANCA-Associated Vasculitis and Declines during Immunosuppressive Treatment Svjetlana Lovric,[1] Torsten Kirsch,[1] Anna Bertram,[1] Hermann G. Haller,[1] Marion Haubitz.[2] *[1]Nephrology and Hypertension, Medical School Hannover, Hannover, Germany; [2]Division of Nephrology, Klinkum Fulda, Fulda, Germany.*

Background: Fms-like tyrosine kinase-1 (FLT1) is one of major receptors for the vascular endothelial growth factor (VEGF). Its soluble form (sFLT1) may act as decoy receptor for VEGF in the circulation thereby reducing VEGF bioavailability. Hence, increased levels of sFLT1 may cause inactivation of VEGF and therefore leads to endothelial dysfunction.

Elevated serum levels of sFLT1 have been found in sepsis and preeclampsia and correlated with disease severity. In ANCA-associated vasculitis higher VEGF levels have been reported in patients with major disease activity compared to those with minor activity. The present study was therefore intended to determine the role of sFLT1 serum level in ANCA-associated vasculitis.

Methods: Plasma sFLT1 levels were measured with a commercially available ELISA in patients with ANCA-associated vasculitis at initial diagnosis and after 1, 3, 6 and 12 months (n=38). Disease activity was assessed in accordance with the Birmingham Vasculitis Activity Score (BVAS). Proteinuria, BVAS, C-reactive protein, creatinine and ANCA titres were recorded at baseline and during follow-up.

Results: In patients with active ANCA- associated vasculitis median levels of sFLT1 are elevated (108 (96.5- 165.5) pg/mL, $p < 0.05$) compared to healthy controls (86 (71.88-100.5) pg/mL). sFLT1 correlates with proteinuria ($r=0.5$, $p <0.01$), disease activity (BVAS) ($r= 0.6$, $p<0.05$) and CRP levels levels ($r=0.6$, $p< 0.05$) in active vasculitis patients. Compared to those without activity median level of sFLT1 were increased ($p<0.05$) and declined during follow-up.

Conclusions: sFLT1 serum level correlate with proteinuria in severe ANCA-associated vasculitis. sFLT1 is elevated before treatment and declines during immunosuppressive therapy. We therefore conclude that sFLT1 may act as a robust marker for disease activity.

FR-PO1881

Long-Term Observation of Clinicopathological Characteristics and Outcome of Japanese Patients with Pauci-Immune Crescentic Glomerulonephritis Kiyoki Kitagawa,[#1] Yasuyuki Shinozaki,[#2] Tadashi Toyama,[#2] Shinji Kitajima,[#2] Akinori Hara,[#2] Kengo Furuichi,[#1] Shuichi Kaneko,[#2] Takashi Wada.[#3] *[1]Division of Blood Purification, Kanazawa University Hospital, Kanazawa, Japan; [2]Department of Disease Control and Homeostasis, Kanazawa University, Kanazawa, Japan; [3]Department of Laboratory Medicine, Kanazawa University, Kanazawa, Japan.*

Background: Clinicopathological characteristics and outcome of Japanese patients with pauci-immune crescentic glomerulonephriris (CreGN) are presumed to vary among periods. We examined characteristics and outcome of patients with CreGN according to the periods.

Methods: From 1968 to 2010, a total of 100 patients who were diagnosed as CreGN by renal biopsy in Kanazawa University Hospital and Kanazawa collaborative group was examined in this study. All cases were divided into group I (1968-1988, 18 cases, mean age 52.9±3.7, female 6, male 12), group II (1989-2001, 37 cases, mean age 66.4±2.5, female 21, male 16) and group III (2002-2010, 45 cases, mean age 67.5±1.5, female 24, male 21).

Results: Mean follow-up period was 1367±130 days. Neither blood pressure, the degree of hematuria, renal function nor titer of ANCA had statistical difference among three groups. On the other hand, the degree of proteinuria was reduced in group II and III compared with group I. The rate of total and fibrous crescentic formation was significantly lower in group III (total crescent : group I 82.9±3.8%, group II 60.6±4.7%, Group III 48.9±4.3%, fibrocellular and fibrous crescent : group I 46.9±4.0%, group II 34.8±3.5%, group III 22.8±3.7%, $p<0.05$). Furthermore, 1- and 5-year renal and patient survival rate improved significantly in group III compared with group I and II (One-year renal survival rate: group I 50%, group II 75%, group III 89%. 5-year renal survival rate: group I 22%, group II 56%, group III 75%. One-year patient survival rate: group I 67%, group II 81%, group III 95%. 5-year patient survival rate: group I 33%, group II 65%, group III 76%).

Conclusions: In conclusion, the patients with CreGN were diagnosed at early phase of crescentic formation and outcome was improved in recent years.

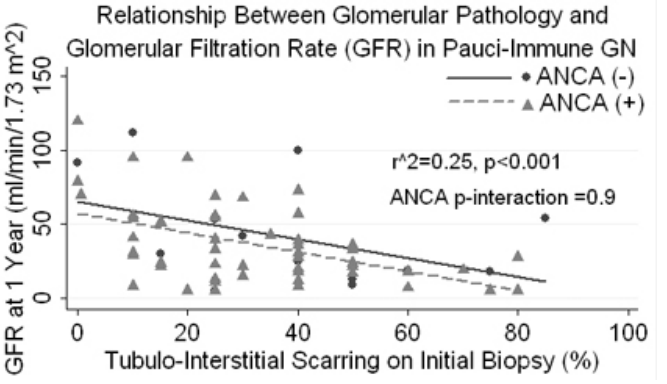
FR-PO1882

ANCA Negative Pauci-Immune Glomerulonephritis in a U.S. Cohort John Havill, Michael Kuperman, Abigail Thompson, Duvuru Geetha, Stephen M. Sozio. *Johns Hopkins Medical Institutions, Baltimore, MD.*

Background: ANCA antibodies are thought to play an important role in the pathogenesis of ANCA associated vasculitis. The lack of ANCA antibodies may indicate a variation in clinical presentation and outcomes of this disease.

Methods: We identified 78 patients from the Johns Hopkins Renal Pathology database between 1995 and 2008 with the diagnosis of pauci-immune glomerulonephritis (GN). Comparing ANCA negative and positive patients, we examined demographics, histological features at presentation, and subsequent treatment and correlated this with renal function at presentation and follow-up. Renal histology was reviewed by a pathologist blinded to clinical data.

Results: 60 patients had positive ANCA serology, and 18 were ANCA negative. At presentation, overall mean age was 54 years, 49% male, 77% Caucasian, and mean Birmingham Vasculitis Score of 8; these were similar between ANCA negative and positive patients. Overall mean eGFR at presentation was 32.5 ml/min/1.73 m^2 with 37% requiring acute hemodialysis; there was a trend toward more renal severity in the ANCA negative group which did not reach statistical significance ($p=0.07$ and 0.2 respectively). Long-term renal function was similar between ANCA negative and positive patients, with mean overall eGFR at one year 39.8 ml/min/1.73 m^2, $p=0.7$. Tubulo-interstitial scarring was a good negative predictor of eGFR at 1 year in both groups (Figure). There were similar relationships between predictors of eGFR at 1 year between ANCA negative and positive patients.



Conclusions: Ours is the first USA based study to compare patients with ANCA negative and positive serology, and we showed these two groups had similar demographics, presentation, and outcomes in our 13 year experience. Percentage of tubulo-interstitial scarring was a strong negative, graded predictor of renal outcomes at 1 year for both groups.

FR-PO1883

Persistent or New Onset Microscopic Hematuria in Vasculitis Patients in Remission: Findings on Renal Biopsy Duvuru Geetha, John Havill, Michael Kuperman. *Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: Hematuria is considered a sign of active disease in patients with small vessel vasculitis requiring aggressive immunosuppressive therapy. In patients who are in apparent clinical remission, the presence of persistent or new onset microscopic hematuria after completion of induction therapy and years after completion of induction and maintenance therapy may be due to active renal vasculitis, chronic and healed vasculitis or other glomerular pathology

Methods: We identified 78 patients from the Johns Hopkins Renal Pathology database between 1995 and 2008 with the diagnosis of pauci-immune glomerulonephritis (GN). Among these 78 patients, we indentified patients who were in apparent clinical remission and underwent a renal biopsy for evaluation of persistent or new onset microscopic hematuria. Renal histology was reviewed by a pathologist blinded to clinical data.

Results: 8 patients with small vessel vasculitis, 7 ANCA positive and 1 ANCA negative underwent a renal biopsy at variable time periods after remission of vasculitis (6 months to 14 years) for persistent microscopic hematuria (n=5) or new onset microscopic hematuria (n=3). All patients were in apparent clinical remission at the time of renal biopsy. Of the three patients presenting with new onset hematuria, two patients had crescentic IgA nephropathy and one patient had healed crescentic pauci-immune glomerulonephritis with fibrous crescents. Of the five patients with persistent hematuria, two patients had arteriosclerosis with no evidence of active vasculitis, two had focal segmental glomerulosclerosis and no signs of active vasculitis and one had global and segmental glomerulosclerosis.

Conclusions: Microscopic hematuria in patients with renal vasculitis otherwise in remission could represent chronic glomerular injury from prior episode of vasculitis or may represent new glomerular pathology. We conclude that patients with small vessel vasculitis who are in apparent clinical remission presenting with persistent or new onset microscopic hematuria should undergo renal biopsy prior to escalation of immunosuppressive therapy.

FR-PO1884

Long-Term Outcome of Severe Alveolar Haemorrhage in Small Vessel Vasculitis: A Retrospective Cohort Study Alina L. Casian,[2] Zdenka Hruskova,[1] Pavel Konopásek,[1] Vladimir Tesar,[1] David R.W. Jayne.[2] *[1]Dpt of Nephrology, First Faculty of Medicine, Charles University, Prague, Czech Republic; [2]Vasculitis and Lupus Clinic, Addenbrooke's Hospital, Cambridge, United Kingdom.*

Background: Alveolar haemorrhage (AH) is an important manifestation of small vessel vasculitis (SVV) and the most common vasculitic cause of early death. We describe the characteristics and long-term outcome of severe AH due to SVV from two European centres.

Methods: A retrospective analysis of hospital notes on 867 patients with SVV presenting between 1990 and 2011. AH was defined as the presence of bilateral alveolar infiltrates on radiological imaging without an alternative explanation plus at least one of

the following: haemoptysis, increased CO diffusing capacity, bronchoscopic evidence of haemorrhage, or an unexplained drop in haemoglobin >2g/dL. AH was considered severe if either hypoxia or the need for blood transfusions were recorded.

Results: 59 patients (M/F 36/23; median age 58 [18-81] years) were identified. Most (89.8%) were diagnosed with ANCA-associated vasculitis, six with anti-GBM disease. PR3-ANCA was positive in 38 (64.4%), MPO-ANCA in 15 (25.4%, p<0.05 for MPO vs PR3-ANCA), and anti-GBM in 9 patients (3 were also ANCA-positive). AH was the first disease manifestation in 54/59 (91.5%). Invasive assisted ventilation was required in 24 patients (40.7%). Renal involvement was present in 57 (96.6%), 33 (55.9%) required dialysis. Forty-six (78.0%) were treated with plasma exchange (PE). At 6 months, 50/59 (84.7%) were alive, six died of active refractory vasculitis and 3 of infection. The mean follow-up was 50 months when 37 (62.7%) were alive. Mortality was higher in those requiring dialysis at entry (48.5 vs. 23.1%, p<0.05). No significant difference in mortality was found between PE and no-PE (39.1 vs. 30.8%, p=0.3).

Conclusions: Severe AH was more commonly associated with PR3- than MPO-ANCA or anti-GBM and strongly correlated with renal vasculitis. Current treatment of severe AH with combined immunosuppression (with or without PE) helps to overcome the acute period in most patients but long-term mortality remains high. Concurrent renal failure was associated with worse outcome. *Supported by ERA-EDTA Fellowship.*

Funding: Private Foundation Support

FR-PO1885

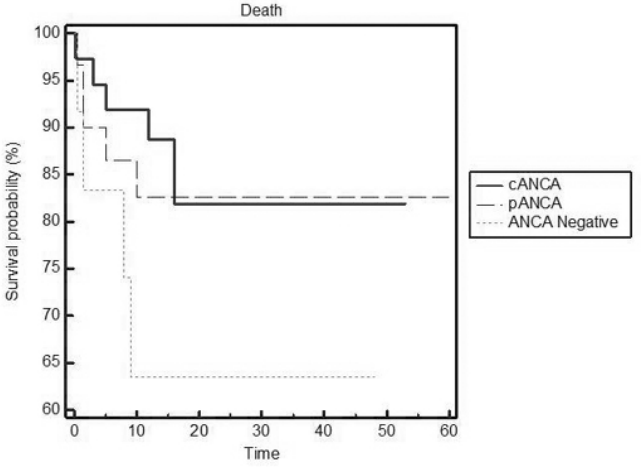
Outcomes of Renal Vasculitis in Preston, UK: 2005-2010 Cheralathan Arunachalam, Olumide Ogundare, Harish B. Shetty. *Department of Renal Medicine, Royal Preston Hospital, Lancashire Teaching Hospitals, Preston, United Kingdom.*

Background: We report outcomes of renal vasculitis in a group of patients treated with standard induction therapy containing pulse cyclophosphamide plus prednisolone followed by Azathioprine and prednisolone for maintenance treatment

Methods: All new patients diagnosed with renal vasculitis at our centre from 2005 until 2010 were evaluated from presentation until last visit or death

Results: 79 patients received standard treatment. Median age was 63 ± 14 years. 36 (45%) patients were PR3- ANCA positive, 31(40%) MPO-ANCA positive and 12 (15%) were ANCA negative. Presenting Creatinine(Cr) was 448±392 mmol/l and mean follow up period was 26 ±13 months. All patients started on pulse intravenous (i.v) cyclophosphamide dose as per the Cyclops protocol. 30% patients received oral pulses later during induction while the rest continued with iv pulse. Patients with presenting serum Cr >500 and or pulmonary haemorrhage received seven plasma exchanges in addition. 15% patients had pulmonary haemorrhage and 39% received plasma exchange.

28 (35%) patients needed renal replacement therapy (RRT) at diagnosis. Of these 28 patients, 10 died, 12 recovered renal function and 6 patients were RRT dependent. Overall 8 (10%) patients reached end stage renal disease (ESRD) and 15 (19%) died. At 2 years, 85% of survivors were dialysis independent. In the ANCA positive group, 1 year and 5 year survival rates were 88% and 82% respectively.



PR3 or MPO ANCA did not influence the major outcomes. ANCA negative patients had a higher mortality rate at 25%. 12% patients developed life threatening infections. We noted a low relapse rate at 13 % and less cumulative cyclophosphamide dose, 5414±2642gm.

Conclusions: In renal vasculitis with less cumulative dose of cyclophosphamide we report survival rates comparable to other treatment regimes and a low relapse rate.

FR-PO1886

Increased Incidence of Venous Thromboembolism in ANCA Associated Vasculitis Sehrash Noor, Issaam Oozeerally, Olumide Ogundare, Ajay Prabhakar Dhaygude. *Renal Medicine, Royal Preston Hospital, Preston, United Kingdom.*

Background: ANCA associated systemic vasculitis (AASV) is a heterogeneous chronic inflammatory relapsing autoimmune disease. Dutch and American studies have suggested that there is an increased incidence (1.8 to 7 per 100 person-years) of venous thromboembolism (VTE) in AASV. Recently it has been shown that there is an increased prevelance of anti-plasminogen antibodies and also anti tissue plasminogen activator antibodies in AASV patients and these antibodies were associated with increased severity of renal involvement .In this retrospective study we analysed incidence of VTE in 165 British patients with AASV and compared it with incidence of VTE in the general population.

Methods: Retrospective analysis of case records of 165 patients with AASV was performed to identify patients with evidence of VTE (deep vein thrombosis and/ or pulmonary embolism).

Results: One hundred sixty-five patients were followed for 3.35 person-years. Seventeen patients experienced 19 events of VTE; three of these events predated the diagnosis of AASV by at least 12 months and were consequently excluded. The overall incidence of VTE in AASV was 2.9 events per 100 person years. Nine patients had active vasculitis and one patient was in partial remission at the time of diagnosis of VTE. VTE occurred between 0 months to 95 months from the diagnosis or relapse of AASV but 10 out of 16 VTE occurred within 6 months of active disease. Age at the time of diagnosis of vasculitis was slightly higher (65.8 years) in patients with VTE; compared with non VTE patients (59.9years). Nine patients with VTE were PR-3 positive.

Conclusions: This study has confirmed previous published findings of increased incidence of VTE in AASV in British population. In general population incidence of VTE is 0.05 per 100 person years but the risk increases with age to 0.2 per 100 person years for age group of 70-79 years. Increased incidence is likely to be multifactorial, related to inflammation, endothelial dysfunction, pro-coagulant state, presence of anti plasminogen antibodies in AASV and chemotherapy. This study has strengthened the association between AASV and VTE and may help the clinicians treating AASV.

FR-PO1887

Serum Polyclonal Free Light Chain Levels in Patients with Vasculitis Lakhvir Assi,[1] Andrew McClean,[2] Gemma Webb,[1] Lorraine Harper,[2] Colin A. Hutchison.[2] *[1]The Binding Site Group Ltd, United Kingdom; [2]Renal Institute of Birmingham, United Kingdom.*

Background: Serum concentrations of polyclonal free light chains (FLCs) have been shown to be elevated in autoimmune conditions (Sjogrens syndrome and systemic lupus erythematosus). In these diseases, FLCs correlated with disease activity and clinical outcomes. The purpose of this study was to evaluate FLCs in patients with vasculitis and to determine if their levels correlated with established markers of disease activity.

Methods: 42 patients were assessed; their average age was 55years and median GFR was 36ml/min/1.73m2. 38% were female and 90% were white. The following laboratory assessments were made at presentation: FLCs (Freelite™ assay, normal range: κ: 3.3-19.4mg/L, λ: 5.71-26.3mg/L), high sensitivity CRP (hsCRP), cystatin C (cys C) and immunoglobulins (Igs). One month follow up (FU) samples were also analysed (N=38). Patients predominately received induction therapy consisting of steroids and cyclophosphamide.

Results: Total FLCs (TFLCs) were elevated in the cohort (median: 39.2mg/L, range 3.75-343.5mg/L).

Disease	N=42	TFLCS (mg/L) median	TFLCs/cys C median
Wegeners granulomatosus	23	36.9	18.8
Churg strauss	2	49.4	39.3
ANCA associated vasculitis	2	72.6	25.4
ANCA -ve vasculitis	2	33.7	20.1
Microscopic polyangitis	10	71.9	32.4
Other	3	51.9	41.9

To determine rates of FLC production, TFLCs were corrected for renal function using cys C (TFLCs/cys C median: 19.7mg/L, range: 4.6-94.6mg/L). There was a weak association between TFLCs and total Igs (GAM): Spearman r=0.32, p=0.04; significance increased when TFLCs/cys C were correlated with Igs (r=0.72, p<0.001). TFLCs also correlated with CRP (r=0.47, p=0.002) although there was no association with autoantibodies (MPO/PR3) (r=0.09, p=0.59). TFLCs increased with decreasing GFR (p<0.03) and increasing age (p=0.01). TFLCs were higher at baseline: 39.21mg/L vs 26.3mg/ml (p=0.007). When analysing patient outcome, there was no difference between TFLCs in patients admitted to hospital over 12 months (p=0.39), nor was there a difference in patients who had suffered an infection (p=0.88).

Conclusions: Further FU samples will be analysed to further elucidate the role of TFLCs in vasculitis.

Funding: Pharmaceutical Company Support

FR-PO1888

Clinical Outcomes of Japanese Myeloperoxidase (MPO) Anti-Neutrophil Cytoplasmic Antibody (ANCA) Related Nephritis: Significance of Initial Renal Death for Survival Kimio Watanabe, Yoshihiro Tani, Kenichi Tanaka, Yoshimitsu Hayashi, Koichi Asahi, Masaaki Nakayama, Tsuyoshi Watanabe. *Nephrology and Hypertension, Fukushima Medical University School of Medicine, Fukushima, Japan.*

Background: MPO-ANCA related vasculitis constitutes 60% of the rapidly progressive glomerulonephritis that occurs among Japanese. The reported 1-year survival rate of such patients is >80%, but the long-term prognosis remains unknown. Therefore, we investigated the prognosis and the clinical factors affecting the survival of patients with MPO-ANCA related nephritis.

Methods: We retrospectively investigated 44 patients (female, n = 26; mean age, 70.2 ±11.4 y) who were diagnosed with MPO-ANCA related nephritis between 2000 and 2010 at our hospital.

Results: The serum creatinine value was 3.7 ± 2.6 mg/dL, the MPO-ANCA titer was 568 ± 731 EU and the Birmingham Vasculitis Activity Score (BVAS) was 17.1 ± 4.4. At the initial therapy, 38 (86.4%) patients achieved remission (non-renal death group: NRD), whereas the remaining 6 (13.6%) did not and required permanent dialysis therapy (renal death group: RD). After 8 weeks of initial prednisolone therapy, six patients relapsed and four developed kidney failure progression in the NRD group, whereas two patients relapsed in the RD group. Eleven (25.0%; NRD, n = 5; RD, n = 6) patients died during a mean observation period of 36.6 ± 33.7 months due to infection (n = 4), recurrent vasculitis (n = 4), cancer (n = 1) and other causes. The 1-and 2-year survival rates were 100.0% and 96.3%, respectively, in the NRD group and 33.3% and 0.0%, respectively, in the RD group. Multiple logistic analysis revealed that renal death at the initial phase was the only significant risk factor for all-cause death (OR, 29.0; 2.4 - 351.8), BVAS at the initial visit for the relapse (OR, 1.52; 1.09 - 2.14) and BVAS at week 4 for death (OR, 2.33; 1.02 - 5.37).

Conclusions: Renal death at the initial phase was a powerful risk factor for all-cause death in patients with MPO-ANCA related nephritis. Patients at high risk of recurrence and death could be stratified according to BVAS during initial therapy.

FR-PO1889

Outcomes of Two Different Cyclophosphamide Regimes for Treatment of ANCA Associated Vasculitis Issaam Oozeerally,[1] Ajay Prabhakar Dhaygude,[1] Edmond O'Riordan.[2] *[1]Renal Medicine, Royal Preston Hospital, Preston, United Kingdom; [2]Renal Medicine, Salford Royal Hospital, Salford, United Kingdom.*

Background: Cyclophosphamide (Cyp) treatment has transformed ANCA associated systemic vasculitis (AASV) from a potentially fatal condition to a chronic remitting, relapsing disease though it is associated with significant mortality and morbidity. Recently published meta-analysis of outcome of 535 AASV patients from four different clinical trials suggested that 48% of the mortality was due to infections in the first year and 19% mortality due to active vasculitis. RAVE and RITUXIVAS trials showed that Cyp. therapy is as effective as Rituximab therapy in majority of the patients. Pulsed arm of CYCLOPS trial protocol recommends 10 pulses of cyp given over 6 months, initially at 2 weekly and subsequently 3weekly intervals. Lupus nephritis regimes consists of 6 pulses given at monthly intervals. In North-west of England, clinicians have used these two regimes for the treatment of AASV and in this retrospective study we analysed the outcomes of these two different regimes.

Methods: Data was collected for 103 AASV patients treated with pulse cyp, regarding cumulative cyp dose, rate of major infections, rate of relapses and renal outcomes. All the patients had at least 2 years follow up.

Results: Seventy-nine patients were treated with CYCLOPS protocol and 24 patients were treated with monthly (lupus nephritis) protocol. Cumulative cyp dose was high in CYCLOPS regime (7.14 grams) compared to monthly regime (4.63 grams). Outcomes are presented in Table-1

Table-1:Outcomes of the two different regimes

Outcomes	**Monthly Cyp 24 patients**	**CYCLOPS Cyp 79 patients**	**p Value**
Infections	7 (29.3%)	29 (36.7)	0.3
Relapses	4 (16.7%)	28 (35.4%)	0.04
Reduction in Creatinine	21.4%	46.5%	0.0001

Conclusions: These results suggests that- CYCLOPS regimes is associated with-

1] Higher cumulative dose

2] Significant improvement in renal function

3] Increased rate of infections though statistically not significant.

Limitations:

1]Retrospective study.

2] Small sample size for monthly regime patients which may explain the lower rate of relapse in this group in spite of low cumulative dose of Cyp.

FR-PO1890

Outcome of Anti GBM Disease: A Single Center Review Arvind Ponnusamy, Edmond O'Riordan. *Renal, Salford Royal NHS Trust Foundation, United Kingdom.*

Background: The incidence of anti-GBM disease is reported to be 0.5 per million per annum. It is associated with significant morbidity and mortality.

Methods: We retrospectively reviewed the case notes of all the patients(n= 19) who were diagnosed with anti-GBM disease over a period of 10 years (1999 – 2009). Creatinine and presence of 100% crescents at presentation were co-related to outcome (End stage Renal Disease and Death)

Results: The average age at diagnosis was 61 with bimodal peaks at decade 30-40 and those over 60 years of age. Male to female ratio was 10:9.18 out of the 19 had a serum creatinine above 500 umol/l (5.6 mg/dl). 7 (36.84%) of them were dialysis dependent at presentation. Only 2 had clinical and radiological evidence of pulmonary haemorrhage. 4 (22.2%) patients were 'double positive' for pANCA (MPO) & anti GBM. Renal biopsy was performed in 14 patients (73.7%) 7 of those had crescentic glomerulonephritis in 100% of glomeruli seen. At 6 months 15 patients (78.9%) were dialysis dependent, 4 patients were dialysis independent with a mean creatinine of 265umol/l (3 mg/dl)+[/underline]117 (1.3). At last follow up mortality was 52.63%. Causes for death include pulmonary haemorrhage, sepsis, and cardiac related. The mortality figures were better for patients who were double positive for anti GBM and pANCA compared to patients who had positive anti GBM and negative ANCA (25% vs 60%)

Outcome according to serum creatinine and crescents at presentation:

Serum creatinine at presentation	Number of patients	Renal survival at 6 months (%)	Patient survival at last follow up
<500 umol/l (5.6mg/dl)	1	1 (100)	1 (100)
>500 umol/l (5.6mg/dl)	18	3 (16.67)	8 (44.44)

Renal biopsy	Number of patients	Renal survival at 6 months (%)	Patient survival at last follow up (%)
less than 100%	7	2 (28.57)	4 (57.14)
Cresentic GN (100%)	7	0 (0)	4 (57.14)

Conclusions: Our data confirms previous studies in which creatinine more than 500umol/l and presence of 100% crescent were associated high mortality and poor renal survival. Patients with double-positive (ANCA and anti GBM) appear to have better patient survival rate although the renal survival appear to be same in both between the double positive group and anti GBM group. Only 16% of our patients with creatinine >500 had renal survival at 6 months underlining the potential risk-benefit balance of aggressive therapy in this group

FR-PO1891

Anti-Glomerular Basement Membrane Disease; a Review of the Experience in an Irish Centre over 21 Years Yvonne C. Ryan,[1] Thomas J. McEnery,[1] Mary T. Keogan,[1] Peter J. Conlon,[1] Anthony M. Dorman,[1] Mark Donald Denton.[1] *[1]Nephrology, Beaumont Hospital, Dublin, Ireland; [2]Histopathology, Beaumont Hospital, Dublin, Ireland.*

Background: We present a series of 64 patients with anti-GBM disease managed at Beaumont Hospital in Dublin, Ireland.

Methods: The biopsy records from January 1st 1990 to April 1st 2011 were reviewed and patients with biopsy proven anti-GBM disease identified. Patients with anti-GBM who did not get a renal biopsy were identified using a database of immunology sera. We conducted a chart review to ascertain demographic data, presenting features, anti-GBM titre, treatment, renal replacement therapy and outcome. Statistical package used was Stata (version 10, College Station, Texas).

Results: 56% of patients were female. The mean age of males and females was 46 and 54 years respectively. 17% had concurrently positive ANCA at presentation. 22% had lung involvement. The mean anti-GBM titre was 265 iu/ml, ref <10iu/ml (range 0-1000) and 26% patients were anuric at presentation. 62/64 patients had a renal biopsy. Mean crescents on biopsy 80% (SD 27), mean percentage fibrosis was 52% (SD 30). Three patients (4.7%) died during the initial admission.

Patient survival was 78%, 67%, 60% and 46% at 1, 5, 10 and 15 years respectively. Age at presentation HR 1.09 (CI 1.04-1.44) and log serum creatinine at presentation HR 5.59 (CI 1.81-17.25) were significantly associated with survival. The renal survival was 26%, 21%, 16% and 16% at 1, 5, 10 and 15 years respectively. Four patients had recurrence within 1 year of presentation (2 lung involvement, 2 renal involvement). One patient had a renal recurrence 9 years after initial presentation. Thirty two percent of patients had at least one renal allograft during the study period. None had recurrent disease post-transplant. Two patients had anti-GBM disease on biopsy for work up of microscopic haematuria with normal creatinine and no lung disease. Neither progressed.

Conclusions: This is a large series of anti-GBM disease with long term follow up. This series emphasises the devastating consequences of anti-GBM disease on patient and renal survival. We also describe an indolent form of anti-GBM disease which is not well understood.

FR-PO1892

Laser Capture Microdissection (LCM) Followed by Tissue Proteomics Identifies Differences in Protein Expression in Biopsies from Class IV and V Lupus Nephritis (LN) Brad H. Rovin,[1] Samir Parikh,[1] Michael A. Freitas,[3] Anjali A. Satoskar,[2] John P. Shapiro,[3] Tibor Nadasdy,[2] Lee A. Hebert.[1] *[1]Internal Medicine, Ohio State University; [2]Pathology, Ohio State University; [3]Molecular Virology, Immunology and Medical Genetics, Ohio State University.*

Background: We postulated LCM of kidney biopsies followed by proteomics of the captured tissues can identify disease-specific protein expression that will inform diagnostic biomarker development and provide a better understanding of disease pathogenesis.

Methods: To test this hypothesis, LCM was used to isolate glomeruli or tubulointerstitium from baseline transplant biopsies (control, n=3), Class IV (n=3) and V (n=2) LN biopsies. Tissue was digested, mass analyzed with an LTQ Orbitrap XL mass spectrometer, and proteins identified using MassMatrix. For each protein, peptide spectral counts reflect abundance. Differences in spectral counts were compared by the G-test to determine significant differences in abundance (G-score >5).

Results: Class IV and V LN glomeruli showed 91 and 26 differentially-expressed proteins respectively, compared to control, while Class IV and V LN interstitium showed 164 and 144 differentially-expressed proteins. Direct comparison of Class IV and V glomeruli showed 38 differentially-expressed proteins, while direct comparison of Class IV and V interstitium showed 56 differentially-expressed proteins. Glomerular and interstitial proteins mainly restricted to either Class IV or V LN are shown in the Table, with average spectral counts and G-scores (G).

0p7	IV	V	G	Interstitial Proteins	IV	V	G
Exostosin-1	0	13	8	Uromodulin	2	24	23
Exostosin-2	0	29	23	Glutathione S-transferase	23	1	15
C1Q	24	0	10	Dipeptidase-1	19	0	12
Complement 8	0	16	10	Fibrinogen	18	0	11
IFN-induced GTP-Binding Protein	27	5	6	IFN-induced GTP-Binding Protein	13	0	7

Conclusions: These data demonstrate that LCM/proteomics identify tissue compartment specific, differentially-expressed proteins in kidney biopsies of glomerular diseases. This can focus diagnostic biomarker development to increase probability of success. Differentially expressed proteins can also provide clues as to the pathogenesis of different types of GN.

Funding: NIDDK Support

FR-PO1893

The Impact of Partial Remission on Renal and Patient's Survival in Severe Lupus Nephritis

Mamdouh N. Albaqumi, Lutfi Alkorbi, Dania Alkhafaji. *KFSHRC, Riyadh, Saudi Arabia.*

Background: Few studies have addressed the value of partial remission on long term renal and patient's survival. In this study, we describe the effect of partial remission (PR) in comparison to complete remission (CR), and treatment failure (TF) on long term renal outcome in a cohort of patients with severe lupus nephritis.

Methods: We retrospectively reviewed our patient's archived files and identified all patients with the diagnosis of lupus nephritis from 2003 until 2008. All renal biopsies were reviewed blindly and reclassified according to ISN/RPS 2003 classifications.

Results: A total of 63 patients with Lupus Nephritis class IV were identified. 31 patients (49%) achieved PR, while 13 (21%) patients had CR, and 19 (30%) patients had TF. Serum creatinine at presentation was higher in patients with TF (178.0 ± 126.0 umol/L) compared to PR (128.4 ± 97.0 umol/L), however, when GFR is estimated, there was no statistical difference between PR and TF groups at presentation (PR: 94.4 ± 84.5 ml/min, TF: 57.8 ± 39.3 ml/min, P= 0.117) . proteinuria was also similar between PR and TF groups (PR:4.9 ± 3.9 mg/24hr, TF: 5.4 ± 4.9 mg/24hr, P= 0.126). Renal survival over the next four years was significantly higher in the CR and PR groups compared to TF (CR: 77% PR: 55%, TF: 29%. P= 0.033).

Conclusions: Despite having a similar GFR and proteinuria at presentation to TF, PR group had a better renal survival in four years, highlighting the favorable impact of partial remission in severe lupus nephritis.

FR-PO1894

Efficacy and Safety of Double Filtration Plasmapheresis Therapy in Severe Lupus Nephritis

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Background: To investigate the clinical efficacy and safety of double filtration plasmapheresis (DFPP) accompanied with corticosteroid in patients with severe lupus nephritis(LN).

Methods: 8 patients (5 females and 3 males, average age 23.9±10.3) with severe LN including class IV (n=5),III,V+IV and V+IIIl were studied. Among them, 7 cases showed rapidly progressive glomerulonephritis(RPGN) with elevated serum creatinine(SCr) (3.9±2.8mg/dl), and 3 of them needed renal replacement therapy. DFPP was performed with two-fold plasma volume on each session using membrane type plasma component separator (EC50W and EC20W, Asahi Kasei Kuraray, Japan).

Results: ***1)Clinical efficacy*** DFPP treated 2.8 times(2~3)for each patient. SLE-DAI was reduced significantly from 18.9±3.3 to 10.5±0.9 (P<0.01)and SCr decreased from 3.9±2.8 to 2.3±2.2mg/dl (P=0.13)after DEPP. 2 patients got off dialysis by 1 and 2 weeks, respectively. ***2)Immunologic parameters*** Serum IgG decreased from 10.3±5.4 to 4.8±3.1g/L(P<0.01). The titers of anti-dsDNA and anti C1q antibody were significantly declined after DFPP, while the levels of complement C3 and C4 had no change. ***3)Improvements of vascular endothelial cell function*** Increases of circulating endothelial cell_(CEC) number and vascular cell adhesion molecule (VCAM) were observed in 6 out of the 7 patients with RPGN, and elevation of thrombomodulin level was found in 5 cases before therapy. After DFPP, CEC count was decreased in 4 cases, VCAM and thrombomodulin levels were lowered in 5 and 3 patients, respectively. ***4)Follow up study*** 8 patients were followed up for 2~16 months(4.3±4.9). 5 patients achieved complete remission, and 2 had partial remission. 5***)Adverse effect*** 1 patient showed transient hypotension at the beginning of DFPP.

Conclusions: DFPP can rapidly and effectively clear autoantibodies in severe LN, thus improving renal function and protecting the endothelial cells. DFPP accompanied with corticosteroid is an effective therapeutic method for LN.

FR-PO1895

Random Spot Urine P/C Ratio Is Unreliable in Estimating 24-h Proteinuria Magnitude in Both SLE GN Patients and CKD Patients, However the Type of Inaccuracy Is Different between These Conditions

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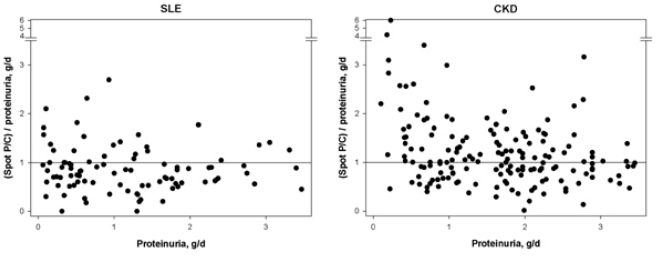
Background: We have previously shown that random spot P/C ratio (spot P/C) is highly inaccurate in estimating 24-h proteinuria in individual SLE GN patients (pts), particularly in the sub-nephrotic range. Here we extend this work to CKD pts and compare the results to those of SLE GN pts.

Methods: For the SLE GN analysis, we added a third cohort to our two previously reported cohorts. The individual data were obtained from our own database and from a previously published work. For the CKD analysis we identified from the published literature three CKD studies of spot P/C and 24-h proteinuria in which the data were presented on linear plots. The individual data were extracted by computer graphic analysis. For all the data, only those with sub-nephrotic proteinuria (< 3.5 g/d), the most common level of proteinuria encountered clinically, are included in this analysis.

Results: The SLE GN pts (N = 93) showed a mean (spot P/C)/(24-h proteinuria) ratio of 0.86 ± 0.48 (p < 0.001 compared to 1.0). The CKD pts (N = 162) showed a mean (spot P/C)/(24-h proteinuria) of 1.21 (p = 0.021) compared to 1.0. The difference in the ratios was significant (p < 0.0001). The figure below shows a calibration plot of the individual data.



Conclusions: Spot P/C is unreliable in estimating 24-h proteinuria in the sub-nephrotic range. In addition, in SLE GN it tends to underestimate and in CKD it tends to overestimate 24-h proteinuria. The mechanism and pathophysiologic significance of this difference is unclear.

Funding: NIDDK Support

FR-PO1896

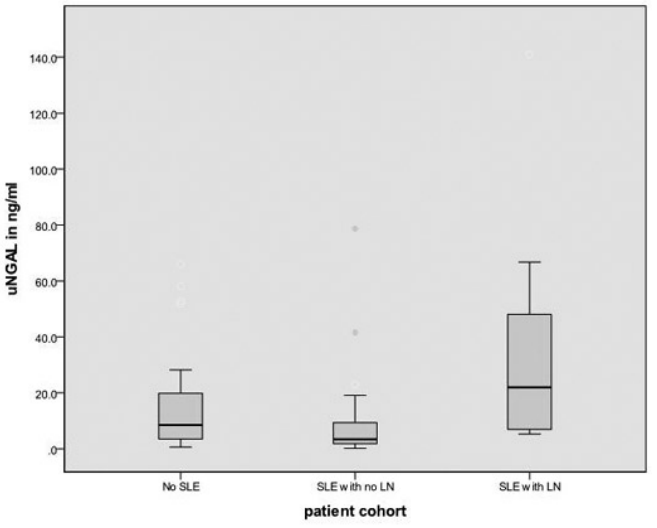
Urine Neutrophil Gelatinase Associated Lipocalin Is Increased during Active Lupus Nephritis

Li Ping Tan, Soo Kun Lim, Tee Chau Keng, Yip-Boon Chong, Wan Ahmad Hafiz Wan Md Adnan, Kok Peng Ng. *Department of Medicine, Division of Nephrology, University of Malaya Medical Center, Kuala Lumpur, Wilayah Persekutuan, Malaysia.*

Background: Urine Neutrophil Gelatinase Associated Lipocalin (NGAL) is a novel marker of acute kidney injury. While it's expression is upregulated chiefly in response to ischemic reperfusion injury, it can also be produced by glomerular mesangial cells and inflammatory cells. Patients with systemic lupus erythematosus (SLE) are often complicated by lupus nephritis, during which the glomeruli can be infiltrated by inflammatory cells. We postulate that urine NGAL levels will rise in patients with active lupus nephritis (LN).

Methods: A cross sectional study was performed over a period of 8 months. Urine NGAL levels were compared between patients with biopsy proven active ILN and SLE patients without LN. Normal healthy volunteers served as a negative control group. Patients were excluded if they had a glomerular filtration rate of <30ml/min/1.73m2.

Results: A total of 72 subjects were recruited. 12 with biopsy proven active lupus nephritis (LN), 27 with SLE but without LN and 33 healthy controls. 67% of the LN group had WHO Class IV disease. Mean GFR was similar in all 3 groups. Patients with LN had significantly higher levels of urine NGAL (median 21.95 ng/ml IQR 6.35- 52.2) compared to SLE patients without LN (median 3.4 ng/ml IQR 1.75 – 10.8)(p<0.01) and the healthy individuals (median 8.5 ng/ml IQR 3.5 – 20.4) (p<0.05). Correcting for the serum creatinine also produced similar results. With the exception of ESR (r=0.268 p<0.05), no correlation was found betweem urine NGAL levels and activity markers of LN.



Conclusions: Urine NGAL is raised in active LN. Urine NGAL may be a promising biomarker in the detection of active LN. Further studies need to be done on larger sample sizes to verify these findings.

Funding: Government Support - Non-U.S.

FR-PO1897

Effect of N-acetylcysteine on Proteinuria, Parameters of Oxidative Stress and Markers of Kidney Damage in Patients with Lupus Nephropathy

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Background: Excessive generation of reactive oxygen species modulates expression of inflammatory chemokines thereby inducing tissue damage in autoimmune diseases. Patients with lupus nephritis present impaired oxidative stress status even without clinical signs of renal activity. N-acetylcysteine (NAC) has a strong antioxidant activity. The aim was to evaluate the effect of NAC treatment on proteinuria and serum markers of oxidative stress and kidney injury in patients with lupus nephritis.

Methods: In a cross-over placebo-controlled study 14 patients (3M, 11F; mean age 35±8 years) with diagnosis of lupus nephritis (class II,III or IV, mean time from diagnosis 8.5±4.2 years) with stable serum creatinine (1.1±0.4mg/dl) and persistent low-range proteinuria 480±550 mg/l were randomly assigned to two 3-month treatment periods with 2 week wash-out. During the active treatment phase the patients received NAC 600 mg per day. Most patients were on stable low doses of steroids (n=12) and azathioprine (n=3) or mycophenolate mofetil (n=2).

Results: The treatment with NAC caused a significant decrease of proteinuria. hsCRP tended to decrease after NAC (p=0.07). None of other biomarkers of lupus nephritis activity (C3, C4 and monocyte chemoattractant protein-1 – MCP-1) changed significantly during the treatment. While serum cystatin C remained unchanged there was a tendency of neutrophil gelatinase-associated lipocalin (NGAL) to decrease during the treatment (p=0.08).

	baseline	active treatment phase (NAC)	control phase
proteinuria [mg/l]	524±627	309±471 (p=0.02 vs baseline)	376±474
hsCRP [ug/ml]	4.0±2.3	3.3±1.9	5.1±2.6
C3 [g/l]	0.89±0.26	0.86±0.31	0.94±0.25
C4 [g/l]	0.25±0.3	0.18±0.09	0.18±0.09
MCP1- [pg/ml]	936.9±641.4	829.4±502.3	663.5±302.5
cystatin C [ng/ml]	1534.6±515.7	1345.7±462.1	1399.9±463.4
NGAL [ng/ml]	103.3±57.3	86.7±49.0	110.8±60.4

Conclusions: In this small pilot study the treatment of clinically stable lupus nephritis patients with NAC led to a decrease of proteinuria but showed neither renoprotective effect nor any significant influence on serum markers of disease activity and oxidative stress.

FR-PO1898

Complement Activation in Ten Patients with C3 Glomerulonephritis without MPGN

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Background: Complement activation is the key pathogenetic factor in C3 glomerulonephritis (C3GN) without membranoproliferative glomerulonephritis (MPGN). It has been well accepted that the alternative pathway possibly plays an important role in it, but the classic pathway is not involved, and involvement of the lectin pathway is still unknown.

Methods: From January 1999 to June 2010 in Renal Division, Peking University First Hospital, ten biopsies were consistent with the definition of C3GN without MPGN (isolated mesangial C3c deposition in the absence of IgG, IgA, IgM and C1q by immunofluorescene). Concentration of plasma C4a, factor B, Ba, C3, C3a and C5a was detected by ELISA. Glomerular C4d and MAC were detected by immunohistochemistry.

Results: The concentration of plasma complement in patients with C3GN without MPGN was shown in table 1.

Table 1

	C3GN without MPGN	Normal controls	t/z	P
C4a(ug/ml)	11.73±7.23	1.96±0.78	4.052	0.004
Factor B(ug/ml)	6.78±0.53	12.07±7.02	-2.374	0.041
Ba(ng/ml)	1149.0±543.2	403.2±108.4	4.102	0.003
C3(g/L)	0.84±0.41	0.80±0.23	0.406	0.684
C3a(ug/ml)	2.61±2.30	0.12±0.06	3.257	0.012
C5a(ng/ml)	24.87±26.79	8.59±6.98	2.262	0.024

	Proteinuria (g/ d)	Plasma creatinine (umol/L)
C4a(ug/ml)	r= 0.015 P=0.969	r= 0.084 P=0.829
Ba(ng/ml)	r= 0.706 P=0.033	r= 0.193 P=0.619
C3a(ug/ml)	r= 0.437 P=0.239	r=-0.017 P=0.966
C5a(ng/ml)	r=-0.127 P=0.744	r=-0.209 P=0.590

The analysis between concentration of plasma complement and proteinuria, plasma creatinine was shown in table1.Glomerular C4d was positive in 8 biopsies in mesangial areas and glomerular capillary wall, and negative in 2 biopsies. MAC was detected in 8 biopsies in a mesangial pattern and negative in 2 biopsies(one patient was negative for C4d, and the other one was positive for C4d).

Conclusions: Alternative pathway in circulation might play an important role in the pathogenesis of C3GN without MPGN. There might also be complement activation via both the lectin and alternative pathway in glomeruli, which needs further investigations.

FR-PO1899

The Terminal Complement Complex (TCC) – A Potential Biomarker for Disease Activity in Patients with Membranoproliferative Glomerulonephritis

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Background: The role of complement (C) in membranoproliferative glomerulonephritis (MPGN) has been investigated in more detail lately. Mutations in C regulator genes, antibodies against C proteins and a common persistent hypocomplementemia (low C3) in many patients support the role of C in the pathogenesis. No biomarker for monitoring disease activity is available yet.

Methods: Here we present data on the concentration of the soluble terminal complement complex (TCC, sC5b-9) in 11 pediatric patients with MPGN. Seven patients were classified as MPGN type I and 4 as DDD (MPGN II) by their renal biopsies, 6/11 were tested positive for C3NeF. In 3 patients multiple tests were performed. 98 healthy adult blood donors were used as controls. The measurement of the TCC, generated as a potentially lytic end product of C activation, was performed by a sandwich ELISA technique. Mann-Whitney-U-test was used for statistical evaluation.

Results: Patients with MPGN showed a statistically significant higher TCC concentration in plasma (3.9 ± 3.0µg/ml vs 1.5 ± 1.2µg/ml, p<0.01) than controls. Patients with an active disease (hematuria, gross proteinuria, arterial hypertension) had a significant increased TCC value (4.8 ± 3.3 µg/ml) compared to patients in remission (1.9 ± 0.6 µg/ml, p<0.03). No significant difference of C3 levels during active disease (37.2 ± 16.1mg/dl) and patients in remission (30.0 ± 14.7mg/dl, p=0.15) was discovered.

Conclusions: This work emphasises the role of C activation in DDD as well as in MPGN I. Thus, complement inhibition therapies targeting the terminal C cascade, such as Eculizumab, should also be considered in patients with MPGN and elevated TCC. The TCC concentration may represent a good biomarker for monitoring disease activity in patients with MPGN.

Funding: Government Support - Non-U.S.

FR-PO1900

Membranoproliferative Glomerulonephritis and Mixed Cryoglobulinemia after Hepatitis C Virus Infection Secondary to Glomerular NS3 Viral Antigen Deposits

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Background: Hepatitis C virus infection is the main etiology of type I membranoproliferative glomerulonephritis. We report on three cases of type I membranoproliferative glomerulonephritis associated with type II cryoglobulin in patients with hepatitis C virus (HCV) antibodies but with a negative viral load.

Methods: We searched for occult HCV infection in B cells or kidney glomeruli using PCR assays and immuohistochemistry.

Results: Hepatitis C virus infection-associated lymphoma was excluded by computed tomodensitometry, bone marrow phenotyping and histology but indirect features of B cell proliferation were present in the three patients. Using ultrasensitive PCR assays, we did not evidence occult hepatitis C infection in peripheral blood mononuclear and bone marrow cells, and in the cryoprecipitates but found HCV-NS3 antigen the kidney in the patient tested using immunohistochemisty 6years after viral PCR was negative. Liver tissue specimens were not available. Remission occurred spontaneously in one patient and after rituximab treatment in one patient. The third patient was lost for follow up.

Conclusions: Persistence of viral antigen HCV-NS3 at least in kidney, even many years after HCV PCR is negative, may explain immunological stimulation and persistence of cryoglobulinemia with a predominant renal clinical expression.

FR-PO1901

Eculizumab for Dense Deposit Disease (DDD) and C3 Glomerulonephritis (C3GN) Andrew S. Bomback,[1] Jai Radhakrishnan,[1] Pietro A. Canetta,[1] Yuzhou Zhang,[2] Carla Nishimura,[2] Nicole Meyer,[2] Kathy Frees,[2] Michael Jones,[2] Richard J. Smith,[2] Gerald B. Appel.[1] *[1]Columbia University; [2]University of Iowa.*

Background: The principle immune defect in DDD and C3GN is excessive alternative complement activation. Eculizumab, a monoclonal Ab to C5 that prevents formation of the membrane attack complex, may provide targeted therapy for DDD and C3GN.

Methods: We present a 6-month interim analysis of an open label, proof-of-concept efficacy/safety study of eculizumab in DDD or C3GN (NCT01221181). Six pts are treated with eculizumab 1200 mg IV every other week for 1 yr. All had proteinuria ≥1 g/day and/or AKI (serum creatinine ≥150% baseline) at enrollment. All underwent biopsy at enrollment and will have repeat biopsies at 1 yr. Additional testing included: mutation screening of CFH, CFI, MCP, CFB, CFHR5 and THBD; autoAb screening for C3 nephritic factors and factor H autoAbs; and functional analysis of complement activity.

Results: We enrolled 3 pts with DDD (2 native, 1 recurrent) and 3 pts with C3GN (1 native, 2 recurrent). One pt (DDD) carried a CFH mutation; 4 pts were C3Nef(+) (2 DDD, 2 C3GN); and 2 pts (1 DDD, 1 C3GN) had elevated sMAC. Total complement levels declined to 0-1 CAE units by week 4 in 5/6 pts (in 1 pt, level was 4 CAE units by week 8). At 6 mos, two pts with AKI (native DDD and recurrent C3GN) had sustained improvements in creatinine (2.1→1.4 and 2.0→1.5 mg/dl, respectively) with consistently low proteinuria. Three other pts (recurrent DDD, native C3GN, and recurrent C3GN) had significant declines in proteinuria (10579→1417, 2279→520, and 4455→2441 mg/g, respectively) with rising serum albumin (2.9→4.1, 3.2→3.9, and 3.4→4.2 g/dl, respectively) and stable creatinine. One pt (native DDD) had no improvement in creatinine or proteinuria. Response to therapy was associated with normalization of sMAC. No C3Nef(+) pt has become C3Nef(-). No adverse events have been reported.

Conclusions: Eculizumab is well-tolerated in pts with DDD and C3GN. Interim results at 6 mos suggest effective inhibition of the terminal complement cascade. For most pts, a clinical response was observed. Laboratory and repeat biopsy data at 12 mos will be done to validate these preliminary results.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-PO1902

Successful Treatment of Membranoproliferative Glomerulonephritis Type I with Monoclonal Anti C5 Antibody (Eculizumab) Arnaud Garnier,[1] Anne Modesto,[2] Stephanie Tellier,[1] Flavio Bandin,[1] Stéphane Decramer.[1] *[1]Pediatric Nephrology Unit, Hopital des Enfants, CHU Purpan, Toulouse, France; [2]Division of Anatomic Pathology, Hôpital Rangueil, CHU Rangueil, Toulouse, France.*

Background: Membranoproliferative glomerulonephritis (MPGN) is a heterogeneous group of nephropathy, characterized on histology by mesangial hypercellularity with increased matrix, splitting of the glomerular basement membrane and different pattern of deposit (type I, II and III). MPGN type I represent less than 5% of primary glomerulonephritides and can be related to complement dysregulation.

Results: We report the case of a 7 years old boy referred to our unit for nephrotic syndrome with hematuria. Renal biopsy displayed classical MPGN type I with subendothelial deposits of C3, C1q, IgG and IgM. Serum C3 level was low (0.11g/L) and a C3 nephritic factor (C3NF) was present. No mutation was found in the complement alternative pathway factors H and I. A 6 months course of oral steroids together with angiotensin converting enzyme inhibitor (ACEi) and angiotensin II receptor blocker (ARB) did not improve proteinuria. After 3 years of sustained nephrotic syndrome, glomerular filtration rate (GFR) slowly decreased and a second renal biopsy was performed. Histological and immunological findings were similar to the first biopsy with global sclerosis concerning 20% of the glomeruli. Low serum C3 level and C3NF were still present. A second course of steroids together with the maintenance of the ACEi and ARB did not succeed to reduce proteinuria. After tetravalent meningococcal vaccine and initiation of prophylactic penicillin V therapy, treatment with the anti C5 monoclonal antibody eculizumab was initiated as follows: 900mg once a week for 4 weeks, then 1200mg every 2 weeks. 3 months after the beginning of treatment, proteinuria is nearly normal (0.2g per 24h) with normal GFR and albuminemia (35g/L).

Conclusions: This is the first report of successful treatment of MPGN type I with eculizumab, a monoclonal anti C5 antibody that blocks the terminal complement activation. In primary MGPN, dysregulation of complement activation is a frequent feature, and the treatment with eculizumab should be discussed in the most severe forms.

FR-PO1903

Lymphangiogenesis and Tertiary Lymphoid Neogenesis in Progression of IgA Nephropathy Guangchang Pei, Rui Zeng, Min Han, Lily Liu, Gang Xu. *Devision of Nephrology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, wuhan, Hubei, China.*

Background: The lymphangiogenesis and formation of tertiary lymphoid organs in renal interstitium have been previously identified in human chronic renal diseases and in human kidney transplants. However, they are not specially described in IgA nephropathy(IgAN). In this prospective study, the lymphangiogenesis and lymphoid neogenesis process and the prognosis of IgAN were evaluated.

Methods: 176 patients with biopsy-proven IgAN were selected. We graded renal biopsy specimens according to the classification of Haas. We assessed tubular lesions, interstitial fibrosis and abnormalities of the arteriolar wall by a semi-quantitative scoring system. The presence of lymphatic vessels, macrophages, dendritic cells and lymphocytes was examined by IHC.

Results: Lymphatic vessels density(LVD) in renal interstitium inversely correlated with the severity of tubular lesions and interstitial fibrosis according to Haas grades. It was also significantly associated with the amount of infiltrating macrophages, dendritic cells, lymphocytes and tertiary lymphoid organs. LVD and tertiary lymphoid organs density in renal interstitium were significantly associated with the renal function. The higher the LVD and tertiary lymphoid organs density, the higher the serum creatinine at the time of biopsy and 12 months after biopsy. The more remarkable discovery was both of the lymphangiogenesis and lymphoid neogenesis correlate with vasculopathy. Lymphangiogenesis and lymphoid neogenesis were found frequently adjacent to the vascular lesion. LVD was higher in IgAN patients with severe arterial lesions and hyaline changes (43.92mm^{-2}) than in IgAN patients who had mild / moderate arterial lesions. (20.92 mm^{-2}, p=0.000358)

Conclusions: Lymphangiogenesis and formation of tertiary lymphoid organs in renal interstitium reflected the renal activity state of chronic inflammatory and they were associated with the IgAN progression. Lymphangiogenesis participated in formation of tertiary lymphoid organs in renal interstitium.Vasculopathy might play a part in lymphangiogenesis and formation of tertiary lymphoid organs in renal interstitium of IgAN patient.

FR-PO1904

Novel Diagnostic Approach for IgA Nephropathy Hiroyuki Yanagawa,[1] Hitoshi Suzuki,[1] Yusuke Suzuki,[1] Keiichi Matsuzaki,[1] Satoshi Horikoshi,[1] Jan Novak,[2] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Dapartment of Microbiology, University of Alabama at Birmingham, AL.*

Background: Galactose-deficient IgA1 (Gd-IgA1) is found in elevated amounts in the circulation and in the mesangial deposits of patients with IgA nephropathy (IgAN). Moreover, serum levels of Gd-IgA1-specific antibodies, responsible for the formation of immune complexes (IC) with Gd-IgA1, are also elevated in IgAN. However, due to the clinical heterogeneity of IgAN, there is no biomarker to replace the diagnostic renal biopsy.

Methods: A cross-sectional study was performed using serum samples collected from 2006 to 2010 at the time of renal biopsy from 121 patients with IgAN and 73 patients with other renal diseases, such as lupus nephritis, diabetic nephropathy, and membranous nephropathy. We measured serum IgA, IgA-IgG IC, Gd-IgA1 and Gd-IgA1-specific IgA by ELISA to assess whether these biomarkers can be used for diagnosis of IgAN. ELISA data from healthy volunteers (n=74) were used to establish baseline for each assay and calculate quantitative scores for each biomarker for IgAN patients and disease controls by principal component analysis using JMP software.

Results: Serum levels of IgA, Gd-IgA1, IgA-IgG IC and Gd-IgA1-specific IgA were elevated in IgAN patients compared with disease controls (P<0.0001) and healthy controls (P<0.0001). However, no biomarker alone could effectively differentiate IgAN from disease controls, due to the overlapping values for these markers in many IgAN patients and disease controls. To test a combination of these four biomarkers as a potential differential diagnostic method for IgAN, these four biomarkers were quantitated and combined by principal component analysis based on data from healthy volunteers. This scoring system showed high sensitivity and specificity of 75% and 81%, respectively.

Conclusions: Our results suggest that serum Gd-IgA1, Gd-IgA1-specific antibodies and IC might be may be useful as combined disease specific biomarkers of IgAN. This novel quantitative scoring system can be used to complement renal biopsy in the diagnosis of IgAN.

FR-PO1905

Beneficial Effect of Steroids and Immunosuppressants in the Treatment of IgA Nephropathy May Be Due to Modifying Local Production or Activation of Multiple Cytokines Maria Stangou,[1] Aikaterini A. Papagianni,[1] Christos Bantis,[1] Maria Skoularopoulou,[1] Afroditi Pantzaki,[2] Nicoletta-Maria Kouri,[3] George Efstratiades,[1] Demitrios Memmos.[1] *[1]Department of Nephrology, Aristotle University of Thessaloniki, Hippokration Hospital, Thessaloniki, Greece; [2]Department of Pathology, Hippokration Hospital, Thessaloniki, Greece; [3]Department of Biochemistry, Hippokration Hospital, Thessaloniki, Greece.*

Background: Steroids and immunosuppressants can reduce proteinuria and delay progression of renal function in IgAN, possibly by interfering with local cytokines, which lead to inflammation and fibrosis.

Methods: Histology in 53 IgAN patients [M/F 35/18 age 40.5yrs (17-65)] was evaluated by Oxford classification system and renal biopsies were classified as MEST score (Mesangial, Endocapillary hypercellularity, Segmental glomerulosclerosis, Tubular atrophy) 1, 2, and 3. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, INF-γ, MCP-1, MIP-1β, TNF-α were measured by multiplex cytokine assay in first morning urine samples at day of renal biopsy. Steroids±azathiopine were introduced in 18/53 proteinuric patients, while 35/53 were treated with angiotensin inhibitors (RAASi)±fish oils(FO).

Results: Screat1 at time of diagnosis correlated with proteinuria (p=0.02), MEST score (0.0001) and urinary levels of IL-1β, IL-2 and MCP-1 (p=0.04, p=0.03 and p=0.04 respectively). At the end of follow up [5.5 (1-12years)], Screat2 was increased significantly in RAASi+FO (from 1.6±0.9 to 3.3±3.7mg/dl, p=0.004) and remained stable in steroids±aza treated patients.

In RAASi+FO patients, Screat2 at the end of follow up had positive correlation with MEST score (p=0.006), IL-1β (p=0.007), IL-2 (p=0.01), IL-6 (p=0.02), IL-10 (p=0.04), IL-12 (p=0.01) and MCP-1 (p=0.03) urinary levels. In steroids±aza patients, the only parameters correlated with Screat2 were IL-1β (p=0.01), IL-6 (p=0.01) and MCP-1 (p=0.01).

Conclusions: In conclusion, several cytokines are excreted in the urine of patients with IgAN, and their levels predict outcome of the disease. Treatment with steroids+aza seems to have a beneficial effect in renal function outcome, and this is probably due to their influence in local cytokine production or activation.

Funding: Private Foundation Support

FR-PO1906

Clinical Features and Long-Term Outcomes of Nephrotic Syndrome in Patients with IgA Nephropathy Hyung Jung Oh, Dong Eun Yoo, Seung Jun Kim, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Nephrotic syndrome(NS) is a rare manifestation of IgA nephropathy(IgAN), which has been reported to behave similarly as minimal change disease. Accordingly, glucocorticoids have been frequently prescribed in IgAN patients with NS. In contrast, a recent study demonstrated that spontaneous remission(SR) of NS was commonly observed in these patients. This study was conducted to investigate the clinical features and long-term outcomes of NS in patients with IgAN.

Methods: A total of 1,076 patients with biopsy-proven IgAN were included from 4 centers in Korea. The primary outcome was regarded as doubling of the baseline serum Cr, and the secondary outcomes as ESRD or death. Cox regression analysis was performed to identify independent risk factors for the development of primary and secondary endpoints and to evaluate the predictive factors of the occurrence of SR.

Results: Among 100 patients(10.2%), who presented with NS, glucocorticoids were prescribed in 65(65.0%). Complete remission(CR), partial remission(PR), and no response(NR) were observed in 48.0%, 32.0%, and 20.0%, respectively. During the median follow-up of 44.0 months, 24 patients(24.0%) in the NS group reached the primary endpoint compared to 63(7.2%) in the non-NS group(P<0.001). Compared to the CR group, the risk for attaining the primary endpoint was significantly higher in the PR(HR, 14.49; 95% CI, 1.14-183.7; P=0.039) and the NR groups(HR, 215.97; 95% CI, 15.63-2983.6; P<0.001). Among patients with NS, 24(24.0%) underwent SR. Multivariate Cox regression analysis revealed that a >50% decrease in proteinuria within 3 months after the onset of NS, serum Cr≤1.2 mg/dL, and female gender were associated with a significantly increased likelihood of SR. None of these patients reached the primary endpoint, and they had fewer relapses during follow-up.

Conclusions: This study shows the prognosis of NS in patients with IgAN is not favorable unless CR or PR is achieved. In addition, SR occurs more frequently in patients with preserved renal function and a prompt decrease in proteinuria after the onset of NS, and these patients have excellent outcomes.

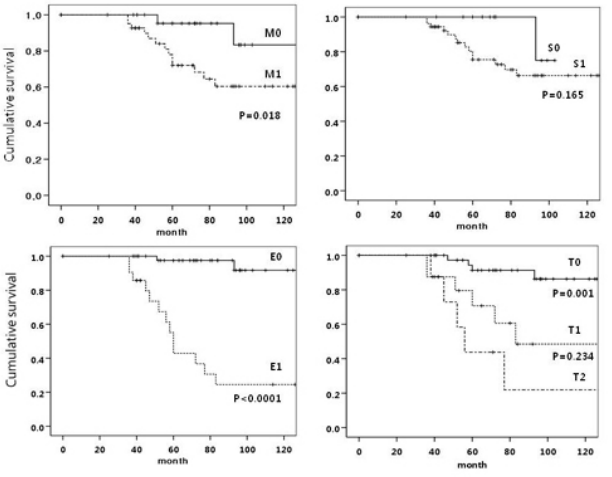
FR-PO1907

Validation of the Oxford Classification of IgA Nephropathy in Korean Adults Soon Hyo Kwon,[2] So-Young Jin.[1] *[1]Department of Pathology, Soon Chun Hyang Univiersity Hospital, Seoul, Korea; [2]Department of Internal Medicine, Soon Chun Hyang University Hospital, Korea.*

Background: The recently published Oxford classification of IgA neuropathy (IgAN) proposed a split system for histologic grading based on prognostic pathologic features. The new classification system must be validated in different cohorts. We investigated whether these pathologic features are applicable in the adult Korean population.

Methods: One hundred seven adult Korean patients with IgAN were analyzed with the Oxford classification system at Soon Chun Hyang University Hospital, Seoul, Korea. Renal biopsies from all patients were scored by a pathologist who was blinded to the clinical data for pathological variables. Inclusion criteria were age greater than 18 years and at least 36 months follow-up. We excluded cases with secondary IgAN, diabetic nephropathy combined other glomerulopathies, less than 36 months of follow-up, and rapidly progressing cases.

Results: The median age of patients was 34 years (range, 27–45 years). Mean arterial blood hypertension (MAP) was 97±10 mmHg at the time of biopsy. The median follow-up period was 85 months (range, 60–114 months). Kaplan-Meier analysis showed significant prognostic prediction with M, E, and T lesions.



E and T lesions also revealed prognostic prediction in the Cox proportional hazards regression analysis.

Cox regression analyses between pathologic features and a 50% delcine in eGFR: univariatie and multivariate pathologic determinants

	Univaritate hazard ratio (95% CI),p	Multivariate hazard ratio	
E0	1	E0	1
E1	12.61(2.47-64.35),p=0.002	E1	12.21(2.23-66.71),p=0.004
T0	1	T0	1
T1	3.60 (0.87–14.81), p=0.75	T1+T2	3.58 (1.03–12.40), p=0.044
T2	14.01 (1.61–121.74) p=0.017		

a: One pathologic feature + mean arterial pressure, proteinuria, and eGFR

Conclusions: In the Oxford classification of IgAN, E and T lesions predict renal outcome in Korean adults after taking clinical variables into account.

FR-PO1908

Long-Term Outcome of Biopsy-Proven IgA Nephropathy Presenting with Mild Proteinuria and or Isolated Microhematuria. A Multicenter Study Eduardo Gutierrez,[1] Isabel Zamora,[2] Teresa Olea,[3] Sara Jiménez álvaro,[4] Carmen Bernis,[5] Manuel Praga.[6] *[1]Nephrology Department, Hospital 12 de Octubre; [2]Nephrology Department, Hospital La Fe; [3]Nephrology Department, Hospital La Paz; [4]Nephrology Department, Hospital Ramon y Cajal; [5]Nephrology Department, Hospital La Princesa; [6]Nephrology Department, Grupo de Estudio de las Enfermedades Glomerulares, Spain.*

Background: Renal biopsies are rarely performed in patients presenting with mild proteinuria and/or isolated microhematuria. Clinical information about long-term outcomes of IgA nephropathy (IgAN) presenting with such benign manifestations is very scarce.

Methods: Retrospective and multicenter study to collect patients with biopsy-proven IgAN who presented at renal biopsy a normal renal function, microhematuria and proteinuria lower than 0.5 g/d.primary endpoint was renal survival, defined as an increase >50% or >100% of baseline serum creatinine (sCr).Secondary endpoints were the occurrence of remission, the development of >1 gr/d proteinuria and the development of ESRD. Thebiopsies were classified according to the new Oxford Classification.

Results: 141 patients were collected.Mean follow-up was 108 months.Clinical characteristics at baseline were:63.4% males, age 23,7±14,8 yr, sCr 0,8±0,2 mg/dl and a median proteinuria of 0.2 gr.Renal biopsies showed mesangial proliferation in 32.6% of patients, focal and segmental glomerulosclerosis in 15.6% ,and endocapillary proliferation in 8.5%.No patient received any immunosuppressive treatment.59 patients were treated with ACEI/ARB during follow-up.Renal survival (> 50% sCr) was 96,2% and 93,6% after 10 and 15 yr,and 97.6% at both 10 and 15 yr when it was defined by >100% sCr increase.Only 6 patients (4.2%) had a e-GFR <60 ml/m and none reached ESRD.Remission was observed in 37.6% and proteinuria increased to >1g/d in only 6 patients (4.2%).By multivariate analysis only age (HR 1,1;95% CI 1,01-1,3;p 0,03) and FSGS lesions in renal biopsy (HR 10,3, CI: 0,9-120; p 0,04) were significant risk factors for renal survival .

Conclusions: Long-term renal outcomes in patients with benign clinical presentations are excellent.Spontaneous clinical remission occurred in more than a third of patients.

FR-PO1909

Down-Regulated Peripheral Lymphocyte miR-155 Is Related to IgA Nephropathy Wei Qin, Mian Wei, Ping Fu. *Division of Nephrology, Department of Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China.*

Background: MicroRNA-155 (miR-155) is a very important immune regulatory factor, which involved in the lymphocyte homeostasis and adjusting of multiple immune related genes. Immune abnormalities, such as lymphocytes dysfunctions and impaired

homeostasis, are key pathogenesis of IgA nephropathy (IgAN). Therefore, we studied the expression level of miR-155 in the peripheral lymphocytes of IgAN.

Methods: Forty biopsy-proven IgAN patients and 15 unrelated healthy controls were included. Expression of miRNAs in peripheral lymphocyte was first determined using Exiqon microRNA microarray in 3 IgAN patients and 3 healthy controls. Realtime RT-PCR of miR-155 was performed. The expression level of Foxp3, a regulator of miR-155, was also measured. Correlation between miR-155 and Foxp3 expression level as well as clinical indexes was analyzed.

Results: microRNA microarray indicated that the expression level of miR-155 in IgAN patients was dramatically lower than that in normal control (2.12 vs 3.21), which was confirmed by realtime RT-PCR examination (IgAN 0.19±0.07 vs Control 0.74±0.22, p=0.003). Further study showed that baseline proteinuria (24 hour quantification) and hematuria (RBC per high power view) level was significantly correlated to the miR-155 expression level (proteinuria: r=-0.490, p=0.007, hematuria: r=-0.648, p<0.001). Significantly correlation between miR-155 and Foxp3 expression level was also noticed. However, no apparent correlation was observed in baseline serum creatinine level, serum albumin level, serum IgA concentration as well as pathological grade (Lee's).

Conclusions: Remarkable lower expression of miR-155 in peripheral lymphocytes was observed in IgA nephropathy patients, which was significantly correlated with severity of proteinuria, hematuria and Foxp3 expression level. These results suggested that miR-155 might play important roles in the pathophysiology of IgAN.

FR-PO1910

Urinary Proteome Analysis by MALDI-TOF-MS with Magnetic Beads Can Identify the Pathologic Presentation of Clinical Early IgA Nephropathy
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Background: To test if pathologic presentation of early IgA nephropathy could be identified by proteomic patterns in the urine from patients using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) protein chip array technology.

Methods: Total 56 patients with IgA nephropathy were included in this study, with 23 patients having severe pathologic presentation, and the other 33 having mild ones. The control group is 14 normal subjects. The urinary proteomic spectra from those three groups were generated by MALDI-TOF-MS with weak cationic exchange magnetic beads. The total experiment data was handled by the Zhejiang University ProteinChip Data Analysis System.

Results: The urine protein/peptide spectra patterns were established to distinguished severe IgA nephropathy from the mild IgA nephropathy group and the normal group. Several potential biomarkers were found and the diagnostic system distinguished the severe IgA nephropathy from the mild IgA nephropathy with a sensitivity of 90.48% and a specificity of 96.77%.

Conclusions: Using MALDI-TOF-MS with magnetic beads to detect urine proteomic patterns shows great potential in identifying early IgA nephropathy with different pathologic prognosis.

FR-PO1911

Evaluation of Serial Serum Levels of IgA-IgG Immune Complexes Prior To Diagnosis of IgA Nephropathy
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Background: Galactose-deficient IgA1 (Gd-IgA1) kidney deposits in IgA nephropathy (IgAN) are likely derived from IgA-containing circulating immune-complexes (IgA-IgG IC). There is no previous study of the presence or levels of circulating IgA-containing IC prior to the biopsy diagnosis of IgAN.

Methods: We compared serial serum levels of IgA-IgG IC preceding diagnosis in 6 patients with biopsy-proven IgAN to the levels in 18 healthy age-, sex-, race-, and age-of-serum-sample-matched healthy controls (HC) using the Department of Defense Serum Repository. IgAN cases had a 25-100% elevation in serum creatinine from baseline, 1-3 g/d proteinuria, or moderate tubulointerstitial disease without crescents on biopsy at the time of diagnosis. IgA-IgG IC were detected by cross-capture ELISA.

Results: IgAN patients had higher mean IgA-IgG IC level compared to HC at less than 500 days prior to diagnosis (0.86 vs. 0.66 OD units; p=0.03) but not greater than 500 days prior to biopsy diagnosis (0.84 vs. 0.75 OD units; p=0.16). More IgAN patients had multiple longitudinal serum samples with IgA-IgG IC level greater than 0.58 OD units than did matching HC (100% vs. 50%; p=0.05).

Conclusions: IgAN cases had a higher average circulating level of IgA-IgG IC prior to diagnosis compared to matching HC. Multiple serial circulating levels of IgA-IgG IC greater than 0.58 OD were associated with the future diagnosis of IgAN. Serial measurements of circulating levels of IgA-IgG IC may improve the prospects for a serologic non-invasive diagnosis of IgAN.

Funding: Other U.S. Government Support, Private Foundation Support

FR-PO1912

***ACE* Insertion/Deletion (I/D) Polymorphism Predicts Renoprotective Effectiveness of Renin-Angiotensin System Brockade (RASb), along with Progression, in IgA Nephropathy (IgAN)**
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Background: Although many studies identified multiple gene polymorphisms as genetic prognostic factors of IgAN, little information is available about genetic predictors of renoprotective effectiveness of RASb.

Methods: The present multicenter longitudinal study included 257 IgAN patients, who participated in our previous study PREDICT-IgAN. To identify genetic predictors of progression in IgAN, we assessed the association between progression (50% increase in serum creatinine at renal biopsy) and RAS-related gene polymorphisms (*ACE* I/D, *AT1R* A1166C, *AGT* M235T and *CYP2C9* A1075C), in multivariate Cox proportional-hazards (CPH) models. To indentify renoprotective effectiveness of RASb in IgAN, effect modifications between RASb and the gene polymorphisms were assessed in multivariate CPH models. P for interaction <0.10 was regarded as statistically significant. As sensitivity analysis, we also assessed these associations with the slope of eGFR during the observational period in multivariate linear regression (LR) models.

Results: During median 10.2 (interquartile range 6.7 - 13.4) yr of the observational period, 50.2% received RAS inhibitors and 27.6% developed progression. Among 4 gene polymorphisms, only *ACE* I/D predicted progression (DD vs. non-DD, hazard ratio (HR) 1.82 [95%CI 1.04 - 3.18]) and also effectiveness of RASb (P for interaction (*ACE* I/D * RASb) = 0.066)). HR of DD patients with RASb was remarkably lower than DD patients without RASb, whereas not in non-DD patients (non-DD without RASb as a reference; non-DD with RASb, 1.48 [0.78 - 2.79]; DD without RASb, 2.96 [1.42 - 6.15]; DD with RASb, 1.38 [0.50 - 3.80]). Multivariate LR models also ascertained the results described above.

Conclusions: *ACE* I/D polymorphism predicts renoprotective effectiveness of RASb, besides progression, in IgAN.

FR-PO1913

A Nationwide Questionnaire on Treatments for IgA Nephropathy in Japan
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Background: Since annual check-ups with urinalysis are routine patients with various stages of IgA nephropathy (IgAN) are managed in Japan. A wide variety of treatments for IgAN including tonsillectomy with steroid pulse therapy (TSP) is performed because there is no single clear treatment approach to all IgAN. The objective of the present study was to investigate the current status of treatments for IgAN in Japan via Progressive Renal Diseases Research, Research on intractable disease, from the Ministry of Health, Labour and Welfare of Japan.

Methods: The nation wide questionnaire survey was conducted in 1,194 teaching hospitals by the Japanese Society of Nephrology in 2008.

Results: Answers from 376 hospitals (31.4%) showed that TSP was performed in 223 hospitals (59.3%). The histological severity and levels of urinary protein excretion (73.5%) were the most cited indications for TSP. Patterns of steroid pulse therapy in TSP were mainly grouped as follows: 1) three times in three consecutive weeks (47.8%) and 2) three times every two months (18.9%). No major differences were found in clinical efficacy between the two groups. The rate of clinical remission by TSP, a condition with no continuing or transient abnormalities in urinalysis was better than that for steroid pulse therapy alone in each hospital. Combination therapy with corticosteroids, immunosuppressants and anticoagulants/antiplatelet agents was performed in about 70% of pediatric departments. Renin-angiotensin system inhibitors (RAS-I) and antiplatelet agents were used in most hospitals.

Conclusions: Besides popular treatments such as RAS-I and antiplatelet agents, TSP is a current treatment for IgAN mainly with two different patterns of steroid pulse therapy in Japan.

FR-PO1914

Mesangial IgG Deposition Is Not Associated with Outcome in Pediatric IgA Nephropathy
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Background: Anti-glycan IgG antibodies to *N*-acetylgalactosamine have been implicated in the pathogenesis of IgA nephropathy (IgAN) [PMID 19478457]. However, many patients with IgAN do not have IgG in their glomerular deposits, suggesting different

pathogenic mechanisms in those patients. Recent analysis of data from Oxford classification cohort (OCC) suggested that the presence of IgG in mesangial deposits is associated with progression to end stage kidney disease (ESKD) in children and adults with IgAN [PMID 21273233]. The purpose of this study was to determine whether the presence of IgG deposits associates with progression to ESKD in the Le Bonheur Children's Hospital (LBCH) IgAN cohort.

Methods: Renal biopsy reports with immunofluorescence data for IgA, IgG and IgM were available for 99 patients diagnosed with IgAN prior to age 18 at LBCH in Memphis, TN since 1974. Kaplan-Meier curves were generated using SAS v9.2. P-values of <0.05 were considered statistically significant.

Results: This cohort included 1 Native American, 2 Asians, 23 African Americans, and 73 Caucasians with a mean age of 10.6 ± 4.0 years at time of diagnosis and a median length of follow up of 5.4 (IQR 2.0 - 15.0) years. Male:Female ratio was 70:29. Twelve patients reached the endpoint of ESKD, defined as date of initiation of chronic dialysis or primary renal transplantation. Overall kidney survival was 92%, 86%, and 83% at 5, 10, and 15 years, respectively. IgA was the only immunoglobulin in 33%, occurred in combination with IgG in 51%, and with IgM in 36%. For those with IgG deposition, survival was 92%, 83% and 83% and without IgG was 91%, 83% and 83% at 5, 10, and 15 years. For those with IgM deposition, survival was 89%, 79%, and 79% and without IgM was 93%, 88%, and 84%. Kidney survival did not differ based upon gender or race.

Conclusions: Survival data from the LBCH IgA cohort does not support the suggestion based upon the OCC that the presence of IgG in glomerular deposits is a risk factor for progression to ESKD.

FR-PO1915

Elevated Soluble Flt-1 Was Associated with Clinical and Pathological Severity in IgAN Patients Li Zhu,[1,2] Sufang Shi,[1,2] Lijun Liu,[1,2] Jicheng Lv,[1,2] Hong Zhang.[1,2] *[1]Renal Division, Peking University First Hospital, Beijing, China; [2]Peking University Institute of Nephrology, Beijing, China.*

Background: Patients with IgA nephropathy often displayed vascular injury, such as clinical hypertension and histological arteriolar hyaline and arterial intimal thickening. Till today, factors for vascular injury in IgAN patients remain incompletely understood. Soluble Flt-1 is a splice variant of VEGF receptor. For lacking of trans-membrane and cytoplasmic domains, sFlt-1 can bind and sequester VEGF to act as a VEGF antagonist. High levels of sFlt-1 were reported in many diseases with proteinuria and hypertension symptoms, including preeclampsia and essential hypertension. Recently, CKD patients were also reported to have elevated sFlt-1, indicating its involvement in kidney disease. In the present study, we investigated the effect of sFlt-1 in IgAN.

Methods: A total of 122 individuals (100 IgAN patients and 22 healthy volunteers) were enrolled. Clinical manifestations at the time of renal biopsy and pathological characteristics were collected from the clinical record. Plasma sFlt-1 levels were determined using commercial ELISA kits.

Results: Plasma sFlt-1 level were significantly elevated in IgAN (patients Vs controls: 101.06±24.84 Vs 79.73±18.85 pg/ml, p<0.001). Furthermore, IgAN patients with hypertension showed significant higher plasma sVCAM-1 levels than those without (106.68±28.30 Vs 96.27±20.54 pg/ml, p=0.029), although IgAN patients, with or without hypertension, presented with higher plasma sFlt-1 levels than controls (p<0.001 & p=0.006), indicating the correlation between sFlt-1 and clinical phenotype of hypertension in IgAN patients. Similar correlation was also found about the phenotype of proteinuria. IgAN patients with proteinuria more than 1g/d, showed significant elevated sFlt-1 to those with less than 1g/d (p=0.025). According to the pathological lesions, IgAN patients were grossly grouped to five groups. Interestingly, we can find the gradually elevated sFlt-1 level from mild to severe pathological group.

Conclusions: Elevated sFlt-1 was associated with hypertension, proteinuria phenotype and pathological severity in IgAN patients, which indicated the involvement of sFlt-1 in IgAN.

Funding: Government Support - Non-U.S.

FR-PO1916

IgA Nephropathy: A Long Term Follow-Up of 132 Cases Doloretta Piras, Patrizia Melis, Maura Conti, Riccardo Cao, Andrea Angioi, Antonello Pani. *Nefrologia e Dialisi, Azienda Ospedaliera Brotzu, Cagliari, Italy.*

Background: Since the beginning of the 1990s our medical practitioners have extensively prescribed angiotensin-converting enzyme inhibitors for patients with mild IgA nephropathy (IgAN) and steroids for those with severe IgAN. The purpose of this retrospective study was to analyze the probability of renal survival in medical practice and to study risk factors for kidney disease progression in a cohort of patients with IgAN.

Methods: We included 132 patients with primary biopsy proven IgAN first diagnosed between 1987 and 2008 in our department. They were observed for an average of 8,4 years (0,14-23,2). 89 patients had been followed up in our centre; 43 were found through databases or telephonic interview. The primary endpoint was the achievement of a glomerular filtration rate (GFR)<15 ml/min or end-stage renal disease (ESRD). Renal survival was estimated using Kaplan Meier plots, logrank test and Cox proportional-hazard models.

Results: At diagnosis, GFR was less than 60 ml/min in 45,1% of patients. The renal survival rate following biopsy at 5th, 10th,15th, and 22nd year was respectively 85% (95% confidence intervals, CI 78-91%), 67% (CI, 58-77%), 50% (CI, 39-63%), and 37%(CI, 20-69%). Univariate analysis showed that patients who had hypertension, proteinuria, and GFR<60 ml/min were associated with poor prognosis. Age, sex, and macroscopic hematuria at initial presentation did not influence prognosis. We created several models of multivariate analysis, each of which included 4 variables (44 events). Hazard ratio of the final model were: 2.6 (CI 1.21-5.6;p=0.014) for patients with hypertension; 1.27 (CI 1.12-1.46;p=0.0002) for each increase of proteinuria of 1 g/day; 0.98 (CI 0.97-0.99;p=0.006) for each increase of eGFR of 1 ml/min.

Conclusions: In medical practice, renal survival rate was poor despite therapy. This is partially explained by lead-time bias: as a matter of fact diagnosis was made when renal function was impaired in nearly half of patients. Moreover, "real" population is more heterogeneous and complex than cohorts enrolled in randomized controlled trial. Patients with renal impairment, hypertension and proteinuria had the highest risk for disease progression.

FR-PO1917

Treatment of Early Immunoglobulin A Nephropathy by Angiotensin Converting Enzyme Inhibitor – A Randomized Controlled Trial Philip K.T. Li,[1,2] Cheuk-Chun Szeto.[1] *[1]Department of Medicine & Therapeutics, Chinese University of Hong Kong, Shatin, Hong Kong; [2]Department of Medicine & Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

Background: The treatment of IgA nephropathy with normal renal function and minimal proteinuria is unknown.

Methods: We randomly assigned 60 patients with IgA nephropathy, proteinuria <0.5 g/day, normal blood pressure and renal function, to ramipril 2.5 mg daily or no treatment. Patients were followed for 5 years for the development of hypertension, proteinuria, or impaired renal function.

Results: The blood pressure of the treatment group was marginally lower than the control group throughout the study period. At 60 months, the event-free survival was similar between the treatment and control group (81.1% and 70.5% respectively, p = 0.3). Similarly, the proteinuria-free survival was 82.9% and 79.3% for the treatment and control groups, respectively (p = 0.6); hypertension-free survival was 86.4% and 79.3% (p = 0.2). None of the patient developed impaired renal function. In general, the study medication was well tolerated, although 2 patients needed to stop prematurely because of cough and dizziness.

Conclusions: For IgA nephropathy patients with minimal proteinuria, blood pressure and normal renal function, treatment with ACE inhibitor does not offer any benefit.

Funding: Clinical Revenue Support

FR-PO1918

Clinical Characteristics and Outcome of Patients with Diffuse Crescentic IgA Nephropathy Yihe Yang, Jicheng Lv, Lijun Liu, Min Chen, Zhao Cui, Sufang Shi, Yuqing Chen, Ming-Hui Zhao, Hong Zhang. *Renal Division, Department of Medicine, Peking University First Hospital, Peking University Institute of Nephrology, Beijing, China.*

Background: Guidelines recommended immunosuppressive therapy for crescentic IgA nephropathy (IgAN). However, the response to such treatment and clinical outcomes were still unclear.

Methods: In this study, 52 patients with diffuse crescentic IgAN(crescent affected>50% glomeruli) were collected and followed up at least 12 months. Crescentic glomerulonephritis due to anti-GBM disease (n=38) and ANCA associated systemic vasculitis (AASV, n=44) served as controls. Logistic regression was used to assess the outcome of all patients with ESKD as the end-point.

Results: Mean initial serum creatinine(SCr) was 413.0±304.7μmol/L and percentage of crescents was 68.5%±15.4%. Cumulative renal survival rate was 59.6%, 53.8% and 31.1% at 1st, 3rd and 5th year. On multivariate Cox analysis, initial SCr was the only independent risk factor of ESKD. Cumulative probability of ESKD at one year by initial SCr fitted an "S" shape curve. Risk of ESKD was relative low when SCr < 360μmol/L (<21.1 %), then grew rapidly with the increase of SCr. While for those with initial Scr >599μmol/L, none recovered from dialysis. This S shape curve was similar to that observed in anti-GBM disease, while it was not observed in AASV.

Conclusions:

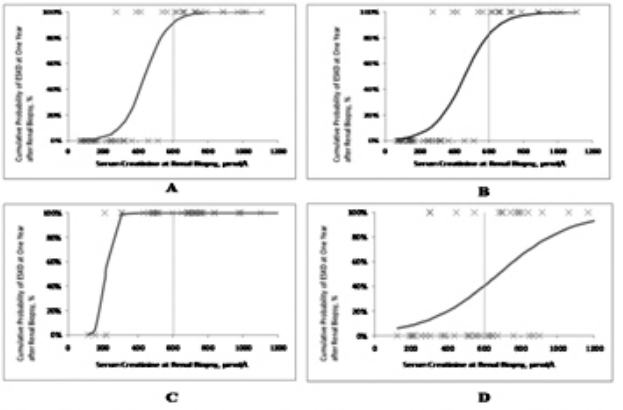

Figure 1: Logistic curve of crescentic IgAN, anti-GBM disease and AASV
Red line: Logistic curve of each disease
Green line: Scr=599 μ mol/L
Blue cross: renal survival at one year after renal biopsy. 0% means the patient did not reach ESKD, 100% means the patient had reached ESKD.
A: Logistic curve of crescentic IgAN fitted an S shape. For patients with Scr<360 μ mol/L, cumulative probability of ESKD was lower than 21.1%. None of the patients with Scr>599 μ mol/L recovered.
B: Similar result was observed in patients with crescentic IgAN received immunosuppressive therapy.
C: Logistic curve of anti-GBM disease fitted an S shape.
D: Logistic curve of AASV. We did not observe such an S shape.

Conclusions: Serum creatinine at presentation was the strongest predictor for ESKD in patients with crescentic IgAN. Patients should receive immunosuppressive therapy as early as initial presentation (SCr < 360μmol/L) at an early stage. Patients with initial renal function severe impairment (Scr >600μmol/L) seldom recover even after aggressive immunosuppressive therapy.

FR-PO1919

The Association of Complement 3 (C3) and Proteinuria in IgA Nephropathy Tsuyoshi Miyagi, Kentaro Kohagura, Yusuke Ohya, Kunitoshi Iseki. *Department of Cardiovascular Medicine, Nephrology and Neurology, University of the Ryukyus, Nishihara-cho, Okinawa, Japan.*

Background: Adipocytokine such as Complement 3 (C3) is associated with metabolic abnormality. However, the roles of C3 in the association with the metabolic marker and proteinuria are not clear. Moreover, its relationship in IgA nephropathy (IgAN) has not been investigated.

Methods: We examined 142 patients with IgAN (54% male) and analyzed clinical and pathological markers among tertile of C3 by sex.

Results: The mean (SD) age were 36.3 (16.3) years, amount of urine protein (UP) 1.1 (1.0) g/gCr, and eGFR 88.5 (12) ml/min/1.73 m^2, C3 88.8 (26) mg/dl. Although the mean age, eGFR, histological change were similar among both sexes, UP was significantly higher in women than in men (1.2 v.s. 0.8 g/gCr, P <0.025). In both sexes, the higher the tertiles of C3 and triglyceride (TG) increased. In the 3rd tertile of C3, TG was significantly higher than that of the 1st tertile and highest proteinuria and percentage of crescent in biopsy specimen was observed in women. Multiple logistic analysis adjusted for age, eGFR, percentage of crescent showed that the 2nd and 3rd tertiles of C3 were significantly associated with higher risk for the presence of above the mean value of UP than that of the lowest tertile only in women. The adjusted odds ratios (95% CI) were 6.0 (1.3 to 28.7) and 7.3 (1.6 to 33.7), respectively.

Conclusions: In conclusion, increased level of C3 was associated with higher UP only in women among IgAN patients. The present study suggested the clinical significance of C3 among patients with IgAN. Reasons of gender difference remained speculative.

FR-PO1920

Henoch-Schonlein Purpura Nephritis in Older Adults Hiroyuki Ueda, Yoichi Miyazaki, Yasunori Utsunomiya, Tetsuya Kawamura, Tatsuo Hosoya. *Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.*

Background: Whereas Henoch-Schonlein purpura nephritis (HSPN) has been extensively studied in children, its natural history in adults, especially elderly patients, is not fully understood. We have analyzed 15 individuals with biopsy-proven HSPN ≥ 50 years of age to clarify the clinical characteristics in this group.

Methods: Patients older than 50 years (older adults, OA) at the time of biopsy were compared with those from 18 to 50 year of age (younger adults, YA) in regard to clinical and pathological features, and outcome of treatment. All patients showed typical renal histology consistent with HSP (predominant IgA mesangial deposits) associated with palpable purpura.

Results: Of 23 patients included, 8 were classified in YA (age at diagnosis: 29.2 ± 9.4 years) and 15 in OA (age at diagnosis: 67.3 ± 8.5 years), with an overall follow-up duration of 32.69 ± 28.69 months. OA had a higher prevalence of complications (e.g., diabetes mellitus, hypertension, atrial fibrillation and malignancy) than YA at presentation. OA was likely to develop rapidly progressive glomerulonephritis (33.3%) and nephrotic syndrome (40.0%) at presentation. Histologically, in OA, cellular and fibrocellular crescents (18.33% vs. 6.66%), interstitial fibrosis and tubular atrophy (20.0% vs. 5.0%), and arteriosclerosis (1.28 vs. 0.0) were seen in a great degree than in YA. More than 70% of patients in both groups received corticosteroid. Finally, renal damage improved in most case of both groups at last visit. However, adverse events including severe infection and cardiovascular events occurred more frequently in OA than YA leading to four deaths in OA group.

Conclusions: HSP in OA is characterized by severe renal involvement, and is susceptible to treatment-related adverse events. Adjustment of treatment regimens is necessary in this age group to reduce the risk of adverse events.

FR-PO1921

Henoch-Schönlein Purpura Nephritis: Comparison of Two Therapies (azathioprine vs Mycophenolate) and it´s Effect on the Urinary Excretion of MCP-1 Yolanda Fuentes, Saul Valverde, Ana M. Hernández, Lourdes M. Ortiz, Mara Medeiros. *Hospital Infantil de México Federico Gómez, Mexico.*

Background: Renal expression of MCP-1 promotes infiltration of monocytes and macrophages in Henoch-Schönlein Purpura Nephritis (HSPN). There is no consensus in the treatment of severe forms of HSPN. Azathioprine (AZA) has proved an effective drug. There is litlle experience using mycophenolate (MMF). Primary outcome is to compare the effectivness of theese drugs in the remission of proteinuria, excretion of MCP-1 and regressions of the histologic lesions in children with HSPN.

Methods: Prospective, randomized, open trial in children with renal biopsy proven HSPN. Two treatment groups: I. Oral prednisone + AZA and II. Oral prednisone + MMF. Monthly visits for serum creatinine, proteinuria, liver function test, urine MCP-1 by ELISA. Renal biopsies and immunohistochemistry with CD68 at baseline and 12 months after treatment. SPSS v16 was used for statistical analysis. We present the preliminary results of 22 patients.

Results: Median age of 7 years (4 - 11 years). 15 males. Hematuria and no nephrotic protienuria was present in 11 patients (50%), 10 patients (45%) had nephrotic syndrome. Mean 12 hour proteinuria in all patients were 89.27 ± 94.1 mg/sqmeter/hour, mean urine MCP-1 was 919 ± 1149.94.1 pg/ mL. Twelve patients (54%) had class III lesion and CD68 was positive in 56% of the glomerulus at baseline in renal biopsies. Ten patients received AZA and twelve patients received MMF. After one year of treatment only 8 patients had remission of proteinuria in the AZA group; all patients in the MMF group had remission of proteinuria without significant statistical difference. In both groups urine MCP-1 decreased. In renal biopsies 5 patients in the AZA group presented regression of the initial histologic lesion vs eigth patients in the MMF group without statistical difference between tehm. There was also a reduction in CD68 on glomerulus of 35%.

Conclusions: There are more remissions of proteinuria and in the histologic lesions in MMF grup, the difference is not statistically significant. More patients and prolonged follow up is needed.

Funding: Government Support - Non-U.S.

FR-PO1922

The Soluble VEGF Receptor sFLT-1 Correlates with Inflammation but Not Glomerular Filtration Rate or Proteinuria in Patients with Acute Glomerulonephritis Anna Bertram, Svjetlana Lovric, Hermann G. Haller, Marion Haubitz, Torsten Kirsch. *Nephrology, Hannover Medical School.*

Background: The soluble vascular endothelial growth facter (VEGF) receptor Fms-like tyrosine kinase-1 (sFLT-1) is known to bind and sequester VEGF in the circulation, thereby antagonizing its regulatory function on endothelial integrity. Elevated levels of sFLT-1 cause endothelial dysfunction and enhanced proteinuria. Furthermore, elevated sFLT-1 levels have been found in patients with chronic kidney disease, where they were correlated with impaired renal function and proteinuria. This study aimed to determine sFLT-1 levels in patients with acute kidney disease and to correlate them with parameters of kidney function and inflammation.

Methods: Plasma levels of sFLT-1 were assessed using a commercially available ELISA in patients with clinically active membranous glomerulonephritis (N = 18), clinically active ANCA associated pauci-immune glomerulonephritis (N = 14), or healthy subjects as a control (N = 18). Serum creatinine, serum C-reactive protein (CRP) and serum cholesterol levels, blood cell counts and proteinuria were examined at the same time. Glomerular filtration rate was estimated using the CKD-EPI formula.

Results: Mean plasma levels of sFLT-1 were significantly elevated in patients with active membranous glomerulonephritis (104.2 ± 5.2 pg/mL, p = 0.028) and ANCA-associated pauci-immune glomerulonephritis (121.7 ± 15.0 pg/mL, p = 0.025) compared to healthy subjects (88.1 ± 4.7 pg/mL). This increase significantly correlated with parameters of inflammation, i.e. white blood cell counts (r = 0.578, p = 0.001) and CRP levels (r = 0.518, p = 0.002). Different to previous findings in chronic kidney disease, sFLT-1 levels did not correlate with glomerular filtration rate (r = -0.244, p = 0.179) and proteinuria (r = 0.122, p = 0,63).

Conclusions: In contrast to chronic kidney disease, sFLT-1 levels do not correlate with renal function in clinically active glomerulonephritis. In these forms of acute kidney disease, sFLT-1 seems to be a marker for inflammatory processes rather than renal function.

FR-PO1923

Impact of a Functional Polymorphism of Vascular Endothelial Growth Factor (VEGF) Gene on Primary Glomerulonephritis Christos Bantis, Peter J. Heering, Nicoletta-Maria Kouri, Maria Stangou, Christina Schwandt, Nicola Kuhr, Lars C. Rump, Katrin Ivens. *Department of Nephrology, Heinrich-Heine University, Düsseldorf, Germany.*

Background: Vascular endothelial growth factor (VEGF)regulates endothelial cell proliferation and participates in interstitial remodelling. In the kidney, VEGF is mainly expressed by podocytes. We evaluated the influence of C-2578A polymorphism, located in the promoter of the VEGF gene, on primary glomerulonephritis.

Methods: We studied 284 patients with biopsy proven primary glomerulonephritis (IgA nephropathy: n=143, focal segmental glomerulosclerosis: n=82, membranous glomerulonephritis: n=59) followed up for 7.0 ± 5.7 years. According to the slope of reciprocal serum creatinine (≥or< -0.1 dl * mg-1 * year-1) group A (slow progressors, n=192) and group B (fast progressors, n=92) were defined. One hundred volunteers were analysed as controls. The biopsies of 156 patients were analysed by the same pathologist. VEGF polymorphism was determined by PCR. VEGF serum levels were determined by ELISA in 105 patients with chronic kidney disease.

Results: VEGF serum levels correlated to the C-2578A genotype: CC/CA: 396 ± 251, AA: 558 ± 425 pg/ml (p=0.018). The genotype frequencies were similar in patients and controls (ns). The initial renal function correlated to the degree of glomerular sclerosis (r=0.520, p<0.001), tubulointerstitial fibrosis (r=0.557, p<0.001) and arteriosclerosis (r=0.469, p<0.001). The percentage of sclerosed glomeruli was higher in group B (44.0 ± 31.1% vs 32.9 ± 28.7% in group A, p=0.051) as was the degree of tubulointerstitial fibrosis (34.1 ± 26.3% vs 24.7 ± 20.2% in group A, p=0.043). There was no significant difference regarding the histological parameters between patients with different genotypes (ns). VEGF gene polymorphism influenced the progression as shown by the genotype distribution in group A (CC/CA: 70.3%, AA: 29.7%) compared to group B (CC/CA: 81.5%, AA: 18.5%, p=0.05). There was also a significant difference in the actual rate of progression (CC/CA genotypes: -0.160 ± 0.417, AA: -0.085 ± 0.132 dl*mg-1 *year-1; p=0.021).

Conclusions: The functional VEGF C-2578A polymorphism is a progression marker in primary glomerulonephritis.

FR-PO1924

Acute Manifestation and 1-Year Follow-Up of a Big Cohort of Patients with Atypical Hemolytic Uremic Syndrome (aHUS) Magdalena Riedl,[1] Johannes Hofer,[1] Alejandra Rosales,[1] Reinhard Würzner,[1] Therese C. Jungraithmayr.[1,2] *[1]Pediatrics, Medical University, Innsbruck, Austria; [2]Gemeinschaft für Pädiatrische Nephrologie (GPN).*

Background: The atypical HUS is a form of thrombotic microangiopathy. Dysfunction of complement proteins are associated with the pathogenesis of the disease. Long-term prognosis is poor.

Methods: Since 2002 the HUSnet Registry investigates the role of complement in aHUS and collects clinical data on long-term outcome. Here we present data of 116 aHUS patients at diagnosis and the 1 year follow-up of 72 patients.

Results: During acute phase the hemoglobin value dropped to 6.03±1.4 mg/dl, the platelet count to 51.3±43.8x109 and mean creatinine was elevated to 4.8±3.5mg/dl. Oliguria/anuria was seen in 59% (mean duration: 13±15 days) of patients. Dialysis was performed in 66% (mean duration: 26±44 days) of patients, of which 30% required chronic dialysis. Arterial hypertension was seen in 79%. Other organ involvement was reported as follows: GI (44%), CNS (28%), cardiac (12%) and pancreas (8%). Treatment of first episode included plasma infusions (PI, 42%) and plasma exchange (PE, 50%). PT was initiated in patients with a higher rate of CNS involvement (p<0.05, X^2) and a tendency towards an increased need for dialysis (p=0.057, X^2).HUS recurrences were reported in 68% (mean 2.6±2.2) of the patients. The first recurrence occurred in median after 4.5 months (range 1-26 months).

One year after diagnosis arterial hypertension was seen in 65%, dialysis in 33% and chronic renal insufficiency in 13% of patients. 51% of the patients had a normal renal function. Two patients died within the first year, due to cardiopulmonary insufficiency. CNS sequel was reported in 1 patient. Patients treated with plasma therapy (PT) showed comparable outcomes to patients without PT after 1 year as measured by the incidence of hypertension, kidney function, CNS sequel and recurrences.

Conclusions: In the acute phase aHUS presents as a multisystem disorder, but in the long term impaired renal function is the main concern. PT is considered as first line treatment, it was especially used in severe cases. Outcome after 1 year was comparable between patients with vs without PT.

Funding: Government Support - Non-U.S.

FR-PO1925

Transcianocobalamin C Deficiency: A Common Cause of Neonatal Thrombotic Mycroangiopathy Gianluigi Ardissino,[1] Francesca Tel,[1] Sara Testa,[1] Fabio Paglialonga,[1] Cristina Felice Civitillo,[1] Francesca Menni,[2] Marta Cerutti,[2] Gabriella Chiarelli,[2] Lorenza Pugni,[3] Fabio Mosca,[3] Alice Monzani.[1] *[1]Pediatric Nephrology Unit, Fondazione Ca'Granda Osp. Maggiore Policlinico, Milano, Italy; [2]Dept. of Pediatrics, Fondazione Ca' Granda Osp. Maggiore Policlinico, Milano, Italy; [3]Neonatal Intensive Care Unit, Fondazione Ca' Granda Osp. Maggiore Policlinico, Milano, Italy.*

Background: Thrombotic mycroangiopathy (TMA) in neonates is extremely rare but when it occurs, transcianocobalamin C deficiency should be suspected.

Methods: We reported 4 cases admitted in our hospital over the past five months.

Results: In all cases symptoms started very early in life with feeding difficulties, failure to thrive and severe hypotonia. In one case left ventricular dilatation had been detected antenatally.

Hereafter, we report the main clinical characteristics of patients at onset.

Patients' characteristics

	SP	MP	DC	MD
Age (days)	21	30	21	19
Weight (Kg)	2.79	3.38	3.30	3.49
PLT (10^3/mm^3)	142	76	120	32
Hb (g/dl)	6.5	7.3	7.8	9.0
LDH (IU/l)	768	911	1101	818
Haptoglobin (mg/dl)	<20	<20	<20	<20
Homocysteine (µmol/l)	17	36	28	>50
sCr (mg/dl)	0.7	0.4	0.6	0.3
uPr/uCr	2.0	10.3	3.8	n.a.
uHb	++++	++	++	+++

uPr/uCr: urinary protein over creatinine ratio - n.a.: not available

The finding of hypomethioninemia, homocystinuria and methylmalonic aciduria led to the diagnosis of methylmalonic acidemia with homocystinuria. Intravenous hydroxocobalamin, oral betain and folic acid were immediately started. During the course of the disease, the first 3 patients developed AKI stage F pRIFLE criteria). Remission of TMA and the recovery of kidney failure took place within next 10 days. Presently, at a mean age of 6 months, all four children are alive and well.

Conclusions: The described "cluster" of TMA due to transcianocobalamin C deficiency, points out that this disease might be a lot more common than diagnosed. Whenever neonatal TMA is detected homocysteinemia should be determined and the disease ruled out. We expect that this report will contribute to an increased awareness regarding this disease among pediatric nephrologists.

FR-PO1926

Mass Spectrometry as a Novel Method for Detection of Podocyturia in Preeclampsia Iasmina Craici,[1] Steven Wagner,[1] Stephen T. Turner,[1] Joseph P. Grande,[2] Vesna D. Garovic.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Background: Increasing evidence suggests that podocyturia may serve as both a reliable diagnostic tool for preeclampsia and as a marker of active disease in proteinuric renal diseases. Reservations exist regarding both the research and clinical utilities of the current method to detect podocyturia, mainly due to its technical complexity, time commitment, and the level of expertise required for interpretation of the results.

Methods: The aim of this study was to develop a new technique for the identification of urinary podocytes based on the detection of podocyte specific tryptic peptides by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which will provide an operator-independent and highly reproducible method. Urine samples collected within 24 hours prior to delivery were centrifuged. One half of the sediment was cultured for 24-hours and then stained with podocin antibody followed by a FITC-labeled secondary antibody to identify viable podocytes. The second half of the pellet was solubilized, digested and analyzed by LC-MS/MS using an internal standard.

Results: We have recruited 12 patients with preeclampsia and 6 patients with HELLP. The diagnosis of preeclampsia was confirmed by the presence of hypertension (>140/90 mm Hg) and proteinuria >0.3 g/24 hour. The diagnosis of HELLP was confirmed based on the accepted clinical criteria of Hemolysis, Elevated Liver enzymes, and Low Platelet count. The presence of podocytes was confirmed in all patients by the podocyte culture method. With the LC-MS/MS technology, we documented the presence of a podocin-specific tryptic peptide in all samples.

Conclusions: LC-MS/MS technology may facilitate the use of podocyturia, as confirmed by the presence of podocyte-specific proteins in the urine, both as a diagnostic test and as a research tool in studying renal injury in human disease and animal models of preeclampsia. In addition, if validated in preeclamptic patients, this technology may be used in future studies to assess both disease activity and response to treatment in a variety of proteinuric renal diseases.

FR-PO1927

Renal Outcome in Patients Presenting with Dialysis Dependent ANCA Associated Vasculitis Treated with Pulsed Intravenous Cyclophosphamide
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[1]UCL; [2]Birmingham University Hospital; [3]Addenbrookes Hospital Cambridge; [4]McMaster; [5]Imperial College London.

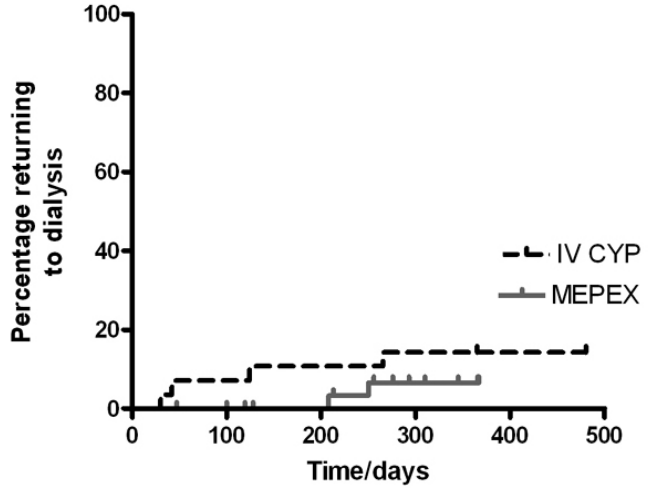
Background: Oral cyclophosphamide (CYP) is the standard of care for patients with severe renal failure (SCr>500 µmol/l) secondary to ANCA associated vasculitis (AAV), while pulsed IV CYP is beneficial for those with less severe disease and induces less toxicity.

Methods: We retrospectively analysed patients with AAV requiring dialysis, who presented between 2005-10 at 2 renal units. All patients were treated with plasma exchange, steroids and IV CYP. We assessed the rate of dialysis independence, as well as the adverse effects of CYP.

Results: Forty-one patients included, 27 male. Mean age 59.7 years. 20 patients MPO-ANCA, 19 PR3-ANCA, 1 ANCA negative, 1 positive for both. 13 patients had concurrent pulmonary haemorrhage. Median number of plasma exchanges 7 (range 2-14); median number of CYP doses 6 (range 1-10) total mean dose 4.75g. Median number of HD days 14 days (range 3-120 days).

12 patients remained dialysis dependent from the time of presentation, including 3 patients who died. 29 patients initially recovered function. 4 patients returned to HD, median time 83 days (range 30 to 265 days). 12 patients had leucopenia, transient in 7. 4 patients relapsed in the 1st 12 months.

At 3 months, 3 dead, 26 patients HD free with 12 on HD (63.4% alive and HD free). At 1 year, 59% patients alive and HD free. This is comparable with the MEPEX study which used oral CYP, in which 52% of patients reaching 1 year were alive and HD free (p=0.58, log rank test).



Conclusions: IV CYP is an effective alternative to oral CYP in dialysis dependent AAV,and results in an equivalent clinical response .

On behalf of the European Vasculitis Study group

FR-PO1928

A Candidate Gene Approach to Genetic Contributors to Development of IgA Nephropathy
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[1]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [3]Department of Nephrology, Osaka Rosai Hospital, Sakai, Osaka, Japan.

Background: Genetic factors contributing to development of IgA nephropathy remains to be elucidated.

Methods: Present multicenter cross-sectional case-control study measured genotype frequencies of 65 atherosclerotic disease-related gene polymorphisms in 230 Japanese patients with IgA nephropathy and 262 apparently healthy volunteers with estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73m^2 and negative or trace for proteinuria and hematuria by dipstick test (non-chronic kidney disease (CKD) participants). Clinical characteristics at kidney biopsy of patients with IgA nephropathy and those at the study recruitment of non-CKD participants were included as covariates in multivariate logistic regression models.

Results: Among 31 gene polymorphisms with ≥5% of minor genotype in non-CKD participants, methionine synthase *MTR* A2756G (D919G) was significantly associated with IgA nephropathy using χ^2 test even after controlling for family-wise error rate by the method of Bonferroni (P = 0.044). A multivariate non-conditional logistic regression model identified *MTR* A2756G as a significant contributor of IgA nephropathy (2756AG and GG vs. AA, odds ratio 0.42 [95%CI 0.25 - 0.69] and 0.21 [0.06 - 0.68], $P_{trend} < 0.001$). After each patient with IgA nephropathy was randomly matched to a non-CKD participant on age (±5 years), gender, mean arterial pressure (±5 mmHg), and eGFR (±5 mL/min/1.73m^2), a multivariate conditional logistic regression model also verified their significant association (0.42 [0.18 - 1.00] and 0.09 [0.01 - 0.73], $P_{trend} = 0.004$). *MTR* A2756G was not associated with slope of eGFR (mL/min/1.73m^2/year) in 230 patients with IgA nephropathy.

Conclusions: Methionine synthase *MTR* A2756G was associated with development, not progression, of IgA nephropathy.

FR-PO1929

Characteristics of Patients with Systemic Lupus Erythematosus Admitted to the Intensive Care Unit
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Department of Nephrology and Transplantation, Guy's and St Thomas' Hospitals NHS Foundation Trust, London, United Kingdom.

Background: Systemic Lupus Erythematosus (SLE) is a multisystem inflammatory disorder associated with significant morbidity and mortality. Affected patients may become critically ill as a complication of disease or treatment. Our aim was to describe the epidemiology and characteristics of SLE patients admitted to the Intensive Care Unit (ICU) in a tertiary teaching hospital.

Methods: Retrospective review of the medical notes of all SLE patients admitted to ICU between 2008-2011 detailing emographics, disease duration and previous treatments alongside reason for ICU admission, APACHE and SOFA scores, level of organ support, immunosuppression and patient mortality.

Results: 25 patients were admitted (88% female; mean age 47±16.8 years). 11 patients were Caucasian, 10 were black and 4 were from other ethnic groups. The most common reasons for admission to ICU were infection (48%), respiratory failure (32%) and renal failure (24%). 64% of patients had been treated with immunosuppression prior to admission (steroids 64%, mycophenolate mofetil 20%) in the preceding 3 months. 44% of patients needed mechanical ventilation, 56% had renal replacement therapy and 76% were treated with antimicrobial therapy. Mean length of stay in ICU was 8.8 days ± 10.1 with a mean hospital length of stay of 30.7 days ± 26.3. 16% died in ICU due to overwhelming sepsis. Among ICU survivors, 1 year survival was 100%.

Conclusions: Infection was the most common reason for admission to ICU. The majority of this group had already been on immunosuppression. Despite a high need for organ support and health care resources, ICU mortality was 16%. The most common cause of death was overwhelming sepsis. 1 year outcome of ICU survivors was excellent.

FR-PO1930

A Novel Murine Model of Arteriovenous Fistula Failure
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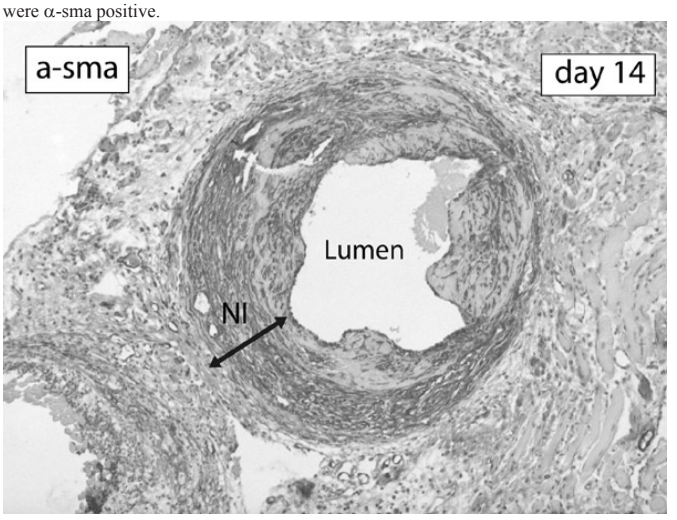
Background: The arteriovenous fistula (AVF) still suffers from a high number of failures caused by insufficient remodeling and neointimal hyperplasia. We developed a mouse model of AVF-failure to unravel the underlying pathophysiology. Since the hemodynamic profile including flow turbulence is an important determinant for the vascular response to fistula insertion, we configured the AVF in an end-to-side manner similar to what is frequently performed in humans.

Methods: AVFs were created by connecting the end of the external jugular vein to the side of the common carotid artery using interrupted 10.0 sutures. Animals were sacrificed at 14 or 28 days. AVFs were processed for histological analysis and sections were stained for hematoxillin, Phloxin and Saffron (HPS), Weigert's Elastin staining and α-smooth muscle actin (α-sma).

Results: At day 14 and 28 after surgery, vessel size increased 20-fold and 32-fold respectively when compared to control veins. Progressive venous neointima formation was observed. At day 14, a luminal stenosis of 65% was observed and at 28 days this number increased to 75%. The majority of the cells that were present in the neointima were α-sma positive.



α-sma staining of venous outflowtract; [NI] neointima

Conclusions: The AVF-model, which resembles the AVF configuration most frequently used in humans, shows that despite substantial outward remodeling, progressive stenotic lesions develop as a result of rapid neointimal hyperplasia in the venous outflow tract. Similar to failed human AVFs, the neointimal hyperplasia is mainly composed of α -sma positive cells. These lesions make this model suitable for intervention studies using e.g. genetically modified mice. We conclude that this murine AVF-model is a good addition to the AVF animal model arsenal.

Funding: Private Foundation Support

FR-PO1931

The Effect of Temporal Variation in Wall Shear Stress on the Remodeling of Arteriovenous Fistulae Ehsan Rajabi-Jaghargh,[1] Prabir Roy-Chaudhury,[2] Yang Wang,[2] Kyuran Ann Choe,[2] Paul Succop,[2] Rupak Banerjee.[1] *[1]CEAS-Schl Dynamic Systems, University of Cincinnati, OH; [2]Dialysis Vascular Access Research Group, Division of Nephrology, University of Cincinnati, OH.*

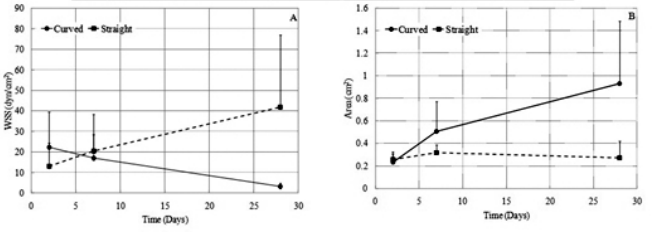
Background: Non-maturation of arteriovenous fistulae (AVF) is in many ways the "Achilles Heel" of hemodialysis. The surgical configuration of the AVF and the subsequent wall shear stress (WSS) are key players in the remodeling of the AVF. This study aimed to investigate the temporal effect of WSS on the maturation of AVFs created in two different configurations.

Methods: AVFs were created between the femoral artery and vein of three pigs in a curved (n=3) and straight (n=3) configuration. Reconstructed geometry of the AVF obtained from CT-scans and flow data from Doppler ultrasound were utilized to numerically evaluate WSS at 2D (D: days), 7D, and 28D post-surgery. Time dependent WSS-area data was regressed using a random effects model: Area = $\beta 0 + \beta 1 time + \beta 2 \Delta WSS/\Delta time$, where βi and Δ were the regression coefficients and the gradient, respectively. A p-value < 0.05 indicated statistical significance.

Results: For the curved AVF, the slope of temporal gradient of WSS (-0.019) had a statistically significant effect (p = 0.022) on the dilation of the vein, while it was not significant for the maturation of the straight AVF (p = 0.53). Also, time had a positive effect ($\beta 1 = 0.337$) on the maturation of the curved AVF (p < 0.05), while it had a negative effect ($\beta 1 = -0.071$) for the straight AVF (p < 0.05).

Conclusions: Our results document a temporal linkage between an increase in diameter and a decrease in WSS in the curved AVF, with an opposite interaction in the straight AVF. Creation of an AVF in a surgical configuration which results in a favorable linkage between WSS and diameter could result in a significant improvement in AVF patency rates.

	Straight Fistula		Curved Fistula	
	regression coefficients	p-value	regression coefficients	p-value
β_0	0.498	0.041	-0.166	0.041
β_1	**-0.071**	<.0001	**0.337**	<.0001
β_2	-0.025	**0.53***	-0.019	**0.022**



Funding: Veterans Administration Support

FR-PO1932

Treatment with Recombinant Human Type 1 Pancreatic Elastase (PRT-201) Does Not Alter the Safety of Angioplasty in a Porcine Arteriovenous Graft Model Dirk M. Hentschel,[1] Steven K. Burke.[2] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Proteon Therapeutics, Waltham, MA.*

Background: PRT-201 is being developed as a treatment for newly created arteriovenous grafts (AVGs) and fistulas to promote access patency. It is not known if degrading elastin fibers in the access outflow vein impacts the safety of subsequent angioplasty procedures.

Methods: Eleven male Yorkshire swine underwent bilateral femoral artery to vein ePTFE grafts insertion (Bard Carboflo 4-6 mm taper) followed by application of PRT-201 6 mg (n=11) or vehicle (n=11) to the external surface of the venous anastomosis and outflow vein. Acute effects were measured using digital photography. At 28 days, AVGs underwent angiography, measurement of graft blood flow (Transonic ReoCath), and angioplasty (Bard Conquest 8mm x 4cm). The animals were euthanized and anastomoses/veins were excised for histology.

Results: Two animals were euthanized early: one due to intestinal torsion, the other due to bilateral AVG occlusion. PRT-201 treated vessels (n=11) increased in diameter by 12 ±14% (p=0.01). In the nine animals surviving to Day 28, 17 of the 18 AVGs had some anastomotic and venous stenosis, one was occluded. Stenosis was > 50% for 5 of 8 PRT-201 and 6 of 9 of vehicle. In this study there were no significant differences in average and minimum lumen diameters and blood flow. Angioplasty was successful in all cases resulting in significant increases in lumen diameter and blood flow. The pressures required to efface stenoses were similar between PRT-201 (10.5±2.6 atm) and vehicle (10.2±3.1 atm). No venous ruptures were documented even after a final inflation of 20atm held for 30sec. Histopathology of the excised veins/anastomoses demonstrated elastin degradation by PRT-201 and a trend to increased mean vein (+18%) and lumen (+26%) areas and decreased mean neointimal area (-25%) for PRT-201 vs. vehicle. There was no apparent adverse effect of PRT-201 with respect to fibrosis, endothelialization, inflammation, and wound healing.

Conclusions: In comparison to vehicle, PRT-201 applied to the venous anastomosis and outflow vein immediately following AVG creation did not impair the safety of subsequent angioplasty.

Funding: Pharmaceutical Company Support

FR-PO1933

Prospective Clinical Investigation of Vascular Structural and Functional Changes Following Hemodialysis Vascular Access Surgery Andrea Remuzzi,[1,2] Anna Caroli,[1] Marko Malovrh,[5] Katia Passera,[1] Luca Antiga,[1] Stefano Rota,[3] Giuseppe Remuzzi,[1,3] Aron Bode,[4] Jan Tordoir.[4] *[1]Mario Negri Institute, Bergamo; [2]Univ. of Bergamo, Italy; [3]Ospedali Riuniti di Bergamo, Italy; [4]Maastricht Univ. Hospital, Netherlands; [5]Univ. Medical Center Liubljana, Slovenia.*

Background: Vascular access (VA) complications represent a major cause of morbidity and hospitalization in hemodialysis (HD) patients and are the major limitation of HD treatment. The ARCH project (EU FP7-ICT) aims to computational modelling tools for surgical planning of VA. These tools are developed using ultrasound (US) measurements and multi-scale computational model of blood flow (BF). Given the high inter-subject variability, the modelling tools must be patient-specific and need calibration and validation using clinical data.

Methods: To this purpose, a prospective observational clinical study has been conducted to collect anatomic, physiologic and clinical data to quantify structural-functional relation between patient vasculature and and its changes after surgery. 93 consecutive patients with ESRD awaiting VA creation have been enrolled in the study (63/30 M/F, age 62Y [18-85]). Pre- and post-operative clinical data and US measurements have been collected for a two years period.

Results: Mean artery diameter in distal (radial artery, RA) and proximal (brachial artery BA) VA and BF before (V0) and after surgery (V1-V4) are as follows.

Visit	time	N	RA Diam mm	RA BF ml/min	N	BA Diam mm	BA BF ml/min
V0	0	52	2.7±0.6	39±33	37	4.2±0.8	89±45
V1	1d	42	3.6±0.9	549±418	22	4.6±0.8	890±554
V2	1w	41	4.0±1.0	676±430	32	4.7±0.7	1288±606
V3	6w	41	4.4±1.1	933±472	28	5.0±0.8	1760±629
V4	>8w	32	4.5±1.1	1057±437	23	5.4±1.0	2229±985

Data are mean+SD. All patients in V4 were on HD treatment.

A large variability in both arterial size and BF was observed during VA maturation. As expected, larger changes in artery diameter were observed in distal than in proximal VA while BF increase was higher in proximal as compared to distal VA.

Conclusions: The clinical data set obtained is currently used to calibrate the model and to simulate BF before and after surgery to investigate major determinants of BF in VA and to predict flow related VA complications.

FR-PO1934

Computational Model for Simulation of Vascular Adaptation Following Hemodialysis Vascular Access Surgery Andrea Remuzzi,[1,2] Simone Manini,[1] Katia Passera,[1] Lorenzo Botti,[1] Wouter Huberts,[3] Luca Antiga.[1] *[1]Mario Negri Institute, Bergamo, Italy; [2]Univ. of Bergamo, Italy; [3]Eindhoven Univ. of Technology, Netherlands.*

Background: Up to 50% of surgical procedures for autologous vascular access (VA) in hemodialysis (HD) patients result in inadequate increase in blood flow volume (BFV). The required increase in BFV after arteriovenous anastomosis of native vessels depends on the ability of the vasculature to dilate and remodel. These changes ultimately determine VA maturation and subsequent use for HD treatment. We have previously reported changes in radial artery (RA) diameter and BFV over time after end-to-end distal fistula creation in 28 ESRD patients. The aim of the present study was to use these data to develop and validate a 1-D computational model of arterial and venous circulation able to simulate changes in vessel diameter in response to surgically induced increase in BFV.

Methods: Blood vessel dimensions and elastic properties have been assumed according to a set of rules defined for generation of patient-specific vascular network models that are dependent on gender, age and body surface area. Arterial and venous diameters, as well as BFV, have been calculated before and after VA creation by assuming constant peak wall shear stress in the arm vasculature.

Results: The best comparison between experimental measurements and computed results of RA diameter and BFV during VA maturation was obtained for a reference arterial peak wall shear stress of 40 dynes/cm2. These results are as follows.

	Time (days)	t=0	t=10	t=40	t=100
RA diameter (mm)	Measurements	2.4	3.7	4.1	4.4
	Computed results	2.5	3.1	4.0	4.3
RA blood flow (ml/min)	Measurements	18	329	476	584
	Computed results	18	380	480	578

t = 0, before surgery. Values are mean of 28 patients.

These results show that the computational model has the ability to accurately simulate flow induced vascular dilatation and the BFV increase in arterial and venous segments that develop with time after VA surgery.

Conclusions: The use of this modelling approach to simulate vascular changes responsible for VA maturation may allow more accurate planning of vascular surgery with the aim to ameliorate surgery outcomes and to increase the rate of VA maturation.

Funding: Government Support - Non-U.S.

FR-PO1935

Hemodialysis Treatment Factors Associated with Outcomes of Arteriovenous Fistula: International Comparison between Facilities Using Data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Manabu Asano,[1] Jyothi R. Thumma,[2] Kenichi Oguchi,[1] Tadao Akizawa,[3] Ronald L. Pisoni,[2] Takashi Akiba,[4] Kiyoshi Kurokawa,[5] Akira Saito.[6] *[1]Renal Unit, Ikegami General Hospital, Tokyo, Japan; [2]Arbor Research Collaborative for Health, Ann Arbor, MI; [3]Division of Nephrology, Showa University, Tokyo, Japan; [4]Tokyo Women's Medical University, Tokyo, Japan; [5]National Graduate Institute for Policy Studies, Tokyo, Japan; [6]Tokai University School of Medicine, Isehara, Japan.*

Background: Various factors have been supposed to associate with the fate of vascular access. Our objective was to clarify the relationship between the dialysis treatment factors and AVF patency across the three regions of Japan, North America (North Am) and Europe/Australia/New Zealand (EUR/ANZ).

Methods: Analyses included 1,288 incident hemodialysis patients on dialysis [[] le[]]7 days and using an AVF at DOPPS II and III entry and 1,046 prevalent patients (on dialysis > 7 days at study entry) who used a new AVF created during study observation for hemodialysis and for which the date of first AVF use was reported. The association of AVF survival with predictable factors (patient characteristics and treatment factors) were compared by the three regions using Cox regression models.

Results: A meaningful relationship appeared to exist between higher rates of primary/final AVF failure and prior catheter use (primary: HR 1.27, 95%CI 1.03-1.57, final: HR 1.38, 95%CI 0.98-1.92). Facilities having higher median blood flow rate (mBFR) were seen to consistently display substantially higher rates of final AVF failure (HR=1.22, 95% CI 1.06-1.40). mBFR varied greatly by region: North Am (mBFR=400 mL/min, IQR=360-448), EUR/ANZ (mBFR=300 mL/min, IQR=300-343), Japan (mBFR= 200 mL/min, IQR=180-200). Analysis not adjusted for mBFR showed higher rate of primary/final AVF failure in North Am (primary: HR 2.14, 95%CI 1.47-3.12, final: HR 2.76, 95%CI 1.34-5.69) and EUR/ANZ (primary: HR 1.75, 95%CI 1.27-2.41, final: HR 2.13, 95%CI 1.13-4.03) relative to Japan.

Conclusions: Of all considered factors, first catheter use and median blood flow rate during hemodialysis only had a significant association with primary/final AVF failure.

FR-PO1936

Causes and Consequences of Arteriovenous Access Failure in Hemodialysis Patients: A Prospective Cohort Study Gurbey Ocak,[1] Joris I. Rotmans,[2] Carla Y. Vossen,[1] Frits R. Rosendaal,[1,3] Raymond T. Krediet,[4] Elisabeth W. Boeschoten,[5] Friedo W. Dekker,[1] Marion Verduijn.[1] *[1]Department of Clinical Epidemiology, Leiden University Medical Center; [2]Department of Nephrology, Leiden University Medical Center; [3]Department of Thrombosis and Haemostasis, Leiden University Medical Center; [4]Department of Nephrology, Academic Medical Center; [5]Hans Mak Institute Naarden, Netherlands.*

Background: Arteriovenous access dysfunction is an important cause of morbidity in patients receiving hemodialysis treatment. The aim of this study was to investigate causes and consequences of loss of primary functional patency within two years in a large Dutch cohort of incident hemodialysis patients.

Methods: We followed 919 incident hemodialysis patients with a functional arteriovenous access, and calculated hazard ratios (HRs) for putative risk factors of primary functional patency using Cox regression. Furthermore, HRs were calculated using time-dependent Cox regression to study the effect of primary functional patency loss on two-year all-cause, cardiovascular (CV), and non-CV mortality.

Results: Age ≥65 years (HR 2.2; 95%CI 1.8-2.8), female sex (HR 1.3; 95% CI 1.1-1.6), CV disease (HR 1.9; 95% CI 1.5-2.4), diabetes mellitus (HR 1.7; 95% CI 1.3-2.1), prior catheter use (HR 1.6; 95% CI 1.3-1.9), albumin (lowest versus highest tertile, HR 1.4; 95% CI 1.1-1.8), high-sensitivity C-reactive protein (hsCRP) (highest versus lowest tertile, HR 1.7; 95% CI 1.2-2.6), and fetuin-A (lowest versus highest tertile HR 2.6; 95% CI 1.7-4.1) were associated with primary functional patency loss after adjustment. Primary functional patency loss was associated with a 2.3-fold (95% CI 1.5-3.5) increased two-year all-cause mortality risk, an 1.6-fold (95% CI 0.9-3.0) increased two-year CV mortality risk, and a 3.1-fold (95% CI 1.7-5.8) two-year non-CV mortality risk after adjustment.

Conclusions: Increased age, female sex, cardiovascular disease, diabetes mellitus, prior catheter use, albumin, hsCRP, and fetuin-A were associated with primary functional patency loss. Primary functional patency loss appears a serious condition with a marked effect on survival.

Funding: Government Support - Non-U.S.

FR-PO1937

Hypercalcemia as a Risk Factor for Vascular Access Failure in Chronic Hemodialysis Patients Myung Jin Choi,[1] Jong-Woo Yoon,[1] Ja-Ryong Koo,[1] Jung-Woo Noh,[1] Eun Young No,[2] Hyun Ok Kim,[2] Man Gyu Lee.[2] *[1]Department of Internal Medicine, Hallym Research Institute, Collage of Medicine, Hallym University, Chuncheon, Republic of Korea; [2]Dialysis Center, Chuncheon Sacred Heart Hospital, Chuncheon, Republic of Korea.*

Background: Chronic kidney disease-mineral bone disorder (CKD-MBD) is known to be an important risk factor for cardiovascular morbidity and mortality. However, it is not known whether vascular access outcomes in hemodialysis (HD) patients are associated with CKD-MBD. We evaluated the relationship between CKD-MBD and vascular access failure in HD patients.

Methods: Sixty patients (age 57 ± 11 years, male 55%, diabetes 43%, arteriovenous fistula 88%, HD duration 59 ± 50 months) who underwent HD for at least 3 months were enrolled. Vascular access failure was defined as thrombosis or hemodynamically significant stenosis requiring intervention. During 2 year follow-up period, the effects of each components of CKD-MBD (mean value of monthly measured corrected calcium, phosphorus and quaterly measured intact parathyroid hormone) on vascular access failure were retrospectively evaluated using Cox proportional hazards regression analysis.

Results: During follow-up periods, the incidence of vascular access failure was 46.7 % (n=28). Among components of CKD-MBD, only corrected calcium level was a significant risk factor for vascular access failure in univariate Cox analysis. Lower serum albumin, hemoglobin and higher predialysis systolic blood pressure (SBP) were also significant risk factors in univariate analysis. After adjustment for dialysis access type, diabetes, aspirin ingestion, serum albumin and predialysis SBP, corrected calcium (hazard ratio, 1.96; 95% confidence interval, 1.038-3.703; p = 0.038) and hemoglobin (hazard ratio, 0.472; 95% confidence interval, 0.230-0.967; p = 0.040) were significant independent risks of the development of vascular access failure.

Conclusions: Higher level of serum calcium was associated with higher incidence of vascular access failure. Calcium loading in CKD-MBD could be a major risk factor of vascular access failure in HD patients.

FR-PO1938

Isometric Handgrip Exercises Improve Success Rates in Arteriovenous Fistula (AVF) Placement in Unsuitable Candidates Alice L. Uy,[1] Christina M. Yuan,[1] Rahul Jindal,[2] Frank P. Hurst.[1] *[1]Medicine (Nephrology), Walter Reed Army Medical Center, Washington, DC; [2]Surgery (Organ Transplant), Walter Reed Army Medical Center, Washington, DC.*

Background: Incident AVF rates remain low. AVF placement is often not attempted because of small vein diameter. We postulated that isometric handgrip exercises would increase forearm vein diameter and allow successful AVF creation in patients who would otherwise receive a synthetic graft.

Methods: Adult subjects without prior vascular access (eGFR< 25; cephalic vein < 2.5mm) were prospectively enrolled. They performed daily handgrip exercises in the preferred access arm (EA), with the non-exercised arm (NEA) as control. Adherence was assessed by exercise logs and grip strength. Vein diameter was measured at baseline, 4 and 8 weeks by duplex ultrasound by the same technician. Primary endpoint was the mean increase in vein diameter (EA vs. NEA). Secondary endpoints were vein diameter increase from baseline, successful AVF placement and maturation.

Results: 16 subjects (7 male and 9 female, median age 77) were enrolled. At present, 11 completed the study. EA grip strength increased significantly. For the primary endpoint, mean vein increase in the EA v. NEA in the distal and proximal veins were 0.31mm v. 0.69mm (p=0.235) and 0.44mm v. 0.66mm (p=0.614) respectively, with a greater increase in NEA. For the secondary endpoints, at 0 to 4 weeks all vein diameters increased significantly. Distal EA veins increased from 1.57mm to 2.14mm and proximal veins increased from 2.23mm to 2.91mm. In the NEA, distal veins increased from 1.58mm to 2.42mm and proximal veins increased from 2.24mm to 3.09mm. Over 90% (10 of 11) of patients achieved at least one vein diameter > 2.5mm at 4 weeks. 3 patients received a successful placement of AVF.

Conclusions: Our data suggested that isometric handgrip exercises resulted in significant increase of cephalic vein diameter with 4 weeks of exercise, in both EA and NEA and may allow AVF creation in those previously not suitable for primary AVF.

The views expressed are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense, or the United States government.

Funding: Other U.S. Government Support

FR-PO1939

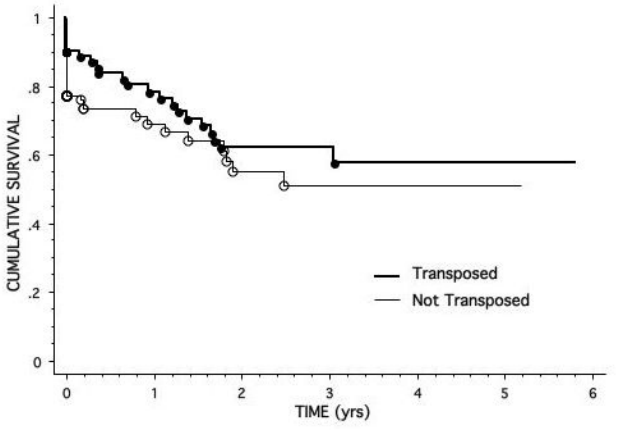
Transposed Brachio-Basilic Fistulae Have Long-Term Survival Comparable to Non-Transposed Arm Fistulae Renu Gupta, Peter Van, Neville R. Dossabhoy.

Background: Transposed brachial-basilic fistulae (TBBF) are not the first choice for all dialysis patients, but may be an attractive option in those patients who cannot get a radio-cephalic or brachio-cephalic fistula (non-transposed, NT). They are a better option than arteriovenous grafts in many respects. However, studies on transposed fistulae are limited. In this study we compared the maturation and survival of TBBF and NT fistulae.

Methods: Our prospective, computerized clinical database was queried retrospectively to identify the clinical outcomes of all upper extremity (UE) fistulae placed at our VA hospital over a 6-year period (2005-10) in CKD and ESRD patients. Patient demographics and comorbid conditions were noted. Primary end points of this study were failure of

maturation and failure of the fistula. Proportions were compared by chi square test. Kaplan-Meier survival curves were also plotted.

Results: 180 UE fistulae were placed in the 6 year period: 81 were transposed (TBBF), 99 non-transposed (NT). Mean age at placement 63 years. African American 64%, Male 99%, Diabetics 61%, HTN 99%, CVD 54%. Patients were already ESRD in 83% cases. Failure of maturation was noted in 9.8% in TBBF and 18.8% in NT group (P=0.11). Fistula failure was 22.5% for TBBF, and 12.2% for NT group (P=0.09). Cumulative survival was 76% and 62% at 1 and 2 yrs for TBBF; and 69% and 55% respectively for NT. The figure shows the Kaplan-Meier survival curves for the two groups (P=0.27).



Conclusions: Transposed brachial-basilic fistulae in our study had an overall survival that was comparable to non-transposed UE fistulae. This study confirms that TBBF remains a viable option in patients who have lost options for non-transposed fistulae.

FR-PO1940

Impact of Past Peripherally Inserted Central Catheters on Prevalence of Functioning Arterio-Venous Fistula in an Outpatient Hemodialysis Unit – Single Center Study Mireille El Ters,[#1] Andrew D. Rule,[#1] Bernice (Bonnie) M. Jenson,[#1] Amy Mahon,[#2] Sanjay Misra,[#2] Robert C. Albright,[#1] Amy W. Williams,[#1] Sandra J. Taler,[#1] Marie C. Hogan.[#1] *[1]Div of Nephrology, Mayo Clinic; [2]Dept of Radiology, Mayo Clinic, Rochester, MN.*

Background: Practice guidelines recommend native arteriovenous fistula (AVF)as the hemodialysis (HD) vascular access of choice. One barrier to successful AVF function is prior central or peripheral venous injury/thrombosis.The impact of prior peripherally inserted central catheter(PICC) on AVF patency among chronic hemodialysis patients has not been systematically examined.

Methods: Medical records of all end stage renal disease (ESRD)patients undergoing HD in the Mayo Clinic HD network and receiving their care in our medical system(n=205) were examined. Baseline characteristics (age, gender, race,history of diabetes, congestive heart failure, peripheral vascular disease & coronary artery disease) & history of PICC line placement were obtained. Patients were considered cases if they did not have a functioning AVF & controls if they did. Those who had PICC placed after AVF creation were excluded. Likelihood of functioning AVF with past history of a PICC was assessed using logistic regression models, with & without adjustment for other clinical characteristics.

Results: Of 205 ESRD patients, 90 did not have functioning AVF. Mean ± SD age was 69 ± 16, 57% were male, 86% caucasian, 56% diabetic, 24% had peripheral vascular disease, and 55% had coronary disease. History of PICC was present in 41% (37/90) of the ESRD patients without a functioning AVF compared to only 14% (16/115) of the ESRD patients with a functioning AVF(OR=4.3, p<0.001).This association increased after adjustment for age, gender & race (OR=4.7, p<0.001),& increased further with additional adjustment for DM, peripheral vascular disease,coronary disease & heart failure (OR=5.5, p<0.001).

Conclusions: Prior PICC placement is an important predictor of lack of functioning AVF even after adjustment for traditional risk factors. More studies are needed to determine whether this association is due to direct venous injury following PICC placement, as its use continues to grow due to perceived convenience and cost-effectiveness.

FR-PO1941

Ipsilateral Dialysis Catheter Use Is Associated with Decreased Long-Term Vascular Access Survival Roman A. Shingarev, Michael Allon. *Department of Medicine, Division of Nephrology, University of Alabama at Birmingham, AL.*

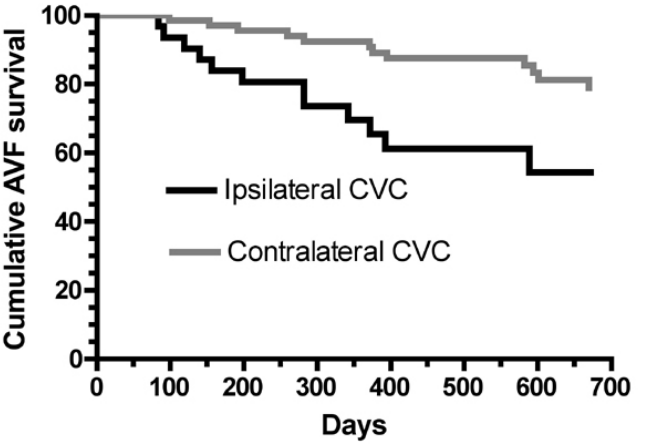
Background: Central venous catheters (CVC) are frequently used for hemodialysis (HD) access while patients await arteriovenous fistula (AVF) or graft (AVG) placement and maturation. CVC may result in central vein stenosis, which could adversely affect vascular access outcomes. We compared the vascular access outcomes in patients with an ipsilateral vs contralateral CVC in place.

Methods: We retrospectively queried a prospective, computerized vascular access database to identify 322 patients who initiated HD with a CVC and had no prior access surgery. Of 233 pts who subsequently received an AVF, 69 had an ipsilateral CVC and 164 a contraleral CVC. Of 89 pts who received an AVG, 27 had an ipsilateral CVC and 62 a contralateral CVC. We calculated primary access failure and cumulative access survival for each subgroup.

Results: Among pts receiving an AVF, primary failure was similar in those with ipsilateral and contralateral CVC, but cumulative survival was inferior in those with an ipsilateral CVC (Table, Figure). Likewise, among pts receiving an AVG, primary failure was similar, but cumulative survival tended to be lower in those with ipsilateral CVC (Table).

Vascular access outcomes in pts with ipsilateral and contralateral CVC

Outcome	Ipsi CVC	Contra CVC	HR	95% CI	p-value
AVF prim fail	50%	53%	0.94	0.70-1.26	0.69
AVF cum surv	54% at 2 yr	74% at 2 yr	2.48	1.33-7.33	0.009
AVG prim fail	35%	38%	0.92	0.49-1.73	0.80
AVG cum surv	22% at 2 yr	58% at 2 yr	2.04	0.92-5.38	0.07



Conclusions: The primary failure rate of AVF and AVG is not affected by the presence of an ipsilateral CVC. However, cumulative AVF and AVG survival is inferior in pts with prior ipsilateral CVC. Avoidance of ipsilateral CVC may improve long-term vascular access survival.

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FR-PO1942

Clinical Determinants of Arteriovenous Fistula Patency, Primary Failure and Longevity in Hemodialysis Patients Srikanth Kunaparaju, Kambiz Kalantarinia. *Nephrology, University of Virginia, Charlottesville, VA.*

Background: Arteriovenous fistulae(AVF) are considered the preferred vascular access(VA). Complications such as primary failure and thrombosis have been barriers in achieving higher AVF rates. In this study in addition to the effect of traditional risk factors, we looked at local factors such as the effect of hemodialysis facility or surgeons' expertise on their outcomes.

Methods: Electronic Medical Records are used for collecting patient demographic, co morbidy and VA related data. The main outcomes studied were overall patency, primary failure(PF) and longevity of AVF. Univariate analyses and multivariate analysis in three different models was performed.

Results: From 695 VA, 428 AVF were studied. Mean age was 60.7 years, 35% females and 55% African American. Patency: Was 70%. The rate was higher for the expert surgeon (76% vs. 67%, p = 0.046) and for those with an AVF as their first VA ever (84% vs. 42%, p<0.0001). In multivariate analysis, hypertension (OR 2.1, 95% CI 1.1 – 4.2), first VA ever (OR 13.1, 95% CI 6.7 – 25.7), expert surgeon (OR 2.4, 95% CI 1.2 – 0.84) and HD facility were associated with higher patency. AVF-PF: 18% had PF and was significantly higher for those created after initiation of HD (20.9% vs.11.8%, p = 0.02) and those with multiple accesses (30.3% vs. 11.9%, p <0.0001).In multivariate analysis, multiple accesses & male gender were associated with better outcomes. Longevity: Median was 16 m. Longevity was lower for women(12.5 vs. 17 mo., p =0.05), those placed after HD initiation (20.8 vs. 33.8 mo., p <0.0001), upperarm AVF (21.8 vs. 32.8 mo., p = 0.01), and those with at least one other HDVA compared to those with first HDVA (16.0 vs. 29.0 mo., p <0.0001). In the multivariate analysis, gender and HD facility were the only associated factors.

Conclusions: Hypertension, first access ever, expert surgeon and HD facility were associated with higher AVF patency. For AVF-PF, male gender and first access ever were significantly associated with better outcomes. First access ever, forearm location and HD facility were significantly associated with longer AVF life span. A history of multiple access surgeries was negatively associated with all three outcomes.

FR-PO1943

Plasma 25-Vitamin D, 1,25-Vitamin D, Parathyroid Hormone (iPTH), and Fibroblast Growth Factor 23 (FGF23) Levels Do Not Predict Vascular Access Thrombosis Shailendra Sharma,[1] Michel B. Chonchol,[1] James S. Kaufman,[2] Alfred K. Cheung,[3,4] Jessica B. Kendrick.[1] *[1]University of Colorado Denver; [2]VA Boston Healthcare System, Boston; [3]VASLCHCS; [4]University of Utah.*

Background: Assessment of risk factors for vascular access failure may provide insight into therapeutic targets for stenosis and thrombosis. Aim of the study was to evaluate the associations of abnormalities of mineral metabolism with vascular access thrombosis in patients requiring hemodialysis.

Methods: We investigate the longitudinal association between 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 levels with vascular access thrombosis in 654 patients requiring hemodialysis, aged, 67± 12 years who participated in a randomized clinical trial evaluating the effects of folic acid and B vitamins on death in subjects requiring dialysis. Thrombosis events were collected only for vascular accesses that were actually being used for dialysis and did not include events that occurred before dialysis initiation or that resulted in failure of access maturation. We used Cox proportional-hazards models to examine the association between abnormalities of mineral metabolism levels with vascular access thrombosis.

Results: Participants had a mean age of 60±11 years and median dialysis vintage of 1.4 years.Vascular access thrombosis occurred in 73 of the 336 patients (22%) with fistulae and 101 of the 221 patients (46%) with grafts at baseline. Increasing log for 1,25D levels (HR 0.77; 95% CI 0.40-1.47; p=0.43) and increasing log FGF23 levels (1.03; 95% CI 0.79-1.34;p=0.82) were not associated with vascular access thrombosis in unadjusted analyses. In multivariate analyses including all predictor variables, age, gender, race, smoking, BMI, treatment assignment, hypertension, diabetes and serum calcium and phosphate levels the associations between log 1,25-vitamin D, land log FGF23 were not significant (p>0.05 for each). Increasing log 25-vitamin D and iPTH were not associated with vascular access thrombosis in unadjusted and adjusted analyses.

Conclusions: In patients requiring hemodialysis, plasma levels of 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 were not independently associated with vascular access thrombosis.

Funding: NIDDK Support, Veterans Administration Support, Pharmaceutical Company Support

FR-PO1944

Erythropoietin Use Is Associated with Increased and ACE-Inhibitor Use Is Associated with Decreased Risk for Failure of Primary Arteriovenous Hemodialysis Fistulae Sylvia Stracke,[1] Lena Hoven,[2] Friedlinde Ernst,[1] Christian Aymanns,[1] Frieder Keller.[2] *[1]Nephrology, University Medicine Greifswald, Greifswald, Germany; [2]Nephrology, University Hospital Ulm, Ulm, Germany.*

Background: Hemodialysis treatment requires a well-functioning vascular access. Access patency is limited by the development of venous intimal hyperplasia, which predisposes to fistula stenosis and subsequent thrombosis. We prospectively followed 100 consecutive patientes receiving a primary arteriovenous hemodialysis fistula.

Methods: At the time of fistula creation, a 5 mm segment of the future shunt vein was removed and examined by histology and histomorphometry. Intima-to-media ratio (IMR) and intimal thickness indices (ITI) were documented. Clinical data were followed for at least one year.

Results: 31 patients showed histologically normal vessel walls (IMR 0.3 ± 0.15; ITI 0.13 ± 0.05). These patients were 70 ys old (range 24-85 ys), 29% were female. The other 69 patients showed intimal hyperplasia with a significantly increased IMR (1.2 ± 1.04; ITI 0.35 ± 0.22; both p<0,001); median age was 67 ys (range 19-86 ys); 29% females. Intimal hyperplasia was seen in 66% of lower arm veins and in 87% of upper arm veins. In 31 cases, stenoses that needed intervention were seen. Stenoses developed at a median of 9.7 mo after operation. Patency of fistulae was 60% after 6 mo, 57% after 9 mo and 56% after 12 mo. Intimal hyperplasia did not predict the development of stenosis, nor did the localization of fistula, sex, age, comorbidities, hyperparathyroidism, CRP or Hb levels. In a cox regression model, we found a hazard ratio (HR) of 8.3 for the use of erythropoietin (p=0.04), HR of 5.3 for comorbid peripheral arterial disease (p=0.009), HR of 2.5 for comorbid coronary artery disease (p=0.03) and HR of 0.2 for the use of ACE-inhibitors (p=0.02).

Conclusions: The patency rate in our 100 primary arteriovenous hemodialysis fistulae of 56% after 12 months is in accordance with the literature. Erythropoietin use was associated with an increased and ACE-inhibitor use with a decreased fistula failure rate in the first twelve months after fistula creation in our cohort.

Funding: Clinical Revenue Support

FR-PO1945

Vein Mapping by Angiography: Findings That Prevent Arterio-Venous Fistula Maturation Zahidul H. Mondal, Candace D. Grant, Mary C. Mallappallil, Fasika M. Tedla, Moro O. Salifu. *Internal Medicine, Division of Nephrology, SUNY HSCB Downstate, Brooklyn, NY.*

Background: Vein mapping is recommended prior to creation of Arterio-Venous Fistula (AVF) in hemodialysis (HD) patients. While ultrasound is sometimes used, it may not be sensitive to the presence of accessory veins and central vein (CV) stenosis that may interfere with AVF maturation. We analyzed results of preoperative vascular imaging with angiography (ANGIO) in 178 HD patients.

Methods: Bilateral ANGIO was performed on 178 patients using 15-20 ml of contrast by our interventional nephrologists. Data were retrospectively abstracted to identify (i) luminal diameter (LD) and (ii) presence of accessory branches in right (R) and left (L) radiocephalic (RC), elbow-cephalic (EC) and elbow-basilic(EB) veins. Presence or absence of CV stenosis was also documented. Veins with LD ≥2.5mm were considered usable. Statistical comparisons were made with chi-square or t-test as appropriate.

Results: Mean age was 57±16[SD] years (range: 18-94), and HD vintage ranged from 0 to >5years. 87% of patients were hypertensive; 53% were diabetic; 49% were men, and most were African American. Only 21/178 (12%) of RRC veins were usable and of these 7/21 (33%) had branches; on the L side, only 12/178 (9.6%) were usable, of which 4/12 (18%) had branches. 42/178 (24%) of REC veins were usable, and 5/42 (12%) had branches; on the L, 39/178 (22%) were usable, while 6/39 (15.4%) had branches. Among the REB veins, 80/175 (45%) were usable, of which 5/80 (6.3%) had branches; on the L, 97/178 (54.5%) were usable, but 11/97 (11%) had branches. CVs were visualized in 96.6%, showing stenosis in 6% of patients. Diabetes was evenly distributed in all categories; patients with usable veins were significantly younger (mean age difference 6-12 years, p<0.05).

Conclusions: ANGIO detected branch-veins in 9.3-33% (average 13%) of usable veins, and central stenosis in 6% of patients --findings less likely to be detected with ultrasound. Usable veins were less likely to be identified in the forearm or in older individuals. These findings underscore the need for vein-preservation strategies in patients with chronic kidney diasease.

FR-PO1946

Arteriovenous Anastomosis Creation Using the Optiflow Anastomotic Connector Lajos Matyas, Istvan Mogan, Adrian Ebner, Mark Mantell, Roberto Manson, Eric Chemla, Sandip Mitra, Milind Nikam, Afshin Tavakoli, Prabir Roy-Chaudhury. *Bioconnect Systems, PA.*

Background: Early arteriovenous fistula (AVF) maturation failure is a major clinical problem. AV fistula failure is frequently preceded by the occurrence of aggressive stenosis in the perianastomotic (PA) region. The Optiflow™ implant device is designed to protect the PA region from the development of stenosis and improve AVF hemodynamics. Clinical data for patients treated with the Optiflow™ was collected and analyzed in order to evaluate safety and clinical performance.

Methods: End to side AVF were created with a novel anastomotic device (Optiflow™). The study population included fifty three patients treated in Paraguay, Hungary, Greece, and the United Kingdom. Forty seven patients were evaluable for patency (5 were lost due to immediate technical failures and 1 patient due to a cardiac event). Unassisted primary patency was evaluated in two different study groups with follow-up time points of 42 (n=10) and 90 days (n=38) respectively. The safety endpoint was freedom from serious adverse events (SAE's) and unanticipated adverse device events (UADE's) during the follow-up period.

Results: Of the 47 patients, 47% were male and 39% were diabetic. Table 1 describes the study results at different time points. Aside from the technical failures, there were no device related SAE's or UADE's.

Study Results

14d Primary Patency	42d Primary Patency	90d Primary Patency	42d ultrasound Vein Size (mm)
100%	91%	82%	6.6 +/- 2.2

Conclusions: The results from the interim data support the safety and effectiveness of the Optiflow™. The 82% unassisted primary patency rate at 90 days for Optiflow™ is favorable when compared to published unassisted primary patency rates of 70% (Falk, 2006) for sutured AVF. Additionally, 42 day ultrasound follow-up performed on 31 patients demonstrated an average vein diameter of 6.6mm. These initial results suggest that the Optiflow™ could potentially play an important role in enhancing AVF maturation.

The Optiflow™ received the CE mark in August 2010.

Funding: Pharmaceutical Company Support

FR-PO1947

Far Infrared (FIR) Therapy – An Effective Treatment for AV Fistula Maturation and Maintenance Iain Moore, Jennifer H. Adam, Debbie Sweeney, Jonathan Murray, Sean Fenwick, Saeed Ahmed. *Renal Unit, City Hospitals Sunderland, Sunderland, United Kingdom.*

Background: Having a functioning arterio-venous fistula (AVF) placed in a timely manner before commencement of haemodialysis (HD) is associated with reduction in patient morbidity, mortality and length of hospital stay. UK renal units are now focused on achieving better than 85% incidence of permanent vascular access rates in their HD population. At the renal unit in Sunderland, we have been using Far Infrared (FIR) therapy to improve our vascular access outcomes since December 2008.

Methods: FIR therapy involves a non-invasive 40 minute heat treatment onto the AVF area. This induces expression of endothelial Heme Oxygenase-1, reducing monocyte adhesions in the endothelium cells, thus inhibiting inflammatory responses that cause further endothelial injury.

From July to December 2010, we used FIR therapy to help maintain AVF function by treating haematomas and pain on needling in our HD unit.

We then extended our FIR therapy usage to the pre-dialysis population, treating patients immediately after AVF formation. We assessed AVF function by Doppler ultrasound.

Results: In one 6 month period, from July to December 2010, 88 HD patients benefited from the use of FIR therapy. 257 individual sessions were completed (median FIR sessions 2, range 1-5).

14/17 patients had improvement of pain score on AVF needling. 23/34 needle-site haematomas resolved more quickly and had improved pain scores compared with those not using FIR therapy.

15/20 AVFs matured with demonstrably better blood flow rates on Doppler, including patients with previous AVF maturation failure.

Conclusions: Currently, we are using FIR therapy across all our HD units as well as on the renal ward. We have instituted a twice weekly, nurse-led Heat Treatment clinic on our Ambulatory Care unit for all patients with new AVFs.

We have found FIR therapy to be of use in AVF maturation, particularly in patients with challenging access, as well as in treatment of haematoma formation and those with AVF pain during HD. We believe FIR therapy offers our patients improved access function, more comfortable treatment provision and decreased access-related morbidity and mortality.

FR-PO1948

Outcomes of Partial Aneurysmectomy in Managing Aneurysm-Associated Complications of Hemodialysis Arteriovenous Fistulae Ammar Almehmi, Shouwen Wang. *Interventional Nephrology, University of Arizona- AKDHC, Phoenix, AZ.*

Background: Fistula aneurysm formations are frequently encountered and their associated complications may affect the viability and function of dialysis vascular access. The key feature of aneurysm-associated complications is that the diseased tissues usually involve only part of the aneurysm, which provides a localized target for intervention. The aim of this study is to describe a targeted approach, "partial aneurysmectomy", for treating these complications and to assess its effects on fistula patency rate.

Methods: This study included 36 dialysis patients who presented with aneurysm-associated complications. All procedures were performed under conscious sedation in an outpatient ambulatory surgery center. Partial aneurysmectomy involved the resection of the diseased skin and aneurysm wall followed by suture repair of the fistula wall and the overlying skin. Kaplan-Meier survival analysis was utilized to calculate the aneurysm intervention-free patency and fistula primary patency rates.

Results: Of the 36 patients: 20 (56%) were males, mean patient age was 54.5±14.6 years, 15 (42%) were diabetics, 35 (97%) were hypertensive, 26 (72%) had upper arm fistula and 10 (28%) had forearm fistula. The average age of the fistula was 72.8±39.5 months. The indications for partial aneurysmectomy were: active bleeding (14%), skin scab or necrosis with fistula defect in imminent danger of bleeding (33%), skin necrosis/erosion (28%), and thin-walled aneurysm in danger of rupture (25%). The procedure was successful in all cases and the patients continued dialysis therapy using their fistulae. The patency rates at 6 months were: 97% for the aneurysm intervention-free patency, 64% for the fistula primary patency, and 97% for the fistula primary-assisted patency.

Conclusions: Partial aneurysmectomy is a simple, safe, and effective intervention for managing aneurysm-associated complications and preserving dialysis fistulae. Further studies with long term follow up are needed to validate the results of this targeted approach.

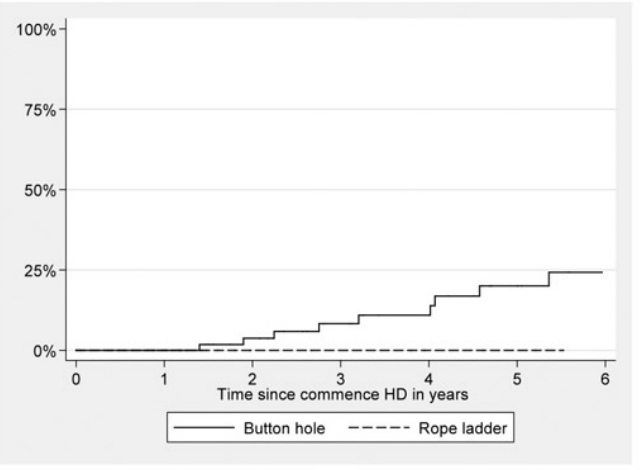
FR-PO1949

Arterio-Venous Fistula Buttonhole Cannulation Technique: A Review of Infectious Complications Frank J. O Brien, Hong Kuan Kok, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background: There are two main methods of arterio-venous fistula (AVF) access; 'buttonhole' cannulation technique and 'rope-ladder' cannulation technique. Several small-scale international studies have hypothesized that the buttonhole technique is associated with increased rates of fistula infection. This study aims to examine this hypothesis.

Methods: A retrospective review of all patients attending a large out patient haemodialysis clinic was performed. Data was collected on cannulation modality, infection rates, organisms grown in the microbiology laboratory, complications of infection, and time on haemodialysis.

Results: 135 patients were noted to undergo haemodialysis via an AVF, 74 in the buttonhole group and 61 in the rope-ladder group. Nine episodes of clinically significant bacteraemia were noted in the buttonhole group.



p=0.02

This equates to a rate of 0.22 bacteraemia events per 100 patient dialysis months. These were attributed to AVF cellulitis. There were no episodes of bacteraemia in the rope ladder group.

8 infections were due to methicillin-sensitive staphylococcus aureus (MSSA); one was secondary to staphylococcus epidermidis infection. Three patients with MSSA bacteraemia subsequently developed infective endocarditis as a complication. Five patients who developed bacteraemia events were undergoing home haemodialysis.

Conclusions: This study highlights the infectious complications associated with buttonhole cannulation techniques. All organisms isolated in our cohort are known skin colonisers. The reason for the increased rates of infection is unclear; however as this is a relatively new technique, further patient and nursing education may help reduce infectious complications. This has particular implications for patient education and training in those doing home haemodialysis, as these patients self needle their fistulae.

FR-PO1950

Buttonhole Versus Sharp Needle Cannulation: Clinical Outcomes in a Home Haemodialysis Cohort Christopher A. Muir,[1] Sradha S. Kotwal,[2] Carmel M. Hawley,[4] Martin P. Gallagher,[2,3] Paul Snelling,[3] Meg J. Jardine.[2,3] *[1]University of Sydney, Australia; [2]The George Institute for Global Health, Sydney, Australia; [3]Statewide Renal Services, Sydney, Australia; [4]Princess Alexandra Hospital, Brisbane, Australia.*

Background: Buttonhole (BH) cannulation is increasingly popular within home haemodialysis (HHD) programs, although recent reports have raised concern about associated infection rates.

Methods: A retrospective review was conducted of consecutive HHD patients dialysing via an arteriovenous fistula (AVF) from training commencement between 01/01/2003 and 31/12/2009 until cessation of HHD, death or 31/12/2010. Co-primary outcomes were systemic access infections (culture-positive infections attributed to dialysis access) and surgical access interventions (AVF surgical intervention or abandonment). Secondary outcomes included all access infections, initial HHD training time, and total staff time requirement (number of days of training, home visits and returns to the unit for dialysis). Incidence-rate ratios (IRR) by cannulation method were calculated using Poisson regression clustered by patient.

Results: There were 35 access infections (9.30/1000 AVF months [AVFmths]), including 17 systemic infections (4.52/1000AVFmths), in 90 patients followed for 3765 AVFmths. BH was associated with a non-significantly higher rate of systemic access infections (IRR 2.71; 95% CI 0.66-11.09; p=0.17) and a significantly higher all-access infection rate (IRR 3.85 (1.66-12.77); p=0.03, absolute increase 8.6 (3.3-13.8) infections/1000AVFmths) compared with sharpneedle cannulation (SN). Surgical access interventions were not different for BH compared with SN (IRR 1.08; 95% CI 0.33-3.55; p=0.90). Adjustment for age and diabetes did not alter the results. BH was associated with longer initial training (mean 51 vs. 41 training days, p=0.01) and increased staff time requirements (1 encounter/13.2 days followup (BH) vs 1 encounter/19.0 days (SN), p<0.001).

Conclusions: In a single unit, BH cannulation was associated with increased rates of infectious events and prolonged initial and ongoing staff support compared with SN. There was no reduction in the requirement for surgical access interventions.

FR-PO1951

Multiple Specialists Perform Percutaneous Hemodialysis (HD) Access Interventions Michael P. Lilly,[1] Nancy Jean Carlson,[2] Janet R. Lynch,[1] Edwin D. Huff.[2] *[1]Mid-Atlantic Renal Coalition (NW5), Richmond, VA; [2]Fistula First Breakthrough Initiative (FFBI) Data Committee, IPRO ERSD Network of NY, Lake Success, NY.*

Background: Interventions to improve maturation or prolong the function of HD access are common. Interventional radiologists (IR) have traditionally done these services in hospitals. Recently, internists/interventional nephrologists (IN) and access surgeons (AS) have begun to perform these access salvage procedures. The extent of this shift in practice is not well documented.

Methods: We reviewed CY 2009 claims submitted to the Centers for Medicare & Medicaid Services (CMS) for dialysis-related access procedures done in NW5. We grouped practitioners by self-designated CMS specialty codes: AS (gen surg 2, thorac surg 33, vasc surg 76+77), IN (int med 11, nephrology 39) and IR (diagnostic radiol 30, interventional radiol 94). We grouped procedures by CPT code: fistulography (36145), percutaneous (precut) interventions (angioplasty 35476, G0392, G0393; and percut thrombectomy 36870), tunneled catheter (HD cath) placement (36558), HD cath exchange (36581), AVF or AVG construction (36818, 36819, 36820, 36821, 36825, 36830) and open thrombectomy/ revision (36831, 36832, 36833, 36834, 37607).

Results: Fistulograms and percut interventions accounted for 67.1% of procedures. IR performed the greatest share of these procedures, but 54% were performed by AS and IN. AS did nearly all open procedures and placed the most of HD caths, while IR or IN did most HD cath exchanges.

Procedure grouping	Surgeons	%	Internists	%	Radiologists	%	Total
Fistulogram	3369	28.6	2951	25.1	5458	46.3	11778
Percut Interventions	3890	23.0	5309	31.4	7714	45.6	16913
Insert HD cath	3138	56.1	441	7.9	2012	36.0	5591
Exchange HD cath	513	27.3	598	31.8	767	40.8	1878
AVF/AVG Construction	4757	99.7	14	0.3	0	0.0	4771
Open AVF/ AVG Revisions	1789	98.6	17	0.9	8	0.4	1814

Conclusions: Changing practice patterns influence the methods used to modify practice behavior. FFBI has focused its efforts on AVF construction on surgeons who still perform nearly all such procedures. Efforts to enhance AVF maturation rates and prolong AVF function must be directed to a broader range of physicians.

Funding: Other U.S. Government Support

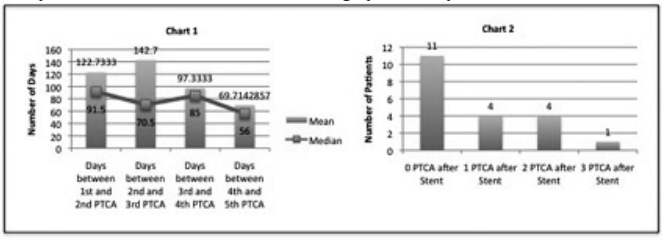
FR-PO1952

Cephalic Vein Arch Stenosis with and without Stent Placement in Hemodialysis Patients Ramanath B. Dukkipati,[1] Luani Lee,[1] Raahil Kajani,[1] Kamyar Kalantar-Zadeh,[1] Naveen K. Atray.[2] *[1]Nephrology, Harbor-UCLA Medical Center, Torrance, CA; [2]Nephrology, Capital Nephrology Associates, Sacramento, CA.*

Background: Cephalic vein arch (CVA) stenosis is a common feature leading to dysfunction and/or failure of brachial artery to cephalic vein fistula. The value and timing of percutaneous transluminal angioplasty (PTA) versus placement of a stent in management of CVA stenosis is not known.

Methods: We examined the risk of recurrence of CVA stenosis after PTA or after stent placement determined by angiography of the involved upper extremity over time in a contemporary cohort of MHD patients treated from March 2008 through May 2011.

Results: We retrospectively identified 45 MHD patients with evidence of CVA stenosis on elective angiograms. The median number of days until another PTA was required decreased (see chart). An association was found between the number of angioplasties and a decreasing median number of days between each subsequent PTA. However, the median number of days between stent placement and the subsequent PTA was much greater (152 days) than the median number of days between the first two angioplasties of a patient who did not have a stent placed. An association was found between increased patency and stent placement. Of the 20 patients who had stents placed, the mean number of angioplasties required after stent placement was 0.75. Of the 25 patients who did not have a stent placed, the mean number of total angioplasties required was 2.76. An association was found between stent placement and a decreased number of angioplasties required.



Conclusions: PTA seems to hasten the recurrence of CVA stenosis. Compared to PTA alone the placement of intravascular stent shows a trend towards a prolonged patency of the CVA. Clinical trials with a larger sample size will better elucidate the value and timing of PTA versus stent placement in CVA stenosis.

Funding: Clinical Revenue Support

FR-PO1953

Cardiac Rhythm Devices and Arteriovenous Access Dysfunction in Hemodialyzed Patients: Experience in a Single Urban Vascular Access Center Brinda Desiraju, Neal Mittman. *Brooklyn Vascular Access, Heights Nephrology Medical Group, Brooklyn, NY.*

Background: Recent attention has been focused on the incidence of hemoaccess-related complications with cardiac rhythm devices (CRD) in hemodialyzed pts. Cardiac co-morbidities are common, and the use of CRD in this population has increased in recent years. The use of contralateral vessels is generally recommended, but the concomitant use of central venous dialysis catheters (CVC) and the presence of unrecognized or developing central venous stenosis and occlusion can complicate this option.

Methods: Retrospective chart review identified forty-three patients with arteriovenous hemoaccess and indwelling cardiac rhythm devices (CRD) referred to our access center for evaluation.

Results: Reasons for referral included arm swelling in 38%, increased venous pressure 14%, thrombosed access 14%, decreased access flows 8%, and prolonged post-dialysis bleeding 6%. Thirty pts had AV fistulae (14 forearm, 16 upper arm) and twenty had AV grafts (5 forearm, 15 upper arm), seven with a second access after prior access losses. CRD were ipsilateral in 21 pts, all of whom had angiographic evidence of central vein stenosis or occlusion, and 71% were symptomatic (arm swelling). Four with ipsilateral fistula received contralateral access, of which two had recurrent arm swelling. CRD were contralateral in 29 pts, of whom 23 (79%) had central vein stenosis or occlusion. Of these 23, 30% had arm swelling, 39% had prolonged bleeding or high venous pressures, and 22% presented with access thrombosis. Arm swelling was more common in pts with upper arm accesses. Fourteen pts had concomitant CVC during a visit.

Conclusions: Pts with ipsilateral CRD are at greater risk of developing symptomatic central vein stenosis. However, a substantial number of pts with contralateral CRD developed symptoms requiring intervention and even access failure. Alternative approaches, including the use of epicardial leads, subcutaneous implantable cardioverter-defibrillators or of peritoneal dialysis have been suggested, but a re-evaluation of the risk-benefit ratio and indications for CRD in this population is in order.

Funding: Clinical Revenue Support

FR-PO1954

Pacemakers & Implantable Cardiverter-Defibrillators in Hemodialysis Patients: Prevalence and Relationship to Ipsilateral and Contralateral Arteriovenous Access Interventions Theodore F. Saad. *Nephrology, Christiana Care Health System, Newark, DE.*

Background: Cardiac rhythm management devices (CRMD), including pacemakers & implantable cardioverter-defibrillators are commonly used for treatment of rhythm disorders in HD patients. CRMD leads may induce central vein stenosis resulting in venous hypertension with ipsilateral arteriovenous (AV) access.

Methods: We surveyed all chronic HD patients in our practice & retrospectively reviewed procedure records to determine the presence of CRMD and the effect of CRMD leads on intervention rates. The study cohort included 1233 HD patients from Jan-Mar, 2011. Data collected included demographics, type & side of AV access & CRMD, dates of access interventions, & sites of lesions treated in the access circuit. Interventions were classified as being performed anywhere in the access circuit & in central veins related to CRMD leads. Rates of intervention were calculated by dividing the number of interventions by the number of access-years (AY) using the current AV access & CRMD.

Results: HD access was a fistula in 767 (62.1%); graft in 271 (21.9%); catheter in 195 (15.8%). 127 patients had a CRMD; ICD's in 69 (5.6%) and pacemakers in 58 (4.7%). Mean age of patients with a CRMD was 72 years, 79 male and 48 female. All CRMD leads were inserted via subclavian or cephalic vein; there were no jugular, femoral, or epicardial leads. 93/127 (73.2%) had left-sided & 34/127 (26.8%) had right-sided leads. Six patients with contralateral access and CRMD had failed previous ipsilateral access due to CRMD-related stenosis. No patient with either ipsilateral or contralateral access was found to have significant SVC stenosis. Rates of interventions are shown in the table:

Rates of Intervention

	Contralateral	Ipsilateral
Number of Patients	80	47
Total Access Years	133.7	109.5
Access Circuit Interventions	204 (1.50/AY)	168 (1.53/AY)
CRMD lead-related interventions	0	66 (0.60/AY)

Conclusions: Prevalence of CRMDs in these chronic HD patients is 10.3%. Access circuit intervention rates were similar for CRMD ipsilateral and contralateral to the AV access; CRMD-lead related interventions were more frequent in the ipsilateral group. SVC stenosis was not demonstrated in any patient.

FR-PO1955

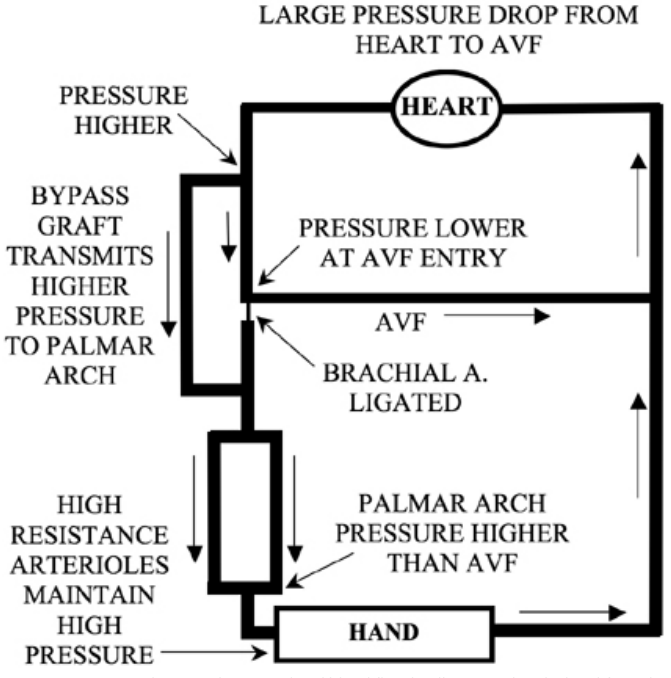
Mathematical Model Explains Why DRIL Procedure Is Effective in Treating Hand Ischemia in Brachiocephalic Fistula William D. Paulson,[1] James J. Wynn,[1] Jan Malik,[2] Vladimir Tuka,[2] Tushar J. Vachharajani,[3] Todd D. Merchen,[1] Steven A. Jones.[4] *[1]Georgia Health Sci. Univ., Augusta, GA; [2]Charles Univ., Prague, Czech Republic; [3]Wake Forest Univ. School of Med., Winston-Salem, NC; [4]Louisiana Tech Univ., Ruston, LA.*

Background: The high flow brachiocephalic fistula (AVF) may be complicated by hand ischemia. The DRIL procedure increases blood flow to the hand by anastomosing a bypass graft to the brachial artery above and below the AVF anastomosis; the brachial

artery distal to the AVF is ligated. Although it is widely accepted that DRIL is effective, there is no consensus on its mechanism.

Methods: We used a mathematical model of the AVF to study effect of DRIL on hand arterial pressure and blood flow. Equations from the engineering literature were used to predict circuit pressures and flow.

Results: The AVF is a high flow shunt with a large drop in pressure by the time brachial artery flow reaches the AVF. This pressure drop reduces hand flow. In the DRIL, the proximal anastomosis of the bypass graft is placed upstream to the AVF where pressure is higher than at the AVF. This higher pressure is transmitted to the hand, which increases hand flow. The narrower the brachial artery, the greater the pressure drop in the artery; this effect is offset by placing the proximal bypass anastomosis further upstream where pressure is higher. The bypass graft and hand are part of a high resistance circuit that is controlled by arteriolar resistances. Pressure is high in this circuit with a minimal drop until the arterioles are crossed.



Conclusions: The DRIL increases hand blood flow by disconnecting the hand from the low pressure AVF circuit and connecting it to the proximal brachial artery where pressures are higher. The model explains why the DRIL is effective, and shows how to optimize application of the DRIL in individual patients with hand ischemia.

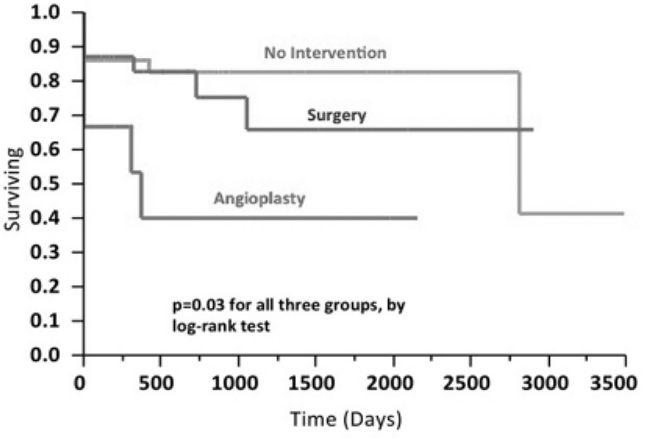
FR-PO1956

Prolonged Cumulative Survival in Fistulas with Surgical Interventions To Promote Maturation Compared to Endovascular Interventions Timmy C. Lee,[1,2] Arshdeep Tindni,[1] Prabir Roy-Chaudhury.[1,2] *[1]Internal Medicine, University of Cincinnati, OH; [2]Dialysis Vascular Access Research Group, Cincinnati, OH.*

Background: Due to high primary failure rates, arteriovenous fistulas (AVF) frequently require either endovascular or surgical interventions to promote maturation (defined as the ability of an AVF to support hemodialysis). The objective of this study was to compare the impact of surgical versus endovascular interventions to promote AVF maturation on cumulative AVF survival.

Methods: We evaluated 89 patients with new AVF placement from a Veterans Affairs patient population. We calculated and compared cumulative survival between AVFs requiring no intervention, and surgery or endovascular intervention to promote AVF maturation.

Results: Among the 89 patients with new AVF placement, 46 AVFs required intervention to promote maturation (31 surgical and 15 endovascular). One year cumulative survival was 86% vs 83% vs 40% for no intervention vs. surgery vs. angioplasty, respectively. Cumulative survival was worse in angioplasty group compared to the no intervention and surgery groups (p=0.03).



Cumulative survival was longer in AVFs receiving surgical intervention compared to angioplasty to promote AVF maturation (p=0.05).

Conclusions: In AVFs that required interventions to promote maturation, AVFs with surgical intervention had longer cumulative survival compared to AVFs treated with endovascular intervention. AVFs with surgical intervention to promote maturation had similar one-year cumulative survival to those AVF that did not require intervention to promote maturation. Our results emphasize the huge need in this area for hard scientific data (from large clinical trials evaluating biology of vascular injury and vascular access outcomes) that could be used to guide clinical practice.

Funding: NIDDK Support

FR-PO1957

Reversible Endovascular Occlusion with Thermo-Sensible Polymer LeGoo® in Arterovenous Fistula Confection Roberto Palumbo,[1] Michele Ferrannini,[1] Annalisa Noce,[2] Simone Manca di Villahermosa,[2] Fulvio Fiorini,[3] Mariapaola Canale,[2] Nicola Di Daniele.[2] *[1]Nephrology and Dialysis Department, S.Eugenio Hospital, Rome, Italy; [2]Nephrology and Dialysis Unit, Tor Vergata University, Rome, Italy; [3]Nephrology and Dialysis Unit, S.Maria della Misericordia Hospital, Rovigo, Italy.*

Background: The temporary interruption of blood flow, using clamps or rubber loops stretching or kinking vessels, is necessary in the confection of arterovenous fistula (AVF). However the vascular trauma may jeopardize the outcome of the procedure. LeGoo® (Pluromed, Woburn, MA), a thermosensitive polymer, is a viscous liquid at room temperatures and changes into a firm occlusive plug instantaneously when exposed to body temperatures. We report five cases of AVF construction using LeGoo® to temporary interrupt the arterial and vein blood flow.

Methods: Five uremic patients (4 male, 1 female) were submitted to AVF construction.

Patients submitted to AVF construction

M/F, age	AVF	Artery caliber (mm)	Renal disease
M, 78	Right radio-cephalic AVF	2.1	hypertension
M, 81	Left radio-cephalic AVF	1.9	hypertension
M, 68	Left radio-cephalic AVF	2.2	ADPKD
F, 72	Left proximal foream AVF	2.1	unknow
M, 84	Left proximal foream AVF	2.4	diabetes

carhacteristics of patients and surgical approach

LeGoo® were employed to occlude the vein and the arteries without clamps or loops. After completing the anastomosis, we employed cold saline on the outer vascular wall to make fluid the polymer.

Results: We obtained a rapid, safety and prolonged emostasis. LeGoo® application is simple and effective. In all cases, the application of cold saline on the outer vascular wall just after completing the anastomosis, made the polymer fluid and promptly cleared by the re-established blood flow.

Conclusions: LeGoo® allows effective hemostasis in distal and proximalized AVF surgery; its use is simple and safe and gives the advantages of a temporary complete, highly selective, non-traumatic, totally reversible endovascular occlusion.

FR-PO1958

Pilot Study Evaluating the Safety and Efficacy of Drug Eluting Balloons in the Treatment of Recurring Shunt Stenosis Deborah Weiss,[1] Beata Lux,[2] Birgit Doris Bader,[1] Christiane M. Erley.[1] *[1]Nephrology, St. Joseph Hospital, Berlin, Germany; [2]Radiology, St. Joseph Hospital, Berlin, Germany.*

Background: With a rising number of HD-patients of an continually increasing age, the percutaneous transluminal angioplasty (PTA) as a treatment option for insufficient dialysis shunts is gaining importance. Though many PTAs are successfull, there remain patients, who suffer from a recurring shunt stenosis. Drug eluting balloons (DEB) could provide a feasible alternative to the standard balloons; even more so since the use of drug

eluting stents in these venous vessels have been considered too problematic by most interventionalists.

Methods: We undertook a pilot study applying new drug eluting balloons (IN.PACT Admiral=DEB coated with paclitaxel, made by KRAUTH cardiovascular GmbH) in patients with recurring shunt stenosis

Results: 11 patients with recurring shunt stenosis were treated with the DEB between 9/2009-12/2009. 1 patient had an AV-fistula, 2 a fistula which had already been corrected by the implantation of a short synthetic graft, and 8 had always had a synthetic graft. All patients had a type III-stenosis, in addition to that 6 patients a type II-stenosis and two patients had a type I-stenosis and a type IV-stenosis, respectively. Apart from 1 patient who took only phenprocoumon all patients were on acetylsalicylic acid, 2 patients received clopidogrel on top, 1 patient phenprocoumon on typ and 1 patient switched to ASS/Dipiridamol. 9 patients underwent a further PTA during the next 4 months. Only one re-stenosis seemed to have developed more slowly. We found, though, that not during all the procedures the special positioning device protecting the paclitaxel-coat hat been used. In the following two years, no apparent increase of restenosis rate, shunt aneurysm or other-possibly procedure related- adverse events have been observed. 2 patients died due to unrelated causes (1 breast cancer, 1 myocardial infarction).

Conclusions: The use of DEB during PTA in shunt stenosis is not primarily associated with a higher rate of complications. The benefit for the patients, especially the prevention of recurring shunt stenosis by using these balloons should be the aim of further investigations.

Funding: Pharmaceutical Company Support

FR-PO1959

A New Prosthetic Graft (RAPIDAX™) for Arteriovenous Access and Early Cannulation: A Single Center Experience Concetta Gangemi,[1] Marta Proglio,[1] Maria Firpo,[2] Michele Giuseppe Messa,[1] Vittorio Ortalda,[1] Antonio Lupo.[1] *[1]Division of Clinical Nephrology, AOUI, Verona, Italy; [2]Division of Vascular Surgery, AOUI, Verona, Italy.*

Background: The AV prosthetic graft is indicated whenever there is a recurrent failure of the native access or the venous bed is not suitable. The main advantage in the use of vascular prosthetic graft is the faster healing time compared to a native fistula, an avarage of 2-3 weeks. In particular, the new ePTFE graft (Rapidax™; Vascutek-Terumo, Renfrewshire, Scotland, UK) thanks to its trilaminar conformation with an elastomer, allows an early cannulation (until 24 hours) and reduces time of bleeding, limiting HD catheter use.

Methods: In the Division of Clinical Nephrology of Verona, a vascular access with Rapidax™ graft was carried out in seven patients. The graft efficacy, patency and post-operative complications were evaluated. Six-month to eighteen-month follow-up data are presented.

Results: in six patients successful first cannulation was achieved within 48 hours, with a mean of 21,7 hours; just one graft was used after 1 week, because the patient had already a HD catheter. At six months graft patency was 100% in all patients. The primary efficacy was evaluated by the eKt/V at three months: mean value was 1.41; at six month 1.58. The mean blood flow at first use was 200 ml/min, at one month 300 ml/min. Time of bleeding was normal. Only one patient had an acute thrombosis 2 hours after graft implantation. Although the small number of patients, no pseudoaneurysm nor loss of graft occurred, even in the one case at eighteen-month follow-up.

Conclusions: this kind of graft permits an early cannulation, reducing the use of HD catheter, preventing also pseudoaneurysm and seroma formation. New ePTFE graft can be a good alternative to long term HD catheter in patients who cannot receive an autologous graft.

FR-PO1960

The Safety and Efficacy of Bedside Tunneled Catheter Removal by Physician-in-Training in an Academic Teaching Setting Vikram R. Beemidi,[1] Naseem A. Qureshi,[1] Albert W. Dreisbach,[1] Eva Csongradi,[2,3] Luis A. Juncos,[1] Tibor Fulop.[1] *[1]Department of Medicine, University of Mississippi Medical Center, Jackson, MS; [2]Department of Medicine, 1st University of Debrecen, Hungary; [3]Department of Physiology & Biophysics, University of Mississippi Medical Center, Jackson, MS.*

Background: Anecdotal experience suggests that procedural aspects of Nephrology training may be endangered. Safety and efficacy of bedside removal of tunneled dialysis catheter (TDC) is relatively little studied in a purely training setting.

Methods: We performed a retrospective cohort review of our consecutive 3-year experience (01/2007 - 12/2009) with bedside TDC removal at the University of Mississippi Renal Fellowship Program. We collected data on patients and procedure-related variables, success and complications rates. The study was reviewed and approved by the University of Mississippi Human Research Office. Data was analyzed with PAWS Statistics 18.

Results: During the index period, we had 55 inpatient TDC removals at bedside under supervision in our teaching log. Of these, 50 (90.9%) were accomplished by Nephrology Fellows with Attending's supervision and the rest of them with Attending/Medical Resident team. 36 (65.5%) TDC was removed from right internal jugular (IJ), 14 (25.5%) from left IJ, and 5 (9.1%) from femoral vein location. Indication at the time of removal included bacteremia in 36.4%, fever in 41.8%, or clinical sepsis with hemodynamic instability or with respiratory failure in 20%. Only 1 complication was observed with prolonged local bleeding, controlled with local pressure.

Conclusions: Our results suggest that bedside removal of TDC is a safe and effective procedure and should be part of competent Nephrology Fellowship Training.

Funding: Clinical Revenue Support

FR-PO1961

Physical Examination of Dysfunctional Arteriovenous Fistulae by Non-Interventionalists: A Skill Worth Teaching Luis Coentrao,[1] Manuel Pestana,[1] Bernardo Faria.[2] *[1]Nephrology Research and Development Unit, Hospital S. Joao, Faculty of Medicine University of Porto, Portugal; [2]Nephrology Department, Hospital S. Teotónio, Viseu.*

Background: Physical examination of arteriovenous fistulae (AVF) has recently emerged as an important element in the detection of stenotic lesions. This study examines the accuracy of physical examination in the assessment of AVF dysfunction by non-interventionalists, in comparison with angiography.

Methods: A prospective observational blinded study was performed among 177 haemodialysis patients with AVF dysfunction consecutively referred to our centre for an angiography procedure. Eleven referring general nephrologists completed a form reporting the physical examination findings regarding their patients' AVFs. Before angiography examination was carried out, a trained nephrology resident performed a physical examination in all the cases. Angiography of the AVFs was then performed by an interventionalist. Cohen's κ value was used as the measurement of the level of agreement beyond chance between the diagnosis made on physical examination and angiography.

Results: There was a moderate agreement beyond chance between the general nephrologists' physical examination and angiography in the detection of AVF inflow problems (κ = 0.49), outflow problems (κ = 0.58) and thrombosis (κ = 0.52). On the other hand, physical examination performed by the trained nephrology resident strongly agreed with angiography in the detection of AVF inflow problems (κ = 0.84), outflow problems (κ = 0.92), and thrombosis (κ = 0.98). The agreement between physical examination and angiography in the detection of coexisting AVF inflow-outflow problems was poor for the general nephrologists and moderate for the trained nephrology resident (κ = 0.14 vs. κ = 0.55, respectively).

Conclusions: Physical examination may provide an accurate means of diagnosis of AVF dysfunction. Theoretical and hands-on training in physical examination of dysfunctional AVFs should be provided for nephrologists in-training and for the dialysis staff.

Funding: Government Support - Non-U.S.

FR-PO1962

Utility of Non-Invasive Arteriovenous Access Monitoring in Children Isa F. Ashoor, Elizabeth Hughson, Michael J. Somers. *Nephrology, Children's Hospital Boston and Harvard Medical School, Boston, MA.*

Background: Limited data exist regarding the use and efficacy of ultrasound dilution monitoring (UDM) as a technique to assess arteriovenous (AV) access patency and reduce AV access complications in children.

Methods: We studied all permanent AV access pts in our pediatric dialysis unit by monthly UDM between 2009-2011. Fistulagrams were prompted by UDM for flow rate <600 ml/min or >20% drop from baseline. Rates of AV access complication (thrombosis, hospitalization, temporary access placement) during the UDM period were compared to baseline rates manifested in the 2 years prior. To account for differences in length of time on HD, rates were determined for individual pts in both groups based on episode count relative to months on HD.

Results: No differences existed between the UDM (n=16) and baseline (n=14) groups with regards to age, gender, weight or age at ESRD diagnosis. In the UDM group (50% boys; median age at ESRD diagnosis 15 yo; median weight 51 kg; median AV access age 12 mos), 162 UD measurements were obtained over median follow-up of 5 mos (range 1-29 mos). Rates of access-related complications (hospitalization, new access formation, or temporary access placement) fell from 4 events/100 pt-mos at baseline to 2.5/100 pt-mos with UDM. Mean rate of thromboses in the UDM group (3.5/ 100 pt-mos on HD) was significantly lower than the baseline group (13.5/ 100 pt-mos on HD) ($p<0.04$). Within the UDM group, mean blood flow rate was lower in AV accesses that went on to thrombose compared to those without thrombosis (1203 ml/min/1.73m2 vs. 1683, $p <0.0001$). In the UDM group, 29 fistulagrams were done, 18 prompted by decreased UDM flow. 55% had hemodynamically significant stenoses requiring angioplasty +/- stenting. UDM was 94% sensitive and 77% specific in detecting hemodynamically significant stenoses with positive and negative predictive values of 83% and 91% respectively.

Conclusions: We conclude that in children on dialysis UDM: 1) reduces adverse outcomes requiring emergent intervention; 2) detects evolving hemodynamically significant stenoses; 3) reduces thrombosis rates in permanent AV access; and 4) has high sensitivity and predictive values as a screening tool.

Funding: Clinical Revenue Support

FR-PO1963

Mapping Segmental AVF Pressure & Resistance with the BlueDop Imager David H. King,[1] Graeme Taylor, Mo Al-Qaisi, Sumith C. Abeygunasekara,[1] Anthony Chan,[1] Yiannis Panayiotopoulos,[3] Abdelgalil Abdelrahman Ali.[1] *[1]Renal Unit, Broomfield Hospital, Chelmsford, Essex, United Kingdom; [2]Department of Physics, Guy's and St Thomas Hospital Trust, London, United Kingdom; [3]Department of Surgery, Broomfield Hospital, Chelmsford, Essex, United Kingdom.*

Background: A novel device 'BlueDop Imager' uses an IP protected method to estimate mean arterial pressure at multiple locations within the body

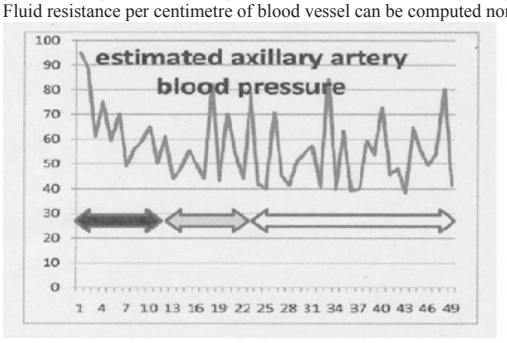
Methods: Data values are derived from the incident pressure and resultant blood flow wave. An algorithm gives mean pressure.Incident data is derived from an arm BP.

The resultant flow wave is displayed on a pc which processes Bluetooth audio transmitted from the Doppler probe and video from a webcam.

Aims: Correlate venous needle pressure(pump off) with BlueDop pressure:Analyse pressure gradients in AVF:Video imaging of probe position makes fluid resistance per centimeter of blood vessel possible.

Results: After nominal correction for CVP(6mmHg)the following pressures were obtained from 14 AVF patients: Needle = 38.4+/-14.8mmHg, BlueDop = 37.4+/-9.9mmHg,corr coeff =0.48. Pressure gradients recorded from 41 AVF (49 gradients), indicate an axillary artery pressure minimum of 40mmHg, (Fig 1a).KEY:failing AVF-black arrow,narrowed AVF-grey,normal AVF=white (true pressure=40+CVP). This may be the first indication of a control loop mediated by the central venous system, effectively protecting the truncal arteries from ischaemia.(Fig 1b) shows probe position plus pressure image.A single flow entry can generate a resistance map.

Conclusions: Averaged BlueDop and direct needle pressure data agree. Labile CVP may be a confounding factor, principally affecting the correlation coefficient at low pressures: A previously unsuspected vasoactive venous control loop has been identified: Fluid resistance per centimetre of blood vessel can be computed non invasively.



a

b

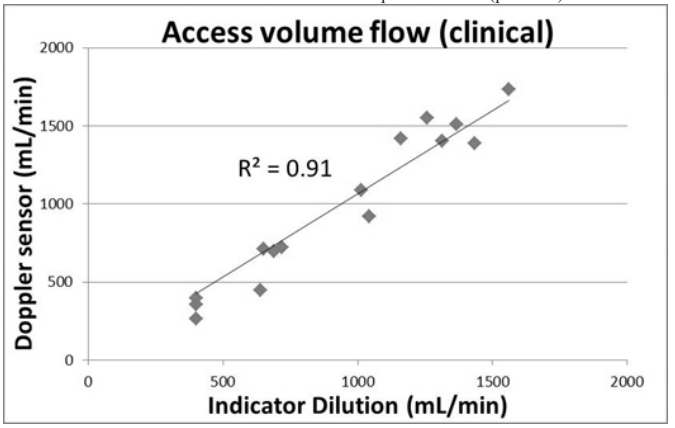
FR-PO1964

Doppler Smart-Sensor for Vascular Access Monitoring William Weitzel,[1] Grant H. Kruger,[1] Brian Thelen,[2] Benjamin Walter Koziol,[2] Benjamin Carpenter,[1] Mainak Mitra,[1] Leo Koziol,[1] David E. Conway,[1] Christopher R. Diroff,[1] Andre Preston,[1] Doug Swaney,[1] Dae Woo Park,[1] Robert Dodde.[1] *[1]Medicine and Engineering, University of Michigan, Ann Arbor, MI; [2]Michigan Technical Research Institute, Ann Arbor, MI.*

Background: Monthly access flow monitoring strategies aiming to reduce morbidity, cost, and improve access decision making have been met with mixed results. We hypothesize that flow measurements monitored throughout every dialysis with functional regression modeling for each patient will allow improved access decision making and reduce costs.

Methods: To investigate this hypothesis we developed a low-cost, low-profile, compact Doppler smart-sensor system with scalable modular hardware and software architecture that can be integrated into the dialysis clinic for continuous patient monitoring. Laboratory testing was conducted using a pulsatile pump (simulating patient access flow) and blood mimicking fluid through phantoms with mock dialysis pump to assess the spectral velocity profile for linearity and determine optimal digital signal processing strategies for access flow ranges from 0 to 2000 ml/min. The prototype was evaluated clinically for performance using indicator dilution measurements as reference measurements.

Results: Laboratory testing showed the Doppler smart-sensor velocities were linearly related to the vascular access phantom volume flow with an R-squared of 0.988 ($p < 0.01$). Clinical comparison of the optimized Doppler digital signal processing model with indicator dilution measurements was correlated with an R-squared of 0.91 ($p < 0.01$).



Conclusions: We conclude it is feasible to obtain accurate flow measurements in the laboratory and clinical setting using this Doppler smart-sensor. Functional regression data analysis is in progress preparing to test this device and monitoring strategy in an upcoming prospective study.

Funding: Other NIH Support - NIH RC1 HL101881

FR-PO1965

The Importance of the Presence of Reverse Color Flow and Collapsibility in Doppler Ultrasound Examination To Evaluate Central Venous Stenosis

Hoon Suk Park, Yu Ah Hong, Sun Ryoung Choi, In O Sun, Byung Ha Chung, Bumsoon Choi, Young Ok Kim, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: The absence of variation in venous diameter or flow pattern during respiration in doppler ultrasound(DU) examination is known as a useful clue to predict central venous stenosis(CVS). However, the variation in venoous diameter or flow pattern during respiration may exist in the cases of CVS. There may be the findings that subclavian vein isn't collapsed when pressed with the probe or the significant amount of reverse color flow exists during expiration in the cases of CVS. We performed this study how exactly DU detect CVS with these additional findings.

Methods: Flow patterns in the both pulsewave and color modes, collapsibility at distal subclavian vein in 2D mode were analyzed in 28 patients. Sixteen patients on chronic hemodialysis underwent subsequently substraction angiography for the evaluation of CVS while the other 12 patients underwent subsequently conventional venography for the creation of access. We define the case with CVS in DU examination that satisfies all of the findings including the variations in venous diameter and flow pattern exist during respiration, venous collapse exsits when pressed with the probe and the significant amount of reverse color flow during respiration doesn't exist. The image findings between DU and substraction angiography or conventional venography were compared.

Results: Kappa between DU and angiography was 0.593(p=0.001). Positive predictive value was 55%, negative predictive value was 100%, sensitivity was 100% and specificity was 77%.

Comparison of DU with angiography

	No stenosis in angiography(N)	Stenosis in angiography(N)	Total(N)
Unsuspicious stenosis in DU(N)	17	0	17
Suspicious stenosis in DU(N)	5	6	11
Total(N)	22	6	28

N: numbers

Conclusions: DU examination with our additional criteria showed favorable power to screen the cases wih possibility of CVS. Therefore, DU can screen asymptomatic patients on hemodialysis with CVS and also detect CVS more efficiently if used with venography prior to access creation.

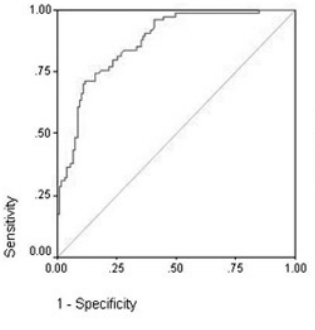
FR-PO1966

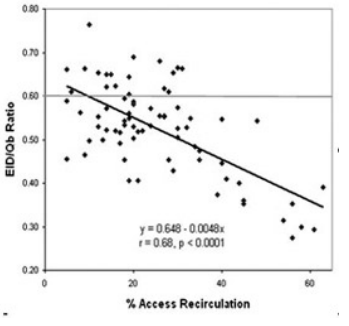
Using Effective Ionic Dialysance/Blood Flow Rate Ratio To Detect Access Recirculation in Hemodialysis Catheters Jennifer Tan,[1] Sumit Mohan,[2] David B. DaRocha-Afodu,[1] Leroy Herbert,[1] Herman L. Anderson,[1] Jen-Tse Cheng.[1] *[1]Div. of Nephrology, Harlem Hospital, NY, NY; [2]Div. of Nephrology, Columbia University Medical Center, NY, NY.*

Background: Vascular catheter use for dialysis remains highly prevalent. Access recirculation is a complication of catheter use and adversely impacts dialysis delivery. We previously reported the utility of effective ionic dialysance(EID)/blood flow rate(Qb) ratio in identifying significant(≥5%) access recirculation (sAR) in AV fistulas. We now present data from 63 patients (59% men, age 53±15.5 yrs, 1.9±2.6 yrs on HD), receiving HD via catheters (86% tunneled catheters, 75±79.8 days of catheter use).

Methods: EID and Qb were measured by Diascan® incorporated in the Gambro Phoenix® HD system during dialysis. Access recirculation was measured at least once monthly by saline dilution technique using Transonic HD-02 monitor.

Results: Among 193 HD sessions when simultaneous measurements of EID, Qb, and access recirculation were performed, we identified 74 instances of sAR. Temporary catheters were associated with a greater incidence of sAR than tunneled catheters(66.7% vs 35.4%, *p=0.019*), as were femoral catheters when compared to IJ catheters(60.9% vs 31.2%, *p=0.002*). No significant difference was seen in the duration of catheter use, yrs on dialysis, and Qb in patients with and without sAR. Patients with sAR had significantly lower EID(191.2±45.9 vs 252.2±35.7ml/min, *p<0.0001*) and demonstrated a significant inverse relationship between the EID/Qb ratio and access recirculation. The area under the ROC curve for EID/Qb was 0.87 and demonstrated a sensitivity of 76% and specificity of 78% at an EID/Qb ratio of 0.60. The highest Youden index was seen at a ratio of 0.58.





Conclusions: Our data show the usefulness of the EID/Qb ratio as an indicator of sAR in dialysis catheters and identifies the practical threshold value for detecting sAR to be an EID/Qb of <60%.

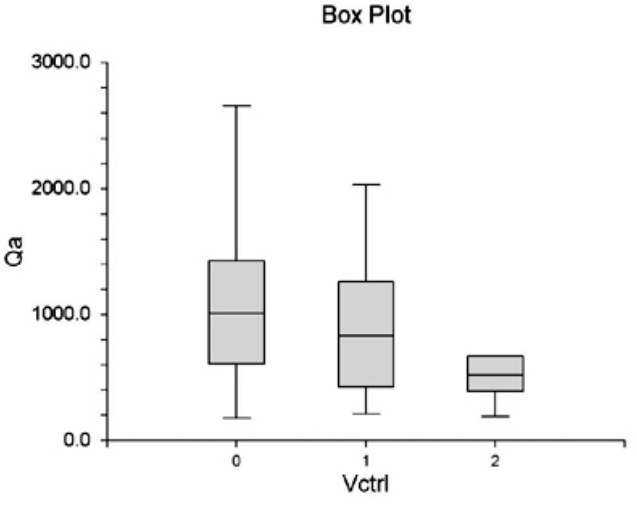
FR-PO1967

Validation of a New Fistula (AVF) Monitoring System by Time Pattern Data Analysis Compared to the Ultrasound Dilution Method (US) in Hemodialysis Patients (HD Pts) Massimo Adorati Menegato. *San Antonio's Hospital, San Daniele, Italy.*

Background: Evaluation of vascular access blood flow (Qa) by US represents the diagnosis gold standard for AVF malfunctions. Unfortunately, US requires sufficient distance between the needles, a dedicated disposable, is time consuming and has a not well-defined Qa threshold. We compared the accuracy of AVF monitoring by US and a new tool (Vascontrol, VCtrl), analyzing the time pattern of parameters, such as arterial and venous pressures (AP, VP), iDy, blood flow (Qb) and Hb.

Methods: We prospectively evaluated 21 HD pts (age 72±18 yrs) with native AVF (thrice-weekly dialysis schedule) for 17 months. The VCtrl approach is based on a supervised learning of a Dynamic Bayesian Networks (DBN) for classifying the dialysis sessions according to a medical risk score (0=good, 1=alert, 2=failed). The DBN system, evaluating the trend of dialytic parameters, calculated a score of AVF status. US test was performed monthly (score 0 Qa≥1000, 1 1000>Qa>600; 2 Qa≤600ml/min).

Results: We assessed 223 AVF monitoring by both US and VCtrl. A significant correlation between VCtrl scores and Qa was found and three different Qa cluster were identified (570±250 vs. 855±196 vs. 1163±50 ml/min, p<0.05 fig.1).



Sexteen angiographies were performed in 12 pts classified by a US score 2, showing 9 stenosis in 8 pts. VCtrl predicted, considering as positive score 1+2, AVF stenosis in 3/9 resulting in a sensitivity of 33.3% and a specificity of 90.4% (Chi^2 test, p=0.023), while US showed a sensitivity of 100% and a specificity of 71.7% (p<0.00005).

Conclusions: US monitoring system confirmed its own validity in AVF stenosis diagnosis, but it requires montly evaluation and presents a relative low specificity, leading in unsuitable angiography prescription. VCtrl seems to be more specific, but it requires further adjustments to increase its sensitivity.

FR-PO1968

Vascular Access Flow Monitoring in Clinical Practice during Maintenance Hemodialysis Improves Graft Survival Eng Kuang Lim,[1] Hui-Lin Choong,[2,3] Manish Kaushik,[2] Soh Theresa,[3] Lay Kwee Chin.[3] *[1]Renal Unit, Department of Medicine, Khoo Teck Puat Hospital, Singapore; [2]Renal Medicine, Singapore General Hospital, Singapore; [3]Kidney Dialysis Foundation, Singapore.*

Background: Ultrasound dilution measurement is one of KDOQI's recommended tools for vascular access surveillance in maintenance hemodialysis (MHD). We studied arteriovenous grafts (AVG) survival after applying this tool in routine clinical practice.

Methods: A retrospective survey of MHD patients using AVG as vascular access in 3 hemodialysis centers was carried out. Data from patients followed up by a single nephrology unit was analyzed. Vascular Access Surveillance (VAS) by ultrasound dilution flow monitoring using the Transonic system (Transonic Inc. Ithaca, NY) had been introduced since 2001. Flow-related problems (AVG flow < 600 ml/min or thrombosis) were promptly referred back to the parent unit for assessment and intervention. The data were divided and analyzed according to 3 study phases: I- Pre-VAS (1997-2000), II-Early VAS (2001-2003) and III-Established VAS (2004-2007).

Results: A total of 119 AVGs were followed in 42 patients (Phase I-16, II-27 and III-40). Etiology of ESRD was Chronic Glomerulonephritis 21 [50%], Diabetic Nephropathy 6 [14.3%], and Lupus Nephritis 4 [9.5%]. There was significant difference in age(I-42.4±9.6yrs, II-46.5±10.5, III-48.8±7.6, p =0.043). There were no significant differences in gender distribution, diabetic and cardiovascular status. Graft loss from thrombosis occurred as follows: Phase I-38, Phase II-29, Phase III-13 events per 100 patient-years. Cox regression survival analysis unadjusted and adjusted for age (using Phase I as reference) showed that AVG survival was significantly better for Phase III (HR 0.35, 95% CI 0.16-0.79, P=0.01 and HR 0.34, 95% CI 0.15 - 0.76, P<0.01 respectively) but not Phase II (HR 0.82, 95% CI 0.37-1.80,P=0.61 and HR 0.73, 95% CI 0.33 - 1.62, P = 0.44 respectively).

Conclusions: Vascular access flow surveillance in maintenance haemodialysis was associated with improved AVG survival despite increasing age of the studied population. There was probably a transition phase after the introduction of access flow monitoring before gains were obvious.

Funding: Government Support - Non-U.S.

FR-PO1969

How To Measure the Access Flow Volume by Doppler Ultrasound in the Cases of Anatomical Variation and Comparison It with Ultrasound Dilution Technique Hoon Suk Park, Sun Ryoung Choi, In O Sun, Byung Ha Chung, Bumsoon Choi, Young Ok Kim, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: In previous studies, comparing doppler ultrasound(DU) with ultrasound dilution technique(UDT) in measuring intra-access flow volume(Qac), Qac by DU was measured at brachial artery or basilic vein at upper arm to avoid anatomical variation that may cause inaccuracy of the measurement. In practice, the sites where the needles are insertetd are different from these sites mostly. We performed this study to investigate how to measure Qac by DU between the needling sites in the cases of anatomical variation and how consistent it is with Qac by UDT.

Methods: We measured Qac by DU only between needling sites in the cases without anatomical variation. But, in the cases with accessory vein or aneurysmal change, we measured Qac at the two needling sites separately and calculate mean of them instead of measuring between the needling sites. Eighty seven Qac were measured in this way by DU and compared it with the Qac by UDT. We also compared the differences of Qac(ΔQac) between these two methods with regard to access location(lower arm VS upper arm), access type(fistula VS graft) and the presence of anatomical variation.

Results: Mean of the Qac measurements in this way by doppler ultrasound was 955.1±569.1ml/min and mean of the Qac measurements by UDT was 1040.4±713.6ml/min. The correlation coefficient(γ) between the two methods was 0.829(p<0.05). ΔQac(expressed by mean±standard deviation) were compared with regard to acecess location, access type and the presence of anatomical variation and there were no differences in ΔQac according to the groups.

Comparison of ΔQac according to the groups

Group comparison	ΔQac(ml/min)	p-value
lower arm VS upper arm	-117.7±436.9 VS -23.5±315.8	0.461
fistula VS graft	-99.3±423.3 VS-48.4±336.4	0.666
presence of anatomical variation VS otherwise	-214±808.46VS-60.4±263.1	0.338

Conclusions: Qac by DU can be measured between or at the needling sites regardless of the presence of anatomical variation, showing the high consistency with the Qac by UDT.

FR-PO1970

Effectiveness of Shower Washing Method without Antiseptics for Exit-Site Care of Noncuffed Hemodialysis Catheters Hiroshi Shibahara,[1] Nami Shibahara,[2] Susumu Takahashi. *[1]Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan; [2]Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan.*

Background: To prevent catheter-related infections with noncuffed hemodialysis catheters (NCC), skin disinfection has long been the mainstay of exit-site care. However, we have reported a care method to prevent infection by restoring the normal skin condition at the exit site, consisting of direct shower-washing without antiseptics. The aim of this study was to evaluate this care method for the exit site of NCCs.

Methods: The subjects were 50 hemodialysis patients (male/female, 33/17; mean age, 69.0 ± 13.3 years; diabetes mellitus/non-diabetes mellitus, 26/24) who had begun to use an NCC and gave informed consent to participate in this study at Sagamihara Kyodo Hospital between January 2008 and January 2010. Our method involved washing the exit site of NCC with physiological saline immediately after catheter insertion. Moisture was wiped away with non-sterile gauze. No antiseptic was applied. Shower washing of the exit-site was continued after every dialysis session until NCC removal. We evaluated the skin condition of the exit site, the incidence of catheter-related infection, and blood examination data [white blood cell (WBC) count, C-reactive protein (CRP), interleukin-6 (IL-6), albumin] at the time of insertion and removal of NCC.

Results: No exit site infection was observed. Slight erythema as compared to the normal skin was observed in two subjects. Three NCCs were removed because of catheter-related blood stream infections. These infections improved immediately following replacement of the NCC together with treatment with antibiotics. There were no significant differences in the values of WBC, CRP, IL-6, and albumin between the times of insertion and removal of NCC.

Conclusions: A shower washing method without antiseptics was effective for management of the NCC exit site. Future studies comparing this approach with conventional care methods in a larger number of subjects are needed.

FR-PO1971

Complications of Vascular Access for Haemodialysis and How To Avoid Them Irena Glowinska,[1] Jerzy Glowinski,[2] Jolanta Malyszko,[1] Edyta Zbroch,[1] Michal Mysliwiec.[1] *[1]Department of Nephrology and Transplantology, Medical University, Bialystok, Poland; [2]Department of Vascular Surgery and Transplantology, Medical University, Bialystok, Poland.*

Background: Maintenance of the vascular access continues to be the Achilles' heel of dialysis therapy. Different strategies have been implemented for monitoring and surveillance of the av fistulae. However, there is still much to do in the field of everyday care and basic monitoring.

The aim of the study was to identify causes of fistula losses that might be saved by the interdisciplanary approach to the problem, and proper cooperation between nephrologist, surgeon, nurse and patient.

Methods: Over 500 cases of reoperation and fistula losses out of total 1500 operation of vascular access, performed on patients from 11 dialysis centers, in 10-year period, made by one vascular surgeon were analyzed.

Results: The most common cause of early dysfunction of fistula was maturation failure. Majority were the result of inadequate blood vessels but some of them were caused by patients' non-compliance. Inappropriate cannulation technique of av fistula led to thrombosed aneurysms or hematomas with subsequent fibrosis. A few patients had symptoms of central or peripheral venous hypertension, mostly treated with endovascular procedure. Many patients required trombectomy and only a few underwent reconstruction of still functioning av fistula. Collateral ligation within 1-2 months after primary operation was rarely performed. Six grafts of total over 70 implanted were removed due to infection and 3 due to post-cannulation damage. Two fistulas were ligated due to steal syndrome (both within 2 months after primary procedure).

Conclusions: Proper understanding of the problem by all people involved (nephrologist, dialysis nurse, surgeon and the properly informed patient) could save many of those fistulas losses. Policy Treat the *failing*, not the *failed* arteriovenous fistula should be implemented more often. Clinical examination of arteriovenous fistula and more frequent use of ultrasonography to detect an early failure might give a chance for better survival. A vascular access should stop being a Cinderella and start its life of first-born son.

FR-PO1972

The Outcome of the Primary Dialysis Access and Its Translation in Prevalence Rates in Chronic Hemodialysis Patients Claudia Praehauser,[1] Tobias Breidthardt,[2] Cora Nina Moser-Bucher,[2] Thomas Wolff,[3] Katrin Bächler,[2] Michael Dickenmann,[2] Lorenz Gurke,[3] Michael Mayr.[1,2] *[1]Medical Outpatient Department, University Hospital Basel, Switzerland; [2]Clinic for Transplantation Immunology and Nephrology, University Hospital Basel, Switzerland; [3]Clinic for Transplantation Surgery, University Hospital Basel, Switzerland.*

Background: In chronic hemodialysis, the creation of a native arteriovenous fistula (AVF) as the primary vascular access is recommended because of higher patency rates and fewer access-related complications. The aim of our study was to investigate the long term outcome of the primary access and its impact on prevalence rates.

Methods: We conducted a retrospective analysis of all patients who had a primary dialysis vascular access created from 1995 to 2006. Primary endpoints were the 'primary access failure free survival' defined as the time from creation of the permanent vascular access to its complete loss, and the prevalence rates of the different access types.

Results: 246 patients were included in the study. 211 (86%) received a native arteriovenous fistula (AVF), 16 (6%) a synthetic arteriovenous graft (AVG) and 19 (8%) a permanent tunneled catheter (PC) as the first permanent vascular access. 1, 3 and 5-year primary access failure free survival rates were 72%, 61% and 51%. Primary access failure free survival was significantly worse for female gender (HR 1.96 (CI 1.24-3.12)), peripheral artery disease (PAD) (HR 1.99 (CI 1.16-3.43)), AVG (HR 2.99 (CI 1.57-5.68)) and PC (HR 3.05 (CI 1.32-7.06)). When stratified by gender, AVG and PC were the only significant risk factors in female patients, while in male patients PAD, malignoma and AVG were associated with a poorer outcome. The rate of patients needing a second vascular access was 32% for patients primarily dialysed by AVF, 75% by AVG and 37% by PC. The overall median prevalence was 80% for AVF, 14% for AVG and 6% for PC.

Conclusions: A high rate of primary AVF contributes to a high prevalence rate of AVF, indicating that in most patients the creation of a native AVF also leads to its sustained use. In consequence, the consistent implementation of the vascular access guidelines achieves their goals.

Funding: Pharmaceutical Company Support

FR-PO1973

Changes in Incident Dialysis Patient Mortality through 2010 Vincent Mor,[1] Franklin W. Maddux,[2] Mahesh Krishnan.[3] *[1]Brown Center for Gerontology & Health Care Research; [2]Fresenius Medical Care; [3]DaVita, Denver, CO.*

Background: The Performance Excellence and Accountability in Kidney Care (PEAK) Campaign is an initiative sponsored by Kidney Care Partners, a organization representing the vast majority of the kidney care community. Its goal is to reduce incident patient mortality by 20% from 2008 through 2012. As part of this program, incident patient mortality was calculated for day 90, day 120 and at one year for all patients entering dialysis.

Methods: In order to get the most recent results, data were obtained from CMS from the Renal Management Information System (REMIS) through December 2010. Beginning dates of entry of patients (from Medical Evidence form 2728), Patient demographic and clinical characteristics (2728/Patient Master file), and Date of death (From Patient Master file, Form 2746 merged with Social Security Master Death File (SSMDF)) were obtained. The 1-year mortality rate, calculated as number of deaths in the first year after dialysis initiation/total number of patient years was calculated. Similar methods were used to calculate the 90 and 120 day mortality rates. Any individual who began dialysis in any given time period January 1, 2007-December 31, 2007 (Cohort 1 - 2007), Jan 1, 2008-December 31, 2008 (Cohort 2 - 2008), January 1, 2009-December 31, 2009 (Cohort 3 - 2009) was included.

Results: Results for each year's 90 day, 120 day, and 1 year mortality rates are displayed below.

	Incident_2007	Incident_2008	Incident_2009
90-day	0.389	0.383	0.372
120-day	0.374	0.369	0.356
1-year	0.267	0.259	0.250

Conclusions: All three measures of incident patient mortality appear to be decreasing. This may be in part due to efforts to reduce catheters and improve the biochemical markers in these patients on the part of nephrologists and dialysis providers. However, other improvements influencing cardiovascular and infectious complications in the general population may also be having an effect. The PEAK Campaign continues to provide insights and interventions to achieve this important goal for the nephrology community and further analysis is required to understand the genesis of these results.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

FR-PO1974

Arteriovenous Fistulas Should Really Be the First Choice Joao Albuquerque Goncalves, Rui Alves Filipe, Raquel Chorão, Ernesto F. Rocha. *Nephrology, Hospital Castelo Branco, Castelo Branco, Portugal.*

Background: The survival of hemodialysis (HD) patients (pt) depends on the long-term functioning and patency of the vascular access. The goal of this investigation is to emphasize the use of "Fistula First" guideline in a region of Portugal, checking the reality of long-term HD access patency, comparing arteriovenous fistula (AVF) to graft and its characteristics.

Methods: Retrospective review of all the HD accesses built from Jan 1st 2008 to June 30th 2010 in a region of Portugal. Follow-up until 15th Jan 2011. Pts were categorized according to demographics, co-morbidities, access date, type and location. Follow-up was considered until pt death or permanent access failure. Patency was calculated. Kaplan-Meyer, Cox-regression and Log Rank test used to analyze data with SPSS®. CI - 95% and $p<0.05$ considered. Access endpoints and complications were documented.

Results: The study reviewed 217 accesses in 181 pts, age 72.1+/-15.4 years, 53% male, 41,5% diabetics; 68.2% AVF and 31.8% grafts; 84,3% proximal, 15,7% distal. Median patency time was 18.37 [16.48; 20.26] months (m), 19.96 m for AVF and 12,5 m for grafts, with 37.4% difference of patency time (p=0,01). The femoral graft had an estimated survival time of 6,9m (p=0,03). There were 44,7% access thrombosis - 55% of grafts (n=38) and 39,8% of AVF (n=59). At 6 months there were 76,6% working AVFs and only 66,7% working grafts (p=0,17). At 1 year of follow-up 68,8% of AVF were working versus 55,3% of grafts (p=0,07). At 18 months there was a significant difference between still functioning AVFs -58,9% and functioning grafts-only 31,7% (p=0,008). There was no difference between diabetics and no diabetics, distal and proximal accesses.

Conclusions: Although both AVF and grafts are useful for pts requiring HD, our data shows that fistulas should really come first because of their longer term patency. This study also reveals the extreme importance of a HD access database.

FR-PO1975

Malnutrition-Inflammation Syndrome in Hemodialysis Patients Associated with Vascular Access Luis Alberto Evangelista-Carrillo,[1] Hildelisa Ordaz Solis,[1] Trinidad Orlando Lugo Lopez,[1] Yeny Pescador,[1] Salvador Mendoza Cabrera,[1] Jorge Andrade-Sierra,[1] Enrique Rojas-Campos,[2] Benjamin Gomez-Navarro.[1] *[1]Department of Nephrology, Hospital de Especialidades, IMSS, CMNO, Guadalajara, Jalisco, Mexico; [2]Medical Research Unit in Renal Diseases, IMSS, CMNO, Guadalajara, Jalisco.*

Background: The Malnutrition-Inflammation Complex Syndrome (MICS) is high prevalent in dialysis. The MICS include anorexia, loss of muscular mass, hypoalbuminemia, refractory anemia and atherosclerosis, also increase morbi-mortality. The association between the type of vascular access and MICS was not been evaluated. **Aim:** To determine the association in patients with MICS and type of vascular access.

Methods: Transversal analytic with 78 prevalence hemodialysis patients. All were evaluated for Malnutrition-Inflammation Score (MIS).

Results: Mean age was 42 years, female was 61%, 20% had Diabetes as cause of CKD, 46% had arteriovenous fistula, 61% received 12,000 UI of EPO (erythropoietin) but only 18% were receiving intravenous iron, 42% had >4 pts in MIS. Age, hospitalization times, antihypertensive drugs were significantly higher in patients with >4pts in MIS compare with <4pts. In logistic regression analysis, had >4pts in MIS were predicted by hospitalization times (p=0.02), low Hb (p=0.04), low alkaline phosphatase (p=.01). Other results are shown in Table.

Comparison between types of vascular access

	All n=78	Catheter n=39	Fistula n=39
MIS (pts)	6(±3.2)	6.3(±4)	5.7(±2.4)
Mean age	42(±20)	43 (±22)	41(±18)
Time in dialysis	45(±35)	28(29)	62(32)*
Hb (gr/dl)	9.6(±1.8)	9.3 (±1.7)	9.3 (±1.7)
EPO (UI/week)	9743(±3832)	10564(±2936)	8923(±4444)
EPO index	1143(±411)	1199(±406)	1080(±413)
Ferritin (ng/ml)	503(±624)	394(±499)	608(±718)
Kt/V	1.4 (±0.7)	1.3(±0.6)	1.4(±0.8)
Phosphorus (mg/dl)	5.6(±1.5)	5.2(±1.2)	6(±1.6)*
Alkaline phosphatase	322(±448)	331(±596)	313(±262)
Albumin (gr/dl)	3.9(±0.5)	3.8 (±0.6)	4.1(±0.4)*
CRP (mg/L)	14(±18)	18.6(±23)	10(±11)
PTHi (U)	890(±804)	643(±502)	1112(±955)*
Hospitalization times	1.18	1.77	0.59*

p=<0.05 between catheter and fistula

Conclusions: According to vascular access was not difference in prevalence of MICS. Although, hyporesponsiveness on EPO and hospitalizations were most frequent in catheter group.

FR-PO1976

The Impact of Vascular Micro-Calcification of Radial Artery on Cardiovascular Mortality in Hemodialysis Patients Yu-Seon Yun, Young Ok Kim, Hyun Gyung Kim. *Nephrology Department, Catholic University of Korea, Seoul, Korea.*

Background: Vascular gross calcification by imaging study is common in hemodialysis (HD) patients, and it is a significant predictor for cardiovascular mortality in HD patients. But vascular micro-calcification (VMC) by pathologic study has been rarely reported. Recently, we have reported that VMC is associated with vascular access failure as well as aortic stiffness (2010 ASN). The aim of this study was to determine the impact of VMC of radial artery on cardiovascular mortality in uremic patients receiving vascular access operation.

Methods: One-hundred forty nine HD patients (Mean age; 59.0 ± 13.9 years, Male/Female; 86/63, Percent of diabetes mellitus; 65.8%) receiving vascular access operation were included in this study. During the operation, we obtained partial arterial specimen and performed pathologic examination by von Kossa stain to identify VMC. We investigated cardiovascular mortality for at least 1 year after the operation. Finally we compared clinical and laboratory findings, and cardiovascular mortality between the patients with VMC and those without VCM.

Results: Mean duration of follow-up was 37.8 ± 34.5 months and the incidence of VCM was 38.8% (n=57). Diabetes (OR 4.138, 95% CI 1.631-11.830, p=0.002) and peripheral artery disease (OR 9.958, 95% CI 1.021-1339.97, p=0.048) were independent predictors for VCM. Serum parameters were not significantly related to VMC. During the period of flow up, there were 27 cardiovascular deaths. Kaplan-Meier analysis showed an increased cardiovascular mortality risk (HR 2.613, 95% CI 1.196-5.711, p-value= 0.016) in VMC group, and Cox regression analysis confirmed that VMC was an independent predictor for cardiovascular mortality (HR 2.352, 95% CI 1.087-5.088, p-value= 0.030).

Conclusions: This study demonstrates that VMC is a strong risk factor of cardiovascular mortality in HD patients.

Funding: Private Foundation Support

FR-PO1977

Novel Blood Access for Home Hemodialysis Frank Prosl.[N/A] [1]*Koestenberg, Austria;* [2]*Duxbury, MA.*

Background: HDA is called Achilles Heel of dialysis from poor safety & performance & reliability. We describe a novel Hemodialysis Access (HDA) system which meets critical needs for improved outcomes & helps patients perform home hemodialysis.

Methods: HDA problems spanning 4 decades seemed intractable. Patients report strong dissatisfaction with HDA in several newly published surveys. Patients achieve best QoL with home hemodialysis(HD)which further detracts on HDA performance & safety requirements. Forecasts indicate increasing numbers of HD patients cannot be sustained forcing policy consideration towards patient personal care. Authors performed fundamental analysis of a HDA system seeking to better performance for critical characteristics & added requirements for patient self-accessing. Additional mandate was to satisfy patient's important self-image concerns of HDA & enable easy secure self-accessing.

Results: The approach was to design elements for solving various problems & integrate them into an elegant system. Our HDA system comprises an implantable port, a novel tissue tract construct, new prophylaxis composition & method, new ancillary tools & methods for accessing and forming the engineered tissue tract. Performance benefits include: 1)"Fail-Safe" design from needle & bloodline dislodgement 2) Simple accessing without pain, bleeding, docking mistakes or tissue damage or infection 3) Increased fluid integrity during HD & between times & comprehensive effective prophylaxis 4) Preserved patient self- reliance & self –image, no stigma or loss of arm function & no bleeding 5) Novel engineered tissue tract comprising new etiology and tissue morphology suitable for connector tissue penetration in precise alignment without guessing (i.e., not buttonhole) & 6) Substantial robustness & system life time, low costs.

Conclusions: Test models demonstrate superior safety and ease of patient accessing. Worst case blood loss is limited to volume in the extracorporeal circuit. Air embolism is prevented in case of dislodgement. The system will help attract patients for home HD & remove the major cause of partner burnout. This HDA should help create conditions favoring HD at home.

FR-PO1978

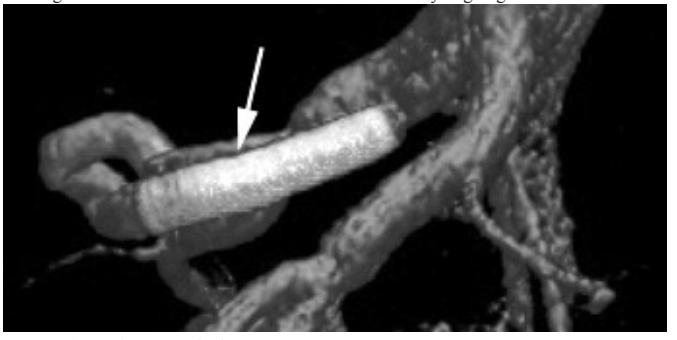
"Hybrid" Graft Reduces Venous Stenosis in a Pig Arteriovenous Graft Model Prabir Roy-Chaudhury,[1] Yang Wang,[1] Meenakshi J. Mistry,[1] Begoña Campos,[1] Timmy C. Lee,[1] Kyuran Ann Choe.[1,2] [1]*Dialysis Vascular Access Research Group, Division of Nephrology and Hypertension, University of Cincinnati, OH;* [2]*Department of Radiology, University of Cincinnati, OH.*

Background: Arteriovenous graft (AVG) stenosis due to neointimal hyperplasia (NH) remains an important cause of hemodialysis vascular access dysfunction. Despite the magnitude of the clinical problem there are currently no effective therapeutic interventions. The "Hybrid" graft (manufactured by WL Gore) is a heparin bonded graft with a nitinol reinforced section (NRS) at the venous end. Insertion of the NRS through a venotomy followed by expansion results in a sutureless end to end anastomosis (see Figure). The biological rationale for the "Hybrid" graft is that the functional end to end anastomosis will optimize hemodynamic profiles resulting in a reduction of NH.

Methods: Standard AVGs and the "Hybrid" grafts were placed between the femoral artery and vein on opposite sides. Animals were auscultated every 3 days to document patency. A 64 slice CT angiogram was performed at 6 weeks to assess for stenoses within the arteriovenous circuit. The maximal degree of stenosis within the access circuit was used for the statistical analysis. A thrombosed graft was considered to constitute a 100% stenosis.

Results: The majority of stenoses in both the control and "Hybrid" arms were at the graft-vein or NRS-vein junction, or within the first 3 cms of downstream vein (in a cranial direction). Control grafts had a mean percentage stenosis+/-SE of 83.3+/-12 % as compared to 45+/-10% for the "Hybrid" grafts (p=0.024;paired t test).

Conclusions: Our results suggest that the "Hybrid" graft reduces venous segment stenosis as compared to control grafts in our pig model of arteriovenous stenosis. Further investigations as to the mechanisms involved are currently ongoing.



Funding: Pharmaceutical Company Support

FR-PO1979

Geographic Variation and Trends in Vascular Access-Related Infection Rates in the United States Rajiv Saran, Joseph M. Messana, Erik Roys, N. A. Lueth, Casey Parrotte, T. H. Shearon, John Kalbfleisch. *UM-KECC, Univ. of MI, Ann Arbor, MI.*

Background: The 2011 Dialysis Facility Reports will include information on dialysis access-related infection (ARI) rates for Medicare Hemodialysis (HD) patients for 2007-2010. These metrics were derived from ICD-9 codes for dialysis ARI for HD patients (996.62 - Infection and inflammatory reaction due vascular device, implant and graft) and will help dialysis providers compare their infection rates to national, state, and ESRD Network averages.

Methods: We describe variation in ARI rate across dialysis facilities and geographic regions. ARI rates per 100 patient months were calculated using ICD-9 codes reported in Medicare claims 2007-2010 and data from other national ESRD data. Poisson regression (log link, offset=ln of patient months) was used to assess the association of facility characteristics with infection rates and to establish expected values and standardized infection rates.

Results: Significant facility variation exists in vascular ARI rates across the country. Over the 4 years of observation, these infection rates have significantly declined overall ($p<0.001$ average decrease 0.05). The new measure of vascular ARI rates is strongly correlated with known predictors of infection such as patient age, percent of patients with diabetes, and percent of patients using catheter at those facilities ($p<0.001$). Mortality associations with vascular ARI were completely abrogated by adjustment for percent facility use of catheter as vascular access ($p<0.01$ to $p=0.71$).

Conclusions: The decreasing trend in vascular ARI is reassuring, but requires continued monitoring. Decreasing use of dialysis catheters should remain a national priority. Our study helps to validate the calculation of infection rates using ICD-9 codes derived from Medicare claims. Lowering the proportion of catheters as vascular access can reduce vascular ARI and potentially mortality resulting therefrom.

Funding: Other U.S. Government Support

FR-PO1980

Development of an Adult-Like Model of Autosomal Dominant Polycystic Kidney Disease by Manipulating the Timing and Extent of Pkd1 Deletion in a Conditional Knockout Mouse Thomas A. Natoli, Kelly A. Rogers, Laurie A. Smith, Sarah E. Moreno, Nikolay Bukanov, Herve Husson, Oxana Beskrovnaya. *Cell Biology, Genzyme Corp., Framingham, MA.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) results from mutations in one of two genes, PKD1 or PKD2. Development of effective therapies to slow cyst growth and preserve renal function requires the use of preclinical models that closely mimic ADPKD. Pkd1 conditional knockout mice have been generated to develop such a model. However, several limitations remain. Conditional inactivation of mouse Pkd1 gene prior to postnatal day 14 leads to aggressive kidney cyst formation within the neonatal period and early death. Deletion after postnatal day 14 leads to cyst formation in adults, but with a very long lag period that is inconvenient for therapeutic testing. We set out to develop a more adult-like model of ADPKD to study molecular pathogenesis and to test potential therapies. Here, we use a tamoxifen-inducible Cre fusion protein and a Pkd1 conditional knockout allele to show that altering the timing of deletion during the first 10 days of life modifies the extent of cystogenesis; deletion on postnatal day 1 gives an aggressive cystic phenotype, while progressively milder cystogenesis is obtained with deletion on days 2, 3, 4, 5 or 8. We further demonstrate that altering the dose of tamoxifen also modifies cystogenesis: using doses of tamoxifen ranging from 10-250 mg/kg delivered at postnatal day 1 produces a dose-dependant PKD phenotype, with lower doses producing fewer kidney cysts, slower decline in renal function and survival up to 6 months of age. Molecular analysis of cystic kidneys reveals changes in a number of signaling pathways, proliferation and apoptosis similar to human ADPKD. The kidneys from these animals also demonstrate signs of fibrosis, which begins after cyst formation. By manipulating the timing and extent of Pkd1 deletion, we have now developed an adult-like model of ADPKD that recapitulates many of the features of human disease and can be used for therapeutic testing.

Funding: Pharmaceutical Company Support

FR-PO1981

Treatment of Embryonic Pkd1 Mutant Mice with the HDAC Inhibitor Trichostatin-A Reduces Cyst Growth Carol G. Carlton,[1,2] Binu M. Paul,[1,2] Lynn Magenheimer,[2] Madhulika Sharma,[1,2] Gregory B. Vanden Heuvel.[1,2] *[1]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [2]Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

Background: Cux1 is a homeobox gene involved in cell cycle regulation and kidney development. Cux1 represses the cyclin kinase inhibitor p27 during early kidney development, promoting cell proliferation in the nephrogenic zone. Promoter reporter analysis of p27 revealed that Cux1 represses p27 in a concentration dependent manner, and chromatin immunoprecipitation showed that Cux1 interacts with the co-repressor Grg4 and the histone deacetylases HDAC1 and HDAC3 on the p27 promoter in vivo. To determine whether HDACs are required for Cux1 repression of p27 we analyzed p27 promoter activity in the presence of the HDAC inhibitor trichostatin A (TSA). TSA treatment completely relieved the repression of p27 by Cux1 and Grg4, demonstrating that Cux1 represses p27 in an HDAC dependent manner. Cux1 is upregulated in the kidneys of both mice and humans with ADPKD. However, Cux1 transgenic mice do not develop cystic kidneys, indicating that upregulation of Cux1 alone is insufficient to develop PKD. Rather, mice carrying both a collecting duct deletion of Pkd1 (Pkd1CD) and a targeted deletion of Cux1 showed that Cux1 is required for cyst growth. Moreover, p27 was upregulated in the cyst lining cells. To begin to test whether HDAC inhibitors could be used to target Cux1 repression of p27 for the treatment of PKD, we treated Pkd1CD mice with TSA or vehicle beginning at embryonic day 10.5 until embryonic day 18.5. Newborn Pkd1CD mice that received vehicle exhibited extensive collecting duct cysts, while newborn Pkd1CD mice that received TSA showed a significant reduction in cysts. Taken together, these results suggest that HDACs are required for cyst growth, and raise the possibility that HDAC inhibitors may be an effective treatment for PKD.

Funding: NIDDK Support

FR-PO1982

Cilia Defects and Polyploidy Linked to Low PC1 Expression in Kidney Epithelia Cells Are Mediated by Increased Expression of Sirt2 and HDAC6 Xia Zhou,[1,2] Lucy X. Fan,[1,2] Wei Liu,[1,2] William E. Sweeney,[1,2] James P. Calvet,[4] Ellis D. Avner,[1,2] Xiaogang Li.[1,2,3] *[1]Children's Research Institute, Children Hospital of Wisconsin, Milwaukee, WI; [2]Pediatrics, Medical College of Wisconsin, Milwaukee, WI; [3]Physiology, Medical College of Wisconsin, Milwaukee, WI; [4]Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS.*

Background: The association of PC-1 and PC-2 with the primary cilium has led to the ''primary cilia'' hypothesis in ADPKD. However, how polycystin(s) levels affects ciliogenesis is still unknown.

Results: In this study, we found that HDAC6 and its binding partner, Sirt2 were upregulated in *Pkd1* mutant mouse kidney epithelial (MEK) cells and tissues compared to Pkd1 wild type MEK cells and tissues. To examine the role of HDAC6 and Sirt2 in ciliogenesis we over-expressed either HDAC6 or Sirt2 in mouse inner medullary collecting duct (mIMCD) cells. Over-expression of either HDAC6 or Sirt2 significantly reduced the percentage of cells with cilia. Inhibition of HDAC6 with either trichostatin (TSA), a general HDAC inhibitor, or tubacin, a specific HDAC6 inhibitor, or inhibition of Sirt2 with nicotinamide, prevented cilia disassembly and increased the acetylation of a-tubulin in both Pkd1 wild type and mutant renal epithelia. Nicotinamide treatment also resulted in a significant increase in the percentage of Pkd1 mutant renal epithelial cells that developed cilia. These results suggest that while HDAC6 may regulate cilia assembly and disassembly during the normal cell cycle, Sirt2 levels appear to be critical for ciliogenesis in cystic epithelial cells. In addition, we found that overexpressing Sirt2 but not HDAC6 induced polyploidy and centrosome amplification similar to that found in the Pkd1 knockdown mIMCD cells.

Conclusions: In summary, reduced PC1 levels results in increased HDAC 6 and Sirt2. While HDAC6 is important in cilia assembly and disassembly, Sirt2 plays a central role in cilia development and increased Sirt2 levels lead directly to polyploidy and centrosome amplification. This is the first report that mechanistic links reduced PC1 levels to ciliogenesis, polyploidy and centrosome amplification.

Funding: NIDDK Support

FR-PO1983

The Microtubule-Associated HDAC6 and Sirt2 Regulate Epidermal Growth Factor Receptor (EGFR) Endocytic Trafficking and Degradation Wei Liu,[1,2] Lucy X. Fan,[1,2] Xia Zhou,[1,2] William E. Sweeney,[1,2] Ellis D. Avner,[1,2,3] Xiaogang Li.[1,2,3] *[1]Children's Research Institute; [2]Pediatrics, ; [3]Physiology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Apical mislocalization, over-expression and hyperactivity of the epidermal growth factor family of receptors and ligands (EGFR axis) has been shown to create an autocrine/paracrine loop of aberrant proliferation in human ADPKD epithelia *in vivo* and *in vitro,* as well as in rodent models of ADPKD and ARPKD. However, the mechanism(s) involved in regulating apical EGFR stability and endocytic trafficking in cystic epithelial cells is unknown.

Results: In this study, we present evidence for the first time that both histone deacetylase 6 (HDAC6) and its binding partner, Sirtuin family protein 2 (Sirt2), regulate EGFR degradation and trafficking along microtubules in Pkd1 mutant kidney epithelial cells. HDAC6 and Sirt2, microtubule-associated deacetylases with tubulin deacetylase activity, demonstrate increased expression in Pkd1 mutant mouse embryonic kidney (MEK) cells. We found that targeting HDAC6 or Sirt2 with a general HDAC inhibitor, trichostatin (TSA), a specific HDAC6 inhibitor, tubacin, or a Sirt2 inhibitor, nicotinamide, increased the acetylation and therefore stability of α-tubulin and downregulated the expression of EGFR in Pkd1 mutant kidney epithelial cells. To examine the role of deacetylation on EGFR expression we treated MEK cells with epidermal growth factor (EGF). HDAC6 or Sirt2 was co-localized with EGF induced endocytic EGFR and endosomes, respectively. Within 10 minutes following EGF stimulation of MEK cells there was a random distribution of EGFR that co-localized with early endosomes. Thirty minutes following EGF stimulation, a significant rearrangement of EGFR along microtubules was evident with EGFR co-localized to late endosomes.

Conclusions: These data indicate that the Pkd1 mutation induces upregulation of HDAC6 and Sirt2, leading to increased deacetylation of α-tubulin and therefore decreased degradation and endocytic trafficking of EGFR. Targeting HDAC6 and/or Sirt2 to downregulate EGFR activity may provide a potential therapeutic approach to treat polycystic kidney disease.

Funding: NIDDK Support

FR-PO1984

mTOR Kinase Inhibition in a Model of Polycystic Kidney Disease Atif A. Kidwai,[1] Hyunho Kim,[2] Michael Martin,[3] Yi Liu,[3] Jian Wang,[1] Kevan Shokat,[1] Christian Rommel,[3] Feng Qian,[2] David Pearce.[1] *[1]Division of Nephrology, UCSF, San Francisco, CA; [2]Center for Polycystic Kidney Disease, Johns Hopkins University, Baltimore, MD; [3]Intellikine, La Jolla, CA.*

Background: Polycystic Kidney Disease is a relatively common cause of renal failure with no current acceptable therapies. There has been some success with treating the disease in rodent models with rapamycin, an mTOR inhibitor; however, success in clinical trials has been poor. This may be in part due to the fact that phosphorylation of several mTOR targets is insensitive to rapamycin.

Methods: We examined the effects of a new class of ATP competitive mTOR kinase inhibitors, termed "torkinibs", on mTOR activities, as well as on cyst volume in PKD(V/V) homozygous mutant mice. We treated V/V mutants, heterozygous (V/+), or wild type (+,+) mice from postnatal days 5-11 with oral torkinib or vehicle (n=6 in each group). Mice were sacrificed 2 hrs after their last dose on P11, and kidneys harvested for immunoblot and histologic analysis.

Results: Total body mass and average kidney mass were lower in treated than in untreated mutants (5.42 gms vs 3.34 $p < 0.01$, 301mg +/- 39 to 172mg +/- 43 $p = 0.02$, respectively). The reduction in kidney mass was greater than reduction in body mass, and the combined kidney mass/body mass ratio in the torkinib-treated mutants was significantly lower than in vehicle-treated mice (0.081 +/- 0.012 vs. 0.113 +/- 0.008; $p = 0.02$). In non-mutant mice, body mass was lower in torkinib-treated than in vehicle-treated animals ($p = 0.02$), but there was no difference in kidney mass ($p = 0.22$) or combined kidney mass/body mass ratio ($p=0.5$). Kidney lysates immunoblots were probed with phospho-specific antibodies to assess the phosphorylation status of mTOR targets; mTOR targets had markedly lower phosphorylation in torkinib vs vehicle-treated controls. In preliminary histologic analysis, cyst volume was lower and parenchymal volume greater in torkinib-treated V/V mice than in vehicle treated mice.

Conclusions: These data demonstrate that torkinibs can inhibit mTOR in the kidneys of V/V mice, and suggest that they may have a beneficial effect on cystogenesis in this model of PKD.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-PO1985

Kirsten-Ras GTPase Isoform as a Target in ADPKD Ayesha Irtiza-Ali,[1] Richard N. Sandford,[2] Dorien J.M. Peters,[3] Patricia D. Wilson,[4] Claire C. Sharpe,[1] Bruce M. Hendry.[1] *[1]King's College London; [2]Cambridge University; [3]Leiden Univ Medical Center; [4]University College London.*

Background: Our previous studies show that inhibition of Kirsten (Ki)-Ras GTPase isoform using antisense oligonucleotide (ASO) has marked anti-proliferative and anti-fibrotic effects in different models of proliferative renal disease. Autosomal dominant polycystic kidney disease (ADPKD) is characterized by abnormal cell proliferative responses that lead to cystogenesis and progressive fibrosis, resulting in loss of renal function. Ras-GTPase signalling may be implicated in these processes. We aim to examine this further.

Methods: Renal cystic disease in an orthologous PKD1nl/nl hypomorphic mouse model, bred on a C57B6xCD1 genetic background, was characterized in detail using histochemistry, qPCR and immunoblotting, and compared to wt (n=4/group). We then employed modified ASO technology to study the effect of Ki-Ras inhibition in vitro and in vivo.

Results: PKD1nl/nl kidneys exhibit altered cystic epithelial cell morphology and E-cadherin expression, peri-cystic and interstitial fibrosis, increased tubular and cystic epithelial cell proliferation and phospho-ERK expression, and upregulation of Ras-GTP. Ki-Ras expression is increased by 4-fold in cystic kidney compared to wt ($p=<0.0001$). Two Ki-Ras ASOs each transfected into mIMCD3 cells at a concentration of 100nM cause selective knockdown of Ki-Ras by >80%, and inhibit cell proliferation by 29 and 38% compared to a control ASO ($p=<0.05$). In C57B/6 mice, these ASOs selectively knockdown Ki-Ras in the kidney by 50% ($p=<0.001$), without adverse effect. Antibody detection of ASO localizes distribution to within the tubular epithelia of the proximal and distal nephron.

Conclusions: Our results indicate that Ki-Ras is important in renal epithelial cell proliferative responses, and may be involved in ADPKD pathogenesis. These studies are also the first to demonstrate use of 2 active ASOs in mouse that safely and specifically target Ki-Ras in the kidney, supporting their use for therapeutic study. Our current work investigates the role of Ki-Ras as a potential common link in the abnormal proliferative and fibrogenic responses that occur in ADPKD.

Funding: Private Foundation Support

FR-PO1986

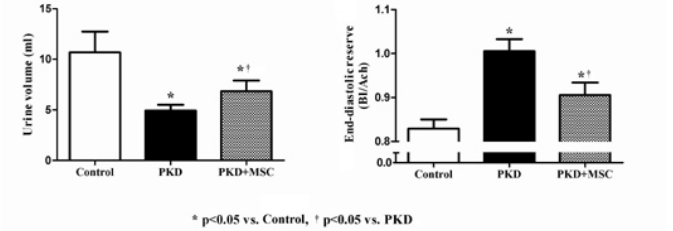
Cell Therapy in Polycystic Kidney Disease Rende Xu, Karen M. Peterson, Peter J. Psaltis, Peter C. Harris, Lilach O. Lerman, Amir Lerman, Martin G. Rodriguez-Porcel. *Department of Internal Medicine, Mayo Clinic, Rochester, MN.*

Background: Polycystic kidney disease (PKD) is the most common genetic renal disease and is characterized by the development of parenchymal cysts and impaired renal vascular and tubular structure and function. In previous studies, cell therapy ameliorated renal dysfunction in a model of chronic renovascular disease. Thus, we hypothesize that cell therapy may ameliorate the renal vascular and tubular dysfunction in PKD**.**

Methods: Bone marrow derived mesenchymal stromal cells (MSCs) ($2.5x10^5$) were transplanted to a rat model of autosomal recessive PKD (ARPKD, n=6) through direct injection into the left renal artery at 6 weeks of age, and compared to wild type. Mean arterial pressure (MAP) was measured via tail-cuff. Fifteen-hour urine collections were performed and kidney volume was measured with High Resolution Ultrasonography (frequency: 30MHz). Renal vascular function was calculated by measuring end-diastolic Doppler flow velocities before and after injection of the endothelial dependent vasodilator Acetylcholine (Ach, 4μg/kg), and expressed as end-diastolic reserve (Baseline/Ach). Measurements were performed at 9 weeks of age.

Results: PKD and controls were similar in body weight (196.8±9.2 vs. 190.3±10.8 grams) and MAP (121.9±9.7 vs. 118.9±11.6 mmHg), while kidney volume was higher in PKD rats compared to wild type (1419.1±176.6 vs. 962.9±90.3 mm^3, $p<0.05$). Compared to controls, PKD rats had lower urinary output and higher end-diastolic reserve, both of which were partially preserved in PKD rats that received MSCs (Figure).

Conclusions: Cell therapy partially restores urinary volume and vascular function in a rat model of ARPKD. While the potential mechanisms of the beneficial effect deserve further study, cell therapies appear as a potential therapeutic intervention in PKD.



Funding: NIDDK Support

FR-PO1987

Urinary Secretion and Extracellular Aggregation of Mutant Uromodulin Isoforms: New Insight for the Understanding of Uromodulin-Associated Kidney Diseases Pathogenesis Celine Schaeffer,[1,2] Angela Cattaneo,[2] Matteo Trudu,[1,2] Sara Santambrogio,[1,2] Ilenia Bernascone,[1,2] Daniela Francesca Giachino,[3] Gianluca Caridi,[4] Corrado Murtas,[4] Mario De Marchi,[3] Antonio Amoroso,[3] Gian Marco Ghiggeri,[4] Francesco Scolari,[5] Angela Bachi,[2] Luca Rampoldi.[1,2] *[1]Dulbecco Telethon Institute, Milan, Italy; [2]San Raffaele Scientific Institute, Milan, Italy; [3]University of Turin, Italy; [4]G. Gaslini Institute, Genoa, Italy; [5]Montichiari Hospital, Brescia, Italy.*

Background: Uromodulin is the most abundant protein secreted in urine where it is found as high molecular weight polymers. Mutations in uromodulin lead to tubulo-interstitial disorders collectively referred to as uromodulin-associated kidney diseases (UAKD). Previous studies pointed at retention in the endoplasmic reticulum (ER) as a common feature of uromodulin mutant isoforms. In this work, we assessed if mutant protein can partly escape the ER quality control and enter the secretory pathway.

Methods: Our studies were carried out in MDCK cells stably expressing wild type and mutant protein and in a transgenic mouse model for UAKD. Secretion of mutant uromodulin was also assessed by mass spectrometry analysis on urine samples from UAKD patients.

Results: In cellular models, mutant uromodulin could partly reach the plasma membrane where it formed large extracellular aggregates. Interestingly, mutant isoforms co-assembled with wild type protein and exerted a dominant negative effect on polymerisation. This was confirmed in transgenic mice, where mutant uromodulin not only accumulated in the ER of thick ascending limb epithelial cells, but was also found in urine and in large lumenal casts. Notably, urinary excretion of mutant uromodulin was also detected in patients carrying uromodulin mutations (R212C, C256Y, C317Y), supporting the relevance of our findings in UAKD.

Conclusions: These results demonstrate that mutant uromodulin can be trafficked to the cell membrane where its aggregation interferes with the formation of uromodulin matrices and possibly affects the function of other membrane proteins. This suggests a new extracellular gain of function effect of uromodulin mutations that has implications for therapeutic strategies.

Funding: Private Foundation Support

FR-PO1988

Initiation and Progression of Fibrosis in a Pkd1 Hypomorphic Mouse Model of Autosomal Dominant Polycystic Kidney Disease Kandai Nozu,[1,2] William E. Sweeney,[1,2] Dorien J.M. Peters,[4] Ellis D. Avner.[1,2,3] *[1]Children's Research Institute, Children's Hospital of Wisconsin, Milwaukee, WI; [2]Pediatrics, Medical College of Wisconsin, Milwaukee, WI; [3]Physiology, Medical College of Wisconsin, Milwaukee, WI; [4]Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development of bilateral fluid filled cysts and intraparenchymal renal fibrosis. The C57Bl6/J-*Pkd1*$^{nl/nl}$ is a mouse model of ADPKD where hypormorphic expression of *Pkd1* results in a model with 2 distinct phases of disease progression. Phase 1 is the formation and growth of cysts followed by phase 2, the development of renal fibrosis.

Methods: We used Masson's trichrome staining, immunohistochemistry (IHC), Western blotting (WB) and quantitative RT-PCR to examine the phenotypic and molecular changes associated with the development of fibrosis in this model.

Results: We chose PN28 as the best time point to examine the molecular changes associated with early stage fibrosis based on the following: 1)Trichrome (+) areas were first evident at PN28; 2) WB revealed initial decreases in ZO-1 and E-cadherin expression: 3) WB demonstrated increases in αSMA; and 4) IHC revealed a layer of αSMA (+) cells beneath the epithelia layer of trichrome (+) cysts. FSP-1 (+) cells within cystic epithelial were very rare and remained rare even at PN56, a point of reduced kidney size due to fibrosis.

Molecular analysis by RT-PCR revealed the earliest changes included statistically increased expression of *Col1A2*, *Col3A1* and *Col5A2.* Changes in the TGFβ/BMP pathway included increased *Bmp1* (7.5 fold), *Tgf*β*2* (17 fold), and *Tgf*β*3* (5 fold). *Bmp7* expression decreased 3 fold while *Tgf*β*1* and *Smad2* remained statistically unchanged. Wnt pathway demonstrated modest increases in *Gsk3*β, *Wnt5a*, *5b* and *Wnt11.* Transcription factors including *Stat3*, *Tcf3*, *Zeb1, Zeb2* and *Twist1* were all significantly increased.

Conclusions: There is no evidence of EMT in this model. Later time point analysis suggest the αSMA (+) layer of cells below the cystic epithelia are myofibroblasts or precursors that trigger fibrotic changes.

Funding: NIDDK Support, Private Foundation Support

FR-PO1989

Conditional Inactivation of *TGF*β*RI* (*Alk5*) in a *Pkd1*- Deletion Mouse Model for ADPKD Does Not Inhibit Renal Fibrosis Wouter N. Leonhard,[1] Anne Marike Van der Wal,[2] Hester Happe,[1] Martijn H. Breuning,[1] Peter Dijke,[3] Emile De Heer,[2] Dorien J.M. Peters.[1] *[1]Human Genetics; [2]Pathology; [3]Molecular Cell Biology, Leiden University Medical Center, Netherlands.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD), caused by mutations in *PKD1 / 2*, is characterized by progressive cyst formation and renal fibrosis. The latter significantly contributes to loss of kidney function, ultimately leading to end-stage renal failure. Characteristic for fibrosis, our *Pkd1*-deletion models display increased nuclear accumulation of phosphorylated SMAD2 (pSMAD2) and elevated expression

of TGFβ target-genes at advanced PKD and can therefore be used to study therapeutic interventions in order to inhibit fibrosis in PKD.

Methods: Since fibrosis is frequently associated with increased TGFβ signaling, we initially treated mice with a pan-selective neutralizing TGFβ antibody (2G7), but observed no reduction in fibrosis-related parameters. Next, we crossbred our tamoxifen-inducible kidney-specific *Pkd1*-deletion model (cKO) with a mouse in which the type I receptor for TGFβ (*Alk5)* is flanked by Lox P sites. We analyzed tissues by immuno-histology and gene expression by RT-MLPA.

Results: Tamoxifen administration to these double-cKO's disrupted both *Pkd1* and *Alk5* as demonstrated by reduced expression levels of these genes. Double-cKO's developed cystic kidneys within the same time frame as single-cKO's and renal histology appeared not to be different. Of note, also in double-cKO's, nuclear pSMAD2 levels and SMAD signaling-dependent gene expression were elevated at end-stage PKD. These results indicate that initiation of renal fibrosis in PKD does not depend on SMAD signaling via *Alk5* in renal epithelial cells and that alternative signaling pathways have to account for the increased levels of nuclear pSMAD2.

Conclusions: In the present study, loss of *Alk5* in renal epithelial cells did not inhibit renal fibrosis in PKD. It is therefore unlikely that targeting only *Alk5* will be successful to halt fibrosis. We will discuss *in vitro* and *in vivo* data suggesting alternative pathways that can lead to SMAD2 phosphorylation and expression of fibrosis-related genes.

FR-PO1990

Inhibition of Multidrug Resistance-Associated Proteins Reduce Cystogenesis Via ERK-Dependent Pathway Jong Hoon Park, Eun Sun Chang, Eun Young Park, Kyung Hyun Ryu. *Department of Biological Science, Sookmyung Women's University, Seoul, Korea.*

Background: Increased tubular epithelial cell proliferation is a prerequisite for cyst formation and expansion in autosomal dominant polycystic kidney disease (ADPKD). Multidrug resistance-associated protein-1 (Mrp1) and Mrp3 are members of the subfamily of ABC transporters. They are well known as drug-resistant proteins, and their other functions are mostly unknown.

Methods: To profile the genes related with cystogenesis, we carried out microarray analysis using cortex region of PKD2 transgenic mice kidney. We found Mrp3 is overexpressed on cyst lining cells of PKD2 transgenic mice kidney using immunohistochemistry. Next we performed three-dimensional culture to determine the effect of Mrp3 with MK571.

Results: Mrp1 and Mrp3 were upregulated dramatically in the cystic region. To examine the effects of these multidrug resistant proteins on cystogenesis, we treated MK571 for inhibition of Mrp1/Mrp3 in Mardin-Darby canine kidney (MDCK) cell line. We found treatment of MK571 reduced the volume of cysts ($P < 0.001$) via reduction of MAPK/ERK signaling.

Conclusions: Our results demonstrate that Mrp1 and Mrp3 may drive proliferation signal on cyst lining cells. In conclusion, antagonist of Mrp1 and Mrp3 (MK571) may have therapeutic potential in ADPKD.

FR-PO1991

An Imbalance of Lymphangiogenesis Versus Blood Vessel Angiogenesis in Polycystic Kidney Disease Jennifer L. Huang,[1] Maria Kolatsi-Joannou,[1] Adrian S. Woolf,[2] Paul J.D. Winyard,[1] David A. Long.[1] *[1]Institute of Child Health, University College London, United Kingdom; [2]Developmental and Regenerative Medicine Research Group, University of Manchester, United Kingdom.*

Background: Therapeutic strategies to reduce cystogenesis in polycystic kidney diseases (PKD) directly target epithelial proliferation using rapamycin-like drugs, cyclin-dependent kinase inhibitors and vasopressin receptor blockade. An alternative approach could be to target pathways extrinsic to epithelia themselves, such as microvessels, which may modulate cystogenesis by paracrine signalling, oxygen delivery, and access to inflammatory/immune mediators.

Methods: To begin to examine this possibility, we investigated the expression of molecules associated with blood vessel angiogenesis and lymphangiogenesis in congenital polycystic kidney (*cpk*) mice. In this mutant, renal lesions phenocopy human autosomal recessive PKD.

Results: We measured 88 angiogenic genes using real-time PCR profiling of whole kidneys. In early stages, levels of 7 transcripts were modestly altered in *cpk* mice. At three weeks of age, when there is massive nephromegaly caused by collecting duct cystogenesis, 37 angiogenic genes were deregulated. Notably, levels of *Tgfb2*, a key player in vascular remodelling, were increased versus wild-types at both time-points. With disease progression, *Vegfr1*, *Vegfr2*, *Npr1* and *Hif1a* were downregulated, as was the mature blood endothelial marker *Meca32*. The lymphatic system was examined by real-time PCR and immunohistochemistry. *Podoplanin* and *Lyve-1* were significantly upregulated in *cpk* mice from day 10 after birth and continued to be elevated throughout the later stages of disease. The number of vessels with positive podoplanin staining found between cysts was increased with additional ectopic expression in cyst walls. mRNA levels of *Vegfr3* and *Vegfc*, implicated in lymphangiogenesis, were however not altered in *cpk* mice.

Conclusions: This is the first evidence that the balance between blood angiogenic and lymphatic gene expression are altered in ARPKD. Further research into the functional significance of our novel findings is being conducted and it is hoped that targeting these pathways may attenuate disease progression.

Funding: Private Foundation Support

FR-PO1992

Global Gene Expression Profiling in Kidneys of PPAR-γ Agonist-Treated PCK Rats, an Orthologous Model of Human ARPKD Daisuke Yoshihara,[1 and 2] Masanori Kugita,[1] Hiroki Kurahashi,[2] Miwa Morita,[1] Yoshiyuki Hiki,[3] Tamio Yamaguchi,[1] Harold M. Aukema,[4] Darren P. Wallace,[5] James P. Calvet,[5] Takafumi Toyohara,[6] Takaaki Abe,[6] Shizuko Nagao.[1] *[1]Education and Research Center of Animal Models for Human Diseases, Fujita Health University; [2]Molecular Genetics, ICMS, Fujita Health University; [3]Health Sciences, Fujita Health University, Toyoake, Aichi, Japan; [4]Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada; [5]Kidney Institute, University of Kansas Medical Center, KC, KS; [6]Medical Science, Tohoku University, Sendai, Miyagi, Japan.*

Background: In polycystic kidney disease (PKD), kidney enlargement is caused by aberrant proliferation of tubule epithelial cells leading to the formation of numerous fluid-filled cysts, extensive nephron loss, and interstitial fibrosis. Pioglitazone (PIO), a PPAR-γ agonist, decreased cell proliferation, interstitial fibrosis and inflammation, and ameliorated PKD progression in PCK rats (Yoshihara et al, AJP, 2011).

Methods: To examine the beneficial effects of PIO, we analyzed changes in global gene expression by DNA microarray in PCK rats treated with 10 mg/kg PIO for 16 weeks.

Results: By Gene Set Enrichment Analysis that used 30655 genes showing significant signal, six of 25 canonical pathways identified to be down-regulated by PIO-treatment were related to cell cycle and cell proliferation, including EGF, JNK, GSK3 and PDGF pathways. Of 43,379 probes examined, 49 were altered 2-fold or more in PIO- compared to vehicle-treated kidneys. Their relevant pathways were identified using the Kyoto Encyclopedia of Gene and Genomes database. Two key enzymes in fatty acid metabolism and three proteins related to calcium signaling were in the top 15 genes down-regulated by PIO treatment. Immunohistochemical analysis revealed that the gene product of two of these, stearoyl-CoA desaturase 1 and cholinergic receptor, were highly expressed in PCK kidneys, and decreased by PIO-treatment.

Conclusions: These data show that PIO has effects on the expression of renal genes involved in fatty acid metabolism, cholinergic signaling, cell cycle progression and proliferation.

Funding: Government Support - Non-U.S.

FR-PO1993

Effects of *Pkd1* Quantitative Differential Expression on Urinary Metabolic Parameters Potentially Involved in ADPKD-Associated Nephrolithiasis Renato Ribeiro Nogueira Ferraz,[1] Jonathan Mackowiak Fonseca,[2] Gregory G. Germino,[3] Luiz F. Onuchic,[2] Ita Pfeferman Heilberg.[1] *[1]Nephrology, Federal University of Sao Paulo, São Paulo, Brazil; [2]Nephrology, University of Sao Paulo School of Medicine, Brazil; [3]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.*

Background: In order to address whether urinary metabolic abnormalities potentially related to the high prevalence of nephrolithiasis in Autosomal Dominant Polycystic Kidney Disease (ADPKD) are caused by *PKD1* haploinsufficiency or the renal cystic phenotype, urinary lithogenic parameters were determined in non-cystic *Pkd1*-haploinsufficient (*Pkd1*$^{+/-}$) and *Pkd1*-targeted cystic(*Pkd1*$^{cond/cond}$:Bal^{cre}) mice models.

Methods: 24-h urine samples were collected during 3 non-consecutive days from 12 week-old male *Pkd1*$^{+/-}$ (n=12), *Pkd1*$^{+/+}$ controls (n=11), *Pkd1*$^{cond/cond}$:Bal^{cre} cystic (n=17), and *Pkd1*$^{cond/cond}$ non-cystic controls (n=18).

Results: As shown in the table, *Pkd1*$^{+/-}$ showed significantly higher urinary calcium than *Pkd1*$^{+/+}$ (p=0.05), an observation not detected in *Pkd1*$^{cond/cond}$:Bal^{cre}. Sodium and potassium FE% and urinary oxalate were significantly lower in *Pkd1*$^{cond/cond}$:Bal^{cre} vs *Pkd1*$^{cond/cond}$ (p=0.0003, p=0.004 and p=0.003, respectively). Urinary citrate did not differ between test and control groups.

	Pkd1+/+	Pkd1+/-	Pkd1cond/cond	Pkd1cond/cond:BalCre
pH	6.31±0.30	6.52±0.26	6.33±0.38	6.06±0.30
Oxalate (mg/mg creat)	0.16±0.17	0.16±0.10	0.16±0.06	0.10±0.04*
Calcium (mg/mg creat)	0.28±0.17	0.41±0.07*	0.15±0.09	0.15±0.07
Magnesium (mg/mg creat)	1.10±1.23	1.23±0.26	0.63±0.17	0.61±0.11
Citrate (mg/mg creat)	3.1±1.5	4.2±2.0	2.8±1.7	3.2±1.3
Uric Acid (mg/mg creat)	0.39±0.27	0.29±0.15	0.26±0.06	0.22±0.05
Sodium (FE%)	0.75±0.18	0.61±0.20	0.75±0.12	0.59±0.07*
Potassium (FE%)	23.3±5.3	19.8±2.4*	23.4±4.3	19.1±2.9*
Creatinine (mg/L)	35.3±11.7	35.0±8.5	42.5±4.5	45.4±3.6

*statistically different vs respective control; FE% (fractional excretion)

Conclusions: These results did not reproduce the main urinary metabolic pattern reported in ADPKD stone formers. Such findings suggest that more complex mechanisms may lead to the urinary metabolic disturbances associated with nephrolithiasis in ADPKD and evince the limitations of these models to investigate this problem.

Funding: Government Support - Non-U.S.

FR-PO1994

Acceleration of Smad3 Phosphorylation at Linker Regions Via c-Jun NH2-terminal Kinase (JNK) in Cyst-Lining Epithelial Cells in cpk Mouse, a Model of ARPKD Hironobu Mukaiyama,[1] Koichi Nakanishi,[1] Taketsugu Hama,[1] Hiroko Togawa,[1] Yuko Shima,[1] Masayasu Miyajima,[2] Hisahide Takahashi,[3] Shizuko Nagao,[3] Kazumoto Iijima,[4] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayama Medical University, Wakayama City, Wakayama, Japan; [2]Laboratory Animal Center, Wakayama Medical University, Wakayama City, Wakayama, Japan; [3]Education and Research Center of Animal Model for Human Disease, Fujita Health University, Toyoake, Aichi, Japan; [4]Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan.*

Background: Cyst-lining epithelia in PKD is characterized by dedifferentiation and perturbations of polarized phenotype with consequent renal tubular cyst formation and progressive enlargement. Previously, we showed activation of key mediators of EMT, TGF-β/Smad3 pathway in cpk mouse (JASN 21:520A,2010). However, specific mechanisms of this phenotypic alteration during cyst formation remain to be elucidated. TGF-β signaling involves Smad3 phosphorylated at linker regions (pSmad3L) and COOH terminal regions (pSmad3C). Therefore, we examined Smad3 phosphorylation profile in this model.

Methods: Kidneys from 5 male cpk and control mice (each on day 0, 7, 14, 21) were harvested. Formaldehyde fixed, paraffin embedded sections were stained with antibodies to domain-specific phospho-Smad3, TGF-β and JNK. We also evaluated their expressions by western blotting.

Results: pSmad3L expression was increased according to age in cpk, while there was no significant staining of pSmad3L in control. pSmad3L was predominantly expressed in nuclei of tubular epithelia in large cysts, and in cytoplasm and nuclei in small and middle cysts. pSmad3C was expressed in both cpk and control in all age, and expression level showed increased tendency according to age in cpk. TGF-β expression was seen in both cpk and control, and also expression level showed increased tendency according to age in cpk. JNK showed the similar staining pattern to pSmad3L. Western blotting analysis supported the expression profiles in sections. These findings suggest that pSmad3L up-regulated via JNK has a key role in cpk.

Conclusions: The linker phosphorylation of Smad3 may be a key pathophysiology in PKD and a target for a disease-specific intervention.

Funding: Government Support - Non-U.S.

FR-PO1995

Vitamin D Deficiency Exacerbates Renal and Cardiac Enlargement in Rats with Polycystic Kidney Disease Kristina Gjerdrum Schwensen, David C. Harris, Gopala K. Rangan. *Centre for Transplant and Renal Research, Westmead Millennium Institute and The University of Sydney, Sydney, NSW, Australia.*

Background: Vitamin D promotes cellular differentiation and inhibits proliferation, raising the hypothesis that the deficiency of vitamin D promotes cyst growth in polycystic kidney disease (PKD). Therefore, the aim of this study was to determine the effect of dietary vitamin D deficiency on the progression of kidney enlargement in PKD.

Methods: Male Lewis PKD rats (Phillips et al. Kidney Blood Press Res 30:129, 2007) received either a semi-pure control diet (AIN93G, D+) or AIN93G with no added Vitamin D (D-), from postnatal week (wk) 3, and examined on wk10 and wk20 (n=9 per group).

Results: Serum 25-hydroxy vitamin D in the control diet group was normal (wk10:109±9; wk20:151±27ng/ml; mean±SD) whereas it was severely reduced in the D-deficient group (wk10:7±9; wk20:17±10 ng/ml). Kidney enlargement, as determined by combined right and left kidney weight, was increased in the D-deficient group compared to the control group, at both wk10 (D+ 11.0±2.2; D- 15.7±2.3) and wk20 (D+ 22.6±1.9; D- 25.5±3.6 g;P<0.05). The latter was confirmed by serial assessment of total kidney volume by magnetic resonance imaging. At wk20, urine volume was greater in the D-deficient group (by 33%), as was proteinuria (D+ 372±84; D-696±246 mg/mmol), whereas the corrected serum calcium was reduced (D+ 2.73±0.10; D-2.46±0.23 mmol/L) (all P<0.05). Endogenous creatinine clearance was similar in both groups (wk10: D+3.4±0.5; D- 3.7±0.6; wk20: D+1.0±0.4; D- 0.8±0.4 ml/min/cm^2). Weight gain was greater in the D-deficient group (32% higher compared to the control diet group) as was cardiac enlargement (wk10: D+ 0.90±0.03; D- 1.18±0.03; wk20: D+ 1.21±0.12;D- 1.62±0.11 g) (both P<0.05).

Conclusions: Dietary deficiency of vitamin D worsens renal and cardiac enlargement in PKD. Further studies should define if kidney enlargement is mediated by the direct or indirect effects of vitamin D on cyst growth, and whether this can be attenuated by replacement therapy.

Funding: Government Support - Non-U.S.

FR-PO1996

A Novel Mouse Mutant for Cystic Kidney Disease and Defects of Planar Cell Polarity Joseph P. Ly,[1] Yoshiro Maezawa,[1] Tuncer Onay,[1] Robert Harrison,[6] Susan E. Quaggin.[1,7,8] *[1]Samuel Lunenfeld Research Institute, Toronto, ON, Canada; [2]University Hospital Aachen, Aachen, Germany; [3]Centre for Modeling Human Disease, Toronto, ON, Canada; [4]Development and Stem Cell Biology Program, Hospital for Sick Children, Toronto, ON, Canada; [5]Departments of Medicine and Molecular Genetics, University of Toronto, ON, Canada; [6]Department of Otolaryngology, Hospital for Sick Children, Toronto, ON, Canada; [7]Nephrology, St. Michael's Hospital, Toronto, ON, Canada; [8]Medicine, University Health Network, Toronto, ON, Canada.*

Background: Cystic kidney diseases represent the primary genetic cause of ESRD in North America, contributing up to 5% of incident cases. The molecular basis of cyst development is complex; recently, defects in planar cell polarity (PCP) have been associated with cyst formation. Here, we describe a new mouse model of cystic kidney disease with evidence for PCP involvement.

Methods: In an autosomal dominant ENU mutagenesis screen, we identified a heritable mouse mutation that causes renal cysts.

Results: Heterozygous mutant mice demonstrate variable and often striking glomerular cysts that can affect up to 100% of glomeruli. At a lower frequency, cysts are identified along the entire nephron. Homozygous mutants die within the first few hours of birth and already exhibit both tubular and glomerular cysts. In addition, homozygotes have "kinked" tails and disoriented inner ear hair cells, phenotypes specific for PCP defects. Furthermore, the loss of one copy of vangl2, a core PCP gene enhances cyst formation in heterozygotes, further substantiating PCP involvement. In addition, there is a trend towards fewer and shorter primary cilia within the renal tubules of the homozygotes. Using SNP analysis, we show that the mutation resides in a 3 MB critical region of chromosome 6 that contains 25 genes, none of which have been associated with cystic disease. Whole-exome next-generation resequencing is currently underway.

Conclusions: Taken together, we report a novel mouse model of renal cysts, which may further elucidate the role of PCP in cystic kidney diseases.

Funding: Government Support - Non-U.S.

FR-PO1997

Planar Cell Polarity Dependent Regulation of Kidney Tubule Morphogenesis Roy D. Bayly, Jeffrey D. Axelrod. *Pathology, Stanford University School of Medicine, Stanford, CA.*

Background: Failure of proper kidney tubule development can result in polycystic kidney disease (PKD), one of the most common genetic disorders inherited in humans. Genetic analysis has revealed that many of the causative gene products identified from patients with PKD localize to or interact with components of the primary cilium, but how they regulate kidney tubule morphogenesis is the subject of considerable interest. Recent research suggests that kidney tubule morphogenesis may involve convergent extension movements and oriented cell division, two processes that require input from the planar cell polarity (PCP) pathway in other developmental contexts. However, the interconnectivity between components within the primary cilium, the PCP pathway and the developmental processes that regulate kidney tubule morphogenesis is tenuous.

Methods: Using mouse genetics and confocal microscopy, we have analyzed the requirement of Vangl1 and Vangl2 during the development and morphogenesis of kidney tubules.

Results: We present results that demonstrate a definitive role for Vangl1 and Vangl2, core members of the PCP pathway, during kidney tubule morphogenesis. Specifically, we observe the asymmetric localization of Vangl1, Vangl2, Frizzled3, and Frizzled6 along the proximal-distal axis of kidney tubule cells. We show that these core components are required for molecular asymmetry, for the proper orientation of cells with respect to the tubule axis, and for the regulation of kidney tubule diameter. Surprisingly, we also observe that in Vangl1 and Vangl2 mutant mice, levels of Polycystin-2, a gene product that is mutated in a class of PKD, is reduced in primary cilia, and increased along the apical surface of kidney tubule cells.

Conclusions: Taken together, we show that Vangl1 and Vangl2, core components of the PCP pathway, are critically important in the regulation of kidney tubule morphogenesis due in part to their requirement for the proper ciliary localization of polycystic kidney disease associated gene products, such as Polycystin-2.

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FR-PO1998

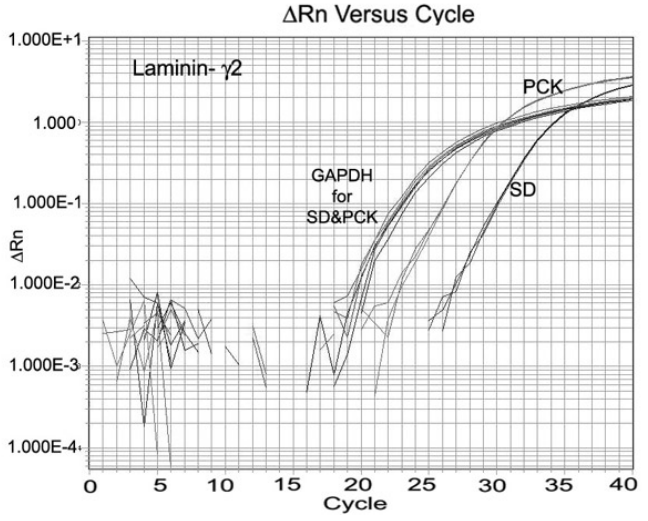
Abnormal Expression and Assembly of Laminin-332 and Laminin-511 in ARPKD Soundarapandian Vijayakumar, Kanchan Parkhi, Jessica Stolar. *Pediatrics, University of Rochester, NY.*

Background: Extracellular matrix abnormalities have been associated with PKD and it is suggested that abnormal upregulation of laminin-332 contributes to ADPKD cystogenesis by inducing proliferation. However, the possible role of aberrant ECM assembly in ARPKD cystogenesis has not yet been investigated. In this study, we addressed this question using PCK rat model of ARPKD and *orpk* cell lines.

Methods: RNA was extracted from 4 adult PCK and control SD rat kidneys and from mutant and rescued orpk cell monolayers. Real-time PCR was performed using Taqman method with GAPDH normalization. For immunoblotting, 300μg of PCK and SD whole

kidney extracts were probed with laminin-γ2 (sc-7652) & laminin γ1 antibodies. Frozen kidney sections were stained with a laminin α5 polyclonal (JH Miner) and a laminin-α3 monoclonal (R&D). Paraffin sections were stained using laminin-γ2 antibody.

Results: Our qPCR results show significant upregulation of laminin-332 chains in PCK kidneys compared to wild-type SD kidneys. Laminin γ2 was upregulated 10 fold, laminin β3 4-fold, and laminin α3, 2-fold.



Immunoblotting confirmed the presence of laminin γ2 in PCK kidneys but not in SD. Immunostaining of frozen and paraffin sections using two different laminin-332 antibodies clearly demonstrated the abnormal expression of laminin-332 in PCK kidney cysts. We also observed a 2 to 4 fold increase in the expression of laminin-332 chains in the cilia defective *orpk* cells. Also, qPCR studies show a modest (1.5-1.8 fold) increase in the expression of laminin-511 chains in PCK kidneys. Preliminary staining results show a weaker laminin α5 staining in PCK cysts but increased staining in nephron tubules.

Conclusions: The abnormal expression and assembly of laminin-332 (and possibly laminin-511) in various ARPKD model systems makes a strong case for further investigation into the role of aberrant laminin assembly in ARPKD cystogenesis.

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FR-PO1999

MicroRNA Regulation of Angiogenesis in Autosomal Dominant Polycystic Kidney Disease Wei Wang, Robert W. Schrier, Berenice Y. Gitomer. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: It is apparent that vascular changes including expansion and remodeling must occur in order to support the massive growth of cysts in patients with autosomal dominant polycystic kidney disease (ADPKD). Evidence for angiogenesis in the form of development of a well-defined vascular capsule around human renal cysts in ADPKD supports this tenet. We have previously reported that the circulating levels of angiogenic growth factors including vascular endothelial growth factor are increased in young patients with ADPKD. Hypoxia is a well established stimulus for angiogenic pathways. The growth of renal cysts with attendant compression of the renal vasculature results in ischaemia within the kidney. We hypothesized that microRNAs (miRs) may play a role in up-regulation of angiogensis in the ADPKD kidneys

Methods: Total RNA was extracted from 5 normal and 6 ADPKD kidneys and cDNA prepared. Renal expression of miR-107, miR-126, and miR-132 was measured by quantitative PCR and compared to expression of the housekeeping genes RNU-6 and SNORD 48.

Results: Expression of miR-107 was significantly decreased (P =0.0006) by 19 fold in the ADPKD kidneys compared to control kidney. There was no significant change in miR-126 expression, while variable increase in expression of miR-132 occured (43 fold increase, P = 01.3) in ADPKD kidneys compared to control kidney.

Conclusions: miR-107 has previously been shown to inhibit hypoxia signaling pathways and inhibit tumor angiogenesis, while decreased miR-107 expression increases HIF-1β, hypoxia signaling and angiogenesis. miR-132 has been shown to facilitate pathological angiogenesis by targeting p120RasGAP in the endothelium. Thus, the observed changes in ADPKD kidney are consistent with upregulation of angiogenesis in response to hypoxia.

Funding: Private Foundation Support

FR-PO2000

NDRG1 Gene Is Involved in the Regulation of Cystogenesis in Transgenic Mice Over-Expressing PKD2 Jong Hoon Park,[1] Bo Hye Kim,[1] Eun Young Park,[1,2] Soo Young Choi.[2] *[1]Department of Biological Science, Sookmyung Women's University, Seoul, Korea; [2]Department of Biomedical Sciences, Hallym University, Gangwon-do, Korea.*

Background: N-myc downstream regulated gene 1 (NDRG1) was chosen as a candidate gene as a regulator of cystogenesis. In this study, relation between PKD2 gene and NDRG1 gene on the cyst formation was uncovered.

Methods: Candidate genes controlled by PKD2 were examined by two dimensional electrophoresis (2-DE) using 18month-old transgenic mice and age-matched wild-type mice. Validation of gene expression was performed by analyzing real time RT-PCR and western blot. To elucidate the effect of gene on cystogenesis, MDCK cells were cultured on the collagen.

Results: Increase of NDRG1 gene expression level in the kidney of PKD2 TG mice was confirmed using real time RT-PCR and western blot. Furthermore, immunohistochemistry result showed that NDRG1 protein highly expressed in the cyst lining epithelial cells. The hypothesis that PKD2 gene will regulate the expression of NDRG1 gene might be supported by *in vitro* experiments. NDRG1 knock down suppressed cystogenesis in 3D culture of Madin-Darby canine kidney cells (MDCK). This result indicated that NDRG1 had impact on the cyst formation. Based on the cell proliferation assay and western blot data, NDRG1 over-expression would accelerate the cellular growth in mIMCD-3 cells.

Conclusions: In conclusion, over-expression of PKD2 promotes the expression level of NDRG1 gene product. This causes the increase in cellular proliferation, aggravating cyst formation.

Funding: Government Support - Non-U.S.

FR-PO2001

Transgenic Expression of Epitope Tagged Pkd2 from Bacterial Artificial Chromosomes Provide a Model for Structure-Function Studies of Polycystin-2 In Vivo Yiqiang Cai,[1] Ming Ma,[1] Xin Tian,[1] Yuehong Wang,[1] Rachel Gallagher,[1] Sorin V. Fedeles,[1] Stefan Somlo.[1,2] *[1]Internal Medicine, Yale University School of Medicine, New Haven, CT; [2]Genetics, Yale University School of Medicine, New Haven, CT.*

Background: Transgenic mice overexpressing Pkd1 or Pkd2 have been reported to develop renal cystic phenotypes. Our recent studies on dual epitope-tagged Pkd1F/H-BAC mouse lines showed that Bacterial Artificial Chromosome (BAC) transgenic expression of PC1 with eight or less copies of the transgene does not cause cystic phenotypes. This observation suggests that low copy BAC-transgenic mouse models are appropriate for the study of polycystin function.

Methods: We modified a BAC containing Pkd2 by homologous recombination in E. coli to introduce a triple-HA epitope tag before the stop codon in the last exon of Pkd2. Transgenic mouse lines were produced using this Pkd2-BAC and tissues from transgenic progeny were analyzed by immunoblotting and immunofluorescent tissue staining using anti-HA and other cellular marker antibodies.

Results: We obtained transgenic mice expressing COOH-terminal triple-HA tagged PC2 from the Pkd2-BAC transgene. Immunoblotting analysis using anti-HA antibodies showed that transgenic PC2 is expressed in all tissues tested in a pattern and at levels comparable to that of native PC2. Immunofluorescent staining of transgenic PC2 by anti-HA in kidney tissue demonstrated a cytoplasmic staining pattern in vivo. Histological examination of tissues from five-month old kidneys of Pkd2-BAC transgenic mice showed normal tubular structure without microscopic cysts. These observations suggest that the BAC-transgenic HA-tagged PC2 recapitulates the expression pattern of native PC2 in vivo. Studies on subcellular localization of transgenically expressed PC2 are ongoing.

Conclusions: The HA epitope tagged Pkd2-BAC can be further modified with site-specific mutation and transgenic mice produced from these BACs will serve as a platform for studying structure-function relationships in PC2 in vivo.

Funding: NIDDK Support

FR-PO2002

Dose-Expression of Exogenous Polycystin-2 Can Significantly Ameliorate the Phenotypic Severity of Pkd2 Knock-Out Mice Guanqing Wu.[1] *[1]Medicine, Vanderbilt University, Nashville, TN; [2]State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China.*

Background: Loss of polycystin-2 (PC2) in mice (Pkd2-/-) results in total body edema, focal hemorrhage, structural cardiac defects, abnormal left-right axis, hepatorenal and pancreatic cysts, and embryonic lethality. To study PC2 functions in vivo, we have produced a Pkd2 transgenic mouse (Pkd2tg7) to test if ectopic overexpression of Pkd2 is able to rescue its disease phenotypes.

Methods: The full-length hPKD2 ORF cDNA was constructed under the control of a CMV immediate early enhancer, a chicken β-actin promoter, and a rabbit β-globin poly A signal downstream of the native termination codon (pCAGGS expression vector). Four founders (Tg7, 9, 10, 14) were found to overexpress PC2, determined by Western blotting using polyclonal antiserum hPKD2-Cp. We selected Tg7 as our founder (PKD2tg7) because these mice have been most extensively studied.

Results: Using a mating strategy, PKD2tg7 mice were crossmated with Pkd2+/- mice to produce PKD2+/tg7:Pkd2-/- and PKD2tg7/tg7:Pkd2-/- mice. Our results showed that both PKD2+/tg7:Pkd2-/- and PKD2tg7/tg7:Pkd2-/- mice can overcome the embryonic lethality seen in Pkd2-/- mice. However, PKD2+/tg7:Pkd2-/- mice were born at a frequency of 10%, much lower than 21% in Pkd2-/- mice with the double Tg7 alleles (PKD2tg7/tg7:Pkd2-/-). In addition, the PKD2tg7/tg7:Pkd2-/- mice exhibited significantly decreased cystic number and volume in the pancreas, liver and kidneys compared to PKD2+/tg7:Pkd2-/- littermates, suggesting that an increased expression level of PC2 can ameliorate ADPKD disease phenotypes seen in Pkd2-/- mouse models. Kaplan and Meier survival analysis also indicated that the PKD2tg7/tg7:Pkd2-/- mice can survive much longer than the Pkd2-/- mice with a single Tg7 allele.

Conclusions: This finding indicates that the functional restoration of PKD2 gene product can rescue disease phenotypes in Pkd2-/- mice and sufficient expression of PC2 can potentially cure PKD2-mutant ADPKD.

Funding: NIDDK Support

FR-PO2003

Expression of Bicc1 Protein during the Mouse Development Guanqing Wu.[1] [1]*Medicine, Vanderbilt University, Nashville, TN;* [2]*State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China;* [3]*Pathology, Yale University, New Haven, CT.*

Background: *Bicc1* is a mouse homologue of Drosophila *Bicaudal-C* (*dBic-C*). Orthologs of *dBic-C* can be identified in many species, from C.elegans to human being. The *Bicc1*-mutant mouse models, *jcpk* and *bpk*, both exhibit cystic phenotype in the kidney that are very similar to human polycystic kidney disease. Yet, the developmental profiles of Bicc1 protein (Bic-C) in the mammalian system remain uncharacterized.

Methods: We have therefore generated two polyclonal antibodies against different portions of Bic-C to examine its spatial and temporal expression patterns during mouse embryogenesis and organogenesis. Two mouse Bicc1-fusion proteins encoding residues from E61 to A199 (mBic-N), and Q711 to D858 (mBic-C) of Bicc1, were used to produce rabbit polyclonal antisera.

Results: Our study demonstrated that Bic-C can be detected in epithelial cells of the neural tube as early as embryonic day 8.5 (E8.5) and positive staining appeared in the myocardial wall of the heart at E10.5. By E11.5, the immature hepatocytes and epithelial cells of the primordial gut, main bronchi and aorta exhibit positive staining at their cytosol. In the kidneys, Bic-C expression is seen in the epithelia of early ureteric bud and mesonephric tubules, as the bud penetrates into the metanephrogenic mesenchyme during E11.5. Significant staining continues in the renal comma-shaped body and the S-shaped body until E12.5. By 1-month of age, increased Bic-C staining extended to the renal juxta-medullary region. At this stage, no significant staining was seen in the medullary and papillary regions and relatively weak Bic-C staining were present in the cortical region of the kidneys. By co-staining adult kidney with Bic-C antibody and renal tubular segment markers, we found that Bic-C is predominantly stained at proximal convoluted tubules in the kidneys.

Conclusions: These results indicate that Bic-C is developmentally regulated and Bic-C exerts an important role in tubulogenesis and organogenesis during the renal

Funding: NIDDK Support

FR-PO2004

Targets and Binding Motif of the Polycystic Kidney Disease-Associated RNA Binding Protein BICC1 Iddo Zeev Ben-Dov, Thomas Tuschl. *Laboratory of RNA Molecular Biology, Rockefeller University, New York, NY.*

Background: Mutations in protein bicaudal C homolog 1 (BICC1) have been shown to cause polycystic kidney disease in mice. We aimed to systematically explore the RNA binding characteristics of BICC1 in-vivo.

Methods: BICC1 was cloned from human podocyte cDNA. Photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) was performed on FLAG-BICC1 expressing HEK293 cells. Recovered small RNA was sequenced (Solexa), genome-aligned and annotated.

Results: Two BICC1 splice isoforms were (inadvertently) cloned from human podocyte cDNA (panel A). Upon expression, both showed cytoplasmic localization in HEK293 cells (panel B). Ample in-vivo crosslinking of BICC1 to RNA was noted with or without the photoactivatable nucleoside (panel C). PAR-CLIP evidence for BICC1 crosslinking to mRNA was widespread, and sporadic associations were noticed with noncoding RNA (tRNA, miRNA). High confidence mRNA clusters were >90% exonic (both isoforms). Of these, 59% (isoform 1) and 82% (isoform 2) mapped to 3'UTRs, while 36% and 17% mapped to coding regions, respectively. Isoform 1 and isoform 2 clusters mapped to 5791 and 2853 genes, respectively (panel D). Subjection of these clusters to motif finder algorithms yielded similar results for both isoforms, with TTGACAA as the chief finding (panel E). Using PAR-CLIP data we were able to substantiate and pinpoint a previously suggested association between BICC1 and PKD2 mRNA near a putative miR-17 binding site (panel F).

Conclusions: PAR-CLIP robustly reveals the in-vivo RNA binding characteristics of BICC1. Nucleotide-level information from these experiments can direct further biochemical studies, while transcript-level data may identify targets involved in the pathogenesis of human polycystic kidney disease.

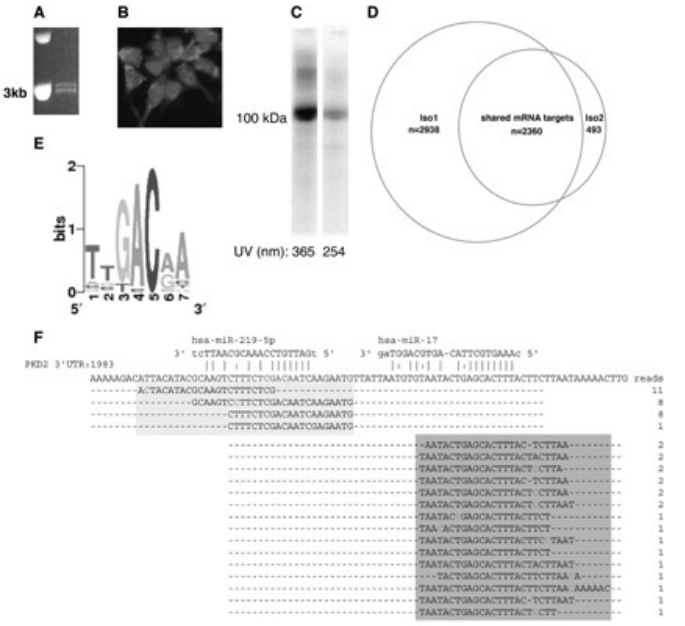

Figure: (A) RT-PCR of the 2 BICC1 isoforms. **(B)** IF staining of FLAG-BICC1 in HEK293 cells. **(C)** SDS gel autoradiography of FLAG IP (RNA is 32P-labeled). **(D)** Venn diagram of isoform 1 and 2 mRNA targets. **(E)** weblogo of the main BICC1 binding motif. **(F)** Example of a BICC1 (isoform 1) PAR-CLIP mRNA cluster aligned with an mRNA cluster from argonaute (AGO) PAR-CLIP experiment. The shown region is within the 3'UTR of the PKD2 gene. The yellow box depicts BICC1 binding reads, while the brown box shows AGO binding reads. Pink 'C's represent PAR-CLIP's crosslinking T->C transition signature. A region similar to the BICC1 binding motif is shown in red. Potential miRNA binding sites are depicted above, with the predominant crosslinked positions in the AGO PAR-CLIP shaded violet.

FR-PO2005

Tissue Engineering Models for Human ADPKD Wei-Che C. Ko, Balaji Karthick Subramanian, Tessa Desrochers, David Kaplan. *Department of Biomedical Engineering, Tufts University, Medford, MA.*

Background: Modeling cystic diseases in vitro presents a unique challenge as cyst morphogenesis, in addition to complex intercellular interactions, is also governed by synergistic spatial, mechanical and temporal effects. We report the development of kidney-like tissue structures for normal and diseased (cystic) states using commercially available human kidney cells.

Methods: Gene silencing is used to simulate autosomal dominant polycystic kidney disease, as inactivating mutations in polycystins -1 and/or -2 are responsible for the disease in vivo. Our system utilizes extracellular-matrix molecules infused in slow degrading porous silk scaffolds, which provides a 3D microenvironment for proper cell polarization (ECM), while exhibiting structural robustness and tension (silk scaffold).

Results: Our results indicate development of cyst-like structures in a 3D environment, while also demonstrating the respective normal and altered phenotypes concurrent with normal tissue and patient-derived ADPKD tissue. The structural and functional features of kidney-like tissue structures were further characterized based on distribution of E-cadherin, N-cadherin, transport phenomena of 6-carboxyfluorescein, and cell-matrix interactions through integrin signaling.

Conclusions: Importantly, this 3D in vitro model may be further extended via perfusion reactor for long term studies of ADPKD or other renal cystic diseases, and may have beneficial use as a therapeutic drug screening tool.

Funding: Other NIH Support - NIBIB, Pharmaceutical Company Support

FR-PO2006

Lonidamine Reduces Cyst Size in Polycystic Kidney Disease Brenda S. Magenheimer, Shirin Sundar, Sumanth Mulamalla, Monica K. Johnson, Gail Reif, Darren P. Wallace, James P. Calvet. *Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.*

Background: Currently, there is no effective treatment to slow kidney enlargement in ADPKD. A lead therapeutic compound for ADPKD should effectively block both abnormal cell proliferation and cyst-filling fluid secretion in addition to having favorable safety/toxicity and biodistribution profiles. Lonidamine, an indazole carboxylic acid derivative, was originally developed to inhibit tumor growth. It is approved for the treatment of prostate, breast, and head and neck cancers and is being tested as a combination therapy with other conventional chemotherapeutic agents. Lonidamine inhibits hexokinase activity, which is often important in the anaerobic glycolysis typical of abnormally growing neoplastic cells; and it inhibits CFTR channel activity. We showed that lonidamine inhibits ADPKD cell proliferation with an LD50 of 5.7 μM, as shown by MTT assay; and at 5 μM, lonidamine inhibits forskolin-induced chloride secretion in M-1 cells as measured by short-circuit current in the presence of benzamil.

Methods: Cell culture, organ culture, and mouse models of PKD.

Results: To determine whether lonidamine alters the cyst-forming process in response to cAMP, kidneys from Pkd1 +/+, +/-, and -/- mice were placed in metanephric culture at E15.5 and treated with 100 μM 8-Br-cAMP with or without lonidamine for 4 days. Lonidamine did not appear to be toxic to the kidneys at these doses, yet it significantly inhibited cystic dilation. 2 μM lonidamine was effective in Pkd1 +/- kidneys, essentially preventing cyst formation, while 10 μM lonidamine resulted in dramatic cyst reduction in Pkd1 -/- kidneys. Lonidamine was also shown to significantly inhibit wound closure in a cell motility scratch assay at 25 μM and 50 μM. Cystic Nphp3 (pcy) mice treated with 50 mg/kg lonidamine or vehicle 6 days a week by oral gavage for 8 weeks showed a decrease in kidney weight to body weight ratio and cystic index upon quantification of midsagittal sections. The drug appeared to be well-tolerated.

Conclusions: These studies suggest that lonidamine or its derivatives may be effective therapeutic agents for long-term use in ADPKD.

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FR-PO2007

Therapeutic Potential of Phospholipase D Inhibitor in Autosomal Dominant Polycystic Kidney Disease Yang Liu,[1] Rudolf P. Wuthrich,[1,2] Andreas L. Serra.[1,2] *[1]Institute of Physiology, University of Zürich, Zürich, Switzerland; [2]Division of Nephrology, University Hospital, Zürich, Switzerland.*

Background: The mTOR pathway is activated in tubular epithelial cells lining cysts in animal and human polycystic kidneys. Phospholipase D (PLD) and the metabolite phosphatidic acid (PA) regulate mTOR activity which is competitive with the classic mTORC1 inhibitor rapamycin. We explored novel and highly specific PLD inhibitors as new therapies for PKD.

Methods: The cellular responses and mechanisms to the PLD1/2 inhibitor Honokiol, a natural compound purified from Magnolia, as well as to the highly specific PLD1, PLD2 and dual PLD1/2 inhibitors were evaluated in primary renal tubular epithelial cells either derived from Han:SPRD rats (Cy/+ TECs) or derived from human ADPKD kidney (HAK).

Results: PLD2 inhibitor decreased cell proliferation Cy/+ TECs and HAK more effective compared with either PLD1 inhibitor or dual PLD1/2 inhibitor. Honokiol reduced total cell numbers, cell viability and DNA synthesis of Cy/+ TECs with inducing complete inhibition at 40μM. Cleavage fragments of caspase 3 could be detected. Phosphorylation of PLD1 (PLD1ser561) decreased upon Honokiol treatment in a dose-dependent way. The phosphorylation of the upstream regulators (AktThr308) and downstream effectors of mTORC1 (4EBPThr37/46, S6KThr421/Ser424, S6Ser235/236) was prevented at a concentration of 30μM. Honokiol suppressed phosphorylation of Akt at Ser473 site, the readout of mTORC2 activity. Phosphorylation of the MAPK (ERK1/2Thr202/204) decreased at the concentration of 40μM.

Conclusions: The PLD2 inhibitor halted primary tubular epithelial cell proliferation derived from human and animal polycystic kidneys, at least in part by inducing apoptosis. The signaling through PLD1, mTORC1/2, and MAPK pathways was blocked by Honokiol. PLD2 but not PLD1 controls proliferation of tubular epithelial cells. PLD2 is a critical regulator of mTOR activity which has been largely overlooked in the rapamycin-based treatment strategies in PKD. Targeting PLD may offer a new therapeutic target for PKD.

FR-PO2008

Periostin, a Soluble Extracellular Matrix Molecule, Activates Focal Adhesion Kinase and Cytoskeleton Reorganization of Human ADPKD Cells Gail Reif, Emily Nivens, Cibele S. Pinto, Corey White, Stephen C. Parnell, Maodong Liu, Darren P. Wallace. *Internal Medicine, University of Kansas Medical Center, Kansas City, KS.*

Background: In ADPKD, abnormal cell proliferation, apoptosis and matrix production suggest that cyst epithelial cells are poorly differentiated, possibly involving an aberrant repair mechanism. Periostin, a soluble extracellular matrix (ECM)-related protein involved in tissue development and repair, is highly over expressed in ADPKD cells and accumulates within the matrix adjacent to cysts. Periostin binds α_V-integrins and accelerates ADPKD cell proliferation. Focal adhesions are sites of integrin clustering and formation of protein complexes involved in ECM communication with intracellular signaling molecules including focal adhesion kinase (FAK). Rho and FAK regulate the assembly and disassembly of focal adhesions by activating pathways that lead to actin polymerization and contraction. We hypothesize that periostin signaling at the focal adhesions causes FAK activation and Rho dependent changes in the cytoskeleton and cell adhesions which contribute to a less differentiated phenotype.

Methods: Human ADPKD cells were treated with recombinant 100-250 ng/ml periostin and levels of phosphorylated Rho (P-Rho), Rac (P-Rac) and FAK (P-FAK) were measured by immunoblot analysis. To determine the effect of periostin on the cytoskeleton, actin filaments were stained with Phalloidin-FITC.

Results: Periostin increased P-Rho and P-FAK levels within 30 min; however these increases were transient. By contrast, periostin did not appear to affect Rac phosphorylation within this time period, consistent with reciprocal activation of Rac and Rho during cell migration. Actin staining indicated that periostin caused bundling of actin filaments into stress fibers, whereas FAK inhibition either in the absense or presence of periostin caused broad web-like extensions.

Conclusions: Periostin binding to integrins at focal adhesions causes activation of Rho and FAK, leading to cytoskeletal reorganization. These effects are consistent with periostin-induced changes in cell adhesion which may play a role in aberrant regulation of cell morphogenesis, repair, and matrix deposition.

Funding: NIDDK Support

FR-PO2009

Ouabain Regulates Expression of Adhesion Proteins and Cell-Cell Interaction in ADPKD Cells Madhulika Sharma,[1] Elsa Bello-Reuss,[2] Gustavo Blanco.[1] *[1]Molecular and Integrative Physiology and Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Internal Medicine, Division of Nephrology and Hypertension, Texas Tech University Health Science Center, Lubbock, TX.*

Background: Epithelial cell proliferation is one of the hallmarks of the development of renal cysts in autosomal dominant polycystic kidney disease (ADPKD). Cell proliferation requires relaxation of the contact between neighboring cells and cell junctions. Cell junction function is controlled by regulation of the expression of adhesion proteins. We have previously shown that the hormone ouabain, at physiological concentrations, stimulates proliferation of cultured human ADPKD cells.

Methods: In this work, we studied the effect of ouabain on adhesion proteins of tight and adherens junctions of ADPKD cells. ADPKD cells were incubated in the absence or presence of 3nM ouabain for 24hrs and the expression levels of a series of adhesion molecules were determined in the whole cells and in plasma membrane fractions by immunoblot and immunocytochemistry.

Results: Ouabain differentially affected tight junction protein expression. While claudin1 and ZO-1 were not significantly modified, occludin amounts were elevated in ouabain-treated ADPKD cells. Ouabain also regulated expression of zonula-adherens proteins in a specific manner, increasing the amounts of vinculin, reducing the levels of E-cadherin and maintaining β-catenin unchanged. In addition, ouabain modulated cell-cell interaction, reducing the ability of ADPKD cells to adhere to each other. In contrast, permeability of ADPKD cell monolayers to dextran showed no significant changes after ouabain treatment.

Conclusions: These results show that in ADPKD cells, ouabain regulates the expression of adhesion proteins in a complex manner to concomitantly favor relaxation of cell-cell junctions while still preserving the permeability of the paracellular pathway of the ADPKD epithelium. These effects are important in contributing to the overall action of ouabain of promoting proliferation of ADPKD cells.

Funding: NIDDK Support

FR-PO2010

Curcumin Analog 2a Inhibits *In Vitro* Proliferation of Autosomal Dominant Polycystic Kidney Disease Cyst Cells Beatriz Adriana Velez,[1] Laura Parra,[1] Moses Lee,[2] Albert C. Ong,[3] Elsa Bello-Reuss.[1] *[1]Internal Medicine, TTUHSC, Lubbock, TX; [2]Hope College, Holland, MI; [3]Academic Nephrology Unit, University of Sheffield Med School, United Kingdom.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive renal cyst development and destruction of the renal parenchyma conducing to kidney failure. Curcumin inhibits proliferation pathways activated in cancer that are common to ADPKD cyst cells. However no therapeutic levels are achieved by oral administration. Analog 2a, a curcumin derivative lacking glucuronidation sites has improved intestinal absorption and is a more potent cytotoxic agent to cancer cell lines. Thus, it may be of value for treatment of ADPKD. Since cyst cells exhibit proliferative pathways not present in normal kidney cells, Analog 2a may have a predominant inhibitory effect on cyst cell proliferation. We addressed the effects of Analog 2a on proliferation of cyst cells,normal kidney cells and cyst cell lines.

Methods: We used the ADPKD-cyst cell lines OX161 and SKI-001, ADPKD-cyst cells in primary cultures, normal cortical tubule cells in primary cultures, and the normal kidney cell line UCL93/9.Cell survival was measured at concentrations between 0 and 3μM.The half-maximal inhibitory concentrations (IC50) of Analog 2a were determined by data fitting to a 4 parameter logistic curve.

Results: Analog 2a was cytotoxic to cyst cells at lower concentrations than to normal kidney cells with a selectivity index larger than 3. The IC50 (mean ± SD) were: ADPKD cyst-cell lines, 0.98 ± 0.13 μM (n=3); cyst-cell primary cultures, 0.73 ± 0.32 μM (n=4); control cell line, 0.87 μM ± 0.09, not different from cyst cells; normal kidney cell primary cultures were unaffected by Analog 2a at 3 μM. P <0.001 vs cyst cells.

Conclusions: 1. ADPKD cell lines and primary cultures of ADPKD cells have high and similar sensitivities to Analog 2a. 2. Normal kidney cells, in primary cultures, are more resistant than ADPKD cells to Analog 2a. 3. Immortalized cells from normal kidney are sensitive to Analog 2a, and hence are not an appropriate control system. These results indicate that Analog 2a has potential for the treatment of ADPKD.

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FR-PO2011

Bidirectional Regulation between Polycystin-2 and Cellular Stress Jungwoo Yang,[1] Qian Wang,[1] Wang Zheng,[1] Carlos Lara,[1] Zuocheng Wang,[1] Guanqing Wu,[2] Xing-Zhen Chen.[1] *[1]Physiol, Univ of Alberta, Edmonton, AB, Canada; [2]Medicine, Vanderbilt Univ, Nashville, TN.*

Background: ADPKD is associated with several cellular abnormalities such as cell over-proliferation and apoptosis. Mutations in polycystin-2 (PC2), a Ca-permeable channel present in the ER membrane, plasma membrane and cilia, account for ~10% of ADPKD cases. How PC2 expression is regulated and how it regulates cell growth remains elusive. We recently reported that PC2 down-regulates cell proliferation and protein synthesis through promoting the phosphorylation of eukaryotic initiation factor eIF2α by kinase PERK.

Results: Here we found that endogenous PC2 protein expression is up-regulated in HEK, HeLa and mouse embryonic fibroblast cells under cellular stress conditions including ER stress, oxidative stress and virus infection-induced stress, which all increase the phosphorylated eIF2α (P-eIF2α). Increased P-eIF2α triggers a number of downstream processes, including global inhibition of translation and proliferation, and regulation of gene expression and apoptosis via translational up-regulation of transcription factor ATF4. Inhibition and stimulation of the activity of protein phosphatase of eIF2α by salubrinal and Gadd34 over-expression respectively up- and down-regulated PC2 expression in cultured cells or live mice. RT-PCR assays indicated that the Pkd2 mRNA level is not affected by stress conditions or P-eIF2α. Further, removal of the 5' upstream ORF (uORF) of Pkd2 mRNA abolishes the regulation of PC2 by P-eIF2α. These data together indicate that P-eIF2α translationally up-regulates PC2 expression through uORF. We found that PC2 binds ER chaperone and ER stress marker GRP-78 and that the binding is substantially reduced during ER stress. In contrast, PC2 also interacted with calnexin, an ER chaperone assisting protein folding, but the binding was increased by ER stress. Finally, using mouse collecting duct cells with Pkd2 knockout and HeLa cells with PC2 knockdown, we found that PC2 is critical for the up-regulation of GRP-78 by ER stress.

Conclusions: In conclusion, PC2 is not only up-regulated by P-eIF2α under stress conditions but is also essential in mediating cellular responses to ER stress.

Funding: Government Support - Non-U.S.

FR-PO2012

Endoplasmic Reticulum Stress in the Pathogenesis and Treatment of TSC Renal Cystic Disease

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Background: Tuberous sclerosis complex (TSC) is an inherited tumor predisposition syndrome in which TSC1 or TSC2 mutations lead to deregulated mammalian target of rapamycin complex 1 (mTORC1) signaling. Nearly 50% of TSC patients develop renal cystic disease, and these patients are at the highest risk to progress to end stage renal failure. ER stress can develop in angiomyolipomas and TSC-mutant mouse embryonic fibroblasts due to increased protein translation stemming from constitutive mTORC1 activity. Recent evidence indicates that TSC solid tumors may experience a cytotoxic response to therapies that potentiate ER stress. However, it is not known whether or to what extent ER stress plays a role in TSC renal cystic disease.

Results: Using the EKT2 renal cystic cell line derived from the Eker rat, we found that mTORC1 inhibition (20 nM RAD001, 1hr, 24hr and 72 hr) reduced levels of ER stress markers glucose related protein-78 (GRP78 or BiP), inositol-requiring enzyme-1 (IRE-1), protein kinase-like ER kinase (PERK), and phospho-eIF2α by western blot. In the HK-2 human proximal tubule cell line, RNAi-induced knockdown of TSC2 increased expression of BiP, IRE1α, and CCAAT/enhancer-binding protein homologous protein (CHOP), a pro-apoptotic transcription factor. ER stress exacerbation by proteasome inhibition with MG-132 (1 μM, 24 hr) induced CHOP expression and cleavage of poly (ADP ribose) polymerase (PARP) in EKT2 cells, indicating pro-apoptotic signaling. This effect was attenuated by pre-treatment with an mTORC1 inhibitor (20 nM RAD001, 72 hr). Co-administration of MG-132 with salubrinal (15 μM), an inhibitor of eIF2α phosphatases that can enhance pro-apoptotic functions of eIF2α under ER stress conditions, was synergistic with respect to CHOP induction and PARP cleavage. Finally, we observed BiP positivity by immunohistochemistry in TSC patient renal cystic epithelia.

Conclusions: These findings indicate that the TSC renal cystic epithelium experiences ER stress due to mTORC1 activation, and that ER stress can be targeted therapeutically toward a cytotoxic endpoint.

Funding: Other NIH Support - NIH 2T32ES7250-21A1

FR-PO2013

mTOR Signaling Is Differentially Affected in Human Renal Cells with *PKD1* or *PKD2* Mutations

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic disorder leading to end stage renal disease (ESRD). It is caused by loss-of-function mutations in either *PKD1* (85%) or *PKD2* (15%) genes which encode polycystin-1 (PC1) and -2 (PC2) respectively. Patients with *PKD2* mutation have a milder phenotype and reach ESRD ~20 years later than those with *PKD1* mutation.

Increased activity of mammalian target of rapamycin (mTOR) pathway has been shown in *PKD1* mutants and is implicated in accelerated cell proliferation. Furthermore, it has been demonstrated that rapamycin is highly effective in reducing renal cystogenesis. However, mTOR activity in PC2 mutants has not been studied thus far.

Methods: We developed conditionally immortalized human proximal tubule epithelial cell lines (ciPTEC) from patients with known *PKD1* and *PKD2* mutations and from healthy controls. Also, we generated stable PC1 and PC2 knockdown (KD) cell lines using lentiviral vectors expressing miRNA-based shRNA. In these models we measured the mTORC1 activity (phosphorylated ribosomal protein S6) via Western blotting.

Results: We confirmed in the PC1 KD cell line and in ciPTEC with *PKD1* mutations an upregulation of mTORC1 activity compared with controls (160% vs 100% respectively; p=0.005). In contrast, in the PC2 KD cells and ciPTEC from patients with PKD2 mutations mTORC1 activity was in the range of control cells (80% vs 100% respectively; p=0.350).

Conclusions: This is the first report that highlights an important difference in the underlying molecular mechanism between *PKD1* and *PKD2* mutations. Using human renal cell models of ADPKD, we demonstrate that mTORC1 activity seemed not to be affected by the downregulation of PC2. This might contribute to the milder renal phenotype in these patients. Our data can have important therapeutic implications for selecting patients for treatment with mTOR inhibitors.

Funding: Government Support - Non-U.S.

FR-PO2014

Pericystic Matrix Interactions Regulate the Rate of Cystogenesis in Autosomal Dominant Polycystic Kidney Disease

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Background: Autosomal Dominant Polycystic Kidney Disease remains a major health care concern affecting several thousand patients worldwide. This is in part attributed to the lack of appropriate tissues for research studies and to assess for therapeutic interventions prior to moving to clinical trials.

Methods: Tissue engineering provides novel approaches to the development of 3D in vitro tissue systems. We have developed a model based on tissue engineering principles for the emulation of cystic structures in vitro, with structural and functional features similar to in vivo. The tissue system was developed by culturing normal or polycystin-1 silenced mouse inner medullary collecting duct cells in extracellular matrix molecules infused into 3D porous silk scaffolds. In the system, silk biomaterial scaffolds were used due to the slow degradation, bio-compatibility, ability to maintain structure and transport and can function in perfusion bioreactor systems for sustained time periods.

Results: The results provide evidence for an increased rate of cystogenesis in the disease system, initiated by abnormal pericystic ECM interactions between matrix molecules and integrin subunit proteins. In addition, molecular signaling analysis showed abnormalities in cyclin proteins and cell-cycle progression. Very importantly, we show that inhibiting the pericystic interaction by double silencing the integrin proteins reverses the abnormalities and reduces the rate of cystogenesis and may be considered for ADPKD therapeutic interventions.

Conclusions: We provide evidence for an autocrine signaling involving abnormal matrix interactions, to regulate the rate of cystogenesis and could be targeted for ADPKD therapeutics.

Funding: Other NIH Support - NIBIB, Pharmaceutical Company Support

FR-PO2015

Reduced Proteoglycans in the Kidney Causes Both Tubule and Glomerular Abnormalities

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Background: Most cells produce some form of proteoglycan(s). The initial assembly of proteoglycans on the core protein requires the transfer of a xylose to a designated serine. The enzyme responsible for this is xylosyltransferase and exists in two isoforms. Xylosyltransferase 2 (XylT2) is ubiquitously expressed in many organs suggesting a significant role of XylT2 dependent proteoglycans in those organs. In our XylT2 knock out mice (Xylt2-/- mice), considerable XylT activity remains in the kidney due to the remaining XylT1 activity. However, despite this remaining activity, considerable renal abnormalities occur including glomerular basement membrane changes, fibrosis, and tubule dilation. Functionally these changes result in increased blood urea nitrogen, proteinuria, and, in a few mice, renal failure. Our previous findings in Xylt2-/- mice has established that proteoglycans are important in cyst development in the liver. The additional findings in the kidney indicate that reduced glycosaminoglycan assembly onto core proteins due to XylT2 deficiency has a pathologic impact as well. Seventy percent of PKD patients develop liver cysts as well as renal cysts. Overall our findings suggest that reduced proteoglycans may have a genetic modifying role in inherited polycystic kidney disease (PKD) the fourth leading cause of renal failure in the United States. PKD patients that develop proteinuria have a much poorer long term prognosis. Since the cause of the proteinuria in PKD patients is unclear, there is no specific therapeutic intervention. Our analyses in the Xylt2-/- mice suggest that one source of the proteinuria is reduced renal proteoglycans.

Methods: PAGE, electron microscopy, Immunohistochemistry, water deprivation test, glycosaminoglycan measurements

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FR-PO2016

A Metabolomic Approach Reveals a Metabolic Switch in PKD

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Background: Polycystin-1 (PC-1), the product of the PKD1 gene, mutated in most cases of Autosomal Dominant Polycystic Kidney Disease (ADPKD), is a very large plasma membrane receptor. It has been described to regulate several cell signaling pathways, in

particular the mTORC1 and Akt pathways, major actors in energy sensing and cellular metabolism regulation.

Results: To investigate the role of PC-1 in regulating metabolism, we performed metabolomic profiling of the extracellular medium of Pkd1-/- and Pkd1+/+ MEF (murine embryonic fibroblast) cells. NMR Spectra were superimposed and have shown differences between Pkd1-/- and Pkd1+/+ cells. In particular, Pkd1-/- cells presented an increase in glucose uptake and lactate production, suggesting an increased anaerobic glycolysis (Warburg's effect).

Consistent with this, intracellular ATP content was enhanced in the mutant cells compared to the wt cells. Measure of the mitochondrial potential has shown that it was normal in Pkd1-/- cells. There was no increased ATP anymore in Pkd1-/- cells compared to the Pkd1+/+ cells after glucose starvation showing that ATP content was esclusively caused by an increased glycolysis. In line with these results, Pkd1-/- cells were hypersensitive to glucose deprivation, with a rapamycin sensitive increased apoptosis. In the same conditions, autophagy was induced in Pkd1+/+ cells as expected while Pkd1-/- cells failed to undergo autophagy in line with the increased mTORC1 activity in these cells. Next we monitored the activation of the energy sensor AMPK which was downregulated in Pkd1-/- cells. Next, we looked if a similar glycolytic switch could be detected in vivo, we found that ATP content was increased and AMPK downregulated in the cystic kidneys of a Pkd1flox/-:Ksp-Cre mouse model. Preliminary results suggest an increase in glucose uptake in these kidneys in vivo.

Conclusions: Our data uncover a previously unrecognized role for the Pkd1 gene in regulation of cellular energy balance.

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FR-PO2017

Renal and Cardiovascular Phenotypes in Mice with Pkd$^{+/-}$ Mice: Evidence for Nephrogenic Diabetes Insipidus and Impaired Urine Acidification but Normal Glomerular Filtration Rate (GFR) Leonidas Tsiokas,[1] Bonnie Eby,[2] Alexander Lau,[2] Uzma I. Hajiyani,[2] Meghan Pantalia,[2] Chris Skaggs,[2] Becky Pennington,[2] Pedro Lozano,[2] Kai Lau.[2] *[1]Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: *Pkd2*$^{+/-}$mice have shortened survivals despite normal BUN & sparse renal cysts in 30% of them. But renal functions in sex- & age-matched mice have not been carefully studied. Abnormal intracellular Ca regulation & lower basal [Ca]i in VSMC are implicated to reduce longevity. *Pkd1*$^{+/-}$mice have endothelial dysfunctions, hypertension, & SIADH, attributed to lower basal [Ca]i, but if *Pkd2*$^{+/-}$mice have similar defects is unclear.

Methods: We tested if renal & cardiovascular phenotypes exist in male *Pkd2*$^{+/-}$mice by measuring tubular functions & GFR [serum creatinine (cre) & clearance (C) by HPLC], abdominal cysts & heart functions by echo, blood pressure (BP) & endothelial function.

Results: Cardiac systolic & diastolic functions were normal in *Pkd2*$^{+/-}$mice at 9 & 15 mon. Direct carotid BP & aortic relaxations were normal at 20 mon. Liver cysts were multiple on echo in 50% at 15 mon, 80% at 18 mon & 100% by 20 mon. Renal cysts were absent except in 1 mouse. Serum cre & Ccre were similar at 8, 11, 15 & 19 mon. At 9 mon, increase in urine osm from dehydration tended to be smaller in *Pkd2*$^{+/-}$mice. By 11 mon, they were polyuric by 20%. Dehydration x 8 h produced similar osm (in mOsm/kg) in serum (299 vs 295) & urine (2,201 vs 1,887). Increases in urine osm elicited by 4 more h of dehydration, however, were blunted by 57 % in *Pkd2*$^{+/-}$mice (657 vs. 1,515), despite similar serum osm (319 vs. 318). Vasopressin elicited 63% smaller rise in urine osm (822 vs. 2,252) despite similar serum osm. pH in urine under oil from 12-20th h of fast was higher in *Pkd2*$^{+/-}$mice (5.5 vs. 4.4).

Conclusions: 1. Contrary to liver, renal cysts are rare & GFR is normal even at 20 mon. 2. Unlike *Pkd1*$^{+/-}$mice, male *Pkd2*$^{+/-}$mice have no cardiovascular phenotypes. 3. They have impaired acidification & partial nephrogenic diabetes insipidus vs. SIADH in *Pkd1*$^{+/-}$mice. These differences may explain the milder diseases in man.

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FR-PO2018

Diabetes Accelerates Cystogenesis and Results in Glomerular and Tubular Damage in the Adult Conditional ift88 Knockout Mouse P. Darwin Bell, Kelli Margot Sas, May Y. Amria, Monika Gooz. *Medicine/Nephrology, Medical University of South Carolina, Charleston, SC.*

Background: Diabetes, the leading cause of end stage renal disease, results in structural and functional hypertrophy in the kidney. We have previously found that renal hypertrophy accelerates cystogenesis following deletion of cilia in the adult mouse. The goal of this study was to determine if the presence of diabetes would modify the time course and degree of cyst formation in the adult mouse with deletion of cilia.

Methods: To examine the role of cilia in diabetes, we utilized a conditional floxed allele for the *ift88/Tg737* gene to produce cilia (+) or cilia (-) adult mice. Mice were administered streptozotocin at 50 mg/kg for 5 days to induce diabetes. After 6 weeks, mice underwent MRI to determine cystic burden and kidneys were removed after 6 or 12 weeks for histological analysis.

Results: Blood glucose concentrations were elevated but not significantly different between groups. MRI and histological analyses identified accelerated cystogenesis mainly in the glomeruli and collecting ducts of diabetic cilia (-) mice. Additionally, diabetic cilia (-) mice exhibited abnormalities in non-cystic regions with signs of tubular stress and glomerular disease, as evidenced by primitive renal tubules, mesangial expansion, and prominent podocytes. These abnormalities did not occur in cilia (+) mice. There was also a marked increase in lymphocytic infiltration and enhanced mTOR, AKT, and B-catenin signaling, as well as decreased E-cadherin and RKIP expression in kidneys from the diabetic cilia (-) mice.

Conclusions: Diabetes may be a significant risk factor for accelerated cyst formation and renal failure in PKD, and cilia may be reno-protective in diabetes.

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FR-PO2019

Projection Structure of the Polycystin-1 Membrane Domains Brian D. Adair. *Massachusetts General Hospital and Harvard Medical School, Charlestown, MA.*

Background: In the majority of cases, polycystic kidney disease arises from a mutation in either of the genes PKD1 or PKD2. PKD2 encodes a nonspecific cation channel (polycystin-2 or PC-2) which possesses six transmembrane spans and is believed to act as a tetramer. In contrast, PKD1 encodes an extremely large membrane protein (polycystin-1 or PC-1) with eleven transmembrane domains and an extensive extracellular N-terminal region possessing numerous structural motifs for binding cell matrix and membrane proteins. The two proteins interact in vivo and are believed to participate in the same signaling pathway with PC-1 modulating the channel activity of PC-2. The nature of the interaction remains elusive, although the region of PC-1 containing the six terminal transmembrane spans shows sequence homology with the transmembrane region of PC-2.

Methods: A region of the PKD1 gene encoding the eleven transmembrane domains has been cloned and expressed in the yeast Pichia pastoris. The construct begins at the GPS domain prior to the first transmembrane span and includes the complete cytosolic C-terminal domain. The recombinant protein forms 2D crystalline arrays in membranes. Electron crystallography has been employed determine the projection structure of the membrane embedded protein.

Results: The 2D crystals have a plane group symmetry of p4 with unit cell parameters a=b=159 Å and γ=90°. The projection structure derived from electron crystallography reveals that the unit cell is composed of four PC-1 dimers related by four-fold rotation symmetry. The dimers are closely associated with direct, protein-protein interactions, while the packing between dimers in the unit cell is much looser with the dimers clearly separated by lipid molecules.

Conclusions: The sequence homology between the transmembrane domains of PC-2 and the terminal transmembrane domains of PC-1 had suggested that the latter might form an equivalent, tetrameric channel. The structure reveals that this is not the case and PC-1 cannot form a channel on its own. However, the dimeric organization suggests that PC-1 might form a heterotetrameric channel with the homologous domains of PC-2, where opposed dimers of PC-1 and PC-2 would form a pseudo four-fold channel.

Funding: Private Foundation Support

FR-PO2020

Mitochondrial Defects and Lifespan Extension in Yeast Model of XPNPEP3 Deletion John F. O'Toole, Erine M. Stames. *Case Western Reserve University, Cleveland, OH.*

Background: *XPNPEP3* is a nuclear gene that encodes a mitochondrial aminopeptidase and mutations have been identified in individuals with heritable kidney failure with tubular atrophy and interstitial fibrosis. The *Saccharomyces cerevisiae* ortholog, *ICP55*, localizes to mitochondria and stabilizes its target proteins. Thus, we used yeast as a genetic model of *XPNPEP3*/*ICP55* deletion to elucidate the underlying mechanisms of tubular atrophy observed in patients.

Methods: Yeast strains were generated with PCR based homologous recombination and grown using standard culture media and conditions. Oxygen consumption was measured with a Clarke-type oxygen electrode. Chronological lifespan (CLS) and resistance to H_2O_2 were determined with trypan blue staining and plating serial dilutions. Reactive oxygen species (ROS) were measured with dihydroethidium staining and flow cytometry.

Results: *ICP55* deletion strains are viable and respiratory competent. Growth in glucose media is comparable to parental strains, but mitochondrial oxygen consumption is significantly decreased. As mitochondrial defects often lead to decrements in lifespan we examined CLS. Surprisingly, *ICP55* deletion increased the CLS. Since TOR1 is known to affect mitochondrial respiration and lifespan, we treated deletion strains with rapamycin, which restored the mitochondrial oxygen consumption to wild-type levels. Deletion of *TOR1* also increased the CLS, but interestingly, combined deletion of *TOR1* and *ICP55* increased CLS additively, suggesting independent mechanisms of CLS extension. Deletion strains were also more resistant to H_2O_2 treatment than parental strains and had lower levels of ROS.

Conclusions: These results suggest that deletion of *XPNPEP3*/*ICP55* results in mitochondrial stress possibly related to dysregulation of mitochondrial protein degradation pathways. The stress response pathways activated in the yeast model may include TOR1 activation, but the resistance to oxidative stress and CLS extension likely occurs through mechanisms that are independent of that observed with *TOR1* deletion. Further characterization of yeast mitochondrial stress pathways should define mechanisms underlying the renal tubular atrophy and interstitial fibrosis observed in patients.

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FR-PO2021

Lymphocyte Subpopulations during Hemodialysis and Hemodiafiltration
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Background: Lymphocytes are central to the adaptive immune response and their function is impaired in maintenance dialysis patients increasing the susceptibility to infection. The effects of different dialysis modes on the course of lymphocyte subpopulations are largely unknown.

Methods: In a prospective, randomized, controlled, cross-over trial, 10 maintenance dialysis patients were subjected to 4 weeks of each low-flux HD (LF-HD), high-flux HD (HF-HD), low- and high-efficiency postdilution hemodiafiltration (Lo-HDF and Hi-HDF, resp.). Identical, highly biocompatible synthetic dialysis membrane materials and ultrapure dialysate were used in all treatment forms (LF-HD, PUREMA® L, 1.8 m^2; HF-HD, Lo-HDF and Hi-HDF, PUREMA® H, 1.9 m^2). Counts of total lymphocytes and lymphocyte subsets (T-lymphocytes ($CD3^+$), T-inducer cells ($CD3^+/CD4^+$), cytotoxic T-cells ($CD3^+/CD8^+$), B-lymphocytes ($CD45^+/CD19^+/CD3^-$), natural killer cells ($CD45^+/CD16^+ 56^+/CD3^-$)) were determined during individual treatments as well as at baseline and at the end of each 4-week period.

Results: At 10 min, total lymphocytes (between 79±12% (LF-HD; $P<0.05$) to 89±11% (HF-HD) of baseline), $CD3^+$ (86±11% (LF-HD; $P<0.05$) to 99±9% (HF-HD) of baseline), $CD3^+/CD4^+$ (91±10% (LF-HD; $P<0.05$) to 104±11% (HF-HD) of baseline), $CD3^+/CD8^+$ (76±16% (LF-HD; $P<0.05$) to 89±15% (HF-HD) of baseline) and $CD45^+/CD19^+/CD3^-$ (82±17% (LF-HD; $P<0.05$) to 90±14% (Hi-HDF) of baseline) cells had only slightly and mostly temporarily decreased. In contrast, $CD45^+/CD16^+ 56^+/CD3^-$ cells steeply and, similar to the course of $CD3^+/CD8^+$ cells, permanently dropped to between 51±14% (LF-HD) to 63±16% (Lo-HDF) of baseline ($P<0.01$). No differences between treatment modes and the predialysis cell counts at baseline and at the end of the 4-week periods were observed.

Conclusions: Different to other lymphocyte subpopulations, $CD45^+/CD16^+ 56^+/CD3^-$ cells are strongly activated during extracorporeal dialysis procedures. Enhanced convective toxin removal, such as in HDF, has no additional effect on the biological behavior of lymphocytes.

Funding: Pharmaceutical Company Support

FR-PO2022

In Acidotic Hemodialysis (HD) Patients, High Dialysate Bicarbonate Concentration Corrects Pre-HD Acidosis but Induces Post-HD Metabolic Alkalosis and Alkalemia Associated with Attenuated Increase in pCO_2
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Background: Acidosis is a common severe problem in HD patients. K/DOQI guideline of pre-HD serum bicarbonate (BIC) level (SBIC) > 22 mEq/L is probably insufficient and frequently not achieved on routinely used dialysate BIC concentration (DBIC) of 33-34 mEq/L. We hypothesized that high DBIC (HDBIC) can correct pre-HD acidosis but may induce intra-dialytic metabolic alkalosis/alkalemia, or due to increased BIC buffering and CO_2 production, severe hypercapnia in pulmonary problems or apneic sleep episodes during HD. This study aimed to assess effects of high (40 mEq/L) vs low (33-34 mEq/L) DBIC on SBIC, pCO2 and pH levels during HD.

Methods: In a prospective bi-center study, 19 chronic HD patients with peripheral access were assessed for consecutive 3-week periods on LDBIC and afterwards HDBIC. Arterial blood gases and electrolytes were assessed once weekly at start, middle and end of first weekly HD, and data after equilibration (3rd week assessment) presented.

Results: Pre-HD SBIC was <22 mEq/L on LDBIC in 11 patients (acidotic-A) and increased in them by HDBIC to 26.6 mEq/L inducing intra-dialytic metabolic alkalosis /alkalemia (post-HD SBIC 35.3 mEq/L, pH 7.52) resembling the non-acidotic patients. On HDBIC, pre-HD SBIC correlated in all patients and in A with post-HD pCO2 ($r=0.47$, $p<0.05$, $r=0.73$, $p<0.05$, respectively) and in A with mid-end HD pCO_2 change ($r=0.7$,$p<0.05$). In A, when pre-HD pCO2 ≤44 mm Hg , it increased at mid-HD (37.5 to 41.5 mm Hg, $p<0.01$) but subsequently partially decreased post-HD (38.5 mm Hg, $p<0.05$) (overall-$p<0.01$).

Hypercapnia >55 mm HG occurred only once.

Conclusions: HDBIC corrected acidosis but induced intra-dialytic metabolic alkalosis/alkalemia, associated in the more acidotic patients with lower pCO_2 post-HD and sometimes even its' partial decrease toward end-HD. To moderate intra-dialytic metabolic alkalosis/alkalemia we suggest gradual individualized increase in DBIC in acidotic HD patients.

FR-PO2023

Role of Advanced Oxidative Protein Products in Breaking Peripheral Th17/Treg Balance in Patients on Maintenance Hemodialysis
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Background: CD4+CD25+ regulatory T (Treg) cells and Th17 cells, subsets of T-helper cells, have been reported to play important roles in peripheral immunity, and their imbalance leads to the development of tissue inflammation and autoimmune diseases. In this study, we investigated the association between levels of serum advanced oxidation protein products (AOPPs) and atherosclerosis and the effect of AOPPs on the Th17/Treg balance in patients on maintenance hemodialysis (MHD).

Methods: 57 consecutive MHD patients and 24 healthy volunteers were included. Common carotid artery intima-medial thickness (CCA-IMT) and carotid plaques (CPs) were determined with non-invasive high-resolution B-mode ultrasonography. Peripheral blood mononuclear cells (PBMCs) collected from atherosclerotic cardiovascular disease (ASCVD), non-ASCVD and healthy volunteers were incubated with AOPPs *in vitro*, and the frequency of Treg and Th17 cells was measured using flow cytometry.

Results: Serum levels of AOPPs were higher in patients with MHD as compared to those in healthy controls. Patients with CPs and ASCVD also showed higher levels of AOPPs as compared with patients without CPs and ASCVD. Serum levels of AOPPs were strongly associated with CRP and CCA-IMT, positively correlated with the Th17 cells frequency and serum IL-17 levels, and negatively correlated with the Treg cells frequency, serum IL-10 and TGF-β1 levels. *In vitro* incubation of PBMC from controls with AOPPs resulted in a significant reduction of Treg cells and elevation of Th17 cells. There were significant changes in the number of Treg and Th17 cells by AOPPs in ASCVD compared to non-ASCVD and healthy controls groups.

Conclusions: Treg and Th17 cells from ASCVD patients were more susceptible to AOPPs-mediated alterations. Increased AOPPs have an effect on Th17/Treg imbalance, pormote the micro-inflammatory state, and may ultimately contribute to the occurrence of ASCVD in MHD patients.

Funding: Government Support - Non-U.S.

FR-PO2024

Tinzaparin for Anticoagulation in Haemodialysis (HD); a Simple Dosing Algorithm Which Is Safe and Efficacious
Christopher J. Kirwan, Neil Ashman, Zahid Farooq Baig. *Royal London Hospital, United Kingdom.*

Background: Bleeding complications when using heparin, essential as anticoagulation during HD, are common. Low molecular weight heparins (LMWH) have reduced their frequency. An iv bolus Tinzaparin (TP), has a 1/2life of 4 hrs in controls but this is longer in renal failure pts allowing potentially dangerous latent anticoagulation. Plasma anti Xa activity (anti-fXa) is used as a surrogate marker for TP activity. TP can be an effective anticoagulation during HD but dosing regimens vary & end HD anti-fXa has not been fully assessed. We studied anti-fXa in pts receiving TP from a fixed dose HD protocol, assessing efficacy & safety aiming to standardise anticoagulation

Methods: Anti-fXa measured during HD in consecutive pts on a single HD unit. 2500,3500 or 4500iu of TP was given based on length of HD and prior coagulation events. Anti-fXa was measured at T0,0.5,1,2hrs & at the end of HD. Anti-fXa<0.2 is considered therapeutically inert. Efficacy & safety data was collected from 5 HD sessions spanning the study day

Results: 43pts on HD (3.5 or 4hrs), had anti Xa profiling. 19,18 & 6pts received 2500, 3500 & 4500iu of TP respectively. 21pts had 3.5hrs HD; 13 received 2500iu, 6/3500iu & 2/4500iu of TP. No pt had detectable anti-fXa at T0. Pts receiving 3500iu weighed more than those for 2500iu (69v56kg; $p<0.005$) & the greater the dose/kg the higher the peak anti-fXa ($p<0.01$). Dose/kg did not predict end HD anti-fXa ($p=0.07$). Higher TP doses correlated to peak anti-fXa ($p<0.05$). 15pts had anti-fXa >0.2 at the end of HD, 5/19 (2500iu), 8/18 (3500iu) & 4/6 (4500iu). Across 260 HD sessions (52 pts) mean AVF compression time post HD was 9 mins & there were 4 incidents of minor bleeding & 1 clotted circuit

Conclusions: Wt based dosing of TP in HD does not add to safety & efficacy of anticoagulation. Higher doses lead to higher peaks & end HD anti-fXa. Adequate anticoagulation can be achieved with a fixed dose of TP in most cases. A higher TP dose causes a significant anti-fXa at the end of HD. We recommend starting TP at 2500iu, increasing to 3500iu if there are signs of circuit coagulation. A single anti-fXa level should be measured at the end of HD guiding dose, if there is a coagulation concern

FR-PO2025

Pharmacokinetics of the Direct Renin Inhibitor Aliskiren in Patients with End-Stage Renal Disease Undergoing Hemodialysis
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Background: Aliskiren represents a new class of orally active direct renin inhibitors and is approved for the treatment of hypertension. The present study compares the pharmacokinetics, safety and tolerability of a single dose of aliskiren in patients with End Stage Renal Disease (ESRD) on hemodialysis (HD)in comparison to matched healthy subjects.

Methods: This open-label, single-sequence study enrolled 6 male ESRD patients undergoing chronic HD and 6 healthy, age and body-weight matched volunteers without HD treatment. The ESRD patients underwent two treatment periods (Period 1: single oral dose of aliskiren 300mg 24 h after the last HD session and 48 h before the next HD session; and Period 2: single oral dose of aliskiren 300mg 1 h before the HD session) with a wash out period of >10 days between them. HD treatment lasted 4 h (blood flow 300ml/min, dialysate flow 500ml/min). Blood and dialysis samples were taken for up to 96 h post-dose to determine plasma concentrations of aliskiren. The healthy subjects received one oral dose of aliskiren 300mg and 96 h plasma pharmacokinetics was determined.

Results: In both treatment protocols, dialysis clearance was low (1-2% of oral clearance) and not significantly altered by timing of dialysis. Fraction of aliskiren eliminated by HD was <0.2% in both settings. Compared to the healthy subjects, administration of 300 mg aliskiren was associated with an increase in AUC (+61%,2031±737ng*h/mL)

and Cmax (+17%,279±203ng/mL) in ESRD patients receiving HD at 48 h and increase in AUC (+42%,1776±1149ng*h/mL) and decrease in Cmax (-16%,200±321ng/mL) in those receiving HD at 1 hr.

Conclusions: Only a minor faction of aliskiren is eliminated by an intermittent HD. The aliskiren exposure is not significantly different in ESRD patients receiving a single oral dose of 300 mg either at 1 h or 48 h before HD. No severe adverse reactions occurred in this study.

Funding: Pharmaceutical Company Support

FR-PO2026

Effective Elimination of Dabigatran with Haemodialysis: A Phase I Single Centre Study in Patients with End-Stage Renal Disease Harm Peters,[1] Dmytro Khadzhynov,[1] Frank-Dietrich H. Wagner,[2] Stephan Formella,[3] Viktoria Moschetti,[3] Andreas Clemens.[3] *[1]Nephrology, Charité, Campus Mitte, Berlin, Germany; [2]Charité Research Organisation GmbH, Berlin, Germany; [3]Boehringer Ingelheim Pharma GmbH Co. KG, Ingelheim, Germany.*

Background: Dabigatran etexilate (DE) is the pro-drug of dabigatran (D), a synthetic low molecular weight orally active reversible direct thrombin inhibitor. DE is approved for the prevention of stroke and systemic embolism in patients with atrial fibrillation and for the primary prevention of thromboembolic events in the orthopaedic setting. A specific antidote for D has been recently preclinically identified and is not yet available for clinical use in patients. Haemodialysis may be a measure in emergency situations requiring fast elimination of D.

Methods: Dialysis modalities (catheter setting, blood flow rate of 200 ml/min, dialysate flow rate of 700 ml/min) are thought to apply for the majority of patients anticoagulated with DE and having a normal renal function. Six patients with end-stage renal disease (ESRD) received DE once daily over 3 days to achieve steady state (Day 1: 150 mg; Day 2: 110 mg; Day 3: 75 mg).

Results: Plasma trough concentrations at Day 3 were 146±58.1 ng/ml and increased to peak concentrations of 176±60 ng/ml 2 h following the final dose of 75 mg. Dialysis was started 8 following the last dose to await absorption and distribution of the drug (D=150±58.9 ng/ml). After the 4-h dialysis, plasma concentrations had dropped sharply to approx. one half (76±48 ng/ml). The extraction ratio calculated from blood entering and leaving the dialyser was 81.3±2.0%. The rebound after stopping dialysis was minor representing a 10% increase in plasma concentration.

Conclusions: The high dialysis extraction ratio of 81.3% in patients with ESRD and the minor rebound of plasma concentration after dialysis indicate that haemodialysis is an efficient method to remove D from the body. Four-hour dialysis under normal blood flow conditions eliminated about 50% of D. Elimination rates with a high blood flow rate of 400 ml/min will be reported in an updated version of this abstract. Haemodialysis can be employed as an effective measure to eliminate D in emergency situations.

Funding: Pharmaceutical Company Support

FR-PO2027

A Multi-Center, Prospective, Open-Label, 8-Week Study To Investigate the Efficacy, Safety and Pharmacodynamics of Certoparin for Anticoagulation during Maintenance Hemodialysis – The MEMBRANE Study Detlef H. Krieter,[1] Oliver Dorsch,[2] Horst-Dieter Lemke,[3] Nima Melzer,[4] Christian Sieder,[4] Peter Bramlage,[5] Job Harenberg.[6] *[1]Nephrology, University Hospital, Würzburg; [2]KfH Renal Center, Kronach; [3]EXcorLab, Obernburg, Germany; [4]Novartis Pharma, Nuremberg, Germany; [5]Cardiovasc. Pharmacology and Epidemiology, Mahlow; [6]Clin. Pharmacology, Univ. of Heidelberg, Mannheim, Germany.*

Background: Anticoagulation is a prerequisite for hemodialysis (HD) to prevent clotting in the extracorporeal circuit. We provide efficacy, safety and pharmacodynamic data on the LMWH certoparin.

Methods: Multicenter, open-label, prospective, 8-week trial in patients (pts) undergoing HD receiving a single dose of 3,000 IU certoparin i.v. at 2-3 sessions per week. Additional titration steps and continuous infusion were allowed.

Results: Of 109 pts enrolled (median 71 (range 26-90) years; mean body weight 82.5±18.3 kg; dialysis duration 265±29 min), 106 pts were available for efficacy analyses. The primary endpoint (% of unsatisfactory HD results at week 8 due to clotting or bleeding) was met in 1.9% (n=2) of patients (95%CI 0.23-6.65); no major bleeding. 1.9% of pts experienced clinically relevant clotting in blood lines/bubble catcher and 2.8% in the dialyzer at week 8. 15.7±14.3% of the dialysis filters' visual surface area showed reddish fibers. 40.4% of pts (n=44) had prolonged shunt compression times at least once (overall 82 cases in 2724 dialyses (3.0%)). 8.3% had at least one minor and none had major bleeding. 85.3% had adverse events, 9.2% were serious; in 29.4% drug-relation was suspected. At baseline, week 4 and 8 in subgroups of pts (n=12, 12 and 36) receiving median doses of 3000 (range 3000–3000), 3000 (2400–6000) and 4200 (3000–6600) IU, plasma aXa levels (C) were determined (geometric mean [95%CI]): C2h was 0.24 [0.21-0.27], 0.33 [0.27-0.40] and 0.38 [0.33-0.45] aXa IU/ml, C48h was 0.01 [0.01-0.02] aXa IU at all visits (below detection limit in 50, 66.7 and 82.4%). At baseline and week 4, the area under curve AUC0-48h was 2.66 [2.19-3.24] and 3.66 [3.00-4.45] aXa IU*h/ml.

Conclusions: Certoparin appears to be safe and effective for anticoagulation in patients undergoing maintenance HD.

Funding: Pharmaceutical Company Support

FR-PO2028

Asymmetric Dimethylarginine Removal in a Hemodiafiltration-Adsorption Technique: Comparison with Bicarbonate Dialyis and Hemodiafiltration Antonino Sidoti,[1] Marina Biagioli,[1] Donella Borracelli,[1] Luisa Sereni,[2] Céline Piekarski,[2] Giuseppe Palladino.[2] *[1]Nephrology, Poggibonsi, Italy; [2]Bellco, Mirandola, Italy.*

Background: Asymmetric dimethylarginine (ADMA) whose plasma levels increase with worsening of kidney failure is a strong independent mortality predictor in ESRD. A large effort to understand which is best dialysis technique to remove it, has not achieved definitive results. A significant protein binding could affect ADMA dialysance.

Methods: SUPRA provides separately, in the same device, hemofiltration, diffusion and adsorption with a 0.7sm super high-flux polyphenilene membrane 43000 D cut–off, a diffusive membrane 1.7sm low-flux polyphenilene and a styrenic resins cartridge to regenerate by adsorption ultrafiltrate for so called endogenous reinfusion.

We studied four patients, three men and a woman, mean age 68.5±15.5, dialytic age 243±119 mo.s, none with residual renal function, each had a 240' SUPRA, on-line Hemodiafiltration(HDF)1.8 sm Polysulfone(PS) hi-flux and bicarbonate dialysis (BD) 1.8 sm PS low-flux treatment as first session of the week. Blood flow was 330±35 ml/min, infusion liters were 13.0±1.9 on SUPRA and 12.9 ±1.29 on HDF. We used ADMA ELISA on plasma samples obtained at 0' and 240'.

Results: ADMA at 0' and 240' were 1.192±0.48 and 0.27±0.09 for SUPRA,1.7±0.355 and 0.625±0.075 for BD, 0.945±0.063 and 0.497±0.063 for HDF. Reduction Ratio Percentage(RR) was 75.9±6.14 for SUPRA, 62.5±5.57 for BD, 47±8.2 for HDF. Differences by pairs were from start to end 0.917±0.418(CI95% 0.25-1.58) p=0.022 for SUPRA, 1.075±0.321(CI 95% 0.56-1.58) p=0.007 for BD, 0.447±0.099 for HDF(CI 95% 0.28-0.60) p=0.003. A two way ANOVA indicate a significant effect due to techniques [F=44.1; p<.001].

Conclusions: SUPRA demostrated a better RR than BD and HDF most likely due to its ability to ultrafiltrate a proportion of plasma protein (e.g. albumin 6g) during each treatment. Substances bound to proteins but also present in plasma water are liable to adsorption during the passage through the cartridge before ultrafiltrate returns to the patient. Further studies are needed to elucidate effectiveness of this technique in a long follow up i.e. in lowering in a permanent way ADMA levels.

Funding: Government Support - Non-U.S.

FR-PO2029

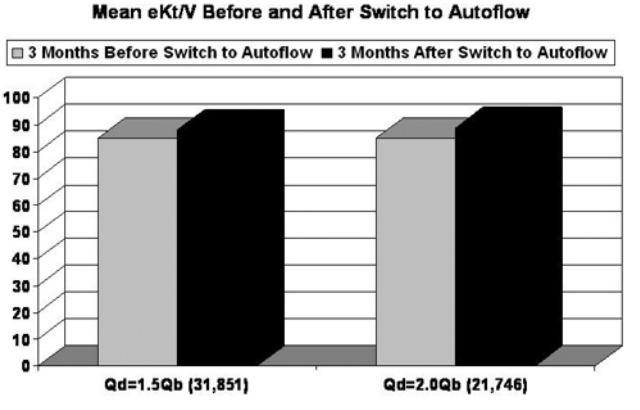
A Blood Flow – Dialysate Flow Ratio Maintains Dialysis Adequacy While Reducing the Quantity of Dialysate Used Per Treatment Linda H. Ficociello, Norma J. Ofsthun, J. Michael Lazarus, Claudy Mullon, Jose A. Diaz-Buxo. *Fresenius Medical Care - NA, Waltham, MA.*

Background: It has been proposed that a blood flow - dialysate flow ratio instead of a fixed dialysate flow would optimize clearance. Fresenius Medical Care NA (FMC-NA) 2008® K and T dialysis machines have an AutoFlow Dialysate Flow feature where dialysate flow is set to either 1.5 or 2.0 times blood flow. Additionally, Autoflow reduces flow rate to 300 ml/min when in idle mode to reduce waste.

Methods: 53,597 patients converted to either AutoFlow 1.5 or 2.0 and had at least 2 measurements of adequacy before switching and at least 2 after the switch. Change in dialysate flow prescription was not allowed during this time.

Results: Of the 31,851 patients who switched from their prescriptive dialysate flow (median =800 ml/min) to Autoflow 1.5, the percentage of patients achieving a mean eKt/V of 1.2 or greater increased by 3% (from 85 to 88%). Of the 21,746 patients who switched from their prescriptive dialysate flow (median =800 ml/min) to Autoflow 2.0, the percentage of patients achieving a mean eKt/V of 1.2 or greater increased by 4% (from 85 to 89%). Comparing before and after eKt/V for each patient, we find a small but statistically significant improvement in patients switched to Autoflow 1.5 (mean increase = 0.3, p<0.0001) and Autoflow 2.0 (mean increase = 0.4, p<0.0001). Average gallons of dialysate per patient dropped by 3 gallons before and after Autoflow use (from 42 to 39 gallons per patient) not including the reduction of dialysate wasted when in idle mode.

Conclusions: The use of Autoflow reduces the amount of dialysate used in each hemodialysis treatment without reducing adequacy of treatment.



Funding: Pharmaceutical Company Support

FR-PO2030

Phosphate Handling by the Kidney in Patients with End-Stage Renal Failure on Hemodialysis Hideaki Iwasawa, Yoshitaka Miyaoka, Ami Hayashi, Toshiyuki Nakao. *Department of Nephrology, Tokyo Medical University, Shinjyuku-ku, Tokyo, Japan.*

Background: Not a few patients with end-stage renal failure on dialysis therapy have residual renal function which plays a significant role on the removal of retained fluids and solutes even after the initiation of dialysis. However, little is known about the excretion mechanism of fluids and solute by end-stage kidneys, such as by those with a glomerulur filtration rate (GFR) of less than 10 ml/min. Thus, we investigated how phosphate is handled and excreted by the end-stage kidneys in hemodialysis patients.

Methods: We studied 67 patients with a urinary output less than 100 ml/day among 189 consecutive chronic hemodialysis patients from 2008 to 2010. Blood specimens were obtained at the start of hemodialysis on the first session of the week, and we measured the serum concentrations of phosphate, calcium, urea nitrogen, creatinine intact PTH and FGF-23. We also collected 24-hour urinary specimens and measured the urinary concentrations of phosphate, calcium, urea nitrogen and creatinine. GFR was calculated from the mean values of creatinine clearance and urea clearance as (Ccr + Curea)/2 based on 24-hour urine collection.

Results: The mean urine volume (UV) and GFR were 1184.9±548.4 ml/day and 3.11±1.94 ml/min, respectively. The mean serum phosphate (sP) and urine phosphate (uP) concentrations and the amount of 24-hour urinary phosphate excretion (PE) were 5.7±1.2 mg/dl, 16.8±6.3 mg/dl and 200.2±122.7mg/day, respectively. sP significantly correlated with uP (r=0.358, p=0.012), but not with PE. The mean maximal tubular reabsorption threshold of phosphate was 2.0±0.9 mg/dl, which correlated with sP (r=0.306, p=0.012), but not with both uP and PE. PE strongly correlated with GFR (r=0.838, p<0.0001) and UV (r=0.791, p<0.0001), and significantly correlated with intact PTH (r=0.533, p<0.0001) and FGF-23, but not with sP.

Conclusions: The daily urinary excretion amount of phosphate by end-stage kidneys in hemodialysis patients mainly depends on GFR. The action of phosphaturic hormones on the kidney remains, even if the GFR is less than 10 ml/min.

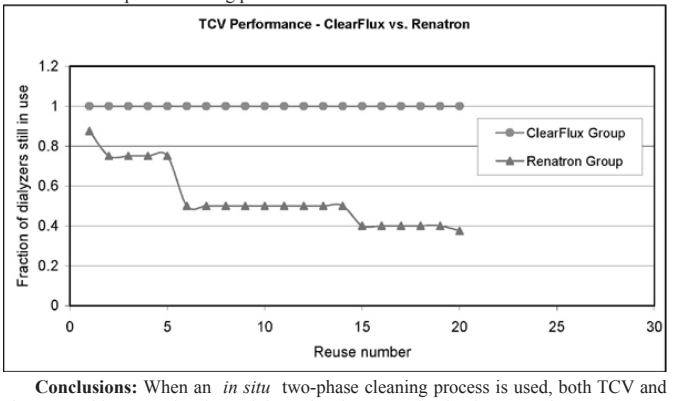
FR-PO2031

Impact of *In Situ* Two-Phase Cleaning on Dialyzer Reuse – Total Cell Volume (TCV) and Middle Molecule Clearance Mohamed E. Labib,[1] Joseph John Murawski,[1] Yacoob Tabani,[1] Dharmeshkumar M. Kanani,[2] Andrew L. Zydney,[2] Toros Kapoian,[3] Richard A. Ward.[4] *[1]Novaflux Technologies, Princeton, NJ; [2]Pennsylvania State University, University Park, PA; [3]Robert Wood Johnson Medical School, New Brunswick, NJ; [4]University of Louisville, KY.*

Background: Reprocessing of dialyzers with peracetic acid has the advantage of easy management of the disinfection solution. However, the clearance of higher molecular weight solutes can markedly decrease after only a small number of reuses and is not reflected in a change in total cell volume (TCV). To address this problem, we studied the effects of an *in situ* two-phase cleaning process (ClearFlux ™ Dialyzer Reprocessing System) on the maintenance of TCV and on the clearance of various molecular weight dextrans under reuse conditions.

Methods: Gambro Polyflux® 21R, Fresenius F80A and Fresenius Optiflux® F200B dialyzers were used for up to 20 treatments, reprocessed using the ClearFlux ™ or conventional peracetic acid reprocessing.

Results: The fraction of dialyzers meeting the criterion for continued use (TCV > 80% of initial value) is shown in the figure. After 20 reuses with the ClearFlux ™, all dialyzers were still usable with TCV > 80% (mean 100% ± 3% SD), while only 40% of those dialyzers treated with conventional processing met the criterion. After 7 patient reuses, clearance of a 10 kDa dextran probe (beta-2 microglobulin = 11.8 kDa) was reduced by 78% in dialyzers with conventional peracetic acid processing, but was unchanged from baseline values with the *in situ* two-phase cleaning process.



Conclusions: When an *in situ* two-phase cleaning process is used, both TCV and clearance of larger molecular weight molecules are maintained to a much greater extent than when using conventional peracetic acid reprocessing.

Funding: NIDDK Support

FR-PO2032

Multi-State Modelling of Serum Albumin and B2-Microglobulin in Hemodialysis Patients Christos Argyropoulos, Maria-Eleni Roumelioti, Mark L. Unruh, Khaled Abdel-Kader. *Renal and Electrolyte, University of Pittsburgh, PA.*

Background: B2-Microglobulin (B2M)and serum albumin (SA) have emerged as predictors of survival in hemodialysis (HD) patients (pts), yet the joint utility of these markers remains unexplored. We examine SA/B2M as determinants of transitory states of health and predictors of mortality in HD pts.

Methods: Prevalent HD pts enrolled in the HEMODIALYSIS (HEMO) trial receiving High Flux dialysis. Panel data of B2M/SA were used to define a Multi-State Model (MSM) of 9 health states by cross classifying patients according to tertiles (Lower, Middle, Upper) of (B2M,SA). We examined instantaneous transitions among these states and relative risk of death with Markov models.

Results: 902 pts out of 1846 initial study participants had available information for MSM. Pt demographics were as follows: age 57.6 ±14, 56.5% women, 63.2% African Americans, 44.7% diabetics SA(nephelometric) and B2M tertiles were L: <3.43, M: 3.43-3.74, U: >3.74 and L: <29.1, M: 29.1-36.6, U: >36.6 respectively. Estimated hazard rates among states are shown in Fig(top), so that patients in state 1 (L B2M and SA) were twice (0.43/0.26) as likely to move to state 4 (L B2M and M SA) than state 2 (M B2M and L SA). Hazard ratios (HR) of death relative to the "best" state 7 (L B2M and H SA) are tabulated below.

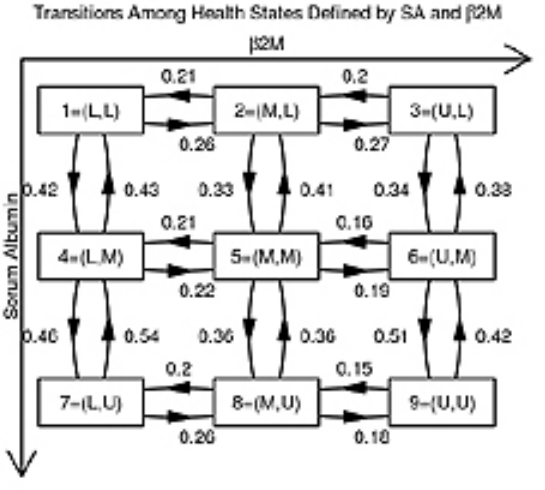
Hazard Rate of Death from Health States Relative to State 7 (L B2M, U SA)

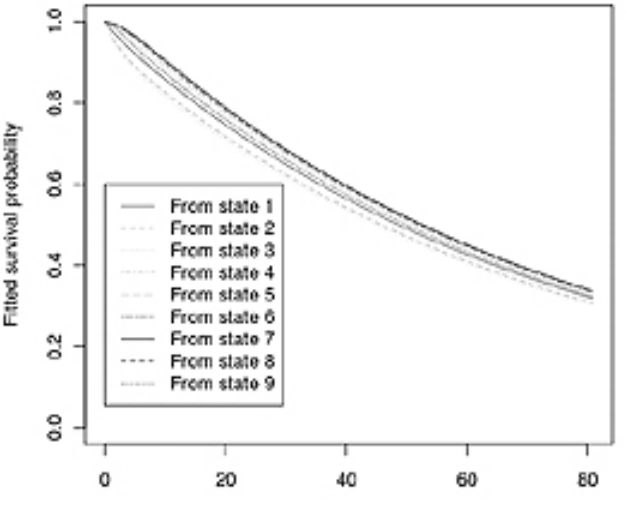
State	HR
1	8.1
2	19.4*
3	16.1*
4	2.3
5	2.0
6	1.8
8	1.7
9	2.4

*p<0.05

For all B2M tertiles, the HR of death appears to worsen with decreasing SA values and the risk was particularly high for pts at the lowest tertile of SA.

Survival estimates from the various states are shown in Fig(bottom).





Conclusions: Joint monitoring of SA and B2M offers a simple clinical rule to identify HD pts with very high risk of death. Future studies should determine optimal management strategies to reduce this risk.

Funding: Pharmaceutical Company Support

FR-PO2033

Sorbent Dialysis: Results of Eight Real World Water Samples James M. Zacharias,[1] Edwin B. Toffelmire,[2,3] Stephen Merchant.[4] *[1]Department of Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]Department of Medicine, Queen's University, Kingston, ON, Canada; [3]Fresenius Medical Care, Toronto, ON, Canada; [4]SORB Technology Inc, Oklahoma, OK.*

Background: Sorbent dialysis has been used for over 30 years, but use has fallen. Newer Sorbent systems have been developed and would be ideal for use in remote areas with poor access to sufficient quantity/quality water. This study analyzed dialysate quality generated by the sorbent cartridge from water samples from 8 remote sites.

Methods: Tap water was taken from 8 remote Canadian sites. These samples were used to prime HISORB®+ cartridges and perform a mock dialysis with the Fresenius 2008 Sorbent System. Analysis of trace minerals was performed. Paired T-Tests and X^2 analysis were used to analyze the results.

Results: Source water failed to meet ANSI/AAMI Standards for dialysate in 22.6% (38/168) prior to treatment with the sorbent cartridge, but only failed to meet ANSI/AAMI standards in 1.4% (2/144) ($p<0001$) instances. One of these results was with SO_4 which is not well removed by the sorbent cartridge and another was with aluminum which was only 0.002 mg/L above AAMI standards of 0.01 mg/L during the prime. Results are shown in table 1.

Source water and post sorbent cartridge treatment mineral comparison

Mineral	Pre(mg/L)	Pre +/- SD	Post (mg/L)	Post +/- SD	P
Cu	0.09	0.1	0.006	0.002	0.1
SO4	161	141	70	133	0.03
N	0.25	0.1	0.2	0.0004	0.2
Al	0.8	0.2	0.009	0.002	0.3
Ba	0.02	0.03	0.001	0.00003	0.051
Ca	60	42	30	0.02	0.08
As	0.002	0.001	0.002	0.000001	0.4
Mg	38	33	0.6	0.9	0.01
Zn	0.04	0.04	0.009	0.01	0.053
F	0.3	0.02	0.1	0.004	0.08

Reductions were seen with all minerals, but due to small sample size and high variability significant reductions were only seen with SO_4 and Mg. Concentrations for the following minerals were below ANSI/AAMI guidelines and below detection limit and therefore statistical analysis was not performed. (Cd, Sb, Tl, Pb, Ag, Be, Se, Hg, Cr) Mineral concentration was stable during the remainder of a 4 hour treatment.

Conclusions: Dialysis using samples from 8 remote Canadian sites showed that the sorbent cartridge is able to remove excess trace minerals and provide ANSI/AAMI quality dialysate from a variety of real world sources.

Funding: Pharmaceutical Company Support

FR-PO2034

Kinetics of Fibroblast Growth Factor 23, Parathyroid Hormone, and Calcium in Hemodialysis Bernhard O. Bielesz, Manfred Hecking, Max Plischke, Gere Sunder-Plassmann. *Nephrology, Medical University of Vienna, Vienna, Austria.*

Background: Fibroblast Growth Factor 23 (FGF23) is rising in the progression of chronic kidney disease. Dialysis induced changes of blood urea nitrogen, calcium, phosphate, β2-microglobulin, and PTH have been studied extensively. Fewer data are available on the kinetics of FGF23 with either minor increases but also decreases reported during a dialysis session.

Methods: 19 adult patients (mean age: 58 yr, range: 26-80 yr) on maintenance hemodialysis not on cinacalcet or any active Vitamin D agent for at least 2 months were selected for determination of serum calcium, phosphate, blood urea nitrogen, β2-microglobulin, PTH (Roche), and intact FGF23 (Kainos). 4 samples per patient and session were drawn at initiation, 1, 2, and 3 hours of dialysis. 11 patients were treated with dialysate calcium of 1,25 mM (6 HDF, 5 HD) and 8 patients with 1,5 mM (4 HDF,4 HD).

Results: PTH showed a decline in the high calcium group (0 hrs 230,98 pg/ml vs. 3 hrs 100,65 pg/ml; p=0,0057) whereas there was no significant change albeit trend to an increase in the low calcium group (0 hrs 336,45 pg/ml vs. 3 hrs 460,46 pg/ml). PTH changes differed in the high vs. low calcium group (-130,33 pg/ml vs. 124,01 pg/ml, respectively; p=0,0383). Correspondingly, serum calcium increased in the high calcium group (0 hrs 2,14 mM vs. 3 hrs 2,35 mM; p=0,0160) whereas it was virtually unchanged in the low calcium group. Changes in serum calcium were significantly different in the high compared to the low calcium group (0,21 mM vs. -0,04 mM, respectively; p=0,0077). Ln(FGF23) dropped significantly in the whole study population (0 hrs vs. 3 hrs, p=0,0009) but was not affected by dialysate calcium. Blood urea nitrogen, phosphate, and β2-microglobulin showed expected and significant decreases over the course of dialysis, which did not differ with respect to dialysate calcium.

Conclusions: PTH and serum calcium were differentially regulated by dialysate calcium content. FGF23 decreased during dialysis but was unaffected by different dialysate calcium concentrations.

Funding: Pharmaceutical Company Support

FR-PO2035

Exhaled Breath Analysis of Patients with End Stage Renal Disease Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) Sevag Demirjian,[1] Kelly Marie Paschke,[2] Riana E. Naude,[1] Leslie Leonard,[1] David E. Grove,[2] Alquam Mashir,[2] Malina Kate Storer,[3] Robert J. Heyka,[1] Raed A. Dweik.[4] *[1]Nephrology, Cleveland Clinic, Cleveland, OH; [2]Pathobiology, Cleveland Clinic, Cleveland, OH; [3]Syft Technologies Ltd., Christchurch, New Zealand; [4]Pulmonary, Allergy, and Critical Care Medicine, Cleveland Clinic, Cleveland, OH.*

Background: End stage renal disease (ESRD) is characterized by the accumulation of numerous compounds, many of which may have adverse biologic effects (uremic toxins). Exhaled breath analysis is a non-invasive method which can measure volatile uremic markers and/or their metabolites. Our aim was to measure the concentration of volatile organic compounds in the breath before and after intermittent hemodialysis (IHD).

Methods: We analyzed the exhaled breath using selected ion flow tube mass spectrometry (SIFT-MS). Pre- and post-IHD comparisons were made using dependent t-test analysis.

Results: Pre- and post-IHD exhaled breath was analyzed in 59 individual ESRD subjects in a single outpatient dialysis unit. Post-IHD exhaled breath concentration was significantly lower for ammonia, acetaldehyde, dimethylsulfide, carbon disulfide, ethanol, 2-propanol, benzene, pentane and higher for isoprene.

Exhaled breath analysis before and after dialysis:

Compound (ppb)	Pre-dialysis (mean ± SD)	Post-dialysis (mean ± SD)	P-value
Ammonia	189±205	117±62	0.001
Trimethylamine	24±43	15±11	0.1
Triethylamine	1.2±1.8	0.8±0.2	0.1
Acetaldehyde±	147±172	90±84	0.0003
Dimethylsulfide	4.6±2.4	3.2±1.8	0.0001
Carbon disulfide	5.2±6.7	3.3±3.2	0.007
Ethanol	720±412	839±527	0.03
2-Propanol	266±160	204±106	0.002
Isoprene	34±18	46±33	0.002
Benzene	6.6±2.5	5.6±1.6	0.0001
Pentane	36±46	22±11	0.02

ppb, parts per billion; SD, standard deviation

Conclusions: Exhaled breath analysis provides a non-invasive method to measure markers of uremia, and their clearance during IHD. Further investigation is needed to examine the agreement between changes of these compounds in exhaled breath and established measures of dialysis adequacy.

Funding: Clinical Revenue Support

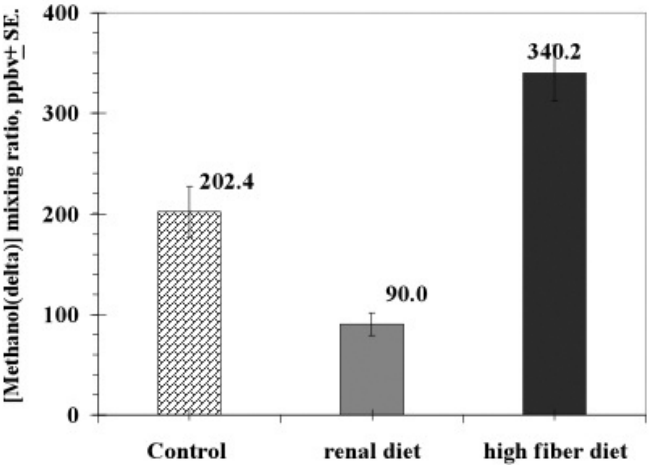
FR-PO2036

Effect of Hemodialysis and Diet on the Exhaled Breath Methanol in Patients with ESRD Hyun Ji Julie Lee,[2] Madeleine V. Pahl,[1] Nosratola D. Vaziri,[1] Donald R. Blake.[2] *[1]Nephrology and Hypertension, University of California, Irvine, CA; [2]Chemistry, University of California, Irvine, CA.*

Background: While the effect of ESRD and dialysis on solute composition is well known, little is known on their effect on the chemical contents of breath. Methanol is the gaseous byproduct of the breakdown of un-absorbable complex carbohydrates by the gut microbiome. Strict dietary restrictions results in an unintended reduction of dietary fibers in ESRD patients. This can reduce production of methanol by the gut microbiome and curtail its appearance in the exhaled breath. Therefore, we investigated the inter- and intra-dialytic changes in the breath methanol levels in a group of hemodialysis patients (HD).

Methods: Ten patients with ESRD were studied during HD at 3- and 2-day-inter-dialytic intervals. Ten normal subjects served as controls. On each occasion, 20 breath and 18 room air samples were collected and analyzed on a unique six column/detector gas chromatography system.

Results: Seven ESRD patients consuming the prescribed renal diet had lower methanol concentration (90 ±29 ppbv) than three ESRD patients consuming a high fiber diet (340 ± 48 ppbv, $P< 0.0006$) and the ten controls consuming unrestricted diets (202 ± 80 ppbv, $P< 0.001$).



HD resulted in a significant (60 ± 12 %) fall in breath methanol concentration which paralleled the fall in serum urea concentration (70 ± 6 %). The average pre-dialysis methanol concentration was slightly higher at the 3-day than the 2-day inter-dialytic interval.

Conclusions: Dietary restriction of fruits and vegetables aimed at limiting potassium intake results in diminished methanol production by the gut microbial flora in ESRD patients. Perhaps methanol is a reliable breath biomarker to monitor individuals' daily fiber intake. Breath methanol concentration is dramatically reduced by HD due to its efficient removal.

Funding: Clinical Revenue Support

FR-PO2037

Serum Soluble Alupha-Klotho Levels in Haemodialysis Patients Tanaka Kenji,[1] Yoko Oyama.[2] *[1]Suiyukai, Kashihar, Nara, Japan; [2]Laboratory and Vascular Medicine, Kagoshima University Graduate School, Kagoshima, Japan.*

Background: Alpha-Klotho (αKl) is a cell surface protein with an extracellular domain that can be released as a cleavage product: soluble-type αKl (sαKl). αKl regulates calcium and phosphate homeostasis. Phenotypes of αKl deficiency resemble clinical features of aging and chronic haemodialysis (HD) patients. So we hypothesized that low levels of sαKl might be related to abnormality of mineral metabolism and vascular calcification in HD patients. The aims of this study were to measure serum sαKl levels in HD patients, and to determine correlation with indices of mineral metabolism, age, HD vintage, and coronary artery calcification (CAC).

Methods: A total of 57 patients, 25 males and 32 females, undergoing maintenance HD were investigated. Their mean age was 69.3 ±11.1 years (range from 50 to 75 years). The mean duration of HD was 196.8 ±101.7 months (range from 34 to 397 months). Blood samples were taken from the arteriovenous fistula before HD on the first dialysis day of the week. Serum sαKl levels were assayed using by ELISA methods (soluble α-Klotho ELISA Kit). Serum levels of intact fibroblast growth factor 23 (FGF23) were measured by ELISA methods (FGF-23 ELISA Kit). Kidney size and CAC score were evaluated by multidetector-row CT.

Results: The serum sαKl level was 742.8 ±280.1 pg/ml, the serum level of FGF23 was 7495.1 ±9800.1 pg/ml, CAC score was 1482 ±1577 in HD patients. The serum sαKl level was not significantly lower than that in healthy controls (n=16, aged 69.3 ±11.1 years) (665.3 ±260.7 pg/ml). In HD patients, no significant correlations were found between serum sαKl levels and calcium ion, inorganic phosphate, intact PTH, 1,25 dihydroxyvitamin D, intact FGF 23, age, but strong correlations were found between serum sαKl levels and HD vintage (P<0.003). The serum sαKl levels was not correlated with CAC score (P= 0.115).

Conclusions: The serum sαKl level in chronic HD patients could be found to be compatible to that in age matched healthy controls and to correlate strongly with HD vintage. Although, underlying mechanisms by which circulating levels of sαKl are maintained in long-term HD patients are unknown, we believe that it deserve of further investigation.

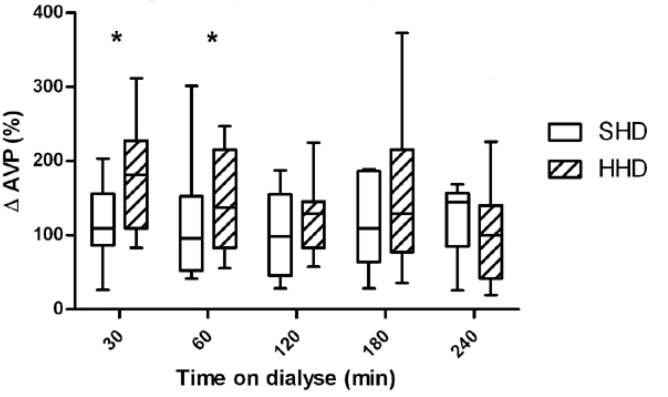
FR-PO2038

Vasopressin Release Is Enhanced by Hemocontrol Biofeedback and Could Contribute to Better Hemodynamic Stability during Hemodialysis Esmée M. Ettema,[1] Johanna J. Kuipers,[2] Henk Groen,[3] Ido P. Kema,[4] Ralf Westerhuis,[2] Paul E. de Jong,[1] Casper F. Franssen.[1] *[1]Nephrology; [2]Dialysis Center Groningen; [3]Epidemiology; [4]Laboratory Medicine, University Medical Center Groningen, Netherlands.*

Background: Hemodialysis (HD) with Hemocontrol biofeedback (HHD) is associated with improved hemodynamic stability compared with standard HD (SHD). Although this beneficial effect of HHD is generally explained by its effect on blood volume (BV), the actual BV change during the second half of HD is comparable between HHD and SHD. Therefore, other factors than BV preservation must play a role. Since HHD is associated with higher initial dialysate sodium concentrations and ultrafiltration rate, we studied whether the beneficial effect of HHD on hemodynamic stability may be explained by increased release of the vasoconstrictor arginine vasopressin (AVP).

Methods: Fifteen HD patients underwent SHD and HHD in random order. All other treatment factors were identical. Plasma levels of AVP and hemodynamic parameters were measured pre-HD and at 30, 60, 180 and 240 min HD.

Results: Median (IQR) pre-HD AVP levels did not differ between SHD (0.94 pg/ml; 0.62-2.10) and HHD (1.0 pg/ml; 0.84-1.60). The Figures shows the percentage change of AVP during HD. AVP levels did not change significantly during SHD whereas AVP levels rose significantly (p<0.01) at 30 min of HHD compared with pre-HD levels. AVP levels were significantly higher at 30 and 60 min of HHD than SHD. Dialysis hypotension occurred significantly less frequent during HHD than during SHD.



Conclusions: HHD is associated with higher AVP levels compared with SHD. The enhanced AVP release with HHD likely contributes to the lower frequency of dialysis hypotension by stimulating plasma refill and facilitating fluid removal during the first part of the HD session, permitting lower ultrafiltration rates during the remainder of the HD session.

Funding: Government Support - Non-U.S.

FR-PO2039

Prevalance of High Predialysis Serum Bicarbonate and Its Effect on Biochemical Parameters in Maintenance Hemodialysis Patients Kawin Tangdhanakanond, Daranee Chewaproug, Eric J. Bloom, Rasib Raja. *Department of Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

Background: Due to the routine use of high-bicarbonate dialysate bath, metabolic alkalosis occurs in hemodialysis patients. Some studies suggest that metabolic alkalosis could be detrimental due to its association with various metabolic derangements, e.g., low serum ionized calcium, hypophosphatemia, and hypokalemia, but there is limited data on these parameters in hemodialysis patients.

Methods: We conducted a retrospective study to assess the prevalence of high predialysis serum bicarbonate and its effect on biochemical parameters on 94 hemodialysis patients at an outpatient hemodialysis unit. All patients received hemodialysis 3 times a week with 35 mmol/L bicarbonate bath. We stratified patients into 2 groups based on predialysis serum bicarbonate: the higher bicarbonate group (bicarbonate ≥28 mmol/L, n=25) and the lower bicarbonate group (bicarbonate <28 mmol/L, n=69). Demographic and laboratory data including serum potassium, phosphorus, total calcium, as well as anion gap were analyzed.

Results: Of 94 patients, 51 (54.26%) and 25 (26.60%) patients had predialysis serum bicarbonate ≥26 mmol/L and ≥28 mmol/L, respectively. The higher bicarbonate group (bicarbonate ≥28 mmol/L) was found to have lower mean serum potassium (mmol/L) (4.56 ± 0.73, 5.01 ± 0.99, p=.041), lower mean serum phosphate (mg/dL) (4.61 ± 1.33, 5.56 ± 1.60, p=.01), and lower mean anion gap (mmol/L) (13.84 ± 2.49, 15.41 ± 2.66, p=.012). Other parameters including serum total calcium, alkaline phosphatase, intact parathyroid hormone, albumin, prealbumin, magnesium, iron studies, vitamin B12, folic acid, lipid profiles, urea reduction ratio, and Kt/V were not statistically different between the 2 groups. Mean C-reactive protein (mg/L) was lower in the higher bicarbonate group but not statistically significant (17.33 ± 29.42, 29.39 ± 69.02, p=.30).

Conclusions: In contrast to some pre-existing data, our study demonstrated more favorable biochemical parameters in hemodialysis patients with higher predialysis serum bicarbonate. A future large randomized control trial looking at hard clinical endpoints is warranted.

FR-PO2040

Management of Anemia with CERA in Routine Clinical Practice: Results around 6 Months of the HORTENSIA Study in Hemodialysis David Verhelst,[1] Sébastien Koné,[2] Michel R. Godin.[3] *[1]CH Avignon; [2]Roche, Neuilly sur Seine; [3]CHU Rouen.*

Background: Correction and stability of hemoglobin (Hb) level is a major goal of anemia treatment. CERA, continuous erythropoietin receptor activator, corrects anemia and maintains the stability of Hb level with a once monthly administration. This French non interventional study is conducted to describe in routine clinical practice the management of anemia with CERA in dialysis.

Methods: HORTENSIA is a one-year prospective study conducted in 112 dialysis centers between 2010 and 2011. Eligible patients (pts) were on dialysis for more than 3 months, treated or not with erythropoiesis stimulating agent (ESA) and initiating CERA

at baseline. Primary endpoint was the proportion of patients with Hb level within [10–12] g/dL around the 6th month (M6) of treatment with CERA.

Results: 372 hemodialysis pts (men: 59%) were analyzed (age: 66±15 years). Median duration of dialysis was 4.3 years. 63% of pts were treated in heavy center. Mean Kt/V was 1.5±0.3. At baseline, 4% of pts were ESA-naive and 96% previously treated : 53% with darbepoetin alfa, 34% with epoetin beta and 13% with epoetin alfa. Mean dose was 39±37 µg/week for darbepoetin α and 9033±7212 IU/week for epoetins. 70% of pts had an adequate iron status. Mean Hb level was 11.3±1.2 g/dL at baseline and 11.3±1.1 g/dL around M6. 64% of pts had Hb level within [10-12] g/dL around M6 and 84% within [10-13] g/dL. 78% of pts had a variation of Hb ≤ 1 g/dL from baseline or Hb within [10-12] g/dL around M6. Mean monthly dose of CERA was 136±76 µg at baseline and 141±91 µg around M6. 18 pts (4.8%) had a total of 20 adverse drug reaction (ADR). The most common ADR was the decreased number of platelets/thrombocytopenia in 7 pts (1.9%). Other ADR were uncommonly reported with a frequency <1%. 6 ADR led to treatment modification or discontinuation. 3 pts (<1%) had a serious ADR (ischemic stroke, allergic reaction, thrombosis of the fistula).

Conclusions: A once monthly administration of CERA can maintain stable the Hb level in hemodialysis patients.

Funding: Pharmaceutical Company Support

FR-PO2041

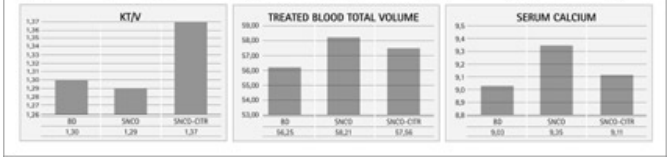
Improving the Dialysis Efficacy in Single Needle by Using Citrasate®: Preliminary Data Roberto Ervo, Sandro Angeletti, Tosca Turrini Dertenois. *Nephrology and Dialysis, Bordighera Hospital, Ventimiglia, Italy.*

Background: The dialysis population shows a growing age, with an increase in clinical problems as malnutrition and cardiovascular diseases. The mortality risks are: ageing, cardiovascular diseases, diabetes, caloric-protein malnutrition and a low serum albumin. Moreover, in those patients the vascular access problems sometimes make it difficult to perform double needle HD, with a serious risk for the dialysis efficacy.

Methods: During a short observational pilot cross over study on 6 patients, we have tested an innovative single needle system where the two pumps run continuously with no interruption of the flux to the dialyser (SNCO - Cross Over B. Braun). The study design shifts patients from standard dialysis (2 months), to SNCO with standard concentrates (3 months), and last to SNCO-CITR with dialysate containing very low acetate (0,3 mmol/l) and 0,8 mmol/l of citrate (Citrasate®) (10 months). The aim was to evaluate the dialysis adequacy (Kt/V, URR, Treated blood volume) with respect to double needle therapies (BD) and in accordance with the KDOQI standards. Furthermore, the evaluation analyzed whether the presence of citrate improved the Kt/V without any change in calcium needs.

Results: Despite the limited number of patients, as indicated in the table below, the three groups are comparable, both as for exchanges (treated blood volume) and Kt/V

	BD		SNCO		SNCO-CITR	
	AVERAGE	ST_DEV	AVERAGE	ST_DEV	AVERAGE	ST_DEV
Kt/V	1,30	0,22	1,29	0,22	1,37	0,19
URR	0,67	0,03	0,66	0,05	0,68	0,05
Nitrogen pre	169,38	59,65	165,75	60,80	146,79	52,62
Nitrogen post	58,13	22,0	60,88	31,63	48,86	26,45
Creat pre	8,71	0,92	9,32	1,84	12,95	10,49
Calcium	9,03	0,7	9,35	1,05	9,11	1,09
B_M	35,91	14,35	33,54	21,30	37,19	16,83
Hb	10,84	0,89	10,38	1,19	11,03	0,72
Htc	35,96	3,98	34,25	4,70	36,52	2,23
Blood Volume	56,25	6,98	58,21	11,93	57,56	5,82
Qb	259,88	11,43	273,13	15,76	260,65	6,04



Conclusions: The SNCO can represent a valid alternative for elderly, diabetic patients and for those with vascular problems who require the native AV fistula preservation over the time or have no chance to use double needle mode and/or a central venous catheter. Citrasate® was introduced to observe whether an increase in the dialysis efficiency may take place due to the higher fibres patency. The study has shown this trend with no significant incidence of the patients' calcium balance.

FR-PO2042

A Potential Use of Polyclonal Free Light Chain Levels for Monitoring in a Chronic Dialysis Population Bertrand Gondouin,[1] Juergen E. Scherberich,[2] Anne Bevins,[3] Paul Cockwell,[1] Colin A. Hutchison.[1] *[1]Renal Unit, University Hospital Birmingham, Birmingham, United Kingdom; [2]Department of Nephrology, Ludwig-Maximilians-University Hospital, Munich, Germany; [3]The Binding site Ltd, Birmingham, United Kingdom.*

Background: Polyclonal free immunoglobulin light chains (FLCs) are uremic toxins. With two isotypes, kappa and lambda, FLCs provide a potential method for the measurement of middle molecular weight uremic toxins (MW 22.5 and 45kDa respectively). The purpose of this study was to determine if FLC levels in a chronic dialysis population are determined by treatment variables and residual renal function (RRF).

Methods: Serum concentrations of polyclonal FLCs were measured in two stable chronic dialysis populations, pre and post dialysis. Levels were compared for different treatment modalities and RRF (>500ml/d).

Results: 112 patients were recruited, 60 were anuric. In both populations anuric patients had higher total FLC levels than those patients with RRF (See table).

	Centre 1	Centre 2
All Patients	316.1 (18.76-738.5)	202,7 (85.1-448)
Anuric	349.3 (125-597)	223 (85.1-448)
RRF	285.6 (18.76-738.5)	153.9 (131.6-322)
P value (anuric vs. RRF)	<0.05	<0.02

Serum total FLC concentrations (mg/L) [Median(range)]

HDF resulted in greater percentage reduction in FLCs compared with regular hemodialysis: 61% [range 43-81] and 39% [14-70], respectively P<0.001.

Patients at the two centres were comparable in terms of age, sex, vascular access, but patients from centre 1 had higher CRP (7.55mg/L [0.12-170]) and FLC levels (316.1mg/L [18.76-738.5]) compared with those from centre 2: 0.7mg/L [0.3-6.6] and 202,7 mg/L [85,1-448.0] respectively (both: P<0.0001).

CRP did not correlate with the serum FLC levels. There was no difference in CRP levels between anuric and RRF patients.

There was no correlation between the amount of FLC removed during the dialysis session and the ultrafiltration rate used.

Conclusions: In conclusion, this study demonstrates polyclonal FLC levels are significantly influenced by both RRF and the dialysis modality used, they could therefore potentially provide a simple target for monitoring 'difficult to remove' middle molecular weight uremic toxins in chronic dialysis populations.

FR-PO2043

Limitations of Serum Cystatin C as Estimate of Residual Renal Function in End-Stage Renal Failure Enric Vilar,[1,2] Capella Boltiador,[1] Ashwini Machado,[1] Adie Viljoen,[1] David Wellsted,[2] Andrew Garrett,[2] Ken Farrington.[1,2] *[1]Lister Hospital, Stevenage, United Kingdom; [2]University of Hertfordshire, Hatfield, United Kingdom.*

Background: Residual renal function (RRF) is inconvenient to measure in HD. Following an HD session, levels of Cystatin C, an alternative marker of GFR, plateau by 24h post dialysis. This suggests that pre-dialysis levels may be a useful marker of RRF in HD.We explored the relationship between serum cystatin C and RRF to determine whether pre-dialysis cystatin C predicts RRF.Additionally, we explored the use of cystatin C as a marker of dialysis adequacy.

Methods: We prospectively analysed serum taken pre- and post- dialysis over two consecutive sessions in 343 patients on high-flux HD or haemodiafiltration. Analyses were performed for urea, creatinine and cystatin C. GFR was measured using body surface area-adjusted mean creatinine and urea clearance from timed urine collections. The relationship of cystatin C to RRF and Kt/V_{urea} was analysed.

Results: GFR ranged from 0-17ml/min/1.73m²BSA. 1/(pre-HD cystatin C levels) had a linear relationship with GFR. For GFR<10ml/min variance increased, with 1/cystatin C explaining only 53% of GFR variance. Inter-dialytic cystatin C rise was inversely related to GFR, explaining 41% of its variance. To investigate poor level of agreement between 1/cystatin C and GFR, we studied pre-dialysis cystatin C in patients without RRF. In this group, cystatin C level (equal to the ratio of generation rate to non-renal clearance) was 6.1mg/L with a wide standard deviation (0.7mg/L), and had no relationship with body size parameters, age and sex. Cystatin C reduction ratio had different linear relationships with Kt/V_{urea} in high-flux HD and HDF(r^2 0.52 and 0.50). A Kt/V_{urea} of 1.2 corresponded to a reduction ratio of 0.46 and 0.53 in high-flux HD and HDF respectively.

Conclusions: Pre-dialysis cystatin C levels have limited usefulness for RRF estimation in patients with GFR<10ml/min due to variation in the generation to non-renal clearance ratio.We were unable to identify parameters which would improve prediction of GFR with cystatin C. Cystatin C reduction ratio correlates with Kt/V_{urea} and may be useful as a marker of middle molecule clearance.

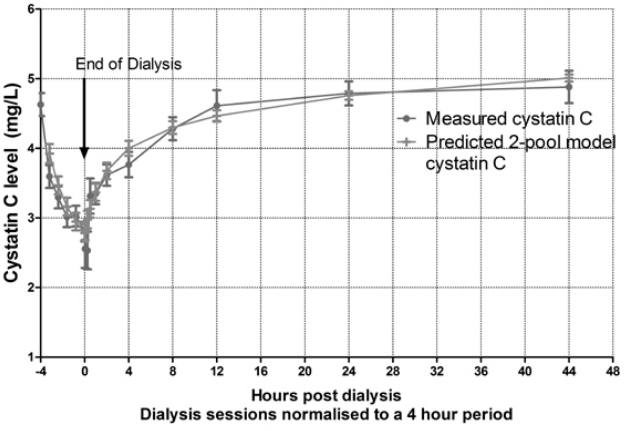
FR-PO2044

Removal Kinetics of Cystatin C during High-Flux Hemodialysis and Hemodiafiltration Enric Vilar,[1,2] Capella Boltiador,[1] Ashwini Machado,[1] Adie Viljoen,[1] Andrew Garrett,[2] David Wellsted,[2] Ken Farrington.[1,2] *[1]Lister Renal Unit, Stevenage, United Kingdom; [2]University of Hertfordshire, Hatfield, United Kingdom.*

Background: Cystatin C has been proposed as a potential alternative marker of GFR since levels correlate more closely than with creatinine. Its 13kDa size allows removal by high-flux dialysis membranes. Serum levels may be useful in estimating residual renal function without the need for urine collection.We studied removal and rebound kinetics of cystatin C during high-flux HD and haemodiafiltration. Using a two-pool computer model, we studied whether serum cystatin C levels are predictable and can be used to determine residual renal function without the need for urine collection.

Methods: We selected 25 patients undergoing high-flux HD or hemodiafiltration. We prospectively collected serum samples at intervals during dialysis and the inter-dialytic period for up to 48 hours. Serum was analysed for urea, creatinine, cystatin C and β2-microglobulin. Cystatin C was measured using an immunoassay. Glomerular filtration rate was measured using mean urea and creatinine clearance.

Results: The mean reduction ratio for each molecule was urea 63%, creatinine 58%, β2-microglobulin 51%, cystatin C 40%. Rebound kinetics differed for all molecules. Cystatin C and β2-microglobulin demonstrated similar kinetics, with 2 hours post-HD levels rebounding to 48% and 38% respectively. A two-pool model demonstrated close goodness-of-fit to actual cystatin C levels, with a median standard deviation of 0.26mg/L between the model and cystatin C data.



Conclusions: Cystatin C levels may be estimated with reasonable accuracy using a two-pool kinetic model. Parameters estimated from this model (internal mass-transfer coefficient, generation rate, non-renal clearance) will allow this model to be tested for prediction of residual renal function in a large dataset.

FR-PO2045

The Difference in Metabolites Disturbance between High-Flux and Low-Flux Hemodialysis: A Metabonomic Study Jiayuan Gao,[1] Zhaohui Ni,[1] Renhua Lu,[1] Jia Qi Qian,[1] Yucheng Yan.[1] *[1]Department of Nephrology, Ren Ji Hospital of Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2]School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.*

Background: Using GC-TOF-MS metabonomic approach, We try to obtain the difference metabolites profiles in both drained dialysate and plasma and analyze the difference in metabolites disturbance between high flux and low flux hemodialysis.

Methods: 25 anuric patients on low flux maintenance hemodialysis and 25 paired high flux dialysis patients were included, Drained dialysate, pre-dialysis and post-dialysis plasma were collected. The concentration of metabolites in drained dialysate and the reduction ratio (RR) in plasma between high flux and low flux were compared to identify different metabolites.

Results: 10 metabolites concentration showed significant differences in drained dialysate between high and low flux dialysis patients, which were tryptophan, Alpha-Ketoisovaleric acid, Gamma-Aminobutyric acid (GABA), Benzoic acid, Ketoleucine, Succinic acid, Palmitic acid, P-Hydroxyphenylacetic acid, Indoleacetic acid and 3-Aminoisobutanoic acid. The concentration of all 10 metabolites was higher in high flux group than low flux group. While in plasma, the RR of 10 metabolites showed significant differences between high and low flux, of which 8 metabolites were higher in high flux group than low flux group, including Alpha-Ketoisovaleric acid, GABA, Allantoin, Citric acid, 4-Hydroxy-L-proline, P-Hydroxyphenylacetic acid, Hippuric acid and Alpha-Tocopherol. Surprisingly, the RR value of Ketoleucine was negative in high flux group but positive in low flux group. On the contrary, the RR value of Hypoxanthine was positive in high flux group but negative in low flux group.

Conclusions: Different metabolites exist in drained dialysis, and metabolites disturbances are different between high flux and low flux hemodialysis patients. Difference in fatty acid metabolism between high flux and low flux exists. Difference might exist in Xanthine metabolism between high and low flux.

Funding: Government Support - Non-U.S.

FR-PO2046

Dialyzer AN69ST – A Valid Alternative in Patients with Hemorrhagic Risk? Mário Raimundo, Catarina Calado Teixeira, Edgar A.F. de Almeida, Antonio Gomes da Costa. *Nephrology and Renal Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

Background: Heparinization of the extra-corporeal circuit (ECC) during hemodialysis (HD) is unadvisable in patients with hemorrhagic risk. Usually pre-dialyzer saline flushes are employed, which carry potential disadvantages. Polyethylenimine coated polyacrylonitrile dialyzers (AN69ST), which promote heparin adsorption, allow heparin-free HD and obviate the need of frequent saline flushes providing the priming is performed with a heparinized solution. Our aim was to evaluate if systemic anticoagulation occurs with this procedure and compare the efficacy of the AN69ST with a polysulphone (PS) dialyzer with similar characteristics.

Methods: Prospective, open-label, cross-over study. We examined 31 patients during 2 mid-week HD sessions with the AN69ST and the PS dialyzer (without systemic heparinization and with saline flushes). Plasma anti-Xa activity, activated partial thromboplastin time (aPTT), prothrombin time (PT) and urea were determined prior to HD, at 60 and 240min and 180min after HD. A semi-quantitative evaluation of dialyzer clotting was performed at the end of HD.

Results: We found a statistically significant increase in anti-Xa activity at 60min compared to baseline (mean 0,044±0,079 vs 0,025±0,059; p<0,001), which disappeared at the end of the session (mean 0,032±0,049; p=0,145) with the AN69ST. There was no difference in TP and aPTT, although there was a trend to increased PT at 60min (12,63s±4,08 vs 12,43s±3,61s; p=0,061). No differences in anti-Xa or coagulation times were found with the PS dialyzer and it was associated with better dialytic efficacy (URR 73,22±7,67% vs 67,15±11,1%; p=0,01) despite a trend to higher dialyzer clotting in a 0-4 semi-quantitative evaluation (2,29±1,20 vs 1,57±1,08; p=0,065).

Conclusions: The AN69ST dialyzer primed with a heparinized solution is associated with a low-grade and short-lived systemic anticoagulation effect, without significant repercussion in standard coagulation times. The clinical significance of this effect in patients with hemorrhagic risk still needs to be clarified, although the lack of relevant clinical benefit (i.e. improved dialytic efficacy) and higher price may hamper its routine use.

FR-PO2047

Utility of Procalcitonin in Delineating Sepsis in Patients on Haemodialysis Arghya Majumdar,[AMRI] Mukesh Kochar.[AMRI] *[1]Nephrology, AMRI Hospital, Kolkata, India; [2]Nephrology, AMRI Hospital, Kolkata, India.*

Background: Few studies have suggested that procalcitonin (PCT) levels may be elevated in haemodialysis patients in the absence of sepsis. Therefore there is uncertaainity regarding it's utility in diagnosing bacterial sepsis in these patients.

Objectives: To determine the baseline levels of PCT in haemodialysis patients without sepsis and the accuracy of PCT in diagnosing sepsis in these patients.

Methods: PCT levels were estimated in haemodialysis patients with sepsis (according to ACCP/ ISCCM criteria and/or isolation of microorganism). PCT levels were also checked in a control population: haemodialysis patients without sepsis, other inflammatory conditions, acute myocardial infarction, cirrhosis of liver, malignancy, burns, recent surgery or trauma. patients recieving immunomodulatory or anti- inflammatory drugs were excluded. PCT concentrations were measured by Chemiluminiscence assay in pre- dialysis samples. All patients underwent intermittent haemodialysis with a low flux polysulfone membrane.

Results: The patients in both groups were well matched for age, gender and co-morbidities; duration on dialysis.and adequacy. In controls (n= 19), mean PCT was 0.3611 (95% CI 0.2215 to 0.5006). In haemodialysis patients with sepsis (n= 17), mean PCT was 8.94 (95% CI 3.54 to 14.35). Focus of sepsis: UTI 2, Pneumonia 8, CRBSI 1, SSTI 2, Peritonitis 2, Undetermined – 1. Micro-organisms were isolated in 12 and 5 were diagnosed on ACCP/ ISCCM criteria. Of patients on haemodialysis 95 percentile patients without infection have PCT values <0.9. On taking 0.45 as a cut off value, below which the test was considered negative for sepsis in haemodialysis patients and a value of 0.93 above which the test was considered positive for sepsis: Sensitivity 94.4% and Specificity 87.5%. So diagnostic algorithm which may be formulated: <0.5: unlikely, 0.5- 0.9 possible, >0.9 most likely to have sepsis.

Conclusions: Procalcitonin is useful in diagnosing bacterial sepsis in haemodialysis patients

FR-PO2048

Safety and Efficacy of High Cut-Off Hemodialysis in Chronic Dialysis Patients: A Randomized Controlled Trial Bertrand Gondouin,[1] Anne Bevins,[2] Paul Cockwell,[3] Colin A. Hutchison.[3] *[1]Centre de Néphrologie, Hôpital de la Conception, Marseille, France; [2]The Binding Site Group Ltd, Birmingham, United Kingdom; [3]Renal Unit, University Hospital Birmingham, United Kingdom.*

Background: High cut off hemodialysis (HCO-HD) membranes provide increase removal of middle molecular weight uremic toxins by their increased membrane permeability. This study investigated the safety and efficacy of their use in a stable chronic dialysis cohort.

Methods: Patients (n-29) were randomized into groups (A or B) in a cross-over study design: (A) received 1 HCO (Gambro HCO1100) and 2 standard HD sessions (Polyflux 170H) per week for 8weeks, followed by 3x HCO for 8weeks; (B) received these treatments in reverse. Safety was defined by serum albumin levels, Kt/V and adverse events. Patients were withdrawn if albumin loss was >25% or a thrombotic event occurred. Efficacy was assessed by removal of middle molecules, phagocyte function.

Results: The use of HCO 3x weekly caused a significant reduction in serum albumin levels (40.6±3.4g/L to 36.7±2.6; p<0.01) during the first 4weeks, but this remained stable from weeks 4 to 8 (37.4±3.4). No significant reduction in serum albumin levels was seen with 1x HCO. No patients were withdrawn for albumin loss, and normal albumin levels were restored 4 weeks following trial completion (41.2±2.7). There was no difference in adverse events between 1x and 3x weekly HCO-HD. Serum CRP concentrations did not change with 3x or 1x HCO. Mean Kt/V for HCO was decreased compared to standard dialysis: 1.03±0.17 and 1.31±0.21 (p<0.05). There was a significant reduction in middle molecules in the 3x weekly group compared to the 1x weekly, κFLC (median 11.59% vs -2.03%; p<0.01), λFLC (15.43 vs -2.68%; p<0.01) and β2M (2.02 vs -8.201%; p=n/s). Phagocyte function increased over the 3x weekly period from 91.2%±9.4 to 98.1%±2.1 (p=0.02).

Conclusions: HCO-HD provides increased removal of middle molecules and the albumin loss associated with its use appears to be tolerated. Possible clinical benefit could be suggested by the increased phagocytic function. Further work is now required to assess potential clinical benefit of HCO-HD in a larger population.

FR-PO2049

Continuous Online Monitoring of Ionic Dialysance in Acute and Chronic Maintenance Hemodialysis Pei-Chen Wu,[1] Ravindra L. Mehta.[2] *[1]Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [2]Medicine, University of California San Diego, San Diego, CA.*

Background: Hemodialysis (HD) adequacy is strongly correlated with clinical outcomes. Continuous online monitoring of ionic dialysance, using sodium flux rather than urea, allows HD sessions to be modified on a real-time and individual basis. We compared single-pool ionic dialysance (Kt/V_{ID}) with traditional blood-side measurements (urea Kt/V and urea reduction ratio [URR]).

Methods: We analyzed 2242 HD sessions from 418 hospitalized patients at an academic medical center. The patients were treated with HD with or without heparin anticoagulation for AKI (n=906 sessions) or as chronic maintenance therapy (n=1327 sessions). Kt/V_{ID} was obtained from Fresenius 4008® machine, and blood-side urea Kt/V was calculated using the second generation formula of Daugirdas.

Results: Mean and SD Kt/V_{ID} was 1.25±0.47, mean urea Kt/V was 1.40±0.46, and mean URR was 67.8±0.3%. Both urea Kt/V and URR moderately correlated with Kt/V_{ID} (r=0.668, p<0.001 and r=0.652, p<0.001, respectively). The discrepancy between Kt/V_{ID} and urea Kt/V in acute and chronic settings was -0.13±0.40 and -0.15±0.36, respectively, and there existed a significant difference between these 2 clinical conditions (p=0.021). AKI (r=0.143), larger post-HD weight (r=0.444), lower blood flow (r=0.383), and higher post-HD urea (r=0.499) were related to a lower Kt/V_{ID} (all p<0.001). In addition, the total body water derived from anthropometric formula, which is incorporated in Kt/V_{ID} monitoring, and that from urea Kt/V (spV) only weakly correlated with each other (r=0.18, p<0.001). The anthropometric volume was significantly larger than spV (46.6±14.9 L and 37.7±21.3 L, respectively, p<0.001).

Conclusions: Our results show that Kt/V_{ID} is lower than urea Kt/V and these 2 measurements are moderately correlated. The difference between the two could be accounted for by the overestimation of total body water by the anthropometric method. Moreover, the discrepancy is present in both acute and chronic settings, suggesting that dialysis delivery can be monitored with Kt/V_{ID} and periodically confirmed with urea Kt/V in hospitalized patients.

Funding: Government Support - Non-U.S.

FR-PO2050

Improvement of the Removal of Medium-Sized Molecules in Hemodiafiltration by the Use of a Very High Permeability Dialyzer Piotr Seniuta, Thierry Baranger, Valerie Drouillat, Emmanuelle Rosier, Frank Bergé, Carlos Frangié. *Dialysis, PBNA, Bordeaux, France.*

Background: The removal of uremic toxins of a medium molecular weight (MW) is one of the main objectives in hemodialyzed patients. The appearance of a very high permeability dialyzer which presents in vivo ultrafiltration coefficients (UCF) greater than 100,led to us testing it on 16 of patients in on-line pre-dilution hemodiafiltration.

Methods: 16 patients,8 men and 8 women, average age 75.5 years were dialyzed with on-line HDF,240 minutes per session, for 6 months period on the Xevonta 20 dialyzer,(polysulfone,2.0 m2,UCF of 110.They had previously been dialyzed on a polysulfone dialyser, with UCF of 80. During this period,each dialysis session was analyzed and recorded on a data checklist for information concerning the clinical and technical parameters.The extraction coefficients of urea, creatinine, phosphorous, beta2-microglobulin, myoglobin, prolactin and Retinol Binding Protein(RBP) were recorded 3 times.The nutritional and inflammatory parameters which were protein, pre-albumin, albumin and CRP were recorded for the same period.

Results: The extraction coefficients were not statistically different for small compounds: urea, creatinine and phosphorous. The removal of molecules of mean MW demonstrates, an improvement for a mean MW of 11800 Kd (Beta 2M, p < 0.05) was very significative for the molecules of MW greater than 17800 Kd (Microglobulin, p<0.000001) until 21000 Kd (Prolactin, p<0.00001), it is reduced for the high MW, 230000 Kd (RBP, p< 0.01. Concerning the nutritional parameters, only the loss of albumin is statistically significant (p<0.001) to 4 grams, without a reduction of pre-albumin.

Conclusions: The superiority of the Xevonta dialyzer could, therefore, come from its own structure, diameter and thickness. This will lead to a higher cut-off, making it possible to improve the removal of uremic toxins of a mean MW. Although albumin loss was significative, it does not accompany a total protein loss.

This study, carried out on elderly patients of a dialysis unit, demonstrates the interest in the use of a very high permeability membrane in pre-dilutional HDF, in order to improve the removal of uremic toxins of a mean MW.

FR-PO2051

Impact of Hemodialytic Procedures and Dialytic Doses on Erythrocyte Glutathione s-tranferase (e-GST) Activity Roberto Palumbo,[1] Annalisa Noce,[2] Michele Ferrannini,[1] Mariarita Dessì,[3] Simone Manca di Villahermosa,[2] Nicola Di Daniele.[2] *[1]Nephrology and Dialysis Department, S.Eugenio Hospital, Rome, Italy; [2]Nephrology and Dialysis Unit, Tor Vergata University, Rome, Italy; [3]Department of Chemical Science and Technologies, Tor Vergata University, Rome, Italy.*

Background: Glutathione-S-Transferases (GST) represent a superfamily of ubiquitous enzymes devoted to the cell protection and play an important role in the detoxification of both endogenous and exogenous compounds. Previous study demonstrated an increased erythrocyte-GST (e-GST) activity in uremic patients. The aim of this preliminary study is to compare e-GST activity in normal and uremic subjects, and to correlate e-GST activity with different hemodialysis technique and with dialytic dose.

Methods: Eighty healthy controls, 44 uremic patient on bicarbonate hemodialisis (HD group) and 59 on online-hemodiafiltration (ol-HDF group) were investigated. E-GST activity was assayed using an automated procedure. Dialytic dose was expressed as Kt/Vurea and weekly Kt/Vurea. To correlate e-GST activity with dialytic dose, all 103 uremic patients (HD and ol-HDF groups) were stratified into two subgroups with a cut-off Kt/Vurea of 1.3.

Results: E-GST activity was significantly increased in the 103 uremic patients compared to controls (9.0±3.1 vs. 5.6±1.7U/grHb respectively, p<0.0001). In ol-HDF group we observed a lower e-GST activity (p=0.0036) and an higher Kt/Vurea (p=0.0007) and weekly Kt/Vurea (p=0.0004), than in HD group. Conversely, there was no difference in e-GST activity between patients with Kt/Vurea <1,3 compared to patients with Kt/Vurea ≥1.3 (9,67±3,23 vs. 8,65± 2,96U/grHB, p=0,156).

Conclusions: Our results suggest that e-GST could be a potential biomarker of uremic status and of dialysis adequacy.

FR-PO2052

Comparison of Na-Analysis during Hemodialysis: Reflection of a Decrease in Plasma Water Adrianus L.H.J. Aarnoudse,[1] Jurgen A. Riedl,[2] Gijs M.T. De Jong.[1] *[1]Internal Medicine, Albert Schweitzer Hospital, Dordrecht, Netherlands; [2]Clinical Chemistry, Albert Schweitzer Hospital, Dordrecht, Netherlands.*

Background: Na and K can be measured by different methods: flamephotometer (NaF, KF), direct ion-selective electrode (NaDSE, KDSE), indirect ion-selective electrode (NaISE, KISE) or Na can be derived from the conductivity of blood (NaC). Hemodialysis (HD) may change plasma water (PW) and Na. NaDSE and NaISE are differently influenced by PW allowing a computational estimate of PW. Changes in Na en PW may be of clinical relevance in HD.

Methods: In 45 HD-patients blood was drawn and NaC was registered before, halfway and after HD. Simultaneously, blood was analyzed for NaDSE and -ISE, KDSE and -ISE, hematocrit, total protein (TP), albumin, cholesterol, triglycerides (TG) and urea. PW was computed from the difference between NaDSE and –ISE. Mean Na was compared between methods and between sampling times using paired tests. A backward stepwise linear regression model was used to explain NaC from NaF, NaDSE, NaISE, PW and measured variables.

Results: Mean(SD) concentrations at start, during and after HD were for NaC 141.2(2.6), 140.2(1.8), and 139.4(1.3) , NaF 136.6(2.6), 136.8(1.7) and 136.2(1.7), NaDSE 136.9(2.8), 136.9(2.0) and 137.3(1.7), NaISE 135.9(3.0), 135.4(1.9) and 135.3(1.7) mmol/l, respectively. PW was 0.93(0.01), 0.92(0.01) and 0.91(0.01) respectively. NaC was always higher than NaF, NaDSE or NaISE and declined during dialyses. NaDSE and –ISE were stable during HD and NaDSE was always higher than -ISE. Using stepwise backward regression NaC before HD without adjusting for PW was best explained using DSE by NaDSE, KDSE, TP and TG (r2=0.72). Including PW in the model improved this to r2 = 0.74. Doing the same with NaISE R2 improved from 0.63 to 0.73 when including PW in the model.

Conclusions: NaC is strongly affected by HD and is substantially higher than NaDSE and -ISE. This may be relevant to HD practice. NaF was structurally higher then NaDSE and –ISE. This can be explained by the fact that flamephotometry measures total Na whereas DSE and ISE measure active Na. NaISE is progressively lower than NaDSE during HD, indicating a decreasing PW. This may be relevant for understanding the effects of HD.

FR-PO2053

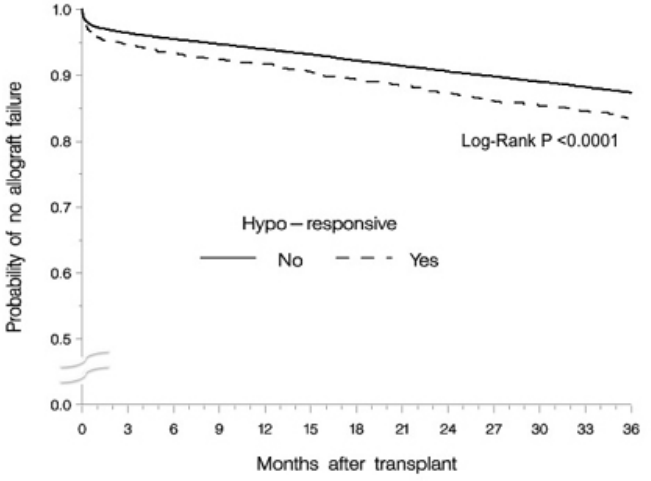
Pre-Transplant Erythropoiesis Stimulating Agent Response as a Predictor of Post-Transplant Allograft Failure & Mortality Nadiesda A. Costa, Abhijit V. Kshirsagar, Lily Wang, Randal K. Detwiler, M. Alan Brookhart. *University of North Carolina, Chapel Hill, NC.*

Background: The effect of dialysis-dependent factors on post-kidney transplant outcomes has not been well studied. We examined whether erythropoiesis stimulating agent (ESA) response during hemodialysis was associated with allograft failure and mortality.

Methods: We identified 36,450 adults from the United States Renal Data System who had received a kidney transplant during years 2000-2007 and at least 6 months of hemodialysis prior to the transplant date. ESA response during the 6-month pre-transplant period was determined using Medicare billing claims for Epoetin alpha and associated hematocrit labs. We defined ESA hypo-responsive patients to be those who experienced three consecutive months with a monthly ESA dose ≥75,000 units while hematocrit remained ≤33%. Cox proportional hazards models and Kaplan-Meier methods were used to estimate the association between ESA hyporesponsiveness and allograft failure and

mortality. Time to allograft failure was examined with and without censoring by death. Models were adjusted for age, gender, race/ethnicity, dialysis duration, dialysis catheter, diabetic status, and donor type.

Results: Baseline characteristics were similar in both hypo-responsive and comparison groups. The adjusted hazard ratios for allograft failure and all-cause mortality post-transplant were 1.26 (95%CI 1.10, 1.45) & 1.63 (95%CI 1.46, 1.82), respectively. A sensitivity analysis showed similar results. The probability of allograft failure was higher in the ESA hypo-responsive group compared to those not hypo-responsive (Figure).



Conclusions: Dialysis patients with decreased response to ESAs are more likely to experience post-transplant allograft failure and mortality. ESA response during dialysis may be used to identify high-risk kidney transplant recipients both before and after transplant.

Funding: NIDDK Support

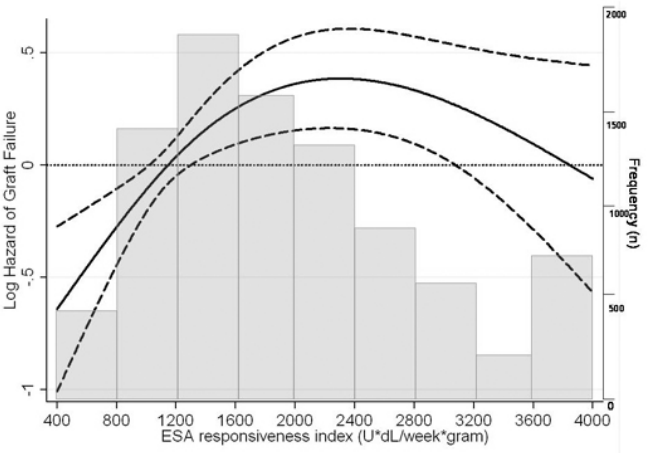
FR-PO2054

Association of Pre-Transplant Erythropoiesis Stimulating Agent Responsiveness with Post-Transplant Graft Loss and Delayed Graft Function Miklos Z. Molnar,[1,2] Suphamai Bunnapradist,[3] Edmund Huang,[3] Mahesh Krishnan,[4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; [2]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]DaVita, Inc, Denver, CO; [5]Salem VA Medical Center, Salem, VA.*

Background: The role of pre-transplant erythropoiesis stimulating agent (ESA) responsiveness in affecting post-transplant graft loss and delayed graft function is not clear.

Methods: Linking the 5-year patient data of a large dialysis organization to the *Scientific Registry of Transplant Recipients*, we identified 9,281 hemodialyzed patients who underwent first kidney transplantation. Graft failure, delayed graft function (DGF) and acute rejection risks were estimated by Cox regression (hazard ratio [HR]) and logistic regression, respectively.

Results: Patients were 48±14 years old and included 38% women and 36% diabetics. Compared to renal allograft recipients who were in first quartile of pre-transplant ESA responsiveness index (ERI) i.e., ESA dose divided by hemoglobin, recipients in second, third and fourth quartiles had higher death-censored graft failure HR of 2.1 (1.3-3.5), 2.3 (1.4-3.8) and 2.1 (1.2-3.5), respectively.



However the OR of DGF was similar in the second 1.10 (0.87-1.39), third 1.13 (0.88-1.45) and fourth 1.28 (0.97-1.67) quartiles. No significant association between pre-transplant ESA responsiveness index and post-transplant acute rejection was noticed.

Conclusions: Higher pre-transplant ESA responsiveness index during hemodialysis treatment period was associated with worse post-transplant risk of graft failure.

Funding: NIDDK Support

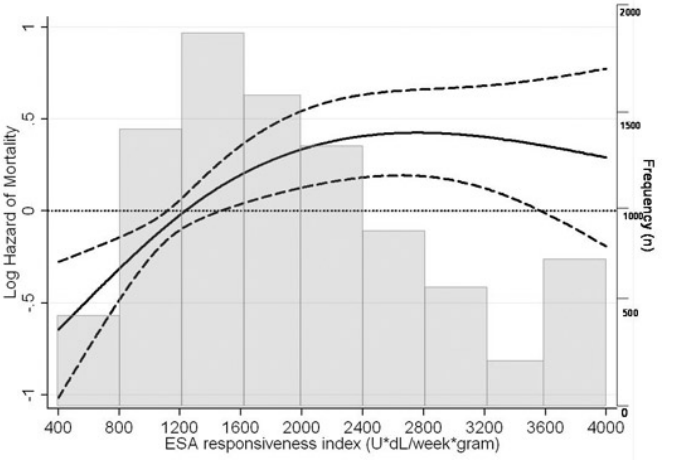
FR-PO2055

Association of Pre-Transplant Erythropoiesis Stimulating Agent Responsiveness with Post-Transplant Mortality Miklos Z. Molnar,[1] Edmund Huang,[2] Suphamai Bunnapradist,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA.*

Background: Studies have shown an association between erythropoiesis stimulating agent (ESA) responsiveness & mortality in CKD patients (pts), but the role of pre-transplant ESA responsiveness & post-transplant mortality is unknown.

Methods: Linking the 5-year patient data of a large dialysis organization to the *Scientific Registry of Transplant Recipients*, we identified 9281 hemodialyzed pts who underwent kidney transplantation. All-cause & CV mortality were estimated by Cox regression (hazard ratio [HR]).

Results: Pts were 48±14 years old and included 38% women and 36% diabetics. The crude all-cause mortality rate was increasing across pre-transplant ESA responsiveness index quartiles, it was 26.2/1000 pt-years (95% confidence interval [CI]: 22.4–30.6)) in the first quartile and 29.9/1000 pt-years (25.6–35.0), 33.0/1000 pt-years (28.5–38.2), 44.5/1000 pt-years (39.1–50.6) in the second, third and fourth quartiles, respectively. Compared to renal allograft recipients who were in first quartile of pre-transplant ESA responsiveness index (ERI) i.e., ESA dose divided by hemoglobin, recipients in second, third and fourth quartiles had higher adjusted graft-censored death HR (and 95% confidence intervals) of 1.7(1.0-2.8), 2.2(1.3-3.5) and 2.4(1.4-3.9), respectively. Figure shows the cubic spline models for the association of the entire range of pre-transplant ERI with post-transplant mortality.



Similar results were found for cardiovascular mortality.

Conclusions: Higher pre-transplant ERI during hemodialysis treatment period was associated with worse post-transplant mortality.

Funding: NIDDK Support

FR-PO2056

Anemia Control in Kidney Allograft Recipients with Once Monthly C.E.R.A.: Results from the BEAT Study Thomas Rath,[1] Klemens Budde.[2] *[1]Hospital, Kaiserslautern, Germany; [2]Charite University, Berlin, Germany.*

Background: Post-transplant anemia (PTA) is highly prevalent in kidney transplant recipients (KTxR). Yet, course and etiology of renal anemia in these patients (pts) differ from the type seen in chronic kidney disease (CKD) pts not on dialysis with respect to overall morbidity and immunosuppressive therapy. The BEAT-study was designed to capture data about efficacy and safety of Continuous Erythropoietin Receptor Activator (C.E.R.A.) therapy in KTxR.

Methods: This single-arm, non-interventional study was conducted in 37 centres in Germany. 288 pts were enrolled, with 276 pts representing the safety population (SP). The analysis was performed in a descriptive way on the efficacy population (EP) (definition: at least one Hb measurement and one C.E.R.A. dosing available at months 7-9).

Results: 180 pts formed the EP. Mean age was 50.8 ± 14.3 years (48.9% male) and pts had a weight of 71.3 ± 13.7 kg and a BMI of 24.8 ± 4.23 kg/m^2, respectively (SP). The mean time since kidney transplantation was 7.2 ± 6.1 years, with 19,6% living donations. Previous ESA -treatment was documented as follows (21.4% missing): darbepoetin alpha (96; 34.8%), epoetin alpha (7; 2.5%), epoetin beta (61; 22.1%), epoetin delta (11; 4.0%), C.E.R.A. (42; 15.2%). During the study the average C.E.R.A dose was 95.3 ± 54.6 µg, given every 35.4 ± 15.8 days (SP). Overall, 73 (40.6%) of pts were within the 10-12 g/dL Hb range during EP (Table1).

Percentage of pts within pre-defined Hb-ranges

Hb-range (g/dL)	10-12	10-13	11-12	11-13
(%)	40,6	63,9	20,0	40,0

27.9% of pts with a GFR < 30ml/min vs. 42.1% of KTxR with a GFR > 60ml/min were within the 10-12-corridor; in parallel, 37.4% of recipients of cadaveric grafts vs. 54.6% of patients with transplants from live donors.

Mean Hb deviation from intra-individual mean was 0.50±0.6 g/dL and in 157 (87.2 %) pts the intra-individual Hb cycled by ≤ 1.0 g/dL. The pts stayed on average 8.6 ± 6.5 months on the actual C.E.R.A. dose. Overall, there were no specific drug related safety events throughout the study.

Conclusions: Recipients of kidney allografts with PTA benefit from a once-monthly C.E.R.A. regimen, arriving at stable Hb-targets with few dose adaptations and an excellent tolerability

Funding: Pharmaceutical Company Support

FR-PO2057

Interstitial Fibrosis at 0-Hr Biopsy as a Useful Predictor for Early Post Transplant Anemia Akihiro Tsuchimoto,[1] Kosuke Masutani,[1] Naoki Haruyama,[1] Hideko Noguchi,[1] Hidehisa Kitada,[2] Kazuhiko Tsuruya,[1] Takanari Kitazono.[1] *[1]Medicine and Clinical Science, Kyushu University, Fukuoka, Japan; [2]Surgery and Oncology, Kyushu University, Fukuoka, Japan.*

Background: Anemia is a common complication that can lead to cadiovasucular events in renal transplant recipients. So far, several studies have determined predictors for post transplant anemia (PTA). However, the association between pathological features at 0-hr biopsy and PTA was not intensively reported.

Methods: This is a retrospective cohort study consisted of 167 consecutive patients who underwent renal transplantation (RTx) in Kyushu University Hospital from January 2006 to December 2009. The patients who received 0-hr biopsy were divided into the two groups according to the percentage of fibrosis area in cortical area at 0-hr biopsy. Patients with fibrosis area > 5% were defined as Fibrosis group (F group) (n=82), and patients with fibrosis area ≤ 5% as Non-fibrosis group (Non-F group) (n=85). We examined hemoglobin (Hb) levels of the recipients at 1, 3, 6, 12, 24 and 36 months after RTx. We compared Hb levels between the two groups. The statistical significance of differences between two groups was assessed by nonparametric tests. Univariate analyses followed by multivariate logistic regression analyses were performed to clarify association between interstitial fibrosis at 0-hr biopsy (IF-0h) and severe PTA (Hb < 10.0 g/dL).

Results: In F group, Hb levels were significantly lower than those in Non-F group at 1, 3, 6, 12 months after RTx. Differences in Hb level at 24 and 36 months were not statistically significant between the two groups (p value is 0.08 and 0.07, respectively). Logistic regression analyses showed that IF-0h was a significant risk factor for severe PTA at 1 and 12 months [Odds ratio (OR) is 3.26 and 3.45, respectively]. In multivariate analyses, even after adjustment for several confounders, IF-0h was an independent risk factor for severe PTA at 1 month [OR, 2.55; 95%CI, 1.04-6.44].

Conclusions: IF-0h is an useful predictor for PTA, especially at an early stage after RTx. Early treatment of PTA should be considered in some patients when we recognize graft fibrosis at 0-hr biopsy.

FR-PO2058

Donor Factors Determining Anemia at 1 Year Post Living Donor Kidney Transplant Richard A. Fatica, Milen Amde, Titte Srinivas, Jesse D. Schold, Emilio D. Poggio. *Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

Background: Post transplant anemia (PTA) is common (30-80% prevalence) and attributed to a variety of factors including persistent malnutrition-inflammation complex, iron deficiency, impaired GFR, and immunosuppressant medications. Donor factors are important to the overall outcome of kidney transplant recipients. We sought to relate donor features with the presence of PTA.

Methods: Living kidney donors between Jan 2005 and Dec 2009 at our institution were studied. Donor data included age, gender, race, and donated kidney volume. Recipient data included age, sex, race, kidney volume, delayed graft function (DGF), clinical acute rejection in the first year, and pre-transplant hemoglobin. Continuous variables were compared between groups using independent samples t test, categorical variables were compared using chi square and Fisher's exact tests utilizing a 2 sided p value < 0.05. The relationship between risk factors and post transplant anemia was further explored using linear regression.

Results: 340 live kidney donors were identified. Donor factors associated with recipient PTA at 1 year were donor age (p = 0.001) and donated kidney volume adjusted for recipient body surface area (BSA), (p = 0.005). Recipients who were female were more likely to have PTA (p < 0.0001). Recipients with clinical acute rejection and delayed graft function displayed a trend towards more anemia, but these findings were not statistically significant.

Clinical variables associated with PTA at 1 year

Parameter	Estimate	p value
Donor age	-0.05	0.001
Adjusted kidney vol	-0.003	0.005
Female recipient	-0.53	< 0.0001

Conclusions: Recipient factors are frequently cited as causes of persistent post transplant anemia, however we have identified several donor factors associated with PTA as well. Donor age and adjusted kidney volume are most likely associated with the nephron mass donated to the recipient. Recipient gender however does not have an immediately identifiable association with donated GFR or persistence of anemia. These findings deserve further investigation and mechanistic explanation.

Funding: Clinical Revenue Support

FR-PO2059

Blood Pressure Follows the Living Donor Kidney: Role of Donor GFR Laura V. de Vries,[1] H. Tent,[1] Johannes S. Sanders,[2] Hendrik Sijbrand Hofker,[1] Stephan J.L. Bakker,[1] Gerjan Navis.[1] *[1]Nephrology, UMCG; [2]Surgery, UMCG.*

Background: Hypertension is common in renal transplant recipients. It has been suggested that hypertensive traits of the donor may be transferred with the transplanted kidney. Direct proof for this "hypertension follows the kidney" hypothesis is, however, lacking. Here we study the effect of systolic blood pressure (SBP) and GFR of living donors on SBP in recipients.

Methods: We included 367 consecutive donor-recipient pairs transplanted between 1991 and 2010 at our center. GFR (^{125}I-iothalamate) was measured two months pre-donation in the donor, and three months and one year (n=267) post-transplantation (tx) in the recipient. Donors were allowed to donate with well controlled blood pressure on a maximum of two antihypertensive drugs. Donor and recipient demographics, transplantation characteristics and immunosuppressive and antihypertensive drug use were adjusted for in multivariate analysis.

Results: Donor and recipient characteristics are shown below. 22 donors used one antihypertensive drug, 15 used two drugs. Short term recipient SBP associated negatively to donor GFR (R -0.13). At one year post-tx, recipient SBP associated with both donor GFR and SBP (R -0.24 and 0.16). In a multivariate analysis, short term recipient SBP was predicted by recipient age and cyclosporine use. At one year post-tx, recipient age and donor GFR gave the best prediction (R^2 0.16). Recipient GFR was influenced by donor GFR (R 0.31 and 0.34) and donor SBP (R -0.18 and -0.25); at both short term and one year post tx.

Conclusions: Higher donor GFR and lower donor systolic blood pressure are associated with lower recipient blood pressure, independent of recipient renal function. Thus, regulatory impact of the kidney on blood pressure appears to be an intrinsic renal property within kidneys from generally normotensive donors. In living kidney donation, hypertension follows the kidney.

	Donor	Recipient	
		3 months post-tx	1 year post-tx
N (% female)	367 (54)	367 (38)	267 (40)
Age at tx (years)	51±11	44±15	44±15
BMI (kg/m^2)	27±15	25±5	26±4
Systolic BP (mmHg)	126±13	139±16	140±15
Diastolic BP (mmHg)	76±9	85±10	85±11
GFR (ml/min)	116±21	61±19	60±19
No. antihypertensive drugs	0.1±0.5	1.3±0.9	1.5±1.0

FR-PO2060

Ammoniagenesis Is Associated with Blood Pressure in Renal Transplant Recipients Else van den Berg,[1,2] Marielle Francis Engberink,[1,3] Reinold O.B. Gans,[2] Gerjan Navis,[2] Stephan J.L. Bakker,[1,2] Elizabeth Brink.[1,4] *[1]Top Institute Food&Nutrition, Wageningen, Netherlands; [2]Kidney Center Groningen, University Medical Center Groningen, Netherlands; [3]Division of Human Nutrition, Wageningen University, Wageningen, Netherlands; [4]Pharmacokinetics & Human Studies Group, TNO, Zeist, Netherlands.*

Background: Hypertension is common in renal transplant recipients (RTR) and a risk factor for graft failure and mortality. Animal data suggest that high dietary acid load contributes to hypertension by stimulating renal ammoniagenesis, leading to tubulo-interstitial damage. This may provide a new target for intervention. Therefore, we studied the association between dietary protein intake, urinary ammonium and blood pressure in a large single center RTR cohort.

Methods: Dietary intake was assessed in 580 outpatient RTR (≥1 y after transplantation) by a food-frequency questionnaire focusing on differentiation of animal and vegetable protein, since animal protein is the main source of dietary acid. Ammonium excretion was assessed from 24h urine collections. Blood pressure was measured automatically for 15 min and last 3 measurements were averaged

Results: Mean age was 53±13 y, 58% was male. Estimated total protein intake was 83±21 g/d (52±16 g/d from animal sources). Urinary ammonium excretion was 19.3 [IQR:12.4-29.4] mmol/24h. Ammonium excretion correlated positively with animal protein intake (r=0.12; p<0.01), but not with vegetable protein intake. Mean arterial pressure (MAP) was 100±12 mmHg on a median of 2.1 [IQR:1-2] anti-hypertensives. In multivariable linear regression analysis, ammonium excretion was significantly associated with MAP (β=0.12 mmHg per mmol/24h; p=0.007) independent of age, sex, BMI, renal function and use of anti-hypertensives.

Conclusions: Higher intake of animal protein is associated with more renal ammoniagenesis. Higher urinary ammonia excretion is positively associated with blood pressure. These cross-sectional data suggest that modulation of renal ammonia production, either by reducing intake of acidifying animal protein or by increasing alkali by diet or bicarbonate suppletion, may be a new therapeutic strategy with the potential to reduce blood pressure and renal damage in RTR.

FR-PO2061

Blood Pressure Treatment Lowers Mortality in Kidney Transplant Recipients Geir Mjøen,[1] Hallvard Holdaas,[1] Gudrun E. Norby,[1] Alan G. Jardine,[2] Bengt C. Fellstrom.[3] *[1]Medical Department, Rikshospitalet, University of Oslo, Norway; [2]Department of Nephrology, University of Glasgow, United Kingdom; [3]Department of Nephrology, Uppsala University, Uppsala, Sweden.*

Background: There are uncertainties regarding cardiovascular medication in renal transplant recipients and all-cause mortality. We assessed possible associations in a post-hoc observational analysis of the ALERT trial.

Methods: ALERT was a randomized, double-blind, placebo-controlled study to investigate the effect of fluvastatin on cardiovascular and renal outcomes in 2102 renal transplant recipients, followed by a 2-year extension. Patients were recruited at a median time of 4.5 years after transplantation with a stable renal function. We investigated the relationship between cardiovascular medication at baseline and all-cause mortality using Cox regression adjusted for demographic variables, other medication and known cardiovascular risk factors.

Results: In total, 1868 out of 2102 patients were available for analysis. During a median follow-up of 7.4 years, there were 334 deaths. In multivariate analysis, significantly reduced mortality was seen in relation to treatment with Calcium antagonists (HR 0.72, CI 0.56-0.92), beta blockers (HR 0.64 CI 0.51-0.81), or ACE/ARB (HR 0.58 CI 0.46-0.73). Treatment with diuretics was associated with increased mortality (HR 1.79 CI 1.38-2.33). No associations were seen for nitrates or warfarin. Borderline significance was seen for ASA (HR 0.80 CI 0.63-1.02).

Conclusions: Blood pressure treatment at baseline seems associated with a favourable outcome in kidney transplant recipients.

Funding: Clinical Revenue Support

FR-PO2062

Renal Allograft Survival Is Decreased in Patients with Thrombotic Disorders Steven Wagner,[1] Sandra Herrmann,[1] Iasmina Craici,[1] Andrew D. Rule,[1] Fernando G. Cosio,[1] Hatem Amer.[1] *[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Health Sciences Research, Mayo Clinic, Rochester, MN.*

Background: Kidney allograft survival in patients with thrombotic disorders pre-transplant is not well studied. We used a matched cohort study design to examine the impact of thrombotic disorders on kidney allograft survival.

Methods: We identified kidney allograft recipients in a single center, transplanted between 2001 and 2010 who had: Lupus anticoagulant (LAC), anti-phospholipid antibodies (APLA), Factor V Leiden (FVL), or a prothrombin gene mutation (PGM). For each case 2 controls were chosen. Proportions were compared by the Chi-square test. Death censored graft loss (DCGL) was assessed with Cox proportional hazard models.

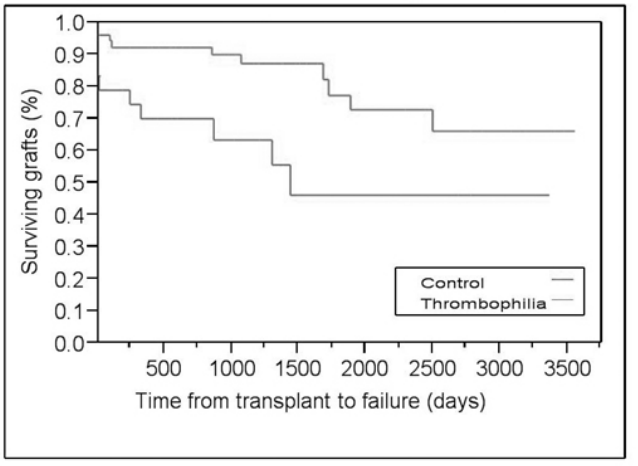
Results: Twenty nine patients were identified with a thrombotic disorder (LAC=8, APLA=8, FVL=10, PGM=4). Between patients and controls, there was no difference in gender (55% vs 59% male, p=0.76), age (46.3 vs 46.5 years, p=0.9), or follow-up (4.8 vs. 4.2 years, p= 0.42).

In the first 30 days, 5 grafts were lost in cases (17%), with four due to thrombosis; only 2 grafts (3.5%) were lost in controls, one due to thrombosis (p=0.03).

Thrombophilic patients had higher overall DCGL (HR 3.06, p=0.02), as well as higher risk for DCGL in the first 30 days (HR 5.73, p=0.03) and in the first year (HR 3.10, p=0.02). After 30 days, however, there was no difference in DCGL (HR 2.22, p=0.18).

There were no differences in DCGL between patients with LAC or APLA compared to those with other disorders.

Figure 1: Death censored graft survival



Conclusions: Patients with thrombotic disorders pre-transplant have higher DCGL. This is entirely due to graft loss in the first 30 days, mostly due to graft thrombosis. Further research is necessary to determine whether anticoagulant use might affect the rate of early graft loss in thrombophilic patients.

FR-PO2063

CUBN as a Novel Locus for ESRD: Insights from Renal Transplantation Anna Reznichenko,[1] Harold Snieder,[2] Marc Seelen,[1] Gerjan Navis.[1] *[1]Internal Diseases, Division of Nephrology, University Medical Center Groningen; [2]Epidemiology, Unit of Genetic Epidemiology & Bioinformatics, University Medical Center Groningen, Groningen, Netherlands.*

Background: Chronic kidney disease (CKD) is a complex disorder with an important genetic component. As urinary protein loss is a risk factor for progressive CKD and a recent genome-wide association study identified the cubilin gene CUBN as a locus for albuminuria, we investigated whether common genetic variance in CUBN is associated with end-stage renal disease (ESRD).

Methods: A total of 1271 patients with ESRD, admitted for renal transplantation between 1993 and 2008, with their 1271 donors as a control population were genotyped for SNPs rs7918972 and rs1801239 in CUBN (case-control study). After transplantation the recipients were followed for a median 5.5 [IQR 2.9-8.9] years and time to graft failure (GF) was documented (longitudinal study).

Results: The minor allele frequency (MAF) of rs7918972 was significantly higher in ESRD patients as compared to kidney donors, implicating an increased risk for ESRD (OR 1.38, p=0.0002).

During follow-up 215 (16.9%) cases of death-censored GF occurred. Donor rs7918972 MAF, reflecting genetic make-up of the kidney, was 14% in GF versus 11% in cases with functioning graft. Consistently, a multivariate Cox regression analysis showed that donor kidney rs7918972 is an independent predictor of GF (HR 1.41, 95% CI 1.00-1.98, p=0.05, per copy of the minor allele). There was no association of recipient rs7918972 with GF.

Conclusions: In the case-control study we identified CUBN SNP rs7918972 as risk locus for ESRD. Independently, follow-up data after transplantation showed direction-consistent association of donor rs7918972 with GF. Thus, rs7918972 was associated with susceptibility to develop ESRD in two settings in a single population, namely ESRD in native kidneys and GF in transplanted kidneys. Kidney genotype was associated with increased risk, suggesting impact of intra-renal pathways on organ damage. Our study set-up – analyzing both donor and recipient genotypes – provides a powerful design for hypothesis-driven studies on risk loci for renal damage enabling differentiation between intra-renal and systemic influences.

FR-PO2064

Body Mass Index and Outcomes after Deceased Donor Kidney Transplantation Joseph Kim,[1] Olusegun Famure,[1] Daniel C. Cattran,[1] Edward H. Cole,[1] Jeffrey Schiff,[1] Kathryn J. Tinckam,[2] Carl J. Cardella.[1] *[1]Medicine (Nephrology), Toronto General Hospital, University Health Network, Toronto, ON, Canada; [2]Laboratory Medicine and Pathobiology, Toronto General Hospital, University Health Network, Toronto, ON, Canada.*

Background: Increased body mass index (BMI) has been associated with adverse kidney allograft outcomes but its effects on delayed graft function and acute rejection are less clear.

Methods: We studied deceased donor kidney transplant recipients from 1 Jan 2000 to 31 Dec 2010 at the Toronto General Hospital . BMI (kg/m2) at transplant was categorized as < 20, 20 to 24.9 [referent], 25 to 29.9, 30 to 34.9, and ≥ 35. Outcomes included delayed graft function (DGF; dialysis in the first week post-transplant), biopsy-proven acute rejection (BPAR), death-censored graft failure (DCGF), and death with graft function (DWGF). Recipient, donor, and transplant factors were included in logistic and Cox regression models.

Results: There were 42, 225, 190, 92, and 43 patients in the first to fifth BMI groups (N = 592). Higher proportions of females were seen at the extremes of BMI. More Whites, diabetics, and male donors were noted in the highest BMI group. DGF rates were 35.7%, 32.0%, 36.3%, 48.9%, and 65.1%, respectively. The table shows results from the logistic and Cox models. Odds ratios for DGF were significantly increased in higher BMI groups. Hazard ratios for BPAR showed a similar trend. Most of this effect was due to an increase in acute cellular rejections in the higher BMI groups. There was a trend to an increased risk of DCGF and DWGF in the highest BMI group.

Conclusions: Larger BMI is associated with a greater risk of DGF and BPAR. The mechanisms by which BMI increases the risk of DGF and BPAR require further study.

Outcome Measures by Body Mass Index Category

Outcomes	Outcome Measures	BMI < 20	BMI 20 to 24.9	BMI 25 to 29.9	BMI 30 to 34.9	BMI > 35
DGF	OR (95% CI)	1.37 (0.65, 2.91)	1	1.19 (0.78, 1.83)	1.96 (1.16, 3.32)	4.33 (2.07, 9.08)
BPAR	HR (95% CI)	0.57 (0.21, 1.53)	1	1.03 (0.61, 1.77)	2.05 (1.17, 3.62)	2.10 (1.04, 4.26)
DCGF	HR (95% CI)	0.92 (0.32, 2.61)	1	1.02 (0.52, 1.99)	0.97 (0.40, 2.33)	2.13 (0.84, 5.40)
DWGF	HR (95% CI)	3.75 (1.22, 11.5)	1	0.80 (0.44, 1.82)	0.87 (0.35, 2.11)	2.05 (0.72, 5.86)

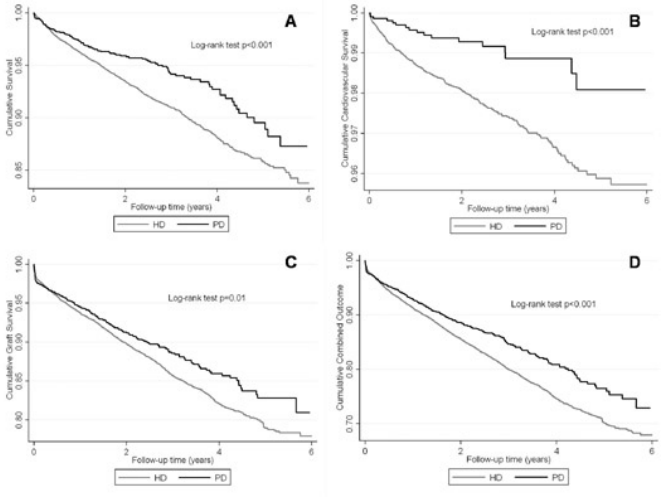
FR-PO2065

Dialysis Modality and Outcomes in Kidney Transplant Recipients Miklos Z. Molnar,[1,2] Rajnish Mehrotra,[3] Uyen Duong,[1] Suphamai Bunnapradist,[3] Lilia R. Lukowsky,[1] Mahesh Krishnan,[4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]DaVita, Inc, Denver, CO; [5]Salem VA Medical Center, Salem, VA.*

Background: The role of pre-transplant dialysis modality in affecting post-transplant outcomes is not clear. We examined associations of pre-transplant dialysis modality with short- and long-term post-transplant outcomes.

Methods: Linking the 5-year patient data of a large dialysis organization to the *Scientific Registry of Transplant Recipients*, we identified 12,416 hemodialysis (HD) and 2,092 peritoneal dialysis (PD) patients who underwent first kidney transplantation. Mortality or graft failure, and delayed graft function (DGF) risks were estimated by Cox regression (hazard ratio [HR]) and logistic regression (odds ratio [OR]), respectively.

Results: Patients were 48±14 years old and included 39% women and 35% diabetics. Patients treated with PD pre-transplant had 43% (HR:0.57[0.38-0.87]) lower adjusted death risk compared to those treated with HD. Similar association was found for cardiovascular death (adjusted HR:0.34[0.14-0.88]).



Conclusions: Compared to HD, PD modality before transplantation appears associated with lower all-cause and cardiovascular mortality. This outcome mirrors the survival benefit previously attributed to the selection bias for patients on PD, and as such confounding by residual selection bias cannot be ruled out.

Funding: NIDDK Support

FR-PO2066

Common Genetic Variance in Glucocorticoid Receptor Locus Associates with Graft Failure in Renal Transplant Recipients Anna Reznichenko, Laura V. de Vries, Stephan J.L. Bakker, Gerjan Navis. *Internal Diseases, Division of Nephrology, University Medical Center Groningen, Groningen, Netherlands.*

Background: Glucocorticoid receptor, encoded by the gene NR3C1, functions as a responsive element forcorticosteroid hormones. We hypothesized that common genetic variance in NR3C1 influences tissue sensitivity and responsiveness to glucocorticoid effects of corticosteroids. Since corticosteroids are used in clinical practice as immunosuppressive treatment in kidney transplantation, we investigated whether polymorphisms in NR3C1 are associated with development of renal graft failure (GF) in renal transplant recipients (RTR) treated with corticosteroids.

Methods: A total of 1271 patients, admitted for renal transplantation between 1993 and 2008, and their 1271 donors (kidney genotype) were genotyped for SNPs rs852977, rs17209258, rs852978, rs10482672 and rs10482682 in NR3C1. After transplantation, the RTR were followed for a median of 5.5 [IQR 2.9-8.9] years. Time to GF, which was censored for death, was documented. For the subsequent graft survival analyses we selected a subset of 1096 RTR receiving corticosteroids.

Results: During follow-up, 175 (16.0%) RTR developed GF and 191 (17.4%) died with functioning graft. Minor allele frequency and genotypes distribution of donor rs852978 were 0.14 and 3/43/129 in cases with GF versus 0.12 and 14/201/705 in cases without GF, respectively. In a multivariate Cox regression analysis, donor rs852978 genotype appeared to be an independent predictor of GF (HR 2.93, p=0.035, for the minor allele homozygote genotype) in a model adjusted for donor and recipient age and sex, donor type (living or deceased) and episodes of acute organ rejection. There was no association of recipient genotypes with GF or of either donor or recipient genotypes with patient survival.

Conclusions: We found that donor NR3C1 genotype impacts long-term graft survival in RTR treated with corticosteroids. Donor kidney rs852978 genotype was associated with significantly increased risk for GF, highlighting involvement of intra-renal pathways in organ damage.

FR-PO2067

Distinct Morphological Features of Acute Tubular Injury (ATI) in Renal Allografts: Relation to Clinical Factors and Outcome Andrea Bohlmann, Anette Melk, Roland Schmitt, Verena Broecker, Jan U. Becker, Irina Scheffner, Hermann G. Haller, Wilfried Gwinner. *Medical School Hannover, Germany.*

Background: ATI is common in kidney transplants within the 1st year of transplantation (approx. 40% in protocol biopsies and 50% in biopsies for cause) and may be associated with an inferior allograft outcome. Commonly used criterions to diagnose ATI are loss of the brush border, flattening and cytoplasmatic lucency of proximal tubular epithelial cells. We examined whether additional features of tubular cell injury and semi-quantification of these alterations can help to classify the injury better.

Methods: Analyses included 204 patients with their protocol biopsies taken at 6 weeks, 3 and 6 months and biopsies for cause. Categories of injury included brush border loss, cytoplasmatic lucency, cell flattening, karyopyknosis, mitoses and loss of nuclei of the tubular epithelium and luminal debris which were graded semi-quantitatively. Scores were related to clinical parameters and allograft function.

Results: Inter-observer reproducibility was good for karyopyknosis (r=0.92), cell flattening (r=0.86), loss of brush border (r=0.6), fair for cytoplasmatic lucency (r=0.54) and poor (r<0.5) for the remaining scores. Delayed graft function was associated with higher scores of brush border loss, cytoplasmatic lucency and karyopyknosis. Organs from deceased donors had higher cytoplasmatic lucency and karyopyknosis scores. Grafts from combined kidney/pancreas recipients had lower scores for brush border loss and epithelial cell flattening. Scores were lower for MMF-treated patients (brush border loss, karyopyknosis) and for organs from female donors (tubular cell flattening). Brush border loss and loss of nuclei in biopsies at 6 months correlated with a worse function at the time of biopsy (p<0.05). The finding of karyopyknosis and cellular flattening was related to a further deterioration at 1 year after transplantation (p<0.05).

Conclusions: Distinct features of tubular cell injury like karyopyknosis and tubular cell flattening appear to be best suited to assess acute allograft damage and the functional consequences of this damage, particularly when used with a semi-quantitative scoring system.

Funding: Government Support - Non-U.S.

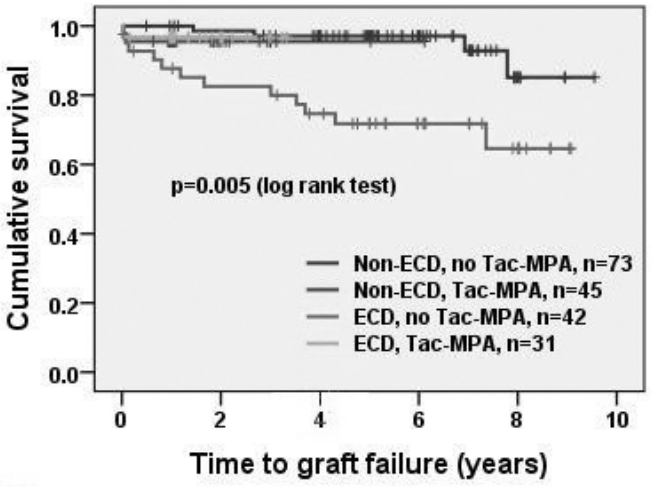
FR-PO2068

Outcome of Expanded Criteria Donor Kidney Transplants in Patients without Donor Specific HLA-Antibodies Claudia Praehauser,[1] Kiymet Saydam Bakar,[2] Patrizia Amico,[2] Eliane Vogler,[2] Patricia Hirt-Minkowski,[2] Stefan Schaub,[2] Michael Mayr.[1,2] *[1]Medical Outpatient Department, University Hospital Basel, Switzerland; [2]Clinic for Transplantation Immunology and Nephrology, University Hospital Basel, Switzerland.*

Background: Due to disparity between organ supply and demand, utilization of kidneys from expanded criteria donors (ECD) has become inevitable. Outcome data of ECD kidneys revealed poorer graft survival but might be confounded by immunological events. Therefore, we investigated outcome of ECD kidneys in an immunological low-risk population.

Methods: We retrospectively analyzed deceased-donor kidney transplantations without pre-transplant donor specific HLA-antibodies (HLA-DSA) performed between 1999-2008. The primary endpoint was graft survival, secondary endpoints were rejection rate and graft function.

Results: Overall, 73 (38%) of 191 transplants were ECD organs. The 1, 3 and 5-year graft survival rates were 98%, 96% and 96% for non-ECD and 91%, 87% and 76% for ECD kidneys (p=0.003). When stratified by IS, graft survival of ECD kidneys treated with tacrolimus-mycophenolate (Tac-MPA) was comparable to non-ECD (96% at 1 and 3-year), whereas graft survival of ECD kidneys not treated with Tac-MPA was significantly decreased (88%, 83% and 72%)(figure).



ECD kidneys not treated with Tac-MPA that experienced rejection showed the worst graft survival (79%, 68% and 43%, p<0.001). At one year, eCrCl was significantly lower in ECD kidneys (38 vs 58 ml/min, p<0.001). When treated with Tac-MPA, however, eCrCl was significantly higher than without Tac-MPA (non-ECD: 65 vs 54ml/min, p=0.010; ECD: 43 vs 35 ml/min, p=0.028).

Conclusions: Patients without pre-transplant HLA-DSA receiving ECD kidneys show excellent graft survival that is similar to non-ECD kidneys when treated with Tac-MPA. Tac-MPA impressively preserves graft function in ECD and non-ECD kidneys.

Funding: Pharmaceutical Company Support

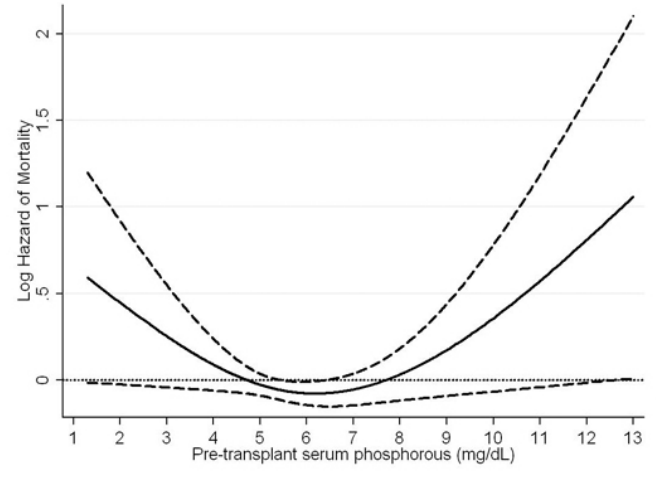
FR-PO2069

Association of Pre-Transplant Serum Phosphorous with Post-Transplant Outcomes Marcelo Santos Sampaio,[1] Miklos Z. Molnar,[1,2] Csaba P. Kovesdy,[3] Mahesh Krishnan,[4] Allen R. Nissenson,[4] John Tayek,[5] Rajnish Mehrotra,[5] Kamyar Kalantar-Zadeh.[1,5] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]Salem VA Medical Center, Salem, VA; [4]DaVita, Inc, Denver, CO; [5]Harbor-UCLA, Torrance, CA.*

Background: Serum phosphorous levels are associated with mortality, cardiovascular disease, & renal function loss in CKD patients. The association of pre-transplant serum phosphorus (13-week interval before transplantation were averaged) levels with transplant outcomes is unknown.

Methods: Data of the *Scientific Registry of Transplant Recipients* up to June 2007 were linked to the database (2000-6) of a US-based large dialysis organization. The selected 9384 primary kidney recipients were divided in five groups according to pre-transplant serum phosphorous levels (mg/dL): <3.5, 3.5-<5.5 (reference group), 5.5-<7.5, 7.5-<9.5 and ≥9.5.

Results: Patients were 48±14 years old & included 37% women & 27% African Americans. Unadjusted & adjusted risks for transplant outcomes were compared. After full adjustment, all-cause & cardiovascular death risk was 2.4 (HR: 2.44,95% CI:1.28-4.65) & 3.6-fold (HR: 3.63,95% CI: 1.13-11.64) higher, respectively in recipients in the ≥9.5 group; & graft loss risk was 1.4 (HR:1.42,95% CI:1.04-1.95) & 2.4-fold (HR: 2.36,95% CI:1.33-4.17) higher in recipients with 7.5->9.5 & ≥9.5, respectively. The association of pre-transplant serum phosphorous level with all cause mortality was U-shaped; both very low & very high phosphorous level was associated with higher mortality risk.



We did not find significant association with delayed graft function.

Conclusions: Pre-transplant phosphate levels >7.5mg/dL and >9.5 mg/dL were associated with increased risk of functional graft failure and increased risk of all-causes and cardiovascular death, respectively when compared to 3.5-<5.5 mg/dL.

Funding: NIDDK Support

FR-PO2070

Depressive Symptoms after Kidney Transplantation Are Associated with Decreased Survival Dorien M. Zelle,[1] Judith Rosmalen,[2] Eva Corpeleijn,[2] Reinold O.B. Gans,[3] Willem Van Son,[1] Gerjan Navis,[1] Stephan J.L. Bakker.[3] *[1]Kidney Center, University Medical Center Groningen, Netherlands; [2]Psychiatry, UMCG; [3]Internal Medicine, UMCG.*

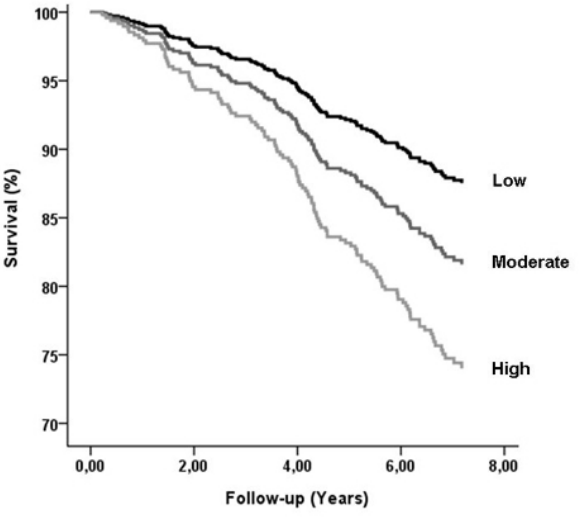
Background: Renal transplantation is the treatment of choice for end stage renal disease; it is nevertheless highly stressful for many patients. We aimed to investigate the association of depression with mortality and graft failure in renal transplant recipients (RTR).

Methods: RTR were investigated between 2001-2003. Depressive symptoms were assessed using the subscale of the Symptom Checklist (SCL-90).SCL-90 scores (SCL-90s) were grouped using standardized scales. We compared SCL-90s of our RTR with a healthy Dutch reference population. Mortality and graft failure were recorded until May 2009.

Results: A total of 527 RTR (age 51±12 yrs,55% men) participated at median time of 6.0 yrs post-transplant. SCL-90s were higher in RTR than in the reference population (24vs.22; p< 0.001). Risk factors for depressive symptoms were being unfit to work, living alone, dialysis duration, low physical activity, female gender and low creatinine clearance (p<0.05). During median follow-up for 7 yrs, 37 (15%) RTR died in the low SCL-90s group (n=243), while 21 (21%), and 56 (31%) died in the groups with moderate (n=102) and high SCL-90s (n=182)(P=0.01,fig.1). In univariate Cox-regression analyses high SCL-90s was associated with increased risk for mortality (HR=2.22[1.46-3.36],P<0.001), while no association was found for graft failure. Adjustment for potential confounders, including dialyses duration and creatinine clearance did not materially change the association.

Conclusions: Our results show that depression after kidney transplantation is associated with decreased survival. Additional studies on the effect of depression treatment on survival after renal transplantation are needed.

Figure 1: Kaplan-Meier curve of mortality according to SCL-90 score.



FR-PO2071

Incidence of Metabolic Syndrome in the First Year of Kidney Transplant and Its Effect on Graft Function Luis Alberto Evangelista-Carrillo,[1] Jorge Andrade-Sierra,[1] Enrique Rojas-Campos,[2] Salvador Mendoza Cabrera,[1] Celina Mora,[1] Maria Del Carmen Alvarado Velarde,[1] Diana Cristina Soria,[1] Abel Puentes Camacho,[1] Ana Maria Contreras,[1] Alfonso M. Cueto-Manzano,[2] Benjamin Gomez-Navarro.[1] *[1]Department of Nephrology and Transplantation, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [2]Medical Research Unit in Renal Disease, CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

Background: The Metabolic Syndrome (MS) during the first year of kidney transplant (KT) has not been completely evaluated. **Aim:** Describe the incidence of MS at 6/12 (early/late) months after KT and its impact on graft function according to time of development.

Methods: Prospective cohort (Mar/2009-Apr/2010) of 197 kidney recipients. Diabetic patients before transplant were excluded. MS was defined using the ATP III criteria.

Results: The accumulated incidence of MS at 12 months was 32%, 69% were male, 66% had tacrolimus, mofetil and prednisone scheme and 11% were steroids-free. Majority (75%) of patients with early MS receive peritoneal dialysis. In a logistic regression analysis, risk factors to early MS were CRP (RR 4; *p*=0.043) and weight (RR 9.7; *p*=0.002) before KT. Other results are shown in Table.

Comparison according to develop of MS after KT

	6 months (n=39;61%)	12 months (n=25;39%)	Non MS (n=133; 100%)
Mean age (yr)	29(±10)	25(±10)	26(±7)*
Weight pre KT (kg)	67(±14)	62(±13)	56(±12)*
BMI pre KT	24(±4)	23(±3)	21(±3)*
CRP pre KT (mg/l)	8.3	8.7	5.1
Insulin pre KT (mUI/ml)	8(±4)	9(±7)	6(±4)*
BMI 6 mth	24(±4)	23(±2)	21(±3)*
SCr 6 mth (mg/dl)	1.1	1.1	1.2
Insulin 6 mth (mUI/ml)	10(±5)	13(±11)	10(±6)
CrCl measure 6 mth(ml/min)	67(±20)	72(±26)	73(±20)
BMI 12 mth	24(±4)	24(±2)	22(±3)*
SCr 12 mth (mg/dl)	1.1	1.5	1.3
Insulin 12 mth (mUI/ml)	11(±6)	13(±4)	10(±4)
CrCl measure 12 mth(ml/min)	73(±19)	71(±28)	77(±27)

*p=<0.05 between groups; CRP: C reactive protein

Conclusions: Majority of MS incidence was in the early phase. Patients with early MS were older and had more pre transplant weight. Insulin, CRP and BMI at 6, 12 months, were different in all MS (early and late) patients compared to Non-MS. The graft function at first year was not affected by the early or late onset of MS. Further follow is necessary.

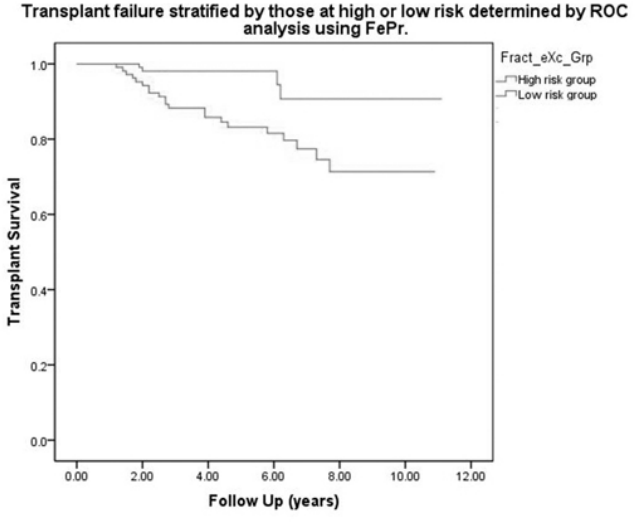
FR-PO2072

Fractional Excretion of Protein May Have Superior Predictive Value over Traditional Proteinuria Measures in Renal Transplant Recipients Kathryn K. Stevens, Rajan Kantilal Patel, Patrick Barry Mark, Colin C. Geddes, Marc J. Clancy, Alan G. Jardine. *Renal Transplant Unit, Western Infirmary, Glasgow, United Kingdom.*

Background: Proteinuria is associated with poorer outcomes in renal transplant recipients. Fractional excretion of total protein (FePr) may better reflect renal excretion of protein than traditional measures. We assessed FePr and protein: creatinine ratio (PCR) as predictors of transplant failure.

Methods: Data were collected from the electronic patient record for recipients of a first renal transplant. FePr and PCR were calculated (FePr = (Serum creatine * Urine protein)/ (Serum protein * Urine creatinine) %, PCR = ((Urinary Protein / UrinaryCreatinine) * 1000). The primary endpoint was transplant failure. ROC analysis was performed for each test and patients were stratified into high/low risk groups. Kaplan Meier and Cox survival analysis was then performed.

Results: 219 recipients were followed up for a median of 4.9 years. 11.4% (n=25) of the transplants failed at a median of 2.7 years. Mean eGFR at 1 year post transplant was 48.5mls/min/1.73m2 ±16.7. Using ROC analysis, both FePr and PCR predict transplant failure. FePr had the higher sensitivity and specificity. In the high risk FePr group the risk of transplant failure was increased by 3.4 fold (p=0.003).



One year eGFR was significantly lower (p<0.001). For PCR, the risk of transplant failure was increased 2.1 fold in the high risk group. In multivariate analysis, both tests remain independently predictive of transplant failure.

Conclusions: FePr and PCR predict transplant failure but FePr is more sensitive and specific. It may be superior at predicting those at risk of transplant failure. Our study is limited by its retrospective nature and the small number of events. Comparison should be made between these tests and measures of albuminuria.

FR-PO2073

Chronic Kidney Disease Stage: Impact on Long-Term Graft Survival Following Kidney Transplantation William Irish,[1] Schiffon L. Wong,[2] Debora Bowers,[1] Digisha Trivedi,[2] Tony Hebden.[2] *[1]CTI Clinical Trial and Consulting Services; [2]Bristol-Myers Squibb.*

Background: Renal function (RF) after kidney transplant has been shown to be a strong predictor of graft survival (GS). We characterized the relationship between early change in post-transplant RF and GS.

Methods: Adult Medicare beneficiaries in the US Renal Data System who received kidney-only transplant (2001 - 2008) were eligible. RF, measured by estimated glomerular filtration rate (eGFR), was calculated by the Modification of Diet in Renal Disease 4-variable formula. eGFR at 6 and 12 months was categorized using the National Kidney Foundation CKD staging (Stage 1 (eGFR ≥90), 2 (60≤eGFR<90), 3 (30≤eGFR<60), 4 (15≤eGFR<30), and 5 (eGFR<15)). GS was estimated using the life-table method. Hazard ratios (HRs) for graft failure (GF) and associated 95% confidence intervals (CIs) were estimated using Cox hazards model.

Results: At 6 months, 77,206 patients (Mean age: 50 years, 29% Black, 61% males) were alive with a functioning graft and eGFR (Median followup: 4 years). Association between change in CKD stage from 6 to 12 months post-transplant and 4 year GS is shown in **Table 1**. Patients in CKD stage 2, 3, or 4 at month 6 who had a one stage worsening in CKD stage by month 12 had a significantly greater risk of GF at 4 years. A majority of patients were in CKD stage 3 at month 6. Those that moved to stage 4 at 12 months had a 3.5 fold increased risk of GF and a 32% lower GS at 4 years (p<0.001).

Table 1: Change in CKD Stage and GS

CKD Stage at Month 6	CKD Stage at Month 12	GS at 4 Years (%)	HR	95% CI for HR
Stage 1 (n=4,695)	Stage 1	88	1.0	
	Stage 2	87	1.2	1.0 - 1.4
	Stage 3	74	2.1	1.7 - 2.7
	Stage 4	61	3.4	1.9 - 5.9
	Stage 5	36	7.4	3.5 - 15.6
Stage 2 (n=27,041)	Stage 2	90	1.0	
	Stage 3	84	1.5	1.4 - 1.6
	Stage 4	45	7.2	5.9 - 8.6
	Stage 5	15	16.5	11.6 - 23.5
Stage 3 (n=40,772)	Stage 3	85	1.0	
	Stage 4	53	3.5	3.3 - 3.7
	Stage 5	20	11.5	9.6 - 13.7
Stage 4 (n=4,218)	Stage 4	55	1.0	
	Stage 5	18	3.7	3.1 - 4.4

Conclusions: Worsening of CKD stage in the first year post-transplant is associated with increased GF. Results suggest that patient management strategies targeted at preserving RF in the early post-transplant period will have a positive impact on long-term GS.

Funding: Pharmaceutical Company Support

FR-PO2074

Metabolic Syndrome Defined by ATP III Classification Predicts Better the Glomerular Filtration Rate Decline Than Its Individual Components after Renal Transplantation Nabil Mohsin, Georges J. Mourad, Magalie Faure, Ilan Szwarc, Fernando Vetromile. *Nephrology Department, University of Montpellier, France.*

Background: Individual components of the metabolic syndrome (MS), especially obesity and hypertension have a deleterious effect on renal graft outcome. Whether MS is better than its individual components to predict the decline of renal function is unknown. We studied the presence of MS and its individual components at 3 and 12 months (m3, m12) after transplantation according to the ATP III Classification, and their influence on graft function.

Methods: A cohort of 322 patients transplanted between 1996 and 2003 who accepted to have their glomerular filtration rate measured by urinary clearance of Tc*-DTPA (mGFR) at 3, 12, 48, 60 and 96 months after transplantation were included. The patients were followed till patient death, graft loss or till December 2009 (Mean F-U: 3 + 2.8 yrs). Linear mixed effect model for longitudinal repeated measures was applied. To compare MS versus its components we used the Akaike information Criterion (AIC) in which a lower value indicates a better model.

Results: MS was present in 34%, 37% and 25% of recipients at m3, m12, and both m3 and m12(m3&12). MS, waist circumference (WC), high triglycerides (TG), high systolic blood pressure (SBP) at m3 and m12 are all associated with a significant decline of mGFR as indicated by the negative sign of the coefficient (Table 1). HDL at m3 has a beneficial effect as shown by the positive sign. Body mass index, glycemia and diastolic BP at m3 and m12, WC and HDL at m12 had no significant effect (p value >0.05. Not shown). MS (m3&12), m12, and m3 had the lowest AIC indicating that they are the best predictors of graft deterioration and that MS is consistently a better model than its individual components.

MS vs its components effect on GFR .

	MS m3/m12/m3&12	TG m3/m12	SBP m3/m12	WC m3	HDL m3
mGFR coefficient	-6.7/-7.8/-8.7	-3.04/-3.85	-0/17/-0.13	-0.20	+12.8
p-value	<0.001/<0.001/<0.001	0.001/<0.001	0.003/0.028	0.012	0.028
AIC	11722/11717/11715	11726/11727	11733/11737	11734	11727

Conclusions: MS is a better predictor of mGFR decline than its individual components. We recommend routine assessment of MS during the clinical visits.

FR-PO2075

De Novo Thrombotic Microangiopathy after Kidney Transplantation: Clinical Features, Treatment and Long-Term Patient and Graft Survival

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Background: Post transplantation thrombotic microangiopathy/hemolytic uremic syndrome (PT TMA/HUS) can occur as a recurrent or *de novo* disease.

Methods: Retrospective single-center observational study to examine the incidence and outcomes of *de novo* PT TMA/HUS among transplants performed between 2000 and 2010. Recurrent HUS or antibody-mediated rejection were excluded.

Results: Seventeen (1.1%) among 1549 KTR fulfilled criteria for *de novo* TMA. The mean follow-up was 572 days (range, 69–1769). All patients received induction therapy. Maintenance immunosuppression was prednisone, tacrolimus (TAC) and mycophenolic acid in 15 patients (88%). Mean age at onset was 40±15 yrs, and serum creatinine was 6.1±4.1mg/dL. TMA occurs at a median of 25 days (range, 1–755) after transplantation. Nine (53%) patients developed TMA within 1 month of transplantation and only 12% after 1 year. Clinical features were anemia (Hb<10g/dL) in 9 (53%) patients, thrombocytopenia in 7 (41%), and increased lactate dehydrogenase in 12 (70%). Decreased haptoglobin was observed in 64% and schistocytes in 35%. CNI withdrawal or reduction was the first step in the management of 10/15 (66%) patients, and 6 (35%) received fresh frozen plasma (FFP) and/or plasmapheresis. The mean trough level of TAC at diagnosis was 11.5 ng/mL (range, 1.2–31). TAC was successfully reintroduced in 6 patients after a mean of 17 days. Eight (47%) patients needed dialytic support after TMA diagnosis and 75% remained on dialysis. Death censored graft survival was worse for TMA group (91.4% vs 84.7%, log rank =0.001; HR= 3.74). There was no difference in patient survival (90.4% vs 92.9%).

Conclusions: *De novo* TMA after kidney transplantation is a rare but severe condition with poor graft outcomes. This syndrome may not be fully manifested and clinical suspicion is essential for early diagnosis and treatment, based mainly in CNI withdrawal and FFP infusions and/or plasmapheresis.

FR-PO2076

Impact of Tacrolimus Variability and *CYP3A5* Genetic Polymorphism on Renal Allograft Outcomes

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Background: Wide intra-individual variation (IIV) in tacrolimus levels was reported to be associated with poor renal graft outcomes in Caucasians. We investigated whether the tacrolimus IIV or tacrolimus trough concentrations can influence kidney allograft outcomes in Korean, and whether *CYP3A5* polymorphism (rs776746) can affect this relationship.

Methods: We enrolled 249 patients that got kidney transplant from 1996 to 2010 in Seoul National University Hospital, Seoul, Korea. The tacrolimus IIV and the mean tacrolimus trough concentrations were calculated from the concentrations between 6 and 12 months after transplantation.

Results: The patients with higher tacrolimus IIV had longer rejection-free survival rate (p=0.002) and higher mean tacrolimus trough concentrations (p<0.001). On the other hand, there was no difference in either rejection-free survival rate or mean tacrolimus trough concentration between CYP3A5 expressers and non-expressers. The tacrolimus IIV was not associated with the *CYP3A5* polymorphism. Interestingly, the association of the tacrolimus variability with the rejection-free survival was significant only in CYP3A5 expressers (p=0.001 in CYP3A5 expressers, p=0.267 in CYP3A5 non-expressers). High IIV of tacrolimus was the independent risk factor (hazard ratio (HR) 1.052, 95% confidence interval (CI) 1.027-1.079) for biopsy-proven acute rejection after adjusting with mean tacrolimus concentration (HR 0.780, 95% CI 0.647-0.939), *CYP3A5* polymorphism (HR 1.139, 95% CI 0.649-2.001), number of HLA mismatch, donor type, and donor gender.

Conclusions: The IIV of tacrolimus trough levels had a significant impact on rejection-free survival independently of mean tacrolimus trough levels. Both *CYP3A5* polymorphism and environmental factors including compliance might contribute to the impact of tacrolimus IIV.

FR-PO2077

Low Dose of Thymoglobulin as Induction Therapy in High Risk Kidney Transplant Recipients

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Background: Acute rejection (AR) in high risk recipients is a concern in renal transplant setting. Information regarding Thymoglobulin (rATG) is scarce in low dose schemes. The aim of this study was to show AR incidence, adverse effects, and renal function after 6 months in a cohort (Jan-2010, Dec-2010), of high risk kidney recipients (KR).

Methods: KR were classified with high risk if they had second transplant, crossmatch or PRA ≥10% and deceased donor. FIfty five KR were prospectively analyzed according to total rATG dose (<4.5 vs 4.5-6mg/kg). The primary endpoint was the rate of acute rejection and graft failure. The secondary endpoints were serum creatinine, hematological adverse effects and infections.

Results: In group 1 the mean accumulated dose was 3.7 vs 5.5mg/kg in group 2. There were no patients with graft failure and there was no difference in AR(biopsy proved) frequency (p = 0.574) as shown in Table 1. Infection was significanttly more frequent in those with higher dose (71 vs 44%; p=0.044), compared to lower dose.

Six month Outcomes according to rATG total dose

Variables	All(n=55)	<4.5mg/kg(n=34)	4.5-6mg/kg(n=21)
Acute Rejection n(%)	6(11)	3(8.8)	3(14.2)
Infections n (%)	30(54.5)	15(44.1)	15(71.4)*
Urinary Tract Infection n(%)	22(40)	11(32.3)	11(52.3)
Sepsis n(%)	2(3.6)	1(2.9)	1(4.7)
CMV n(%)	1(1.8)	1(2.9)	0 (0)
Death n(%)	3(5.4)	1(2.9)	2(9.5)
Serum Creatinine(mg/dl)	1.13±0.45	1.17±0.53	1.04±0.22
Creatinine Clearence (ml/min)	77.90±19.13	75.56±19.16	81.84±18.92
Lymphocyte count(cels/ mm3)	1024±045	1005±079	1057±108
Platlet count(cels/mm3)	210,150±61,485	208,060±10,745	213,790±14,357
Hemoglobin(gr/dl)	13.6±1.1	13.4±0.4	13.8±0.4

* p value < 0.05.

Conclusions: Low dose of rATG was not associated to more AR or graft failure and it appears to be associated to lower infection frequency compared to those with higher dose in high risk kidney transplant receptors.This is one of the first reports of the use of rATG in Latin America.

FR-PO2078

Metabolic Syndrome Occurring during the First Year of Renal Transplantation Is Associated with Impaired Graft Function

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Background: Metabolic syndrome (MS) is a frequent observation in renal transplant recipients (RTR). The aim of this study was to analyze the influence of MS occurring at 3 and 12 months (M3, M12) after transplantation on the long-term renal graft function as well as on the patient and graft survival (PS, GS).

Methods: RTR having had received their transplant between January 1996 and December 2003 underwent prospective and sequential measurements of their renal graft glomerular filtration rate (mGFR) using isotopic methods. The RTR had to be at least 3 months post transplant and to have had at least two mGFR. A cohort of 322 out of 665 RTR were included after informed consent.

Clinical parameters (Body Weight, Height, Waist circumference, Blood Pressure), immunosuppressive and anti-hypertensive drugs, Tc*****-DTPA urinary clearance, proteinuria, serum creatinine, lipids and glycemia were measured at M3, M12, M24, M48, M60 and M96. The patients were followed until their death, graft loss or until December 2009 (Mean F-U: 3 + 2.8 yrs). MS was assessed according to three classifications: ATP III, WHO and IDF. PS and GS were analyzed using the Cox proportional hazard ratio and a linear mixed effect model for longitudinal repeated measures was used to assess the evolution of mGFR.

Results: Using ATP III, MS was present in 34 % and 37 % of RTR at M3 and M12 respectively; MS at M3 or M12 did not have an influence on PS, GS or death-censored GS. In contrast, MS at both M3 and/or M12 was significantly associated with an impaired graft function (ΔGFR: - 6.7 and - 7.8 ml/min/yr at M3 and M12 respectively with a p-value <0.001 for both). MS according to WHO or IDF had no significant influence on mGFR. In multivariate analysis, the deleterious effect of MS on graft function remained significant, in addition to transplant duration, dialysis duration, donor age, acute rejection and delayed graft function]

Conclusions: MS has a negative impact on long term graft function. Whether its correction would improve the renal outcome warrants further investigation.

FR-PO2079

The Impact of Pre-Transplant Dialysis Modality on the Outcome of Kidney Transplantation

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Background: Preemtive transplantation shows the best clinical outcome in kidney transplantation, but majority of the patients should be instituted a renal replacement because of the lack of donor. We hypothesized that the pre-transplant dialysis modality [pre-emptive, hemodialysis (HD), peritoneal dialysis (PD)] may affect the probability of transplantation as well as the clinical outcome.

Methods: We performed a retrospective cohort study of 577 patients who received kidney transplantation between January 1996 and December 2009 in Seoul National University Hospital (SNUH). The three groups were compared with the rates of graft and patients survival. The mean follow-up duration was 58.1 ± 48.4 months. And then we analyzed 975 patients who had maintenance dialysis (HD: 256, PD: 719) between January 1996 and December 2009 in SNUH for the rates of mortality and probability of kidney transplantation.

Results: Patients who had less than 1month of dialysis before kidney TPL (i.e. preemptive group) had survival benefit compared with non-preemptive group (P=0.010), but no difference in graft survival rate (P= 0.966). When non-preemptive group is subdivided into HD and PD groups, there were no significant difference in patient survival and graft survival rate (P=0.392, P=0.226, respectively). Patients with maintenance HD had high mortality rate than in patients with maintenance PD in our center (P=0.006). But the recipients on maintenance PD had higher probability for kidney transplantation in cohort between 2005 and 2009 (P=0.009), yet no difference in patient cohort between 1996 and 2004.

Conclusions: Based on the higher probability of kidney transplantation in patients on PD, we may recommend the patients to institute PD if the patients are suitable for PD and transplantation candidates.

Funding: Government Support - Non-U.S.

FR-PO2080

Hemoglobin Variability Is a Strong Predictor for Mortality in Renal Transplant Patients Alexander Kainz,[1,2] Julia Wilflingseder,[1,2] Rainer Oberbauer.[1,2,3] *[1]Department of Nephrology, Medical University of Vienna, Vienna, Austria; [2]Department of Nephrology, KH Elisabethinen, Linz, Austria; [3]Austrian Dialysis and Transplant Registry, Austria.*

Background: Anemia is a common problem after renal transplantation. Therefore patients are treated with erythropoietin stimulating agents. The varying response to treatment contributes to hemoglobin variability. It remains unclear however, whether hemoglobin variability is negatively associated with outcomes.

Methods: We conducted a retrospective cohort study of all first kidney allograft recipients between January 1990 and December 2008 represented in the Austrian Dialysis and Transplant Registry (OEDTR). We included 1441 patients of which 683 received erythropoietin stimulating agents at any time after transplantation. Analysis was conducted by Cox proportional hazard regression with cubic splines and linear estimates and the purposeful selection algorithm of covariables. The measure of variability was the moving standard deviation meaning it was computed for every possible four quarters of a year in a row.

Results: The hazard ratio (HR) of mortality and graft loss in the spline models increased with hemoglobin variability. In the linear model the slope for mortality was 2.35 (95 % confidence interval 1.75 – 3.17, p<0.001) and functional graft loss 2.45 (1.76 – 3.40, p<0.001). In the clinical expertise Cox model adjusted for ESA use, hemoglobin, age at transplantation, diabetes, days on dialysis, estimated glomerular filtration rate, biopsy confirmed acute rejection and year of transplantation hemoglobin variability was associated with mortality (HR: 2.11; 1.51 – 2.94; p<0.001). No association with functional graft loss could be detected (HR: 1.34; 0.93 – 1.93; p=0.121). The purposeful selection model was also significant when mortality was the outcome (HR: 2.63; 1.70 – 4.08; p<0.001) whereas a nonsignificant result was obtained for functional graft survival (HR: 1.27; 0.63 – 2.54; p=0.509).

Conclusions: These findings suggest that hemoglobin variability accounts for mortality in renal transplant patients.

Funding: Government Support - Non-U.S.

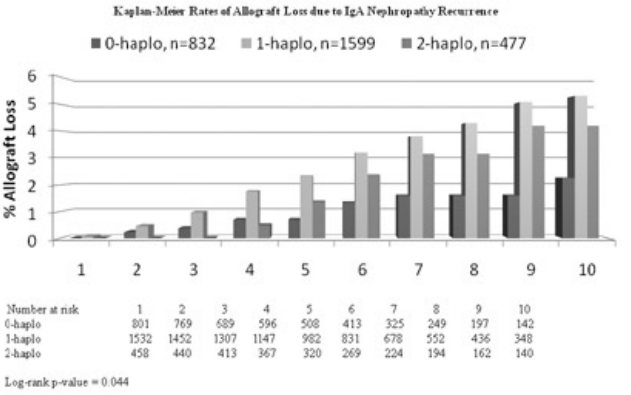
FR-PO2081

IgA Nephropathy Recurrence in Living Related Kidney Transplantation P.T. T. Pham,[1] P.C. T. Pham.[2] *[1]Kidney and Pancreas Transplant, David Geffen UCLA Medical Center, Los Angeles, CA; [2]Nephrology Division, UCLA-Olive View Medical Center, Sylmar, CA.*

Background: Current data suggest that living related kidney transplantation (LRKT) among recipients with the native kidney diagnosis of IgA nephropathy (IgAN) may be associated with an increased risk of histological recurrence. The current study aims to determine whether increasing haplotype match among LRKT affects the rate of actual allograft loss due to IgAN recurrence (IgANR).

Methods: Data were provided by the Organ Procurement and Transplantation Network, United Network for Organ Sharing (OPTN/UNOS). Primary LRKT performed between 1/1/1988-12/31/2007 (as of 10/29/10) with the native kidney diagnosis of IgAN for all patients alive and with functioning graft at discharge were included. The Kaplan Meier rates of allograft loss due to IgANR stratified by haplotype match were obtained.

Results: There were 832, 1599, 477 recipients with 0-, 1-, and 2-haplotype matched kidneys respectively, with mean age: 40.3 +/- 0.4, 37.1 +/- 0.4, 37.1 +/- 0.4 respectively. The Kaplan Meier rates of graft loss due to IgAN recurrence in primary LRKT recipients receiving any kind of immunosuppressive therapy were 2.3% (n=832), 5.3% (n=1599), 4.2% (n=477) for recipients with 0-, 1-, and 2-haplotype matches, respectively, log rank p-value 0.04, at 10-year follow-up.



Chi-square analyses did not reveal any significant differences among the different haplotype groups in terms of gender or ethnic backgrounds.

Conclusions: In accordance with previous reports of increased histological recurrence of IgAN among LRKT, OPTN/UNOS data suggest increased actual graft loss due to IgAN recurrence in living related renal transplant among 1- and 2-haplotype compared with zero match recipients. Potential contributory factors to this observation are not known.

FR-PO2082

Membranous Glomerulonephritis (GN) Is the Most Common Primary GN Associated with Acute Rejection after Transplantation Sanjeev R. Shah, Vijay Vidyasagar, Sumaira Talib Shaikh, Maha A. Mohamed, Brenda L. Muth, Glen E. Leverson, Jose R. Torrealba, Arjang Djamali. *Medicine and Surgery, UW-SMPH, Madison, WI.*

Background: It is unclear whether the type of primary glomerulonephritis predicts acute rejection after transplantation.

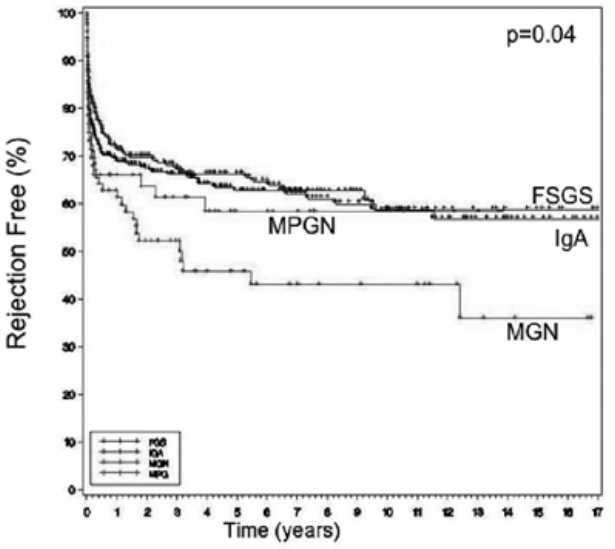
Methods: In a retrospective study of kidney transplant recipients with biopsy confirmed primary glomerulonephritis (n=598 from 1993 to 2009), we compared rejection rates between patients with Membranous GN (MGN), IgA nephropathy (IgA), focal segmental glomerulonephritis (FSGS) and membranoproliferative GN (MPGN).

Results: Baseline characteristics are displayed in Table 1

	MGN	IgA	FSGS	MPGN
N	76	226	239	57
Mean Age	47.1	41.5	47.1	42.9
Male Gender	55 (72.3)	160 (70.8)	137 (57.3)	28 (49.1)
Caucasian	68 (89.4)	201 (88.9)	188 (78.6)	47 (82.4)
Duration disease pre-transplant	11.4	8.5	10.2	14
Donor Age	39.1	40	40.6	39.2
Basiliximab Induction	24 (31.56)	101 (44.69)	94 (39.33)	24 (42.11)
DGF	9 (11.8)	17 (7.52)	47 (19.67)	6 (10.53)
Deceased donor	43 (56.5)	92 (40.7)	150 (64.4)	30 (52.4)
Previous Transplant	21 (27.6)	36 (15.9)	50 (20.9)	22 (38.6)
HLA MM > 2	48 (63.1)	147 (65.3)	161 (67.3)	35 (61.4)

The risk of rejection was 59% greater in patients with MGN compared to other GNs (HR 1.59, 95% CI 1.13 to 2.24, p=0.007). At 3 years, 50% of patients with MGN had an episode of acute rejection (p=0.04)

Figure 1. Rejection was highest in MGN



Univariate analyses including all the variables in Table 1 determined that delayed graft function (DGF) (HR 2.7, 95% CI 1.2 to 6.2, p=0.01) and basiliximab induction (HR 0.39, 95% CI 0.16 to 0.94, p=0.03) were predictors of acute rejection in patients with MGN. Mutivariate analyses retained these covariates as independent predictors of acute rejection.

Conclusions: MGN was the most common primary GN associated with acute rejection after transplantation. DGF and basiliximab induction were independent positive and negative predictors of acute rejection in these patients.

Funding: NIDDK Support

FR-PO2083

Outcomes of Transplantation in Primary Systemic Amyloidosis Horacio E. Adrogue, Jerry D. Estep, Rammurti Kamble, George Carrum, Lawrence Rice, Kelty Ruth Baker, Larhea Nichols, Catherine Frenette, Harish Seethamraju, Luan D. Truong, Lillian W. Gaber, A. Osama Gaber. *Methodist J.C. Walter Jr. Transplant Center at the Methodist Hospital, Houston, TX.*

Background: Primary systemic amyloidosis (AL) is a clonal plasma cell disorder that results in light chain deposition in organ tissues manifesting primarily as nephrotic syndrome, cardiac failure, and neuropathy. Treatment of AL is complex since organ failure may preclude intended treatment with an AHSCT. To address the challenges presented, we initiated an individualized, multi-disciplinary, and multi-specialty Amyloid Clinic at The Methodist Hospital, Houston, Texas.

Methods: Medical charts of patients (January 1998- 2011) with AL receiving solid organ transplants (SOT) and autologous hematopoietic stem cell transplant (AHSCT) were reviewed for patients, disease and transplant related characteristics.

Results: Our table depicts the outcomes of solid organ transplants (SOT) in 8 patients. Overall heart transplant pateint survival was 5/6 (83%) with a median follow-up of 20 months (range 4-90) from the date of SOT (DOT). A total of 18 patients received AHSCT. At median follow-up of 36 months (range 3-150), 15/18 (83%) patients are alive. Treatment related mortality with AHSCT was 5.5%. Mutliorgan transplants are rare for AL. They are possible but must be very carefully screened since they present unique challenges and a higher mortality.

Outcomes of SOT for AL at The Methodist J. C. Walter Jr. Transplant Center

Age/Sex	SOT	DOT	AL treatment	Date of AHSCT	Outcome	SO FU (months)
64/F	Heart	02/09	Len	03/10	Alive	28
62/F	Heart	07/09	Mel/Len	11/10	Alive	30
46/M	Heart/Kidney	01/11	Dex/Len	09/06	Alive	4
57/M	Kidney	09/07	Dex/Thal	06/06	Alive	44
45/M	Heart	06/10	Mel/Len	NA	Alive	12
72/M	Heart	12/04	Mel/Dex	NA	Alive	90
63/M	Heart	04/10	Dex/Thal	NA	Dead	6
63/F	Liver/Kidney	11/10	Dex/Velcade	NA	Dead	1

Conclusions: SOT/AHSCT should be considered an excellent treatment option for selected AL patients. Renewed hope exists especially for AL patients with heart failure. Since the median survival for an AL pateint with end stage heart failure is 6 months, our data show very favorable long-term outcomes for these patients.

FR-PO2084

Low Recurrence Rate of Primary Disease after Kidney Transplantation in Children Yuko Hamasaki,[1] Seiichirou Shishido,[3] Riku Hamada,[1] Tomoyuki Sakai,[1] Kenji Ishikura,[1] Hiroshi Hataya,[1] Hiroyuki Satoh,[2] Masataka Honda.[1] *[1]Nephrology, Tokyo Metropolitan Children's Medical Center, Fuchu, Tokyo, Japan; [2]Urology, Tokyo Metropolitan Children's Medical Center, Fuchu, Tokyo, Japan; [3]Pediatric Nephrology, Toho University Omori Medical Center, Ota-ku, Tokyo, Japan.*

Background: The incidence of acute rejection after kidney transplantion (KTx) has decreased owing to improvement of early immunosuppression but the importance of primary disease recurrence is becoming relatively high. We evaluated that the frequency of the recurrence of primary glomerular disease and outcomes after KTx in our hospital.

Methods: We analyzed that after June 1986, a total of 79 patients who received first KTx and they were treated with calcineurin-based immunosuppressive therapy. Twenty-nine were diagnosed as focal segmental gromeruloscrelosis (FSGS), 16 congenital nephrotic syndrome (CNS), 9 IgA nephropathy, each 6 rapid progressive glomerulonephritis (RPGN) and Henoch-Schonlein purpura nephritis, 5 Alport syndrome, 3 Lupus nephritis, each 2 membranoproliferative glomerulonephritis type1 and atypical hemolytic uremic syndrome, 1 membrenus nephropathy. The original diagnosis was biopsy-proven in every case. Survival curves were estimated by Kaplan-Meier method.

Results: The median age was 12.0 years at the KTx. The mean observation period was 8.5 years. Posttransplant recurrence of primary diseases was confirmed in 10 patients (12.6%): 8 FSGS, 1 RPGN, and 1 CNS. Among the 10 patients who had recurrence of primary disease, 3 lost their graft (FSGS, RPGN and CNS in each 1). Of the 7 patients with FSGS achieved remission by steroid pulse therapy. In contrast, 69 patients who did not have recurrence of primary disease, 8 lost their graft. Graft losses due to acute and choronic rejection were in 2 and 6, respectively. Probability of graft survival at 10-year follow-up was 84%.

Conclusions: Recurrence rate of primary glomerular disease after KTx is lower than the previous reports (30-50%) and graft survival rate is favorable may due to calcineurin-based immunosuppressive therapy.

FR-PO2085

Recurrent Membranous Nephropathy Post-Transplant: Is Rituximab an Option? Tripti Singh, Craig E. Gordon, Laurence H. Beck, Jean M. Francis. *Department of Medicine, Renal Section, Boston University Medical Center, Boston, MA.*

Background: Membranous Nephropathy (MN) is a leading cause of adult nephrotic syndrome. MN recurs in the transplant kidney with recurrence rates between 10 to 45%. Treatment of recurrent MN after transplant remains a challenge since all patients are already on immunosuppressive therapy. Rituximab (RTX), a monoclonal anti CD 20 antibody which targets B cells and decreases antibody production has emerged as a possible treatment option.

Methods: A literature search was performed using PUBMED from inception to Dec 2010. Studies reporting the use of RTX for treatment of recurrent MN after kidney transplant were reviewed and relevant data was extracted. Studies reporting use of RTX for treatment of native MN were excluded. Descriptive statistics were used to determine means (with 95% confidence intervals) for categorical and continuous variables and medians for non parametric variables. The primary outcome was proportion achieving complete remission (CR), defined in source studies as proteinuria<0.3gm/24hr with preserved renal function.

Results: Eight studies met the inclusion criterion. All patients had primary MN and mean time to recurrence was 29.5 months. Mean proteinuria and creatinine on diagnosis were 5.9 g/d and 1.6 mg/dl, respectively. All patients were treated with tight BP control using ACE-I/ARB. RTX was the primary immunomodulator used for treatment. RTX treatment resulted in CR in 72% of reported patients. Mean time to response was 2.6 months. Mean proteinuria and creatinine after treatment were 1.1 g/d and 1.5 mg/dl, respectively. Studies reporting peripheral B cells showed a decrease in the concentration from mean of 97/µl to 5/µl after treatment. Mean follow up after treatment with RTX was 22.6 months. The drug was well tolerated with no reported side effects in the follow up period.

Conclusions: RTX appears to be an effective treatment for recurrent MN with a high rate of response and low adverse event rate. However, this study was limited by both publication and selection bias. The limited toxicity profile of RTX as compared to alkylating agents makes it a preferred option for treatment in this patient population.

FR-PO2086

Limited Utility of Routine Electron Microscopy (EM) in the Diagnosis of Kidney Allograft Dysfunction Maytee Boonyapredee, Jack Moore. *Nephrology, Washington Hospital Center, Washington, DC.*

Background: Biopsy of the transplanted kidney serves a definitive role in elucidating the possible causes of allograft dysfunction. Of the histologic imaging modalities, EM is the most costly and labor-intensive. We have informally noted that EM tends not to modify the findings disclosed by light microscopy with direct immunofluorescence (DIF). We therefore chose to study whether EM results differ from or add to the LM results.

Methods: We compared the LM and EM reports of 65 renal transplant biopsies performed on 60 patients over 2 years. Different pathologists independently interpreted the biopsy specimens; a separate pathologist performed all the EM. We classified biopsy interpretations were into 15 possible diagnoses and categorically by glomerular (e.g. transplant glomerulopathy) versus nonglomerular (cellular rejection) disease. We analyzed agreement between LM and EM reports by kappa statistics and applied the McNemar test

to determine if EM interpretation yielded significantly more glomerular diagnoses on the same biopsy samples.

Results: The biopsies (N=65) represented a sample population with native kidney disease including diabetes (34%), hypertension (36%), FSGS (12%), SLE (5%), chronic glomerulonephritis (1%), and other (12%). There was very good agreement (Kappa= 0.94, 95% CI 0.88-1.00), between the EM- and LM-based interpretations. EM did not detect significantly more glomerular disease than LM/DIF alone (discordance rate 4.6%, 95% CI -1.92% to 4.62%, p=0.25). Furthermore, EM did not add to the diagnosis of rejection. EM described 3 more cases of transplant glomerulopathy than did LM/DIF but did not result in a change in management.

Conclusions: When EM is routinely performed for kidney transplant dysfunction, electron microcopy does not substantially add to LM/DIF evaluation.

FR-PO2087

Corticosteroid Withdrawal in Renal Transplant Recipients: One Year Analysis of the Mycophenolic Acid Observational Renal Transplant Registry V. Ram Peddi,[1] Kimi Ueda Stevenson,[1] Kevin M. McCague,[2] Anne Wiland.[2] *[1]California Pacific Medical Center; [2]Novartis.*

Background: Corticosteroid withdrawal (CSW) is desired by renal transplant recipients (RTRs) given the potential for reduction of CS adverse effects (AEs).

Methods: Using data from the Mycophenolic Acid Observational Renal Transplant (MORE) registry, a prospective study of RTRs receiving mycophenolate (MPA) either as enteric-coated mycophenolate sodium (EC-MPS) or mycophenolate mofetil (MMF) based on local clinical practice at 40 US sites, 12-month CSW (withdrawal of steroids by 3-months posttransplant)outcomes were analyzed. A total of 847 tacrolimus-treated RTRs (352 CSW, 495 CS) were included.

Results: Demographics were similar (mean age 52 yrs, 64% male, 25% AA). Nearly all RTRs received at least one induction agent (CS/CSW: 58.6/63.1% thymoglobulin; 4.7/22.7% alemtuzumab; 26.3/11.1% basiliximab; 9.9/0.9% daclizumab). Tacrolimus trough levels were similar. Biopsy-proven acute rejections (BPAR) were low (9.6% CS/7.4% CSW, p=0.20). Interim results at 1, 3, 6 and 12 months showed that significantly (p=<0.01) more of the CS patients were maintained on full dose MPA (CS/CSW: 83.0/68.5%; 73.1/52.6%; 58.4/37.6%; 51.3/36.1%). In the CS RTRs, a significantly higher % of EC-MPS-treated RTRs were maintained on full MPA doses compared to MMF-treated RTRs but this was not observed in the CSW RTRs. A difference in graft survival (99.4/97.4%, p=0.02) favoring the CSW group was observed. Patient survival was similar. The CS RTRs had a higher mean serum creatinine (CS 1.53/CSW 1.40 mg/dL, p=0.02) and there were no differences in reported infections (including CMV and BK), bone disease, cardiovascular events, diabetes or malignancies. There were more reported GI AEs in the MMF treated CSW RTRs (73.6 MMF/66.1% EC-MPS, p = 0.15). There were more hematological AEs in the CSW RTRs (61.4/28.5%, p=<0.01), mainly driven by leukopenia.

Conclusions: CS allowed for better tolerance of full dose MPA with no difference in BPAR. More RTRs in the CSW group received depleting antibody induction (87/61%). Graft survival was better in the CSW group at 1 year. CS AEs were similar between groups; longer follow-up is needed to assess long-term outcomes of CSW.

Funding: Pharmaceutical Company Support

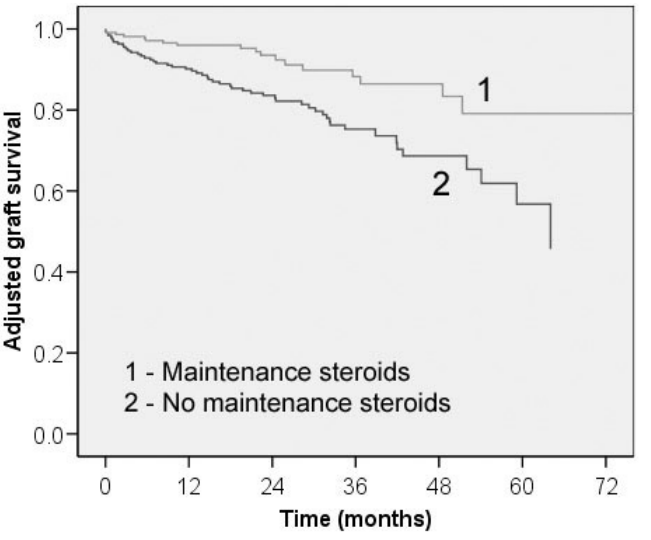
FR-PO2088

Which Repeat Kidney Transplant Recipient Benefits from Maintenance Steroids? Influence of Induction Agent Kalathil K. Sureshkumar, Sabiha M. Hussain, Richard J. Marcus. *Nephrology & Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: Steroid free maintenance immunosuppression is increasingly used following kidney transplantation and many patients will eventually receive a repeat transplant. It is not clear which re-transplant recipient would benefit the most from maintenance steroids. We aimed to look at the graft survival benefits of maintenance steroids in repeat deceased donor kidney transplant (DDKT) recipients with respect to the type of induction agent.

Methods: Using OPTN/UNOS database, we identified patients (≥18 years) who received a repeat DDKT from January 2000 to December 2008 following induction with rabbit antithymocyte globulin (r-ATG), alemtuzumab (ALE) or an IL-2 receptor blocker (IL-2B) and were discharged on a calcineurin inhibitor/MMF regimen with or without maintenance steroids. A multivariate analysis was performed by including confounding variables to evaluate the independent influence of maintenance steroids on graft survival in different induction groups.

Results: Median follow-up was 29.6 months (range 10.7-60.1 months). There were 3643 patients in the r-ATG (steroid = 3157, no steroid = 486), 448 in ALE (steroid= 196, no steroid = 252) and 1543 in IL-2B (steroid 1465, no steroid = 78) groups. In the unadjusted model, maintenance steroids failed to significantly improve graft survival in r-ATG (p=0.103), ALE (p=0.179) and IL-2B (p=0.23) groups. After adjusting for confounding variables, steroid use significantly improved graft survival in the ALE group (p=0.002) but neither in r-ATG (p=0.191) nor IL-2B (p=0.955) groups. Adjusted graft survival in ALE group with and without maintenance steroids is shown in the figure.



Conclusions: Our analysis shows a graft survival benefit for adding steroid to a calcineurin inhibitor/MMF regimen in repeat DDKT recipients who received ALE but not r-ATG or IL-2B induction.

FR-PO2089

Alloantibody Sensitization Does Not Impede Graft Survival in African American Living Donor Kidney Transplant Recipients Basit Javaid, Ahmadshah Mirkhel, Joseph Keith Melancon. *Georgetown Transplant Institute, Georgetown University Hospital, Washington, DC.*

Background: To study the effects of elevated panel reactive antibody (PRA) titers on renal allograft survival in African American (AA) living donor (LD) kidney transplant recipients.

Methods: All patients in the UNOS database were eligible. Patients were categorized as Highly Sensitized (HS) if PRA value was at or above 95th percentile of all PRA values in the cohort, or as Non-sensitized (NS) if PRA value was zero. Graft survival was compared in the HS and NS Caucasian (CC) and AA LD kidney transplant recipients.

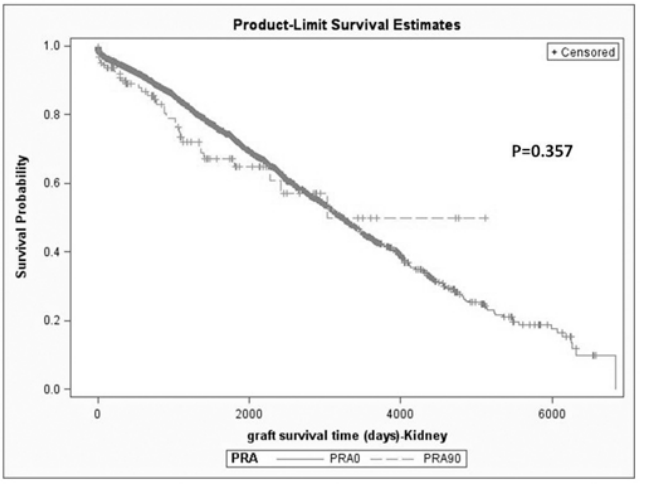
Results: From Oct 1987 to Feb 2009, 271,867 patients underwent a kidney transplant. Of these, 93,899 patients received a kidney from a LD, 64,923 of whom were CC and 13,141 were AA.

AA kidney transplant recipients had longer wait-list time (690.3±663.5 days vs. 434.6±489.2 days; p<0.001) and were less likely to receive a LD kidney transplant (OR=0.41; 95%CI=0.40-0.42; p<0.001), compared with CC recipients. PRA value at 95th percentile was 90.

HS (PRA>=90) CC LD kidney transplant recipients had a higher risk of graft loss compared with the NS CC LD transplant recipients (HR=1.59; 95%CI=1.28-1.97; P<0.001). This difference remained significant after adjustment for confounders (HR=1.50; 95%CI=1.21-1.87; p<0.001).

The risk of graft loss in HS AA LD kidney recipients was similar to NS AA patients who received a kidney from a LD (HR=1.18; 95%CI=0.83-1.66; p=0.358; Fig. 1). The risk difference was unchanged when adjusted for confounders (HR=1.15; 95%CI=0.80-1.64; p=0.394).

Figure 1: Kaplan-Meier Estimates for Post-Transplant Renal Allograft Survival in Highly Sensitized (PRA>=90) and Non-Sensitized (PRA=0) African American Living Donor Kidney Transplant Recipients.



Conclusions: Pre-sensitization did not impair kidney transplant survival in AA LD kidney recipients. Given longer wait-list time and lower odds of LD kidney transplantation, utilization of antibody desensitization techniques in such individuals could improve transplant rates, with expected graft survival comparable to NS recipients.

FR-PO2090

Effect of Immunosuppressive Treatment Withdrawal on Kidney Recipient Sensitization after Allograft Failure Gulnara Rackauskas,[1] Khazenay Bakhsh,[1] Siegmund Teichman,[1] Navin Jaipaul.[1,2] *[1]Nephrology, Loma Linda University Medical Center, Loma Linda, CA; [2]Nephrology, VA Loma Linda Healthcare System, Loma Linda, CA.*

Background: Sensitization to human leukocyte antigens (HLA) and the development of donor specific antibodies (DSA) create barriers to kidney transplantation. Kidney allograft recipients may become sensitized by defining events such as blood transfusion, pregnancy, and previous transplant. Another factor after allograft failure which may cause sensitization is withdrawal of immunosuppressive treatment (IST). However, universally accepted standards for IST after allograft failure do not exist and it is unclear whether withdrawal of IST in this context may independently sensitize patients.

Methods: We conducted a single center retrospective chart review of 242 adult renal allograft recipients with documented allograft failure between January 1, 2000 and January 1, 2010, to determine whether withdrawal of IST after allograft failure is an independent risk factor for sensitization. Sensitization was defined by the presence of Class I or Class II DSA, and/or a panel-reactive antibody level ≥80%.

Results: The 242 patients had the following characteristics: mean age 43.1 (+/- 16.4) years; 58.3% male; 43.0% Hispanic, 40.9% White, 8.3% Black race; 43% hypertensive or diabetic; 66.9% deceased-donor recipient; mean transplant duration 66.9 (+/- 48.9) months; and majority receiving IST with tacrolimus, mycophenolate mofetil, and prednisone. The effects of IST withdrawal after allograft failure and other potentially sensitizing events are shown in Table 1.

Table 1. Sensitization after various exposures

Exposure	Not sensitized	Sensitized	p-value
IST Withdrawal, n(%)	4(0.09)	8(1.0)	0.83
Blood Transfusion, n(%)	25(48.1)	55(56.1)	0.35
Pregnancy, n(%)	6(35.2)	33(66.0)	0.03
Previous Transplant, n(%)	4(0.08)	22(22.2)	0.03
Transplant Nephrectomy, n(%)	10(19.2)	28(30.1)	0.15

Note: Total n varies for each exposure

Conclusions: These findings suggest that withdrawal of IST after allograft failure is not an independent risk factor for sensitization. We believe that our results are valid due to the confirmed risk of other known sensitizing events such as pregnancy and previous transplant.

FR-PO2091

HLA Class II Antibodies and CD4 Peritubular Capillary Deposits in Pediatric Renal Transplantation: Influence on Estimated GFR and Graft Survival Marta Monteverde, Alicia Chaparro, Juan Ibañez, Cintia Marcos, Mario Diaz, Amalia Turconi, Ziomara Balbarrey. *Nephrology, Hosp JP Garraham, Bs As, Argentina.*

Background: C4d peritubular capillary deposits and donor specific HLA class II antibodies (abs) have been associated with vascular rejection, transplant dysfunction and failure. We studied the incidence of C4d deposits and HLA class II abs in kidney transplanted children and the influence of C4d deposits and HLA abs on graft survival and estimated GFR (eGFR).

Methods: We reviewed 78 children (43 males, mean age at RTX 9.9 ± 3.8 y) biopsied at 41.9 months after renal transplantation (RTX, r: 0.33-175.73) because of creeping creatinine n=75, and nephrotic proteinuria: n=3. Fifty two pats received a cadaveric graft; 74 were first RTX. Mean HLA mm: 3.16±098. All were on steroids and MMF/MPS with CYA (n=61), SRL (n=8) or TAC (n=9). Median follow-up after kidney biopsy was 9.16 m (r: 0.33-70.5).Classes II HLA abs were analyzed by Luminex and C4d by monoclonal antibody-immunofluorescence.

Results: Histological findings were: IFTA: n= 40(51%), ACR: n= 25 (32%), ATN: n=6 (8%), de novo membranous glomerulopathy: n= 2 (2.6 %), TX Glomerulopathy: n=(5.2%), TMA: n= 2(2.6%). Positive C4d: 40 pats (51.3%), focal in 10, diffuse 30. HLA class II abs were present in 27 pats (34.6.%). There was a significant association between HLA class II abs and diffuse c4d capillary deposits (p=0,000). The association of ACR with HLA class II abs and diffuse c4d deposits (p=0.000) was also statistically significant. The presence of Class I abs (n=4) was mainly seen during the first year (n=20), always associated to class II atbs All DQ positive pats (n=8) had positive DR. Thirty seven pats (47%) showed no adherence. Eight children lost their graft (10.2%). Graft survival after biopsy in pats without and with diffuse C4d was: 97% and 91% at 12m, 97% and 58% at 36m (p=0.026). HLA clas II abs (p=0.018), diffuse c4d (p=0.002) and ACR (p=0. 001) were associated with transplant failure; eGFR was lower in pats with HLA abs (mean: 79.0±5.9 vs.42.8±6.1;p= 0.0000).

Conclusions: HLA class II abs was associated with diffuse c4d+ peritubular capillary deposits, worst eGFR and graft survival.

FR-PO2092

Serum NGAL and Cystatin C: 2 Early Biomarkers Predicting Delayed Graft Function after Kidney Transplantation Marine Berguignat,[1] Ahmed Jeribi,[1] Fanny Boullenger,[1] Olivier Moranne,[2] Elisabeth Cassuto,[1] Laetitia Albano.[1] *[1]UMC Transplantation Rénale, CHU Nice, Nice, France; [2]Nephrology, CHU Nice, Nice, France.*

Background: Delayed Graft Function (DGF) occurs in 25% of kidney transplant recipients from deceased donors, and is predominantly caused by ischemia-reperfusion injury. While urinary neutrophil gelatinase-associated lipocalin (uNGAL) has been shown to be predictive of DGF, Cystatin C (Cyst C) has not yet been studied as a DGF biomarker. The aim of our work was to assess whether serum NGAL (sNGAL) and Cyst C could predict DGF.

Methods: We conducted a monocentric, prospective cohort study in our transplantation unit, collecting serial serum samples of deceased-donor kidney recipients to investigate these potential biomarkers. We analyzed serum levels of creatinine, sNGAL and Cyst C at day 1 post-transplantation in recipients with immediate graft function (IGF) compared to those with DGF. DGF was defined as the need for dialysis within the first 7 days after transplantation.

Results: Our cohort consisted of 54 recipients, including 13 with DGF. Medians (min-max) of serum creatinine (µmol/L), sNGAL (ng/mL) and Cyst C (mg/L) in IGF vs DGF were 597 (318-1269) vs 700 (354-1098) (p=ns), 307 (59-813) vs 514 (383-1010) (p =.0022), 2.89 (1.35-5.92) vs 3.93 (2.70-5.73) (p =.0004), respectively. ROC analysis for day 1 serum creatinine, sNGAL and Cyst C predicted DGF with an AUC of 0.69 (95%CI 0.53-0.85), 0.78 (95%CI 0.68-0.91), and 0.84 (95%CI 0.73-0.95) respectively. The cutoff levels predicting DGF were >378 ng/mL (Sp 100%-Se 69%) for sNGAL and >2.69 mg/L (Sp 90%-Se 59%) for Cyst C.

Conclusions: Cyst C and sNGAL measured at postoperative day 1 were found to be more reliable biomarkers than serum creatinine for predicting DGF. While sNGAL is less studied than uNGAL, it could be more relevant to cases of oligoanuria. However, we found Cyst C to be a more specific marker than sNGAL in our study. The major advantage of using Cyst C is that it is already measured by a standardized assay in routine labs. Further studies are needed to confirm our findings on Cyst C using larger transplant cohorts.

FR-PO2093

Creatinine Reduction Ratio on Day Two Is an Objective and Effective Tool To Stratify Delayed Graft Function Mahendra V. Govani,[#1] Alvin Wee,[#2] Jay H. Weiss.[#1] *[1]Medicine/Nephrology, St. Vincent Hospital, Indianapolis, IN; [2]Transplantation, St. Vincent Hospital, Indianapolis, IN.*

Background: Creatinine reduction ratio on day 2 (CRR2) < 30%, a simple, objective and well-defined criterion for diagnosis of DGF (instead of necessity for dialysis within a week of kidney transplantation), has not been used effectively to stratify DGF into mild to moderate DGF (MM-DGF) and severe DGF (S-DGF), each of which may have different prognosis.

Methods: We stratify all adult deceased donor kidney transplants performed at our center begining 1/1/2009 according to CRR2 pattern: Immediate graft function (IGF, CRR2 ≥ 30%), MM-DGF (10% ≥ CRR2 < 30%) or S-DGF (CRR2 < 10%). We study variables including demographics, cold ischemia time (CIT), SCD/ECD/DCD status, patient survival (PS), graft survival (GS), dialysis need with a week of transplantation (DGFRD), acute rejection rate (ARR), serum creatinine (SCR), and GFR. We present interim results of 57 transplants performed within the first 2 years and followed until 3/31/2011. Median follow-up was 12.1 ± 6.5 (range 4.2-27.1) months.

Results: Twenty-four of 57 (42%) patients had IGF, 13 (23%) had MM-DGF, and the remaining 20 (35%) had S-DGF. Four of 20 (20%) patients with S-DGF had DGFRD. No patients with MM-DGF had DGFRD. PS and GS were 100% at 3 months (57 pts), at 6 months (50 pts), and at 1 year (28 pts). SCR and GFR at follow-up were respectively higher and lower in MM-DGF (p>0.05) and S-DGF (p<0.05) patients compared to those of IGF patients.

Demographics, Risk Factors, and Outcomes

Variable	IGF (N=24)	MM-DGF (N=13)	S-DGF (N=20)
Age in yrs, mean±SD	57.6±12.7	50.3±15.1	57.2±10.7
Gender Male, n(%)	14(58)	9(69)	15(75)
Race AA, n(%)	9(38)	7(54)	7(35)
ECD/DCD, n(%)	8(33)	3(23)	14(70)*#
CIT in hrs, mean±SD	12.7±4.0	15.2±5.0	16.5±5.8*
Donor age in yrs, mean±SD	44.6±14.0	41.3±18.2	52.7±9.4*
ARR, n(%)	0	1(8%)	3(15)*
SCR mg/dl, mean±SD	1.3±0.4	1.7±0.6	2.1±1.3*
MDRD GFR ml/min, mean±SD	60.4±19.3	49.9±13.5	39.9±14.3*

p < 0.05 compared to IGF pts, # p < 0.05 compared to MM-DGF pts.

Conclusions: Our interim results show that CRR2 is an effective tool for DGF stratification, which may be useful in the management of patients and to compare studies objectively.

Funding: Clinical Revenue Support

FR-PO2094

Using Simulect Plus Low Dose Thymoglobulin in Renal Transplant Recipients with Poor Graft Function Ahmad M. Tuffaha, James B. Wetmore, Da Zhang, Connie J. Wang. *Nephrology and Pathology, University of Kansas Medical Center, Kansas City.*

Background: Simulect and thymoglobulin (rATG) are widely used induction agents in renal transplantation. In patients with poor postoperative graft function (PGF), whether using low-dose rATG by targeting CD3+ cell count, can delay calcineurin inhibitor (CNI) introduction has not been studied. We report our experience with an induction protocol using single-dose simulect plus a CD3+ count-based rATG regimen employed when the creatinine (Cr) reduction rate is < 30% over 24 hours (CRR24hr <30%).

Methods: The cohort included 84 consecutive patients who experienced PGF (CRR24hr <30%). Patients received a single dose simulect intra-operatively; rATG was initiated and titrated to keep CD3+ count < 30 cells/ml. CNI was introduced when Cr fell < 4mg/dL or < 50% of pre-transplant level. We examined the initiation, total dose and duration of rATG, resultant CD3+ count, the initiation of CNI; 1-year patient and graft survival.

Results: rATG was initiated by POD 2 in 77% (mean onset POD 2.0 ± 0.8). Mean cumulative dose was 5.1 ± 4.5mg/kg and duration of therapy was 8.5 ± 6.0 days (Fig 1A). Mean CD3+ count was 16.7 ± 17.0 cells/ml; rATG dosing permitted CD3+ counts to be maintained < 30 most of the days(Fig 1B) and in all but 7 patients(Fig 1C). CNI was introduced at POD 10.3 ± 6.2. One year patient and graft survival were 97.6% and 92.9%. Acute rejection occurred in only 3 (8.6%)recipients. Cytomegalovirus and BK virus infection occurred in 3 and 8(3.6% and 9.5%)recipients. No malignancies were diagnosed.

Figure 1A

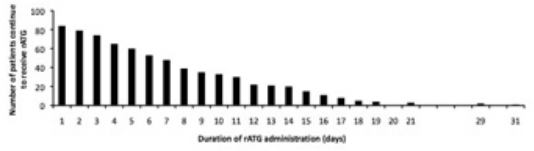


Figure 1B

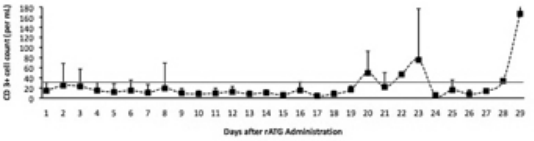
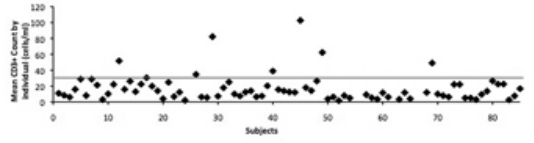


Figure 1C



Conclusions: Our findings suggest that in recipients with PGF, simulect plus a CD3 count-based rATG regimen, reliably leads to sufficiently-low CD3+ counts to prevent rejection. The rATG dose is lower than traditional exposure, yet permits delayed introduction of CNI. Excellent long-term graft outcomes are achieved without an increased risk.

FR-PO2095

Associations between MMP-2 Gene Polymorphisms and Post-Transplantational Diabetes Mellitus in Korean Renal Allograft Recipients Sunwoo Kang, Yang Wook Kim, Hyun Ju Kim, Tae Hee Kim. *Inje University, Republic of Korea.*

Background: Post-transplantational diabetes mellitus (PTDM) is a serious metabolic complication that may follow renal transplantation. Matrix metalloproteinase-2 (*MMP2*) function is indispensable for pancreatic beta islet formation and endocrine cell differentiation. Thus, specific *MMP2* gene polymorphisms are considered to be risk factors for diabetes. In this study, we investigated the association between *MMP2* gene polymorphisms and the occurrence of PTDM in Korean patients who had undergone renal transplants.

Methods: A total of 311 patients who had received kidney transplants without a prior history of diabetes were included. Four single nucleotide polymorphisms (SNPs) of the *MMP2* gene were genotyped from genomic DNA with direct sequencing.

Results: PTDM developed in 56 patients (18.0%). The results showed that the allele frequencies of *MMP2* gene polymorphisms rs1132896*C and rs243849*C were significantly higher in the patients with PTDM than in those without PTDM. In multiple logistic regression analysis, 2 SNPs (rs1132896 and rs243849) of the *MMP2* gene were significantly associated with the development of PTDM in the codominant and recessive or, codominant and dominant models, respectively.

Conclusions: Our results indicated that genetic polymorphisms of the *MMP2* gene were associated with PTDM, suggesting that the *MMP2* gene might confer susceptibility to PTDM in patients who receive renal transplants.

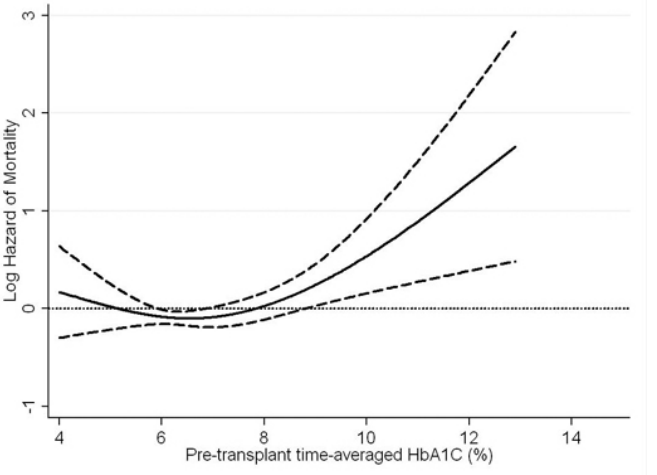
FR-PO2096

Association of Pre-Transplant Glycemic Control with Post-Transplant Outcomes in Diabetic Kidney Transplanted Recipients Miklos Z. Molnar,[1,2] Edmund Huang,[3] Junichi Hoshino,[4] Mahesh Krishnan,[5] Allen R. Nissenson,[5] Csaba P. Kovesdy,[6] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]UCLA School of Public Health, Los Angeles, CA; [5]DaVita, Inc, Denver, CO; [6]Salem VA Medical Center, Salem, VA.*

Background: Studies yielded inconsistent findings regarding the association of hemoglobin A1c (A1c) with survival in diabetic patients (pts) on dialysis. The association between pre-transplant glycemic control & post-transplant outcomes in kidney transplant recipients is not clear.

Methods: Linking the 5-year patient data of a large dialysis organization (DaVita) to the *Scientific Registry of Transplant Recipients*, we identified 2872 diabetic dialysis pts who underwent kidney transplantation. Mortality or graft failure & delayed graft function (DGF) risks were estimated by Cox regression (hazard ratio [HR]) and logistic regression, respectively.

Results: Pts were 53±11 years old and included 36% women and 24% African Americans. In our fully adjusted model, allograft failure censored all-cause death HRs and 95% confidence interval (95% CIs) for time-averaged pre-transplant A1c increments of 7-<8%, 8-<9%, 9-10% & ≥10%, compared to 6-<7% (reference), were 0.89(0.59-1.36), 2.06(1.31-3.24), 1.41(0.73-2.74) & 3.43(1.56-7.56), respectively; and graft failure censored cardiovascular death HRs were 0.38(0.13-1.05), 1.78(0.69-4.55), 1.59(0.44-5.76) & 4.28(0.85-21.64), respectively.



We did not find any difference in risk of death censored graft failure or DGF in different pre-transplant A1c levels.

Conclusions: Poor pre-transplant glycemic control (A1c≥ 9%) appears to be associated with decreased post-transplant survival in kidney transplant recipients although allograft outcomes do not appear to be affected.

Funding: NIDDK Support

FR-PO2097

The Incidence of New Onset Diabetes after Kidney Transplantation in Korea Joon Seok Oh, Joong Kyung Kim, Seong Min Kim. *Division of Nephrology, Internal Medicine, Bong Seng Hospital, Busan, Korea.*

Background: New onset diabetes after transplantation (NODAT) is a major metabolic complication in renal transplant recipients. It is associated with poor graft and patient survival. However, the incidence, risk factors and clinical relevance of NODAT vary among reports from single-center observational studies and clinical trials in Korea. So, we investigate the incidence and clinical correlations of NODAT in Korean renal transplant population.

Methods: We studied 967 patients receiving a living or deceased donor kidney transplant at 12 institutions between 1 January, 1999 and 31 December, 2007. Patients with graft failure or death within 1month post-transplant, multi-organ transplant recipients and patients who had a diagnosis of diabetes mellitus and severe metabolic disease prior to transplant (either as native kidney disease or co-morbidity) were excluded.

Results: The cumulative incidence of NODAT was 7.65%, 10.24%, 11.27%, 11.70%, 12.10%, 13.03% and 13.65% at 1, 3, 6, 12, 24, 36 and 48 months post-transplant, respectively. Using Cox's proportional hazards analysis, risk factors for NODAT included the use of tacrolimus as the initial maintenance immunosuppressive medication (hazard ratio 1.28, p = 0.03). Factors that reduced the risk for NODAT included living donor (0.69, p=0.00) and the use of cyclosporine (0.78, p=0.03).

Conclusions: We conclude that incidences of NODAT may be associated with the use of tacrolimus, cyclosporine and living donor. Efforts should be made to minimize the risk of this important complication.

FR-PO2098

New Onset Diabetes after Transplantation: Incidence and Risk Factors. Experience from a Single Centre Aikaterini K. Nikolopoulou, Thomas Alexander Vale, Catriona Hilton, Edward Sharples. *Oxford Kidney Unit, Headington, Oxford, United Kingdom.*

Background: New Onset Diabetes after Transplantation (NODAT) is associated with increased morbidity and mortality.

Methods: We performed a retrospective review of 199 kidney transplants performed at the Oxford Transplant Centre between 2007 and 2009. Immunosuppression was tacrolimus and mycophenolate with either basiliximab induction and tapered steroid withdrawal or steroid avoidance in association with alemtuzumab induction. NODAT was diagnosed in patients with more than 2 consecutive random plasma glucose concentrations >9 within the first twelve months post transplant, or required treatment for hyperglycaemia at any time during this period.

Results: The incidence of NODAT requiring oral hypoglycaemic agents was 6.32% (11 patients), with 3 recipients diagnosed late (>12 months). These recipients had an average age of 51 years (range 28-70) and average BMI 27.7 (range 24-30) at the time of transplantation. The majority of patients that developed NODAT (81.8%, n=9) received basiliximab at induction and were on steroids at 8 weeks post transplant (RR 1.94 , 95%CI 0.42-8.32). The rest (18.2%) received alemtuzumab induction with no adjunctive steroids. All patients were on Tacrolimus, average level 8.64ng/ml (range 7.6-11.5). Biopsy proven acute rejection episode was noted in 4 patients in the Basiliximab group and three of them received high dose methylprednisolone prior to developing NODAT. Graft survival was not affected [average eGFR at 12months was 44.5mls/min/1.73m2(range 23-61)].

Conclusions: Primary renal diagnosis did not predispose to NODAT. Elevated BMI at transplantation as expected was noted in the majority of patients developing NODAT.

Our findings suggest that NODAT is associated with use of high dose steroids in the context of a rejection episode, and patients with tapered steroid withdrawal were at higher risk than those in whom steroids were not used.

FR-PO2099

Innovator Versus Generic Mycophenolate Mofetil and Mycophenolate Sodium in Renal Allograft Recipients in India Basu Gopal,[1] Vellaichamy M. Annapandian,[1] Binu S. Mathew,[2] Kuppusamy Saravanakumar,[2] Denise H. Fleming,[2] George T. John,[3] Chakko Korula Jacob,[1] Veerasamy Tamilarasi.[1] *[1]Nephrology, Christian Medical College, Vellore, Tamil Nadu, India; [2]Clinical Pharmacology, Christian Medical College, Vellore, Tamil Nadu, India; [3]Renal Medicine, Royal Brisbane and Women's Hospital, Queensland, Australia.*

Background: KDIGO guidelines endorse use of low cost generics. However, risks and benefits of generic (G) vs. innovator (I) brands of mycophenolate acid (MPA) in renal allograft recipients is unclear.

Methods: Renal allograft recipients, who underwent transplantation at our center between January 2003 & June 2010, on innovator brands of Mycophenolate Mofetil / Sodium (MMF-I / MPS-I) or its generic equivalents (MMF-G1, MMF-G2 / MPS-G1) along with prednisolone and tacrolimus underwent extrapolated MPA-$AUC_{0\text{-}12h}$ measurements at specific intervals and adhoc, for MPA dose adjustment to achieve a target AUC of 30-60mg.h/L. The difference in the dose, AUC, dose controlled AUC (Dc-AUC = AUC/ Dose), number of tests, graft function, graft survival, rejections, infections, leucopenia, diarrhea and cost of MPA between the innovator and generic MMF/MPS users were analyzed.

Results: Of the 305 renal allograft recipients (mean age =36.6±11.8 yrs, M:F=3.1:1), 19.3,18.0,20.3,14.8 & 27.5% were on MMF-I,MPS-I,MMF-G1,MMF-G2 & MPS-G1 respectively. The AUC profiles of MMF-I, MMF-G1 & MMF-G2 were similar. MPS-G1 had a lower, delayed C_{max} compared to MPS-I only in the first 3 months (9.8 vs. 17.4ng/ml p=0.01). MPA Dose, AUC and Dc-AUC of I & G forms of MMF were similar, but MPS-G1 had a Dc-AUC lower than MPS-I. The number of AUC tests required during the first 6 months, graft survival, change in graft function, rejections, urinary tract infections, CMV disease, tuberculosis, systemic mycosis, leucopenia and diarrhea were not different between I & G forms of both MMF and MPS. However, the cost of G forms was significantly lower than MMF-I & MPS-I (by 16.0-20.8%; p<0.01).

Conclusions: Among renal allograft recipients in India, generic MMF exhibited comparable pharmacokinetics to innovator brand, but generic MPS did not. However, generic MPA use resulted in similar survival and post transplant events with lower cost of therapy.

FR-PO2100

Outcomes of Pediatric Kidney Transplantation in Korea over the Last Thirty Years Hee Gyung Kang,[1] Se Eun Lee,[1] Yo Han Ahn,[2] Seong Heon Kim,[1] Kyoung Hee Han,[1] Min Hyun Cho,[3] IL-Soo Ha,[1] Hae Il Cheong.[1] *[1]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Center for Pediatric Oncology, National Cancer Center, Goyang, Republic of Korea; [3]Department of Pediatrics, Kyungpook National University Hospital, Daegu, Republic of Korea.*

Background: The first pediatric kidney transplantation in Korea was performed in 1979 and today kidney transplantation is the most common modality of renal replacement therapy for Korean children with end stage renal disease. This study was done to evaluate the outcomes of Korean pediatric kidney transplantations and related factors.

Methods: The experiences of five major transplant centers in Korea were reviewed.

Results: Until 2010, 498 cases of kidney transplantation were recorded in 488 (M: F=311:177, living donor (LD): deceased donor (DD)=386:112) Korean children younger than 18 years (mean 11.7±4.0 years). Common primary diagnoses were focal segmental glomerulosclerosis (19.4%), reflux nephropathy (12.2%), chronic glomerulonephritis (8.2%), and aplasia/hypoplasia/dysplasia (5.3%). Since 2000, induction monoclonal antibodies were used in 69.4% of DD and in 28.6% of LD, and in > 75% of the cases the maintenance immunosuppressive medication regimen was mainly triple therapy of prednisolone, tacrolimus, and mycofenolate mofetil.

Acute rejection occurred in 40.4% of the cases and its significant risk factors were transplants before 2000, dialysis before transplantation, previous hemodialysis, and recurrence of the primary disease. Median graft survival was 14.9 years for grafts earlier than 2000 and undetermined for those since 2000, with 5 year graft survival of 89.7% for LD and 78.2% for DD. A history of acute rejection, recurrence of primary disease, and a recipient age younger than 6 years or older than 12 years were significant hazard factors for graft loss.

Conclusions: Overall, the outcomes of Korean pediatric kidney transplantations are satisfactory. In this homogenous North-Eastern Asian ethnicity population with high proportion of LD, the favorable effect of pre-emptive transplantation and deleterious effect of recurrence of disease on the outcome of pediatric kidney transplantation were confirmed.

FR-PO2101

Outcome of Renal Transplantation in a Developing Country, like Bangladesh Shahidul Islam. *Department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.*

Background: Renal transplantation is a best form of management in the selective ESRD patients. Outcome of renal transplantation has been steadily improving with the advance immunosuppression. Recently interleukin 2 (IL2) receptor antibody specially Basilaximab (Simuleet) use as induction therapy followed by maintenance dose with Tacrolimus, MMF & prednisolone in most of the transplant centre. Though the cost of induction therapy is enormously high but episode of acute rejection is negligible. Steroid withdrawal protocol and introduction of Sirolimus also introduce in some transplant centers of developed countries.

Methods: In our centre 214 ESRD Patients were transplanted from July 1995 to December 2005. All patients were live related donor & received triple immunosuppression (cyclosporine 6mg/kg, MMF 1000 mg & prednisolone 0.5mg/kg per day initially). After one year of transplantation, patients were received Azathioprine 2mg/kg & prednisolone 7.5 to 10 mg per day.

Results: Over all graft survival rate in 1st year 85%, 3rd year 75%, 5th year 65% & 10 year 50%.

Conclusions: In conclusions, out-come of Renal Transplantation in the developing country like our Bangladesh is good as compared to developed county. Through only live related transplantation has been existing now a days.

FR-PO2102

Living Donation – A District General Hospital Experience in the United Kingdom Andrew K. Coutinho, Girish S. Namagondlu, Emma D'amato, Brian Camilleri. *Renal Unit, The Ipswich Hospital NHS Trust, Ipswich, Suffolk, United Kingdom.*

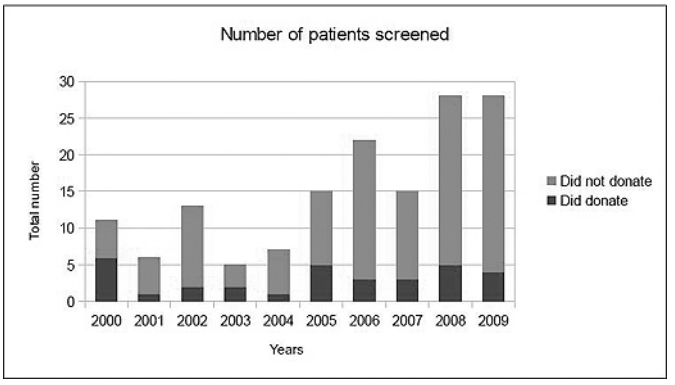
Background: In the United Kingdom the living donor is assessed using the British transplantation society guidelines. A committed time span is not kept for these evaluations. The contribution of a District General hospital (DGH) to donor evaluations are vital as currently many are performed in University teaching hospitals.

Methods: A retrospective study of all potential living donors for kidney transplantation was conducted at Ipswich Hospital from January 2000 to December 2009. Data was got from an electronic database and patient case notes. The Tissue typing (TT) report was taken as the starting point to calculate the duration of time from donor agreement to donation.

Results: 151 potential donors were identified and 32 donated. The ratio of females to males and unrelated to related is 2.02:1 and 1.16: 1 respectively among all the potential donors. Time elapsed at various stages is shown below.

Year	TT report to Nephrologist review in weeks	Nephrologist review to surgical referral in weeks	Surgical referral to donation in weeks	TT to donation in weeks
2000	4.6	21.6	18.5	44.7
2001	3	28	11	42
2002	6	12	11	29
2003	14	23	12	49
2004	6	35	30	81
2005	7	15.3	17.6	39.9
2006	3.5	25	12.5	41
2007	8	15.5	23	46.5
2008	1	18	13	32
2009	6	16.5	12	35
Mean	5.5 (38 days)12.3%	19.75 (138 days)44.8%	16.6 (116 days)37.6%	44 (308 days)

Waiting for appointments and final results before proceeding further accounted for the time elapsed. The number of potential donors evaluated increased over the years but did not affect the donation rate.



Conclusions: Time and resources in evaluation can be saved at the Transplanting centre if done at a DGH. Our study showed that increased donor evaluation does not necessarily contribute to increased number of donations. This study gives a scale of time involved and encourages DGHs to undertake more live donor assessments.

SA-PO2103

Urinary L-FABP and N-Acetyl-β-D-Glucosaminidase Predict AKI after Adult Cardiac Surgery Daisuke Katagiri,[1] Kent Doi,[1] Kenjiro Honda,[1] Kousuke Negishi,[1] Toshiro Fujita,[1] Motoyuki Hisagi,[1] Minoru Ono,[1] Takayasu Ohtake,[2] Shuzo Kobayashi,[2] Takeshi Sugaya,[3] Eisei Noiri.[1] *[1]University of Tokyo, Japan; [2]Shonan Kamakura General Hospital; [3]CMIC, Co, Ltd.*

Background: Urinary L-FABP, a new AKI biomarker, could detect pediatric post-cardiac surgery AKI (KI 2007). However, it is unclear whether urinary L-FABP shows a similar performance in more heterogeneous population of adult patients. Urinary N-acetyl-β-D-glucosaminidase (NAG) is a brush border enzyme and a more established renal injury marker. This study evaluated a biomarker panel consisting of these renal markers for adult post-cardiac surgery AKI.

Methods: 77 adult patients who had cardiac surgery were analyzed. Urinary L-FABP and NAG were measured before surgery, at ICU arrival after the surgery (0 h), 4 and 12 h after. AKI was diagnosed by the AKIN criteria.

Results: 28 patients (36.4%) developed AKI after surgery. Urinary L-FABP (0, 4, 12 h) and NAG (4 and 12 h) in AKI were significantly higher than non-AKI. The highest AUC-ROC values were observed with urinary L-FABP and NAG at 4 h (Table). Urinary L-FABP and NAG showed high sensitivity and high specificity, respectively. The combination can detect AKI with higher accuracy than each biomarker measurement [ROC-AUC 0.81 (0.68-0.90)].

Urinary L-FABP and NAG

		AKI (n=28)	non-AKI (n=49)	AUC-ROC (95%CI)	sensitivity (%)	specificity (%)
L-FABP (ng/ml)	pre	1.7 (0.7-6.8)	1.7 (0.9-4.5)	0.51 (0.37-0.65)	18.5	100.0
	0h	63.3 (27.4-246.9)*	43.1 (11.0-89.4)	0.65 (0.51-0.77)*	42.9	85.4
	4h	120.9 (53.8-274.7)*	49.6 (12.4-149.4)	0.72 (0.59-0.82)*	92.9	44.9
	12h	76.4 (28.6-176.6)*	20.1 (12.7-45.7)	0.76 (0.62-0.86)*	64.3	78.7
NAG (U/l)	pre	5.7 (2.4-12.6)	2.9 (1.9-5.6)	0.63 (0.49-0.76)	53.6	72.9
	0h	9.8 (3.9-23.9)	6.9 (4.0-10.6)	0.60 (0.45-0.73)	35.7	91.2
	4h	22.0 (6.7-30.6)*	6.0 (3.6-11.0)	0.75 (0.61-0.86)*	53.6	100.0
	12h	11.1 (7.2-21.3)*	6.5 (4.8-10.1)	0.67 (0.51-0.79)*	75.0	60.0

Data as median (IQR); *, p<0.05

Conclusions: Urinary L-FABP and NAG can detect AKI accurately in an adult post-cardiac surgery population, when these biomarkers are combined. Combining two markers with different features (sensitivity and specificity) will be a good strategy to improve diagnostic performance of AKI biomarkers.

Funding: Government Support - Non-U.S.

SA-PO2104

Calcitriol Levels in Acute Kidney Injury in the Critically Ill Anitha Vijayan, Adriana S. Dusso, Sanjay Jain, Daniel W. Coyne. *Renal Division, Washington University in St. Louis, St. Louis, MO.*

Background: Acute kidney injury (AKI) is a devastating complication in the critically ill patient and despite advances in renal replacement therapy (RRT), it is associated with hospital mortality of 50%. Low 1,25 OH Vitamin D (1,25VitD) levels are associated with increased mortality in CKD and given the lack of data regarding 1,25VitD levels in the setting of AKI, we conducted a pilot study to evaluate the relationship between 1,25VitD and mortality in AKI.

Methods: We analyzed serum from 34 critically ill patients who had AKI from acute tubular necrosis (ATN) and had a baseline GFR> 60ml/min. Vitamin D (VitD), 1,25VitD, intact PTH (iPTH), calcium (Ca) and phosphorus (P) were measured and compared to 12 healthy controls. Protocol was appoved by human research protection office and informed written consent was obtained. VitD, 1,25VitD and iPTH levels were quantified using radioimmunoassay (Immunodiagostic Systems). ANOVA was performed using Tukey's multiple comparisons test (Graph Pad InStat).

Results: The mean age of AKI patients was 51.9 yrs (controls - 45.3yrs) with 56% female and 91% caucasian.The mean serum creatinine (SCr) was 4.6mg/dL in the AKI group, with mean Ca of 8.1mg/dl±0.8 and P of 5.1mg/dl±1.9. The VitD and calcitriol levels in AKI (12.6ng/ml±1.059 and 41.6pg/ml±5.7 respectively), were significantly lower than controls (29.2ng/ml±2.7, p<0.01 and 76.1pg/ml±5.3, p<0.01, respectively), while iPTH levels were significantly higher (134.4 pg/ml ± 24.7) vs. (12.3 ± 3.7pg/ml, p <0.05). The in-hospital mortality for AKI was 30%. Univariate analysis showed that the 1,25VitD was significantly higher (62pg/ml±13.1) in non-survivors vs. survivors (32.7pg/ml±5.1, p=0.049). There was no difference in Ca levels between the 2 groups. Multiple regression analysis (Ca, P, Vitamin D, iPTH and 1,25VitD levels, age, sex, race, SCr) showed that higher 1,25VitD levels was significantly associated with increased mortality.(p=0.043, adjusted R^2 of 0.47).

Conclusions: In conclusion, increased mortality was inexplicably associated with higher 1,25VitD levels despite controlling for other variables. A large multicenter study is required to examine the effects of 1,25VitD on mortality in AKI.

Funding: Other NIH Support - George O'Brien Center

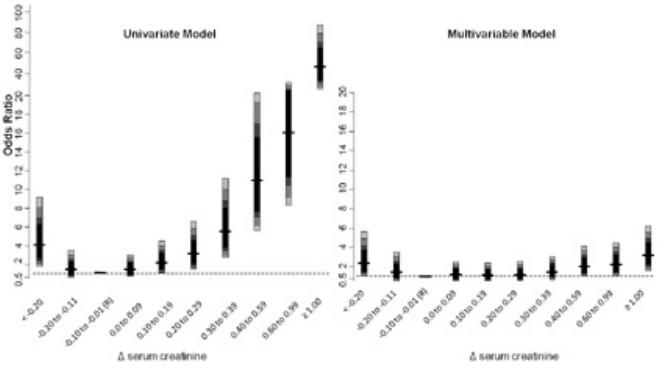
SA-PO2105

Minimal Changes of Serum Creatinine Do Not Predict Prognosis Following Cardiothoracic Surgery If Adjusted for Renal Complications Diana L. Deitzer,[1] David G. Anthony,[2] Jesse D. Schold,[3] Allen Bashour,[2] Sevag Demirjian.[1] *[1]Nephrology, Cleveland Clinic, Cleveland, OH; [2]Anesthesiology, Cleveland Clinic, Cleveland, OH; [3]Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH.*

Background: Severe acute kidney injury (AKI) has been associated with poor prognosis in a myriad of clinical settings; in recent years, even minimal changes in serum creatinine (sCr) have been implicated to carry long term prognostic value. We examined the relationship of sCr change in the post-operative period with in-hospital mortality.

Methods: Prospective cohort of 25,898 subjects who underwent cardiac surgery at Cleveland Clinic between April 2000 and January 2008. Change in sCr (ΔCr) was calculated using peak sCr within 2 weeks following surgery, and divided to 10 intervals (reference interval is ΔCr: -0.1 to -0.01). The multivariable model adjusted for nadir serum bicarbonate, serum sodium, peak serum potassium, and delayed extubation.

Results: Median age was 65 years; 67% were male, and 89% white. In univariate analysis ΔCr less than -0.2, and greater than 0.1 mg/dl were associated with increased in-hospital mortality. Whereas, in the multivariable model, only ΔCr of ≥0.6 mg/dl was associated with higher mortality; and the magnitude of the effect of changes were substantially reduced.



Conclusions: Consistent with prior studies, minimal changes in sCr following cardiothoracic surgery are associated with mortality. However, this association is largely explained by other renal parameters which may suggest a reduced independent impact of creatinine changes alone and reclassification of AKI risk based on combined indicators.

Funding: Clinical Revenue Support

SA-PO2106

Prediction of AKI Is Not Improved by Cystatin C in Critically Ill Patients – Preliminary Results of a Longitudinal Analysis Silvia Coelho,[1] Pedro Fidalgo,[1] Inês Barbosa,[2] Bruno Rodrigues,[1] Ana P. Fernandes,[2] Ana Luisa Papoila,[3] Karina Soto.[1] *[1]Nephrology, Hospital Fernando Fonseca, Lisbon, Portugal; [2]Intensive Care, Hospital Fernando Fonseca, Lisbon, Portugal; [3]Biostatistic, Faculdade de Ciências Médicas, Lisbon, Portugal.*

Background: The utility of Cystatin C (CysC) as AKI biomarker in critically ill patients remains unknown, especially in those with renal replacement therapy (RRT) requirements. The aim of this study was to evaluate the performance of serum CysC associated with severity indexes in a prediction model of AKI and RRT in ICU setting.

Methods: We prospectively studied a cohort of 128 consecutive ICU adult patients over 6m. AKI was defined according to RIFLE criteria. Baseline characteristics, sequential organ failure and severity indexes were record and creatinine and CysC were measured daily. Generalized linear models and linear mixed-effects models for the analysis were used.

Results: Patients, mean age of 57.1y (SD 16.8), 61.7% male, showed median SAPS II and APACHE II scores of 56.5 (CI 50-58) and 24.0 (CI 22-26), respectively. Crude in-hospital mortality was 31.3%. A total of 33.6% developed AKI (91% within 24 hours of admission) and 44.2% were treated with RRT. AKI patients were older (p=0.028), with more commorbidities (p=0.004), higher severity indexes (p<0.001). 76.7% had septic shock and 91% MOF compared to only 26.2% and 16.7% of non-AKI, respectively, p<0.001). Crude in-hospital mortality was 60.5 % in AKI vs. 16.5% in non-AKI (p<0.001).

AKI was associated with both SAPS II and APACHE II index: ROCAUC 0.94 (CI 0.90-0,98) and 0.92 (CI 0.87-0.97). When CysC was added to the model, the discriminative ability was 0.94 (CI 0.89-0.98); 0.93 (CI 0.88-0.97), respectively. When discriminative ability to RRT was analyzed, the performance of both indexes was poor ROCAUC 0.60 (0.43-0.76) and 0.46 (0.29-0.64): without improving by adding CysC to the model (ROCAUC 0.64; CI 0.47-0.80).

Conclusions: Patients with AKI were more severely ill and had a higher mortality. Severity scores showed a good performance for AKI prediction but not for RRT. Cystatin C did not improve discriminative ability. Future larger studies should be done to confirm these results.

SA-PO2107

The Identification of Renal Angina Improves AKI Prediction in Critically Ill Children Rajit K. Basu,[1] Lori Brunner,[1] Derek Wheeler,[1] Lakhmir S. Chawla,[2] Stuart Goldstein.[1] *[1]Center for Acute Care Nephrology, Cincinnati Children's Research Foundation, Cincinnati, OH; [2]Anesthesia and Critical Care, George Washington University Medical Center, Washington, DC.*

Background: Earlier detection of acute kidney injury (AKI) with novel biomarkers holds promise to expedite treatment and improve outcomes. Unfortunately, AKI lacks physical signs and symptoms to identify patients at-risk for AKI to trigger biomarker assessment. We recently proposed the empiric concept of Renal Angina (ANG), to "rule-in" or "rule-out" children at-risk for AKI in the pediatric intensive care unit (PICU). ANG stratifies known AKI risk factors (RF; e.g., intubation, stem cell transplantation) and pairs them with early clinical AKI signs (graded thresholds of small decreases in estimated creatinine clearance (eCCl) or fluid accumulation increase).

Methods: We performed a retrospective review of 135 children admitted to PICU (median age 4.3 [1.2, 12.6]) with a diagnosis of "shock" to determine if ANG on Day 0 or Day 1 improved the ability to predict severe AKI (>50% eCCl decrease; pRIFLE-I or F) on Day 3 or Day 4 over RF alone.

Results: ANG on Day 0 yielded better PPV and NPV for AKI presence on Day 3 than RF alone; Day 1 ANG precision improved further for Day 4 AKI prediction (Table). We observed similar ANG-associated improved prediction for CRRT initiation. ANG was also associated with higher mortality. In summary, we found 1) Lack of ANG was associated with a very low likelihood of AKI development in the next 72 hours, 2) RF alone were poorly predictive of AKI development, and 3) ANG presence was fair at predicting AKI development.

	Day 0		Day 1	
	RF	ANG	RF	ANG
n (%)	95 (70.4)	40 (29.6)	91 (67.4)	44 (32.6)
Age*	2.9 [0.8,10.3]	8.3 [2.3,14.3]	2.9 [0.9,11.3]	7.8 [2.1,14.6]
Male (%)	53 (55.8)	23 (57.5)	56 (61.5)	20 (45.4)
Ventilated	43 (45.3)	17 (42.5)	52 (57.1)	24 (54.5)
Inotropy	31 (32.6)	14 (35)	43 (47.2)	23 (52.3)
Mortality*	5 (5.3)	7 (17.5)	4 (4.4)	8 (18.2)
PPV/NPV to predict pRIFLE-I/F 3 days later	0.14/0.53	0.47/0.86	0.08/0.43	0.57/0.92

* $p < 0.05$

Conclusions: In summary, we suggest fulfillment of renal angina criteria identifies patients for whom biomarker testing would have the highest yield, improving the efficiency of biomarkers to predict severe AKI.

Funding: Other NIH Support - UL1-RR026314-01 NCRR/NIH

SA-PO2108

Role of Statins in Prevention of Contrast Induced Nephropathy Majed Samarneh, Norbert Shtaynberg, Suzanne E. El Sayegh, Morton J. Kleiner. *Nephrology/Cardiology, Staten Island University Hospital, Staten Island, NY.*

Background: Contrast-induced nephropathy (CIN) remains one of the most important clinical complications associated with the intravascular administration of radio-contrast media. A potential role for statins in CIN prevention is suggested by findings in animal models where statins prevent ischemic nephropathy by stabilizing the endothelium and acting as free radical scavengers.

Methods: We carried out a retrospective chart review at our institution to evaluate a possible association between statin pre- treatment and prevention of contrast induced nephropathy. At our institution, 282 charts of patients who underwent a cardiac catheterization or a CT scan with an intravenous contrast were reviewed. Patients presenting with acute renal failure or who have end stage renal disease were excluded. We defined Contrast-induced nephropathy as an increase in serum creatinine by ≥ 0.5 mg/dL or an increase of 25% of the baseline within 72 h following the procedure

Results: Subjects who had CIN (contrast-induced nephropathy) were significantly older (69.6 ± 11.9 vs. 63.2±14.7; p=.0006) and had significantly higher baseline creatinine levels (1.6±0.84 vs. 1.14±0.98; p<.0001). Subjects aged ≥ 65 were twice more likely to develop CIN compared to subjects aged <65 yrs [95% CI: (1.2, 3.2)]. Subjects with baseline creatinine ≥1.5 were 4 times more likely to develop CIN compared to subjects with baseline creatinine<1.5 [95% CI: (2.3, 7.3)] Subjects with HTN were 4.6 times more likely to develop CIN compared to subjects who did not have HTN yrs [95% CI: (2.4, 8.6). Subjects with all three risk factors, including hypertension, diabetes mellitus, and age>65 were 11.6 times more likely to develop post-CIN.

Conclusions: Statin therapy was not effective in prevention of CIN in our study. However, these subjects who were on statins had higher prevalence of risk factors such as hypertension, age >65, and a higher baseline creatinine which may have contributed to the higher incidence on CIN. A larger randomized sample size may elucidate the beneficial effects of statins in prevention of CIN.

SA-PO2109

Safety and Efficacy of Citrate Anticoagulation in Septic Shock Patients Treated with Coupled Plasma Filtration Adsorption (CPFA) Marco Pozzato, Fiorenza Ferrari, Pasqualina Cecere, Paola Mesiano, Antonella Vallero, Sergio Livigni, Francesco Quarello. *Nephrology & Dialysis Unit and ICU, S. Giovanni Bosco Hospital, Turin, Italy.*

Background: From 2001 to 2010 we treated 87 septic shock pts with CPFA, an extracorporeal therapy that combines unselective plasma adsorption resins (MediaSorb) with continuous hemofiltration. CPFA proved to be an effective treatment of septic shock with or without acute kidney injury (AKI), improving hemodynamics, amine reduction and potentially survival.

Study aim was to evaluate a citrate anticoagulation in CPFA to improve the treatment efficiency and simplifying its management. We evaluate the treatment duration, coagulative parameters, bicarbonate and ionized plasma calcium.

Methods: Seven (3 M/4 F) mechanical ventilated with septic shock and multiorgan failure(2/7 had AKI) pts were treated. Prescribed CPFA parameters were: Qb 150 ml/min, plasma flow rate (Qp)30 ml/min, predilution solution (Na+ 136, citrate 10, citric acid 2 in mmol/) infused to keep inlet citratemia at 3mmol/L, postdilution solution (Na+ 139, K+ 1.5, Ca++ 2, 0.75, HCO3- 35, glucose 5.55 in mmol/l) and postdilution CaCl at a rate restoring the plasma Ca++ to 1.1 mmol/l (tab.1) and adjusted according to the pts' need.

Results: We performed 50 treatments accounting for 432 hrs, mean duration 8.5±1.6 hrs, mean plasma volume of 10.8±2.8 l, Qb 142±15 ml/min, Qp 23±2.4 ml/min, a treated plasma dose/kg body weight of 0.88±0.34 l/kg. Mean $CaCl_{10\%}$ infusion of 4.6±1.5 ml/h, with a citratemia, evaluated as total Ca++/iCa++ ratio, always <2.5 (1.96±0.07), also in 3 patients with liver dysfunction (mean 2.06±0.20. Plasma HCO3- was 26±4.8 mmol/l, pH 7.43±0.05, ATIII (median 84%), PTT (49±12 sec) and Ca2+ 1.1±0.1 mmol/l.

Conclusions: Our protocol allowed a high plasma dose, with a safe coagulative status, acid-base balance and calcemia correction. Survival rates at 28 and 90 days were 85.7 and 71.5%, respectively. Citrate CPFA seems a viable and safe treatment for the septic shock pts.

Citrate infusion - Calcium Chloride

Kg	Predilution ml/h	Postdilution ml/h	CaCl2 ml/h
50	2250	100	4
55	2250	150	4
60	2250	200	4
65	2250	250	3
70	2250	300	3
75	2250	350	3
80	2250	400	3
85	2250	450	3
90	2250	500	2
95	2250	550	2
100	2250	600	2

SA-PO2110

Abstract Withdrawn

SA-PO2111

Electronic Alerts To Prevent Contrast-Induced Nephropathy among Hospitalized Patients Ji Hyeon Park, Ajin Cho, Seung Tae Han, Jaeyoung Yoon, Hye Ryoun Jang, Jung Eun Lee, Wooseong Huh, Dae Joong Kim, Yoon-Goo Kim. *Department of Medicine, Samsung Medical Center, Seoul, Korea.*

Background: Contrast-induced nephropathy (CIN) is a common cause of hospital acquired kidney injury. However, CIN preventive strategy using extracellular volume expansion remains still underused. We evaluated the institution of alert program can increase the use of standard prevention for CIN and reduce the incidence of CIN in hospitalized patients with chronic kidney disease (CKD) undergoing computed tomography (CT).

Methods: We developed a computer program in which physicians were alerted to a patient's risk of CIN when they ordered contrast enhanced CT in patients with CKD (defined as estimated GFR <60 mL/min per 1.73 m^2). The physicians were required to acknowledge the alert and order preventive strategy, including hydration with 0.9% saline or sodium bicarbonate, N-acetylcysteine administration, and follow-up serum creatinine level within 24-72 hours. This electronic alert program was applied to all hospitalized patients from March, 2010. We identified 618 hospitalized patients with pre-end stage renal disease who underwent contrast-enhanced CT. CIN was defined as an increase in the serum creatinine level after contrast administration of ≥ 25% or ≥ 0.5mg/dl from the baseline level.

Results: 463 patients were eligible in the study: 258 patients in the pre-alert program group and 205 patients in the post-alert program group. The two study groups were well balanced with respect to baseline characteristics. The post-alert program group were considerably more likely to receive pre- and post-hydration and NAC (55% vs 25%, P<0.001). CIN occurred in 3 patients in the post-alert program group (2%), as compared with 12 patients in the pre-alert program group (8%, P=0.029). Logistic regression models revealed computer electronic alerts reduced the incidence of CIN (Odds ratio 0.26, 95% confidence interval 0.07 to 0.95, P=0.041).

Conclusions: Our results suggest that hospitals with adequate information-systems resources should consider implementing electronic alerts to increase physicians' awareness of the risk of CIN, to increase the use of prophylaxis, and to reduce the rates of CIN.

SA-PO2112

Survival and Mortality Risk Factors in Mexican Patients with Acute Kidney Injury Miguel De Jesus Beltran-Perez,[1] Luis Alberto Evangelista-Carrillo,[1] Salvador Mendoza Cabrera,[1] Jorge Andrade-Sierra,[1] Enrique Rojas-Campos,[2] Miguel Medina Perez,[1] Basilio Jalomo Martínez,[1] Benjamin Gomez-Navarro.[1] *[1]Department of Nephrology, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [2]Medical Resarch Unit in Renal Diseases, CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

Background: Acute Kidney Injury (AKI) information is scarce in Latin American ICU and non ICU patients.

Aim: To determine patient survival, mortality risk factors and treatment in AKI patients from a hospital of the West of Mexico.

Methods: Prospective cohort (Jan-May2011) of 79 patients with AKI (AKIN classification), diagnosed and treated by Nephrologists, were recorded at admission, at AKI diagnosis and daily for 1 month: age, gender, time between AKI onset and Nephrology diagnosis, fluid balance, SOFA, APACHE II, ISI, treatment (IHD, CCRT, conservative), date of death or patient discharge and other clinical and biochemical variables.

Results: Mean age was 52±18 years, 61% were male, 48% were from ICU, 50% had surgery, 25% had sepsis; 59% had AKIN 3, mean time between AKI onset and Nephrology consultation was 59±48 hours, 56% received conservative treatment, 28% IHD and 16% CCRT; mean hospitalization was 15±9 days; Mortality was 51% (according to treatment was 46% conservative, 41% IHD and 92% CCRT) Results are shown in Table. Mortality predictors at day of diagnosis were: Δ SCr, Uresis volume and diuretic use (χ2=11.4; p=0.01); and predictors 24-Hrs after were: Diuretic use and SOFA score (χ2=7.1; p=0.03).

Comparisons according to hospitalization site and mortality

	ICU (n=30)	General ward(n=49)
Fluid balance(Lt)	6.7 (3.3-11.3)	3.1 (0.8-6.4)*
SCr Δ	1.8±1.5	3.6±3.1*
Mortality n(%)	20(67)	20(42)*
	Alive (n=39)	Dead (n=40)
Fluid balance(Lt)	2.08 (-0.38-5.4)	6.8 (3.3-11.4)*
SCr Δ	3.7±3.2	2±1.8*
SOFA (pts)	10±3	13±3*
Diuretic use N (%)	21 (70)	19 (40)*
ISI (pts)	0.37±0.2	0.57±0.3*

* p< 0.05

Conclusions: Mortality was similar to other studies, was high in general ward (42%) and was significantly predicted at diagnosis by small changes in serum creatinine. At 24 hours evaluation, SOFA and conservative treatment significantly also predict mortality.

SA-PO2113

A Real Time Electronic Alert System To Identify and Stage Acute Kidney Injury in a Large Acute NHS Trust Linda H. Bisset,[1] Christine Porter,[1] Irene Juurlink,[1] Mark A.J. Devonald.[1,2] *[1]Nottingham Renal and Transplant Unit, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [2]School of Clinical Sciences, University of Nottingham, United Kingdom.*

Background: Acute Kidney Injury (AKI) is associated with significant morbidity and mortality. The 2009 NCEPOD report highlighted deficiencies in diagnosis and management of AKI in the U.K. To improve detection of AKI, we developed an automated alert system which identifies and stages AKI in our large NHS hospital.

Methods: Algorithms compare admission serum creatinines (SCr) with actual baseline SCr (lowest SCr 7 days to 1 year before admission). Where no actual baseline exists, SCr is calculated assuming eGFR 75 mL/min. Where RIFLE or AKIN criteria are fulfilled, an alert is displayed on the computer screen. All subsequent SCr results are compared with SCr from previous 48 h (AKIN) and previous 7 days (RIFLE); if these differ, the higher stage is reported. Staging is thus continually updated. Sensitivity is increased by use of both AKIN and RIFLE (retrospective analysis of AKI incidence at our hospital revealed that AKIN detects 60% more episodes of stage 1 than RIFLE but RIFLE detects 30% more stage 3 than AKIN). For each patient admission, the worst AKI stage is allocated using a hierarchy: A3, R3, A2, R2, A1, R1. This gives an idea of the numbers at each stage that would be missed if only one staging system were used. Clinical coding is used to exclude patients with known end stage renal disease.

Results: Sample data from Oct-Dec 2010 are reported. A total number of 7699 alerts were generated, representing 3518 patient admissions. The worst overall AKI stage for each admission is shown (table 1). Mean age 67 y, 48% male.

Worst AKIN (A) or RIFLE (R) stage alerted during admission							
	A3	R3	A2	R2	A1	R1	Total
n=	302	71	399	178	2325	243	3518

Conclusions: Our AKI alert system is fully automated, real time and continuously revises staging. Using actual baseline SCr increases specificity. Combining AKIN and RIFLE systems maximizes sensitivity and specificity. Prospective data are collected automatically, facilitating audit and research.

SA-PO2114

The Impact of Early Initiation of Continuous Renal Replacement Therapy on Outcomes of Critically Ill Patients with Acute Kidney Injury Hideo Yasuda,[1] Akihiko Kato,[2] Yukitoshi Sakao,[1] Naro Ohashi.[1] *[1]First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Division of Blood Purification, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

Background: Acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT) is a potent risk factor for mortality in critically ill patients. Accumulating evidences suggested that later initiation of CRRT might be associated with worse outcomes in AKI patients. However, it remains to be fully determined as to the appropriate timing to start CRRT therapy in intensive care unit (ICU) patients. The aim of the present study is to clarify the impact of duration from the diagnosis of AKI to CRRT initiation on fluid overload and total mortality in ICU patients.

Methods: We retrospectively collected the data of 67 ICU patients (age: 66.9 ± 19.3, male/female = 47/20) suffering from AKI requiring CRRT in our hospital from 2006 to 2010. We divided all patients into the two groups according to durations until the start of CRRT; the early group (n = 30) that conducted CRRT treatment within 24 hr after the diagnosis of AKI, and the late group (n = 37) that did 24 hr later. We compared clinical parameters at the start of CRRT and prognosis after CRRT therapy between the two groups.

Results: There was a significant difference in serum urea nitrogen (51.5 ± 30.8 vs. 70.1 ± 35.7 mg/dl, $p < 0.05$) and platelet counts (158 ± 111 x 10^3 vs. 98 ± 69 x 10^3 /μl, $p < 0.05$) . The late group disclosed a higher rate of total mortality at 30-day (33.3 vs. 73.0%), 60-day (43.3 vs. 75.7%), and in-hospital mortality (43.3 vs. 78.4%). No significant difference was found in fluid balance from admission of ICU to initiation of CRRT (1124 ± 2414 vs 2313 ± 4392 ml) and central venous pressure (8.2 ± 5.7 vs 8.8 ± 5.6 cm) between the both groups.

Conclusions: It follows from these observations that early initiation of CRRT within 24 hr may provide a better prognosis independently of fluid balance in ICU patients with AKI.

SA-PO2115

Acute Kidney Injury after Off-Pump Cardiac Surgery Stratified by Stages of Pre-Existing Renal Disease Rachita Sethi Reddy, Michael F. Michelis, Nirav C. Patel, Maria V. DeVita. *Lenox Hill Hospital, New York, NY.*

Background: Acute Kidney Injury (AKI) is common among hospitalized individuals, particularly those undergoing cardiac surgery. Studies have shown ≥20% drop in glomerular filtration rate, prolonged hospital stay, progression to end stage renal disease (ESRD), and increased mortality following cardiac surgery. However, many were unable to adjust for baseline kidney function or specifically assess off-pump cardiac surgery.

Our study investigates the association between preoperative stages of chronic kidney disease (CKD) and AKI after off-pump cardiac surgery, defined as 25% increase in serum creatinine (SCr). We also assessed significant predictors for AKI and mortality.

Methods: Using the cardiac surgery database from Lenox Hill Hospital, we performed a single-center, retrospective analysis on all off-pump cardiac surgery patients from June 2009 to January 2011. Extracted data included: age, sex, race, preoperative SCr, postoperative peak SCr, comorbidities [diabetes, dyslipidemia, hypertension (HTN), peripheral vascular disease (PVD), cardiovascular disease (CVD)], urgent versus elective surgery, complications during postoperative period, use of ACEI/ARBs, mortality. Exclusion criteria included on-pump cardiac surgery, preoperative dialysis (ESRD or prior 60 days), intraoperative death, missing data points.

Results: Of the 441 patients that met inclusion criteria, 23.8% had AKI. Incidence of AKI by CKD stages 1-4 was 32.7%, 17.6%, 20.7%, 28.6%, respectively. There were no stage 5 CKD patients. Chi-squared analyses compared risk factors between patients with and without AKI. Patients with AKI were more likely to be diabetic (p=0.038) and have postoperative complications (p=0.014), mainly atrial fibrillation and prolonged ventilation. Mortality rates were six times higher when compared to those without kidney injury (p=0.08). Age, sex, dyslipidemia, HTN, PVD, CVD, urgent versus elective surgery, and use of ACEI/ARBs showed no significant difference in predicting risk of kidney injury.

Conclusions: Patients with AKI after off-pump cardiac surgery are more likely to be diabetic and have postoperative complications. They also have higher rates of mortality during hospitalization.

SA-PO2116

A Simple Method To Classify Acute Kidney Injury (AKI) in Children Alexandre Braga Liborio,[1] Ticiana Andrade Castelo Branco Diniz,[1] Natalia Albuquerque Rocha,[2] Elizabeth De Francesco Daher.[2] *[1]UNIFOR; [2]UFC.*

Background: A RIFLE criteria adaptation has been proposed to classify AKI in children (pRIFLE). However, pRIFLEcr requires eGFR and consequently patients' height. Besides, eGFR is not routinely performed in daily practice, complicating promptly AKI-diagnosis.

Methods: Through mathematical calculation using Schwartz equation(GFR (ml/min/1.73m2) =0.413(height)/Scr, with height in cm) and considering the decline in eGFR as: (baseline eGFR – lowest eGFR/baseline eGFR)x100, we defined the equation:(0.413(height)/Scr1 – 0.413 (Height)/Scr2 / 0.413 (height)/Scr1) x100

Where,Scr1:Scr at baseline,Scr2:Scr at end-point.

Cutoff values of ΔSCr to maintain same performance of eGFR decline were determined for each RIFLE stage. It was tested in 260 hospitalized children in a general pediatric hospital. In patients with no baseline Cr, it was estimated considering normal GFR(120ml/min/1.73m2).

Results: Using mathematical formulas, values required for classification as RIFLE R(1.3xbaseline SCr);I(2.0xbaseline SCr) and F(4.0xbaseline SCr). Patients used to validate this classification were aged 30 days to 12 years and the main diseases were Kala-Azar(56%),pneumonia(14%) and dehydration(8%). Agreement was 100% between pRIFLEcr classified by reduction in eGFR and by increment in sCR.

Distribution of pRIFLE according to eGFR and ΔScr

	No AKI	Rise to 1,3xbaseline Scr	Rise to 2,0-3,9xbaseline Scr	Rise >4xbaseline Scr
No AKI	161	-	-	-
RIFLE R(eGFR decreased by 25%)	-	52	-	-
RIFLE I(eGFR decreased by 50%)	-	-	38	-
RIFLE F(eGFR decreased by 75%)	-	-	-	9

When the GFR reduced to less than 30ml/min/1.73m2, all patients had an increment of at least four times in Scr. The described increments in Scr are different from those suggested by AKIN and by RIFLE for adult patient.

Conclusions: Mathematically, sCr increment was determined to maintain the same performance when compared with eGFR decline. This demonstrates that eGFR decrease magnitude in children is independent from any constant or height. Scr increments used for adults are not applicable to children. Moreover, this simplified classification facilitates a prompt AKI diagnosis and makes retrospective studies possible when height is missing.

SA-PO2117

Acute Kidney Injury Following Heart Surgery: A Comparison between Infants and Neonates Abdullah E. Alabbas, Andrew I. Campbell, Peter Skippen, Cherry Mammen. *BC Children's Hospital, Vancouver, BC, Canada.*

Background: Acute kidney injury (AKI) is associated with increased mortality in critically-ill children and adults. Heart surgery is a known risk factor for AKI. The incidence of AKI in children following heart surgery ranges from <1 to 30% depending on various AKI definitions. However, there are no AKI studies specific for infants or neonates undergoing heart surgery. Our objective was to compare the clinical characteristics of post-heart surgery infants (>28days-1year) and neonates (≤28days) with AKI, utilizing the AKIN definition.

Methods: Retrospective descriptive study. We included all post-operative heart surgery patients ≤1year old who were admitted to our PICU between January 2006 and May 2009 with AKI defined by their maximal AKIN stage. Patient data was collected from a PICU database and medical charts.

Results: 280 infants and 122 neonates had heart surgery in the study period. We identified 125 (45%) infants and 76 (62%) neonates with AKI. The following variables were significantly higher ($p<0.05$) in neonates: Pediatric Risk of Mortality (PRISM) III scores (median 8 vs 10), ventilation days (median 4 vs 5), cardiopulmonary bypass time (median 102 vs 128 min), need for ECMO (5.6 vs 18.4%), PICU and hospital length of stay (median 6 vs 7.5 & 11 vs 18 days respectively). AKI occurred early as 75% of neonates and 76% of infants reached their maximal AKIN stage within 48 hours of admission. The mortality rates of infants and neonates were 7/280 (2.5%) and 11/122 (9%) respectively. Of those who died, 5/7 infants & 11/11 neonates were classified as AKIN stage 3. 15/16 who died in AKIN stage 3 were defined by urine output criteria only.

Conclusions: The incidence of AKI and mortality rate was higher in neonates (62% & 9%) compared to infants (45% & 2.5%). This may be explained in part by increased severity of illness in neonates. Most patients who died were defined by stage 3 urine output criteria alone. Therefore, AKIN urine output criteria may have a stronger association with mortality as compared to creatinine criteria in this population. Further prospective studies are needed to confirm our findings.

SA-PO2118

Acute Kidney Injury Due to Fibrates in the Absence of Rhabdomyolysis. A Common and Occasionally Irreversible Complication Natalia I. Polanco Fernandez, Eduardo R. Hernandez, Eduardo Gutierrez, Victor Gutierrez-Millet, Esther Gonzalez Monte, Enrique Morales, Manuel Praga. *Nephrology, H. U. 12 de Octubre, Madrid, Spain.*

Background: Fibrates are increasingly prescribed for the treatment of hypertriglyceridemia.Several case reports have described acute kidney injury (AKI) in patients treated with fibrates, although most of them were due to rhabdomyolysis.

Methods: Single-center analysis of patients who showed fibrate-induced AKI (FB-AKI) in the period 2007-2010.Definition of FB-AKI included a >20% serum creatinine (sCr) increase in at least two consecutive measurements after the onset of fibrate therapy, absence of other possible causes of AKI and improvement of renal function after fibrate withdrawal.

Results: We collected 60 cases of FB-AKI (37/23 M/F, aged 69±11 yr). Forty-three (71%) had stage 3 CKD at the onset of fibrate treatment, 52 patients (87%) hypertension, 37 patients (62%) type2 diabetes, 39 patients (66%) hyperuricemia.Clinical or analytical signs of rhabdomyolysis were not detected in any patient.Median sCr increase from baseline values was 53.4%(r 23-286). Baseline sCr was 1.2±0.25 mg/dl, peak sCr 2±0.45 mg/dl and final sCr after fibrate discontinuation 1.26±0.28 mg/dl. Renal function improved in all the patients after fibrate withdrawal but 9(15%) did not recover baseline values of renal function. As shown in the table, these patients had a better renal function at baseline, had shown a greater sCr peak and had tended to receive fibrates for a longer time.

Characteristics of patients with complete or incomplete recovery of baseline renal function after fibrate withdrawal

	Complete recovery (51)	Partial recover (9)	p
Age (yr)	69±11	66±13	0,6
Baseline sCr (mg/dl)	1,2±0,6	1±0,7	0,07
Peak sCr (mg/dl)	1,94±0,26	2,2±0,66	0,43
Final sCr (mg/dl)	1,23±0,29	1,4±0,2	0,04
% increase of sCr (range)±	51.6 (23-141)	86.4 (43.6-286)	0,05
Duration of fibrate treatment (months)	13.5 (2-132)	29 (7-45)	0,27

Conclusions: FB-AKI in the absence of rhabdomyolysis is not an uncommon complication. Most of the cases were due to fenofibrate. Although renal function improved after fibrate withdrawal 15% of patients exhibited a partial recovery of baseline renal function values.

SA-PO2119

The Epidemiology of Acute Kidney Injury in Canadian Critical Care Units Ayodele Odutayo,[1] Sasha P. Litwin,[1] Bonnie R. Richardson,[2] Neill Adhikari,[1] James William Barton,[3] Jan O. Friedrich,[1] Amit X. Garg,[2] Stephen Lapinsky,[1] Rahim Moineddin,[1] Michelle A. Hladunewich,[1] Ron Wald.[1] *[1]University of Toronto; [2]University of Western Ontario; [3]University of Saskatchewan.*

Background: There is limited information regarding the epidemiology and outcomes of acute kidney injury (AKI) in Canadian intensive care units (ICUs). Although AKI is frequently diagnosed early, the ability to predict dialysis and death is limited.

Methods: We conducted a prospective cohort study of consecutive patients admitted to critical care units in five Canadian ICUs over a 30-day period. Each patient was followed until hospital discharge or for a maximum of 30 days. The Acute Kidney Injury Network system was used to identify and classify individuals with AKI. We used descriptive statistics to characterize patients with AKI and their outcomes. Among AKI patients, we used multivariable logistic regression to identify predictors of dialysis and the composite outcome of dialysis or death.

Results: We identified 603 patients of whom 161 (26.7%) developed AKI. Patients with AKI were more likely to die (29.2% vs. 8.6% of those with no AKI, $p<0.001$) and the risk of death escalated with AKIN stage (19.2, 37.9 and 51.5% for AKIN stages 1, 2 and 3, respectively). Nineteen (12%) patients received dialysis a median of 1 (1-2) days after AKI diagnosis. Increments in plasma urea and urine output < 400 mL/day on the day of AKI diagnosis accurately predicted the receipt of dialysis (area under the receiver operator curve 0.85). The composite outcome of death or receipt of dialysis occurred in 57 (35.4%) patients with AKI. The combination of plasma urea, urine output <400 mL/day and decrements in serum bicarbonate on the day of AKI diagnosis predicted this occurrence (AUC 0.83).

Conclusions: AKI is a common complication of critical illness in Canadian ICUs. Even mild AKI is associated with a substantial risk of death. At the time of AKI diagnosis, clinical data may be helpful in identifying individuals with a high likelihood of developing progressive disease.

SA-PO2120

Determinants of Nutritional Support in Patients with Acute Kidney Injury (AKI): The PICARD Experience Soo Young Yoon,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] Talat Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]Univ of California San Diego, San Diego, CA; [2]Stanford Univ, Palo Alto, CA; [3]Univ of Washington, Seattle, WA; [4]Vanderbilt Univ, Nashville, TN; [5]Cleveland Clinic Foundation, Cleveland, OH.*

Background: There is limited information on the timing, route, and amount of nutrition required for ICU patients with AKI. We assessed the pattern of nutritional support (NUTS) and utilization of enteral (EN) and parenteral (PN) nutrition in patients enrolled in the PICARD study (KI, 2004, 66: 1613-1621). We hypothesized that AKI severity and comorbidities would influence the mode of nutrition (MON).

Methods: We analyzed data from 615 of the 618 patients who stayed in ICU >48 hours. We assessed the MON provided in the ICU and its relationship to underlying disease severity and other factors.

Results: Among 615 patients, 199 ate orally without NUTS (Oral), 183 received EN, 66 PN, 91 EN+PN, and 76 no oral or NUTS (None). A subset of the NUTS patients had oral intake for part of their ICU stay (EN n=81; PN n=22; EN+PN n= 27). Overall, nutrition was provided for 65.4% of the ICU stay (range 2-100; NUTS 60.9 vs Oral 73.2%; $p<0.001$).

Parameters on ICU admission	None (n=76)	EN (n=183)	PN (n=66)	EN+PN (n=91)	Oral (n=199)	Total (n=615)
%Sepsis*	58.2	62.0	61.4	62.7	42.9	55.3
%Mech Vent*	84.2	86.4	78.3	87.2	78.4	82.7
%Oliguria**	36.8	27.2	15.3	19.3	25.8	25.3
%CKD*	26.8	19.1	21.2	19.8	41.2	27.5
%Diabetes*'**	32.9	34.5	15.6	20.9	33.2	29.8
%CAD*	33.8	35.9	40.6	26.4	47.2	38.5
%CHF*	24.6	21.1	26.2	21.8	40.7	28.6

*p<.05 among groups when including Oral; **excluding Oral

In logistic regression, significant determinants for each MON (Odds ratio) were: None: oliguria (.999), shorter ICU stay (.859), EN: lower body weight (.986) and medical

ICU (.198), EN+PN: lower creatinine (.598) and CRRT (4.882), and Oral: surgical ICU (3.525), preexisting CKD (2.594), shorter ICU stay (.946) and shorter ventilation days (.896).

Conclusions: Nutritional support was required in over 2/3 of patients with AKI. Oral intake and transition between nutritional modes was common. Further studies should determine the relationship between changes in patient's illness and transition of the nutritional mode.

Funding: NIDDK Support

SA-PO2121

Urinary Liver-Type Fatty Acid-Binding Protein Predicts Mortality in Critically Ill Patients Eunjung Cho, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Department of Internal Medicine, Korea University Hospital, The Institute of Renal Disease, Seoul, Republic of Korea.*

Background: Although several urinary biomarkers including neutrophil gelatinase associated lipocalin (NGAL) have been characterized and validated as useful biomarkers for the early detection of acute kidney injury (AKI), their usefulness as outcome predictors is not well established. Here, we determined the diagnostic and prognostic ability of urinary liver-type fatty acid-binding protein (L-FABP), one of the newly recognized candidate biomarkers for kidney injury, in heterogeneous intensive care unit (ICU) patients, comparing with those of NGAL.

Methods: We prospectively collected data of patients admitted to medical and surgical ICUs from July, 2010 to January, 2011, and urine NGAL and L-FABP at the time of admission to ICU were quantitated.

Results: Among 96 patients enrolled, 35 (36.5%) had AKI and 9 patients required renal replacement therapy. Urinary NGAL and L-FABP were significantly higher in patients with AKI compared to non-AKI ICU patients. The diagnostic performance of these biomarkers, assessed by the area under the receiver operating characteristic curve (ROC-AUC), was 0.811 (95% C.I. 0.718 - 0.903) for NGAL and 0.796 (95% C.I. 0.700 - 0.892) for L-FABP, demonstrating their usefulness in diagnosing AKI. In addition, urinary L-FABP was also found to be useful in predicting in-hospital mortality in multivariate analysis along with SAPS II score, whereas urinary NGAL failed to demonstrate it. The ROC-AUC of urinary L-FABP in predicting in-hospital mortality was 0.743 (95% C.I. 0.635 - 0.851, with a sensitivity of 72% and a specificity of 70% at a cutoff value of 44.5ng/ml).

Conclusions: L-FABP, an emerging urinary biomarker, seems to be promising both for the diagnosis of AKI and the prediction of prognosis in heterogeneous ICU patients. It needs to be further examined and validated for clinical utility. Discovery of biomarkers to stratify patients at risk of poor prognosis might improve ultimate outcomes in critically ill patients.

SA-PO2122

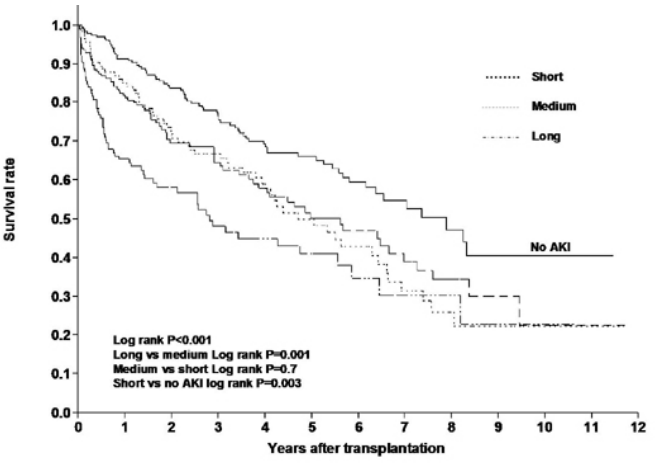
The Duration of Postoperative Acute Kidney Injury and Risk of Long Term Mortality after Lung Transplantation Edgard I. Wehbe,[1] Marie M. Budev,[2] Rachel Lauren Brock,[1] Sevag Demirjian,[1] Martin J. Schreiber,[1] Brian R. Stephany.[1] *[1]Nephrology and Hypertension, Glickman Urological and Kidney Institute; [2]Pulmonary and Critical care, Cleveland Clinic.*

Background: To determine if the duration of Acute kidney injury(AKI)after lung transplantation predicts long term mortality

Methods: We retrospectively evaluated data on 657 patients who underwent lung transplantation from 1997 to 2009. AKI was defined by absolute rise in creatinine by >= 0.3 mg/dl and categorized into three stages by the magnitude rise in creatinine according to the AKIN classification and by the duration from baseline to nadir of creatinine (short (less than 5 days), medium (5–10days) or long (10 days or more)). Outcomes analyzed were all cause mortality.

Results: We identified 424 patients (65 %) who had at least one AKI event in the first 2 weeks after transplantation. 115 (17.5%), 184(28%) and 125(19%) experienced short, medium and long duration AKI respectively. After a median follow up of 2.2 year (0.4-6.9), a total of 277 (42%) died. One year patient survival was 84%, 81%, 65% in the short, medium and long duration AKI respectively. The survival curve (figure 1) showed that long-term survival decreased according to the duration of AKI with significant difference between long duration vs medium or short duration AKI. Adjusting for age, gender, race, type and cause of lung transplant, diabetes and hypertension, the hazard ratio for death was 1.6 (95% CI 1.1-2.3), 1.6(95% CI 1.1-2.2), and 2.7 (95% CI 1.9-3.79) for short, medium and long duration AKI respectively

Conclusions: This study showed that the duration of AKI is independently associated with long-term mortality and may provide additional prognostic information in patients undergoing lung transplantation.



SA-PO2123

Tubular Dysfunction in American Cutaneous Leishmaniasis: Evolution after Treatment Rodrigo Alves de Oliveira,[1] Alexandre Braga Liborio,[2] Geraldo B. Silva,[1,2] Antonio C. Seguro,[3] Elizabeth De Francesco Daher.[1] *[1]School of Medicine, UFC; [2]UNIFOR; [3]School of Medicine, USP.*

Background: Leishmaniasis is an infectious,zoonotic disease.Various types of kidney injury have been reported in visceral type.There are few reports of renal lesions in American Cutaneous Leishmaniasis (ACL). The aim of this study was to determine tubular function in ACL.

Methods: Prospective study,conducted in Brazil.Thirty-seven patients diagnosed with ACL based on histopathological and Montenegro test. Prior and 48 hours after treatment with Glucantime®,tubular function was tested and the results were compared with those obtained for 8 control subjects.Urine and plasma osmolality (Uosm and Posm) were tested before and after intranasal DDAVP.Bicarbonate (sBic), urinary pH (UpH) were evaluated before and after acidification test with CaCl2 (acid test). UpH>5.5 after CaCl2 test and U/POsm<2.8 with UOsm<700mOsm/KgH20 after DDAVP were considered abnormal.

Results: Age was 35±6 years against 29±5 years in control group. The study group comprised 19 men with a mean disease time of 31±22 days. At ACL diagnosis, 27 patients had urinary concentration dysfunction. From these, only seven presented normal urinary concentration after ACL treatment ($\chi 2$ p=0.4). About urinary acidification, 15 patients presented an abnormal acid test at the diagnosis. From these, six had sBic<22mEq/L. After treatment, six patients (40%) maintained urinary acidification defect ($\chi 2$ p=0.01) with three presenting sBic<22mEq/L. Overall, 12 patients had combined tubular dysfunction before treatment and 5 persisted after treatment.

Conclusions: ACL is associated with renal tubular dysfunctions and these are only partially reverted after treatment,especially urinary concentration ability.Glucantime has no detrimental effect.

Renal function before and after treatment vs controls

	Diagnosis	After treat.	Control
ClCr (ml/min/1,73m)²	109,6±31,5	108,4±28,5	116,4±22,7
U/Posm T4	2,19±0,73	1,95±0,73	3,47±0,33
Uosm T4	618±202	552±210	965±81†
UpH T4	5,45±0,64	5,19±0,60‡	4,82±0,20†
FENa(%)	1,15±0,74	1,35±1,51	0,73±0,79
FEK(%)	10±6,6	10±7,6	7,5±2,6†

†before and after vs controls, p< 0,05; ‡Before vs after treatment, p= 0,0066

Funding: Government Support - Non-U.S.

SA-PO2124

Acute Kidney Injury and AKI Biomarkers Are Associated with Fluid Overload in Critically Ill Children Ana Palijan,[1] Prasad Devarajan,[2] Joseph V. Bonventre,[3] Michael R. Bennett,[2] Qing Ma,[2] Venkata Sabbisetti,[3] Michael Zappitelli.[1] *[1]Pediatrics, McGill University Health Centre, Montreal, Canada; [2]Cincinnati Children's Hospital Medical Centre, Cincinnati; [3]Brigham and Women's Hospital, MA.*

Background: Little data exist on fluid overload (FO) of all ICU children and the relation between acute kidney injury (AKI) and FO. We hypothesized that ICU-admitted children with AKI develop worse FO and that urine AKI biomarkers help predict worse FO.

Methods: We prospectively followed 160 non-transplanted children admitted to ICU≥1day. Serum creatinine (SCr) and fluid intake/output were recorded daily. FO% was calculated as (fluid in-out [L]/weight)X100. AKI was defined as≥50% or 27 umol/l SCr rise from baseline. Risk factors for Day 3 FO were evaluated by multiple linear regression. The association of AKI and Day 3 FO on ICU stay was evaluated by Cox regression. Area under the curve (AUC) was used to evaluate clinical factors to predict presence Day 3 FO≥3% (median FO). In 51 pts with biomarkers measured, first ICU urine neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1) were added to the clinical model predicting Day 3 FO≥3%, to determine if this increased prediction.

Results: Mean[SD] age, ICU stay and Day 3 FO% were 4.5[5.7] yrs, 9.3[8.7] d and 4.6[4.9%]; 62% were boys, 4% had sepsis, 65% received pressors, 45% had AKI. Day 3 FO was higher in AKI pts(6.2[5.6] vs. 3.9[3.3]%,p=0.02). AKI was associated with worse Day 3 FO(p=0.03), independent of age, gender, and fluid (ml) per kg in the first 6 ICU hours(p=0.001).AKI and higher Day 3 FO predicted longer ICU stay, controlled for age and gender. A clinical model including AKI, age, gender and fluid in first 6 ICU hrs predicted presence of Day 3 FO≥3% with AUC=0.67. When first ICU urine NGAL, IL-18 and KIM-1 were added to the model, AUC increased to 0.76.

Conclusions: When all ICU children are studied, FO is not as severe as previously described, but is associated with presence of AKI and longer ICU stay. Combining clinical risk factors and AKI biomarkers leads to enhanced prediction of more severe FO development and may assist in FO prevention strategies.

SA-PO2125

Fighting Bloody Diarrhea for HUS Prevention and Mitigation: Lombardy Regional HUS Network Gianluigi Ardissino, Francesca Tel, Sara Testa, Fabio Paglialonga, Stefania Salardi, Silvana Tedeschi, Nicolò Borsa, Rosaria Colombo, Manuela Colosimo, Erminio Torresani, Alberto Edefonti. *Center for HUS Control, Fondazione Ca' Granda Osp Maggiore Policlinico, Milano, Italy.*

Background: Typical hemolytic uremic syndrome (tHUS), although rare, still represents a major public health problem in industrialized countries caused by a Verotoxin-producing Escherichia coli (VTEC) intestinal infection often presenting with bloody diarrhea.

Methods: In order to identify patients at risk of tHUS early in the course of the disease, a network connecting pediatric hospitals in Lombardy Region (10 millions gp) was developed.

Results: Fifty-three units presently partecipate in the network and since May 28, 2010 (founding day) children with bloody diarrhea were centrally tested for Shigatoxin (Stx) 1 and 2 with a rapid immunochromatographic test and multiplex PCR test. The objectives of the project were: 1. to increase the ability of the surveillance system in identifying the sources of VTEC infection and its spreading; 2. to understand the mechanisms of Stx delivery to target organs endothelia; 3. to test the potential role of overhydration and/or leukoapheresis to prevent or mitigate renal and CNS involvement. So far 248 patients have been tested. Hereafter are the preliminary results concerning the 80 samples for which all the procedures were completed. Ten out of 80 (12.5%) were positive for VTEC. Among negative samples Salmonella (25%) and Campylobacter (11%) were the most common identified bacteria. Among patients with negative colture (47%) 1 patient had Henoch Schonlein purpura, 1 ulcerative colitis and 1 Meckel diverticulum.

Conclusions: In conclusion, our findings point out an unexpected very high frequency of VTEC among bloody diarrhea in children in our region. No conclusion can be anticipated on the remaining objectives.

Acknowledgement: the project is feasible thanks to the collaboration of the members of the Regional HUS Network whose complete list is available at www.centroseu.org. The project has been supported by the " PROGETTO ALICE ONLUS – Associazione per la lotta alla SEU"

SA-PO2126

Impact of Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD) on Re-Admissions in Hospitalized Patients with Congestive Heart Failure (CHF) Charuhas V. Thakar,[1] Pratik Parikh,[3] Yan Liu,[3] Anthony Leonard.[1] *[1]Internal Medicine/Nephrology, University of Cincinnati, OH; [2]Medical Service/ Renal Section, Cincinnati VA, Cincinnati, OH; [3]Biomedical, Industrial and Human Factors Engineering, Wright State University, Dayton, OH.*

Background: Reducing readmission rate in patients hospitalized with CHF improves quality of care and reduces healthcare costs. Effect of AKI and CKD on readmissions in patients with CHF is not well understood.

Methods: We examined the state inpatient database (SID; derived from AHRQ-HCUP) for the state of Washington. 6,535 patients were discharged with a primary diagnosis of CHF from all hospitals in the state between 1/1/06 and 9/30/06.

The primary endpoint was re-admission within 30 days from the index discharge with either primary or secondary diagnosis of CHF. Patients were classified based on diagnosis of AKI, CKD and other co-morbid diseases based on ICD-9 codes. Logistic regression analysis was adjusted for demographics, co-morbidites, and procedures, and economic indicators.

Results: Patients in the sample had a mean age of 73.8 years (standard deviation, SD, 14.6). 51.3% were female, 25% had diabetes, 51% had hypertension. Medicare was the payor in 71% of patients. 6.5% of patients developed AKI during the index hospitalization for CHF; 124/424 (29%) of AKI cases developed AKI with underlying CKD. 14% of the sample had CKD. but did not develop AKI. Results are shown in Table 1.

Results

	AKI- CKD-	AKI- CKD+	AKI+ CKD-	AKI+ CKD+	
N (%)	5211 (80)	900 (14)	300 (5)	124 (2)	
Index Hosp Mortality	1.7%	3.3%	11.2%	12.7%	p < 0.0001
30-day Readmission Rate	13.9%	18%	23%	16.9%	p < 0.0001
Mean length of stay per patient within 30 days	5.2 (SD, 4.8)	6.1 (SD, 4.7)	8.5 (SD, 7.6)	9.4 (SD, 7.1)	p < 0.0001
Odds Ratio of 30-day Readmission*	Ref	1.3 (1.1 - 1.6)	1.8 (1.4 - 2.4)	1.2 (0.7 - 1.8)	

*Case-mix adjusted model

Conclusions: AKI in the absence of CKD significantly increases the risk of re-admission within 30 days in CHF patients. This association also raises the possibility of a pathophysiological link between AKI and recurrent admissons for CHF. Prevention or management of AKI in these patients may improve quality of care and reduce healthcare costs.

Funding: Veterans Administration Support

SA-PO2127

Piecewise Analysis of Mortality Hazard and Survival Probability in Patients with Acute Kidney Injury (AKI) Jane Hongyuan Zhang,[1] Paul M. Palevsky,[2,3] Glenn M. Chertow,[4] Bingqing Zhou.[5] *[1]VA Cooperative Studies Program Coordinating Center, West Haven, CT; [2]VA Pittsburgh HCS, Pittsburgh, PA; [3]Univ of Pittsburgh Sch of Med, Pittsburgh, PA; [4]Stanford University School of Medicine, Palo Alto, CA; [5]Yale University School of Public Health, New Haven, CT.*

Background: AKI is a life-threatening complication of critical illness associated with acute mortality rates of 40-70%. However, the mortality-hazard is not constant but decreases over time. We utilized survival data from the 1124 participants in the VA/NIH Acute Renal Failure Trial Network (ATN) study to analyze the hazard-function for mortality in critically ill patients with severe AKI requiring renal replacement therapy.

Methods: We used piecewise log-linear models to examine patient survival and fit the hazard of death utilizing models with no change (inflection) points (1 piece), 1 change point (2 piece) and 2 change points (3 piece).

Results: There was an initial steep decrease in the hazard of death, followed by a more gradual decline, suggesting the existence of a critical change point between an acute and convalescent phase of illness. There was excellent fit of both the 2- and 3-piece models (Table). The change-point for the 2-piece model was at 44 days while the change points for the 3-piece model were at 34 and 145 days. The 3-piece model provided the best fit to the data, with a likelihood value of -3906, representing an increase of 114 from the 1-piece model.

	1 Segment	2 Segments	3 Segments
loglikelihood	-4020	-3914	-3906
1st change point (day)		44	34
2nd change point (day)			145
Log(hazard(t)): 1st piece	-3.78 – 0.021*t	-3.15 - 0.058*t	-3.14 – 0.060*t
Log(hazard(t)):2nd piece		-5.31 – 0.009*t	-4.54 – 0.018*t
Log(hazard(t)):3rd piece			-6.82 – 0.003*t

Conclusions: These results are consistent with a clinical course comprised of an acute phase (days 0-34) followed by an early convalescent phase (days 34-145) and a late convalescent phase (beyond day 145). These results should inform the design of design of future clinical trials of interventions in AKI, supporting the use of an early survival endpoint at 1 month and a later endpoint at 3 to 6 months.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2128

Application of the Acute Kidney Injury Network Definition in Post-Heart Surgery Neonates: A Retrospective Study Abdullah E. Alabbas, Peter Skippen, Andrew I. Campbell, Douglas G. Matsell, Cherry Mammen. *BC Children's Hospital, Vancouver, BC, Canada.*

Background: Heart surgery is a known risk factor for acute kidney injury (AKI) in children. The incidence of AKI post-heart surgery ranges from <1 to 30% depending on various AKI definitions and the population of interest. AKI in neonates following cardiac surgery has not been well-studied. Our objectives were: 1) To describe the clinical characteristics of post-heart surgery neonates with AKI, utilizing the AKIN definition 2) To explore potential risk factors for mortality in this population.

Methods: Retrospective, single center observational design. We included all post-operative heart surgery neonates admitted to our PICU between January 2006 and May 2009 with AKI defined by their maximal AKIN stage. Clinical characteristics were compared between the patients' AKIN stages (1,2 and 3). Patient data were collected from a PICU database and medical charts. Multiple logistic regression analyses were performed to evaluate possible risk factors of mortality.

Results: We identified 76 out of 122 (62%) post-heart surgery neonates with AKI during the study period. Overall mean age was 8.4±7.6 days with 90% being term gestation. When compared to stage 1 and stage 2, AKIN stage 3 patients were younger (mean 9.68 vs 11.63 vs 6.03 days for stages 1, 2, and 3 respectively), had higher ventilation days (median 5 vs 4 vs 7), PICU length of stay (median 7 vs 6 vs 9 days), and Pediatric Risk of Mortality (PRISM) III scores (mean 9.68 vs 8.68 vs 13.49) (all p< 0.05). Eleven neonates died after AKI, all with AKIN stage 3. Age <5 days (OR=25.5, 95%CI=2.3-279) and use of ECMO (OR=53.2, 95%CI=5.4-525.5) were independently associated with mortality.

Conclusions: The incidence of AKI in neonates post-heart surgery is high (62%). Those with severe AKI are younger, require longer ventilatory support, stay longer in the PICU and are more critically ill. The development of severe AKI, age < 5 days and need for ECMO are all potential independent risk factors for mortality in this cardiac population. These findings need to be supported by larger prospective studies.

SA-PO2129

The Risk Factors on the Prognosis of Acute Kidney Injury under AKIN Definition in Critical Ill Patients Yang Lichuan, Ping Fu. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: Despite significant advances in the medical management and therapeutics, acute kidney injury (AKI) is still a familiar and serious complication with enhanced morbidity and mortality in hospitalized patients, especially in intensive care units (ICUs). The primary purpose of this study is to apply the definition proposed by the Acute Kidney Injury Network (AKIN) workgroup to assess the incidence, the short-time (28 days) outcomes and the risk factors on the prognosis of AKI in ICU.

Methods: In this retrospective study, we collected the data from a cohort of 4642 patients admitted in ICUs from Dec 2009 to march 2011. Univariate and multivariate analysis were performed to investigate the risk factors for short-time mortality of AKI (AKI definited by the increase of serum creatinine by 26.4 umol/L) in ICU.

Results: After further exclusion, there were 1036 patients of ICU admissions enrolled in this research. We found AKI occurred in 353 of the 1036 patients (34.1%) under the AKIN criteria and the mortality was 54.4%. The variables that were related to the prognosis of AKI in multivariable analysis were as follows: AKI III [odds ratio (OR), 12.5338), AKI II (OR, 4.6625), **SAP** (OR,2.3522), renal replacement therapy (RRT, OR = 2.1113;), the timing of AKI in ICU (OR = 1.2351), base serum creatinine (OR = 1.0059),the length of stay in ICU (OR = 1.0546) and age (OR = 1.0223).The area under the receiver operator characteristic (ROC) curve for 28 days mortality was 0.7571 for the AKIN criteria ($p < 0.001$).

Conclusions: In these ICU patients, the mortality of AKI is correlated with various risk factors, especially AKI II,AKI III, SAP , the timing of AKI was independent risk factor for ICU mortality. Our results support the utility of the AKIN criteria in predicting outcomes for patients with AKI.

SA-PO2130

Acute Kidney Injury and Its Severity Are Independently Associated with 10-Year Survival and End Stage Renal Disease Following Cardiac Surgery, Resembling a Dose-Response Pattern Alejandro Ferreiro,[1] Raul Lombardi,[1] Emma Schwedt,[2] González-Bedat Carlota María,[2] Nelson Mazzuchi.[2] *[1]National Institute for Cardiac Surgery, Uruguay; [2]Uruguayan Dialysis Registry, Uruguay.*

Background: Acute kidney injury (AKI) has been associated with long-term mortality and ESRD in large medical claim-based databases. The relationship between multivariate adjusted and prospectively collected Scr-diagnosed AKI and long-term outcomes is mostly scarce. The aim of the study is to evaluate the multivariate-adjusted impact of postoperative (PO) AKI severity on long-term ESRD incidence and mortality after cardiac surgery (CS).

Methods: All adult patients submitted to cardiac surgery between 1/1/2000 and 12/31/2009 (n=7773) were enrolled. Co-morbidities, type of CS procedure, and outcomes were prospectively registered. Long-time survival (up to 10 year) was obtained from a telephone survey and/or National Population Registry. The ESRD status was obtained from the Uruguayan Dialysis Registry which includes all dialysis units in the country. Baseline renal function (eGFR) was assessed by the Cockroft-Gault formula. AKI was defined according to RIFLE criteria, and staged according to the peak minus baseline PO Scr (5 categories). Additive EuroSCORE was used for risk-adjustment. Statistical analysis included "t"test, $\chi 2$ test, Kruskal-Wallis test, Kaplan-Meier curves with long-rank test and Cox regression for multivariate-adjusted survival analysis

Results: AKI incidence: 24.2%. Renal replacement therapy (RRT): 1.7%. Operative mortality: 5.2% (no AKI: 3.1%, AKI no RRT 12.6%, AKI-RRT: 62%, $p<0.001$). Long-term (10 years) actuarial survival after hospital discharge was 0.77 in non AKI patients, being progressively worse according to AKI staging in a dose-response pattern ($p<0.0001$). 10-year ESRD free survival was statistically different inΔ Scr 1 to 1.9 mg/dl (0.98) and $\geq$ 2 mg/dl (0.84) groups ($p<0.001$). In multivariate analysis, AKI (OR 4.21), EuroSCORE (OR 1.16) and eGFR (ml/min) (OR 0.991) were independently associated with long-term survival.

Conclusions: AKI is independently associated with long term survival (10 year) and ESRD in cardiac surgery, related with its severity and resembling a dose-response pattern.

Funding: Clinical Revenue Support

SA-PO2131

Serum Metabolomic Profiles from Patients with Acute Kidney Injury(AKI): A Pilot Study Richard D. Beger,[1] Jinchun Sun,[1] Melissa Lamb Shannon,[2] Yosuke Ando,[1] Laura Schnackenberg,[1] Didier Portilla.[2] *[1]Division of Systems Biology, National Center for Toxicological Research, US FDA, Jefferson, AR; [2]Division of Nephrology, Department of Internal Medicine, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare Systems, Little Rock, AR.*

Background: The aim of this pilot study was to demonstrate that systemic metabolic alterations associated with AKI could be evaluated using a rapid metabolomics screening technique.

Methods: AKI was defined by an increase in serum creatinine level of 50% or more from previous baseline value. Serum samples obtained from 17 patients admitted to Central Arkansas Veterans Healthcare System (CAVHS) with newly diagnosed AKI as well as pooled serum samples obtained from individuals with normal renal function from National Institute of Standard and Technology (NIST) were analyzed by MS-based metabolomics procedures and the resulting data was analyzed for potential biomarkers.

Results: Five out of 17 AKI patients required dialysis as a form of therapy. Mortality rate in our study was 47 % (8/17 patients) after a follow up of one year. Amino acids, including homocystine, pyroglutamate, phenylalanine and tryptophan, were significantly increased in the serum from AKI patients. Free carnitine and acylcarnitines were significantly increased (about 30 to 500 times increase) in the serum from AKI patients. The traditional clinical biomarkers, creatinine and uric acid, were also elevated in serum from AKI patients when compared to the serum from the NIST standard. LysoPC, citric acid and benzoic acid were significantly decreased at $p<0.0001$ in AKI patients.

Conclusions: 1) Metabolomics data detected increases in homocystine and pyroglutamate in AKI patients, which are commonly recognized as major risks factors for having cardiovascular disease,2) Our metabolomic study is the first one to show a marked elevation in serum carnitine and acylcarnitines concentrations in hospitalized patients with newly diagnosed AKI. These results support the notion of a reduced activity of fatty acid oxidation enzymes during acute kidney injury, a metabolic abnormality we have described in kidney tissue from animal models of acute kidney injury.

Funding: NIDDK Support, Veterans Administration Support

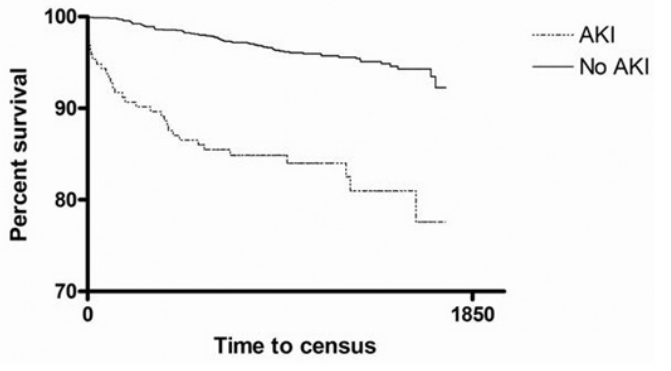
SA-PO2132

Acute Kidney Injury Is Associated with Increased Mortality after Cardiac Surgery in Patients without Pre-Existing Renal Impairment Sean Gallagher, Matt Lovell, Dan A. Jones, Krishnaraj S. Rathod, Akhil Kapur, Andrew Wragg, Magdi Yaqoob, Rakesh Uppal. *Cardiac and Renal, Barts and the London NHS Trust, London, United Kingdom.*

Background: Acute kidney injury (AKI) following coronary artery bypass graft surgery (CABG)has been associated with increased mortality. The incidence and prognostic implications of AKI following CABG in patients without pre-existing renal impairment is unknown.

Methods: An analysis of prospectively collected data was undertaken in 1838 patients without pre-existing renal impairment (eGFR rise >60mls/min) undergoing CABG at a tertiary Cardiac centre between Jan 2003 and Dec 2007. AKI was defined by a 50% increase in serum creatinine post-operatively. All-cause mortality was determined via Office of National Statistics data.

Results: The incidence of AKI was 10.5%. Patients developing AKI were older (67.0vs64.1, $p<0.0001$), with more previous MIs (53.9 vs 40.5%, $p=0.0005$), more LV impairment (47.6vs35.9%, $p=0.002$), more diabetes (45.6vs28.3%, $p<0.0001$), more hypertension (88.6vs79.1%, $p=0.0012$), more PVD (18.7vs11.2%, 0.0047) and Euroscore (a model used to estimate risk of death following cardiac surgery) was higher (5.03vs3.21, $p<0.0001$). In-hospital mortality was significantly increased in patients with AKI (5.6vs0.12%, $p<0.0001$). 5 year survival was also worse among patients with AKI (82.9vs95.7%, $p<0.0001$).



AKI was the strongest independent predictor of long term mortality with an adjusted HR of 3.27 (CI 2.09 to 5.12). Only LV function (adjusted HR1.98 (CI 1.38 to 2.85) and age (adjusted HR 1.07 (CI 1.03 to 1.1)) were also independently associated with 5 year all cause mortality.

Conclusions: The development of AKI following CABG in patients without pre-existing renal imapairment is associated with worsening of 5 year mortality. Patients that develop AKI following CABG are high risk and aggressive subsequent medical therapy may be warranted.

SA-PO2133

Preoperative Prescription of Diuretics, but Not Inhibition of Renin-Angiotensin-System, Predicts Acute Kidney Injury after Non-Cardiac Surgery Miho Tagawa,[1] Takayuki Hamano.[2] *[1]Nephrology, Kyoto Katsura Hospital, Kyoto, Japan; [2]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Pennsylvania, PA.*

Background: Angiotensin converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) are reported to be an independent risk factor for acute kidney injury (AKI) in cardiac surgery. No study has evaluated which kind of preoperative prescription of antihypertensives are risk factors of AKI after non-cardiac surgery.

Methods: This is a retrospective observational study. Inclusion criteria were adult (age ≥ 18) patients who underwent non-cardiac surgery under general anesthesia from 2007-2009 at our institute. Exclusion criteria were urological surgery, those without creatinine (Cre) values, and who had underwent dialysis preoperatively. Exposure of interests are preoperative use of anti-hypertensives including ACE-I/ARB and diuretics. Outcome variable was postoperative acute kidney injury defined by AKI network (increase in Cre ≥ 0.3 mg/dL or 150 %, or urine output < 0.5 ml/kg/hour for > 6 hours). Multivariable logistic regression was performed. We adjusted for 23 covariates including kinds of surgery. A propensity score (PS) of receiving ACE-I/ARB therapy preoperatively was estimated and PS analyses were performed including PS adjustment and PS matching as sensitivity analyses.

Results: There were 123 AKI (5.0 %) among 2472 subjects.

Odds ratio of AKI by multivariable logistic regression analysis

	Odds ratio	95 % CI	p value
ACE-I/ARB	0.833	0.503-1.379	0.477
Diuretics	2.360	1.288-4.325	0.005

PS analyses yielded similar results with regard to ACE-I/ARB therapy. Other independent predictors of AKI included male sex, intrathorasic, intraperitoneal surgery, surgery with large fluid shift, emergent surgery, insulin-dependent diabetes, hypertension, cerebrovascular disease, intraoperative use of pressors.

Conclusions: Not prescription of ACE-I/ARB, but of diuretics was an independent risk factor for postoperative AKI in non-cardiac surgery.

SA-PO2134

The Impact of Early Nephrology Follow-Up among Survivors of Acute Kidney Injury Requiring Dialysis Ziv Harel,[1,4] Ron Wald,[1,4] Muhammad Mamdani,[1,3,4] Joanne M. Bargman,[4] Amit X. Garg,[2,3] Robert R. Quinn,[3,5] Joel Ray,[1,3,4] Jin Luo,[3] Chaim Bell.[1,3,4] *[1]St. Michael's Hospital; [2]London Health Sciences Centre; [3]Institute for Clinical Evaluative Sciences; [4]University of Toronto; [5]Foothills Medical Centre and University of Calgary.*

Background: Acute kidney injury (AKI) is associated with an increased risk of death and kidney disease progression. However, the optimal management of AKI survivors is unclear. We postulate that early follow-up with a nephrologist following a hospitalization for AKI may modify the risk of death.

Methods: Using linked administrative databases for all of Ontario for the period April 1994 to March 2008, we conducted a cohort study of all adults with AKI who received in-hospital dialysis and remained dialysis free at least 90 days after discharge. The exposure of interest was early nephrology follow-up, defined as at least one nephrologist visit within 90 days of discharge. A propensity-score was used to match those who did versus those who did not receive early nephrology follow-up after discharge. A Cox Proportional Hazards model was used to estimate the treatment effect. The primary endpoint was all-cause mortality within 2 years of cohort assembly.

Results: Of the 3877 eligible patients, 1553 (40%) received early nephrology follow-up. We successfully matched 1184 individuals who received early follow-up to 1184 of those who did not. The incidence of death was 0.02 per 100 person-years among individuals receiving early follow-up compared to 0.03 per 100 person years in those who did not (hazard ratio of 0.76, 95% confidence interval 0.62-0.93).

Conclusions: Early nephrology follow-up after a hospitalization with AKI requiring in-hospital dialysis was associated with improved survival. Whether this is a reflection of better care provided by nephrologists, or a biased tendency to bring less morbid individuals to nephrology care, is unknown. In light of these results, further research is needed to evaluate the processes of care undertaken by nephrologists when following-up patients hospitalized with AKI requring in-hospital dialysis.

Funding: Government Support - Non-U.S.

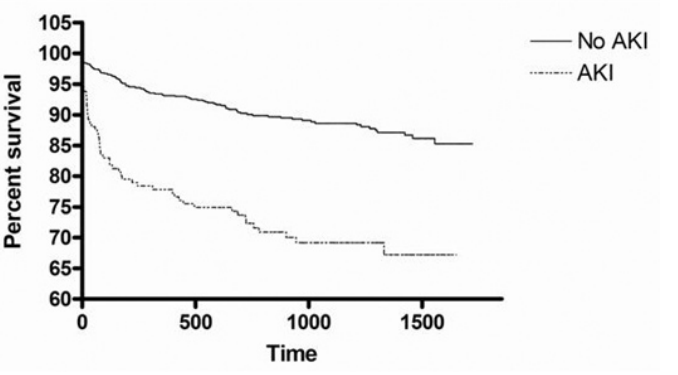
SA-PO2135

Acute Kidney Injury Is Associated with Increased Mortality after Cardiac Surgery in Patients with Pre-Existing Renal Impairment Sean Gallagher, Matt Lovell, Dan A. Jones, Krishnaraj S. Rathod, Akhil Kapur, Andrew Wragg, Magdi Yaqoob, Rakesh Uppal. *Cardiac and Renal, Barts and the London NHS Trust, United Kingdom.*

Background: Acute kidney injury (AKI) following coronary artery bypass graft surgery(CABG)has been associated with increased mortality. Whether AKI is as prognostically important in patients with pre-existing renal impairment remains unclear.

Methods: An analysis of prospectively collected data upon 874 patients with renal impairment (eGFR <60mls/min) undergoing CABG at a tertiary Cardiac centre between 2003 and 2007 was undertaken. AKI was defined by a 50% increase in serum creatinine post operatively. All-cause mortality was determined via Office of National Statistics data.

Results: The incidence of AKI in this cohort was 20.1%. Patients that developed AKI had lower pre-operative eGFR (45.2vs48.0mls/min, p=0.0014), more diabetes (38.1vs28.1%, p<0.0128) and Euroscore (a risk model used to estimate risk of death following cardiac surgery) was also higher (6.7vs5.7, p<0.0004). In hospital mortality was dramatically increased in patients that developed AKI (11.4vs 1.23%, p<0.0001). Moreover, long term survival was worse among patients that developed AKI (68.9vs88.1%, p<0.0001) with an adjusted hazard ratio of 3.57 (CI 2.28 to 5.61) when compared with patients that did not develop AKI.



After multivariate analysis impaired LV function (adjusted HR 1.96 (CI 1.38 to 2.79)) and age (adjusted HR 1.07 (CI 1.03 to 1.1)) were also additional factors independently associated with 5 year all cause mortality.

Conclusions: The development of AKI following CABG surgery is associated with worse 5 year mortality in patients with pre-existing renal impairment. Improved strategies to protect renal function during CABG surgery and more aggressive and timely medical therapy following CABG may be warranted to improve prognosis in this high risk patient group.

SA-PO2136

Kidney Biopsy in Intensive Care Units: Is it Realisable? Is it Useful? Results of the COBRRA Study Group Carole Philipponnet,[1] Saleh Kaysi,[1] Anne-Elisabeth Heng.[1] *[1]Nephrology, CHU G Montpied, Clermont-Ferrand, France; [2]Nephrology, CHU St Etienne, St Etienne, France; [3]Nephrology, CHU Poitiers, Poitiers, France.*

Background: In ICUs, Kidney biopsy (KB) is considered complex and dangerous to achieve. We describe the complications, diagnostic and therapeutic contributions of the KB in ICUs .

Methods: A Retrospective study was done in 5 ICUs. Inclusion criteria: Patients admitted between 01/00 and 09/10 with a Sequential Organ Failure Assessment score (SOFA) ≥ 3 (at least 1 organ other than kidney) and with a KB performed during ICU stay. Exclusion criteria: Autopsy. KB was contributive if it revealed a non-expected diagnosis or changed the patient management (start or stop a treatment, start a renal replacement therapy, or taking the decision of reducing the global medical management).

Results: Forty-six KB were analyzed. The patients Simplified Acute Physiology Score, SOFA at ICU admission was 54+/-19 and 10+/-4, respectively. ICU mortality was.21%. KB was performed as an average 8 days [1-126] after admission: 45 percutaneous and 1 trans-jugular ; 35 echo-guided in ICU and 7 CTscan guided. At the KB day, 16 patients had a SOFA score ≥3 (respiratory or hemodynamic) ; 6 KB content < 5 glomeruli.

Histological finding: 24 Acute Tubular Necrosis (ATN) ; 12 Acute Glomerular Lesions (3 Crescentic , 3 Cryoglobulinemia, 3 Wegner, 1 Lupus, and 2 Membranoproliferative), 7 Acute Vascular Lesions (5 Thrombotic microangiopathy, 1 Cholesterol Embolism Disease, and 1 Malignant Nephroangiosclerosis), 4 Acute Interstitial Nephritis, 2 Myeloma and 1 Amyloidosis. These findings led to start new treatments (n=20), to stop treatments (n=7), to start chronic hemodialysis (n=12) and to reduce global medical management (n=4). KB was contributive in 43 cases (diagnostic n=26 and therapeutic n=34) and not contributive .in 3 (1 expected ATN and 2 inadequate materials). Complications (bleedingt that necessitated transfusion of ≥2 blood units within 7 days of KB) occured in 5 patients, one of these was dead as a result of this complication.

Conclusions: KB is rarely performed in ICUs. However, it could be performed even in very ill patients and its diagnostic and therapeutic contribution could be very important.

SA-PO2137

AKIN (Acute Kidney Injury Network) Score Did Not Predict Survival after Orthotopic Liver Transplantation (OLT) Thais Nemoto Matsui, Maria C.C. Andreoli, Cláudia Tótoli, Maria P.V. Coelho, Nadia K. Guimaraes, Fellype Barreto, Jose Ben-Hur Ferraz-Neto, Bento Santos. *Einstein Dialysis Center & Liver Transplantation Unit, Albert Einstein Jewish Hospital, São Paulo, Brazil.*

Background: Previous studies have shown that perioperative renal failure exerts a significant negative impact on liver transplantation outcomes. However, serum creatinine cut-offs used in those studies tends to underestimate renal failure in patients with cirrhosis. The aim of the present study was to determine the impact of preoperative kidney dysfunction as assessed by the AKIN score on mortality rates within 28 days and 1 year after OLT.

Methods: This retrospective study included 286 patients with chronic liver disease who were consecutively admitted in an urban tertiary medical center to undergo OLT from June 1st, 2005, to December 31, 2009. AKIN score was determined at the moment of admission and immediately before OLT. AKI was defined according to the AKIN criteria as a 50% or greater increase of baseline creatinine level.

Results: From the 286 included patients (52.3±11.7 yrs), 54% had viral-related disease, with MELD of 19.4±9.8, and 21% had diabetes mellitus. AKI at admission and on the day of OLT was observed in 84 (29%) and 73 (26%) subjects, respectively, with the following AKIN scores: 1=48%, 2=23%, 3=29% at admission; 1=48%, 2=22%, 3=30% on the day of OLT. RRT was needed in 81 cases (28%). Mortality rate at 28 days was

6%, with similar rates across AKIN scores (1=7%, 2=5%, 3=4% at admission, p=0.597; 1=6%, 2=12%, 3=5% on the day of OLT, p=0.954). Likewise, mortality rate 1 year after OLT (14%) was not influenced by pretransplant AKIN scores (1=17%, 2=26%, 3=17% at admission, p=0.939; 1=11%, 2=19%, 3=18% on the day of OLT, p=0.462). After adjustment for MELD and diabetes mellitus, RRT (OR 4.036, CI95% 1.793–9.084, p=0.001) and older age (OR 1.039, CI95% 1.002–1.078, p=0.039) were independently associated with death within 1 year of OLT.

Conclusions: Unexpectedly, the AKIN score did not predict the mortality rates within 28 days and 1 year after OLT. The most important prognostic factor for survival was the need for peritransplant RRT.

SA-PO2138

Renal Function in Patients with Multiple Myeloma Treated with Bortezomib-Based Regimens: A Single-Center Cohort Analysis Catarina Calado Teixeira, Mário Raimundo, Jose António Lopes, Antonio Gomes da Costa. *Department of Nephrology and Renal Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

Background: Bortezomib is a proteasome inhibitor that blocks several molecular pathways in a cell and may cause cancer cells to die. When added to standard therapy significantly improves time to progression and overall survival in patients with multiple myeloma (MM). The aim of our study was to evaluate the effect of bortezomib-based regimens in renal function in patients with MM.

Methods: In all, 34 patients (mean age: 63 years; 18 male; 30 Caucasian) with MM treated with bortezomib-based regimens were studied retrospectively. Bortezomib was administered at the dose of 1.3mg/m^2 on days 1, 4, 8 and 11 of each cycle of 21 days. Twenty patients, eligible to stem cell transplantation (SCT), were treated with bortezomib, doxorubicin (40mg/m^2/4thd) and prednisolone (60mg/m^2/d). Six patients, not eligible to SCT, were treated with bortezomib, melfalan (9mg/m^2/d) and prednisolone (60mg/m^2/d). Eight patients, with relapsed or refractory MM, were treated with bortezomib and prednisolone (60mg/m^2/d). Glomerular filtration rate (GFR) was calculated by the Modification of Diet in Renal Disease equation. Comparisons between GFR values determined at the beginning of the first cycle and one month after the last cycle were performed by paired-samples T test. Pre-existing chronic kidney disease (CKD) was considered whenever baseline GFR was lower than 60 ml/min/1.73m^2. A *P*-value <0.05 was considered statistically significant.

Results: There were no statistically significant differences between GFR values determined at the first cycle and GFR values determined one month after the last cycle, both when evaluating all patients (97±49 ml/min/1.73m^2 *vs* 124±138 ml/min/1.73m^2, *P*=0.182) and when evaluating exclusively those patients with pre-existing CKD (N=6) (37±13 ml/min/1.73m^2 *vs* 56±28 ml/min/1.73m^2, *P*=0.077), although there was a trend towards improved renal function associated with bortezomib-based regimens.

Conclusions: Our results suggest that in patients with MM bortezomib-based regimens are not harmful to kidney: On contrary, such regimens can improve renal function mainly in patients with pre-existing CKD.

SA-PO2139

Nephroprotection: PBI-4419, a Novel Orally Active Anti-Fibrotic Agent, Reduces Doxorubicin-Induced Nephrotoxicity in Mice Brigitte Grouix, Kathy Hince, François Sarra-Bournet, André Doucet, Shaun Abbott, Jean-François Bienvenu, Jean-Simon Duceppe, Boulos Zacharie, Christopher Penney, Pierre Laurin, Lyne Gagnon. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: PBI-4419 is a novel, first-in-class, orally active low molecular weight compound which displays anti-fibrotic activity via inhibition of CTGF expression and production. The clinical use of doxorubicin (Dox), a potent anticancer agent; is associated with marked nephrotoxicity characterized by tubulo-interstitial lesions. The aim of this study was to investigate the anti-fibrotic activity of PBI-4419 on Dox-induced nephrotoxicity in mice.

Methods: On day 0, BALB/c mice were immunosuppressed with 10 mg/kg intravenous administration of Dox. The first experiment was undertaken with oral administration of PBI-4419 (1 and 10 mg/kg) from day -3 to day 10, and the second with intravenous administration of PBI-4419 (0.1 mg/kg) on day-3, 3 and 10. Mice were sacrificed at day 11 (oral treatment) or 14 (intravenous treatment).

Results: Treatment with oral (10 mg/kg) or intravenous (0.1 mg/kg) administration of PBI-4419 significantly reduced Dox-induced nephrotoxicity as measured by a reduction of serum albumin loss induced by doxorubicin. Histological lesions were also significantly reduced in PBI-4419-treated mice [Lesion scores determined by HPE staining: reduction of 70% with oral treatment (10 mg/kg, p=0.008); reduction of 50% with intravenous treatment, (0.1 mg/kg, p<0.05)]. Dox induced an increase in CTGF mRNA expression in the kidney. Treatment with PBI-4419 (10 mg/kg, oral daily and 0.1 mg/kg, i.v. administration once a week) resulted in a significant reduction (27%, p=0.007 and 20%, p=0.008, respectively) in the expression of CTGF in the kidney.

Conclusions: These results suggest that PBI-4419 acts as an anti-fibrotic agent in the acute phase of doxorubicin-induced toxicity. The nephroprotective effect of PBI-4419 relies, in part, on PBI-4419's ability to reduce CTGF expression and hence fibrosis.

Funding: Pharmaceutical Company Support

SA-PO2140

Nephroprotection: PBI-4050, a Novel Orally Active, Anti-Inflammatory/ Anti-fibrotic Agent, Reduces Doxorubicin-Induced Nephrotoxicity in Mice Brigitte Grouix, Kathy Hince, André Doucet, Nathalie Julien, Liette Gervais, Valérie Perron, Jean-Simon Duceppe, Abdallah Ezzitouni, Boulos Zacharie, Christopher Penney, Pierre Laurin, Lyne Gagnon. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: PBI-4050 is a novel, first-in-class, orally active low molecular weight compound which displays its anti-inflammatory/anti-fibrotic activity via inhibition of TGFβ and CTGF expression and production.

Methods: The anti-inflammatory activity was evaluated in the LPS-induced air pouch rat model. The anti-fibrotic activity was studied using the doxorubicin-induced nephrotoxicity mouse model. The clinical use of doxorubicin (Dox), a potent anticancer agent; is associated with marked nephrotoxicity characterized by tubulo-interstitial lesions. BALB/c mice were randomized in three groups; Control, Dox (10 mg/kg) and Dox (10 mg/kg) + PBI-4050 (200 mg/kg). Mice were treated with oral administration of PBI-4050 from day -3 to day 10. On day 0, mice were immunosuppressed with intravenous administration of Dox.

Results: In LPS-induced air pouch model, PBI-4050 (200 mg/kg) inhibits PGE2 production to the same extend as indomethacin (50 mg/kg). However, in contrast to indomethacin, PBI-4050 has no effect on cyclooxygenase and lipoxygenase pathways. Treatment with PBI-4050 significantly reduced Dox-induced nephrotoxicity as demonstrated by a reduction of serum albumin loss induced by doxorubicin. Histological lesions were also significantly reduced (p<0.05) in PBI-4050-treated mice (Lesion scores determined by HPE staining at Day 7 Dox: 1.03; Dox + PBI-4050: 0.18; at Day 11; Dox: 2.0; Dox + PBI-4050: 0.72). The Dox treated group showed an increase in TGFβ and CTGF mRNA expression in the kidney. Treatment with PBI-4050 gave a significant reduction (p=0.03) of the expression of TGFβ (26%) and CTGF (33%) in the kidney.

Conclusions: These results suggest that PBI-4050 exerts anti-inflammatoy and anti-fibrotic properties in the acute phase of doxorubicin-induced toxicity. The nephroprotective effect of PBI-4050 relies, in part, on the antifibrotic activity as suggested by a decrease of TGFβ and CTGF expression in the kidney.

Funding: Pharmaceutical Company Support

SA-PO2141

Elucidating the Role of Notch Signaling in Acute Kidney Injury Jin Nakamura, Nariaki Asada, Tomomi Endo, Motoko Yanagita. *Graduate School of Medicine, Kyoto University, Kyoto, Japan.*

Background: Notch signaling plays a crucial role in the regulation of cell proliferation, stem cell maintenance and differentiation during embryonic and adult development. Recent studies reported that the activation of Notch signaling in tubular epithelial cells augments interstitial fibrosis. However, the role of Notch signaling in tubule cell maintenance and regeneration remains unclear.

Here we analyzed the role of Notch signaling in the regeneration of proximal tubules during AKI, utilizing RBP-j conditional knockout mice and proximal tubule-specific inducible Cre mice that we recently generated, in which Cre is activated only after the administration of tamoxifen.

Methods: We bred RBP-j conditional knockout mice to the proximal tubule-specific inducible Cre mice (NDRG1-CreERT2 mice) to generate RBP-j knockdown (RBP-j flox/-:NDRG1-CreERT2) and control (RBP-j flox/+ :NDRG1-CreERT2) littermates.

We administered tamoxifen at 4 wks of age, performed ischemic reperfusion (I/R) injury 2 wks after the adminstration of tamoxifen, and analyzed the kidneys 4 days after the operation.

Results: The expression of RBP-j mRNA in the whole kidneys of knockdown mice was reduced to about 30% of control kidneys, indicating the effective deletion of RBP-j in the proximal tubules. Unexpectedly, however, the proliferation of tubular epithelial cells and severity of tubular injury was indistinguishable between both genotypes. The expression of Cyclin D1, HSP47, and osteopontin in the knockdown kidneys tended to be lower compared to that of control kidneys, but the difference was not statistically significant.

Conclusions: Reduction of Notch signaling in proximal tubules during AKI did not cause significant change in the proliferation of tubular epithelial cells and severity of tubular injury. Considering the role of tubular Notch signaling in the progression of interstitial fibrosis, it is likely that epithelial Notch signaling affects the neighboring fibroblasts, but not neighboring epithelial cells.

SA-PO2142

Six2-GDNF Pathway Is Activated during Experimental Acute Kidney Injury and Plays a Crucial Role in Renal Tubular Regeneration Seiki Hirano,[1] Nazuki Okada,[2] Madoka Urushido,[2] Masayuki Bun,[2] Masayuki Hisa,[2] Koji Ogata,[1] Yoshiko Shimamura,[1] Kosuke Inoue,[1] Masayuki Ishihara,[1] Toru Kagawa,[1] Toshihiro Takao,[1] Yoshio Terada.[1] *[1]Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Kochi, Japan; [2]Centre for Innovative and Translational Medicine, Kochi Medical School, Kochi University, Kochi, Japan.*

Background: The transcriptional regulator Six2 emerged as a key factor in kidney development and maintenance of nephron progenitor cells. GDNF (glial cell-derived neurotrophic growth factor) is also reported to play an important role for nephrogenesis.

However, the roles of Six2-GDNF pathway in tubular proliferation and regeneration are poorly understood in acute kidney injury (AKI).

Methods: The aim of this study is to understand the functional roles of Six2 and GDNF in tubular damages in AKI *in vivo* and *in vitro*.

Results: To clarify the significance of Six2-GDNF pathway in AKI, we used a rat AKI model *in vivo* and renal tubular cells (NRK-52E cells) as an *in vitro* model. After clamping left rat renal artery for 1h, kidney homogenates from 3 to 72h after reperfusion were extracted. In Western blot analysis, Six2 and GDNF expressions were increased at 3-12h and 6-24h, respectively. In immunohistological examinations, Six2 positive cells and GDNF positive cells were observed in proximal tubules after AKI. In *in vitro* experiments, hypoxia stimulated mRNA expression and protein expression of Six2. To understand the downstream signaling of Six2, we transfected Six2 expression vector to NRK-52E cells. Overexpression of Six2 caused increments of GDNF promoter activity and the expressions of mRNA and protein. Overexpression of Six2 increased ^{3}H-thymidine uptake. Furthermore, we used 3D gels to examine the role of Six2 for tubular formation. Overexpression of Six2 promoted tubular formation, while tubular formation was inhibited by transfection of Six2 siRNA.

Conclusions: Six2 and GDNF were up-regulated in AKI at proximal tubular cells *in vivo*, and Six2 could regulate cell proliferation and tubular formation by regulating GDNF expression. The current study therefore unveils pathophysiological significance of Six2-GDNF pathway in AKI *in vivo* and *in vitro*.

SA-PO2143

Protective Effect of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells on Experimental Kidney Warm Ischemia-Reperfusion Injury in Mice Hye Ryoun Jang,[1] Ji Hyeon Park,[1] Seung Tae Han,[1] Jaeyoung Yoon,[1] Soo Jin Choi,[2] Wonil Oh,[2] Yoon Sun Yang,[2] Jung Eun Lee,[1] Wooseong Huh,[1] Dae Joong Kim,[1] Ha Young Oh,[1] Yoon-Goo Kim.[1] *[1]Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Biomedical Research Institute, MEDIPOST Co., Ltd., Seoul, Korea.*

Background: Kidney ischemia-reperfusion injury (IRI), the leading cause of acute kidney injury (AKI), is characterized with robust inflammatory response. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have been reported to show hypo-immunogenic property and exert anti-inflammatory and regenerative effects in a cerebral IRI model. We investigated the effects of hUCB-MSCs on renal injury following warm IRI in mice.

Methods: C57BL/6 mice were randomly allocated into 2 groups: control group (IRI without medication, n=13) and hUCB-MSCs group (n=15). A total of $1.5X10^{6}$ hUCB-MSCs were injected into the peritoneal cavity twice: 24 hour prior to IRI and right before reperfusion. Serum creatinine was measured for 48 hours. The trafficking of PKH-26 labeled hUCB-MSCs was assessed with immunofluorescence staining.

Results: Renal functional impairment was attenuated in the hUCB-MSCs group compared with the control group (serum creatinine mean ± SE, Day 0: 0.49±0.044 in the control group, 0.50±0.014 in the hUCB-MSCs group, Day 1: 2.16±0.037 in the control group, 1.75±0.097 in the hUCB-MSCs group, Day 2: 2.68±0.115 in the control group, 1.89±0.184 in the hUCB-MSCs group). PKH-26 labeled hUCB-MSCs were found in the post-ischemic kidneys of the hUCB-MSCs group on both day 1 and day 2 after IRI.

Conclusions: The hUCB-MSCs were trafficked into the post-ischemic kidneys and attenuated renal injury following IRI.

SA-PO2144

Improving Ischemia-Reperfusion-Induced Acute Kidney Injury by Treating with Induced Pluripotent Stem Cells Pei-Ying Lee,[1] Der-Cherng Tarng.[1,2] *[1]Institute of Physiology, National Yang-Ming University, Taipei, Taiwan; [2]Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.*

Background: Induced pluripotent stem (iPS) cells are novel stem cell populations induced from somatic cells, but the effects of iPS cells on acute kidney injury (AKI) are not currently known.

Methods: To investigate effects of iPS cells on AKI, iPS cells were injected via the renal artery into rats with ischemia-reperfusion (I/R) AKI and compared with PBS-treated AKI rats. Renal function,histopathological findings, blood reperfusion ratio, and the levels of ROS-related factors and inflammatory cytokines were measured and analyzed.

Results: Our findings showed that iPS cells injected into kidney of AKI rats reduced the impairment of renal functioning and tissue injury compared to PBS-treated rats, and that maximal improvement was observed at a cell dose of iPS 5×10^{5}. However, transplantation of large number (5×10^{7}) of iPS cells may decrease blood flow recovery, which was measured by laser Doppler imaging. At 48 hours after I/R, the iPS cells had mobilized to peritubular area and significantly diminished the histopathological changes associated with AKI, such as macrophages infiltration and the presence of apoptotic cells; in addition there was an enhancement of cell proliferation. Notably, iPS cell transplantation reduced the response to I/R injury in relation to ROS-related factors and inflammatory cytokines.

Conclusions: Injection of iPS cells into AKI rats provided a significantly beneficial effect by diminishing the impairment of renal functions, reducing tissue injury and protecting rats from the lethal effects of AKI. The effect to rescue AKI may be mediated by a reduction in ROS production and inflammation.

Funding: Government Support - Non-U.S.

SA-PO2145

Effects of Adipose-Derived Mesenchymal Cells on Ischemia-Reperfusion Injury in Kidney Kengo Furuichi,[1] Yasuyuki Shinozaki,[2] Shuichi Kaneko,[2] Takashi Wada.[3] *[1]Division of Blood Purification, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; [2]Department of Disease Control and Homeostasis, Kanazawa University, Kanazawa, Ishikawa, Japan; [3]Department of Laboratory Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan.*

Background: Acute kidney injury (AKI) is critical condition for kidney and other remote organs including lung. However, currently available treatments for AKI are limited. In this study, we explored the effect of adipose-derived mesenchymal cells on a mouse model of AKI.

Methods: Adipose-derived mesenchymal cells was isolated from mouse subcutaneous and peritoneal adipose tissue by digestion with collagenase type I. The left renal artery and vein of C57BL/6 mice were clamped for 45 minutes to induce ischemia, and were injected with the adipose-derived mesenchymal cells from GFP mice(1 x 10∧5 cells/0.2 mL PBS) or 0.2 ml PBS via the tail vein on Days 0, 1 and 2. In each group, mice were sacrificed on Day 2, 4 and 7. Blood was collected from the inferior vena cava of each sacrificed mouse, accompanied by collection of kidney, lung, brain, spleen and liver.

Results: The adipose-derived mesenchymal cells had stem cell surface markers and multi-lineage differentiating potentials. Administered adipose-derived mesenchymal cells were mainly to home into lung. Interrestingly, repeated administration of adipose-derived mesenchymal cells reduced acute tubular necrosis and interstitial macrophage infiltration in the injured kidney accompanied with reduction in cytokine and chemokine expression in the injured kidney. Moreover, Ischemic kidney injury affected lungs. The number of F4/80-positive cells was lower in the adipose-derived mesenchymal cells administration group than in the control group 7 days after kidney ischemic injury. In vitro study indicated that adipose-derived mesenchymal cells produced anti-inflammatory cytokines such as TGF-β and GCSF, as well as others inflammatory cytokines and chemokines under stimulation of pro-inflammatory cytokine; IL-1.

Conclusions: Adipose-derived mesenchymal cells can be used as cell-based therapy for ischemic kidney injury.

SA-PO2146

Hydrogel-Embedded Endothelial Progenitor Cells Evade LPS and Mitigate Endotoxemia Tammer N. Ghaly, May Rabadi, Mia Weber, Seham Rabadi, Michael Bank, John Grom, Michael S. Goligorsky, Brian B. Ratliff. *New York Medical College, Valhalla, NY.*

Background: Sepsis and its complications are associated with poor clinical outcomes. The circulatory system is a well-known target of lipopolysaccharide (LPS). Recently, several clinical studies documented mobilization of endothelial progenitor cells (EPCs) during endotoxemia, with the probability of patients' survival correlating with the rise in circulating EPCs. This fact combined with endotoxemia-induced vascular injury lead us to hypothesize that the developing functional EPC incompetence could impede vascular repair and that adoptive transfer of EPCs could improve hemodynamics in endotoxemia.

Methods: We used LPS injection to model endotoxemia.

Results: EPCs isolated from endotoxemic mice exhibited impaired clonogenic potential and LPS exerted TLR4-mediated cytotoxic effects toward EPCs, which was mitigated by embedding them in hyaluronic acid (HA) hydrogels. Therefore, intact EPCs were either delivered intravenously or embedded within pronectin-coated HA-hydrogels. Adoptive transfer of EPCs in LPS-injected mice improved control of blood pressure and reduced hepatocellular and renal injury. Specifically, EPC treatment was associated with the restoration of renal microcirculation and reduction in renal dysfunction. EPC therapy was most efficient when cells were delivered embedded in HA-hydrogel.

Conclusions: These findings establish major therapeutic benefits of adoptive transfer of EPCs, especially when embedded in HA-hydrogels, in mice with LPS-induced endotoxemia, and argue that hemodynamic and renal abnormalities of endotoxemia are in significant part due to developing incompetence of endogenous EPCs.

Funding: NIDDK Support

SA-PO2147

Intravenous Renal Cell Transplantation (IRCT) for Renal Failure Katherine J. Kelly,[1] Jesus H. Dominguez.[1,2] *[1]Medicine Nephrology, IUMC, Indianapolis, IN; [2]Nephrology, VAMC, Indianapolis, IN.*

Background: AKI is unpredictable and its outcome ranges from minor to end-stage renal disease (ESRD). Multiple therapies have failed. We now propose to test in rats the hypothesis that AKI can be markedly improved by intravenous renal cell transplantation (IRCT) with primary renal epithelial cells.

Methods: The cells were derived from normal male rat tubules transfected with empty vector, and plasmids designed to express cytosolic GFP and nuclear BFP ("A" cells), or with plasmid expressing tubulogenic Serum Amyloid A1 protein (SAA) plus the other tracking vectors ("B" cells). AKI was induced by 50 min ischemia and reperfusion (I/R) injury in 2 groups of Sprague Dawley female rats.

Results: After 24 hrs post I/R serum creatinine (CR) was 2.62 ±0.29 mg/dl (N = 14 M±SE) in group A and 2.79 ±0.19 in B (N= 12), and "A" cells were infused to A group and "B" cells to group B. The following day CR was 3.94 ±070 in A rats and much lower, 1.01 ±0.29 in B rats, $p<0.007$. The severity of AKI is emphasized by 5 deaths in A rats and zero deaths in B rats, $p = 0.02$. BUN followed same pattern for A and B rats and remained higher in A rats for the 7 day study, at this point rats in group A had more fibrosis and tubular atrophy. There was substantial B cell engraftment on surfaces vacated by dead tubular cells

as indicated by co-expressed cytosolic GFP, nuclear BFP and SAA. Also male SRY gene was retained by female kidneys.We then studied a model of CKD from platinum (Plt, 1.5 mg/kg daily X 3) and three weeks later BUN was 115±3 in A and 121±13 in B rats (mg/dl; N =3). The rats were uni-nephrectomized, tubules transfected and cultured X 7 days with growth promoters and cells infused (auto-transplant). After 12 days BUN was 101±15 in A and 53±5 in B rats, p <0.02.

Conclusions: IRCT is an effective therapy to promote recovery of renal failure and is subject to amplification, where one rat donates to six rats. IRCT acts acutely by paracrine mechanisms and long-term by structural integration of transplanted cells. IRCT can also be used as auto-transplantation, where damaged cells are recovered, healed in vitro, and injected with successful improvement of renal function.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2148

Adipose-Derived Stem Cells Transfected with Hypoxia Inducible Factor-Lα Could Lessen the Injury of HK-2 Cells Indcued by Cisplatin Weiwei Wang, Jinyuan Zhang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: Adipose-derived mesenchymal stem cells (ADSCs) maybe an alternative cell source in cell therapy for some advantages. We hypothesize that ADSCs transfected by HIF-1α gene will have a positive effect on the cisplatin-induced HK-2 cells.

Methods: The hADSCs were transfected by lentiviral vector containing EGFP-HIF-1α genes. Cells expressing EGFP was sorted by flow cytometer. The expression of HIF-1α was examined by immunocytochemical method. The following groups were made: I: Cisplatin-induced group: Cisplatin (3μg/ml)-induced HK-2 cells; II: Transfected group: HIF-1α-transfected hADSCs and cisplatin-induced HK-2 cells were incubated; III: Non-transfected group: hADSCs and cisplatin-induced HK-2 cells were incubated; IV: Control group: HK-2 cells. The ultrastructural changes of HK-2 cells was observed by electron microscopy. Apoptosis index of HK-2 cells was tested by TUNEL. Western blot was performed to examine the expression of caspase-3 and Bcl-2 in HK-2 Cells and also the expression of EPO, HO-1 and VEGF in hADSCs cells. The concentration of NGAL, KIM-1, EPO, HO-1, VEGF and HIF-1α in media were tested by ELISA.

Results: The apoptosis in coincubation groups were decreased compared with the cisplatin group. The expression of caspase-3 in HK-2 cells in coincubation groups was lower than that in cisplatin group and the expression of Bcl-2 in HK-2 cells in coincubation groups was increased. The injury of HK-2 cells coincubated with hADSCs tranfected with HIF-1α were lessened. The concentration of KIM-1 increased markedly in cisplatin-induced HK-2 group and that was decreased after coincubated with hADSCs. The protein expression of EPO, HO-1 and VEGF in hADSCs cells tranfected with HIF-1α were increased compared with the vector transfected group. The concentration of HO-1 and VEGF in media of gene-transfected group were higher than in vector transfected group and the variation of HIF-1α and EPO were not obvious.

Conclusions: The hADSCs could lessen the apoptosis of HK-2 cells induced by cisplatin, especially after transfected with HIF-1α and the protection may be related to the higher expression of growth factors. Specific mechanism needs further experimental study.

Funding: Government Support - Non-U.S.

SA-PO2149

The Alteration of Macrophage Polarisation and Phenotype Via Mesenchymal Stem Cells In Vitro and In Vivo Sharon D. Ricardo, Maliha A. Alikhan, Junli Zhuang, Andrea Wise. *Monash Immunology and Stem Cell Laboratories, Monash University, Clayton, Victoria, Australia.*

Background: Monocyte-derived macrophages comprise a heterogenous population of cells that govern tissue remodeling and repair following acute kidney injury. Infiltrating monocytes can differentiate into a pro-inflammatory/deleterious (M1) or anti-inflammatory/reparative (M2) polarisation state depending on the inflammatory stimuli. Mesenchymal stem cells (MSCs) may exert therapeutic effects through their unique immunomodulatory properties, which enable them to interact with various cells of the immune system. This study determined whether human bone marrow derived MSCs (hMSCs) can modulate the polarisation state of macrophages in vitro, following macrophage and hMSC co-culture, and in vivo, following the exogenous administration to mice with induced ischemia-reperfusion (IR) injury.

Methods: C57BL6/J mice (20-25g; n = 5/group) underwent 40 minutes of unilateral IR injury and following reperfusion were injected with 1x106 hMSCs (i.v) or vehicle. Flow cytometry was used to assess macrophage polarisation following hMSC therapy at 1, 3, 5 and 7 days post-treatment. In vitro, hMSCs and bone marrow-derived macrophages were co-cultured both directly and indirectly for up to 72 hours and macrophage phenotype was assessed using qPCR.

Results: In comparison to vehicle-treated IR kidneys that exhibited widespread damage, hMSC-treated mice with IR injury demonstrated structural repair showing reduced interstitial matrix expansion and cellular replacement at 7 days post-hMSC treatment. IR resulted in a significant infiltration of CD45+CD11b+F4/80+ macrophages expressing mannose receptor and MHC class II. hMSC treatment did not significantly alter the number of infiltrating inflammatory cells in IR injury in vivo, however in vitro evidence suggests they modulate macrophage phenotype via paracrine mechanisms.

Conclusions: The administration of hMSCs to mice with induced IR ameliorates injury and promotes renal regeneration and repair. hMSC treatment does not alter the number of macrophages infiltrating the kidney post-IR however, may promote the polarization of a M2 macrophage that leads to downstream tissue remodeling.

SA-PO2150

Mesenchymal Stem Cells and Endothelial Progenitor Cells Improve Renal Function in Experimental Swine Renal Artery Stenosis through Different Mechanisms Xiang-Yang Zhu,[1] Alfonso Eirin,[1] James Krier,[1] Stephen C. Textor,[1] Amir Lerman,[2] Lilach O. Lerman.[1] *[1]Nephrology & Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic.*

Background: Both endothelial progenitor cells and mesenchymal stem cells can augment tissue repair. Whether cell phenotype affects the efficacy of cell therapy in the stenotic kidney is unclear.

Methods: Peripheral blood EPC and adipose derived MSC were expanded and characterized by cell surface markers (e.g. CD34 and KDR, or CD44 and CD90 respectively). Single kidney hemodynamics and function were assessed using multi detector CT in pigs after 10 weeks of renal artery stenosis treated 4 weeks earlier with an intra-renal infusion of vehicle EPC (10x10^6, RAS+EPC, n=6) or MSC (10x10^6, RAS+MSC, n=6), and normal controls. Kidney microvascular structure, growth factors, apoptosis, oxidative stress and inflammatory pathways were evaluated in vitro.

Results: The degree of stenosis and hypertension were similar in RAS, RAS+EPC and RAS+MSC. Renal blood flow and glomerular filtration rate in RAS were lower than normal controls, but similarly improved in RAS+EPC and RAS+MSC. EPC mainly improved renal growth factor expression and oxidative stress, whereas MSC significantly attenuated inflammatory cytokines, endoplasmic reticulum stress related protein expression, and apoptosis.

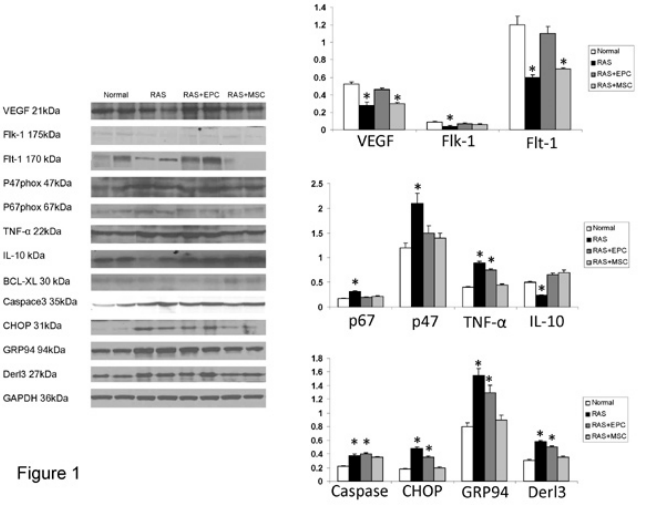


Figure 1

Conclusions: Intra-renal delivery of EPC and MSC similarly improved renal function, but through different pathways. These results support development of selective cell based approaches for management of kidney disease.

Funding: Other NIH Support - DK73608, DK77013, HL77131, and HL085307

SA-PO2151

Human Umbilical Cord Blood CD133$^+$ Cells Exacerbate Acute Kidney Injury in NOD-SCID Mice Dylan Burger, David Allan, Alex Gutsol, Anthony Carter, Rhian Touyz, Kevin D. Burns. *Kidney Research Centre, Division of Nephrology, Dept. of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada.*

Background: Acute kidney injury (AKI) occurs in 5% of hospitalized patients, with a 50% mortality. An increase in circulating vascular progenitor cells occurs in humans with AKI, and certain blood-derived progenitor cells facilitate recovery in animal models of tissue injury, including AKI. CD133$^+$ cells isolated from human umbilical cord blood are associated with improvement following acute injury in several settings. We examined the effects of CD133$^+$ progenitor cells in a mouse model of AKI, induced by ischemia-reperfusion (I/R).

Methods: In non-obese diabetic severe combined immunodeficient (NOD-SCID) mice, I/R was induced by bilateral clamping of the renal artery and vein for 60 min, followed by clamp release. Human cord blood CD133$^+$ cells or a mixed population of CD133$^-$ cells (10^6/mouse) were injected via the jugular vein at reperfusion.

Results: Fluorescently-labeled CD133$^+$ cells were detected in blood at 2 min following i.v. injection (35% bioavailability) but numbers decreased rapidly (2% at 1 hr). Homing to the kidney, liver, spleen, brain, lung, bone marrow, or heart was not reliably detected. Administration of CD133$^+$ cells did not alter plasma urea or creatinine in sham-operated mice. In contrast, in mice subjected to I/R, CD133$^+$ cells significantly increased plasma urea at 24 hrs (CD133$^+$: 63 vs control: 34 mM, $p<0.01$, n=11-12), and serum creatinine at 6 and 24 hrs (respectively, CD133$^+$: 47 and 95 vs control: 18 and 43 μM, $p<0.05$). Administration of CD133$^+$ cells exacerbated histologic tubular necrosis. No change in blood pressure occurred at 6 hrs in mice treated with CD133$^+$ cells. CD133$^-$ cells did not alter blood urea, creatinine or histologic indices of injury compared with I/R alone ($p>0.05$). Administration of CD133$^+$ cells to immunocompetent mice (FVB/N) with I/R also increased renal injury.

Conclusions: These data indicate that human cord blood-derived CD133$^+$ cells exacerbate murine I/R renal injury, possibly via release of soluble factors. Our study highlights the importance of organ-specific responses and caution in any cell-based therapies for AKI.

Funding: Clinical Revenue Support

SA-PO2152

Adult Renal Progenitor Cells Revert Acute Renal Tubular Cells Injury by Means of TLR2-Driven Specific Paracrine Factors Fabio Sallustio,[1,2] Vincenzo Costantino,[1] Sharon N. Cox,[1] Antonia Loverre,[1] Marco Rizzi,[3] Francesco Paolo Schena.[1,2] *[1]Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]Department of Human Anatomy and Histology, University of Bari, Bari, Italy.*

Background: Acute Kidney Injury (AKI) is emerging as a public health problem worldwide, characterized by acute tubular apoptosis and necrosis. Tubular damage may recover, although a critical number of surviving cells is required to reconstitute structural integrity. Recently, many researchers focused their attention on the possibility of using adult renal progenitor cells (ARPCs) to improve regeneration in AKI. We studied the influence of ARPC on the regenerative process of cisplatin-injured renal proximal tubular epithelial cells (RPTECs) and injured Human Kidney 2 (HK2) cells.

Methods: We set up an in vitro model of cisplatin-induced toxicity, in which RPTECs were co-coltured with ARPCs, and performed proliferation and apoptosis assays to study regenerative effect of ARPCs on RPTECs. Moreover, we performed multiplex ELISA assays to identify molecules responsible for regenerative processes.

Results: Exposure of RPTECs or HK2 cells to cisplatin markedly reduced cell number and their viability, but co-culture with tubular ARPCs (TARPCs) provided a protective effect by promoting tubular cell proliferation of survival cells and inhibiting cisplatin-induced apoptosis. Tubular cell regeneration process was specific of TARPCs and occurred only following the sensing of a damage. On the contrary, when glomerular ARPCs were cocultured with damaged tubular cells, any effect of regeneration was observed. Surprisingly, regenerative effect was completely cancelled blocking the TLR-2, expressed by TARPC. By bioinformatic analyses on microarray data and by multiplex cytokine assays, we identified some specific citokines, growth factors and microvescicle-shuttled mRNAs, secreted by ARPCs and dependent from TLR2 activation, that worked synergistically and were essential in the regenerative process.

Conclusions: In conclusion, we identified for the first time a regenerative mechanism, driven by the TLR2, by which ARPCs induced regeneration of tubular epithelial cells.

SA-PO2153

Modulation of Kidney Injury by Mesenchymal Stromal Bone Marrow Cells (MS-BMC) in a Novel Puromycin Single Kidney Injury Model Dileep Kumar, Kang Cheng, Partab Rai, Rivka Lederman, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Puromycin has been demonstrated to induce podocyte apoptosis. Mesenchymal stromal bone marrow cells (MS-BMCs) have been demonstrated to provide cytoprotection by the modulation of cytokine production in several cytotoxic models. In the present study, we evaluated the effect of MS-BMCs on puromycin-induced podocyte injury.

Methods: MS-BMCs were harvested from bone marrows of mice and their profile was characterized (reported in another abstract). Mice in groups of six were administered either buffer (group A), puromycin alone (group B, 150 mg/Kg, subcutaneously), intracapsular instillation of MS-BMCs in left kidney 24 hours prior to puromycin administration (group C, 150 mg/Kg, subcutaneously). All mice were sacrificed on 5th day; urine and blood samples were collected for BUN and albumin: creatinine ratio. Kidneys were harvested for histology and TUNEL staining. Immunoblots were prepared and probed for nephrin and CD2AP expression. Immunohistochemical studies were carried out to study interstitial inflammatory milieu. In parallel sets of experiments, conditioned media of MS-BMC was collected. The effect of conditioned media of MS-BMC was evaluated on puromycin-induced podocyte apoptosis in in vitro studies.

Results: Group C mice displayed decrease($P<0.05$) in albumin: creatinine ratio vs. Group B mice. Immunoblotting studies revealed decreased ($P<0.01$) nephrin and CD2AP expression in renal tissues from group B and right kidneys of group C vs. group A. On the other hand, renal tissues of the Left kidneys from Group C displayed increased ($P<0.01$) expression of renin and CD2AP when compared to contralateral kidneys. Similarly, left kidneys from the group C displayed decreased number for TUNEL +ve glomerular cells when compared to the contralateral kidney of the same group and kidneys from group B. In in vitro studies, conditioned media from MS-BMC provided partial protection to podocytes from pro-apoptotic effect of puromycin.

Conclusions: These findings indicate that MS-BMCs provide protection from injurious effect of puromycin by modulating pro-apoptotic milieu.

Funding: NIDDK Support

SA-PO2154

Therapy of Acute Kidney Injury in Pigs with Porcine Mesenchymal Stromal Cells Is Ineffective Because They Possess Inadequate Immune-Modulating Activity Anna Gooch,[1] Barbel Brunswig-Spickenheier,[2] Janna Boche,[2] Frauke Peimann,[2] Achim Gruber,[2] Kai Jaquet,[2] Korff Krause,[2] Jozef Zustin,[2] Axel Zander,[2] Claudia Lange,[2] Christof Westenfelder.[1,3] *[1]Medicine/Nephrology, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Clinic for Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [3]Physiology, University of Utah and VA Medical Centers, Salt Lake City, UT.*

Background: We demonstrated that allogeneic mesenchymal stromal cells (MSC) are highly renoprotective in rats with AKI, and promising in a Phase I Trial (*Nature Rev Nephrol* 2010), and this without eliciting an antibody response. We showed earlier that human MSCs in rats with AKI afford significant renoprotection, while followed by an antibody response to human MSCs. In vitro, we confirmed substantial phenotypic overlaps between rat, human, and porcine MSCs (pMSC), all of which exhibited tri-lineage differentiation, antigen profiles, and secretion of renoprotective VEGF-A and IGF-1. However, in striking contrast to human and rat MSCs, pMSCs failed to inhibit the mixed lymphocyte reaction and induced robust production of pro-inflammatory IL-6.

Methods: We then tested the hypothesis that the full expression of the immune-modulating/anti-inflammatory activities of MSCs is critical for their protective actions in experimental AKI. Groups of female pigs with bilateral ischemia/reperfusion AKI were infused with autologous or male allogeneic pMSCs.

Results: Strikingly, MSC therapy had no beneficial effects on kidney function and histopathology.

Conclusions: Summary: in contrast to allogeneic or xenogeneic rodent models (human MSC into rats), infusion of pMSCs into pigs with AKI was not kidney-protective. Conclusion: This therapeutic ineffectiveness is due to the inadequate immune-modulating activity of pMSCs, clearly demonstrating that effective therapy of AKI with MSCs depends critically on both their anti-inflammatory and trophic actions, rendering the pig model of AKI and its therapy with pMSCs not informative for human studies. We expect, however, that treatment of human AKI with immune-modulating, allogeneic MSCs will be as effective as in rodent models.

Funding: Veterans Administration Support, Private Foundation Support

SA-PO2155

Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSC) Provide Partial Protection Against Kidney Injury Kang Cheng,[1] Kuldeep K. Bhargava,[2] Christopher J. Palestro,[2] Ashwani Malhotra,[1] Sanjeev Sanjeev Gupta,[3] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Nuclear Medicine, North Shore LIJ Hofstra Medical School, New Hyde Park, NY; [3]Medicine, Albert Einstein College of Medicine, Bronx, NY.*

Background: The immunomodulatory potential of BM-MSC is of much interest for cell-based therapies in multiple disorders, including acute kidney injury (AKI). To define the therapeutic potential of BM-MSC in cisplatin-induced AKI, we studied syngeneic mice.

Methods: Primary mouse MSC were isolated from compact bone of healthy syngeneic C57BL/6-GFP donors. To determine biodistributions of transplanted MSC, we injected ^{111}In-oxine labeled MSC i.v. into healthy and cisplatin (12.7mg/kg) -treated mice. Since only limited numbers transplanted MSCs were localized in the kidneys, we examined whether MSC served roles through paracrine signaling. The conditioned medium from BM-MSC was probed by high density protein arrays for 97 cytokines.

Results: Cellular phenotype was verified by FACS for CD11b, CD14, CD45, CD86, CD90, CD105, and their differentiation into adipocytes or chondrocytes. Transplanted cells were localized by GFP immunostaining and DNA PCR. BM-MSC accumulated primarily in the lungs (67%), followed by liver (13%), spleen (11%), and kidneys (5%). These cells were rapidly destroyed in pulmonary capillaries and other organs after 7 days. In mice treated with cisplatin, significant AKI was observed. By contract, BM-MSC transplantation provided partial protection to cisplatin-treated mice (on day 5, BUN 56mg/dl vs 44mg/dl; $P<0.05$; severe vs moderate tubular cell apoptosis). Protein arrays of the condition media identified 30 cytokines, including osteoponin, osteoporotegerin, galectin-1, decorin MMP2, gas6, RANTES, sTNFR1, CXCL1, CXCL16, VCAM-1, MCP1, MIP1 alpha, MIP2, IL6, etc.

Conclusions: Targeting of BM-MSC via i.v. injection resulted in cells confined largely to pulmonary capillaries, most of which were rapidly destroyed. BM-MSC did exert beneficial effects on cisplatin-induced AKI due to release of soluble factors capable of modifying pathophysiological processes

Funding: NIDDK Support

SA-PO2156

The Bone Marrow-Derived Mesenquimal Stem Cells Repair the Acute Kidney Injury Induced by Acyclovir Joelma Santina Christo, Paulo Maciel Lopes, Waldemar S. Almeida, Luciana Aparecida Reis, Nestor Schor. *Medicina, UNIFESP, São Paulo, Brazil.*

Background: The Acyclovir is an antiviral drug used to treat herpes simplex type 1 and 2 and varicella zoster. This drug is widely distributed in all body tissues, being very high in the kidneys, which may induce nephrotoxicity characterized by an acute injury. Several groups have reported the contribution BMSC in repair processes employing different animal models.

Methods: The BMSC were characterized for FACS analysis and then, differentiation into adipocytes and osteocytes. After, the cells were cultured and used 4th passages for all experiment. The female Wistar rats received acyclovir (80mg/Kg/BW) (Acyclovir group) or water (CTL) in a dose intraperitoneally (N=10) during 5 days. After 48, 72 hours, the female rats received iv BMSC (1X10^{6}cells). Then, blood and 24 hours blood were collected for urea (U), creatinine (Cr) evaluations. The animals were sacrificed and kidneys were perfused and removed for histology

Results: It was observed that Cr increased in acyclovir group (1.7± 0.1 mg/dl) and U (174.5±0.2 mg/dl p<0.05) when compared to CTL (0.7±0.01cr and U 56.0±0.1 mg/dl p<0.05) after 5 days of treatment with acyclovir. The Acyclovir+BMSC groups, observed a decrease Cr and U serum after 48 hours (Cr 1.3±0.1 Acyclovir vs Acyclovir+ BMSC 0.9±0.2 and U 143.5±0.2 vs Acyclovir+ BMSC 89.2±0.2 P< 0.05), 72 hours (Cr 1.7±0.1 Acyclovir vs Acyclovir+ BMSC 1.0±0.11 and U 174.5±0.2 Acyclovir vs Acyclovir+ BMSC 112.9±0.01 P< 0.05) when compared to in acyclovir groups. After 5 days of treatment with acyclovir was observed glomerular congestion, cell damage, tubular dilation and pyknotic nuclei. The acyclovir+BMSC after 48 and 72 hours, histological analysis was observed glomerulos and tubule changed less with dilation of the lumem and less cell.

Conclusions: These results strongly suggest that BMSC minimize AKI induced by acyclovir. This protocol can be a potential tool for treatment of this disease with impressive high morbid- mortality.

SA-PO2157

Therapeutic Potential of Human Placenta-Derived Mesenchymal Stem Cells for Ischemia/Reperfusion Acute Kidney Injury Dong Ho Yang,[1] So-Young Lee,[1] Yoon Hee Lee,[2] Hoon Jung.[3] *[1]Internal Medicine, Bundang CHA General Hospital, CHA University, Seongman-ci, Gyeonggi-do, Republic of Korea; [2]Pathology, Gangnam CHA Hospital, CHA University, Seoul, Republic of Korea; [3]Internal Medicine, Seoul Bukbu Geriatic Hospital, Seoul, Republic of Korea.*

Background: Mesenchymal stem cells derived from both bone marrow and umbilical cord blood have been shown to be beneficial in animal models of acute kidney injury. Human placenta-derived mesenchymal stem cells (hPMSCs) are free of ethical concerns, easily accessible, abundant, and strongly immunosuppressive. Recently, transplantation of hPMSCs into rodents with lung or liver cirrhosis was shown to reduce disease activity. The aim of this study was to evaluate the therapeutic potential of hPMSCs in rats subjected to ischemia/reperfusion (I/R).

Methods: Male Sprague-Dawley rats (200±10 g) were randomly assigned into three groups: hPMSC-treated (n=14), PBS-treated controls (n=14), and sham-operated (n=6). The treated groups underwent 45 min of ischemia by renal artery clamping and contralateral nephrectomy. hPMSCs [1×10^6 (or 0 PBS control)] were then intravenously injected into the rats subjected to ischemia. Serum creatinine (SCr) was measured 24, 48, and 72 hours after surgery, and renal tissue was obtained the first 2 days post-surgery.

Results: hPMSC treatment significantly improved renal function [48 hour SCr: 2.00±0.14 mg/dL (control group) vs. 1.16±0.69 mg/dL (hPMSC-treated group), p<0.05]. Histological analysis demonstrated a significant decrease in tubular casts and necrosis in hPMSC-treated rats [Day 2 cast scores: 3.34±0.02 (control) vs. 0.21±0.08 (hPMSC-treated); Day 2 necrosis scores: 5.67±0.01 (control) vs. 0.81±0.05 (hPMSC-treated), p<0.05]. In renal tubular epithelial cells, hPMSC treatment significantly increased the expression of PCNA (p<0.05) and significantly reduced apoptosis (p<0.05). Caspase-3 levels were significantly lower (p<0.01) and Bcl-2 levels were significantly higher (p<0.01) in the hPMSC-treated group compared to the controls.

Conclusions: These results suggest the use of hPMSCs as a novel renoprotective therapy for patients who develop ischemic renal injury.

SA-PO2158

Effect of Erythropoietin on Mesenchymal Stem Cells Proliferation In Vitro under Acute Kidney Injury Microenvironment and Its Mechanism Nanmei Liu, Weiwei Wang, Jinyuan Zhang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: To investigate the effect of erythropoietin (EPO) on mesenchymal stem cells(MSCs) proliferation under acute kidney injury (AKI) microenvironment, and to study its possible mechanism.

Methods: C57BL/6 mice's mMSCs had been successfully isolated by Percoll density gradient centrifugation and adherence cultivation, surface markers were identified by flow cytometry. To make AKI mice models by clamping bilateral renal pedicles 30 minutes and reopening 30 minutes. Then immediately drew both renal cortex to make ischemia/reperfusion(I/R) kidney homogenate supernatant.P3-mMSCs were treated with different group: ①Group A:low glucose DMEM medium with 10% fetal bovine serum; ②Group B: low glucose DMEM medium with 10% fetal bovine serum plus I/R kidney homogenate supernatant; ③Group C: low glucose DMEM medium with 10% fetal bovine serum plus I/R kidney homogenate supernatant and different concentrations of EPO(1,5,10,50IU/ml). Each group was incubated for 1d, 3d, 5d, 7d. Proliferation of mMSCs was detected by CCK-8, apoptosis was detected by TUNEL. The protein expression of erythropoietin receptor (EPOR) and the proteins of proliferation/apoptosis related signal pathway were examined by Western blot.

Results: Under I/R kidney homogenate supernatant, the proliferation ability of mMSCs decreased significantly, while the apoptotic percentage was significantly higher than Group A. After intervention of EPO, their proliferation enhanced, at the same time, the apoptotic percentage decreased, present dose-dependent manner (P<0.05 or P<0.01). P3-mMSCs were positive for EPOR. EPO decreased the expression of Caspase-3 in mMSCs under AKI microenvironment in a dose and time dependent manner, but it increased the expression of Bcl-2.The expression of phosphor-Janus kinase2 (pJAK2) and phosphor-signal transducer and activator of transcription (pSTAT-5) was significantly higher in 10 IU/ml EPO cultured 5d.

Conclusions: Erythropoietin can promote proliferation of mMSCs in vitro under AKI microenvironment, which is mediated by EPOR, and related with proliferation/apoptosis signal pathway.

Funding: Government Support - Non-U.S.

SA-PO2159

The Effect of Bone Marrow Mesenchymal Stem Cells (BMSC) or Conditioned Medium (CM) in Rats with Acute Kidney Injury (AKI) Induced by Lipopolysaccharide (LPS) Luciana Aparecida Reis,[1] Joelma Santina Christo,[1] Nestor Schor.[1] *[1]Nephrology, UNIFESP/EPM, Sao Paulo, SP, Brazil; [2]Morphology Department, UNIFESP/EPM, Sao Paulo, SP, Brazil.*

Background: Sepsis is characterized by a severe inflammatory response to infection, and its complications, including AKI, can be fatal. BMSC can act on several levels of endogenous repair to bring about resolution of diseases and its mechanism of action may be due to paracrine modulation and conditioned medium. The aim of this study is to evaluate the BMSC or CM effect at cellular modulation and/or repairment on LPS in rats.

Methods: BMSC were collected from male Wistar rats, characterized by FACS and differentiated into osteocytes and adipocytes. Female Wistar rats received LPS (10mg/Kg/BW) (LPS group) or water (CTL) in a single dose *i.v.* (N=10). After 24 or 72 hr, the female rats received *i.v.* BMSC (1X10^{6}cells) injection or CM (500µl), 1 or 3 doses via the tail vein. Blood and urine 24 hours were collected to creatinine (Cr), urea (U) and FE_{Na} evaluations. The kidneys were perfused and removed for HE, KI67, caspase 3 and Y chromosome analysis.

Results:

GROUPS	Cr [mg/dl]	U[mg/dl]	FENa [%]
CTL24h	0.6±0.04	25±3.1	2.6±0.02
CTL72h	0.7±0.08	27±1.8	2.5±0.01
LPS24h	1.7 ±0.14*	87.7±2.1*	0.7±0.03*
LPS72h	2.2±0.04*	146±4.6*	0.6±0.02*
LPS72h+BMSC1x	0.9±0.03#	59±3.1#	1.7±0.04#
LPS72h+BMSC3x	0.9±0.01#	50±4.3#	1.9±0.04#
LPS72h+CM1x	1.1±0.03#	66±4.9#	2.0±0.03#
LPS72h+CM3x	0.9±0.1#	49±2.7#	2.2±0.02#

*p<.05 vs. CTL24,72h; #p<.05 vs. LPS24, 72h

In LPS-group the kidneys showed a small marked KI67 and intensive caspase 3 expression but it was highly marked for KI67 and lower expression for caspase 3 in LPS+BMSC and LPS+CM groups. However, a striking difference was observed in the BMSC or CM treated animals where the presence of KI67 and Y chromosome was detected and no histological ATN lesions were observed. This effect was maximized when the doses of BMSC or CM were higher.

Conclusions: These results strongly suggest that BMSC or CM can minimize AKI in this sepsis model. This therapeutics effects have a significant impact on renal function observed holds substantial promise for its use in this pathological situation with high morbidity and mortality.

Funding: Government Support - Non-U.S.

SA-PO2160

Angiopoetin-2 – A Dose Dependent Modulator of Syngeneic Murine EPCs in Acute Ischemic Kidney Injury Daniel Patschan,[1] Susann Patschan,[1] Gerhard A. Mueller.[1] *[1]Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany; [2]Göttingen; [3]Göttingen.*

Background: Exogenously administered endothelial progenitor cells (EPCs) can protect the kidney from acute ischemic injury (iAKI). The proteins Angiopoietin-1 and -2 play essential roles in regulating vascular regeneration and function. Aim of the study was to analyze to possible effects of Ang-2 on EPCs in an EPC-based therapeutic regimen of iAKI.

Methods: EPCs were isolated from male C57BL/6N mice and, after 5-7 days of culturing, incubated with Ang-2 at different concentrations for one hour, respectively. Pretreated, dye-labelled cells were systemically injected into recipient animals after bilateral renal ischemia of 40 minutes. Two days later, renal function and histology were investigated. In vitro studies with cultured EPCs were performed in order to analyze the cells´ migratory activity under the influence of Ang-2.

Results: Systemic injection of 0.5 × 10^6 untreated EPCs did not prevent mice from acute renal failure. The consequences of cell pretreatment with Ang-2 were dose-dependent: at 200 ng/ml renal function remained unaffected. At 400 ng/ml, renoprotective effects of EPCs were significantly stimulated, indicated by lower postischemic serum creatinine levels. Incubating the cells with Ang-2 at 800 ng/ml dramatically reduced renoprotective effects of the cells, renal outcome was the worst of all four groups. Ang-2 did not have any influence on the migratory activity of cultured EPCs.

Conclusions: Ang-2 acts as dose-dependent modulator of syngeneic murine EPCs in iAKI. Renoprotecive effects of the cells can either be stimulated or reduced. Although the exact mechanisms responsible for these diametral actions remain speculative at the moment, Ang-2 at least does not modulate EPC migration.

SA-PO2161

A Phase I Trial of Human Allogeneic Mesenchymal Stem Cells for the Prevention of Acute Kidney Injury in Cardiac Surgery Subjects John R. Doty,[1] David G. Affleck,[2] Benjamin D. Horne,[1] Joseph Brent Muhlestein,[1] Matthew Psioda,[3] Viken Paragamian,[5] David G. Warnock.[4] *[1]Surgery, Intermountain Medical Center, Salt Lake City, UT; [2]Surgery, St. Mark's Hospital, Salt Lake City, UT; [3]Kendle International, Cincinnati, OH; [4]Medicine, University of Alabama at Birmingham, AL; [5]Allocure, Inc, Burlington, MA.*

Background: Acute kidney injury (AKI) is common and has no effective treatment. Pre-clinical studies have shown that mesenchymal stem cells protect kidney function and stimulate repair. AlloCure has developed a proprietary process to isolate and expand allogeneic human MSC (AC607).

Methods: In this dose escalation phase I study, 16 subjects undergoing on-pump CABG and/or valve surgery who were at high risk for developing AKI were treated with 3 doses of AC607 [7 x 10E5 (n=6), 2 x 10E6 (n=5), and 7 x 10E6 (n=5) cells].

Results: All subjects were Caucasian, mean age and weight (± SD) were 72.1 yrs (±7.8), and 92.1 Kg (±15.9), respectively. Fifteen subjects had CKD (II-III), 13 were hypertensive, and 6 had diabetes. The mean cardiopulmonary bypass time was 135.7 min (±45.5). The primary endpoint for the study was safety. None of the study subjects developed adverse or serious adverse events attributable to AC607 therapy, at any dose delivered. To enable a preliminary assessment of efficacy, study subjects were matched to historical controls (n=64). Overall, 12.5% of AC607 subjects developed AKI (AKIN criteria), compared to 29.7% of historical controls (p=0.214). Additionally, hospital length of stay was reduced in the AC607 group [6.5 days (±3.1) vs. 9.3 (±5.4), p=0.049]. Hospital readmission rates were 6.3% vs. 12.5% for AC607 subjects and historical controls respectively (p=0.679). One subject died within 30 days of surgery in the treated group (unrelated to AC607) vs. 2 of the historical controls. No subjects in either group received dialysis.

Conclusions: In this study, AC607 was safe and well tolerated. Furthermore, this study provides initial evidence that AC607 may be effective for the management of AKI in this population. Phase II studies are under development.

Funding: Pharmaceutical Company Support

SA-PO2162

I KappaB Kinase Alpha Promotes Repair after Ischemic Acute Kidney Injury Changchun Cao, Xin Wan, Li Fan, Bo Hu. *Nephrology, Nanjing First Hospital Affiliated to Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Understanding the mechanisms of repair and regeneration of the kidney after injury is of great interest because there currently are few therapies that promote repair, and kidneys frequently do not repair adequately. We studied the capacity of I kappaB kinase alpha (IKKα) on the recovery phase to promote kidney repair and regeneration using an established ischemia/reperfusion injury model in mice.

Methods: Renal ischemia/reperfusion injury was induced by clamping bilateral renal artery for 25 min in C57BL/6J mice (wild type), $IKKa^{TKO}$ mice (tubule epithelial cells-specific IKKa knockout mice), and $IKKa^{TG}$ mice (KAP-IKKa transgenic mice). The reperfusion time was 1, 2, 4, 7 and 30 days respectively. Blood was harvested for biochemistry; and kidney tissues for histopathological examination, and for study of renal expression of IKKa, intercellar adhesion molecule-1 (ICAM-1), interleukin-10 (IL-10), colony- stimulating factor-1 (CSF-1), and Ki67.

Results: Postischemic kidneys of $IKKa^{TG}$ mice expressed higher IKKa compared with wild-type and $IKKa^{TKO}$ mice, associated with enhanced repair of the tubule epithelial cells, and enhanced functional recovery.

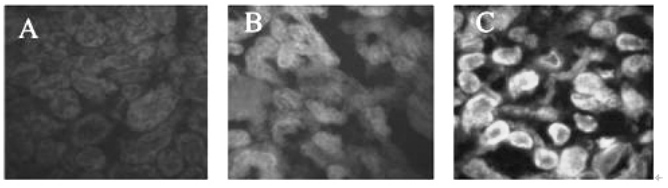


Fig1. Expression of IKKα in postischemic mouse kidneys. The reperfusion time was 96h. A, $IKKα^{TKO}$ group; B, wild type group; C, IKK^{TG} group

$IKKa^{TG}$ mice synthesized higher IL-10, which was an anti-inflammatory cytokine, and less ICAM-1, which was regarded as an pro-inflammatory cytokine, and expressed higher levels of CSF-1 and Ki67, which promoted proliferation of tubule epithelial cells. While $IKKa^{TKO}$ mice espressed higher ICAM-1 and reduced tubular proliferation compared with wild-type and $IKKa^{TG}$ mice, indicating that IKKa mediated renal repair is by limiting inflammation and promoting proliferation.

Conclusions: Our data confirms that IKKa promotes kidney repair and regeneration during the healing process after ischemia/reperfusion injury, likely through modulation of inflammatory cytokine and increasing tubule epithelial cell proliferation.

Funding: Government Support - Non-U.S.

SA-PO2163

A Model of Tubular Regeneration after Surgical Injury in Adult Rat Kidney Natalia O. Litbarg,[1, 2] Snezana Vujicic,[1, 2] Suman Setty,[1] George Dunea,[3] Jose A.L. Arruda,[1] Ashok K. Singh.[3] *[1]U of Illinois Chicago, Chicago, IL; [2]JB VAMC, Chicago, IL; [3]Hektoen Institute, Chicago, IL.*

Background: Wound repair after surgical injury is used to investigate mechanisms of tissue regeneration. The mechanisms of surgical wound regeneration in the adult kidney have not been explored.

Methods: To address this issue we subjected adult rats to uninephrectomy and surgical polectomy of the contralateral kidney. We enclosed the remnant kidney in an inert plastic pouch to prevent adhesions. Control rats underwent uninephrectomy and the uninjured contralateral kidney was placed in a pouch. We examined serial kidney sections by immunohistology at 1, 2 and 4 weeks after surgery.

Results: As compared to controls, tubular cells of the wounded kidney edge lost terminal differentiation markers (*Phaseolus vulgaris* hemagglutinin and aquaporin2) and acquired markers of both mesenchymal transition (vimentin and FSP1) and embryonic nephrogenesis (cadherin6, NCAM and Pax2). Similar changes, suggestive of cell dedifferentiation, have been described in tubular epithelium after acute tubular necrosis (ATN). However, as opposed to classical ATN models, we observed new growth of injured tubules. Tubular epithelial outgrowths (TEOs) extended from the wound edge into adjacent granulation tissue and formed branching tubular structures. TEOs were abundant in markers of proliferation (PCNA, Ki67 and phosphohistone H3), and were positive for markers of epithelial differentiation (pancytokeratin and E-cadherin). They lacked aquaporin2 and vimentin expression thus distinguishing them from normal adult collecting ducts as well as injured tubules. Notably, TEOs strongly expressed developmental markers Pax2 and *Dolichos biflorus* agglutinin. Overall their branching and staining pattern resembled that seen in primitive branching epithelium of ureteric bud. The morphologic connection between TEOs and wounded tubules suggested that TEOs were derived from injury-induced dedifferentiated tubular epithelium.

Conclusions: In conclusion, these results suggest that the adult rat kidney has the potential for *de novo* tubulogenesis. Our model provides a simple and reproducible tool for exploring this potential.

SA-PO2164

Role of the BMP-7/Gremlin Pathway in the Tubular Epithelial-Mesenchymal Transition Induced by TGF-β1 Mirian A. Boim, Edgar Maquigussa, Luciana G. Pereira, Carine Prisco Arnoni. *Medicine - Renal Division, Federal University of Sao Paulo, Sao Paulo, Brazil.*

Background: TGF-β1 is a potent inducer of the epithelial-mesenchymal transition (EMT). Tubular cells undergoing to EMT, invade the interstitium and synthesize interstitial matrix contributing to the tubulointerstitial fibrosis. Bone morphogenetic protein-7 (BMP-7) plays a critical role in the repairing process of the damaged tubular cells by a inhibiting EMT. Gremlin is an endogenous antagonist of BMP-7. Our objective was to evaluate the role of BMP-7/Gremlin pathway in the induction and reversion of EMT induced by TGF-β1 *in vitro* by stimulating immortalized human proximal tubular cells (HK-2) with TGF-β1.

Methods: HK-2 cells were treated with TGF-β1 (3ng/ml) for up to 72 hours. The role of BMP-7/gremlin pathway was analyzed by Gremlin mRNA silencing technique (siRNA). The expression of EMT markers (a-SMA, E-cadherin and FSP1), fibronectin, collagen, TGF-b1, BMP-7 and gremlin were estimated by real time PCR and western blot.

Results: TGF-β1 induced EMT was evidenced by upregulation in a-SMA and fibronectin expressions with a decrease in E-cadherin. Gremlin levels were increased in TGF-β1 stimulated cells and Gremlin siRNA was able to prevent the increase in the EMT markers including FSP1 and fibronectin. In contrast the addition of BMP-7 to the culture medium had no effect on the expression of these markers. Results suggest that BMP-7 was unable to prevent cells to undergo EMT and gremlin may have direct effect contributing to TGF- β1 inducing EMT in HK-2 cells.

Conclusions: In conclusion Gremlin suppression may be more efficient than BMP-7 treatment to prevent cells to undergo EMT induced by TGF-β1.

Funding: Government Support - Non-U.S.

SA-PO2165

Important Repair/Protective Process of Papillae in Renal Function and Morphology Demonstrated by Use of 14 Days-UUO-Released Model and Small Synthetic Compounds in Rats Michio Ishibashi,[1] Yoshikatsu Kanai,[2] Masayuki Iwano.[3] *[1]Department of Urology, Nara Medical University, Kashihara, Nara, Japan; [2]Department of Pharmacology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Department of Internal Medicine, Nara Medical University, Kashihara, Nara, Japan.*

Background: Using 14 days-UUO released model established in rats and small newly synthetic gammalactone compounds (#1376 or #NK0075), the important repair or protective process of papillae in renal morphology associated with renal function and involved in activation of protein molecules of rBAT, E-cadherin and galectin-3 after ureteral obstruction were presented.

Methods: In UUO-release model, complete ureteral obstruction was released by ureterouretrostomy and contra lateral nephrectomy (CNx) was done. After release of 14-days UUO with CNx in control animals (n=16), 8 weeks old male SD rats, plasma creatinine (pCr) level was recovered from 4.8± 0.6 mg/dl on day 2 to 2.2±0.1mg/dl on day 6. Treatment of #1376 at the dose of 30mg/kg/day s.c. throughout the 21 days of period ameliorated renal recovery to a better pCr level to 1.45± 0.25 mg/dl.

Results: Using pCr category of recovered renal function from UUO, two kinds of animals treated with #1376 were classified into group-1 (excellent pCr: less than 1.5mg/dl) and group-2 (intermediate pCr:1.5-1.7mg/dl). Renal morphology of group-1 animals was well preserved having almost normal transitional cell layer (TCL) covering papillae, and showed dominant collagen fiber response at the base of papillae. In group-2 cases, TCL was proliferated into more than four or five layers with loose connection, and the dominant cellular response at the base of papillae, close to TCL, with large amount of mononuclear cell accumulation, including angiogenesis detected by E-PHA or tubular regeneration, was observed. A strongly positive staining of galactin-3 concomitant with rBAT-positive intertubular mononuclear cells, E-cadherin-positive of TCL and distal tubulus, and E-PHA-positive angiogenesis were remarkable.

Conclusions: In conclusion, the repair process of papillae might be important to preserve renal function and morphology in the disease progression of UUO model.

Funding: Government Support - Non-U.S.

SA-PO2166

NF-E2 Related Factor-2 (Nrf-2) Signaling Promotes Survival and Healing of Tubular Epithelial Cells

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Background: Oxidative stress is a clinically relevant trigger for tubular cell death and insufficient tubular repair both commonly leading to tubular atrophy and renal failure. The transcription factor Nrf-2 induces a number of genes that counterbalance oxidative stress in injured cells. Hence, we hypothesized that Nrf-2 activation can promote tubular epithelial cell survival and repair.

Methods: We assessed three elements of tubular survival and healing: 1. Cell survival during cell isolation (oxidative stress): primary tubular epithelial cells were isolated from wild type C57BL6 mice at 2-3 weeks of age and then cultured in fresh-made hormone-conditioned medium. The cells were characterized by immunofluorescence microscopy, flow cytometry, and qPCR for the expression of tubular marker genes. 2. Regenerative expansion of the surviving cells: confluence was assessed every 24h for a period of 6 days by means of digital morphometry; and 3. Repair by scratch assay experiments: Cells were grown to complete confluence either with the Nrf-2 agonist Sulforaphane/DMSO or DMSO for 4 hours prior to artificial scratch wounding. Wound closure was analyzed by digital morphometry after different timepoints.

Results: Isolated cells in culture extensively expressed epithelial markers such as E-Cadherin and Cytokeratin 7 (immunostaining), and were positive for the tubular cell marker FXYD2, a specific ion channel protein. Sulforaphane increased tubular cell survival at 30 hours after the isolation procedure in a dose-dependent manner. In vitro expansion as well as wound closure upon scratching monolayers were 30% faster upon Sulforaphane stimulation. By contrast, a cell cycle inhibitor (p53 agonist) lead to dramatic decrease of the regeneration capacity in our model.

Conclusions: Nrf-2 activation with Sulforaphane promotes renal epithelial healing in-vitro after ischemic or mechanical injury. Therefore, Nrf-2 seems to be a potential therapeutic target to increase healing and regenerative response after ischemic injury in the kidney in order to prevent tubular atrophy.

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SA-PO2167

Urinary Thioredoxin 1 Is an Oxidative Stress-Specific Biomarker of Acute Kidney Injury

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Background: Oxidative stress is an important etiology of acute kidney injury (AKI); however, few existing AKI markers are directly involved in oxidative stress or redox regulation.

Methods: We investigated urinary levels of redox regulatory protein thioredoxin 1 (TRX1) in patients with various kidney diseases and mice with renal ischemia/reperfusion (rI/R) injury.

Results: Urinary TRX1 levels were markedly increased in patients with AKI as compared to chronic kidney disease and healthy subjects. In receiver operating characteristic curve analysis to differentiate between AKI and other renal diseases, the area under the curve of urinary TRX1 was 0.90, and the sensitivity and specificity were 0.90 and 0.82, respectively, at the optimal cutoff value of 35.2 ng/mg creatinine. Immunostaining of TRX1 was observed diffusely in the tubules of normal kidney, whereas it was shifted to brush borders in injured tubules which abundantly expressed 8-hydroxydeoxyguanosine. In cultured human proximal tubular epithelial cells, the supernatant levels of TRX1 were specifically increased by addition of hydrogen peroxide in a dose dependent manner. In animal experiments, the kidney of TRX1-transgenic mice contained lesser amount of an oxidative stress marker, protein carbonyl, than that of wild-type mice.

Conclusions: These findings suggest urinary TRX1 is a novel biomarker useful in distinguishing AKI from chronic kidney disease and healthy conditions, and the source of urinary TRX1 is renal tubules injured by oxidative stress.

Funding: Government Support - Non-U.S.

SA-PO2168

Identification and Validation of microRNAs as New Biomarkers of Acute Renal Failure

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Background: miRNAs are small non-coding RNAs which regulate gene expression. They are crucial regulators of cell responses to external stimuli such as ischemia/reperfusion and nephrotoxic compounds. Recently, it has been demonstrated that miRNAs could be detected in body fluids, including serum, where they have revealed a strong stability. Moreover, changes in miRNAs profiles in serum have been associated with several pathologies. All these features join to the non-invasive extraction method to obtain serum, have unveiled miRNAs as potential biomarkers of acute kidney injury (AKI).

The aim of this work is to identify and validate microRNAs as potential AKI biomarkers in serum from acute renal failure patients.

Methods: RNA was extracted from ARF patient serum using an optimized protocol for these samples. Firstly, we have performed a massive screening experiment using TLDA platform in serum samples from ischemic, toxic and septic ARF patients. A pool of healthy volunteers was used as control. Next, the expression of selected miRNAs was confirmed by qRT-PCR in a larger ARF patient cohort with different aethiologies. Samples from Day 0 (diagnosis) to day 7 were used for miRNA detection and serum creatinine levels were estimated in all the analyzed samples.

Results: TLDA analysis revealed several altered miRNAs in ARF patients vs healthy controls. We have chosen miR-101-1, miR-127-3p and miR-210 for further confirmation because all these miRNAs were significantly downregulated in ARF patients. miR-101-1 recovered healthy-like expression levels correlating with normalized creatinine values. Expression of miR-127-3p and miR-210 is restored in ischemic ARF patients who exhibited almost normal levels of creatinine.

Conclusions: In summary, miRNAs could be easily detected in serum and could be used for diagnosis and prognosis of ARF. In particular, miR-101-1, miR-127-3p and miR-210 could be considered as new biomarkers of AKI. Moreover, miR-127-3p and miR-210 appeared as specific markers of ischemic ARF outcome.

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SA-PO2169

The Molecular Phenotype of Acute Kidney Injury Is Also Found in Progressing Chronic Kidney Disease: A Human Kidney Transplant Study

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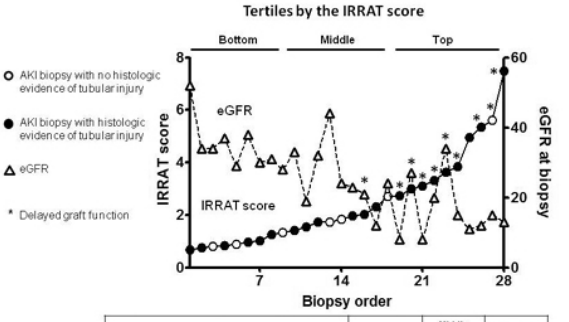
Background: Because of frequent biopsies and follow-up, early kidney transplants without rejection provide a unique opportunity to study molecular features of human acute kidney injury (AKI) that is a major impediment to transplant function.

Methods: We performed microarrays on early transplant biopsies with AKI and compared them to protocol biopsies of stable kidney transplants, Our aim was to tease out the molecular component of AKI as opposed to injury of transplantation procedure alone.

Results: Transplants with AKI expressed transcripts reflecting the injury-repair response (IRRAT), including some known (ITGB6, LCN2), but also novel kidney injury molecules e.g. OLFM4, SERPINA3. Many represented tissue remodelling, reminiscent of wound repair and cancer e.g. VCAN, CADH6. In transplants with AKI, IRRAT expression correlated with depression of function (r= -0.77), need for dialysis, and future recovery of function (r=0.62), whereas histologic features claimed to reflect tubular injury showed no correlations with these features.



	Bottom Tertile AKI biopsies	Middle Tertile AKI biopsies	Top Tertile AKI biopsies
eGFR at time of biopsy (mean ±SE)	35 ±2	27 ±3	15 ±2**
Delta eGFR from biopsy to 6 months (mean ±SE)	10 ±3	25 ±6	40±8**
Delayed / immediate graft function	0 / 9	0 / 8	8 / 1**
Histologic evidence of tubular injury	6	6	9

Figure 1. Relationship between the IRRAT scores, histopathology, delayed graft function and eGFR in transplants with AKI. ** significant difference between the top tertile versus remaining tertiles.

IRRAT were also expressed in rejecting kidneys, indicating that rejection triggers injury-repair. Surprisingly, IRRAT selected in acute kidney injury were induced in transplants with chronic kidney diseases (glomerulonephritis, antibody-mediated rejection) and correlated with progression to failure and with the previously published molecular risk score.

Conclusions: Transcripts strongly induced in AKI are also induced in progressive chronic kidney diseases. Thus both AKI and chronic progressive kidney diseases induce the same signal, analogous to wound repair. The extent of this response correlates with the extent of dysfunction and future recovery in pure acute kidney injury, but indicates serious ongoing nephron damage in progressive chronic kidney disease.

Funding: Government Support - Non-U.S.

SA-PO2170

Kidney Injury Biomarkers Kim-1, NGAL, Clusterin, and Cystatin C in Mouse Models of Ischemia Reperfusion Injury Venkata Sabbisetti, Chang Wang, Kazumi Ito, Joseph V. Bonventre. *Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: The application of kidney biomarkers to mouse models of kidney injury and nephrotoxicity will enhance our understanding of pathophysiology and facilitate the development of safe drugs. Kim-1, NGAL, clusterin, & cystatin C are capable of detecting kidney toxicity at early stages. However, the lack of reliable assays and urine volume requirements have hampered the utilization of these markers in preclinical studies. The goal of the current study was to establish a high-through multiplex bead based assay to measure these biomarkers, characterize their kinetics in urine and serum, and validate them vs histopathology after ischemia/reperfusion (I/R).

Methods: BALB/C mice were subjected to I/R injury for 10, 20, & 30 min and urine and serum specimens were collected for 14 days and evaluated for Kim-1, NGAL, clusterin, & cystatin C using microbead-based ELISA assays which we developed. Serum creatinine, BUN, NAG and total protein were measured spectrophotometrically.

Results: Microbead-based ELISA assays were developed that can measure the four biomarkers in duplicate in 10 µl of biological sample. The lower detection limits for Kim-1, NGAL, clusterin & cystatin C assays are 12, 10, 290 & 10 pg/ml with dynamic ranges of 0.01-50, 0.01-50, 0.29-1200, 0.01-50 ng/ml respectively. In mice exposed to 10 and 20 min I/R injury serum creatinine levels did not change, but there was a robust ischemic time-dependent increase in each of the novel markers and histopathology reflecting tubular injury. With 10 & 20 min I/R injury, NAG & total protein levels returned to baseline within 12 hours, while Kim-1, NGAL and cystatin C levels remained elevated for 5 days after the injury. Similarly, in mice subjected to 30 min I/R injury, NAG and total protein levels returned to baseline within 48 hours after injury, while Kim-1 & NGAL levels remained elevated for the entire duration of the study.

Conclusions: An assay panel for mouse urinary and serum biomarkers has been developed and validated which can measure four biomarkers in duplicate in very small sample volumes. This will greatly facilitate kidney injury studies in the mouse.

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SA-PO2171

Soluble RAGE Prevents Sepsis-Induced Acute Kidney Injury Sun Ha Lee, Jisun Paeng, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: The receptor for advanced glycation endproducts(RAGE) is involved in the pathogenesis of inflammatory diseases. Soluble RAGE(sRAGE) competitively inhibits the binding of RAGE ligands and is proposed as a potential therapeutic agent. However, little is known about the efficacy of sRAGE in septic acute kidney injury (AKI), which is mainly mediated by inflammation. This study was undertaken to investigate the effect of sRAGE on renal function, epithelial-mesenchymal transition(EMT), and inflammatory cells infiltration in septic AKI.

Methods: In vivo, C57/BL6 mice were subjected to cecal ligation and puncture(CLP) or sham operation(control, C) and were maintained for 24 hours. CLP mice were pretreated either with diluent or sRAGE intraperitoneally(CLP+sRAGE) at 1 hr before operation. In vitro, NRK-52E cells were cultured in DMEM media with or without lipopolysaccharide(LPS). To examine the effect of sRAGE on LPS-induced tubular cell injury, LPS-treated NRK-52E cells were also incubated with sRAGE or RAGE siRNA. Western blot analysis was performed to evaluate E-cadherin, α-smooth muscle actin(α-SMA), and ICAM-1 protein expression. Immunohistochemistry was carried out with renal tissues.

Results: The increase in serum BUN levels in CLP mice was significantly abrogated by the administration of sRAGE($p<0.05$). The ratios of α-SMA/E-cadherin protein expression and the expression of ICAM-1 protein were significantly higher in CLP model compared to C mice($p<0.05$). These changes in CLP model were significantly abrogated by sRAGE pretreatment($p<0.05$). sRAGE also significantly reduced the number of infiltrated inflammatory cells within kidney in CLP mice. In vitro, RAGE protein expression was significantly increased in LPS-stimulated tubular epithelial cells, and this increase was ameliorated by sRAGE and RAGE siRNA($p<0.05$). The increases in the ratios of α-SMA/E-cadherin and the expression of ICAM-1 protein in LPS-stimulated cells were also significantly attenuated by RAGE inhibition($p<0.05$).

Conclusions: These findings suggest that RAGE plays a role in the pathogenesis of septic AKI and its inhibition by sRAGE may be a potential therapeutic target for AKI in severe sepsis.

SA-PO2172

Sepsis-Induced Glomerular Endothelial Dysfunction Mediates Reductions in GFR and Increases in Protein Filtration Ruben M. Sandoval,[1] George Rhodes,[1] Exing Wang,[1] Silvia B. Campos-Bilderback,[1] Sarah E. Wean,[1] Bruce A. Molitoris.[1,2] *[1]Medicine/Div of Nephrology, Indiana Univ. School of Medicine, Indianapolis, IN; [2]Roudebush VAMC, Indianapolis, IN.*

Background: Sepsis is now the leading cause of acute kidney injury (AKI) known to decrease Glomerular filtration rate (GFR) and increase proteinuria. There also exists a discrepancy between renal perfusion and GFR.

Methods: To evaluate the potential role of the glomerulus in the overall pathogenesis of these abnormalities, we studied surface glomeruli in 8-10 week old Munich Wistar Frömter rats using intravital 2-photon microscopy in a cecal ligation and puncture (CLP) model of sepsis to ask targeted questions and compare the metric of measured GFR to serum creatinine changes at 24 hours post CLP.

Results: Male rats undergoing CLP showed an increase in serum creatinine from 0.23 +/- 0.06 mg/dl to 0.80 +/-0.17 ($P\leq0.01$) and a decrease in real time GFR from 0.69 +/- 0.06 ml/min/100gm body wt to 0.34 +/-0.15 ($P\leq0.01$). Hemodynamic monitoring revealed normal and hyperdynamic cardiac status within the CLP group. Quantitative analysis of 15 glomeruli in three CLP septic rats revealed a reduction in red blood cell flow rates within capillary loops from 1,771 +/- 467 to 576 +/- 327 um/sec ($P\leq0.01$); an increase in WBC adherence to glomerular capillary endothelial cells from 0.42 +/-0.33 to 7.25 +/- 5.82 WBC's/standardized glomerular volume ($P\leq0.05$) in CLP rats; and an increase in the glomerular sieving coefficient (GSC) of a 150kD dextran from 0.007 +/- 0.003 to 0.097 +/- 0.046 ($P\leq0.05$). Rouleaux formations were seen only in septic rats.

Conclusions: These data indicate glomerular endothelial-WBC interactions during sepsis, in part, explain the reduction in GFR and increased filtration of large molecular weight proteins. The results from real time GFR accurately detected the drop in renal function for this model of sepsis.

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SA-PO2173

Chronic Kidney Disease Amplifies Sepsis-Induced Acute Kidney Injury and Spleen Apoptosis Via Toll-Like Receptor 4 Signaling Takayuki Tsuji, Ana Carolina Souza, Xuzhen Hu, Taro Horino, Peter S.T. Yuen, Robert A. Star. *Renal Diagnostics and Therapeutics Unit, NIH/NIDDK, Bethesda, MD.*

Background: Patients with chronic kidney disease (CKD) have increased risk of morbidity and mortality from sepsis and acute kidney injury (AKI), an effect mimicked in our mouse model of sepsis following CKD. To further study the mechanism of amplification by CKD, we studied Toll-like receptor 4 (TLR4), which plays a major role in systemic inflammation after endotoxemia, and has been implicated in sepsis-AKI and in renal fibrosis.

Methods: We used C3H/HeJ (TLR4-) and C3H/HeOuJ (TLR4+) mice as matched controls. We performed sham(CKD) or 5/6 nephrectomy (5/6Nx) to induce CKD, waited 8 weeks, then performed sham(sepsis) or cecal ligation and puncture (CLP). Outcomes were measured at 24h.

Results: In both TLR4+ and TLR4- mice, 5/6Nx induced mild CKD at 8 weeks [BUN 42-52.5 mg/dl; serum creatinine (Scr) 0.265-0.300 mg/dl; histologic fibrosis], although urinary albumin excretion was significantly higher in TLR4+ than TLR4- mice (186.1 vs 34.5 ug/mg Cr, $p<0.05$). CKD intensified sepsis-AKI, as SCr, BUN, and tubule damage score (TDS) were significantly higher after CKD-sepsis vs sham(CKD)-sepsis in TLR4+ mice ($p<0.05$). However in TLR4- mice, SCr ($p>0.05$), BUN ($p<0.05$), and TDS ($p<0.05$) increases were blunted [CKD-sepsis vs sham(CKD)-sepsis]. Spleen apoptosis (by active caspase 3) was significantly increased in CKD-sepsis vs sham(CKD)-sepsis ($p<0.001$) in TLR4+ but not in TLR4- mice. Interestingly, sepsis increased TNF-α and HMGB1, but pre-existing CKD had no further effect in either TLR4+ or TLR4- mice.

Conclusions: Much of the CKD-driven amplification of sepsis-AKI is TLR4-dependent in this milder version of the mouse acute-on-chronic kidney disease model. Sepsis-induced spleen apoptosis was greatly amplified by CKD, which was TLR4-dependent. The amplification occurred without changes in TNF-α and HMGB1 (classic early and late inflammatory mediators). Therefore spleen apoptosis is driven by TLR4 signaling, and may represent an important amplifier in acute-on-chronic kidney disease.

Funding: NIDDK Support

SA-PO2174

Distinct Pathophysiology of Septic Acute Kidney Injury – Role of Immune Suppression and Apoptosis So-Young Lee,[1] Sang-Kyung Jo,[2] Won-Yong Cho,[2] Hyoung-Kyu Kim.[2] *[1]Nephrology, Eulji university hospital, Seoul, Republic of Korea; [2]Nephrology, Korea University Hospital, Seoul, Republic of Korea.*

Background: Sepsis is the most common cause of acute kidney injury(AKI) in critically ill patients. However, the mechanisms leading to AKI in sepsis remain elusive. Although, sepsis is traditionally considered an excessive systemic inflammatory response, according to recent observations, sepsis induced organ dysfunction might be associated with paradoxical immune suppression. The purpose of this study was to examine the pathophysiology of septic AKI focusing on immune suppression and apoptosis of kidney and immune cells by providing on-site comparison between septic vs ischemia/reperfusion(I/R) induced AKI, a well known disease mediated by activation of innate immunity.

Methods: At 24 h after cecal ligation & puncture (CLP) or I/R injury, biochemical, histologic kidney injury and cytokine profiles were compared. Apoptosis of immune cell

and renal cell was assessed by TUNEL staining and measurement of caspase 3 activity. We also examined the effect of caspase 3 inhibition and IL-10 blocking on renal function. Finally, we observed $CD4^+CD25^+$regulatory T cells(Tregs) frequency and the effect of depletion of these cells in renal function.

Results: Acute tubular necrosis or inflammation were hardly observed in septic kidneys. However, tubular cell apoptosis was prominent and caspase 3 activity showed a positive correlation with plasma cr. Pretreatment with caspase 3 inhibitor resulted in attenuation of renal dysfunction in septic AKI with reduced apoptosis. Septic AKI was associated with increased IL-10, and massive immune cell apoptosis with increased percentage of Tregs. In contrast to I/R injury that depletion of Tregs aggravates renal injury, depletion of these cells resulted in significant renoprotection and IL-10 blocking was also associated with renoprotection in septic AKI.

Conclusions: Our data showed a link between apoptosis, immune suppression and kidney dysfunction during sepsis and suggest that inhibition of apoptosis and recovered immune balance might be useful to decrease mortality or organ dysfunction. Future studies are needed to clarify the exact pathophysiology of this devastating disease.

Funding: Pharmaceutical Company Support

SA-PO2175

Role of Renal Cell Apoptosis in Pathogenesis of Septic Acute Kidney Injury Sung Yoon Lim, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Korea University Anam Hospital.*

Background: The presence of acute kidney injury (AKI) in septic patients is known to be associated with worse outcome. However, mechanisms of septic AKI remains elusive and several recent reports suggest that excess immune suppression characterized by massive lymphocytes apoptosis might be causally linked to sepsis mortality or sepsis induced organ dysfunction. In addition to immune cell apoptosis, renal cell apoptosis has also been demonstrated in animal models of sepsis or some autopsies. However, the exact role of renal cell apoptosis in the development of AKI in sepsis has never been assessed.

Methods: To examine the role of renal cell apoptosis in septic AKI, we used cecal ligation and puncture (CLP) method in mice.

Results: In C57/BL6 mice, plasma creatinine increased on day 1 and despite lack of overt tubular necrosis, significantly increased numbers of renal cell apoptosis were observed and kidney caspase 3 activity showed positive correlation with plasma creatinine. Although pretretreatment with caspase 3 inhibitor markedly attenuated functional impairment, this renoprotective effect was accompanied by decreased apoptosis of both immune cells and renal cells. Therefore, to further dissect out the role of renal cell apoptosis independent of immune cell apoptosis, we performed CLP in mature T and B lymphocyte deficient RAG-1 deficient mice. Similar with WT mice, CLP also evoked mixed gram negative and positive bacterial peritonitis induced sepsis with multiple organ dysfunction in RAG-1 deficient mice. Furthermore, in the absence of mature T and B lymphocytes, that are known to undergo apoptosis in sepsis, pretreatment with caspase 3 inhibitor still partially rescued RAG-1 deficient mice from development of AKI with significant reduction of plasma cytokines (IL-6, IL-10, TNF-a, MCP-1).

Conclusions: These results demonstrated the important role of renal cell apoptosis in the development of AKI and strategies that suppress renal cell apoptosis might be useful in preventing or treating AKI associated with sepsis.

Funding: Private Foundation Support

SA-PO2176

Lipopolysaccharide Causes Marked Changes in Tubular Zonula Occludens-1 Michael T. Eadon, F. Gary Toback, Patrick Cunningham. *Section of Nephrology, University of Chicago, IL.*

Background: Tight junctional (TJ) proteins maintain an integral role in tubular ion transport and waste excretion. Disruption of TJs contribute to decreased GFR in acute kidney injury (AKI) via the tubular backleak mechanism. TJ protein derangements have been shown following a variety of insults to intestine, lung, and kidney *in vivo*, as well as in response to TNF-α in cultured MDCK cells. The lipopolysaccharide (LPS) model of AKI yields subtle pathologic findings on light microscopy, raising concern that this model's injury is chiefly hemodynamic, as opposed to structural. We hypothesized that LPS would cause a disruption in tubular TJ proteins such as Zona Occludens-1 (ZO-1) and Occludin.

Methods: C57/BL6 mice were injected with LPS (10 mg/kg) and sacrificed 6, 24, and 48 h after injection. Kidney cortex was harvested and prepared for analysis by light microscopy, immunoblot, immunofluorescence, and RT-PCR for occludin, ZO-1, and claudin-1. AKI was confirmed via measurement of blood urea nitrogen and creatinine.

Results: Immunoblot showed significantly decreased ZO-1 expression by densitometry 24 h after LPS (decrease of 56.0 +/- 4.6%, $p = 0.009$), with recovery at 48 h. However, ZO-1 mRNA was unchanged at 24 h, with a trend for increase at 48 h (increase of 68.0 +/- 11.9% from baseline, $p = 0.12$). Immunofluorescence 24 h after LPS revealed a marked change in ZO-1 localization from its usual circumferential pattern to one with greater fragmentation, decreased basolateral staining, and greater apical distribution. Staining showed decreased tubular co-localization of occludin and ZO-1. Occludin and claudin-1 protein and RNA expression were not significantly altered at 24 h.

Conclusions: ZO-1 protein expression after LPS administration was markedly decreased at 24 h, with subsequent recovery at 48 h. Membrane localization of TJ proteins was disrupted by LPS. As RNA expression was unchanged, the results suggest disruption of renal tubular epithelial TJs in LPS-induced AKI is not mediated by transcriptional regulation, but may involve degradation or protein trafficking. This study provides important evidence that LPS-induced AKI is associated with structural injury and not merely hemodynamic changes.

Funding: NIDDK Support

SA-PO2177

Dexamethasone Attenuates Septic Acute Kidney Injury by Reducing Apoptosis of Spleen Immune Cells and Renal Tubule Cells Hye Min Choi,[1] So-Young Lee,[2] Sang-Kyung Jo,[1] Won-Yong Cho,[1] Hyoung-Kyu Kim,[1] Young Youl Hyun,[3] Dae R. Cha.[3] *[1]Nephrology, Korea University Hospital, Seoul; [2]Nephrology, Eulji University Hospital; [3]Nephrology, Korea University Hospital, Ansan.*

Background: Sepsis is the most common cause of acute kidney injury (AKI) in hospitalized patients and the clinical outcome is very poor, whereas our understanding of pathogenesis and treatment for septic AKI has remained limited. Low-dose glucocorticosteroids (GCs) has been clinically recommended in refractory septic shock patients through Surviving Sepsis Campaign, and in rat endotoxemia model, GCs were shown to ameliorate renal dysfunction. However, the mechanisms for the beneficial effects of GCs on septic shock and possibly on septic renal injury are still unclear. In this study, we purposed to investigate the pathophysiology of septic AKI and the effect of GCs using septic mice.

Methods: We induced AKI by polymicrobial sepsis using a CLP (cecal ligation and punture) model in 8~10wk-old C57BL/6 mice. Saline or dexamethasone (DEX) dissolved in saline was administered right after CLP surgery. We examined hemodynamic, biochemical and histological changes in a time-course manner.

Results: Mean arterial Blood pressure (BP) significantly decreased starting at 3hr after CLP. Fractional shortening which estimate cardiac systolic function significantly increased and remained high until 24hr, suggesting hyperdynamic "warm shock" state. Serum Creatinine started to increase after 12hr. Many apoptotic cells were observed even at 3hr after CLP in spleen and mainly located in lymphoid tissue. Renal tubular apoptosis was also prominent in cortex and outer medulla although acute tubular necrosis or infiltration of neutrophils and macrophages was not distinct.

We compared hemodynamics, renal function and apoptosis of tissues between CLP and CLP+DEX. BP, Heart rate and fractional shortening was not significantly different between CLP and CLP+DEX. However mice with CLP+DEX had a significant reduction in serum creatinine and decreased apoptosis of spleen and kidney compared to CLP.

Conclusions: DEX attenuates septic AKI, and the beneficial effect might be associated with reduced apoptosis of renal tubule cells and spleen immune cells.

SA-PO2178

Calpain 10 Loss Mediates Increased Susceptibility to Nephrotoxicity Ryan Whitaker, Marisa D. Covington, Matthew Allen Smith, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: Age and certain disease states such as diabetes predispose humans to AKI; however, few studies have examined the mechanism of increased sensitivity to AKI in these patient populations. Our laboratory has determined that calpain 10 protein and mRNA is down-regulated in aged kidneys from multiple species. In addition, recent studies showed that mitochondrial calpain 10 is decreased in a rat model of diabetes and renal calpain 10 mRNA is decreased in human diabetics. Furthermore, knock down of mitochondrial calpain 10 in renal proximal tubular cells (RPTC) alone disrupts mitochondrial functions as evidenced by accumulation of mitochondrial calpain 10 substrates, and decreased basal and uncoupled respiration. The goal of these experiments is to test the hypothesis that decreased calpain 10 sensitizes RPTC to toxicant injury.

Methods: We used adenovirus delivered shRNA to knock down calpain 10 in RPTC. Controls received adenovirus delivered scrambled shRNA. Two days later, RPTC were exposed to paraquat (100-300 μM), or $HgCl_2$ (0.1-3 μM) for 24 hours. Cell death was measured via propidium iodide staining. Additionally, RPTC were cultured in high (17 mM) glucose as a cellular model of diabetes for 48 hours. Control cells were cultured in normal (5 mM) glucose. RPTC were then exposed to 100 uM paraquat μM for 24 hours. ATP levels were measured by using an ATP Determination Kit (Invitrogen).

Results: RPTC treated with adenovirus delivered calpain 10 shRNA exhibited 62% and 84% increases in cell death with paraquat and $HgCl_2$ respectively, compared to controls. RPTC cultured in 17 mM glucose media demonstrated a 75% greater reduction in ATP levels when exposed to paraquat compared to 5 mM glucose controls.

Conclusions: We hypothesize that the loss of renal calpain 10 in aging or diabetes leads to accumulation of mitochondrial calpain 10 substrates, causing mitochondrial dysfunction; thereby, sensitizes RPTC to a "second hit" such as I/R or drug/toxicant-induced injury that initiates AKI. Because calpain 10 is ubiquitously expressed, these findings may have broader implications in other tissues and disease states.

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SA-PO2179

STAT3 Activation Via Tyrosine Phosphorylation Is Cytoreductive during Cisplatin-Induced Acute Kidney Injury Robert L. Safirstein,[0071] Judit Megyesi,[0071] Adel Tarcsafalvi,[0071] Rawad Hodeify,[0071] Peter M. Price.[0071] *[1]Medicine Service, Central Arkansas Veterans Healthcare System, Little Rock, AR; [2]Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: The Stat3 transcription factor is a member of a family proteins that play a significant role in signal transduction from cytokine and chemokine receptors. We showed that Stat3 is activated during cisplatin-induced acute kidney injury at both serine

and tyrosine residues. Transduction of proximal tubule cells with a dominant negative (DN) Stat3(tyr) construct renders them resistant to oxidant stress showing that the Stat3 tyrosine phosphorylation is cytoreductive during oxidant-induced injury.

Methods: We transduced proximal tubule cells with a DN-Stat3(tyr) construct and created a transgenic mouse strain in which the DN-Stat3(tyr) cDNA was conditionally expressed in proximal tubule cells under the control of the testosterone-responsive KAP2 promoter.

Results: Transduced cells were resistant to cisplatin-induced apoptosis. The transgenic mouse strain showed significant protection from cisplatin-induced AKI, as creatinine levels in serum 3 days after cisplatin exposure were reduced from (mean and SE) 2.37 ± mg/dL (wt) to 0.84 ± .42 in transgenic mice ($p<01$). Similarly, morphologic protection from cisplatin cytotoxicity was also conferred by transgene expression.

Conclusions: These studies identify the Stat3 protein as a significant target of both cisplatin and oxidant injury to kidney cells. Furthermore, these transgenic animals should help to identify the link between proximal tubule damage, cytokine expression, leukocyte activation and physiologic events downstream of Stat3(tyr) phosphorylation.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2180

Essential Role for Apoptosis Signal-Regulating Kinase 1 in Free Light Chain-Mediated Apoptosis Wei-Zhong Ying,[1,2] Pei-Xuan Wang,[1,2] Paul W. Sanders.[1,2] *[1]Medicine, University of Alabama at Birmingham, AL; [2]Medicine, Birmingham Veterans Affairs Medical Center, Birmingham, AL.*

Background: A major attendant complications of multiple myeloma is renal failure, which is related to deposition of monoclonal immunoglobulin free light chains (FLC) and directly contributes to morbidity and mortality in this disease. Because our prior studies demonstrated that FLC generated intracellular oxidative stress, the present studies focused on the redox-sensitive mitogen-activated protein kinase kinase kinase known as Apoptosis Signal-regulating Kinase 1 (ASK1).

Methods: The mechanism of cytotoxicity of monoclonal FLC was determined by incubating human proximal tubular epithelial cells (HK-2) in medium containing two human monoclonal FLC (termed κ2 and λ3). Cytoplasmic caspase 3 and 9 activities were determined using ELISA. The percentage of apoptotic cells in each population of HK-2 cells was determined by flow cytometry using MitoTracker® Red and annexin V conjugated to Alexa Fluor 488. RNA interference was accomplished using small interfering RNA (siRNA) that targeted human ASK1. Western analyses determined ASK1 protein levels, using rabbit-anti-human polyclonal antibody to ASK1, and activity, by use of an antibody that detected phosphorylated ASK1 at residue T845.

Results: Incubation of HK-2 cells with each FLC, 1 mg/ml, promoted activation of caspase 9 and caspase 3. Therefore, the intrinsic (mitochondrial) pathway mediated FLC-induced apoptosis. A time-dependent increase in phosphorylation of ASK1 at T845, indicating activation of this enzyme, was also observed. Incubation of HK-2 cells for 24 and 48 hours with either FLC increased the apoptotic rate. siRNA designed to reduce ASK1 expression in HK-2 cells successfully decreased ASK1, which was confirmed by western blot analysis. Incubation of ASK1-depleted HK-2 cells with the two FLC prevented the increase in apoptosis, while pre-treating HK-2 cell with non-targeting siRNA did not prevent FLC-mediated apoptosis.

Conclusions: Monoclonal FLC triggered the intrinsic apoptotic pathway in renal epithelial cells by activation of ASK1. The ASK1 pathway provides a potential therapeutic target in renal failure in multiple myeloma.

Funding: NIDDK Support, Veterans Administration Support

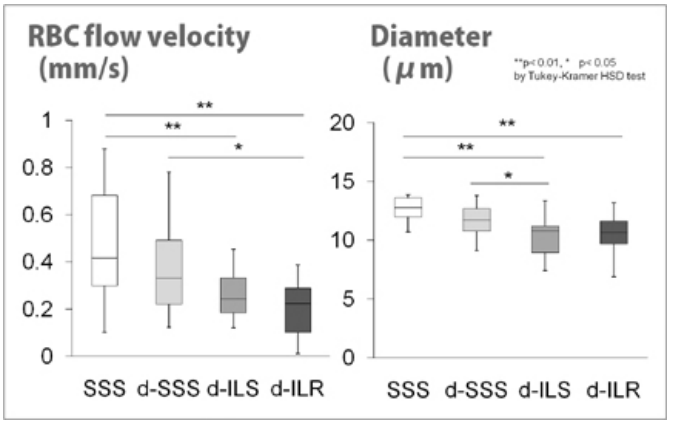
SA-PO2181

Renal Peritubular Hypoperfusion in Subcapsular Cortex Is Inversely Correlated with Urinary L-FABP in Mouse Radiocontrast-Induced Acute Kidney Injury Kousuke Negishi,[1] Eisei Noiri,[1] Kent Doi,[1] Toshiro Fujita,[1] Takeshi Sugaya.[2] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]CMIC Co., Ltd., Tokyo, Japan.*

Background: Radiocontrast-induced AKI (RC-AKI) is characterized by maldistribution of renal microcirculation. We recently demonstrated that L-type fatty-acid binding protein (L-FABP) expressed in human and mouse proximal tubules was markedly upregulated in ischemic conditions, and shed into the urine via hypoxia response element in its promoter region. The aim of this study is to evaluate the correlation between urinary L-FABP and renal peritubular capillary (PTC) blood perfusion in RC-AKI.

Methods: Mice were subjected unilateral nephrectomy 7 days before RC injection. After water restriction/ dehydration for 24 h, mice were intraperitoneally injected indomethacin (I), L-NAME (L) and low-osmolar RC iohexol (3g Iodine/kg, R). Equivalent volume of saline was injected as control for each reagent (S). In addition to serial urine and blood sampling, renal PTC blood flow of subcapsular cortex at 12 h was directly monitored by intravital CCD video-microscope.

Results: Urinary L-FABP in dehydrated (d-) mice with I, L, and R injection (d-I-L-R) at 12 h significantly increased up to 3000-fold from the baseline. BUN was also peaked at 12 h. PTC flow velocity and diameter in the d-I-L-R group was significantly decreased than that in mice injected saline alone (S-S-S). Moreover, log converted urinary L-FABP was inversely correlated with the lowest PTC flow velocity at 12h ($r2 = 0.248$, $p = 0.022$). Histological analysis revealed vacuolation and increased L-FABP expression in proximal tubules were limited to the d-I-L-R group.



Conclusions: Not only direct tubular toxicity but PTC hypoperfusion in subcapsular cortex is suggested to induce shedding of L-FABP into the urine in RC-AKI.

Funding: Government Support - Non-U.S.

SA-PO2182

Afferent Arterioles but Not Efferent Constrict to Iodinated Contrast Media in Mice – A New Feature in the Pathophysiology of Contrast Induced Nephropathy Zhizhao Liu, Vinicius Urbano Viegas, Andrea Perlewitz, Pontus Persson, Andreas Patzak, Mauricio Michalak Sendeski. *Institut für Vegetative Physiologie, Charité-Universitätsmedizin, Berlin, Germany.*

Background: Contrast induced nephropathy (CIN) is an important cause for in-hospital acute kidney injury. A key feature of the pathophysiology of CIN is the reduction in renal blood flow. We investigated the influence of iodinated contrast media (CM) on afferent and efferent arterioles which help explain disturbances of renal hemodynamic present in CIN.

Methods: Afferent and efferent arterioles were isolated from C57Bl6 mice and perfused with either vehicle solution or 23mg iodine/ml CM. L-NAME (10-4 mol/l), a non-selective nitric oxide synthesis inhibitor, was also applied to investigate a possible role of nitric oxide. Angiotensin II concentration responses (10-12 to 10-6 mol/l) were performed after 20 minute treatment. Changes in luminal diameter were used for analysis of vessel tone and reactivity.

Results: Diameters of afferent arterioles were significantly reduced by treatment with CM (86.3%), L-NAME (70.7%), and CM+L-NAME (52.9%) compared to the control group. CM+L-NAME induced a significantly more pronounced constriction than CM alone. Moreover, subsequent angiotensin II application showed a further more pronounced constriction. This constriction was concentration dependent and increased after CM and CM+L-NAME treatment, but not after L-NAME alone. In contrast, neither the diameters of efferent arterioles after 20 minute treatment nor the responses to angiotensin II were significantly affected by CM.

Conclusions: The result shows a stronger effect of CM on afferent arterioles tone and reactivity compared to efferent arterioles. Moreover, CM+L-NAME constricts afferent arterioles more than CM alone, reinforcing that other mechanisms besides nitric oxide contribute to the CIN blood flow disturbances. Our results suggest an important contribution of afferent arterioles for the reduction in renal blood flow and in glomerular filtration rate observed in CIN.

SA-PO2183

Deletion of Longevity Gene p66ShcA Rescues Cisplatinum-Induced Acute Renal Failure Rungwasee Rattanavich, Fnu Washdave, Partab Rai, Dileep Kumar, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Cisplatimum has been demonstrated to induce acute renal failure both in human and animal experimental models. Cisplatinum directly causes tubular cell apoptosis. This cytotoxic effect of cisplatinum has been attributed to the induction of reactive oxygen species (ROS) generation. p66ShcA$^{-/-}$ mice have been demonstrated to have ROS resistance phenotype and have diplayed a longer life span when compared to normal mice. In addition, deletion of p66ShcA in both cardiac and renal genomes has been demonstrted to provide protection agaist glucose induced injury in diabetic animal experimental animal models. On that account, we hypothesized that p66ShcA$^{-/-}$ mice would also display resistant to cytotoxic tubular cell injury induced by cisplatinum. In the present study, we evaluated whether cisplatinum would be able to induce acute renal failure in p66ShcA$^{-/-}$ mice.

Methods: Control and p66ShcA$^{-/-}$ mice in groups of four were administered either vehicle or cisplatinum (12.5 mg/Kg, intraperitoneal). All mice were sacrificed on day 3; urine and blood samples were collected for BUN and albumin: creatinine ratio. Kidneys were harvested for renal histology and TUNEL staining. Immmunoblots were prepared from renal tissues and evaluated for the activation of the redox-sensitive stress response program in the form of phosphorylation of Foxo3A.

Results: Control mice receiving cisplatinum displayed elevated BUN (68.0 ± 7.0 mg/dl) when compared to control mice receiving vehicle (40.9 ± 2.0 mg/dl). However, p66ShcA$^{-/-}$ mice receiving-cisplatinum displayed nomrla levels of BUN (34.0 ± 8.0 mg/dl). Number of TUNEL +ve cells was many fold greater in control mice-receiving cisplatinum when

compared to p66ShcA$^{-/-}$ mice-receiving cisplatinum. Control mice-receiving cisplatinum displayed inactivation of redox-sensitive stress response program; whereas, p66ShcA$^{-/-}$ mice-receiving cisplatinum showed adequate response of the stress response program.

Conclusions: These findings indicate that deletion of p66ShcA from renal cell genome provides protection against cisplatinum induced acute renal failure.

Funding: NIDDK Support

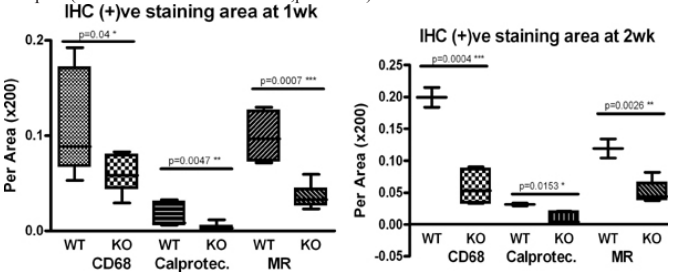
SA-PO2184

The Role of Mannose Receptor in Acute Folic Acid Nephritis Hsu-Han Wang,[1] Sally Hamour,[2] Ruth J. Pepper,[1] Mark Little,[1] H. Terence Cook,[2] Alan D. Salama.[1] *[1]Centre for Nephrology, University College London, United Kingdom; [2]Renal Medicine, Imperial College London, United Kingdom.*

Background: Folic acid (FA) induces acute tubular injury, characterised by tubular apoptosis/necrosis, followed by both repair and scarring and is associated with macrophage infiltration. FA nephritis is therefore a good model of acute renal failure secondary to tubular damage. We have previously demonstrated requirement of mannose receptor (MR) for induction of immune mediated glomerulonephritis, and we have now evaluated the role of MR in acute kidney injury.

Methods: MR+/- mice and control WT C57BL6 mice were used at 8-12 weeks age. Folic acid 240mg/kg was injected intraperitoneally with 0.2ml $NaHCO_3$ as vehicle. Mice are sacrificed at 7 or 14 days after injection. We assessed renal function, histological damage (by acute tubular injury score) and degree of macrophage/cell infiltration (by staining for CD68, calprotectin, and mannose receptor) which was enumerated using Image Pro software.

Results: At day 7 following injury, serum urea/creatinine were similar between WT and MR+/- mice; however, acute tubular injury score was higher in WT mice (46.0±1.4 vs 20.5±3.7,p=0.009). WT mice had greater activated macrophage infiltration than MR+/-, assessed by CD68 staining (relative area 0.113±0.025 vs 0.061±0.006,p=0.04) and greater levels of calprotectin (0.016±0.006 vs 0.004±0.0009,p=0.0047) and predictably mannose receptor (0.099±0.012 vs 0.036±0.004,p=0.0007).



Two weeks following injury, there was increased staining in the WT mice (0.199±0.016 for CD68, 0.031±0.002 for calprotectin and 0.119±0.015 for mannose receptor) compared to MR+/- animals (0.058±0.010, 0.009±0.004, 0.049±0.007, respectively), all p<0.05.

Conclusions: Mannose receptor insufficiency protects mice from acute macrophage mediated kidney damage and may be a novel non-immunosuppressive therapeutic target for acute kidney injury.

SA-PO2185

Adrenomedullin Has Protective Effects on Renal Descending Vasa Recta and Endothelian Cells Against Functional and Morphologic Impairment by Iodinated Contrast Media Mauricio Michalak Sendeski,[1] Anja Bondke Persson,[1] Pontus Persson,[1] Andreas Patzak.[1] *[1]Institut fuer Vegetative Physiologie, Charite Universitaetsmedizin Berlin, Berlin, Germany; [2]Uppsala University, Sweden.*

Background: Contrast induced nephropathy (CIN), a complication of iodinated contrast media (CM), is a frequent cause of acute kidney injury. Renal medullary ischemic damage is a hallmark of CIN. CM causes functional impairment of isolated outer medullary descending vasa recta (DVR) and lower endothelial nitric oxide production. We investigated the effect of adrenomedullin—an endogenous peptide with endothelial protective effects—in the prevention of CM deleterious effects on DVR and endothelial cells from renal interlobar arteries.

Methods: We studied isolated, perfused DVR and interlobar arteries from Sprague-Dawley rats. Iodixanol, a dimeric non-ionic CM, was applied intraluminally to DVR and interlobar arteries using microperfusion techniques (23mg iodine/ml). Control experiments used vehicle solution. Diameter changes of DVR were serially monitored. The reactivity to angiotensin II was tested after 20 minutes of CM application. The inner surface of fixated interlobar arteries was studied with scan electron microscopy (SEM). Adrenomedullin (10-7M) was applied intra- and extra-luminally to DVR and interlobar arteries, with and without concomitant use of CM.

Results: Perfusion with CM caused a marked constriction of DVR (nearly 50% of initial diameter). CM induced anatomical damage of endothelial cells from interlobar arteries (nuclear protrusion, cell shrinking, fenestration of the endothelial layer, and "blebbling"). Adrenomedullin significantly prevented DVR constriction and increased DVR response to angiotensin II. Structural damage of endothelial cells was also effectively prevented by adrenomedullin.

Conclusions: Adrenomedullin is effective in preventing structural damage and vascular functional changes caused by CM. Our results point out that avoiding the structural damage of endothelial cells may also prevent functional changes caused by CM on DVR. This suggests that prophylaxis of CIN should focus on the prevention of cell damage by CM in order to diminish overall renal tissue injury by CM.

SA-PO2186

Bactericidal Antibiotics Temporarily Increase Inflammation and Worsen Acute Kidney Injury in Experimental Sepsis Anan Chuasuwan,[1] Zhiyong Peng,[1] Hongzhi Wang,[1,2] Nattachai Srisawat,[1] Xiao-Yan Wen,[1] Thomas Rimmelé,[1] Jeffery Bishop,[1] Kai Singbartl,[1] Raghavan Murugan,[1] John A. Kellum.[1] *[1]Department of Critical Care Medicine, Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), University of Pittsburgh, Pittsburgh, PA; [2]Beijing Cancer Hospital & Institute, Beijing, China.*

Background: Acute kidney injury (AKI) is a common disorder in critically ill patients, and mortality reaches 70% when combined with sepsis. Bactericidal antibiotics release bacterial products including pathogen-associated molecular patterns (PAMPs) which could trigger inflammation and induce or worsen severity of AKI.

Methods: Sepsis was induced by cecal ligation and puncture (CLP) in fifty-two Sprague-Dawley rats and was treated with either bactericidal antibiotics (ampicillin/sulbactam) or placebo (saline). Serial blood specimens were obtained from 18 hrs up to day 7 after CLP for serum creatinine, interleukin (IL)-6, and neutrophil gelatinase-associated lipocalin (NGAL) concentrations. RIFLE criteria were used to assess severity of AKI. All animals were observed for survival at one week. In a separate experiment, 12 animals were sacrificed 2 days after CLP for histology.

Results: Survival in placebo-treated animals was 50% compared to 82% with antibiotics (P <0.05). Most animals (93%) without antibiotics developed AKI, of which 39% exhibited greater than a 3-fold rise in serum creatinine (RIFLE-F). Furthermore, survival decreased as AKI severity increased. Surprisingly, all animals treated with antibiotics developed AKI, of which 68.6% reached RIFLE-F. However, renal dysfunction was less persistent in antibiotic-treated animals indicated by both serum creatinine and plasma NGAL concentration. Patterns of plasma IL-6 were similar to creatinine. Histological findings were consistent with functional parameters showing that antibiotics worsened AKI.

Conclusions: Bactericidal antibiotic therapy during sepsis improved survival but resulted in higher IL-6 concentration and more severe AKI. Animals without antibiotics had less evidence of early inflammation and AKI but failed to recover. Mortality was associated with failure to recover from AKI.

Funding: Other NIH Support - National Institutes of Health (NHLBI)
Systems Engineering of a Pheresis Intervention for Sepsis (SEPsIS) HL080926

SA-PO2187

The Constant Region Contributes to the Antigenic Specificity and Potential Nephritogenicity of Anti-DNA Antibodies Chaim Putterman, Yumin Xia. *Albert Einstein College of Medicine.*

Background: Certain IgG subclasses are particularly enriched in kidney Ig eluates in active lupus nephritis, suggesting that antibody (Ab) isotype is one important feature determining the outcome of Ab binding to renal antigens. Although the variable region was believed to be the sole determinant of antigenic specificity, more recent studies have shown that isotype switching leads to altered binding of a protective Ab. Our hypothesis was that Ab isotype may affect the renal pathogenicity of anti-DNA Ab, by influencing antigen binding.

Methods: PL9-11 is an IgG3 anti-DNA Ab isolated from a MRL/+ mouse. To obtain IgG1, IgG2b and IgG2a forms of this Ab, the PL9-11 hybridoma clone was isotype switched in vitro by stimulation with IL-4 and TGF-β. The PL9-11 (IgM) variant was generated by cloning the PL9-11 VDJ into an IgM expression vector, followed by transfection into a cell line expressing only the PL9-11 light chain. The affinity/specificity of the PL9-11 Ab panel were analyzed by ELISA, surface plasmon resonance, and cross-inhibition. Antigenic specificity was studied by binding to mesangial cells, isolated glomeruli, and glomerular proteome arrays. Finally, renal deposition and pathogenicity were assayed by analyzing kidneys of SCID mice injected with the PL9-11 Ab panel.

Results: We found that PL9-11 and its isotype switched variants had differential binding to ssDNA, dsDNA, chromatin and mesangial cells in order of IgG3>IgG2a>IgG1>IgG2b>IgM. This order of relative affinities of the different IgG isotypes for dsDNA was confirmed in a competition ELISA. In contrast, in binding to Matrigel, laminin, and collagen IV the IgG2a isotype actually had the highest affinity, followed by IgG3>IgG1>IgG2b>IgM. In concert with ELISA assays, assessing antibody specificity in glomerular proteome arrays also revealed significant differences between the members of the PL9-11 panel in binding to multiple antigens.

Conclusions: Our data suggest that the constant region plays an important role in the affinity and specificity of anti-DNA Ab, and that IgG2a and IgG3 isotypes may be more nephritogenic due to higher potential for binding to multiple glomerular and nuclear antigens.

Funding: Other NIH Support - NIAMS

SA-PO2188

TWEAK/Fn14 Pathway Blockade Attenuates Renal Disease in Autoantibody-Induced Nephritis Chaim Putterman,[1] Jennifer Michaelson,[2] Linda Burkly.[2] *[1]Albert Einstein College of Medicine; [2]Biogen Idec.*

Background: TNF-superfamily members are instrumental in the pathogenesis of lupus nephritis. Previously, we found that TWEAK (TNFSF12)-mediated activation of its receptor, Fn14, stimulates the secretion of MCP-1, RANTES, IP-10 and KC by mesangial

cells and podocytes. TWEAK also modulates renal cell survival and proliferation. Thus, we hypothesized that TWEAK blockade may be therapeutically beneficial in autoantibody-mediated nephritis.

Methods: Nephrotoxic serum nephritis (NTN), a murine model for lupus nephritis, was used to study the role of the TWEAK/Fn14 pathway in the pathogenesis of renal disease induced by pathogenic antibodies.

Results: We induced NTN by passive transfer of pre-formed nephritogenic rabbit antibodies into 129 Fn14 knockout (KO) and wildtype (WT) mice that had been preimmunized with heterologous rabbit IgG. On days 7, 14, and 21 after antibody transfer, Fn14KO mice had significantly decreased levels of proteinuria as compared to Fn14 WT mice (day 7: 61±24 vs 220±42 mg/dl, $p<0.01$; day 14: 99±50 vs. 678±205 mg/dl, $p=0.02$; day 21: 101±49 vs 678±205 mg/dl, $p=0.02$). Moreover, crescent formation and tubular dilatation were significantly decreased in Fn14KO mice, as were MCP-1, RANTES, and IP-10 kidney mRNA expression levels. To confirm the protective effect of TWEAK inhibition with a pharmacological approach, we induced nephrotoxic nephritis in 129 Fn14 WT mice and initiated treatment with an anti-TWEAK mAb or isotype matched control Ig. Similar to results in Fn14KO mice, significant amelioration of proteinuria and improvement in renal histology was observed in mice with induced nephritis receiving treatment with anti-TWEAK mAb. Anti-TWEAK mAb treatment did not appear to affect the systemic immune response, as no alteration in murine anti-rabbit IgG subclass antibody titers was evident.

Conclusions: TWEAK/Fn14 interactions promote the pathogenesis of nephritis in the NTN model, likely playing a role in pathologic events locally in the kidney rather than impacting the systemic immune response. Thus, disrupting TWEAK/Fn14 interactions may be an innovative approach for the treatment of lupus and other antibody-induced renal diseases.

Funding: Other NIH Support - NIAMS, Pharmaceutical Company Support

SA-PO2189

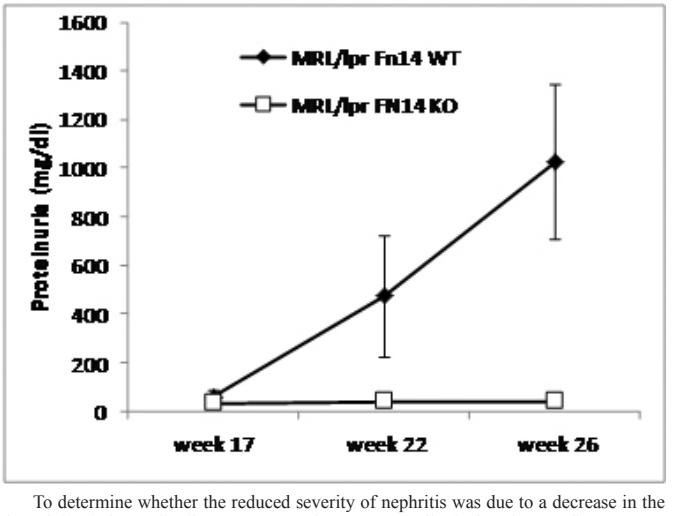
Deficiency of the TWEAK Receptor Fn14 Is Protective in the MRL/lpr Mouse Model of Lupus Nephritis Chaim Putterman,[1] Jennifer Michaelson,[2] Linda Burkly.[2] *[1]Albert Einstein College of Medicine; [2]Biogen Idec.*

Background: Renal mesangial cells and podocytes express Fn14, the receptor for TWEAK (TNFSF12). TWEAK engagement of Fn14 induces kidney resident cells to produce multiple inflammatory mediators including MCP-1 and RANTES, which have been linked to the pathogenesis of lupus nephritis. In cGVH induced nephritis, genetic deficiency of Fn14, or treatment with an anti-TWEAK mAb, decreases kidney inflammation and proteinuria without affecting autoantibody titers. These studies suggested that inhibition of TWEAK/Fn14 might be efficacious in spontaneous models of lupus.

Methods: We assessed the role of the TWEAK/Fn14 pathway in the pathogenesis of lupus nephritis (LN) by evaluating the effect of Fn14 deficiency in the MRL/lpr spontaneous mouse model of lupus.

Results: We found that kidney Fn14 was significantly increased in MRL/lpr mice at 26 as compared to 7 weeks of age, while splenic Fn14 expression actually decreased over time. Kidney TWEAK expression also increased over time in MRL/lpr mice, and was significantly higher by 19 as compared to 7 weeks, and when compared to MRL/+ mice.

At 26 weeks MRL/lpr Fn14 WT mice (n=10) had significantly higher levels of proteinuria as compared to MRL/lpr Fn14KO (n=9).



To determine whether the reduced severity of nephritis was due to a decrease in the titer of circulating antibodies, we compared serum autoantibody titers in MRL/lpr Fn14WT and KO mice. There were no differences between the groups in anti-dsDNA antibody titers, suggesting that TWEAK likely acts by modulating events locally in the kidney.

Conclusions: TWEAK/Fn14 interactions are instrumental in the pathogenesis of LN in the MRL/lpr mouse model. Our results suggest that blocking the effects of TWEAK may be a novel therapeutic approach to the treatment of the kidney disease associated with SLE, without inducing systemic immunosuppression.

Funding: Other NIH Support - NIAMS, Pharmaceutical Company Support

SA-PO2190

Activated Protein C Attenuates Systemic Lupus Erythematosus and Lupus Nephritis in MRL-Fas(lpr) Mice Julia Lichtnekert, Khader Valli Rupanagudi, Onkar Kulkarni, Hans J. Anders. *Medizinische Poliklinik, LMU, Klinikum der Universitat Munchen, Munich, Germany.*

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease leading to inflammatory tissue damage in multiple organs, e.g. lupus nephritis. Current treatments including steroids, antimalarials, and immunosuppressive drugs have significant side effects. Activated protein C (APC) is a natural protein with anticoagulant and immunomodulatory effects and its recombinant version has been approved by the FDA to treat severe sepsis. Given the similarities between overshooting immune activation in sepsis and autoimmunity, we hypothesized that recombinant APC would also suppress SLE and lupus nephritis.

Methods: Autoimmune female MRL-Fas(lpr) mice were injected with either vehicle or recombinant human APC from week 14 to 18 of age.

Results: APC treatment significantly suppressed lupus nephritis as evidenced by decrease in activity index, glomerular IgG and complement C3 deposits, macrophage counts as well as intrarenal IL-12 expression. Further, APC attenuated cutaneous lupus and lung disease as compared to vehicle-treated MRL-Fas(lpr) mice. In addition, parameters of systemic autoimmunity, such as plasma cytokine levels of IL-12p40, IL-6, CCL2/MCP-1 and numbers of B cells and plasma cells in spleen were suppressed by APC. The latter was associated with lower total plasma IgM and IgG levels as well as lower titers of anti-dsDNA IgG and rheumatoid factor.

Conclusions: Together, recombinant APC suppresses the abnormal systemic immune activation in SLE of MRL-Fas(lpr) mice which prevents subsequent kidney, lung and skin disease. These results implicate that recombinant APC might be useful for the treatment of human SLE.

Funding: Private Foundation Support

SA-PO2191

Serum IgG Binding to Human Mesangial Cells in Patients with Lupus Nephritis and Its Correlation with Disease Activity Desmond Y.H. Yap, Susan Yung, Owen Chan, Qing Zhang, Daniel Tak Mao Chan. *Department of Medicine, University of Hong Kong, China.*

Background: Mesangial immunoglobulin deposition is a hallmark in lupus nephritis. We previously demonstrated that human anti-dsDNA antibodies could bind to human mesangial cells (HMC). In this study we investigated the binding activity of serum IgG to HMC and its correlation with clinical parameters in lupus patients.

Methods: Serial serum samples were obtained from 23 patients with biopsy-proven diffuse proliferative lupus nephritis over a mean follow-up of 74 months. Binding activity (expressed as OD) of total serum IgG and its subclasses (IgG_1, IgG_2, IgG_3, IgG_4) to HMC was measured using a cellular ELISA and its correlation with clinical or laboratory parameters investigated. Sera from 23 healthy individuals were used as controls.

Results: A total of 189 samples were collected - 48 samples during active and 141 during inactive disease, defined according to clinical assessment. Binding of serum total IgG to HMC was 0.12±0.09, 0.59±0.37 and 0.74±0.43 OD for healthy controls, inactive lupus, and active lupus respectively (P=0.023 active vs inactive, P<0.001 controls vs active or inactive disease). Binding of serum IgG_1 to HMC was 0.05±0.05, 0.41±0.38 and 0.55±0.40 OD for the three groups respectively (P=0.037 active vs inactive, P<0.001 controls vs active or inactive disease). Controls and lupus patients did not differ in the binding of serum IgG_2, IgG_3 or IgG_4 to HMC. HMC-binding activity of total IgG and IgG_1 correlated with anti-dsDNA antibody levels (r=0.26 and 0.39 respectively, P<0.001 for both), and inversely correlated with C3 levels (r=-0.17 and -0.45 respectively, P<0.05 for both). No correlation was observed between IgG binding to HMC and clinical parameters such as serum creatinine, albumin, or proteinuria. Sensitivity/specificity of total IgG or IgG_1 binding to HMC in the prediction of renal flare in the patients was 81.3%/39.7% (ROC AUC 0.61, P=0.03) and 83.3%/41.8% (AUC 0.63, P=0.009) respectively.

Conclusions: We conclude that total IgG and IgG_1 in the serum of patients with lupus nephritis bind significantly to mesangial cells, especially during flare, and this binding correlated with the level of anti-dsDNA antibodies.

Funding: Government Support - Non-U.S.

SA-PO2192

Involvement of $CD11b^+$ $GR\text{-}1^{low}$ Cells in Autoimmune Disorder in MRL-*Fas^{lpr}* Mouse Yasunori Iwata, Kengo Furuichi, Takashi Wada. *Nephrology, Kanazawa University, Kanazawa, Ishikawa, Japan.*

Background: Myeloid derived suppressor cells (MDSCs) have been identified as immunosuppressive cells in tumor related inflammation. However, the pathogenesis of MDSCs for autoimmune disease has not been investigated yet. The aim of this study is to address whether MDSCs contribute to autoimmune organ injury in lupus prone mice.

Methods: MDSCs were analyzed by flow cytometric staining of $CD11b^+$ $GR\text{-}1^+$ in MRL-*Fas^{lpr}* mice. $CD4^+$ T cell proliferation assay was performed by the co-culture with $CD11b^+$ $GR\text{-}1^+$ splenocytes. The percentage of immunosuppressive cells was examined during disease progression. The expression of chemokine receptor on immunosuppressive cells was analyzed. Moreover, chemotaxis assay was performed.

Results: $CD11b^+$ $GR\text{-}1^{low}$ cells had a suppressive effect on $CD4^+$ T cell proliferation, which was restored by an arginase-1 inhibitor. $CD11b^+$ $GR\text{-}1^{low}$ cells increased in percentage during disease progression in kidney and blood. The number of migrated $CD11b^+$ $GR\text{-}1^{low}$ cells increased in the presence of monocyte chemoattractant protein (MCP)-1/CCL2.

Conclusions: We assessed the involvement of $CD11b^{+}$ $GR\text{-}1^{low}$ cells in autoimmune disorder in MRL-*Fas*^{lpr} mice. These cells regulate immunological responses via CCL2/CCR2 signaling. The regulation of immunosuppressive monocytes may provide novel therapeutic strategy for organ damage in autoimmune diseases.

Funding: Government Support - Non-U.S.

SA-PO2193

Anti-Glomerular Basement Membrane Antibodies Against Certain Linear Epitopes on Goodpasture Antigen Are Associated with Clinical Phenotypes and Disease Severity Zhao Cui, Xiao-Yu Jia, Rui Yang, Ming-Hui Zhao. *Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.*

Background: Anti-glomerular basement membrane (GBM) disease could be initiated by nephrogenic linear epitopes which stimulate both T and B cell responses in experimental autoimmune glomerulonephritis. Linear epitopes involved in human anti-GBM disease have not been fully defined. In this study, we investigated the linear epitopes recognized by circulating antibodies in sera from patients with anti-GBM disease, aiming to identify the potential nephrogenic linear epitopes and their clinical and pathological significance.

Methods: 68 patients with anti-GBM disease were enrolled. A panel of 23 overlapping linear peptides was synthesized across the whole sequence of human α3(IV)NC1. Among them, P14 contained the previous known immunodominant regions E_B and a T cell epitope of patients. Circulating antibodies against linear epitopes were detected by enzyme-linked immunoabsorbent assay. The associations between linear epitopes recognition and clinical and pathological data of patients were further analyzed.

Results: Antibodies against linear peptides were detected in sera from 55 (80.9%) patients. Three major epitopes were identified with high frequencies: P14 (amino acid residues 131-150) 41%, P16 (amino acid residues 161-180) 36.8% and P18 (amino acid residues 181-200) 57%. Antibodies against P14 were frequently detected in patients with positive ANCA (39.3% vs. 12.5%, P=0.010). Patients with antibodies against P16 presented with higher concentration of serum creatinine on diagnosis (665.5±227.2 vs. 443.7±296.8 μmol/L, P=0.001). The levels of antibodies against P18 were positively correlated with the percentage of crescentic formation in glomeruli (r=0.54, P=0.008).

Conclusions: Antibodies against certain linear epitopes could be detected in patients with anti-GBM disease and were associated with clinical phenotypes and disease activity. Antibodies against P14 were associated with coexistence of ANCA. Antibodies against P16 and P18 were associated with the severity of crescentic glomerulonephritis.

Funding: Government Support - Non-U.S.

SA-PO2194

Extensive Antigen Receptor Editing Modifies Autoimmunity in Anti-Glomerular Basement Membrane Disease Inge Maria Schudel, Melissa L. Weston, Amy G. Clark, Mary H. Foster. *Medicine, Duke University Medical Center and DVAMC, Durham, NC.*

Background: In anti-glomerular basement membrane disease autoantibodies against the NC1 domain of the alpha3 chain of type IV collagen can lead to autoimmune nephritis and severe kidney damage. To determine the origins of and tolerance mechanisms regulating the pathogenic autoantibodies, we developed an anti-alpha3(IV)NC1 IgM/kappa autoantibody transgenic (Tg) mouse model. Initial analysis of immunoglobulins (Ig) in serum and on spleen B cell surfaces revealed that anti-alpha3(IV)NC1 B cells are regulated by deletion and receptor editing that replaces Tg with endogenous Ig receptors. To further examine the extent and nature of editing and to dissect independent Ig chain contributions within individual anti-alpha3(IV)NC1 B cells, we used a combined genetic and cell fusion approach.

Methods: Anti-alpha3(IV)NC1 Ig Tg mice were bred with mice carrying a knockout of both endogenous kappa alleles, such that any kappa light chain derives only from the transgene. Spleen B cells from four mice then were fused with myeloma cells. The resulting hybridoma cells were analyzed by ELISA and PCR for expression of Tg (IgMa, kappa) and endogenous (IgMb, lambda) Ig.

Results: Of 65 IgM-secreting hybridomas recovered (mean 16 clones/spleen), 60% secrete variable amounts of transgenic IgMa and/or kappa chain. Strikingly, 94% of hybridomas show editing, detected as secretion of endogenous IgMb, lambda, or both (82%). In addition, differentiation of the four murine lambda subtypes by PCR shows restricted lambda chain usage.

Conclusions: These results indicate that extensive heavy and light chain editing occurs at the single cell level to modify expression of pathogenic anti-GBM autoantibodies. Ongoing studies should shed light on the molecular basis for this unexpected requirement for multiple editors and provide insights into disease origins.

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SA-PO2195

Modifiers of Susceptibility to Anti-Glomerular Basement Membrane Antibody Disease in Fc Gamma Receptor 2B Deficient Mice Ruth M. Tarzi,[1] Phoebe E.H. Sharp,[1] Javier Martín Ramírez,[2] Sara M. Mangsbo,[2] John Reynolds,[1] Charles D. Pusey,[1] H. Terence Cook,[1] Sjef Verbeek.[2] [1]*Renal Department, Imperial College, London, United Kingdom;* [2]*Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.*

Background: Anti-glomerular basement membrane antibody disease (anti-GBM disease) is an autoimmune disease, leading to crescentic glomerulonephritis. Fc gamma receptor 2B (Fcgr2B) is the only inhibitory Fc receptor for IgG in humans and mice. The purpose of this study was to compare the susceptibility to anti-GBM disease of mice lacking Fcgr2B on a pure C57BL/6 background (Fcgr2B-B6), with mice lacking Fcgr2B created in 129 embryonic stem cells and backcrossed to C57BL/6 (Fcgr2B-129/B6), therefore having 129 sequences flanking the Fcgr2B deletion. We then compared the susceptibilty of mice with cre-mediated conditional deletion of Fcgr2B on B cells and monocytes/macrophages to anti-GBM disease.

Methods: Anti-GBM disease was induced by subcutaneous immunization with a recombinant peptide of the NC1 domain of alpha 3 Type IV collagen at day 0, with boosts at day 7, 14 and 21. Mice were sacrificed 20 weeks after immunization.

Results: In two experiments, there was a higher incidence of crescentic glomerulonephritis in Fcgr2B-129/B6 mice compared with Fcgr2B-B6 mice (Experiment 1: 6/8 (75%) vs 2/8 (25%) vs WT (0/4) (0%); Experiment 2: 8/12 (66%) vs 4/11(37%) vs WT 1/9 (11%). However, in those mice that developed disease, there was no difference in the severity of the crescentic glomerulonephritis.

In the second part of the experiments, 4/11 (37%) Fcgr2B-B6 mice developed crescentic glomerulonephritis, compared to 2/9 Fcgr2B-CD19cre mice (22%), 1/12 Fcgr2B-LysM mice (8%) and 1/9 WT mice (11%).

Conclusions: The incidence of crescentic glomeruonephritis was higher in the Fcgr2B-129/B6 mice than the Fcgr2B-B6 mice, indicating that genetic modifiers located near to Fcgr2B on the 129 genome increase the tendency of Fcgr2B-/- mice to develop anti-GBM disease.

Secondly, Fcgr2B-LysM mice had the same incidence of anti-GBM disease as WT mice. Our preliminary results indicate that loss of Fcgr2B on B cells alone did not increase the incidence of anti-GBM disease as much as the full deletion.

Funding: Private Foundation Support

SA-PO2196

Pathogenesis of IgA Nephropathy: Characterization of the Kinetics and Site-Specificity of GalNAc-Transferase 2, the Enzyme Initiating O-Glycosylation of IgA1 Kazuo Takahashi,[1] Milada Horynova,[2,1] Milan Raska,[2,1] Stacy D. Hall,[1] Bruce A. Julian,[1] Zina Moldoveanu,[1] Jiri F. Mestecky,[1] Matthew B. Renfrow,[1] Jan Novak.[1] [1]*University of Alabama at Birmingham, AL;* [2]*Palacky University in Olomouc, Olomouc, Czech Republic.*

Background: IgA1 with galactose-deficient hinge-region (HR) *O*-glycans (Gd-IgA1) plays a key role in IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential *O*-glycosylation sites occupied; some Gd glycans consist of terminal *N*-acetylgalactosamine (GalNAc). As *O*-glycosylation of IgA1 is initiated by a GalNAc-transferase, namely GalNAc-T2, understanding the kinetics and site-specificity of this enzyme will provide insight into the pathogenesis of IgAN.

Methods: We used recombinant GalNAc-T2 in an *in vitro* system to study kinetics of site-specific glycosylation using high-resolution mass spectrometry (MS). We used two synthetic HR peptides (sHR) as acceptors: VPSTPPTPSPSTPPTPSPSC (short sHR) and VPSTPPTPSPSTPPTPSPSCCHPR (long sHR)

Results: Time-course analysis showed that the number of added GalNAc residues increased with time. GalNAc-T2 showed higher activity, *i.e.,* faster rate of glycosylation, with long sHR than with short sHR. The reaction reached plateau when seven sites were glycosylated in the long sHR and eight sites were glycosylated in short sHR. Thus, the sequence and length of acceptor substrate affect *O*-glycosylation, particularly the maximal number of GalNAc residues attached and the kinetics of the reaction. sHR *O*-glycoforms were subjected to tandem MS to localize glycosylated sites. GalNAc-T2 added GalNAc residues in an ordered fashion: to T7 first and then to T15, followed by S11, T4, S9, S17 and S19. Glycosylation sites on sHR were consistent with the sites previously described on IgA1, except the last two sites, S17 and S19, that are not usually glycosylated in serum IgA1. S230, the dominant site with terminal GalNAc in Gd-IgA1 myeloma proteins that mimic Gd-IgA1 from patients with IgAN, corresponds to S9 in sHR; this site is ineffectively glycosylated by GalNAc-T2.

Conclusions: Detailed studies of GalNAc-T2 kinetics and specificity using this new tool will yield new information relevant to the pathogenesis of IgAN.

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SA-PO2197

Effect of gp130 Cytokines on IgA1-Producing Cells from Patients with IgA Nephropathy Koshi Yamada,[1,2] Milan Raska,[1,5] Zina Moldoveanu,[1] Hitoshi Suzuki,[2,1] Krzysztof Kiryluk,[3] Bruce A. Julian,[1] Robert J. Wyatt,[4] Yasuhiko Tomino,[2] Jiri F. Mestecky,[1] Ali G. Gharavi,[3] Jan Novak.[1] *[1]University of Alabama at Birmingham, AL; [2]Juntendo University, Tokyo, Japan; [3]Columbia University, New York, NY; [4]University of Tennessee, Memphis, TN; [5]Palacky University, Olomouc, Czech Republic.*

Background: A recent GWAS study identified five loci associated with IgA nephropathy (IgAN). One locus was Chr. 22q12.2 that includes genes encoding Leukemia Inhibitory Factor (LIF) and Oncostatin M (OSM), IL-6-related cytokines using gp130 for signal transduction and implicated in mucosal immunity and inflammation. As Chr. 22q12.2 has been associated with serum IgA, LIF and OSM are candidate cytokines to be tested for their effect on IgA1-producing cells.

Methods: Using EBV-immortalized IgA1-secreting cell lines derived from the circulation of patients with IgAN (IgAN cells) and healthy controls (HC cells), we assessed the effects of LIF and OSM, and IL-6 as a control, on IgA1 production and its *O*-glycosylation. Expression of genes encoding the corresponding receptors (IL-6R1, 2, 3; OSMR1, 2; and LIFR) and glycosyltransferases (C1GalT1, GalNAc-T2, GalNAc-T14) was measured by RealTime RT-PCR. IgA level and IgA1 galactose (Gal) deficiency were determined by ELISA.

Results: IgAN and HC cells expressed IL-6R1, 2, 3, OSMR1, and low levels of LIFR, but not OSMR2. Cytokines LIF, OSM, and IL-6 affected IgA1 production in all cells, but increased Gal deficiency of IgA1 only in IgAN cells. In IgAN cells, but not in HC cells, all three cytokines influenced the expression of glycosyltransferases: decreased for C1GalT1 and increased for GalNAc-T2. The expression of GalNAc-T14 has not changed in IgAN cells, but decreased significantly in the HC cells in response to stimulation with each cytokine.

Conclusions: In summary, gp130 cytokines in Chr. 22q12.2 locus affected IgA1-producing cells from IgAN patients and induced differential gene expression of specific glycosyltransferases that may lead to aberrant *O*-glycosylation of secreted IgA1.

Funding: NIDDK Support

SA-PO2198

Characterization of Galactose-Deficient IgA1 Secreted by IgA1-Producing Cell Lines: Implication for Pathogenesis of IgA Nephropathy Kazuo Takahashi,[1] Hitoshi Suzuki,[2,1] Koshi Yamada,[1] Stacy D. Hall,[1] Zina Moldoveanu,[1] Bruce A. Julian,[1] Knud Poulsen,[3] Jiri F. Mestecky,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, AL; [2]Juntendo University, Tokyo, Japan; [3]Aarhus University, Aarhus, Denmark.*

Background: IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans plays a key role in IgA nephropathy (IgAN). We established IgA1-producing cells derived from the circulation of IgAN patients and healthy controls (HC). IgA1 secreted by cells from IgAN patients has more Gal-deficient *O*-glycans with terminal or sialylated *N*-acetylgalactosamine (GalNAc) compared to IgA1 from HC cells. To define the glycosylation patterns, including the sites of glycan attachment, we analyzed IgA1 produced by cells derived from an IgAN patient (IgAN-IgA1) and a HC (HC-IgA1).

Methods: IgA1 was treated with bacterial IgA-specific proteases (AK183, TIGR4, or HK50) followed by trypsin and HR glycopeptides were characterized by high-resolution mass spectrometry (MS). Relative abundance of each glycopeptide was expressed as % of total HR.

Results: The number of *O*-glycans per HR ranged from 3 to 6, with 4 to 6 being most abundant on IgAN-IgA1 and 3 to 5 predominating on HC-IgA1. HR variant with 3 *O*-glycans was fully galactosylated; it was the predominant variant in HC-IgA1 but not in IgAN-IgA1 (17.9% vs. 0.8%). N-terminal HR (H208-P227) released by TIGR4 was glycosylated in 98.2% as a disaccharide in IgAN-IgA1 compared to 80.2% in HC-IgA1. This indicated that a disaccharide attached at S224 or T225 was present in the HR with 4 to 6 *O*-glycans, as there was no *O*-glycan at S224/T225 in HR with 3 *O*-glycans in HC-IgA1. Gal-deficient HR variants were more common in IgAN-IgA1 than in HC-IgA1 (24.5% vs. 15.9%). A Gal-deficient GalNAc was detected in N-terminal HK fragment of HR (T228 or S230) and in C-terminal HK fragment of HR (S232 to R245). These findings were corroborated by lectin blotting.

Conclusions: Thus, we confirmed the glycosylation abnormality of *O*-glycans on IgA1 in IgAN using high-resolution MS. This new tool provides useful information relevant to IgAN pathogenesis through the identification of distinct glycosylation patterns.

Funding: NIDDK Support

SA-PO2199

Role of GalNAc-Transferases in the Synthesis of Aberrant IgA1 *O*-glycans in IgA Nephropathy Milan Raska,[1] Koshi Yamada,[1] Milada Horynova,[2,1] Kazuo Takahashi,[1] Hitoshi Suzuki,[3,1] Zina Moldoveanu,[1] Jana Novakova,[2] Alena Kasperova,[2] Bruce A. Julian,[1] Krzysztof Kiryluk,[4] Jiri F. Mestecky,[1] Matthew B. Renfrow,[1] Ali G. Gharavi,[4] Jan Novak.[1] *[1]University of Alabama at Birmingham, AL; [2]Palacky University, Olomouc, Czech Republic; [3]Juntendo University, Tokyo, Japan; [4]Columbia University, New York, NY.*

Background: IgA nephropathy (IgAN) is an autoimmune disease in which IgA1 with galactose (Gal)-deficient *O*-glycans in the hinge region (HR) plays a key role as the autoantigen. The mechanisms involved in the formation of the aberrant glycans are not well understood, but likely include changes in the expression, activity, and/or localization of enzymes involved in the individual glycosylation steps. *O*-glycans of normal IgA1 consist of *N*-acetylgalactosamine (GalNAc) and Gal, with possibly one or both residues sialylated, whereas IgA1 in the circulation and renal deposits of patients with IgAN has some *O*-glycans deficient in Gal. *O*-glycan formation is initiated by attachment of GalNAc to the serine or threonine in IgA1 HR, catalyzed by a GalNAc-transferase (GalNAc-T).

Methods: It has been speculated that GalNAc-T2 is responsible for initiation of *O*-glycosylation in IgA1. However, our recent *in vitro* studies with recombinant GalNAc-T2 demonstrated that some sites with Gal-deficient glycans are added by this enzyme ineffectively, suggesting involvement of another GalNAc-T. Therefore, we analyzed by RealTime RT-PCR the expression of GalNAc-T1 to -T14 in EBV-immortalized IgA1-producing cells derived from blood of IgAN patients and healthy controls.

Results: GalNAc-T14 was among the major GalNAc-Ts transcribed in IgA1-producing cells and its expression was 5-fold greater in the cells from the patients *vs.* the cells from healthy controls. The expression of GalNAc-T2, and other GalNAc-Ts, did not differ between patients and healthy controls.

Conclusions: Based on these results, we speculate that the expression profile of a specific GalNAc-T, such as over-expression of GalNAc-T14, contributes to production of Gal-deficient IgA1 *O*-glycans in patients with IgAN.

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SA-PO2200

Activated Innate Immunity and Involvement of the CX3CR1-FKN Axis in Promoting Hematuria in IgA Nephropathy Patients Sharon N. Cox,[1] Fabio Sallustio,[1,2] Grazia Serino,[1,2] Antonia Loverre,[1] Francesco Pesce,[1] Patrizia Stifanelli,[3] Nicola Ancona,[3] Gianluigi Zaza,[1] Francesco Paolo Schena.[1,2] *[1]Dept. of Emergency and Organ Transplant, Univ. of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]ISSIA, CNR, Bari, Italy.*

Background: The hallmark of IgA nephropathy (IgAN) is gross hematuria (GH) coinciding with or immediately following a respiratory or gastrointestinal tract infection and can represent the disease triggering event.

Methods: Therefore, a whole genomic screening of IgAN patients during the GH was done to clarify the link between mucosal encountered antigens and the occurrence of glomerular hematuria.

Results: The modulated genes during GH showed a clear involvement of the interferon signalling, antigen presenting pathway, and the immuno-proteasome. The gene characterizing cytotoxic effector lymphocytes (*CX3CR1*), implicated in vascular endothelial damage, was found up-regulated at both mRNA and protein level. *In vitro* antigenic stimulation of PBMCs on an independent set of IgAN patients and healthy blood donors (HBD) demonstrated that patients upregulate specifically CX3CR1 in an enhanced and dose dependant manner, while an expected down-regulation occurred in HBD. This enhanced activation occurred in patients both characterized by recurrent GH and by permanent microscopic hematuria (MH). We then analyzed glomerular fractalkine (FKN) expression, since this ligand is involved in the vascular gateway for $CX3CR1^+$ cells towards the inflamed tissues. A significantly higher FKN expression on the capillary vessels and podocytes was found in IgAN patients with recurrent GH compared to permanent MH, suggesting a predisposition for cytotoxic cell extravasation in recurrent GH patients.

Conclusions: Taken together, our findings demonstrate, for the first time, a defect in antigen handling in PBMCs of IgAN patients with a specific up-regulation of CX3CR1. Furthermore, the constitutive up regulation of glomerular FKN, suggests an involvement of the CX3CR1-FKN axis in the exacerbation of GH.

SA-PO2201

IgA-IgG Immune-Complex Formation by Marginal Zone B Cells Is Crucial for the Progression of Murine IgA Nephropathy Keiko Okazaki,[1] Yusuke Suzuki,[1] Mareki Ohtsuji,[2] Qingshun Lin,[2] Sachiko Hirose,[2] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Second Department of Pathology, Juntendo University Faculty of Medicine, Tokyo, Japan.*

Background: Splenic marginal zone B cells (MZB) are potentially poly-reactive and their expansion might be associated with autoimmune diseases, such as SLE or rheumatoid arthritis. Although the pathogenesis of IgA nephropathy (IgAN) has not been clarified, the recent studies have suggested that IgAN may be related to autoimmune disorders including immune-complex (IC) formation with aberrantly glycosylated IgA1 as an auto-antigen. The objective of the present study is to verify whether MZB contribute to the pathogenesis of murine IgAN.

Methods: Two IgAN prone mice, high IgA mice (HIGA) and grouped ddY mice (gddY) that we recently established as 100% onset model (J Am Soc Nephrol, 2005), were employed for present study.

Results: When we evaluated HIGA (N=5) and gddY (N=5) at 12 weeks of age, only gddY showed severe glomerular damage with increased massive albuminuria. Flow cytometry analysis showed that the numbers of MZB ($B220^+CD21^{high}CD23^{low/negative}$) and their precursors ($B220^+IgM^{high}CD21^{high}CD23^+$) in gddY were significantly higher than those in HIGA (gddY vs. HIGA; MZB: 6.1±0.9x106 vs. 3.8±0.6x106, P=0.002, precursors: 2.6±0.6x106 vs. 1.4±0.2x106, P=0.002), suggesting that MZB may be involved in the progression of murine IgAN. For further confirmation, MZB in gddY at 4 weeks of age were selectively depleted by anti-LFA and anti-CD49d monoclonal antibodies. Clinical outcomes (depleted mice; N=5 vs. isotype-control mice; N=5) at 14 days after the treatment were as follows; albuminuria (ng/day): 40.9±19.2 vs. 230.6±146.4 (P=0.02), serum IgA (mg/dl): 47.1±8.5 vs. 43.2±6.4 (P=0.44), serum IgG (mg/dl): 40.9±7.4 vs. 71.1±14.4

(P=0.003), serum IgA-IgG IC (OD): 0.16±0.03 vs. 0.2±0.03 (P=0.05) and intensity of glomerular depositions: IgA, 97.6±13.0 vs. 103.5±9.8 (P=0.49) and IgG: 79.4±11.1 vs. 105.2±11.8 (P=0.02).

Conclusions: MZB may contribute to the progression of murine IgAN via nephritogenic IgA-IgG IC formation.

SA-PO2202

B Cell Depleting Therapies Protect Mice from Adriamycin Nephropathy by Reducing Natural Antibody IgM Joshua M. Thurman, Derek Strassheim, Magdalena Glogowska. *Medicine, University of Colorado School of Medicine, Aurora, CO.*

Background: Recent reports suggest that rituximab, a monoclonal antibody that depletes B cells, may be an effective treatment for some patients with focal segmental glomerulosclerosis (FSGS). It has long been noted that IgM and C3 are detectable in the mesangium of patients with FSGS. We hypothesized that natural antibody IgM binds to specific epitopes expressed in the injured mesangium, and that depletion of this antibody may be a mechanism by which immunosuppressive therapies are protective in FSGS.

Methods: To test this hypothesis, we induced adriamycin nephropathy (AN) in Balb/c mice after injecting them with a monoclonal antibody to murine CD20 or with vehicle control. FACS analysis of peritoneal B cells confirmed that the antibody depleted B-1 cells (the primary source of natural antibody).

Results: Mice treated with anti-CD20 displayed reduced glomerular IgM and C3 when assessed by quantitative immunofluorescence microscopy. The degree of albuminuria was also reduced in the anti-CD20 group compared to the control group (10,500 vs. 19,000 mcg alb/mg creatinine; P = 0.056). To confirm that protection was due to depletion of B-1 cells, we induced AN in another cohort of mice after depleting peritoneal B cells by hypotonic shock. This treatment did not reduce the levels of circulating IgM compared to control animals, but it did reduce glomerular IgM, C3, and collagen IV deposition. It also significantly reduced the degree of albuminuria (16,916 vs. 31,860 mcg alb/mg creatinine; P < 0.05).

Conclusions: Regarded together, these results demonstrate that therapies that deplete peritoneal B-1 cells reduce binding of IgM within the mesangium of mice with AN and prevent glomerular complement activation. These B cell targeting therapies reduce glomerular injury and albuminuria. These experiments reveal a novel mechanism of glomerular injury in FSGS. Our results also suggest that therapies that can specifically target B-1 B cells or that block the glomerular epitope(s) may be effective for the treatment of FSGS.

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SA-PO2203

Anti-LAMP-2 Antibody Responses Correlate with Clinical Relapse and Demonstrate Epitope Spreading during the Evolution of ANCA Associated Pauci-Immune FNGN Andrew J. Rees, Renate Kain. *Clinical Department of Pathology, Medical University of Vienna, Vienna, Austria.*

Background: Autoantibodies to lysosome associated membrane protein-2 (LAMP-2) are highly prevalent in patients with active untreated ANCA associated pauci-immune FNGN but their pathogenicity is uncertain. Autoantibodies to LAMP-2 commonly bind two well-characterised epitopes as well as other less frequently recognised epitopes. The present study was designed to answer two questions: do antibodies to LAMP-2 correlate with clinical relapse; and does the antibody response demonstrate epitope spreading.

Methods: We analysed 274 sera from 39 patients with ANCA associated piFNGN (mean: 6.9 sera per patient). Seventeen were recruited at presentation and 22 later in the disease and subsequently followed for 4 to 12 months. The sera were assayed for antibodies to LAMP-2 by our standard ELISA with recombinant human LAMP-2 expressed in E.coli as substrate. The range of epitopes they recognised was assessed in 3 parallel ELISA with overlapping HIS-tagged fusion proteins expressed in E.coli spanning the hLAMP-2 extracellular domain as substrates. These were designated: hL2/1 (187 amino acids), hL2/2 (181 amino acids) and hL2/3 (111 amino acids) respectively.

Results: Antibodies to LAMP-2 were detected in 56 of 62 (90.3%) sera from patients with active disease - 15 at onset and 41 during relapse - but in only 7 of 212 (3.3%) sera collected from those without signs of active disease. There was a highly significant difference in the breadth of specificity of anti-LAMP-2 antibodies detected at presentation and those detected during relapse: at presentation 8 of 15 (53.3%) reacted with a single peptide; 5 reacted with 2 peptides; and 2 (13.3%) reacted with all three peptides. Whereas during relapse: 15 (36.6%) of 41 sera reacted with all three peptides; 5 bound to 2 peptides and 16 (39%) reacted with a single peptide. Six sera with antibodies to LAMP-2 failed to react with individual peptides, possibly because of conformational differences between them and full length LAMP-2.

Conclusions: Antibodies to LAMP-2 correlate with active disease in piFNGN and demonstrate epitope spreading from presentation to relapse. This suggests they may be pathogenic.

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SA-PO2204

Strain Differences in Vasculitis Development upon Administration of Polyclonal and Monoclonal Anti-MPO Antibodies Mirjan M. Van Timmeren, Betty S. Van der Veen, Arjen H. Petersen, Coen A. Stegeman, Peter Heeringa. *Medical Biology, UMCG, Groningen, Netherlands.*

Background: Polyclonal antibodies against myeloperoxidase (MPO) induce vasculitis and crescentic glomerulonephritis (GN) in C57BL/6 (B6) mice. Whether the pathogenic potential of anti-MPO IgG is restricted to a specific subclass and/or epitope is unknown. Our previously generated panel of monoclonal anti-MPO antibodies (anti-MPO moAbs) did not induce GN in B6 mice. However, genetic variation can determine disease susceptibility, hence other mouse strains may be more susceptible.

Aim

To compare vasculitis development in C57Bl/6 (B6), 129S6 (129) and DBA/1 mouse strains upon polyclonal and monoclonal anti-MPO antibodies.

Methods: Mice were injected with polyclonal anti-MPO IgG (1 mg; iv; n=6/group), a mix of three anti-MPO moAbs of different subclass and recognizing different epitopes (10 mg; ip; n=3-6/group) or isotype control moAbs. This was followed by ip LPS injection (150 EU/g). Mice were sacrificed at day 7.

Results: Polyclonal anti-MPO IgG induced GN in B6 and 129 mice, as evidenced by leukocyturia, albuminuria and crescent formation. DBA/1 mice did not develop GN.

Anti-MPO moAbs also induced GN in B6 and 129 mice. DBA/1 mice did not develop GN, but displayed profound lung hemorrhage with inflammatory lesions (neutrophil and macrophage influx). Also, DBA/1 mice displayed liver inflammation and an increase in serum alanine aminotransaminase (ALAT, 66.4±34.1 vs 19.2±7.2 u/L in isotype controls, p<0.01). Isotype control moAbs did not induce disease.

Conclusions: Polyclonal anti-MPO IgG induce GN in B6 and 129 mice, but not in DBA/1 mice. Also, anti-MPO moAbs induce GN in B6 and 129 mice. DBA/1 mice do not develop GN, but develop lung hemorrhage and liver inflammation. DBA/1 mice can potentially serve as a model for anti-MPO IgG-mediated lung vasculitis.

	B6	129	DBA/1
Polyclonal anti-MPO			
Leukocyturia d7 (0-3)	2.5 (range 2-3)	2	0
Albuminuria d7 (µg/18h)	3800±2509**	2697±650*	33±16
Glomerular crescents (%)	13.8±2.6	7.0±1.5	0
anti-MPO moAbs			
Leukocyturia d7 (0-3)	1 (range 0-1)	2	0 (range 0-2)
Albuminuria d7 (µg/18h)	67±24*	662±279*	65±42
Glomerular crescents (%)	2.0±1.0	8.7±2.1	0
Lung lesions	-	-	+++
Liver lesions	-	-	++

*p<0.05 and **p<0.01 vs baseline

Funding: Government Support - Non-U.S.

SA-PO2205

Is CD177 (NB1) Sufficient for the Membrane Expression of PR3 in Myeloid Cells? Veronique Witko-Sarsat,[1] Arnaud Millet,[1] Magali Pederzoli-Ribeil,[1] Luc Mouthon.[2] *[1]Cochin Institute Immunology-Hematology Department, INSERM U1016- University René Descartes, Paris, France; [2]Internal Medine Department, Cochin Hospital, Paris, France.*

Background: Proteinase 3 (PR3), the auto-antigen in granulomatosis with polyangiitis, is a granular serine-proteinase, which is also present at the plasma membrane in a stable neutrophil subset. ANCA binding to PR3 at the surface of primed neutrophils results in their activation. In neutrophils, PR3 has been shown to be associated with CD177 (also called NB1) is a GPI-anchored glycoprotein, which is expressed on the same neutrophil subset than PR3. It has been proposed that CD177 is essential for PR3 membrane expression in the basal state.

Methods: The rat basophilic cell line (RBL) has been used to study the molecular mechanisms leading to PR3 membrane expression after degranulation or apoptosis but unfortunately, RBL-PR3 do not display PR3 membrane expression under the resting state as neutrophil do. The hypothesis was that CD177 favors PR3 membrane expression by binding to PR3 thus resulting in its membrane targeting and surface expression. We generated stable RBL transfectant expressing either human CD177 or human PR3 or both. Membrane expressions of CD177 or PR3 were studied by flow cytometry under basal conditions or following ionophore-induced degranulation or gliotoxin-induced apoptosis.

Results: Stable human CD177-transfected cells show a membrane expression under the basal state in wild type RBL as well as RBL-PR3. However, in RBL-PR3 CD177 failed to trigger PR3 membrane expression under the basal state. After degranulation or apoptosis PR3 membrane expression was similar in RBL PR3 and RBL-PR3 CD177. However, we confirmed that CD177 is a receptor for extracellular PR3, as demonstrated by the binding of exogenous PR3 to RBL-CD177 in the basal state contrary to RBL WT.

Conclusions: We concluded that CD177 appears to be a partner of PR3, which could play a role as a receptor for extracellular PR3, but it seems to be insufficient for PR3 membrane targeting in the basal state in a cell model of neutrophils.

Funding: Government Support - Non-U.S.

SA-PO2206

De Novo Protein Synthesis of PR3 and MPO Autoantigens in Mature Neutrophils of Patients with ANCA Vasculitis Anshul K. Badhwar,[1] Elizabeth A. Alderman,[1] Akhil Muthigi,[1] Heng Ge,[1,2] Elisabeth Berg,[1] J. Charles Jennette,[1] Gloria A. Preston,[1] Ronald J. Falk.[1] *[1]UNC Kidney Center, University of North Carolina at Chapel Hill, NC; [2]Xian Jiaotong University, Xian City, Shaanxi Province, China.*

Background: Due to a defect in epigenetic silencing, circulating neutrophils from patients with ANCA disease express PR3 and MPO genes which are normally expressed only in bone marrow cells. We examine the processing of transcripts in mature neutrophils and whether increased transcription results in increased PR3/MPO protein.

Methods: A psoralen-biotin RNA probe complementary to sense PR-3 and MPO was used for hybridization on northern blot. Total RNA from 9 patients and 9 healthy donors was used to characterize the transcripts present. Quantitative RT-PCR of PR3 and MPO transcripts was used to confirm expression levels detected by northern blotting. Nascent protein synthesis was metabolically labeled with a methionine analog, selectively biotinylated, purified by magnetic streptavidin beads and detected by western blotting.

Results: Multiple isoforms of PR3 transcripts were observed by northern blotting of leukocyte RNA from patients with PR3-ANCA. Five of nine patients expressed at least one isoform of PR3 mRNA, and of the five, three patients expressed an alternatively spliced variant larger (approx. 100 to 400 additional nucleic acids) than currently annotated size. Unexpectedly, PR3 transcripts were also detected in three of nine healthy controls. Northern blotting was determined to be quantitative and correlated with levels of expression of both PR3 and MPO by standardized qRT-PCR assay. A novel polyadenylation site distal to the canonical site was associated with expression in circulating mature neutrophils and monocytes. Upregulation of PR3 and MPO transcripts was associated with de novo protein synthesis in four of four patients with MPO-ANCA. None of the 8 healthy donors tested produced significant levels of either protein.

Conclusions: The data indicate that neutrophils in the periphery produce both PR3 and MPO protein de novo and that the presence of previously unidentified isoforms of PR3 may lead to the production of altered forms of PR3 protein.

Funding: NIDDK Support

SA-PO2207

Membrane Association of Proteinase 3, the Autoantigen in Granulomatosis with Polyangiitis (GPA), Expressed at the Membrane of Apoptotic Neutrophils, Is Essential for Impairing Their Phagocytosis by Macrophages Veronique Witko-Sarsat,[1] Arnaud Millet,[1] Magali Pederzoli-Ribeil,[1] Luc Mouthon.[2] *[1]Cochin Institute Immunology-Hematology Department, INSERM U1016- University René Descartes, Paris, France; [2]Internal Medicine Department, Cochin Hospital, Paris, France.*

Background: The removal of apoptotic neutrophils is a key event in the resolution of inflammation, its failure has been incriminated in chronic autoimmune diseases. We described that proteinase 3 (PR3) the autoantigen in granulomatosis with polyangiitis (GPA) was externalized during apoptosis and impaired the phagocytosis of apoptotic neutrophils by macrophages thus acting as a dont eat me signal (Kantari et al, Blood 2007). The aim of the study was to investigate whether PR3 membrane expression and/or its enzymatic activity was essential for this activity

Methods: Stable transfectant in RBL cells expressing a mutant of PR3 (PR34H4A) inable to insert into the plasma membrane was generated. The phagocytosis of apoptotic RBLPR34H4A by human monocyte-derived macrophages was studied in comparison with wild type RBLPR3. The enzymatic activity of apoptosis-induced membrane PR3 was studied for its ability to cleave extracellular matrix proteins such as fibronectin.

Results: The mutations of four hydrophobic (F180, F181, L228, F229) amino acids abrogated PR3 membrane anchorage and cells expressing this hydrophobic patch-deficient PR3 mutant (PR34H4A) did not inhibit macrophage phagocytosis thus confirming this importance of PR3 membrane association in this phenomena. We demonstrated that this "dont eat me" activity of membrane-associated PR3 was independent of its serine proteinase activity because 1) the enzymatically-dead mutant PR3S203A displayed the same activity and 2) that apoptosis-induced PR3 externalization did not result in an increased ability to cleave extracellular matrix proteins such as fibronectin.

Conclusions: Our conclusion is 1) that the molecular basis of PR3 "dont-eat-me signal" relies more on PR3 membrane anchorage but not on its enzymatic activity and ii) that PR3 "dont-eat-me" activity might potentiate the mechanisms of autoimmunity and be involved in the pathophysiology of ANCA-associated vasculitis.

Funding: Government Support - Non-U.S.

SA-PO2208

Endothelial Lineage Impairment and Increased PR3 Expression on Peripheral Cells of Endothelial Phenotype in Wegener´s Granulomatosis Susann Patschan,[1] Daniel Patschan,[1] Sabine Blaschke,[1] Gerhard A. Mueller.[1] *[1]Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany; [2]Göttingen; [3]Göttingen.*

Background: Wegener´s Granulomatosis (WG) is characterized by microvascular endothelial damage and by alterations of the endothelial progenitor cell (EPC) system. Interactions between anti-Proteinase 3 antibodies and their respective antigens (PR3) on neutro-phils are pathogenetically relevant in WG. Aim of this study was (I) to analyze total circulating EPCs and regenerative activity of blood-derived EPCs, and (II) to evaluate PR3 expression patterns on circulating myelomonocytic and endothelial cells in WG.

Methods: Blood samples from WG patients were analyzed for total and for Flk-1+ myolomono-cytic cells. Healthy donors served as controls. For evaluating the proliferative activity of EPCs, a colony forming unit assay (CFU) was performed. PR3 expression by the cells was quantified by cytometric analysis. Serum Angiopoietin 1 and serum TNF-α were measured by ELISA.

Results: A total of 21 healthy donors (12 female, 9 male [40.3 ±9.2 years]) and 31 WG patients (13 female, 18 male [59.2 ±15.3 years]) were included into the study. The total percentages of EPCs were not different between the two groups. WG patients displayed lower proliferate activity of EPCs. In addition PR3 expression was significantly higher in the total as well as in the Flk-1+ (sub)population of myelomonocytic cells in WG. Finally, WG patients showed lower mean serum levels of Angiopoietin 1 and higher mean serum levels of TNF-α as compared to controls, the serum levels of both cytokines did not linearily correlate with either clinical activity or the total number of circulating EPCs or the numbers of colonies formed (EPC regeneration).

Conclusions: In addition to reduced EPC regeneration and decreased serum levels of Angiopoietin 1, both indicating impairement of the endothelial system, patients with WG show sig-nificantly increased expression of PR3 in the total and in the Flk-1+ myelomonocytic cell population. These data imply, that PR3 could be involved in the pathogenesis of microvascular endothelial damage in patients with WG.

SA-PO2209

C4d in Thrombotic Microangiopathy: Cause or Consequence? Jamie S. Chua, Hans J. Baelde, Ingeborg M. Bajema, Jan A. Bruijn, Danielle Cohen. *Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Background: Complement activation, whether caused by excessive activation or inadequate regulation, is known to play a major role in thrombotic microangiopathy (TMA). We previously showed that glomerular C4d deposition is associated to development of TMA in patients with lupus nephritis and antiphospholipid syndrome (APS). The aim of this study was to investigate whether C4d is also present in other forms of TMA and whether this marker for classical complement activation could identify patients with an antibody- or immune complex mediated TMA.

Methods: We investigated the presence of C4d and MBL depositions on 47 renal biopsies or autopsies with histologically proven TMA. Patients were divided into 2 groups: A first group with TMA in association with auto- or alloimmune disease (including SLE, APS, renal transplantation and stemcell transplantation) (n=27) and a second group of patients with clinically confirmed Hemolytic Uremic Syndrome (HUS) (n=20). Deposition patterns of C4d and MBL were scored blindly and semi-quantitatively in glomeruli, peritubular capillaries (PTC), arterioles and arterial branches.

Results: In general, C4d deposition was found in 94% of TMA cases, independent of the underlying clinical setting. Glomerular C4d deposition was present in 85% of allo- and autoimmune cases and in 80% of HUS cases (P=0,456). Arteriolar C4d deposition was found in 48% of allo- and autoimmune cases, in 60% of HUS cases (P=0,421) and was mainly observed in vessels obstructed by microthrombi. Diffuse C4d depositions in PTCs were only present in two cases of *de novo* TMA in renal allografts. Co-localization of C4d with MBL never occurred.

Conclusions: C4d is found in virtually all TMA cases, independent of the underlying clinical condition. Since C4d and MBL do not co-localize, C4d seems to represent classical pathway activation. However, rather than reflecting the cause, the classical pathway may be the consequence of severe endothelial damage or vascular remodeling in TMA. In addition, these data suggest that C5-inhibition (Eculizumab) could benefit the full spectrum of TMA patients, which is in line with recent successful results of C5 inhibitors in Shiga-toxin associated HUS.

SA-PO2210

Membranoproliferative Glomerulonephritis: Identification of New Diseases Associated Complement Genes Qian Chen,[1] Christoph Licht,[2] Gunter B. Wolf,[3] Christine Skerka,[1] Peter F. Zipfel.[1] *[1]Infection Biology, Leibniz Institue for Natural Product Researach and Infection Biology, Jena, Germany; [2]Hospital for Sick Children, Toronto, Canada; [3]Internal Medicine, University Hospital, Jena, Germany.*

Background: Membranoproliferative glomerulonephritis (MPGN) is a rare kidney disease characterized by hematuria, proteinuria and complement deposit formation, particularly at the glomerular basement membrane of the kidney. In order to define the mechanisms of this severe renal disease, we set up a European MPGN registry. Factor H-, CFHR1-, and Factor B-sequence variations, as well as copy number variations in the Factor H-CFHR gene cluster were assayed for 34 MPGN patients, as well as 67 healthy individuals. Two patients had a three nucleotide deletion causing absence of Lysine 224 in SCR4 Factor H. For the Factor B gene two major allelic variants, i.e. c.95G>A; R32Q- and c.672C>T; Y224Y appeared with higher frequencies in the patients vs controls (0.106 vs. 0.076 and 0.025 vs 0.008, respectively). The CFHR gene cluster shows different CFHR1 haploptypes as well as copy number variations. In the patient group homozygous deletion of a chromosomal segment which includes the CFHR1-CFHR3 genes was more frequent among patients as compared to the control group (Delta CFHR1 17.7%; n=6) vs the control group (Delta CFHR1 3.3%; n=2). The overall frequency of this allelic deletion was similar in both groups, which is explained by a higher frequency of the heterozygous alleles in the control group, suggesting that one copy of CFHR1 has a protective effect. Additional copy number variations were identified in MPGN patients. One patient had three allelic copies of the CFHR3/CFHR1 segment and two related patients showed a novel heterozygous deletion

in exon IV of CFHR2. This deletion correlated with the presence of a new plasma protein, which appeared as a doublet of 65 and 70 kDa bands and which apparently represent different glycosylated forms of a CFHR2-CFHR5 hybrid protein. Thus new gene variations in the Factor H-CFHR gene cluster were identified in the European MPGN registry, indicating a major role of CFHR proteins for disease pathology.

SA-PO2211

Complement-Induced Hemolysis Requires Purinergic Signaling Helle A. Praetorius, Marianne G. Skals, Jens G. Leipziger, Julie L. Hejl. *Dept. of Physiology and Biophysics, Aarhus University, Aarhus, Denmark.*

Background: The complement system is a key element of the innate immune system. The system elicits efficient responses against cells identified as non-self via insertion of Membrane Attack Complexes. It is associated with many types of hemolytic anemia including typical and atypical hemolytic-uremic syndrome as well as paroxysmal nocturnal hemolglobinuria. Recently, we found that hemolysis caused by other types of membrane pore-formers such as α-hemolysin (HlyA) from *Escherichia coli* and α-toxin from *Staphylococcus aureus* inflict their cytotoxic effects through P2 receptor activation.

Methods: To address if P2 receptors are involved in complement-induced lysis, we used simple hemolysis assays of sensitizes human and sheep erythrocytes, combined with time-lapse microscopy.

Results: Here we show that similarly to HlyA-induced hemolysis, red cell lysis caused by complement activation is amplified through ATP release and subsequent P2 receptor activation. Ovine and human erythrocytes were incubated with anti-sheep erythrocyte antibodies or anti-RhD antibodies respectively, with either human plasma or guinea pig serum as complement donors. Non-selective P2 antagonists (PPADS and suramin) concentration-dependently inhibit complement-induced hemolysis. More specific P2 receptor antagonists imply that $P2X_7$ and $P2X_1$ are the main receptors involved in this response. Moreover, complement-activation produced a sustained increase in the $[Ca^{2+}]_i$, which triggered significant erythrocyte shrinkage that preceded swelling and lysis. This early volume reduction is likely to result from activation of the K^+ channel $K_{Ca}3.1$ as TRAM34 and clotrimazole augment the complement-induced hemolysis.

Conclusions: These results indicate that complement, similar to HlyA, requires purinergic signaling for full hemolysis, and that activation of the erythrocyte volume regulation protracts the lysis. This finding points to several new pathways to interfere with hemolytic diseases and implies that P2 receptor antagonists potentially can be used in more broad terms to prevent intravascular hemolysis.

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SA-PO2212

A New Model of Atypical Haemolytic Uraemic Syndrome Katherine Anne Vernon, Talat H. Malik, Marina Botto, Matthew C. Pickering. *Centre for Complement and Inflammation Research, Imperial College London, London, United Kingdom.*

Background: Complement factor H (CFH), the major regulator of the alternative pathway, is synthesised by both hepatic and extra-hepatic cells. The physiological role and contribution of extra-hepatic CFH is unknown. Rodent studies suggest that renal-derived CFH is important, with rat podocytes increasing CFH synthesis in membranous nephropathy. To define the physiological roles of hepatic and extra-hepatic CFH we utilised a conditional *CFH* gene-targeted mouse line to generate mice with hepatocyte-specific CFH deficiency.

Methods: Gene targeted mice were generated using recombineering technology in embryonic stem cells. Nephrotoxic nephritis (NTN) was induced using a sheep anti-mouse glomerular basement membrane antibody in pre-immunised animals. Serum CFH, complement component 3 (C3) and urea levels were detected and measured by enzyme-linked immunosorbent assay or Western blot. Haematocrit and peripheral blood film examination were used to assess haematological parameters, whilst histology was examined using light microscopy and immunofluorescence.

Results: Conditional *CFH* gene-targeted animals (in which exons 2 and 3 of the *CFH* gene were flanked by loxP sites) had normal plasma CFH and C3 levels indicating that the conditionally targeted *CFH* gene remained intact. Hepatocyte-specific CFH-deficient mice were generated by inter-crossing these animals with mice expressing Cre recombinase under the control of the murine albumin promoter. Hepatocyte-specific CFH-deficient mice had reduced plasma CFH and C3 levels (15% and 40% of normal respectively). Renal histology (in particular glomerular C3 staining) and urea levels were normal despite the low levels of plasma CFH. However, these animals developed haemolytic uraemic syndrome following induction of NTN, characterised by haemolytic anaemia, thrombocytopaenia and renal failure.

Conclusions: These novel data (1) define the contribution of extra-hepatic CFH to circulating CFH and C3 levels; (2) demonstrate that remarkably low levels of CFH are sufficient to prevent spontaneous glomerular C3 accumulation and (3) provide a new model of CFH-associated atypical haemolytic uraemic syndrome.

Funding: Private Foundation Support

SA-PO2213

Genetic Background Influences VEGFR Pathways and Glomerular Thrombotic Lesions in Nephrotoxic Nephritis Laurent Mesnard,[1, 2] Sophie Vandermeersch,[1, 2] Patrice Callard,[2] Chantal Jouanneau,[1] Alexandre Hertig,[1, 2] Eric Rondeau.[1, 2] *[1]INSERM UMR S702, Paris, France; [2]APHP, Hopital Tenon, Paris, France; [3]APHP, HEGP, Paris, France.*

Background: As genetic background (GB) may play a role in various experimental models, we evaluated its impact on glomerular pathology.

Methods: Mice were evaluated during passive mouse anti-glomerular basement membrane glomerulonephritis (anti-GBM-GN) in leading inbred strains (C57BL/6J, BALB/cbyJ, 129S2SvPas) and their F1 offspring. Glomerular DNA microarray were also performed at early time point (day 4)

Results: Mice exhibited different severity of renal failure, hypertension and pseudocrescentic lesions according their GB. Proteinuria correlated with GB-dependent podocytic lesions, dramatic in 129S2SvPas, intermediate in BALB/cbyJ and some F1, mild in C57BL/6J.

Originally, we discovered that glomerular thrombotic microangiopathy (TMA) was a histopathological hallmark of this model, along with its classical biological abnormalities (anemia, thrombocytopenia, schistocytosis). TMA parameters were also remarkably GB-dependent: major in 129S2SvPas, far less in C57BL/6J, intermediate in F1. Subsequent glomerular DNA microarray analysis comparing C57BL/6J-resistant to 129S2SvPas-disease-prone mice indicated major differences in VEGF/VEGFR pathways, which have already been involved in TMA pathophysiology. Glomerular VEGF-A expression was not different and exogenous vegf165 failed to rescue TMA. Further analysis revealed a glomerular VEGFR2 signaling defect in 129S2SvPas disease-prone mice compared to C57BL/6J.

Conclusions: Differences, and sometimes discordances, between studies about mouse anti-GBM-GN are classically inferred to GB; our results identify glomerular TMA lesions as a GB-dependant hallmark of anti-GBM-GN, which could be related to a genetic difference in VEGFR2 signaling.

SA-PO2214

The Growth Factor Midkine Ameliorates Crescentic Glomerulonephritis through Inhibiting Thrombosis Hiroshi Kojima, Waichi Sato, Tomoki Kosugi, Yuka Sato, Kayaho Maeda, Shoichi Maruyama, Yukio Yuzawa, Seiichi Matsuo. *Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: The growth factor Midkine (MK), a heparin-binding protein, has been implicated in neuronal survival and differentiation, cancer development and inflammation-related diseases. We have previously demonstrated that MK plays deleterious effects for ischemic renal injury, diabetes and hypertension. However, little is known about the kinetics of MK in pathophysiological state. Using mass spectrometry method, a large number of MK-binding proteins such as coagulation- and fibrinogenolysis-related factors were identified. Indeed, MK activates fibrinolysis in bovine endothelial cells. In order to clarify the molecule mechanism of thrombosis *in vivo*, we examined the role of MK in crescentic glomerulonephritis (GN) model.

Methods: MK deficient mice ($Mdk^{-/-}$) or wild-type mice ($Mdk^{+/+}$) were treated with unilateral nephrectomy and the pre-immunization of rabbit IgG, and then injected with rabbit anti-mouse glomerular basement membrane (GBM) antibody (Ab) after 7 days. Mice were sacrificed at 1, 3 and 7 days after injection of anti-GBM Ab. Kidney, serum and urine were collected for immunohistochemistry and biochemical analysis.

Results: There were no differences in rabbit IgG, mouse IgG and C3 depositions of glomeruli in both groups. Blood urea nitrogen levels in $Mdk^{-/-}$ were higher than $Mdk^{+/+}$. Similarly, $Mdk^{-/-}$ developed severer crescentic GN accompanied with marked thrombosis over time. Obvious tubulointerstitial injuries were also found in $Mdk^{-/-}$ compare with $Mdk^{+/+}$. Macrophage and neutrophil infiltrations into glomeruli were more significant in $Mdk^{-/-}$ than $Mdk^{+/+}$. Plasminogen activator inhibitor (PAI)-1 in glomeruli of $Mdk^{-/-}$ was significantly induced, consistent with the result of mass spectrometry.

Conclusions: In contrast to previously reported *in vivo* models such as ischemic reperfusion model, diabetic nephropathy and remnant kidney model, MK might play beneficial effects for crescentic GN though the blockade of PAI-1-mediated thrombosis and be a candidate for preventing or delaying rapidly progressive GN.

SA-PO2215

Endothelial BAMBI: A Novel Modulator of Angiogenesis Dmitrij Kollins,[1] Nicolas Guillot,[1] Victoria Gilbert,[1] Sandhya Xavier,[2] Jun Chen,[2] Maria Pia Rastaldi,[3] Alessandro Corbelli,[3] Detlef O. Schlondorff.[1] *[1]Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Nephrology, New York Medical College, Valhalla, NY; [3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*

Background: BAMBI (BMP and Activin receptor Membrane Bound Inhibitor), was described as a highly conserved protein that can act as a competitive antagonist of type 1 TGFβ receptors. BAMBI is highly expressed in solid tumors, where it has been postulated to contribute to growth and metastasis. There are, however, no data on cell-specific expression of BAMBI in mammalian organs.

Methods: As TGFβ action is highly cell type-specific, we examined the localization of BAMBI in mouse tissues and its function in target cells in-vitro and in-vivo in BAMBI knock-out mice.

Results: By immunohistology, BAMBI is expressed in endothelial cells of the major visceral mouse organs. By EM, the endothelial cell phenotype in BAMBI-/- mice shows signs of cell activation as compared to the wild type mice. In vitro angiogenesis and scratch wound assays using human umbilical vein endothelial cells (HUVEC) show enhanced angiogenesis and cell migration in HUVEC with BAMBI knock-down by siRNA, and the opposite with overexpression of BAMBI. In vivo angiogenesis using s.c. matrigel implantation assays confirmed marked enhancement of angiogenesis in the BAMBI-/- as compared to the wild type mice. To confirm the enhanced angiogenesis in vivo in BAMBI-/- mice, we examined glomeruli as highly-vascularized structures. BAMBI-/- mice have significantly larger glomeruli than wild type mice due to larger capillary endothelial tufts. Furthermore, compensatory glomerular capillary hypertrophy after unilateral nephrectomy was greater in the BAMBI-/- than in wild type mice.

Conclusions: Taken together our data show that BAMBI is expressed in endothelial cells of different organs and support a role for BAMBI in vascular homeostasis. Elimination of BAMBI results in enhanced angiogenesis, which may play a role in neoangiogenesis after renal injury and in renal diseases with capillary loss.

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SA-PO2216

Osteopontin Deficiency Results in Severe Glomerulosclerosis in Uninephrectomized Mice Sandra Schordan,[1] Olaf Grisk,[2] Eric Schordan,[1] Baerbel Miehe,[1] Wilhelm Kriz,[3] Karlhans Endlich,[1] Nicole Endlich.[1] *[1]Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany; [2]Physiology, University Medicine Greifswald, Greifswald, Germany; [3]Center for Biomedicine and Medical Technology, University of Heidelberg, Germany.*

Background: Expression of osteopontin (OPN) in podocytes has been reported to be elevated in mechanical stress and in experimental models of renal injury such as diabetic nephropathy or glomerular hypertension.

Methods: To investigate the role of OPN in the development of focal segmental glomerulosclerosis (FSGS), OPN+/+ and OPN-/- mice were subjected to uninephrectomy (UNX) and DOCA-salt treatment for 6 weeks, a model of FSGS through elevation of glomerular capillary pressure. Proteinuria and albuminuria were measured and renal morphology was analyzed by scoring glomerulosclerosis and collagen expansion on kidney sections stained with PAS or Masson trichrome.

Results: Following UNX, OPN-/- mice exhibited prominent glomerular damage as evidenced by severe glomerulosclerosis degree and mesangial matrix (collagen) deposition. After DOCA salt-treatment glomerular lesions between OPN+/+ and OPN-/- mice were not strikingly different anymore. Massive podocyte injury was demonstrated by electron microscopy in response to DOCA-salt treatment. WT-1, podocin, synaptopodin and actin expression were reduced upon UNX in OPN-/- kidneys as evaluated by immunofluorescence and real-time PCR. Integrin αV and β1 mRNA, identified as receptor of OPN mediated mechano-protection, were up-regulated by more than 2-fold in DOCA-salt treated rats.

Conclusions: We conclude that OPN plays a crucial role in adaptation following renal ablation and is renoprotective towards increased mechanical load.

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SA-PO2217

GSK3β Inhibition Protects Against NSAID Induced Acute Kidney Injury: Effects on Mitochondrial Permeability Transition and ATP Production Hao Bao,[1] Zhi-Hong Liu,[1] Shougang Zhuang,[2] Lance D. Dworkin,[2] Rujun Gong.[2] *[1]Research Institute of Nephrology, Nanjing University, Nanjing, China; [2]Brown Medical School, Providence, RI.*

Background: Clinical use of non-steroid anti-inflammatory drugs (NSAID) like Diclofenac (DCLF) is limited by renal toxicity and acute kidney injury (AKI). This study examined the role of glycogen synthase kinase (GSK)3β, a newly recognized modulator of kidney injury, in NSAID induced acute nephrotoxicity.

Methods: Cultured mouse renal proximal tubular epithelial cells were exposed to DCLF in the presence or absence of TDZD-8, a highly selective small molecule GSK3β inhibitor. *In vivo,* mice were administered a nephrotoxic dose of DCLF following pretreatment with TDZD-8 or vehicle. Tubular cell necrosis and apoptosis as well as mitochondrial permeability transition (MPT) and adenosine triphosphate (ATP) production were measured.

Results: *In vitro*, TDZD-8 improved cell viability and suppressed both apoptosis and necrosis in cultured tubular epithelial cells exposed to DCLF. Mechanistically, TDZD-8 normalized the levels of intracellular ATP in DCLF-treated cells and diminished DCLF elicited MPT evidenced by entry of fluorescent calcein from cytoplasm into mitochondrial matrix space as well as blunted calcium-induced mitochondrial swelling. Conversely, ectopic expression of an uninhibitable mutant of GSK3β markedly exacerbated DCLF induced injury and abrogated the protective effects of TDZD-8 on tubular cell apoptosis, necrosis, MPT and ATP production. *In vivo,* DCLF induced a typical pathology of acute tubular necrosis, characterized by epithelial simplification, isometric vacuolization of proximal tubular epithelium, luminal ectasia, epithelial necrosis, sloughing of tubular cell into lumen, loss of brush border, nuclear enlargement and pleomorphism, and prominent inflammation. GSK3β inhibition by TDZD-8 prominently prevented acute kidney dysfunction and ameliorated tubular necrosis and apoptosis. This protective effect was associated with reduced MPT pore opening and preserved ATP homeostasis in DCLF injured kidneys.

Conclusions: Collectively, our findings suggest that GSK3β inhibition ameliorates NSAID-induced AKI by inhibiting MPT and preserving ATP production.

Funding: Private Foundation Support

SA-PO2218

Derangement of Oxygen Sensing for Erythropoietin (EPO) Production by Unfolded Protein Response (UPR) Reiko Inagi, Chih-Kang Chiang, Tetsuhiro Tanaka, Toshiro Fujita, Masaomi Nangaku. *Div of Nephrol & Endocrinol, Univ of Tokyo Sch of Med, Tokyo, Japan.*

Background: In addition to loss of renal EPO-producing cells, derangement of oxygen sensing for EPO production contributes to a low level of EPO in advanced CKD patients. Here, We investigated the possibility of derangement of oxygen sensing by UPR, which is a stress signal induced by endoplasmic reticulum (ER) stress and contributes to various kidney disease progression.

Methods: EPO-producing cell line (HepG2) or rats were treated with UPR inducers (thapsigargin: THG or tunicamycin: TUN) or a suppressor (salubrinal: SAL) under normoxia or hypoxia [0.1% O_2 or 100 μM hypoxia-inducible factor (HIF) activator $CoCl_2$, 16hrs]. EPO transcription or plasma EPO level was measured by real-time qRT-PCR or ELISA. The change in hypoxia-induced EPO 3'-enhancer activity by UPR was assessed by 1) Western blotting for detection of HIF-2α nuclear translocation or 2) luciferase assay for EPO 3'-enhancer region or HIF binding site (HRE) in HepG2 treated with UPR inducers or the cells overexpressed UPR transcription factor, ATF4.

Results: UPR activated by THG or TUN as well as overexpression of ATF4 markedly suppressed hypoxia-induced EPO transcription in HepG2 without affecting the cell viability. In contrast, UPR did not alter other HIF target gene expressions such as VEGF or GLUT-1. UPR suppressor, SAL, restored the suppression of EPO transcription by UPR activation. Of note, the UPR activation, especially ATF4 overexpression, significantly blunted the transcriptional activity of EPO 3'-enhancer region, which includes a putative ATF4 binding site located adjacent to HRE. However, the UPR did not directly affect nuclear translocation of HIF-2α and its subsequent binding to HRE. *In vivo* studies showed that UPR activation on renal interstitial cells by non-nephrotoxic dose of TUN suppressed hypoxia-induced plasma EPO production, together with a reduction of renal EPO mRNA in rats ($P<0.05$).

Conclusions: Imbalance of UPR, especially ATF4 activation, deranges EPO transcription by suppressing EPO 3'-enhancer activity without interaction with HIF directly, suggesting the crosstalk between UPR and HIF pathways on oxygen sensing for EPO regulation.

Funding: Government Support - Non-U.S.

SA-PO2219

Effects of MCP-1 Inhibition by Bindarit Therapy in a Rat Model of Polycystic Kidney Disease (PKD) Carlamaria Zoja,[1] Daniela Corna,[1] Monica Locatelli,[1] Daniela Rottoli,[1] Norberto Perico,[1] Andrea Remuzzi,[1] Angelo Guglielmotti,[2] Ariela Benigni,[1] Giuseppe Remuzzi.[1,3] *[1]Mario Negri Institute, Bergamo, Italy; [2]Angelini Research Center-ACRAF, S. Palomba-Pomezia, Italy; [3]Unit of Nephrology, Ospedali Riuniti, Bergamo, Italy.*

Background: Experimental and clinical evidence suggested that the proinflammatory chemokine MCP-1/CCL2 has a role in the development of interstitial inflammation and renal failure in autosomal-dominant PKD. Here we investigated the effects of the MCP-1 synthesis inhibitor bindarit in the PCK rat, a model orthologous to human autosomal-recessive PKD that has also phenotypic characteristics of human ADPKD.

Methods: PCK rats were treated by gavage from 5 to 15wks of age with vehicle (n=10) or bindarit (100mg/kg bid, n=9). Age-matched SD rats (n=8) served as control.

Results: MCP-1 mRNA was two-fold increased in the kidney of PCK rats on vehicle and significantly ($P<0.05$) reduced by bindarit. Bindarit limited overexpression of MCP-1 protein by epithelial cells of dilated tubules and cysts, and interstitial inflammatory cells. Excessive accumulation of monocytes/macrophages in the kidney of PCK rats was lowered by bindarit (-41%, $P<0.05$ vs vehicle). PCK rats developed hypertension (15wks, SBP: 146±2 vs control 127±1 mmHg, $P<0.01$), that was limited by bindarit (138±1mmHg, $P<0.05$ vs vehicle). Serum creatinine increased in PCK rats (0.72±0.03 vs control: 0.58±0.03 mg/dl, $P<0.05$) and was comparable to controls after bindarit (0.64±0.05mg/dl). In PKC vehicle-rats proteinuria progressively increased ($P<0.01$) compared to controls (13wks,144±40 vs 26±1mg/d; 15wks, 232±36 vs 25±1mg/d). Bindarit reduced proteinuria by 57-46% at 13 and 15wks ($P<0.05$ vs vehicle). The antiproteinuric effect was associated with amelioration of the defective nephrin expression in podocytes of PCK rats. Reduced protein filtration translated in less tubular casts. Kidney and liver cysts were not affected by treatment.

Conclusions: Although bindarit did not prevent renal cyst growth, it limited interstitial inflammation and renal dysfunction and reduced proteinuria in PKD. Thus bindarit could be considered as therapeutic intervention complementary to therapies specifically acting to block renal cyst growth.

Funding: Pharmaceutical Company Support

SA-PO2220

Chronic Kidney Disease-Induced Cardiac Fibrosis Is Ameliorated by Reducing Circulating Levels of a Non-Dialysable Uremic Toxin, Indoxyl Sulfate Suree Lekawanvijit,[1] Andrew Kompa,[1,2] Bing Hui Wang,[1] Fuyuhiko Nishijima,[3] Darren J. Kelly,[2] Henry Krum.[1] *[1]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Vic, Australia; [2]Department of Medicine, University of Melbourne, Melbourne, Vic, Australia; [3]Pharmaceutical Department, Kureha Corporation, Tokyo, Japan.*

Background: Non-dialysable uremic toxins have been under-explored as a contributor of uremic cardiomyopathy. We have recently demonstrated that one such toxin, indoxyl sulfate (IS), directly contributes to cardiac fibrosis. As an oral adsorbent, AST-120, can

lower serum IS levels, we hypothesized that cardiac fibrosis can be abrogated by this agent in an animal model of CKD.

Methods: We performed subtotal-nephrectomy (5/6-STNx) on Sprague-Dawley rats which were then randomized to receive either AST-120 (n=13) or vehicle (n=17) for 12 weeks. Sham-operated rats (n=12) served as controls.

Results: Left ventricular (LV) diastolic dysfunction was observed in STNx+vehicle rats on echocardiography. A 4.5-fold increase in serum IS ($p<0.001$) was noted in STNx+vehicle vs sham as well as elevated tail-cuff blood pressure (BP) ($p<0.001$) and heart weight ($p<0.001$). Increased LV fibrosis ($p<0.001$), gene expression of pro-fibrotic (TGF-β, CTGF) and hypertrophic (ANP, β-MHC and α-skeletal muscle actin) markers, as well as TGF-β and phosphorylated-NF-κB protein expression were observed in STNx+vehicle rats. Treatment with AST-120 reduced serum creatinine ($p<0.05$) and urine total protein ($p<0.05$) vs STNx+vehicle with no effect on BP (AST-120,227±11 vs vehicle,224±8 mmHg, NS) and heart weight. Compared to vehicle STNx, serum IS was reduced by 79% with AST-120 ($p<0.001$), accompanied by reduced LV fibrosis by 35% ($p<0.01$) as well as TGF-β and phosphorylated-NF-κB protein expression ($p<0.05$).

Conclusions: STNx increased cardiac fibrosis and circulating IS levels, which was significantly reduced by AST-120 independent of any change in BP. These findings support IS as being an important contributor to uremic cardiomyopathy and AST-120 a potential complementary treatment.

SA-PO2221

Renal and Systemic Effects of PGE2 on Nephrotoxic Nephritis by Limiting Synaptopodin Degredation and Modulation of Splenic Stem Cells Nino Kvirkvelia, Maggie McMenamin, Kapil Chaudhary, Ryan Layman, Michael P. Madaio. *Medicine, Georgia Health Sciences University, Augusta, GA.*

Background: The mechanisms leading to podocyte injury are incompletely understood, and current treatments insufficiently promotes recovery. Previously, PGE2 promoted recovery following NTN induction. Initiation of PGE2 therapy even on day 4 after NTS injection, when acute inflammation is well established, resulted in attenuation of proteinuria, and the prostanoid prolonged life expectancy. Furthermore PGE2 limited injury and promoted cellular recovery of NTS injured glomerular endothelial cells and podocytes.

Methods: To further obtain mechanistic insights, the systemic and renal effects were evaluated. Splenocytes were evaluated by FACS; synaptopodin expression was evaluated by FACS and IF of cells and tissues.

Results: Systemically, in NTN-PGE2 treated mice, there was 10-fold decrease in spleen progenitor CD45- cells, compared to NTN mice, suggesting that PGE2 promotes transmigration of spleen stem cells into the injured kidney and subsequent recruitment of progenitor splenocytes into kidney epithelial cells. Additionally, PGE2 treatment restored the NTS induced decrease in CD4+ splenocytes, and reduced the number of CD69+ cells in the spleen. CD69 is transiently expressed upon activation on subsets of T and B cells with a net proinflammatory effect, and depletion of CD69+ cells leads to reduced inflammation and immunity. Within the kidney, improvement in proteinuria was at least partly explained by direct effect of PGE2 on podocytes, as there was a 27.32% increase in synaptopodin expression in kidney in PGE2 treated mice. Similarly, in cultured podocytes, NTS induced 32.21 % decrease in mean fluorescence intensity of synaptopodin staining (FACS), which was prevented by pre-treatment with PGE2 (5ug/ml).

Conclusions: The beneficial effect of PGE2 may be accounted for by PGE2/EP2/EP4 receptor mediated activation of cAMP dependent PKA, which is known to decrease calcineurin activity and protect synaptopodin from degradation. Taken together, the results indicate that PGE2 is a candidate to investigate its therapeutic potential to improve cellular recovery and function during the course of glomerulonephritis.

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SA-PO2222

Myosin Expression and Kidney Structure in Older Heterozygous Myh9 Knockout Mice Mostafa Belghasem, Joseph S. Coppola, Philip A. Bondzie, Hui Chen, Julie A. Tomolonis, Joel M. Henderson. *Pathology, Boston University, Boston, MA.*

Background: The gene MYH9 encodes non-muscle myosin heavy chain IIA (NMHCIIA), an essential podocyte contractile protein. The increased incidence of chronic kidney disease in African Americans has been linked to genetic variation near MYH9, and rare hereditary syndromes involving mutations in MYH9 often include renal involvement. The genetic features of these forms of MYH9-associated kidney disease suggest that altered MYH9 expression levels could play a role in disease pathogenesis. The objective of this study is to determine the long-term effect of MYH9 haploinsufficiency on non-muscle myosin expression and kidney structure, using a pre-existing murine model of Myh9 ablation.

Methods: Kidney tissue was harvested from heterozygous Myh9 knockout (Myh9 HET) and wild type mice on 129/Sv background, aged 9 and 17 months. Kidney structure was evaluated by light microscopy and transmission electron microscopy. Immunohistochemistry was used to assess the expression and distribution of NMHCIIA in the kidney. Kidney glomeruli were isolated using a sieving technique to evaluate Myh9 expression using real time PCR.

Results: Glomeruli from Myh9 HET mice showed a significant decrease in Myh9 mRNA transcription (> 50% decrease; n = 3) relative to wild type mice. However, the diminished expression of Myh9 did not appear to be associated with changes in kidney structure in the Myh9 HET mice. Specifically, kidney histology revealed no pathology, and electron microscopy showed no podocyte or glomerular basement membrane pathology, in either Myh9 HET or wild type mice. Furthermore, both age groups of the Myh9 HET and wild-type mice showed similar intense expression of non-muscle myosin IIA in the glomeruli.

Conclusions: We found that Myh9 haploinsufficiency has no pathologic effect on kidney structure under normal physiological conditions, despite a significant decrease in Myh9 transcription in older Myh9 HET mice. We are currently investigating the possibility that conditions of physiologic stress may cause pathologic changes in the kidneys of Myh9 HET mice that are more severe than those seen in wild type mice.

Funding: NIDDK Support

SA-PO2223

Experimental Lupus Nephritis Accelerate Mortality and Arteriosclerosis in Apo-E Deficient Mice Yvonne Riedl, Romy Boehme, Thomas Winkler, Christoph Daniel, Kerstin U. Amann. *University Erlangen-Nürnberg, Erlangen, Germany.*

Background: Chronic kidney disease is shown to aggravate arteriosclerosis in man, but the underlying mechanisms are not completely understood. To investigate such mechanisms in an animal model we combined the classical arteriosclerosis mouse model (Apo-E -/-) with a progressive lupus nephritis model (yaaxFcgammaR2 -/-). In this study we investigated the impact of Apo-E deficiency on survival and renal morphology in lupus nephritic mice.

Methods: First we analysed survival during a period of 40 weeks in: 1. FcgammaR2 deficient mice (n=11). 2. A progressive lupus model yaaxFcgammaR2-/- (n=115). 3. YaaxFCgammaR2 -/-xApo-E+/- (n=19). 4. yaaxFcgammaR2xApo-E double knockout mice (n=40). Renal injury including vascular alterations was analysed on week 22 to determine the impact of renal morphological changes on survival. Using enface preparation, arteriotic plaques were analysed in APO-E deficient mice (n=5) and yaaxFcgammaR2xApo-E double knockout mice (n=7).

Results: YaaxFcgammaR2xApo-E double knockout mice had the shortest lifespan of all investigated groups (max. 26 weeks). In contrast, yaaxFcgammaR2 deficient mice being heterozygous for APO-E had a significant reduced mortality of 57% (p=0,0005) surviving mice at week 40, similar to yaaxFcgammaR2-/-xAPO-E+/+ mice (59% survival. In addition, mortality was further significantly reduced in FcgammaR2 deficient lupus mice lacking the yaa modification (91% survival, p=0,048) compared to those bearing this modification. Arteriosclerotic plaques were still found in 4 of 7 yaaxFcgammaR2xAPO-E double knockout mice on week 22 using enface preparations of aortas, whereas mice lacking only the APO-E gene showed no detectable aortic plaque formation. Analysis of renal pathology revealed a tendency to aggravated renal injury, but significantly enhanced vascular injury in yaaxFcgammaR2xAPO-E double knockout mice compared to APO-E and yaaxFcgammaR2 deficient mice (VSI: 0,95±0,34. vs. 0,28±0,16 and 0,17±0,15).

Conclusions: The lack of Apo-E gene in the progressive yaaxFcgammaR2 -/- lupus nephritis model highly increased mortality. Aortic plaque formation and renal vascular impairment is accelerated by lupus nephritis.

Funding: Government Support - Non-U.S.

SA-PO2224

Renin -Angiotensin System and Mammalian Target of Rapamycin (mTOR) Pathway Modulate the Course of HIV-Associated Nephropathy through HIV Gene Expression Dileep Kumar, Partab Rai, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: The activation of renin -angiotensin system (RAS) and the mammalian target of rapamycin (mTOR) pathway have been demonstrated to play an important role for the development and the progression of HIV-associated nephropathy (HIVAN). We evaluated the role of the RAS and the mTOR pathway on HIV gene expression on the course of HIVAN.

Methods: To activate endogenous RAS, HIVAN mice (Vpr and Tg26) were bred witn angiotensinogen (Agt) transgenic mice having four Agt copies; this strategy allowed us to develop Vpr/Tg26 mice with 2, 3 and 4 copies of Agt.Vpr-Agt-2,-3, and -4 mice were fed either normal saline or doxycycline for six weeks to induce podocyte expression of Vpr. To inhibit the mTOR pathway, Tg26 (n=6) were administered either vehicle or sirolimus (5 mg/kg/day, intra-peritoneally) for 14 days. At the end of experimental periods, kidneys were isolated and prepared for histology and immunohistochemistry. RNA and proteins were extracted and HIV-1 gene expression was determined by real time PCR and immunoblotting. Renal lesions were graded for sclerosis and tubular dilatation. In in vitro studies, human podocytes were transduced with either HIV-1 or vector constructs, followed by treatment with either vehicle, sirolimus, or Ang II for 72 h. Subsequently RNA and proteins were harvested and HIV-1 gene expression was determined by RT-PCR and immunoblotting.

Results: Doxy fed Vpr Agt-4 mice displayed more advanced renal lesions than to doxy-fed Vpr-Agt-2 mice. Vpr-Agt-4 mice displayed 50% increase in Vpr expression. Saline-treated Tg26 showed two-fold advanced glomerular and tubular lesions vs. sirolimus-treated animals. Whereas, Sirolimus decreased transcription of HIV-genes both in renal tissue as well as in HIV-1 transduced podocytes. Renal tissues of Tg26-Agt-4 displayed 2-4 fold increase in gp120, Vpr, Tat, Nef and Vpu genes.

Conclusions: The RAS activation increased HIV gene trancription; whereas, downregulation of mTOR decreased HIV gene transcription. These gene modulating effects of the RAS and the mTOR pathway may be contributing to the altered course of HIVAN.

Funding: NIDDK Support

SA-PO2225

HIV-Induced Human Podocyte Vitamin D Receptor (VDR) Downregulation Enhances Cathepsin L Expression Bipin Sharma, Dileep Kumar, Ashwani Malhotra, Mohammad Husain, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstre Medical School, Great Neck, NY.*

Background: In HIV-associated nephropathy (HIVAN), podocytes detach and proliferate in Bowman's space. We hypothesized that HIV-induced down regulation of VDR enhances upregulation of cathepsin L, which compromises cytoskeletal integrity of podocytes leading to their detachment and proliferation.

Methods: To determine the effect of HIV on renal tissue VDR, synaptopodin, and cathepsin L expression, immunoblotting and immunohistochemical studies were performed on renal tissue from control and Tg26 mice (n=3). To develop in in vitro model of HIV infection, both mouse and human podocytes were transduced with either empty vector (EV/MP/HP) or NL4-3 (HIV/MP/HP) constructs and evaluated for the expression of VDR, cathepsin L, and synaptopodin (immunoblotting and real time PCR studies). To establish causal relationship between VDR and cathepsin L, human podocytes with silenced VDR were evaluated for cathepsin L expression. To confirm relationship between VDR and cathepsin L, EV/HP and HIV/HP were treated with either buffer or vit D (25 nM) for 24 hours and lysates prepared for VDR, cathepsin L, and synaptopodin expression. To determine the functional status of cathepsin L, cells were labeled with phalloidin and evaluated for the integrity of actin filaments . In addition, migration, adhesion, detachment, and proliferation studies were conducted in cells treated under similar conditions.

Results: Immunoblotting and PCR studies revealed attenuated expression of VDR and synaptopodin but enhanced expression of cathepsin L in renal tissues of HIVAN mice. Similarly, both HIV/HPs and siRNA-VDR/HPs displayed attenuated expression of VDR and enhanced expression of cathepsin L. whereas, vit D treatment of HIV/HPs not only increased VDR and synaptopodin expression but also diminished expression of cathepsin L. HIV/HPs displayed disrupted actin filaments, decreased adhesion, increased detachment, and proliferative phenotype.

Conclusions: These findings indicate that HIV enhances podocyte cathepsin L expression via downregulation of VDR. The former decreases adhesion, eahances detachment and proliferation of podocytes.

Funding: NIDDK Support

SA-PO2226

Renin Inhibition Retards the Progression of HIV-Associated Nephropathy Dileep Kumar,[1] Iti Yadav,[1] Deepti D. Torri,[1] Partab Rai,[1] Mohammad Husain,[1] Ashwani Malhotra,[1] Guohua Ding,[2] Praveen N. Chander,[3] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [3]Pathology, New York Medical College, Valhalla, NY.*

Background: Blockade of the downstream effect of angiotensin II has been reported to slow down the progression of HIV-associated nephropathy (HIVAN). In the present study, we evaluated the effect of renin inhibition on the progression of renal lesions in a mouse model of HIVAN (Vpr).

Methods: We have used Vpr transgenic mice; they express podocyte specific Vpr after doxycycline administration. In protocol A, Vpr mice were fed either water (W-VprA) or doxycycline (D-VprA) in their drinking water for six weeks. In protocol B, Vpr mice were fed either doxycylcine (D-VprB) in their drinking water + normal saline (by miniosmotic pump) or doxy + aliskiren (by miniosomotic pump, AD-VprB) for six weeks. In protocol C, Vpr mice were fed doxycycline (doxy) for six weeks followed by kidney biopsy to determine baseline kidney lesions. Subsequently, half of the mice were administered either normal saline (NS-VprC) or aliskiren (A-VprC) for 4 weeks. Protocol D was identical to protocol C, except, Vpr mice were administered either normal saline or aliskiren for 8 weeks after kidney biopsy.

Results: All D-VprA showed 2-3 fold greater expression of renin expression when compared to W-VprA. All D-VprA showed overt HIVAN phenotype in the form of focal segmental glomerular sclerosis (FSGS) and microcystic dilatation of tubules. In protocol B, aliskiren slowed the development of HIVAN. In protocol C, A-VprC showed 24.2% increase in number of sclerosed glomeruli (from their baseline) as compared to 139.2% increase in sclerosed glomeruli in NS-VprC ($P<0.01$) from their baseline. Aliskiren also slowed down increase in size of micorycysts in A-VprC. Aliskiren diminished urinary protein creatinine ratio both in protocol B and protocol C. The attenuating effect of aliskiren on the progression of renal lesions continued in A-VprD.

Conclusions: Renin inhibition has a potential to slow down the progression of renal lesions in HIVAN.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2227

Both Lectin and Classical Complement Pathways Determine the Severity of Murine IgA Nephropathy Azusa Hashimoto, Yusuke Suzuki, Isao Ohsawa, Hiroyuki Ohi, Yasuhiko Tomino. *Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan.*

Background: IgA nephropathy (IgAN) is a common form of primary progressive glomerulonephritis with mesangial cell proliferation and, matrix expansion with mesangial depositions of IgA and complement 3 (C3). Mesangial depositions of circulating aberrantly glycosylated and polymeric IgA1 are discussed as a major paraeters in the pathogenesis of IgAN, but the pathogenic roles of co-deposited C3 or related complement pathways have not been fully clarified.As a basis for confirmation of the role for IgAN, complement activation of two murine IgAN prone models were investigated.

Methods: The high serum IgA mouse (HIGA) and grouped ddY mouse (gddY) which were recently established as 100% onset IgAN prone models, were used in this study. Albuminuria, glomerular injury, complement activation in glomeruli, molecular weight of serum IgA and IgA-IgG2a immune complex (IC), IgA-IgM IC and IgA-mannose binding lectin (MBL)-A/C IC were analyzed in these models.

Results: Levels of albminuria, glomerular injury and complement activation in gddY were significantly higher than those in HIGA. Complements in both classical and lectin pathways of gddY were activated much more than those of HIGA, although glomeruler IgA depositions were similar in both mice. As in the case of human IgAN, gddY had much more aberrantly glycosylated IgA, IgA-IgG2a IC, IgA-IgM IC and IgA-MBL-A/C IC in serum than HIGA, but serum high molecular IgA did not differ in the two models.

Conclusions: These results suggest that levels of aberrantly glycosylated IgA and IgA-IC, but not total IgA, mainly determine the amplitude of activation of both complement pathways, and subsequent glomerular injury. It appears that complement activation of classical and lectin pathways may be one of the critical factors for progression of murine IgAN.

Funding: Government Support - Non-U.S.

SA-PO2228

Green Tea Polyphenol (-)-Epigallocatechin-3-gallate Restores Nrf2 Activity and Ameliorates Crescentic Glomerulonephritis Ting Ye,[1,2] Dinesh Rakheja,[2] Nosratola D. Vaziri,[3] Chandra Mohan,[4] Xin J. Zhou.[2] *[1]medicine, Wuhan Tonji Hospital, Wuhan, China; [2]Pathology, UT Southwestern Medical Center, Dallas, TX; [3]Medicine, UC Irvine, Irvine, CA; [4]Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: Crescentic glomerulonephritis (GN) is the most severe form of GN and is associated with significant morbidity and mortality despite aggressive immunotherapy. Overproduction of free radical species by inflammatory cells can cause further tissue damage, intensify inflammation, promote apoptosis, and accelerate progression of crescentic GN. The green tea catechins, particularly (-)-epigallocatechin-3-gallate (EGCG), are potent anti-inflammatory and anti-oxidant agents shown to inhibit leukocyte chemotaxis, quench free radicals, chelate transition metals, and interrupt lipid peroxidation chain reaction. The present study was undertaken to examine the therapeutic efficacy of EGCG on experimental crescentic GN.

Methods: Crescentic GN is induced in 129/svJ mice by administration of rabbit anti-mouse-glomerular basement membrane (anti-GBM) sera. The anti-GBM antibody-injected mice were allowed to develop full-blown nephritis for 7 days before intervention. On day 7, mice were randomized into either EGCG-treated (50 mg/kg/day, orally x 3 weeks) or vehicle group. Animals were then euthanized on day 28 and routine histology and key molecules involved in inflammatory pathways were studied.

Results: EGCG treatment significantly reduced mortality, decreased proteinuria and serum creatinine, and markedly improved renal histology when compared with vehicle-treated mice. The improvement in renal function and histology were accompanied by the restoration of Nrf2 signaling (which was impaired in vehicle-treated mice) as shown by increase in nuclear Nrf2 and cytoplasmic glutamate cysteine ligase catalytic subunit, glutamate cysteine ligase modifier subunit, and glutathione peroxidase. EGCG-treated mice also showed reduction in p-Akt, p-JNK, p-ERK1/2, PPARγ, and SIRT1 (which were all elevated in vehicle-treated mice).

Conclusions: Our data illustrate the efficacy of EGCG in reversing the progression of crescentic GN in mice by targeting key inflammatory pathways.

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SA-PO2229

p53 Deficiency Exacerbates Immune-Mediated Glomerulonephritis Yuki Hamano,[1] Ryota Kimura,[2] Yuki Udagawa,[2] Yoshihiko Ueda,[3] Osamu Yokosuka,[4] Makoto Ogawa.[1] *[1]Nephrology, Chiba University Hospital; [2]Clinical Education and Research, Graduate School of Pharmaceutical Sciences, Chiba University; [3]Pathology, Dokkyo Medical University Koshigaya Hospital; [4]Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Japan.*

Background: p53 tumor suppressor is a key regulator of the response to cellular stress or DNA damage. p53 activation leads to cell cycle arrest, apoptosis, or senescence. p53 has been shown to be an important regulator of renal tubular cell viability in the animal models of ischemic and toxic acute kidney injuries. In theses models, p53 activation induced proapoptotic pathways in renal tubular cells and contributed to kidney dysfunction. In this study, we investigated whether genetic or pharmacological inhibition of p53 provided a protective effect in a mouse model of immune-mediated glomerulonephritis.

Methods: p53-null (KO) and wild-type (WT) mice were immunized subcutaneously with normal rabbit IgG, followed by intravenous injections of rabbit anti-mouse GBM antibodies. In the experiment to test the effect of pharmacological inhibition of p53, C57BL/6J mice were administered pifithrin-α (a chemical inhibitor of p53) at the injections of the anti-GBM antibodies. On day 6, blood and urine samples were collected and mice were sacrificed to obtain tissues for histological analysis.

Results: Upon induction of anti-GBM glomerulonephritis in WT mice, the expression of p53 was upregulated within the glomeruli and tubulointerstitium. Unexpectedly, serum creatinine (Scr) levels and urine albumin excretion were significantly increased in nephritic KO mice compared with nephritic WT mice. The KO mice displayed significant increase in the numbers of $BrdU^+$ cells and macrophages in the glomeruli and interstitial fibrosis.

The upregulation of cyclin E1 and activation of cyclin-dependent kinase 2 (CDK2) were observed in the KO mice. Moreover, Scr levels, the number of $BrdU^+$ cells in the glomeruli and the expression of cyclin E1 were increased by pifithrin-α, which was also reversed by co-administration of roscovitine (CDK2 inhibitor).

Conclusions: p53 exerts anti-inflammatory effects by inhibiting glomerular cell proliferation during anti-GBM disease.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

SA-PO2230

Ephrin-B1 in Podocyte Is Interacted with Nephrin and Is Phosphorylated by a Signal from Nephrin Md. Murad Hossain, Masayuki Tomita, Mihoko Yamazaki, Yoshiyasu Fukusumi, Hiroshi Kawachi. *Department of Cell Biology, Institute of Nephrology, Niigata University Medicine, Niigata, Japan.*

Background: Eph and ephrin are membrane-bound proteins that function as receptor-ligand pairs. The Eph-ephrin-B family is reported to regulate the paracellular permeability of epithelial cells. We have previously reported that ephrin-B1 was expressed at the slit diaphragm (SD) of podocyte (Kidney Int 72: 954-964, 2007). The aim of this study is to elucidate the function of ephrin-B1 in the SD.

Methods: The expression of ephrin-B is analyzed with an Eph-B2-Fc probe as well as a specific antibody against ephrin-B1. The precise localization of ephrin-B and its interaction with other SD molecules in normal rats and in nephrotic models were analyzed by dual labeling techniques with a confocal laser microscopy. We also analyzed the interaction of ephrin-B1 with other SD molecules by a sequential precipitate/Western blot analysis with glomerular lysates. Next, the binding sites of ephrin-B1 and nephrin were determined with HEK293 cells transfected with full length and several domains of these molecules. Then, the mechanisms of phosphorylation of ephrin-B1 and nephrin are analyzed with HEK293 cells cotransfected with these molecules.

Results: Ephrin-B1 staining at the SD is detected with Eph-B2-Fc probe as a same pattern detected with the specific anti-ephrin-B1 antibody. Ephrin-B1 was co-localized with nephrin, and other SD molecules. The staining of ephrin-B detected by Eph-B2-Fc probe was clearly reduced at early stages of proteinuric models. Analysis with normal rat glomerular lysates revealed that ephrin-B1 interacted with nephrin. The analysis with HEK 293 cells showed that ephrin-B1 interacted with nephrin not via PDZ-binding motif of both molecules. Not only nephrin but also ephrin-B1 was phosphorylated by the anti-nephrin antibody binding to nephrin. The phosphorylation of nephrin was prevented by pretreatment with PP2, a src family kinase inhibitor.

Conclusions: Ephrin-B1 is structurally and functionally interacted with nephrin, and plays a role in maintaining the barrier function of the SD.

Funding: Government Support - Non-U.S.

SA-PO2231

Novel Roles of Notch Signal in the Pathogenesis of Collapsing FSGS Toshiharu Ueno,[1] Namiko Kobayashi,[1] Takamoto Ohse,[2] Taiji Matsusaka,[3] Jeffrey W. Pippin,[4] Stuart J. Shankland,[4] Michio Nagata.[1] *[1]Department of Pathology, University of Tsukuba; [2]Division of Nephrology and Endocrinology, University of Tokyo; [3]Department of Medicine, Tokai University; [4]Division of Nephrology, University of Washington.*

Background: Glomerular epithelial cell hyperplasia is the hallmark lesion in Collapsing Focal Segmental Glomerulosclerosis (CFSGS) and that may be involved in the disease progression. In two different mice models of CFSGS, we have shown that predominant hyperplastic epithelial cells are parietal epithelial cell (PEC) by genetic tagging of Lac-Z with nephrin promoter (Asano. JASN 2005, and Suzuki. Am J Pathol 2009).

Methods: The present study investigated one of the feasible transgenic models of CFSGS (NEP25 with immunotoxin, Asano et al JASN 2005) looking at expression Notch signal in the development of CFSGS.

Results: In this model, severe proteinuria and histological feature of CFSGS associated with migration/proliferation of PEC were observed at day 12 after single injection of immunotoxin (n = 8). Immunostaining revealed progressive podocytepenia with Lac-Z stain, and co-localization of Claudin1 (as marker of PEC) and Notch signals (cleaved Notch1, Jagged1 and Hes1) in PEC lesions. Real time PCR of isolated glomeruli revealed one third of reduced expression of podocyte marker, Nephrin and Synaptopodin, and fourfold increase of Claudin1. Over expressions of Notch signals were also observed; threefold increase of Notch1, Hes1, and HeyL, and twofold of Jagged1, Hes1, and Hes5, respectively. To determine the function of Notch signal on PEC, we incubated g-secretase inhibitor (GSI) as a blockade of Notch signal on immortalized PEC line. In the wound healing assay, GSI significantly prevented wound closure (91±2.6%) compared with control (64±6.9%). Moreover, GSI drastically repressed Notch down-stream, Hes1-, Hey1-, HeyL-mRNA, in the PEC.

Conclusions: Ectopic Notch signals in PEC may play pivotal roles for PEC migration/proliferation which is the pathologic base of CFSGS in this model. And blockade of Notch signal can become a new therapeutic approach for CFSGS.

SA-PO2232

Enhanced Albuminuria in an Adriamycin Nephropathy Model in Acatalasemic Mice Keiichi Takiue,[1] Hitoshi Sugiyama,[1] Tatsuyuki Inoue,[1] Ayu Ogawa,[1] Masashi Kitagawa,[1] Hiroshi Morinaga,[1] Shinji Kitamura,[1] Yohei Maeshima,[1] Akira Nishiyama,[2] Hirofumi Makino.[1] *[1]Okayama University Graduate School, Okayama, Japan; [2]Kagawa University, Kagawa, Japan.*

Background: Catalase is an important antioxidant enzyme that regulates the level of intracellular hydrogen peroxide and hydroxyl radicals. The adriamycin (ADR) nephropathy mouse is considered to be an experimental model of focal and segmental glomerular sclerosis with significant albuminuria. However, the effect of a functional catalase deficiency on albuminuria and progressive renal injury in an ADR nephropathy model has not yet been elucidated.

Methods: ADR (15 mg/kg BW) was administered intravenously to both homozygous acatalasemic mutant mice ($C3H/AnLCs^bCs^b$) and control wild-type mice ($C3H/AnLCs^aCs^a$). The functional and morphological alterations of the kidneys, including albuminuria, renal function, podocytic, glomerular and tubulointerstitial injury, the activities of antioxidant enzymes including catalase and glutathione peroxidase were then compared between the two groups until 8 weeks after disease induction. Moreover, the presence of a mutation of the toll-like receptor 4 (TLR4) gene, which is reported in the C3H/HeJ strain, was also investigated in both groups.

Results: The ADR-treated acatalasemic mice developed significant albuminuria in association with glomerular hypertrophy at week 4. The degree of albuminuria in the ADR-treated acatalasemic mice was significantly higher than that in the wild-type mice. No significant difference was observed in the degree of glomerulosclerosis between the two groups. Unexpectedly, the degree of renal dysfunction and tubulointerstitial injury were not remarkable in the ADR nephropathy model of the C3H mouse strain. The level of catalase activity was significantly lower in the kidneys of the acatalasemic mice than in the wild-type mice without any compensatory upregulation of glutathione peroxidase. In addition, the C3H/AnL strain was not identified to contain the TLR4 mutation.

Conclusions: These data indicate that catalase deficiency plays an important role in the development of albuminuria possibly via the functional alteration of renal tubular reabsorption in an ADR nephropathy mouse model.

SA-PO2233

Impairment of Podocyte Function by Diphteria Toxin – A New Reversible Murine Proteinuria Model Andreas Goldwich,[1] Alexander Steinkasserer,[1] Andre Gessner,[2] Kerstin U. Amann.[3] *[1]Department of Immunemodulation at the Dermatology, University Hospital Erlangen, Erlangen, Germany; [2]Institute for Medical Microbiology and Hygiene, University Regensburg, Germany; [3]Nephropathology, Institute for Pathology, University Hospital Erlangen, Erlangen, Germany.*

Background: Diphteria toxin (Dtx) receptor-mediated conditional cell ablation in transgenic mice is a powerful tool to analyze cell function *in vivo.* Transgenic mice with cell-specific expression of the human DTx receptor allow conditional depletion of these cells through Dtx administration. We carefully analysed mice after DTx injection and found proteinuria as unexpected renal side effect. Since non-genetic mouse models of proteinuric glomerular damage are limited we aimed to characterize the DTX-induced model of transient proteinuria in mice.

Methods: C57/Bl6 and C3H/HeJ mice were treated with 40µg/kg DTx/bw i.p (day 0 and 1) with DEREG mice as positive depletion controls. To exclude undesired LPS-effects heat-treated DTx and LPS-resistant C3H/HeJ mice were used. For comparison the anti-GBM nephritis and LPS-induced proteinuria models were analysed. Kidneys were investigated by immunofluorescence, confocal and electron microscopy. Cell culture studies (HeLa,NIH 3T3,murine podocyte cell line) were also performed.

Results: C57/Bl6 mice tolerated up to 80µg/kg DTx whereas higher doses were lethal. Injection of 40µg/kg DTx led to a marked transient and completely reversible proteinuria morphologically characterized by foot process fusion. 5-9 days after DTx application mice recovered completely. In i*n vitro* analysis DTx treated podocytes showed diminished attachment to basal membrane proteins.

Conclusions: Most animal models of non-genetic proteinuric glomerular disease were induced in the rat. The advantage of genetic manipulation technology favor the use of murine models. There is, however, only a limited number of murine podocyte injury models. Hence there is need for mouse models resembling human glomerular diseases. We suggest DTx-induced kidney dysfunction as a new reversible model of podocyte injury with transient proteinuria. which could be used as an additional approach to complement studies in humans.

SA-PO2234

Semaphorin 3G and Cystatin C Are Expressed in Podocytes of Rat and Are Differentially Regulated upon Toxic Injury Pierre Moulin,[1] Frank Dieterle,[2] Andre Cordier,[1] Valérie Dubost.[1] *[1]Molecular Pathology & Immunology, Novartis Institutes of Biomedical Research, Basel, BS, Switzerland; [2]Molecular Diagnostics, Novartis AG, Basel, BS, Switzerland.*

Background: Experimental glomerulopathies induced by toxic agents offer the unique opportunity to study the sequence of molecular events leading to podocyte alteration and thereby unveil early biomarkers of glomerular injury.

Methods: This study compared the molecular profile of kidney from rats treated with glomerular (e.g. Puromycin) or with tubular nephrotoxicants (e.g. Cisplatin) for various

durations (3 to 22 d). Samples were processed for gene expression profiling, histopathology and in situ hybridization(ISH)/immunohistochemistry(IHC).

Results: The initial transcriptional analysis compared glomerular and tubular toxicants. The resulting signature suggestive of glomerular damage was subsequently confirmed on laser-capture microdissected glomeruli. Major elements of this signature were CystatinC (Cst3) and Semaphorin3G (Sema3g). Interestingly, Cst3 was upregulated whereas Sema3g was downregulated during glomerular injury. ISH showed Cst3 and Sema3g mRNA expression mainly in podocytes. Moreover semi-quantitative image analysis of the ISH signal on samples from treated animals confirmed the differential regulation of both markers specifically in podocytes. Finally, the comparison between Cst3 ISH and IHC reveals temporal sequence of glomerular and tubular injuries.

Conclusions: In conclusion this is the first report of the expression of Cst3 and Sema3g mRNA in podocytes of rats. In our observations, these two transcripts were differentially regulated early in the course of toxicity and were useful markers to establish the temporal sequence between tubular and glomerular lesions. However, whether the differential regulation of these two transcripts represents distinct aspects of podocyte adaptation or reaction to injury is unclear. Sema3G is a novel podocyte marker, mostly known as a regulator of angiogenesis. Its modulation during glomerular toxicity sheds light on new aspects of podocyte involvement in the pathogenesis of glomerular lesions.

Funding: Pharmaceutical Company Support

SA-PO2235

HIV Induces Tubular Cell ROS Generation and DNA Strand Breaks through Down Regulation of Vitamin D Receptor (VDR) Divya Salhan, Shabina Rehman, Ashwani Malhotra, Mohammad Husain, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: HIV infection of tubular cells has been implicated for tubule cell injury in HIV-associated nephropathy (HIVAN). In the present study, we evaluated the role of vitamin D receptor (VDR) in HIV-induced tubular cell injury.

Methods: To develop *in vitro* model of HIV-infected tubular cells, mouse tubular cells (MCT) were transduced with empty vector (EV/MCT) or NL4-3 (HIV/MCT) constructs. To determine the effect of HIV on mitochondrial ROS generation, EV/MCTs and HIV/MCTs were double labeled with Red-CC1 (ROS labeling) and MITOTracker green (mitochondrial labeling). To study occurrence of double strand DNA breaks, EV/MCTs and HIV/MCTs were labeled with H2AX and examined by confocal microscopy. Immunoblots were probed for VDR expression in EV/MCTs and HIV/MCTs. To establish causal relationship between lack of VDR and ROS generation, siRNA-VDR transfected MCTs and control cells were evaluated for ROS generation and DNA breaks. To confirm the relationship between VDR and DNA injury, EV/MCTs and HIV/MCTs were treated with either buffer or vitamin D2 analogue, followed by evaluation of cells for ROS generation (co-labeling with Red CC1 and MITO tracker green) and DNA repair by labeling for RAD1. Immunoblots were probed for VDR from cells treated under similar conditions.

Results: HIV/MCTs displayed increased (P<0.01) mitochondrial generation of ROS as well as higher number of double strand DNA breaks. Interestingly, HIV/MCTs also showed downregulation (P<0.01) of VDR. siRNA-VDR/MCTs also displayed enhanced generation of ROS and higher number of double strand DNA breaks. However, treatment of HIV/MCTs with a vitamin D2 analogue not only enhanced (P<0.01) expression of VDR by HIV/MCTs but also displayed attenuated (P<0.01) generation of ROS as well as enhanced DNA repair.

Conclusions: Since reversal of HIV-induced attenuated VDR expression by a vit D analogue was associated with attenuated ROS generation and enhanced repair of DNA breaks, it would suggest that HIV-induced tubular cells ROS generation and DNA breaks may be caused by HIV-induced downregulation of VDR and associated signaling events.

Funding: NIDDK Support

SA-PO2236

Effects of Sodium Thiosulfate on Urinary Lithogenicity in Adults with Hypercalciuric Nephrolithiasis Onyeka W. Okonkwo,[1] John R. Asplin,[2] David S. Goldfarb.[3] *[1]Medicine, New York VAMC, New York, NY; [2]Litholink, Chicago, IL; [3]Nephrology Section, New York Harbor VAMC and NYU School of Medicine, New York, NY.*

Background: Sodium thiosulfate (STS) has been shown to reduce calcium stone formation in both humans (Yatzidis; Clin Nephrol 1985), and genetic hypercalciuric stone forming rats (GHS; Asplin at al; 2009). We studied the effects of STS on the urine and serum chemistries in hypercalciuric stone forming adults.

Methods: 5 people with idiopathic hypercalciuria and calcium kidney stones with a mean age of 66 years participated. 2 baseline 24-hour urine collections were performed on days 2 and 3 of 3 days of recorded self-selected diets. Subjects then drank STS 10 mmol BID for 7 days and repeated 24h urines while repeating the 3 days of self-selected diet from the first period. Results were compared by non parametric Wilcoxon signed rank test. Results were also compared to those of a prior study involving healthy non-stone-forming subjects administered STS.

Results: STS administration did not cause a significant change in urinary calcium excretion. It increased 24 hour urinary ammonia (P=0.002) and sulfate excretion (P=0.001) and decreased urinary pH (P=0.001) and citrate excretion (P=0.004). 3 of 5 patients had measurement of serum HCO_3 concentration: it did not change. The findings were similar to those from a prior study involving normal adults with the exception that urinary calcium excretion increased in those subjects.

Effects of STS on 24 hour urine chemistry

	Mean baseline	Mean post STS	p-value
Calcium	279 ± 155	277 ± 144	0.918
pH	6.09 ± 0.58	5.76 ± 0.52	0.001
Ammonium	36 ± 6	56 ± 11	0.022
Citrate	606 ± 195	444 ± 221	0.004
Sulfate	44 ± 14	99 ± 16	0.001

Conclusions: Although STS is apparently effective in preventing stones in humans and did prevent stones in GHS rats, the basis for these effects was not reflected by the changes in urine chemistry seen here. Although serum HCO_3 did not change, urine tests (increased urine ammonium, decreased urine citrate and pH) were suggestive of an increased acid load. Although STS did not change urine calcium, evidence of an acid load suggests that the long term safety of STS with respect to bone needs to be studied.

Funding: Veterans Administration Support

SA-PO2237

Reduced Bone Mineral Density in Young Adults with Childhood Onset Idiopathic Hypercalciuria Carlos Cuervo, Carolyn L. Abitbol, Jayanthi Chandar, Wacharee Seeherunvong, Gaston E. Zilleruelo, Michael Freundlich. *Pediatric Nephrology, University of Miami, FL.*

Background: Sustained low bone mineral density (BMD) in children with idiopathic hypercalciuria (IH) may lead to reduced peak bone mass and adult osteoporosis, but to date longitudinal studies of BMD with its predictors are lacking.

Methods: Children (81; 9.6±3.8yr) on cross sectional evaluation had IH; 53/81(66%) including 24>16 yr followed longitudinally 4.0±2.8 yr(range1-12) with ≥2 DXA studies (GE-Lunar) at spine (Sp) and femoral neck (Fn). Clinical measurements, K citrate and/or thiazide treatment (Rx) ≥6 months were analyzed.

Results: PTH, Ca, P, CO2, GFR, and final height and BMI Z-scores (-0.02 and 0.19, respectively) were normal. Calciuria (mg/kg/24 hrs)↓from baseline (7.3±3.0 vs.3.0±1.4; p<0.0001) and Rx significantly↓calciuria 3.58±1.7 to 2.7±1.0 (p=0.01). Cross sectional Z-scores <-1 were evident in 37% of patients at both Sp and Fn. Table summarizes BMD Z-scores in the longitudinal subcohort. By univariate analysis initial age, interval time between DXA studies, and Ca, P, PTH did not associate significantly with final Sp or Fn Z-scores. However, initial and final calciuria associated directly with final Sp Z (r=0.33, P=0.01 and r=0.48, P=0.0001) but not with Fn Z. By multiple regression analysis, initial Sp Z was the strongest predictor of final Sp Z (P<0.0001). Patients>16 yr had lowest final Sp (-0.8±1.5) and Fn Z-scores (-1.26±1.23) vs. children<11 yr (-0.7±1.3 and -0.65±1.3) and adolescents >11<16 yr (-0.24±0.3 and 0.1±1.2).

Conclusions: A high patient proportion with childhood onset IH present with reduced BMD and show progressive decline during adolescence with ultimate reduced BMD, particularly those with initial reduced measurements. Early screening with DXA appears indicated to identify patients at risk for adult osteoporosis.

Longitudinal BMD Z-scores in young patients with Idiopathic Hypercalciuria

Spine (n=53)			Femoral Neck (n=28)		
	Initial	Final		Initial	Final
>-1 Z-score (n=31)	-0.02±0.8	0.04±1.2	>-1 Z-score(n=17)	-0.1±0.62	-0.24±0.9
<-1 Z-score (n=22)	-1.83±0.7	-2.1±1.2**	<-1 Z-score(n=11)	-1.63±0.64	-1.66±1.3*

** P<0.0001 and * P=0.002 vs. final values of patients with initial Z-scores>-1

Funding: Clinical Revenue Support

SA-PO2238

Urine pH in Diabetic and Non Diabetic Stone Formers with Different Body Mass Index Elisa Elena Del Valle, Armando Luis Negri, Francisco Rodolfo Spivacow, Juan Ibañez. *Nephrology, Instituto de Investigaciones Metabolicas, Argentina.*

Background: Patients with type 2 diabetes are at increased risk for nephrolithiasis due to low urine pH. Overweight and obesity is frequent in this population. Higher body mass can contribute to the lower urine pH in these patients as there is a strong inverse association between pH and body weight. The purpose of this study was to identify if stone forming diabetics had a lower urine pH independent of their body mass.

Methods: Two groups of individuals were recruited for our outpatient clinic: patients who have type 2 diabetes and were stone formers (DSFn =31) and patients who were stone formers but did not have diabetes (NDSF; n =56). Both groups were paired for age and creatinine excretion. Participants had a complete stone risk analysis that included urine pH. Both groups were divided according to their BMI (kg/m2) in normal (BMI < 24.9) overweight (BMI 25 to 29.9) and obese (BMI ≥30).

Results: DSF had lower 24-h urine pH than NDSF. Urine pH remained significantly lower in DSF than NDSF in Overweight and obese groups: normals: DSF 5.75; NDSF 5.98 (NS); overweight: DSF 5.32; NDSF 5.76 (p<0.04); Obese: DSF 5.15; NDSF 5.79 (p<0.001)

Conclusions: Although higher body mass can contribute to the lower urine pH in stone formers with type 2 diabetes it cannot entirely account for that finding. An increased acid intake or generation could probably explain the difference in urine pH.

SA-PO2239

Blockade of Megalin Attenuates Nephrocalcinosis in Rats Isao Matsui, Kazunori Inoue, Takayuki Hamano, Akihiro Shimomura, Chikako Nakano, Tomoko Namba, Hiroki Omori, Masaru Horio, Hiromi Rakugi, Yoshitaka Isaka. *Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Megalin is an apical membrane protein of proximal tubular cells that works as a multifunctional endocytic receptor. Because megalin regulates the intracellular localization of type IIa sodium-dependent phosphate cotransporter (Na-Pi IIa), it was suggested that megalin plays some roles in the development of nephrocalcinosis.

Methods: We examined whether the blockade of megalin affects the development of nephrocalcinosis in rats. We inhibited the function of megalin by histidine-tagged soluble receptor-associated protein (His-sRAP). Nephrocalcinosis was induced by continuous injection of PTH 1-34 at a dose of 40 μg/kg/day for 48 hours. Rats were randomly divided into three groups; control (group C), PTH + vehicle (group P+V), or PTH + His-sRAP group (group P+H).

Results: In comparison with group C, group P+V developed extensive calcification in the renal cortex and in the outer stripe of outer medulla. His-sRAP injection dramatically reduced the calcification (group P+H). Because urinary calcium and phosphate levels in group P+H were higher than those of group P+V, it was suggested that the urine of group P+H contained inhibitory molecules against crystallization. To investigate the underlying mechanism, we analyzed whether His-sRAP affected the distribution of fetuin-A, a systemic inhibitor against calcification. Although normal rat kidney did not express mRNA for fetuin-A, immunohistochemistry demonstrated a punctate staining of fetuin-A in the megalin expressing proximal tubular cells. The blockade of megalin by His-sRAP attenuated the fetuin-A staining and resulted in a loss of fetuin-A into the urine. Because fetuin-A prevents a mineral precipitation in a solution of calcium and phosphate, the retention of fetuin-A in the tubular lumen explain the reason, at least partly, why His-sRAP attenuated intratubular crystal formation, hence nephrocalcinosis.

Conclusions: Blockade of megalin by His-sRAP attenuates nephrocalcinosis in rats.

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SA-PO2240

Clinical Characteristics of Hypercalcemia Due to Adrenal Insufficiency in Patients on Long-Term Hemodialysis Yukitoshi Sakao,[1] Akihiko Kato,[1] Hideo Yasuda.[2] *[1]Blood Purification Unit, Hmamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]First Department of Medicine, Hmamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

Background: Hypercalcemia is rarely caused by adrenal crisis. However, the mechanisms for hypercalcemia due to adrenal insufficiency remain to be determined in patients on hemodialysis (HD).

Methods: We examined the clinical characteristics of hypercalcemia caused by acute adrenal failure in 5 HD patients. We also analyzed bone turnover markers before and after the replacement of glucocorticoid agents.

Results: The patients' age and vintage on HD were ranged from 44 to 75 years old and from 7 to 32 years respectively. At the onset of hypercalcemia, all patients were anuric, and under critically ill conditions. The causes of adrenal insufficiency were isolated ACTH deficiency, pituitary apoplexy and glucocortiocoid withdrawal syndrome. Serum calcium (Ca) corrected by serum albumin were maximally increased to 12.9 to 14.9 mg/dl. Intact parathyroid hormone (iPTH) levels were reduced to 4 to 38.5 pg/ml expect for 1 patient with severe secondary hyperparathyroidism. Serum 1,25-dihydroxy-vitamin D3 level was also suppressed to 8.1 to 16.2 pg/ml in 4 cases. Serum levels of type I collagen telopeptides, a marker of bone reabsorption (ICTP), were elevated in all patients. Basal ACTH levels were 2.9 to 40.2 pg/ml, while cortisol levels were as low as <6.5 μg/dl in 4 patients and 15.4 μg/dl in 1 patient. A single corticotropin-releasing hormone injection failed to increase plasma ACTH and cortisol in all patients. Glucocorticoid replacement therapy acutely decreased serum Ca and ICTP levels, while increased serum iPTH. Administration of bisphosphonate also normalized serum Ca without glucocorticoid treatment in 1 patient.

Conclusions: These findings suggest that the adrenal failure-induced hypercalcemia could occur in anuric patients on long-term HD when complicated of acute illness. Increased bone reabsorption due to adrenal insufficiency without any urinary Ca excretion may be related to the development of hypercalcemia.

SA-PO2241

Modulation of FGF23 by Phosphate Binders in Chronic Kidney Disease Stage 4-5 Predialysis (Lanthanum vs Calcium Carbonate) Sagrario Soriano, Raquel Ojeda, Maria Luisa Agüera, Mariano Rodriguez, Alejandro Martin-Malo, Pedro Aljama-Garcia. *Nephrology, Hospital Universitario Reina Sofia, Córdoba, Spain.*

Background: Cardiovascular mortality is increased in Chronic Kidney Disease (CKD) patients with serum phosphate (P) concentration in the upper limit of normality. Increased serum concentration of FGF 23 is also independently associated with cardiovascular mortality. The present study evaluates in CKD 4-5 patients on protein restriction (1g/Kg/day) whether a reasonable dose of calcium carbonate (1.5 g/day) or lanthanum carbonate (1.35 g/day) have an effect on serum FGF23 levels.

Methods: Thirty two patients were selected; after one month wash out they were randomized to receive four months of calcium carbonate or lanthanum carbonate. Patients included had serum P>4 mg/dl, a normal serum calcium, 25(OH) levels >20 ng/ml, no treatment with VDR activators or Cinacalcet. Baseline clinical characteristics of patients were similar in both groups.

Results: Results are presented in the following.

Calcium Carbonate (1) Lanthanum Carbonate(2)

	Baseline(1)	After treatment(1)	Baseline(2)	After treatment(2)
Cr Cl (MDRD-7, ml/min)	15,6±3,6	16.6±7,1	17,8±5,6	17,6±5,5
P (mg/dl)	4,7±0,8	4,5±0,7	5±0,4	4,6±0,5 *
PTH (pg/ml)	195±34	210±87	104±57	130±86
FE (PO4) (%)	46,2±8,9	52,4±17	46,4±9,5	39,2±8,3 *$
Intact FGF23 (pg/ml)	237,8±44	245,9±75	230,4±65	146,2±30 *$

Values are mean ± SD are shown, Cr Cl: Creatinine clearance, P: serum phosphate, FE (PO_4)(%): fractional excretion of phosphate. *P <0,05 vs same group baseline,. **$** P <0,05 vs calcium carbonate after treatment

Conclusions: In CKD 4-5 patients the administration of 1.5 g of Calcium Carbonate produced a non significant decrease serum phosphate levels and, FGF 23 and FE(PO_4) did not change. By contrast, the administration of Lanthanum carbonate (1.35 g) resulted in a decrease in serum phosphate levels together with a reduction of FE(PO_4) and plasma concentration of FGF23. In conclusion, in CKD 4-5 the administration of lanthanum carbonate decreases the phosphate load as reflected by the reduction in FE(PO_4) and the marked decrease in FGF 23.

SA-PO2242

Preliminary Data of the Italian Multicentric Study on the Prevalence of Vascular Calcifications and Vertebral Fractures in Parathyroidectomised Dialysis Patients (CAVE PTX Study) Sandro Mazzaferro.[1,2] *[1]Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences, Sapienza University, Rome, Italy; [2]Mineral Metabolism & Trace Element Study Group, Italian Society of Nephrology.*

Background: The CAVE PTX study is aimed at evaluating, in dialysis patients who received PTX at any time in their life, the biochemical control of divalent ions (phase I), and the prevalence of aortic calcifications and vertebral fractures (phase II).

Methods: We report here data of the phase I. During 2010, values of Ca, P, AP and PTH and pertinent therapies, were asked from 149 dialysis Unit from all over Italy, supplying therapy to 12515 patients (HD = 87.7%; DP = 12.3%)

Results: 123 Units had data of 528 living PTX patients (29 on PD). Prevalence of PTX (4.22%), was different between HD (4.5%) and PD (1.9%). Clinical characteristics were: age 58±13 y.o.; sex: 56% female, dialysis: since 15±8 years; diabetes: 7%; BMI: 25±4, Systolic BP: 126±21 and Diastolic BP: 73±12 mmHg; on antihypertensive drugs: 45%; on EPO: 80%. With the exception of reduced albumin (3.9±0.5 g/dl), biochemical parameters averaged mostly acceptable values: PTH 182 ± 292 pg/ml; Ca 8.8 ± 0.8 mg/dl; P 4.9±1.3 mg/dl; AP 240±210 mU/ml (n.v. 90-270); Hb 11 ± 1 g/dl). Prescribed therapies were: Phosphate binders = 87.8% (Ca based: 67.4%; Sevelamer: 50.5%; Lanthanum 11%; Aluminum: 14.9%); vitamin D = 61.8% (oral calcitriol 71.1%; i.v. calcitriol 6.2%; paricalcitol 22.3%; other D: 1.8%), calcimimetic = 12.8%. PTX had been mostly subtotal (54.8%) or total (38%), with only 4.7% treated with total PTX plus autotransplantation. PTH was optimal (150-300) in a limited 17%; high in 19 and low in 64% of the cases. Serum Ca was optimal (8.5-9.5) in 47%, high in 19% and low in 34%. Serum P was optimal (3.5-5.5) in 54%, high in 33% and low in 14%.

Conclusions: PTX prevalence in Italy is low and is confirmed to involve relatively young patients, mainly female, and with a long history of haemodialysis. These patients show acceptable control of divalent ions, but most of them require active therapies for secondary hyperparathyroidism. Low PTH values are very frequent and associated with low-normal Ca. Evaluation of hard outcomes is mandatory.

Funding: Pharmaceutical Company Support

SA-PO2243

Increased Platelet Count and Marrow Fibrosis in Rats with Renal Failure and Secondary Hyperparathyroidism Cheryl P. Sanchez,[1] Kristen R. Friedrichs.[2] *[1]Pediatrics, LLUMC, Loma Linda, CA; [2]Pathobiological Sciences, UW Sch Vet Med, Madison, WI.*

Background: Bone marrow fibrosis (BMF) has been associated with elevated PTH levels seen in primary and secondary hyperparathyroidism (SPHT), and may contribute to bone disease and anemia. Recent studies have shown that factors associated with megakaryocytes and platelets such as TGF-b may contribute to BMF.

Methods: To evaluate changes in platelet and megakaryocytes in renal failure and SHPT, 23 male weanling rats (48 ± 3 grams) underwent a 2-stage 5/6 nephrectomy (NX, N=16) or sham Nx (C, N=7). Nx rats were fed standard diet (Nx-C, N=7) or high phosphorus diet, 1.2% (Nx-P, N=9). At the end of 4 weeks, blood was obtained for CBC, PTH and creatinine levels. The femur was used for marrow analysis, sternum for marrow histology and tibia for trichome stain.

Results: Tibial length was shorter in Nx-P, 3.0±0.1 cm, versus Nx-C, 3.2±0.06 cm and C, 3.2±0.03, p<0.0003. Hemoglobin (Hgb) decreased in Nx-P and Nx-C, 10±1.7 g/dL and 13±4 g/dL, compared to C, 15±0.4 g/dL, p<0.05. PTH was higher in Nx-P, 1719±599 pg/mL, versus Nx-C and C, 142±67 pg/mL and 66±78 pg/mL, p<0.0001. In the peripheral blood, platelet count increased in Nx-P, 1221±131 $x10^3$/uL, p<0.002, and similar between Nx-C and C, 933±243 $x10^3$/uL and 905±125 $x10^3$/uL. Mean platelet volume did not change. There was an increase in segmenters in Nx-P and Nx-C, 0.6±0.2 $x10^3$/uL and 0.4±0.1 $x10^3$/uL, compared to C, 0.2±0.1 $x10^3$/uL, p<.05; this may suggest an inflammatory state. Nx-P had significant reticulin staining in the sternum and tibia, 2.8±0.4 and 2.8±0.5, compared

to Nx-C, 0.8±0.4 and 0±0, and C, 0.8±0.7 and 0±0, p<0.01. Marrow core analysis showed similar megakaryocyte score in all groups. Qualitatively, trichome staining was present only in the marrow of Nx-P. Platelet count correlated with reticulin, R=0.7, p<0.0003 and PTH, R=0.6, p<0.002. There was an inverse relationship between Hgb and platelet count, R=-0.5, p<0.009.

Conclusions: An increase in platelet count may contribute to BMF in advanced SHPT without any changes in megakaryocte numbers. Further studies are needed to determine the alterations in growth factors associated with platelet and megakaryocytes in renal failure.

SA-PO2244

Skeletal FGF-23 and DMP1 Expression Increase during Treatment of Secondary Hyperparathyroidism R.C. Pereira,[1] Harald Jueppner,[2] Barbara Gales,[1] Isidro B. Salusky,[1] Katherine Wesseling-Perry.[1] *[1]Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Endocrine Unit, Massachusetts General Hospital, Boston, MA.*

Background: Skeletal FGF-23 expression correlates with plasma FGF-23 levels and vitamin D sterol therapy increases plasma FGF-23 levels in dialysis patients; however, it is unknown how therapy affects skeletal expression of FGF-23 and its regulator DMP1.

Methods: 10 dialysis patients (6M, 4F), age 16.9 ± 0.7 years, with secondary hyperparathyroidism underwent bone biopsy before and after 8 months of therapy with vitamin D sterols and phosphate binders. Biochemical values, bone histomorphometry, and skeletal expression of FGF-23 (determined by immunohistochemistry and quantified by Ariol scanning) were measured at baseline and after therapy (Table 1. *p<0.05). Plasma FGF-23 values were determined by the 2nd generation C-terminal assay (Immutopics).

Results: Plasma FGF-23 increased by 226 ±111% from baseline while other biochemical parameters did not change. Bone FGF-23 and DMP1 expression increased by 340 ± 155% (p<0.05) and 134 ± 45% (p<0.05), respectively.

Variable	Baseline	Post-therapy
Biochemicals		
Ca (mg/dl)	8.7 ± 0.3	9.0 ± 0.1
P (mg/dl)	6.4 ± 0.5	5.4 ± 0.3
PTH (pg/ml)	653 ± 81	717 ± 90
Alkaline phosphatase (IU/l)	314 ± 51	385 ± 152
25(OH)vitamin D (ng/ml)	19 ± 3	26 ± 2
FGF-23 (2nd generation C-terminal, Immutopics) (RU/ml)	743 (478, 1196)	1972 (565, 3973) RU/mL * 226 ± 111%
Bone Histomorphometry		
BFR/BS (um²/mm³/d)	54.7 ± 9.7	39.9 ± 11.6
OV/BV (%)	6.7 ± 1.0	4.7 ± 0.9
OS/BS (%)	40.7 ± 0.6	31.9 ± 3.9*
O.Th (µm)	13.6 ± 0.8	11.5 ± 0.8
OMT (d)	15.0 ± 1.7	15.0 ± 2.0
MLT (d)	38.9 ± 8.4	66.0 ± 32.1
BV/TV (%)	39.9 ± 3.4	36.4 ± 2.8
Tb.N (n/mm²)	2.2 ± 0.1	2.1 ± 0.1
Tb.Sp (µm)	279 ± 19	321 ± 46
Skeletal Protein Expression		
Bone FGF-23 (pixels/mm²)	195 (136, 256)	371 (310, 857) *
Bone FGF-23 (pixels/mm²)	3833 (3456, 6015)	8560 (7459, 10362) *

The percent change in bone FGF-23 correlated with the percent change in plasma FGF-23 (r=0.60, p=0.06).

Conclusions: Therapy with vitamin D sterols increases skeletal FGF-23 as well as DMP1 expression in dialysis patients with secondary hyperparathyroidism, suggesting that dysregulation of osteocyte function may be exacerbated by current therapeutic strategies. How these changes affect long-term outcomes, including bone and cardiac morbidity, remains to be determined.

Funding: NIDDK Support, Private Foundation Support

SA-PO2245

Outcomes of a Large Series of Parathyroidectomies Performed in a Single Centre Satish Babu Ramakrishna. *Renal Medicine, University Hospital Birmingham, Birmingham, United Kingdom.*

Background: Secondary Hyperparathyroidism (SHPT) is an important complications of renal failure and its management remains a challenge. Total parathyroidectomy (PTX) is widely used but questions regarding long term outcomes remain unanswered. Low paratharmone (PTH) levels and adynamic bone disease following

(PTX) are considered significant risk factors for fractures.

Methods: Between 2000 to 2007, 131 total parathyroidectomies were performed without re-implantation at a single centre by a single operator. Clinical information and biochemistry data were obtained along with fracture data. Calcium, phosphate and PTH were recorded pre-operatively and at 1, 6 and 12 months after PTX.

Results: Mean age at the time of PTX was 47 years. Time on RRT before PTX varied from 20 to 100 months with an average of 65 months. Mean follow up period is 3.5±2.06yrs. The PTH fell significantly from a median of 995pg/ml pre surgery to 7.9pg/ml at 1 month and remained low at 6.0pg/ml at 6 months and 33.0pg/ml at 12 months. The average calcium level pre-operative was 2.6mmol/L (SD 0.24mmol/L) which dropped to 2.28mmol/L(SD 0.38mmol/L) at 1 month and 2.36mmol/L(SD 0.3mmol/L) at 12 months. There was no significant fall in phosphate levels after surgery. Six patients who had recurrence of SHPT underwent repeat PTX and one patient required 2 repeat operations. Only four patients had fractures following PTX equating to 7.77 fractures per 1000 patient years. All the four patients who had the fractures had recurrence of SHPT and the 63% of patients with PTH suppressed below 70pg/ml at 12 months had no fractures. There were no cases of laryngeal nerve palsy and no significant surgical complications. There were no deaths in the first year following surgery and 19 deaths thereafter.

Conclusions: In our centre PTX plays an important role in the management of severe SHPT. It can be a safe and effective treatment resulting in significant suppression of PTH, a fall in the mean serum calcium and no increased risk of fractures over 1-7 years following surgery.

SA-PO2246

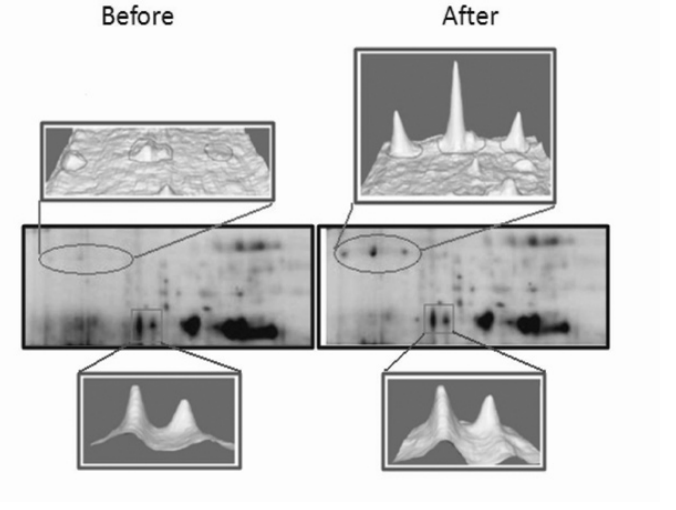
Serum Proteomics Analysis of in a Hemodialysis Patient with Secondary Hyperparathyroidism before and after Parathyroidectomy Operation Itir Yegenaga,[1] Murat Kasap,[2] Gurler Akpinar,[2] Sibel Bek,[1] Sara Yamaner.[1] *[1]Internal Medicine, Nephrology, Kocaeli University Medical School, Kocaeli, Izmit, Turkey; [2]Medical Biology, Kocaeli University Medical School, Kocaeli, Izmit, Turkey.*

Background: Systemic bone and mineral disturbances co exist in CKD patients from the very early stages and effects life quality, morbidity and mortality. Uremic bone disease shows heterogeneity in terms of bone turnover; while most patients suffer with high turnover (secondary hyperparathyroidism-HPT) the others have low turnover (adynamic) bone disease. It is important to know the bone turnover to apply appropriate treatment to these patients.

Methods: We analyzed serum proteome profile pre and post-op of parathyroidectomy operation in a 33 year old male hemodialysis(HD) patient with secondary HPT. We aim to detect some changes in proteome profile correlated PTH level and may be used as a marker to determine the bone turnover. Less abundant protein fractions obtained by microrotofor fractionation were applied to the two D-gel electrophoresis on the pH 3-10 IPG strips, three spots took the attention which appeared after parathyoidectomy and they were cut from the gel and were subjected to MALDI-TOF TOF analysis .

Results: Two of the spots were identified with high confidence and one of them belonged to a protein defined as a zinc finger- FYVE containing domain (ZFYVE). Among ZFYVE Osterix was reported to be involved in bone turnover.This protein might be an Osterix homologue and related to bone turnover.

Conclusions: Since this is only one case and the preliminary results report, this hypothesis needs to be proven and further investigations to understand the relationship and if there is; importance of ZFYVE on bone turnover.



SA-PO2247

Increased Extramedullary Hematopoiesis in Anemic Rats with Renal Failure and Secondary Hyperparathyroidism Cheryl P. Sanchez,[1] Kristen R. Friedrichs.[2] *[1]Pediatrics, LLUMC, Loma Linda, CA; [2]Pathobiological Sciences, UW Sch Vet Medicine, Madison, WI.*

Background: Anemia and secondary hyperparathyroidism (SHPT) are complications of renal failure. Studies have shown that anemia is worst in advanced SHPT. In children, anemia has been correlated with poor growth.

Methods: To demonstrate whether extramedullary hematopoiesis (EMH) is increased in renal failure and severe SHPT, 23 male weanling rats (48 ± 3 grams) underwent a 2-stage 5/6 nephrectomy (NX, N=16) or sham Nx (C, N=7). Nx rats were fed standard diet (Nx-C, N=7) or high phosphorus diet, 1.2% (Nx-Phos, N=9). At the end of 4 weeks, blood was obtained for CBC, PTH, creatinine, calcium and phosphorus. The femur was used for bone marrow analysis and the sternum for bone marrow histology.

Results: Nx-Phos group had lower gain in weight and length, 77±12 grams and 9.8±1.2 cm, compared to Nx-C, 89±7 grams and 11.4±.0 cm and C, 94±9 grams and 12±0.9 cm, p<0.01. Tibial length was shorter in Nx-Phos, 3.0±0.1 cm, versus Nx-C, 3.2±0.06 cm and C, 3.2±0.03, p<0.0003. Hemoglobin (Hgb) decreased in Nx-Phos and Nx-C, 10±1.7 g/dL and

13±4 g/dL, compared to C, 15±0.4 g/dL, p<0.05. Estimated reticulocyte count increased in Nx-Phos, 2.6±1.4 x10^{5}u/L compared to Nx-C and C, 1.4±0.4x10^{5}u/L and 1.0±0.3x10^{5}u/L, p<0.01. Nx-Phos had increased polychromatophil score, 2.0±1.4, compared to Nx-C and C, 1.0±.0 0.7, p<0.01, denoting enhanced peripheral regeneration. When corrected for tibial length, total erythrocyte counts in the marrow were similar in all groups. EMH was more pronounced in the liver of Nx-Phos, 1.33±1.0 versus Nx-C and C, 0.29±0.4 and 0±0, p<0.03, and in the spleen, 2.0±0.4 in Nx-Phos versus Nx-C and C, 1.1±1.4 and 0.3±0.4, p<0.01. Reticulin staining increased in the sternum and tibia of Nx-Phos, 2.8±0.4 compared to Nx-C and C, 0.8±0.4, p<0.01. Hgb correlated with body length, R=0.7, p<0.005. There was an inverse relationship between Hgb and PTH, R=-0.9, p<0.0001.

Conclusions: Findings in the current study suggest that response to anemia in renal failure and severe SHPT may be limited by reticulin in the marrow, and an increase in peripheral regeneration may be due to an enhancement of extramedullary hematopoiesis in the liver and spleen.

SA-PO2248

Decrease of Serum Sphingosine-1-Phosphate Levels in Hemodialysis Patients with Secondary Hyperparathyroidism Treated with Cinacalcet

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Background: Both cinacalcet, a calcium sensing receptor agonist, and sphingosine-1-phosphate (S1P) affect bone mineral homeostasis(Ishii M. Nature 2009). Consequently, we investigated the effects of cinacalcet on serum levels of SIP in hemodialysis (HD) patients with secondary hyperparathyroidism (SHPT).

Methods: Thirty-four chronic HD patients with SHPT for more than 3 months were enrolled in this prospective intervention study. We measured serum S1P by liquid chromatography-tandem mass spectrometry before and after treatment with cinacalcet.

Results: Serum S1P decreased significantly after 6 month-treatment with cinacalcet in all patients (897.5+/-424.1 to 251.9+/-46.4 nmol/l, P<0.001). Serum S1P level showed no significant associations with age, gender, HD vintage, serum creatinine, urea, calcium, phosphorus, intact parathyroid hormone, alkaline phosphatase, tartrate-resistant acid phosphatase 5b or hematocrit.

	Pre	Post	P value
S1P (nmol/L)	897.5+/-424.1	251.9+/-46.4	<0.001
cCa (mg/dL)	9.53+/-0.55	9.02+/-0.90	<0.01
P (mg/dL)	6.05+/-1.63	4.92+/-1.36	<0.01
iPTH (pg/mL)	248.2+/-164.8	81.8+/-59.5	<0.001
ALP (U/L)	256.0+/-121.95	257.5+/-130.6	0.87604
TRAP5b (mU/dL)	442.9+/-263.5	298.6+/-23.2	<0.001

Changes in various parameters after cinacalcet administration

There were no associations between the decrease of S1P and change of iPTH, TRAP, or ALP.

Conclusions: These results suggest that cinacalcet may reduce serum S1P level in HD patients with SHPT. This mechanism of serum S1P level reduction after cinacalcet administration was unknown. However, since S1P has various physiological actions, this finding may explain some physiological effects of cinacalcet hydrochloride in addition to its known suppression of parathyroid hormone secretion.

Funding: Government Support - Non-U.S.

SA-PO2249

Vitamin E Decreases Vascular Calcification in Obese (Zucker fa/fa) Uremic Rats

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Background: Vascular calcification(VC) is a frequent complication in patients with chronic kidney disease(CKD) and diabetes. Hyperphosphatemia is a major pathogenic factor for uremic VC while chronic inflammation and oxidative stress seem to be related to both uremia and diabetic VC. In addition, mitochondrial ROS-NFκB signaling has been recently shown to be involved in phosphate(P) induced VC. According that, antioxidants, such as vitamin E (vit.E), may protect against VC. We hypothesized that a diet with high vit.E content may decrease VC in an in vivo model of metabolic syndrome and uremia.

Methods: Obese Zucker (fa/fa) rats(n=12) were 5/6 nephrectomized and treated with calcitriol (80ng/kg ip/3 times per week) to induce VC. Rats were randomly allocated in two groups that were fed a diet containing Ca=0.6%, P=0.9% supplemented with normal (Control=27mg/kg) or increased (Vit E=30000 mg/kg) amount of vit.E. After 21 days, rats were euthanized to obtain blood and aortic tissue.

Results: Plasma level of insulin,TNF∝ and FGF21, and Aortic Ca and P content were decreased in VitE group (P<0.05 vs Control).

Table 1. *Results*

Determination	Control	Vit E
Ca^{2+} (mmol/L)	1.31±0.02	1.34±0.03
P(mg/dL)	10.9±1.8	10.3±1.4
Creatinine(mg/dL)	1.1±0.2	1.2±0.1
Glucose(mg/dL)	209.5±40.6	185.8±18.2
Insulin(ng/mL)	10.0±1.6	6.9±0.6*
TNFα(pg/mL)	82.1±4.8	70.3±1.7*
Leptin(ng/mL)	81.9±3.9	82.9±4.3
Adiponectin(μg/mL)	2.2 ±0.4	3.1±0.6
FGF21(pg/mL)	2827 ±303	1501 ±195*
Aortic Ca (mg/g tissue)	5.4±1.42	1.4±0.07*
Aortic P(mg/g tissue)	2.5±0.89	0.1±0.02*

Values are means ± SE. *P < 0.05 vs. Control.

Conclusions: In the present model of obese uremic rats, high dietary intake of vit.E decreases VC without changing plasma Ca, P and creatinine. In addition, supplementation with vit.E seems to improve metabolic syndrome (decreased FGF21 and insulin) and to reduce inflammation associated to uremia and metabolic syndrome (decrease in TNFα).

Funding: Government Support - Non-U.S.

SA-PO2250

The Association of Fetuin-A with Coronary Artery Calcification in Community-Living Persons: The Multi-Ethnic Study of Atherosclerosis

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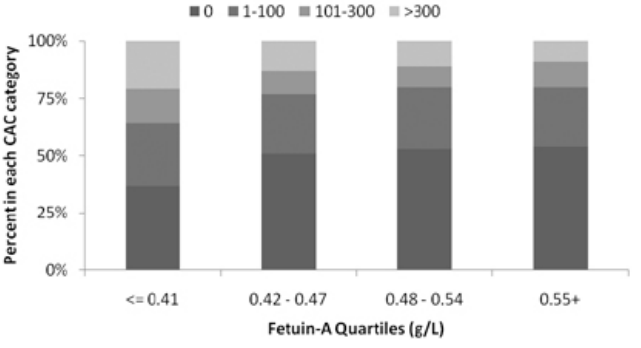
Background: Fetuin-A inhibits arterial calcium deposition *in vitro*. Lower levels associate with CAC in ESRD. The association of fetuin-A with CAC in other settings is unknown.

Methods: We evaluated the association of fetuin-A with CAC prevalence and severity at baseline, and CAC incidence over 3.2 years among 2,457 participants in MESA. Associations were evaluated using relative risk, linear, and Poisson regression, respectively.

Results: Mean eGFR was 94±21ml/min/1.73m^2. 1,200 (49%) had CAC at baseline, and 272 developed incident CAC during follow-up. There was a threshold effect at the lowest fetuin-A quartile with CAC prevalence. In models adjusted for demographics, traditional CVD risk factors, and kidney function, the lowest fetuin-A quartile had 7% (95% CI 1-13%) greater CAC prevalence compared to quartiles 2-4. Similar associations were observed with CAC severity; a more linear association.

Distribution of Coronary Artery Calcification Scores by Fetuin-A Quartiles n Community-Living Individuals: The Multi-Ethnic Study of Atherosclerosis



Each SD (0.1 g/L) lower fetuin-A was associated with a 12% (95% CI 3-21%) greater CAC severity in adjusted models. There was no association of fetuin-A with incident CAC (RR per SD lower 1.06; 95% CI 0.95-1.19). Associations were similar by sex, race/ethnicity, and diabetes status.

Conclusions: Fetuin-A is inversely associated with CAC severity among community-living individuals without severe CKD.

Funding: Other NIH Support - NHLBI, Veterans Administration Support

SA-PO2251

Restoration of Bone Mineralization by Cinacalcet or Parathyroidectomy Is Associated with a Significant Reduction in Calcitriol-Induced Vascular Calcification in Uremic Rats

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Background: The present study investigated to which extent high dose calcitriol-induced vascular calcifications in uremic rats correspond to defined changes in bone histomorphometry.

Methods: Five groups were studied: sham-operated controls (n=7); subtotally nephrectomised (SNX) uremic animals (U; n=12); U + calcitriol (VitD) (0.25µg/kg/day), (n=12); U + VitD + cinacalcet (CIN) (10mg/kg/day), (n=12); U + VitD + PTX, (n=12). Treatment started 2 weeks after SNX and continued for the next 14 weeks.

Results: Calcitriol treatment went along with the development of distinct vascular calcification which was reduced by > 50% in both the CIN treated and PTX animals. Compared to control animals and those of the U group, PTX, CIN and calcitriol treatment were associated with a significant increase ($p<0.05$) in bone area comprising +/- 60% of the total tissue area. However, whereas excessive woven bone accompanied by a dramatically increased osteoid width/area was seen in the U+vitD group, CIN treatment and PTX resulted in a significant decrease in the double labelled perimeter and normalization of both the bone formation rate and amount of osteoid ($p<0.05$) which was accompanied by a significant reduction in serum PTH levels.

Conclusions: These data indicate that the excessive vascular calcification in vitD treated uremic rats is accompanied by less efficient calcium (Ca) and phosphorus (P) incorporation in bone; a process which is reversed by CIN treatment and PTX corresponding to a decrease in Ca and P deposition in vascular calcifications.

SA-PO2252

The Relationship between FGF-23 and Markers of Cardiovascular Lesions

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Background: FGF-23 is secreted by osteocytes and influence vitamin D and phosphate metabolism as well as bone mineralization. It rise following progressive loss of renal function and the highest levels were confirmed in dialysis population.

The aim of the study was to investigate the relationship between FGF-23 and markers of heart lesion (NT-proBNP), vitamin D (25-OH D3) as well as the incidence of vascular calcifications (CCA-IMT, CaCs) in patients with ESRD.

Methods: Studied group consisted of 76 patients (36 female and 40 male) of average age 60 12 years on maintenance hemodialysis (25 5 months). Plasma levels of FGF-23, and serum levels of 25-OH D3 were measured by ELISA technique while NT-proBNP were determined based on immunochemiluminescence. CCA-IMT was evaluated by B-mode ultrasound of carotid arteries using Acuson 128 XP apparatus. Coronary artery calcification score (CaSc) was assessed by multi-slice spiral computed tomography (MSCT).

Results: The obtained results indicate elevated plasma concentrations of FGF-23 in ESRD patients (12086 6269 RU/ml). The mean level of FGF-23 correlates well with NT-proBNP (9.72 9.83 ng/ml), CCA-IMT (0.98 0.41 mm), 25-OH D3 (18.22 7.03 ng/ml) and CaSc (1193.07 1706.71 Agatston units).The obtained correlations were shown in table 1.

Correlation between FGF-23 and studied parameters

FGF-23			
Parameter	r (Spearman's)	r^2	p
NT-proBNP	0.491719	0.241787	<0.000013
25-OH D3	0.260696	0.067963	<0.025906
CCA-IMT	0.312896	0.097904	<0.014926
CaCs	0.470679	0.221539	<0.000129

Conclusions: 1. The strong positive relationship between FGF-23 and tested calcification markers (CCA-IMT, CaSc) indicates that FGF-23 may be one of a causative factors for arterial calcifications in ESRD patients.

2. Correlation of increased FGF-23 with NT-proBNP may indicate that patients with advanced renal failure have signs of myocardial damage caused by calcium phosphate disturbances.

SA-PO2253

Persistently Low iPTH Level Predicts Progression of Aortic Arch Calcification in Incident Hemodialysis Patients

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Background: The aim of this study is to determine the relationship between adynamic bone disease and aortic arch calcification in incident hemodialysis patients.

Methods: From January to December 2008, a total of 94 incident hemodialysis patients were enrolled, whose iPTH level was lower than 300pg/dL throughout the initial one year. They were divided into three groups according to iPTH changing pattern during the initial first year (Group 1: iPTH level persistently below 150pg/dL ; Group 2:iPTH level moves up or down; Group 3: iPTH level persistently over 150pg/dL). Aortic arch calcification was measured on posterior-anterior plain chest X-ray using the specific scale. In each group, aortic arch calcification was scored at the initiation of dialysis and followed up till May 2011. The progression of aortic arch calcification was defined as the increase in the score >50%.

Results: A total of 94 patients were enrolled [Group 1 (N=39), Group 2(N=32), Group 3(N=23)]. Median follow up period was 2.5 years. The prevalence of baseline aortic arch calcification was highest in the persistently low iPTH group (respectively, Group 1, 59.0%; Group2 ,43.8%; Group 3, 21.7%, P=0.017) and , calcification progression was most frequently found in the persistently low iPTH group (repectively, Group 1, 19.1%; Group 2, 7.1%; Group 3, 3.2%, P=0.040). In a multivariate logistic regression analysis, age and persistently low iPTH independently contributed to progression of aortic arch calcification (HR=1.060, 95% CI 1.012-1.110, P=0.013 and, HR=4.89, 95% CI 1.059-22.38, P=0.042, respectively). But, we couldn't find any association between aortic arch calcification and mortality.

Conclusions: Persistently low iPTH and age was an independent risk factor for progression of aortic arch calcification in incident hemodialysis patients.

SA-PO2254

Circulating Vascular Calcification Inhibitors Are Associated with Mortality in Incident Dialysis Patients

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Background: Vascular calcification is common among diabetics and dialysis patients and associated with increased mortality. We evaluated whether circulating calcification inhibitors osteoprotegerin (OPG), osteopontin (OPN), bone morphogenic protein-7 (BMP7) and fetuin-A were associated with mortality in 602 incident dialysis participants from the Choices for Healthy Outcomes in Caring for ESRD Study.

Methods: Calcification inhibitors were measured in blood samples collected < 6 months after dialysis initiation. Risk of mortality associated with tertiles of calcification inhibitors was modeled using Cox proportional hazards models adjusted for demographic factors, comorbidities, serum phosphorus, and corrected serum calcium. Prespecified interactions were tested with diabetes.

Results: Mean age of study participants was 58 yrs; 35% were African American, 57% had diabetes and 6% were treated with peritoneal dialysis. There were 423 deaths over a median follow-up of 3.3 years. Higher OPG and lower fetuin-A were associated with increased mortality overall (p-trend=0.02 for both). In stratified models, highest tertiles of OPG, BMP7, and OPN were associated with mortality among non-diabetics, but not among diabetics.

Hazard Ratio of Mortality (95% CI) by Tertiles of Calcification Inhibitor Compared to Tertile 1

Tertile	Overall	Diabetes	No diabetes
OPG: 2	**1.4 (1.0, 1.9)***	1.2 (0.8, 1.8)	1.5 (0.8, 2.7)
3	**1.5 (1.1, 2.1)***	1.2 (0.8, 1.9)	**2.8 (1.5, 5.3)***
BMP7: 2	1.1 (0.8, 1.5)	1.2 (0.8, 1.7)	1.0 (0.5, 1.7)
3	1.1 (0.9, 1.5)	1.1 (0.8, 1.7)	**2.0 (1.1, 3.7)***
OPN: 2	1.1 (0.8, 1.5)	1.2 (0.8, 1.9)	1.1 (0.6, 2.0)
3	1.2 (0.9, 1.7)	1.1 (0.7, 1.7)	**2.2 (1.1, 4.2)***
Fetuin-A: 2	0.9 (0.7, 1.2)	-	-
3	**0.7 (0.5, 0.9)***	-	-

* $p<0.05$; p-interaction for calcification inhibitor and diabetic status: OPG, $p<0.001$; BMP7, $p=0.41$; OPN, $p=0.93$; Fetuin-A, $p=0.63$

Conclusions: Circulating calcification inhibitors may identify dialysis patients at higher risk for mortality, particularly in the absence of diabetes.

Funding: NIDDK Support, Other NIH Support - NHLBI, Other U.S. Government Support, Pharmaceutical Company Support

SA-PO2255

Overexpression of Muscular Related Proteins and Dowregulation of Lamin A/C Might Be Involved in the Protection Against Vascular Calcification

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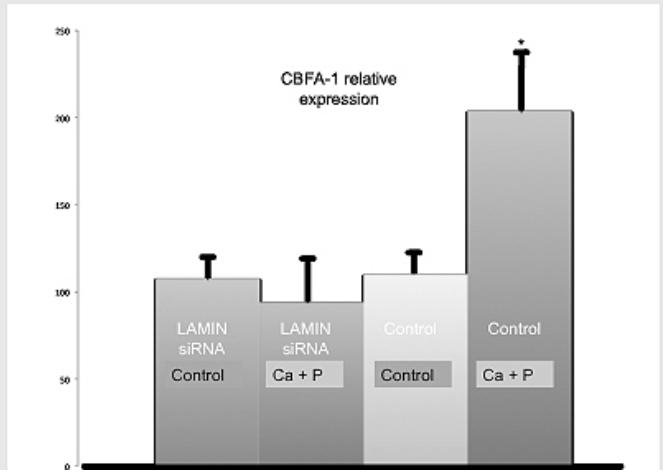
Background: A variable but not negligible proportion of CKD patients seem to be protected to develop vascular calcification; the causes are unknown. Molecular mechanisms modifying the expression of muscle-related or bone-related proteins may explain this advantageous profile. Among them, defects associated to lamin A have been implicated in vascular damage and senescence. The main objective of this study was to compare the differential protein expression in calcified and non-calcified aortas of uremic rats exposed to a strong calcifying stimulus.

Methods: Rats were nephrectomized (7/8) and fed with high P (HP) diet (0.9%) for 20 weeks (n=20); the abdominal aorta was extracted for Von Kossa staining and proteomic analysis. To confirm the results, in vitro studies were performed: Lamin A expression was knocked down in vascular smooth muscle cells (VSMCs) using siRNA, then VSMCs were cultured using normal o calcifying media (2mM Ca and 3mM P) for 2 days. CBFA-1/runx-2 and Lamin A gene expression were measured by qRT-PCR.

Results: The proteomic analysis revealed that the aortas from rats that after 20 weeks with calcifying stimulus did not develop VC, showed a significant upregulation of transgelin, tropomiosin and actin (all well known muscle-related proteins) and significant

downregulation of lamin A. In addition, further experiments in VSMCs, knocking down Lamin A by siRNA showed a reduction in CBFA1 despite the stimulus of the calcifying medium.



Conclusions: The study demonstrates for the first time that muscle related proteins overexpression and Lamin A downregulation could be involved as part of the mechanisms triggered to protect against vascular calcification.

Funding: Government Support - Non-U.S.

SA-PO2256

The Reduction of Vertebral Bone Density (VDB) Is Associated with Progression of Coronary Calcification in Pre-Dialysis Patients Renato Watanabe,[1] Marcelo M. Lemos,[1] Lilian Cuppari,[1] Aluizio B. Carvalho,[1] Carlos Eduardo Rochitte,[2] Raul D. Santos Filho,[2] Maria Eugenia F. Canziani.[1] *[1]Nephrology Division, Federal University of São Paulo, São Paulo, Brazil; [2]Cardiology Division, University of São Paulo, São Paulo, Brazil.*

Background: Vascular calcification and low bone density are common findings in chronic kidney disease (CKD) patients and are associated with mortality in this population. We aimed to investigate whether the reduction of VDB was associated with progression of coronary calcification in pre-dialysis patients.

Methods: In a 24-month prospective study a sample of 72 nondialyzed CKD patients (57.6±10.3years, 62%male, creatinine clearance 39.2±15.5ml/min/1.73m2), underwent multi-slice computed tomography in order to determine coronary calcium score and thoracic VBD. The measurements were performed at baseline and at the end of the study.

Results: At the baseline, coronary calcification was observed in 33 patients (46%) [median 316AU(range 123–861.5AU)-calcified group)]. These patients were older, mostly male and had lower VBD, compared to non-calcified patients. There were no differences between groups regarding renal function, proteinuria, lipid profile, ionized calcium, phosphorus, alkaline phosphatase or iPTH levels. During the follow up, although the mean values of VBD did not change in both groups, VBD decreased in 61% and 48% of the patients (non-calcified and calcified group respectively; p=0.27). Thirty out of 33 (91%) patients of the calcified group showed progression of coronary calcification which was inversely related to the change of eGFR (r=-0.38; p=0.039), and to the relative change of VBD (r=-0.56; p=0.001). In multivariate regression analysis, adjusted for age, sex, diabetes and eGFR, calcification progression was independently associated to the relative change of VBD (p=0.01; βcoefficient=-0.419; 95%CI,-0.036 to-0.004). Despite reduction of eGFR, non-calcified patients have not developed of coronary calcification.

Conclusions: This study showed that reduction of VBD is associated with progression of coronary calcification in calcified pre-dialysis patients but not with the development of coronary calcification in the non-calcified ones.

Funding: Government Support - Non-U.S.

SA-PO2257

A Novel Model of In-Vitro Osteocytogenesis Deborah Mattinzoli,[1] Piergiorgio Messa,[1] Alessandro Corbelli,[1] Masami Ikehata,[1] Cristina Zennaro,[2] Silvia Armelloni,[1] Min Li,[1] Laura Giardino,[1] Maria Pia Rastaldi.[1] *[1]Renal Research Laboratory, Fondazione IRCCS Policlinico & Fondazione D'Amico, Milan, Italy; [2]Renal Physiopathology Laboratory, University of Trieste, Italy.*

Background: Though recent research is more and more highlighting the importance of osteocytes as central players of bone and systemic mineral metabolism, molecular and functional knowledge of osteocyte properties are still incomplete, mostly due to limited availability of in vitro models.

Aim of our study was to find a simple, reproducible method to obtain cultured osteocytes.

Methods: MC3T3-E1 cells were cultivated in α-MEM medium. At 80% confluence, medium was added ascorbic acid/glycerol phosphate (AA/GP) for 5 days, then cells were divided into 3 groups. The 1st group continued in the same conditions (AA/GP). Melatonin (50uM) or All-Trans Retinoic Acid (10uM, ATRA) were respectively added to the 2nd and the 3rd group. Cells were studied from 4 to 25 days.

Morphology was evaluated by light, electron, and atomic force microscopy. Real time RT-PCR arrays were used to compare gene expression between groups. Immunostainings, western blot, and in-cell ELISA were applied to define expression of osteocyte markers. Osteocalcin and FGF23 excretion were determined by ELISA on the supernatant.

Results: Our data show that ATRA, not melatonin, treatment generates a homogeneous population of ramified cells, with distinct features of osteocytes, as we confirm by a detailed morphological and molecular analysis. The phenotype clearly changes as soon as after 4 days of treatment and is completed in 10 days, and consists in the progressive development of ramifications, loss of the ability to produce extracellular matrix, downregulation of osteoblast markers and upregulation of osteocyte-specific molecules, most notably among them sclerostin.

Conclusions: Compared to other published protocols, our method has a number of advantages. It is easy to perform and does not require additional instrumentation, it is highly reproducible, and generates in a ten-day time-frame a mature osteocyte population in complete absence of extracellular matrix, allowing the use of these cells for unlimited biological applications.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2258

Experimental CKD-MBD: A Comparison between 5/6 Nephrectomy and Adenine Models Guaraciaba O. Ferrari,[1] Juliana C. Ferreira,[1] Raquel T. Cavallari,[1] Katia R. Neves,[1] Luciene M. dos Reis,[1] Wagner V. Dominguez,[1] Elizabeth M. Oliveira,[1] Fabiana G. Graciolli,[1] Jutta Passlick-Deetjen,[2] Rosa M. Moyses,[1] Vanda Jorgetti.[1] *[1]Sao Paulo University, Brazil; [2]Fresenius.*

Background: Experimental models are important to understand the physiopathology, as well as the effects of therapy of some diesases. Currently, two models are used to evaluate CKD-MBD: 5/6 nephrectomy (NX) and adenine-induced kidney disease (AD). However, they have never been compared using animals in similar housing conditions. To that end, we compared these two models, focusing on biochemical and bone histomorphometric findings.

Methods: Wistar rats, fed with the same diet, were divided into 3 groups: Control (C), NX and AD(diet + adenine). After 9 wks, animals were sacrificed and biochemical and histomorphometric analyses were performed.

Results: NX group presented a greater final weight (FW) and blood pressure (BP) than did AD group. No differences were seen in serum Creatinine (Cr), phosphate (P), ionized calcium (iCa), PTH and FGF23. However, AD rats had a higher FeP and presented a more severe form of high-turnover bone disease.

Table1

	C	AD	NX
FW(g)	366±19	312±17*	385±60
BP(mmHg)	121(103-131)*	147(131-181)*	228(214-230)
LV(g/100g)	0.19(0.17-0.20)	0.21(0.19-0.24)	0.19(0.15-0.31)
Cr(mg/dl)	0.6±0.2*	1.6±0.3	1.3±0.6
iCa(mmol/l)	1.15±0.08	1.07±0.07	1.10±0.09
P(mg/dl)	5.7±0.9*	7.4±0.7	6.7±1.4
FeP(%)	9.1±4.8	21.2±11.0*	7.6±4.6
PTH(pg/ml)	150(47-681)*	681(163-1,604)	572(197-4,199)
FGF23(pg/ml)	157(106-310)*	784(418-14,476)	893.2(649-2,609)
BV/TV(%)	29.4(20.2-32.9)	32.5(16.0-37.0)	30.5(16.0-37.1)
OS/BS(%)	9.4±5.0	30.1±7.4*	6.9±3.0
ES/BS(%)	14.3±5.7	24.5±6.3*	12.4±5.4
ObS/BS(%)	8.3(1.8-14.8)	25.8(15.4-33.2)*	4.8(4.0-11.3)
OcS/BS(%)	4.0±2.6	7.4±2.8*	3.2±1.6
BFR(μm²/μm³/d)	0.04(0.00-0.11)	0.14(0.00-0.22)*	0.02(0.00-0.03)
MLT(d)	4.1(1.6-8.1)	8.4(1.4-17.7)	4.6(3.4-14.8)

p<0.05; * vs all; FW=final weight; LV=left ventricule; BV/TV=bone volume; OS/BS=osteoid surface(S); ObS/BS=osteoblast S; OcS/BS=osteoclast S; BFR=bone formation rate; MLT=mineralization lag time.

Conclusions: In similar conditions of diet and housing, AD model is associated with a more severe form of bone disease than NX. This should be taken into account when choosing one of these models for future preclinical experiments.

Funding: Government Support - Non-U.S.

SA-PO2259

The Role of a Skeletal Anabolic in the Early CKD-MBD of Stage 2 CKD Keith A. Hruska, Yifu Fang. *Pediatrics, Washington University School of Medicine, St. Louis, MO.*

Background: In CKD, osteoblastic differentiation of cells in the neointima causes calcification of atherosclerotic plaques. Both atherosclerotic and medial vascular calcification (VC) are important causes of CV morbidity in CKD. The early CKD-MBD begins in stage 2 CKD patients as shown by HRqCT (Bacchetta J et al JBMR 2009), increased FGF-23 (Pereira RC et al Bone 2009), and decreases in vascular smooth muscle (VSM) contractile phenotype markers (Kokubo et al, JASN 2009). Abnormalities of bone remodeling and the VSM phenotype before abnormalities of mineral metabolism in the early phases of the CKD-MBD make it necessary to define a regulatory system underlying the condition. Here we tested the hypothesis that CKD induces the CKD-MBD in early CKD and that the skeleton participates in stimulation of VC.

Methods: Ldlr-/- mice fed high fat diets were subjected to renal cortical electrocautery and contralateral nephrectomy at 12 weeks of age to produce CKD-MBD, euthanasia was at 22 or 28 weeks. Treatment with vehicle, DKK1 mab (30 mg/Kg tiw IP), or CaAc (3% w/w mixed in diet) was begun at 14 or 22 wks.

Results: A relatively mild reduction in the glomerular filtration rate was seen with 76% of normal {GFR (ml/min/kg) at 22 weeks of age (stage 2 CKD). BUN, Ca, Pi and PTH levels were normal. CKD stimulated and DKK1 mab inhibited the CKD stimulated

accumulation of aortic Ca levels (0.52±0.19 (CKD) vs. 0.26±0.14 (wt), 0.32±0.23 (sham) and 0.4±0.1 (DKK1 mab) mg/g dry weight, p<0.05). CaAc did not affect CKD stimulated VC. CKD decreased aortic smooth muscle actin and the DKK1 mab had no effect. Micro CT analysis of femurs revealed cortical bone loss prevented by the DKK1 mab. Dickkopf-1 (Dkk1), a circulating inhibitor of bone formation, levels were increased from normal wt 1868±772 or sham 1650±882 to 3132±1590 (pg/ml, p<0.01). Increased serum levels of FGF-23 were also detected but not decreased by the DKK1 mab (554±263 (CKD) vs. 242±56 (wt), 309±64 (sham) and 590±185 (DDK1 mab).

Conclusions: We conclude that the skeleton is affected in response to kidney injury and participates in the stimulation of VC.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2260

Upregulation of Osteocyte Fibroblast Growth Factor 23 with Improvement of the Uremic Mineralization Defect Induced by the Vitamin D Analog Paricalcitol

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Background: It has been shown that $1,25(OH)_2D_3$ stimulates both osteoblast and osteocyte fibroblast growth factor 23 (FGF23) production, but little is known on the skeletal response to FGF23 in chronic kidney disease, particularly following paricalcitol (Pc) treatment.

Methods: We evaluated the bone expression of FGF23 by immunohistochemistry with simultaneous quantitated bone histomorphometry in sham (S) and 5/6-nephrectomyzed rats after treatment during 8 weeks with vehicle (U), Pc (IP 0.3 μg/kg/thrice weekly), or enalapril (E) (5 mg/kg/day in drinking water).

Results: Pc and E attenuated renal insufficiency when compared to U (p<0.05). Plasma calcium (Ca) was higher in Pc and phosphorus (P) was similar in all groups (Table). Bone histomorphometry (Table) revealed decreased eroded surfaces (ES/BS) and improved mineralization after Pc. In contrast, E showed less attenuated OS/BS and ES/BS (p<0.05 vs.Pc). Osteocyte FGF23 expression was markedly increased in U when compared to S or E, and even more prominent following Pc.

Biochemical and Bone Histomorphometric Parameters

	Sham	Uremia	Enalapril	Paricalcitol
Creatinine (mg/dL)	0.58 ± 0.08	1.67 ± 0.75*	0.76 ± 0.06**	0.76 ± 0.1**
Phosphorus (mg/dL)	6.72 ± 0.49	7.31 ± 1.89	6.34 ± 1.7	6.46 ± 1.83
Calcium (mg/dL)	7.34 ± 0.28	8.58 ± 0.78	7.68 ± 0.60	9.9 ± 1.74• ♦
OV/TV %	1.78 ± 0.35	3.5 ± 1.9	2.0 ± 0.2	2.65 ± 2.3
OS/BS %	6.36 ± 0.57	15.4 ± 8.1†	8.5 ± 2.8	7.8 ± 3.8
ES/BS %	13.1± 2.0	22.1± 7.9¶	24± 2.6¶	16± 4∞

*p<0.01 vs. S; **p<0.05 vs. U; • p<0.05 vs. En and ♦ p<0.01 vs. sham; † p<0.01 vs sham and p< 0.05 vs Pc; ¶ p< 0.05 vs S; ∞ p<, 0.05 vs En

Conclusions: Pc therapy upregulated bone FGF23 expression and partially improved the mineralization defect without changes in P levels, suggesting that FGF23 in addition to Pc modulates bone mineralization in uremia.

Funding: Clinical Revenue Support

SA-PO2261

Plasma Serotonin Affects Bone Metabolism in Hemodialysis Patients

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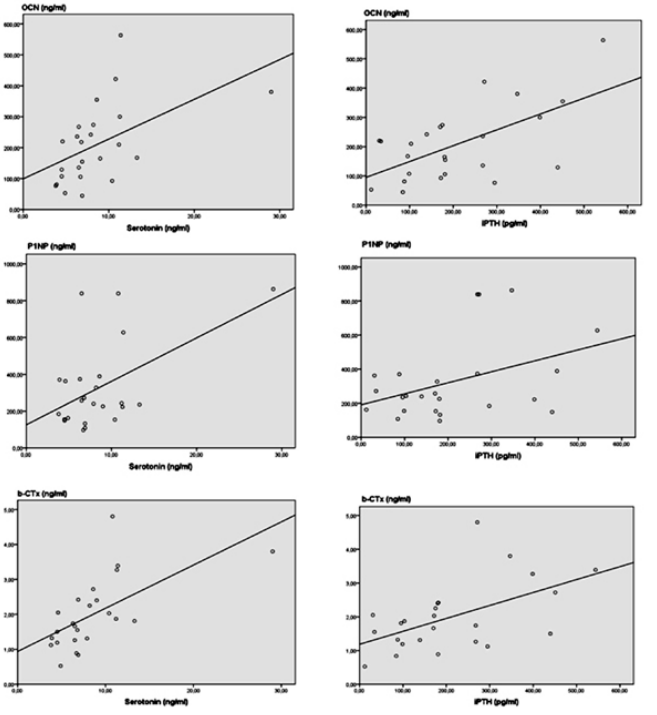
Background: Serotonin receptors are present in osteoblasts and osteoclasts and many experimental studies showed that peripheral serotonin affects bone metabolism. In the present study the effect of plasma serotonin on bone metabolism was evaluated in hemodialysis (HD) patients.

Methods: 24 HD patients (11 diabetics) and 22 healthy volunteers enrolled into the study. Serotonin was assessed in platelet free plasma, whereas the markers of osteoblastic activity N-MID osteocalcin (OCN) and total procollagen type-1 amino-terminal propeptide (P1NP) as well as the marker of osteoclastic activity beta-isomerized C-terminal cross-linked peptide of collagen type I (β-CTx) were measured in serum. Serum intact parathyroid hormone (iPTH) was also assessed.

Results: Serotonin did not differ significantly between HD patients and healthy volunteers (8.50±5.12 ng/ml vs 6.44±1.54 ng/ml, p=n.s.). All evaluated markers of bone metabolism and iPTH were much higher in HD patients. Serotonin was positively related to all evaluated markers of bone metabolism in HD patients, and independently of iPTH, which was also positively related to all evaluated markers of bone metabolism.



Serotonin was negatively related to the patients' age. Serotonin, was much lower in diabetic HD patients (6.42±1.99 ng/ml vs 10.25±6.30 ng/ml, p=0.044), which was the case for OCN, P1NP and β-CTx as well.

Conclusions: Serotonin increases both bone formation and bone resorption in HD patients. The negative relation of serotonin to patients' age as well as its lower levels in diabetic HD patients indicate that low plasma serotonin may contribute to the higher incidence of low-turnover bone disease that characterizes old and diabetic HD patients.

SA-PO2262

Sevelamer Hydrochloride Inhibits Aortic Calcification Regardless of Serum Phosphorus Concentrations

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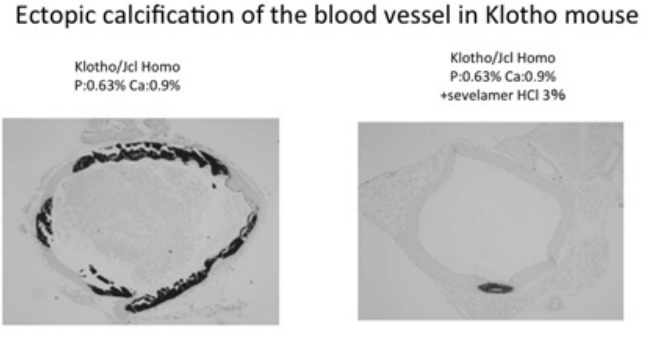
Background: Sevelamer hydrochloride, calcium-free phosphate binder, reduces vascular calcification by preventing episodes of hypercalcaemia and/or hyperphosphatemia in the patients with the CKD. However, the mechanism of this effect on vascular calcification is quite obscure. Pleiotropic effects of sevelamer hydrochloride have been focused. Hyperphosphatemia, high serum FGF23 levels as well as vascular calcification are observed in the patients with the CKD, which were also characteristics of klotho mice. Therefore we examined the pathophysiology of the influences of sevelamer hydrochloride on vascular calcification using klotho mice.

Methods: Klotho mice and control mice(C57BL/jj6) were fed by normal diet group(NPD: Pi 0.6 %) , or low-Pi diet group(LPD : Pi 0.2 %), or NPD with Sevelamer hydrochloride respectively. After 1 week, all mice were sacrificed, then, biochemical analysis of serum and urine was carried out.

Results: Klotho mouse had decreased serum phosphorus concentrations by LPD intake. At the NPD intake with sevelamer hydrochloride administration, in klotho mice, serum phosphorus concentrations decreased similar to LPD. On the other hand, the aortic calcification of klotho mice was inhibited by sevelamer hydrochloride administration although that was not inhibited by a LPD intake.



Conclusions: In our study, sevelamer hydrochloride inhibited aortic calcification regardless of serum phosphorus concentrations. These results might be associated with the pleiotropic effects of sevelamer hydrochloride.

Funding: Other U.S. Government Support

SA-PO2263

Interaction between RANK/RANKL Positive Macrophages Infiltrating around the Amyloid Deposit in the Yellow Ligament and the Oosteoclasts in Destructive Spondyloarthropathy Patients

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Background: Destructive spondyloarthropathy (DSA) is one of the symptoms of dialysis-related amyloidosis and is reported to be observed as a complication in approximately 10% of long-term hemodialysis patients. Histological examination shows β2-microglobulin amyloidal deposits in the intervertebral disk, the synovium of apophyseal joints, and the ligaments, however, the precise mechanism in the development of the destructive change of the bone is still unknown. Hence the aim of the study is to clarify the mechanism of the destruction of the vertebrae, focusing on the macrophages infiltrating around the amyloid deposits, and the cytokines and RANK-RANKL system.

Methods: Yellow ligament of the cervical or lumbar vertebrae in the DSA patients were extracted and collected when the posterior spinal fusions were conducted in each case. These yellow ligaments were fixed by 10% formalin, embedded in paraffin. Immunohistochemical study was performed on 5 μm paraffin section to examine the expression of RANK, RANKL, CD68, IL-1β, IL-6, TNF-α, and IL-17. THP-1 cells, monocyte/macrophage cell line, were cultured with various conditions to investigate the induction o RANK and RANKL.

Results: Histological examination revealed that CD68 positive monocyte/macrophage lineage cells infiltrating around the amyloidal deposit expressed both RANK and RANKL. IL-1β, IL-6, and TNF-α were not detected in the CD68 positive cells, nor were the IL-17 producing T cells either. THP-1 cells were cultured with lipopolysaccharide in vitro, and the up-regulation of both RANK and RANKL expression on their cell surface were detected by flow cytometry.

Conclusions: In this study we reported the possibility that monocyte/macrophage lineage cells would be activated by lipopolysaccharide resulting in the expression of RANK and RANKL on their cell surface and facilitate osteoclastgenesis.

SA-PO2264

Alterations in Osteoblastic Gene Expression in Dialysis Patients: A Potential Role in Skeletal Mineralization

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Background: Abnormal skeletal mineralization is common in pediatric CKD. The mechanisms underlying this defect are unknown but osteocyte dysfunction has been characterized in patients with early CKD.

Methods: Primary osteoblasts were cultured from bone biopsies obtained from 6 pediatric PD patients (5M, 1F), age 16.6 ± 1.0 years, not receiving vitamin D sterols for at least 4 weeks prior to patients bone biopsy with varying types of bone histology. Expression of osteoblastic and osteocytic gene expression was evaluated at passage 0 by qPCR and normalized by endogenous GAPDH expression in each sample. Expression in dialysis patients was then normalized by normal control. Bone histomorphometry was measured and biochemical values obtained in all subjects.

Results: Biochemical variables were: Ca: 8.3 ± 0.5 mg/dl, P: 7.2 ± 0.6 mg/dl, Alk P'tase: 289 ± 90 IU/l, PTH: 595 ± 231 pg/ml, FGF-23: 491 (436, 919) RU/ml.

Parameter	Value	Normal Range
Bone Histomorphometry		
Bone volume (BV/TV) (%)	31.9 ± 3.0	8.9 – 34.4
Bone formation rate (BFR/BS) ($um^2/mm^3/d$)	43.4 ± 19.0	8.0 – 73.4
Osteoid volume (OV/BV) (%)	5.2 ± 2.0	0.2 – 5.8
Osteoid surface (OS/BS) (%)	31.0 ± 9.3	4.3 – 37.0
Osteoid thickness (O.Th) (um)	8.0 ± 1.5	2.0 – 13.2
Osteoid maturation time (OMT) (d)	13.8 ± 1.7	1.2 – 11.5
Mineralization lag time (MLT) (d)	58.7 ± 20.3	2.3 – 63.8
Gene (expressed as fold increase from control)		
Alkaline phosphatase	3.53 ± 1.12	
Type I Collagen	1.43 ± 0.06	
FGF receptor 1	1.90 ± 0.11	
PTH receptor	5.04 ± 1.11	
NHERF1	2.31 ± 0.32	
IGF1	7.26 ± 1.71	
VDR	3.23 ± 0.27	
CYP27	1.25 ± 0.20	
CYP24	2.87 ± 1.07	
Osteocalcin	1.14 ± 0.38	
RUNX	3.53 ± 1.12	
Osterix	6.21 ± 2.2	
Matrix gla protein	2.33 ± 0.40	
FGF23	Undetectable	
CaSR	Undetectable	

Overall, mineralization parameters on histomorphometry were within the normal range. As evidenced by bone alk P'tase, osteocalcin and type I collagen expression and lack of DMP1, FGF23, MEPE and sclerostin, cells cultured from bone biopsy specimens retain osteoblast characteristics. Compared to control, Alk P'tase, PTHR1, NHERF1, IGF1, VDR, BMP2, RUNX, osterix, and matrix gla expression are increased in dialysis patients, suggesting an intrinsic upregulation of factors involved in the mineralization process.

Conclusions: The upregulation of pro-mineralization genes in osteoblasts may be required in order to maintain normal mineralization parameters in dialysis patients. Whether these factors vary according to subtype of renal osteodystrophy and how they relate to matrix mineralization remain to be evaluated.

Funding: NIDDK Support, Private Foundation Support

SA-PO2265

Bisphosphonates Exert Skeletal Benefits without Severe Suppression of Turnover in an Animal Model of Chronic Kidney Disease

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Background: Patients with chronic kidney disease (CKD) have increased fracture rates. Bisphosphonates are routinely used to reduce fractures in non-CDK patients, but limited data exists concerning their efficacy in CKD. There is also concern that bisphosphonates will induce adynamic bone disease due to severe suppression of bone remodeling in patients with CKD. The goal of this study was to test the hypothesis that zoledronic acid (ZOL) exhibited similar skeletal effects in normal and CKD animals.

Methods: AAt 25 weeks of age, normal rats (NL) were treated with saline or 100 mcg/kg of ZOL while animals with CKD (Cy, GFR of approx 30-40 ml/min) were treated with a single dose of saline, low dose ZOL (20 mcg/kg), or high dose ZOL (100 mcg/kg). Skeletal properties were assessed 5 weeks later using micro-computed tomography (CT), dynamic histomorphometry, and mechanical testing.

Results: Trabecular bone remodeling was 92% lower in NL animals treated with ZOL compared to untreated animals. Cy animals treated with low and high doses of ZOL were 80% lower than untreated Cy animals and were higher than NL animals treated with ZOL. By CT, trabecular bone volume in the proximal tibia was significantly higher in Cy rats treated with low (+73%) and high (+93%) doses of ZOL compared to untreated Cy rats. Mechanical testing showed that cortical bone strength (-28%) and energy absorption (-37%) were significantly lower in untreated Cy rats compared to NL; these effects were partially normalized by ZOL treatment. There were no significant differences between the two ZOL doses in Cy rats for any histomorphometric outcome parameter.

Conclusions: Based on these results we conclude ZOL improves bone volume and strength in the absence of severe remodeling suppression, and thus provides bone protective effects in animals with advanced CKD.

Funding: Other NIH Support - NIAMS

SA-PO2266

The Effects of Phosphate Binders on the Wnt Pathway Gene Expression in Adynamic Bone Disease

Juliana C. Ferreira,[1] Guaraciaba O. Ferrari,[1] Raquel T. Cavallari,[1] Wagner V. Dominguez,[1] Elizabeth M. Oliveira,[1] Luciene M. dos Reis,[1] Fabiana G. Graciolli,[1] Katia R. Neves,[1] Shiguang Liu,[2] Yves Sabbagh,[2] Vanda Jorgetti,[1] Susan Schiavi,[2] Rosa M.A. Moyses.[1] *[1]Nephrology Division, Universidade de São Paulo, Brazil; [2]Genzyme Co.*

Background: The Wnt pathway is involved in bone formation, and high serum levels of sclerostin (SOST), a Wnt inhibitor, are associated with osteoporosis and adynamic bone disease (ABD). In this study, we tested the hypothesis that phosphate (P) binders could modulate gene expression of SOST in an experimental model of ABD.

Methods: Nephrectomized (Nx) rats were divided into 4 groups: Ca (calcium carbonate therapy); Sev (sevelamer carbonate therapy); CKD (untreated); and Control. After 8 wks, biochemical, histomorphometric and bone gene expression (TLDA) analyses were performed.

Results: Histomorphometry confirmed ABD in Nx groups, based on decreased bone formation rate, osteoid volume, osteoblast and osteoclast surfaces without fibrosis. We found no differences among the CKD groups in terms of bone histomorphometric parameters, except for greater eroded surface in the Ca group. The gene expression of SOST was increased in CKD and only Sev administration was able to decrease it significantly.

Biochemical and histomorphometric data

	Ca	Sev	CKD	Control
Creatinine(mg/dl)	1.65±1.01	1.33±0.35	1.29±0.23	0.61±0.10[a]
iCa(mmol/L)	0.60±0.14	0.49±0.16	0.55±0.19	1.15±0.09[a]
P(mg/dl)	10.7±2.4	11.3±2.9	11.1±2.7	5.4±0.4[a]
FeP(%)	4.4±7.6[a]	25.1±11.5[a]	49.2±21.2[a]	6.9±3.4[a]
FGF-23(pg/ml)	271±177	206±183	144±108	279±95
BV/TV(%)	29.1±6.4	25.1±7.5	24.0±6.1	22.3±5.7
OS/BS(%)	2.7±2.3	5.7±6.3	2.1±0.8	17.6±8.3[a]
ES/BS(%)	10.4±2.8[c,d]	6.9±1.3	5.7±2.9	19.1±3.0[a]
Ob.S/BS(%)	2.4±2.0	4.1±4.3	1.7±0.7	14.7±7.2[a]
BFR/BS(μ^3/μ^2/day)	0.01±0.01	0.03±0.03	0.02± 0.01	0.08±0.04[a]
SOST(fold change)	3.9±0.1	2.3±0.5[d]	4.1±0.3	1.2±0.4

iCa: ionized calcium; BV/TV: trabecular bone volume; OS/BS: osteoid surface (S); ES/BS: eroded S; Ob.S/BS: osteoblast S; Oc.S/BS: osteoclast S; BFR/BS: bone formation rate; a=p< 0.05 vs. all; b=p<0.05 vs. Ca; c=p<0.05 vs. Sev; d=p<0.05 vs. CKD

Conclusions: Gene expression of SOST is elevated in ABD and is decreased with Sevelamer. Longer treatment periods may be required to observe improve in histomorphometric parameters.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

SA-PO2267

The Expression of Runx2 and Col II in Arteries of Remnant Kidney Rats and Patients with Chronic Renal Failure Yi Yu. *Dept of Nephrology and Hemodialysis, Dongfang Hospital of Fujian Province, Fuzhou, Fujian, China.*

Background: This study is aimed to observe the expression of Runx2 and collagen II (Col II) involved in vascular calcification in remnant kidney rats and patients with chronic renal failure (CRF).

Methods: 1. SD rats underwent 5/6 nephrectomy or sham operation, and rats were fed with high or normal phosphorous diet for 16weeks. They were divided into 4 groups: (1) remnant kidney rats receiving high phosphorous diet (NHP group, n=10); (2) remnant kidney rats receiving normal phosphorous diet (NP group, n=10); (3) sham operation rats receiving normal phosphorous diet (SP group, n=10); (4) sham operation rats receiving high phosphorous diet (SHP group, n=10). At the 16th week, Scr, P, LDL were examined and thoracic aorta was removed. 2. Pieces of radial arteries were taken from 40 CRF patients at the time of arteriovenous fistula operation, while 10 subtotal gastrectomy patients with normal renal function were chosen as control. Serum Ca, P and iPTH were detected. The vessels were examined for calcification by von Kossa stain and for the expression of Runx2 and Col II by immunohistochemistry, RT-PCR or Western Blot.

Results: 1. After 16 weeks, Scr were higher in 5/6 nephrectomy rats, and serum P increased significantly in NHP group ($P< 0.05$). 2. Overt vascular calcification was found in NHP group while NP and SHP group occasionally had vascular calcification; SP group had none. 3. The expression of Runx2 and Col II increased in NHP group ($P<0.05$) by immunohistochemistry. In NHP group, the aorta mRNA expression of Runx2 and Col II, and the aorta protein expression of Runx2 increased significantly ($P<0.05$). 4. In CRF patients, positive staining of Runx2 and Col II was found in the smooth muscular cell plasma of medial layer in the vessels with overt calcification, and was also observed in 78.3% of no calcification group. Serum P was positively correlated with staining score of Runx2 and Col II ($P<0.01$).

Conclusions: Serum P, Runx2 and Col II are involved in the vascular calcification in remnant kidney rats and CRF patients. The incidence of arterial calcification is high in CRF patients; the arterial expression of Runx2 and Col II is an early sign of calcification.

SA-PO2268

No Modifications in Clinical Outcomes after Availability of Guidelines for Mineral Metabolism in Dialysis Patients Walter Guillermo Douthat,[1,2] Carlos R. Chiurchiu,[1,2] Javier De Arteaga,[1,2] Jorge Luis De la Fuente,[1,2] Pablo U. Massari.[1,2] *[1]Renal Service, Hospital Privado-Centro Medico de Cordoba, Cordoba, Argentina; [2]Posgraduate School of Nephrology, Catholic University of Cordoba, Cordoba, Argentina.*

Background: In recent years new guidelines for bone and mineral metabolism (BM) management for patients with ESRD were published, but unfortunately their availability not necessarily guarantees better outcomes.

Methods: We performed a survey that included 1210 patients belonging to 25 dialysis centers, to analyzed witch guide was used, percentage of patients that achieve major BM outcomes and treatment used. Results were compared with a similar survey performed during pre-guide K/DIGO era (2001).

Results: Guidelines used were Local (Argentinian) 68,1%, K/DOQI 33,9%, K/DIGO 21,6%, and in 6,1% cases not guide was used. Comparing results between 2001 to 2010, no difference was found in serum phosphate levels, but an increase in iPTH ($p<0.0001$), and a decrease in mean s.calcium ($p<0.0001$), and CaxP product ($p<0.02$) were observed. Percentage of patients that reach K/DOQI outcomes in 2010 compared to 2001 were: phosphorous 52 vs 51%, calcium 52 vs 43%, CaxP product 77 vs 73%, and iPTH 21 vs 28% respectively. For K/DIGO (iPTH 2 to 9 times up normal levels) 47,3% vs 58% in 2010 vs 2001 respectively. Percentage of patients who achieve any of 4 outcomes of K/DOQI were 9,5% vs 8,4%, and reach all 4 outcomes 5,9 vs 6,6% for 2010 vs 2001.

Treatment of hyperparathyroidism used in 2010 vs 2001 were: parathyroidectomy 8,6 vs 6,1%; oral calcitriol 40,8 vs 71,3%, e.v.calcitriol 5,2 vs 3,3%; paricalcitol 2,1 vs 0%, and doxercalciferol 0,9 vs 0%.Oral phosphate chelators were: calcium salts 86,6 vs 94,8%, aluminium salts 4,0 vs 4,4% and sevelamer 5,6 vs 0% respectively.

Conclusions: In our population during the last decade, no changes were observed in outcomes and procedures despite availability of new BM guidelines.

SA-PO2269

The Impact of Daily Activity and Muscle Strength on Fractures in CKD Sarah West,[1,3] Sophie Jamal,[1,3] A. M. Cheung,[1,2] Charmaine E. Lok.[2,3] *[1]University of Toronto, ON, Canada; [2]Toronto General Hospital, Toronto, ON, Canada; [3]Women's College Hospital, Toronto, ON, Canada.*

Background: Fractures are common in patients with CKD. Tests of neuromuscular function (NMT) discriminate well among fractured and non-fractured patients on dialysis. The ability of daily activity and NMT to discriminate among fracture status in patients with CKDIII-V(non-dialysis) is unknown.

Methods: Baseline data from an ongoing prospective study of adult patients with CKDIII-V were used to determine if NMT (the timed up and go test [TUG] and 6 minute walk test [6MW]), and/or daily activity (sedentary, light, or moderate/vigorous activity measured by triaxial accelerometry [StayHealthy, RT3]) could discriminate among those with and without fractures (self-reported low trauma fractures >40 yrs and/or prevalent vertebral fractures identified by morphometry). Results are expressed as areas under the receiver operating characteristic curves (AUROC) with 95% confidence intervals (CI), adjusted for age and weight.

Results: Of 128 men and 86 women studied, 32 completed accelerometry. Compared to non-fractured patients (n=143), those with fractures (n=70) were older (59±16 vs. 69±14 yrs, $p<0.001$), had a reduced 6MW distance (335.5±87.1 vs. 289.9±142.2 m, $p=0.04$), were more sedentary with light daily activity (min/day, $p<0.05$). No other demographic factors distinguished those with and without fractures. The mean weight of patients was 79.9±19.8 kg, most were Caucasian (70.0%), > 40% had diabetes, and 74% patients were CKD stage 4 or 5. The mean duration of CKD was 8.0±10.3 yrs, and 30.2% reported a fall in the past 12 mos. The TUG (mean 12.3±5.7 sec) was able to discriminate among those with and without fractures (AUROC: 0.70 [95% CI: 0.61-0.80]), as was the 6MW test with an AUROC: 0.69 (95% CI: 0.59-0.80). By accelerometry, sedentary activity (min/day) was able to discriminate fracture status (AUROC: 0.71 [95% CI: 0.51-0.93]), as was light activity (min/day), with an AUROC of 0.76 (95% CI: 0.56-0.95).

Conclusions: NMT and accelerometry are simple, practical measures that can discriminate among CKD patients with and without fractures. Their ability to predict future fractures require further study.

Funding: Government Support - Non-U.S.

SA-PO2270

Bone and Mineral Metabolism in the Course of Secondary Hyperparathyroidism Therapy with Cinacalcet Zbigniew Nowak, Daniel Baczynski, Grzegorz Kade, Zofia Wakowicz, Stanislaw Niemczyk. *Department of Nephrology, Military Institute of Medicine, Warsaw, Poland.*

Background: The evaluation of secondary hyperparathyroidism (sHPT) in everyday practice is based on non-invasive measurements of bone markers such as iPTH, calcium, phosphate. Tartrate-resistant acid phosphatase-TRAP5b is expressed in bone resorbing osteoclasts and has been considered as a useful marker of bone resorption in uremic patients. There are no date related to the usefulness of this marker in the monitoring of sHPT therapy with calcimimetics.

The purpose of our study was to determine the influence of cinacalcet on markers such as TRAP5b, iPTH, Ca, P.

Methods: We studied group of 32 patients with sHPT with PTH> 500 pg/ml(mean 757+/-264pg/ml) (F=14, M=18; peritoneal dialysis 6 patients and HD 26 patients; mean age 66±17). We planned 6 month cinacalcet therapy and 6 month observation without cinacalcet therapy. There were 2 subgroups of patients with sHPT: groupA (n=18) treated with cinacalcet and group B (n=14) trated with traditional methods. The following parameters were determined in serum: iPTH, Ca, P, and tartrate resistant acid phosphatase TRACP5b as a marker of bone resorption. TRACP 5b activity was measured using assay Bone TRAP™ (SBA Finland). These parameters were measured before start of cinacalcet therapy and after 3rd and 6 st month of therapy and after 3rd and 6 st month without cinacalcet therapy.

Results: Patients treated with cinacalcet (group A) had significant lower values of iPTH and TRAP 5b after 3rd and 6st month of therapy in comparison to group B . We observed 66% reduction of iPTH level (from 816±295pg/ml to 278±149pg/ml at 6st month) and 76% reduction of TRAP 5b level(5.6±2,8 to 1,3± 0,5U/L). After stooping cinacalcet therapy we observed significant increase of iPTH (from 266±142pg/ml to 683±292pg/ml) and TRACP 5b (1,3± 0,5U/L to 4,9±1,8U/L). We did not found any significant difference Ca and P values in both groups.

Conclusions: Treatment of sHPT with Cinacalcet in dialysed patients was effective and allowed the significant reductions of iPTH and TRACP 5b levels. Monitoring of TRACP5b might be useful in assessment of bone resorption in the course of cinacalcet treatment.

SA-PO2271

Changes in Serum Phosphorus and Calcium Predict Parathyroid Hormone (PTH) Over-Suppression in Hemodialysis (HD) Patients (pts) Neal Mittman, Swapna Vemulapalli, Brinda Desiraju, Morrell M. Avram. *Avram Division of Nephrology, S.U.N.Y. Downstate University Hospital at L.I.C.H., Brooklyn, NY.*

Background: Secondary hyperparathyroidism is associated with significant morbidity in HD pts. K/DOQI guidelines recommend a therapeutic target for intact PTH (iPTH) between 150-300 pg/ml. Over-aggressive treatment, especially with vitamin D analogs, risks PTH over-suppression and adynamic bone disease (ABD), a low bone turnover state associated with hypercalcemia which has become the most prevalent bone histology. ABD has been associated with increased risk of fractures, cardiovascular calcification and mortality, and requires prompt modification of treatment regimen. iPTH levels are most commonly tested quarterly, potentially delaying identification of ABD.

Methods: Over a period of two years in our urban dialysis center, we found 93 HD pts with quarterly iPTH values both above and below 150 pg/ml on stable doses of paricalcitol (mean dose 5.7±3.5 µg TIW, range 1-20), on whom we collected demographic and biochemical data.

Results: Mean age was 65 yr (range, 22-90). Fifty-four percent were men, and the majority were of African descent (80%). Sixty-one percent were diabetic. Dialysis vintage was 55 months. The results are shown in the Table.

	First sample	Second sample	p
Intact PTH (pg/ml)	327±177	90±40	<0.0001
Calcium (mg/dl)	9.29±0.65	9.93±0.66	<0.0001
Phosphorus (mg/dl)	5.28±1.46	4.60±1.60	<0.0001
CaxP product	49.4±14	45.8±16.8	0.012
Alkaline Phosphatase (U/l)	95.9±68	85.7±45	0.039

PTH=Parathyroid hormone; Calcium was corrected for albumin

PTH over-suppression (327 ± 177 pg/mL to 90 ± 39.6; p<0.0001) was associated with significant elevations in corrected serum calcium (9.29 ± 0.65 mg/dL to 9.93 ± 0.66; p<0.0001) and reductions in alkaline phosphatase (95.9 ± 68 µg/L to 85.7± 45; p= 0.039), as expected. In addition, we found highly significant reductions in serum phosphorus (5.28 ± 1.46 mg/dL to 4.60 ± 1.60; p<0.0001) and in calcium-phosphorus product (49.4 ± 14 to 45.8 ± 16.8, p= 0.012).

Conclusions: In conclusion, falling monthly values of serum phosphorus associated with rising serum calcium may portend the onset of PTH over-suppression, leading to earlier recognition and intervention for ABD.

Funding: Private Foundation Support

SA-PO2272

Normal Serum Phosphate Levels Associated to Carotid Atheromatosis in Chronic Kidney Disease Stages 2-5 Secundino Cigarran,[1] Emilio E. Gonzalez-Parra,[2] Guillermina Barril,[3] Juan J. Carrero.[4] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Fundación Jimenez Díaz, Madrid, Spain; [3]Nephrology, Hospital Universitario de la Princesa, Madrid, Spain; [4]Division of Renal Medicine, Karolinska Institutet, Stockholm, Stockholm, Sweden.*

Background: Atherosclerotic cardiovascular disease is a significant cause of morbidity and mortality in patients with chronic kidney disease (CKD). Phosphate (P) levels are consistently linked with cardiiovascular events and death in chronic kidney disease (CKD) patients and in general population. Fibroblast growth factor 23 (FGF23) is involved in P homeostasis at early stages of CKD and in atheromatosis process.

Methods: We analyze, in cross-sectional study, the role between P levels and carotid atheromatosis in CKD patients stage 2-5. 426 pts with CKD stage 2-5 (mean age 68.08 ± 12.5 yr, 31.1% women, 27.7% diabetic status, GFR-MDRD 51.62± 23.69 ml/min/1.73m^2) were included. Carotid atheromatosis diagnosed by high-resolution B-mode ultrasonography (USBM) and body composition assessment was performed by whole tetrapolar bioelectrical vectorial impedance analysis (BIVA) (EFG, Akern Firenza Italy). Biochemical nutrition, inflammation & mineral bone disease markers were analyzed. ABI test was performed to 56 pts.

Results: 313 (74.4%) had CA and compared with non CA were older (68.66±10.1vs 54.34 ±10.5 years, P<0.001), male gender (65% vs 49% p<0.05).

T paired Test

Variable	Non CA (N=113)	CA (N=313)	P
Na-k Exchange	.96±.13	1.05±.18	.000
ECW (%)	47.47±4.45	49.59±4.82	.000
Phase Angle (º)	5.70±.87	5.30±.91	.000
C Reactive Protein (mg/dl)	.52±.50	.68±1.13	.020
Serum P (mg/dl)	3.35±.55	3.47±.57	.000
P excrertion Index	.79±.34	.94±.39	.000
ABI	.86±.09	1.03±.23	.000

CA=Carotid Atheromatosis; Non CA =Non Carotid Atheromatosis

Conclusions: CA pts are older, lower GFR , diabetic and cellular damage expresed by increased Na-K exchange, lower PA, increased ECW, C reactive protein, serum P and P excretion index. The latter indirectly assess the FGF23 activation, and both of them are directly involved in vascular calcification process. Further studies are required to clarify if P levels and P excretion index may be useful markers for cardiovascular risk in CKD and nonCKD populations.

Funding: Other NIH Support - SERGAS

SA-PO2273

Ionized Calcium and Mortality in Chronic Hemodialysis Jean-Christophe Szelag, Walid Arkouche, Ignace Mpio, Alejandra Lenz, Carlos Cardozo, Elias Abdullah, Nouredine Boumendjel, Denis Fouque, Maurice Laville. *AURAL Lyon.*

Background: Previous studies have emphasized the noxious effects of high serum calcium and its variations on HD-subjects risk of death. Ionized calcium (Ca++) variations are independent of inflammatory, nutritional and hydratation statuses which are other prognosis factors.

Methods: The studied population consisted in 197 subjects (76 women, 121 men, median age 61,2,diabetes=50)on hemodialysis for more than 3 months. The mean follow-up was 30 months (2-39 months). Ca++ was measured in optimal conditions two hours after sampling. We used a single concentration value in 35 subjects and a three month averaged-concentration (3 to 5 samplings) in 162 subjects. The data are expressed as mean+/-SD, median.

Results: Forty patients died during the follow-up. A survival benefit was observed with concentration under 1,14 mol/L (ph 7,40)(low Ca++ group: 65 subjects, 1,08+/- 0,08, 1,10 mol/L , total calcium concentration 2,11+/-0,20, 2,125 mol/L vs. higher Ca++ group: 132 subjects, 1,21+/-0,05, 1,20 mmol/L, (p=0,03), total calcium concentration 2,24+/-0,14, 2,225 mmol/L, (p=0,004)) (log rank test, p=0,0082). Serum phosphorus (1,66+/-0,54, 1,59 mmol/L vs 1,65+/-0,77, 1,53 mmol/L, p=0,48), albuminemia (39,5+/-3, 39,55 vs 40,1+/-2,9, 40,1 g/L), inflammatory status (usCRP, 11,09+/-15,3, 5,35 vs 10,7+/-13,5, 5,23 mg/L) were the same in both groups. iPTH was significantly higher in low Ca++ group (622,4+/-616, 409 pg/ml vs 396+/-369, 285 pg/ml, p=0,01. Active vitamin D prescription was the same in both groups (Low Ca++ 60%, Higher 49,2%, p=0,07), whereas subjects with low Ca++ were more often treated with cinacalcet (27,6 vs 16,6%, p=0,03). Ionized calcium above 1,14 mmol/L was an independent risk factor for death in univariate Cox model (p=0,015, HR=2,9, 95%CI 1,2-7), case-mix (diabetes, dialysis vintage, age and sexe) (p=0,0308, HR=2,6, 95%CI 1-6,37) and multivariate model (inflammatory status, albuminemia, serum phosphorus and iPTH) (p=0,0227, HR=3 , 95% CI 1,17-8,2)

Conclusions: Our small scale, retrospective and non time-dependent study found a survival benefit with Ca++ under 1,14mmol/L in HD-subjects with higher PTH level and cinacalcet treatment.

SA-PO2274

The Relationship between Prevalent Cardiovascular Events, Hip Fractures and Dialysate Calcium Concentration, Stratified by Predialysis Serum Calcium Concentration in Japanese Hemodialysis Patients Shinichi Sueta,[1] Seiji Hashimoto,[2] Satoshi Ogata,[2] Miho Tagawa.[1] *[1]Nephrology, Kyoto Katsura Hospital, Kyoto, Japan; [2]Patient Registration Committee, Japanese Society for Dialysis Therapy, Tokyo, Japan.*

Background: Previous studies showed that higher dialysate calcium (Ca) concentration is associated with higher mortality. However, it is not known whether predialysis serum Ca concentration has effects on the association.

Methods: This is a cross sectional study using the database of the Japan Renal Data Registry in 2008. Predictor variable was dialysate Ca concentration (dialysate Ca ≤ 2.5 mEq/L, dialysate Ca ≥ 3.0 mEq/L). Outcome variables were prevalence of myocardial infarction (MI), cerebral hemorrhage (CH), cerebral infarction (CI), amputation, and hip fracture. Multivariable logistic regression model was employed adjusting for 15 covariates. Stratified analysis was performed by tertiles of predialysis serum corrected Ca (corrected Ca: 8.9mg/dl, 9.0-9.5, 9.6mg/dl).

Results: Of total 271,510 patients in 2008, we used complete data set of 91,533 patients.

Odds ratios (95 % CI) for higher dialysate Ca user of having prevalent cardiovascular events or hip fractureAssociation of higher dialysate Ca and prevalence of cardiovascular events or hip fracture compared with lower dialysate Ca user as a reference.

Tertiles of predialysis corrected Ca	≤8.9	9.0-9.5	≥9.6
MI	0.917(0.844-0.997)	1.063(0.967-1.168)	0.902(0.820-0.993)
CH	0.881(0.785-0.989)	1.003(0.890-1.130)	0.898(0.805-1.003)
CI	0.878(0.822-0.937)	1.008(0.939-1.081)	0.987(0.921-1.059)
Amputation	0.879(0.760-1.017)	0.920(0.783-1.080)	0.921(0.791-1.072)
Hip fracture	0.773(0.668-0.895)	0.935(0.801-1.092)	0.804(0.695-0.930)

Data shown as odds ratio

Conclusions: Higher dialysate Ca concentration was significantly associated with lower prevalence of MI and hip fracture in lowest and highest tertiles of predialysis corrected Ca concentration. It was also significantly associated with lower prevalence of CH and CI in lowest tertile of predialysis albumin corrected Ca concentration. Longitudinal studies of incident hard outcomes are required to delineate the appropriate choice of dialysate Ca concentration based on predialysis serum calcium levels.

SA-PO2275

Increased Risk of Hip Fracture among Hemodialysis Patients Masks the Influence of Ethnic Difference Minako Wakasugi,[1] Junichiro J. Kazama,[1, 2] Masatomo Taniguchi,[2] Atsushi Wada,[2] Kunitoshi Iseki,[2] Yoshiharu Tsubakihara,[2] Ichiei Narita.[1] *[1]Division of Clinical Nephrology and Rheumatology, Niigata University, Niigata, Japan; [2]Renal Data Registry Committee, Japanese Society for Dialysis Therapy, Bunkyo, Tokyo, Japan.*

Background: Patients with end-stage renal disease have an approximately four-fold greater risk of hip fracture than that of the general population in the United States. However, this finding was based on the observation of Caucasians, who have significantly greater risk for hip fracture than Asians in general.

Methods: We conducted a retrospective cohort study using panel data from the Japanese Society for Dialysis Therapy registry. Data from the hemodialysis patients receiving 3 times/week therapy who had no history of hip fracture as of December 31of 2007 was extracted. The observed number of hip fractures was compared to the expected number based on a Japanese national survey. The standardized incidence ratios (SIRs) were calculated as the ratios of observed to expected number of cases.

Results: During the one-year study period, 1,437 hip fractures out of 128,141 hemodialysis patients (61.9% male) were recorded. The overall incidences of hip fracture were 7.57 and 17.43 per 1000 person years in males and females, respectively. The SIRs for male and female patients were 6.2 (95% confidence interval (CI), 5.7 to 6.8) and 4.9 (95% CI, 4.6 to 5.3), respectively, compared to those in the general population. In diabetic patients, the SIRs continuously rose with increasing dialysis vintage (duration after the initiation of dialysis), whereas the SIRs remained nearly constant until 16 years vintage in non-diabetic patients, but steeply increased thereafter.

Conclusions: Japanese hemodialysis patients have an approximately five-fold greater risk of hip fracture than that of the general population. Although the bone fracture in the Caucasians is twice as much as that in the Asians, the overall incidences of hip fracture were comparable among dialysis patients. Since the influence of uremia on fracture risk was so critical, the effect of ethnic difference seemed to have been masked.

Funding: Government Support - Non-U.S.

SA-PO2276

Fibroblast Growth Factor 23 and Bone Metabolism in Elderly Chronic Kidney Disease Patients Eiichiro Kanda,[1] Sei Sasaki.[2] *[1]Tokyo Kyosai Hospital, Tokyo, Japan; [2]Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Osteoporosis is commonly observed among elderly persons. We investigated the relationship between lumbar fracture and bone metabolism markers in elderly chronic kidney disease (CKD) patients.

Methods: 105 elderly CKD patients who had never used calcium or vitamin D supplements were enrolled in this cross sectional study in Tokyo, Japan. We investigated the prevalence of lumbar fracture and measured serum calcium, phosphate, $1,25(OH)_2$ vitamin D (1,25-VD), intact parathyroid hormone (iPTH), fibroblast growth factor 23 (FGF23), serum N-terminal telopeptide (NTX) and urinary NTX. Factors associated with lumbar fracture and FGF23 levels were assessed by regression or multivariate logistic model adjusted for age and gender.

Results: The following results were obtained: female, 32.4%; diabetics, 30.5%; average age (SD), 73.2(7.7) years; estimated glomerular filtration rate (eGFR), 45.7(24.1) ml/min; calcium 9.4 (0.5) mg/dl; phosphate 3.5(0.7) mg/dl; intact PTH 3.5(0.7) pg/ml; 1,25-VD 47.2(21.0) pg/ml; FGF23 78.0(101.7) pg/ml; serum NTX 20.5 (17.4) nmol BCE/L; urinary NTX 35.8 (24.3) nmol BCE/mmol.Cr. Univariate analysis revealed that FGF23 level significantly correlated with eGFR, calcium, phosphate, 1,25-VD, intact PTH, and serum NTX levels (P<0.0001), but not with urinary NTX level. Lumbar fracture was associated with age and High FGF23 levels: more than 81 years group (referred to 61-70 years) odds ratio (OR) 4.00 (95% confidence interval 1.05-15.30); high FGF23 levels (>71.0), adjusted OR 4.37 (1.06-17.97). High FGF23 levels were closely associated with bone mineral factors: increased phosphate level (>4.3), adjusted OR 4.18 (1.13-15.40); decreased 1,25-VD level (<20), 19.38 (3.71-101.29); increased serum NTX level (>16.5), 15.08 (4.72-48.15); increased urinary NTX level (male>66.2, female >89.0), 9.15 (1.37-60.99). None of the patients showed both high intact PTH and low FGF23 levels.

Conclusions: Increased FGF23 level was observed with the progression of CKD before intact PTH level increased, and was associated with lumbar fracture and osteoporosis markers. FGF23 is a valuable marker to detect and prevent abnormality of bone metabolism at an early stage of CKD.

SA-PO2277

Tests of Bone Density and Structure Are Associated with Fractures in Stage 3-5 Chronic Kidney Disease Sophie Jamal,[1,3] Sarah West,[1,3] A. M. Cheung,[1,2] Charmaine E. Lok.[1,2] *[1]University of Toronto, ON, Canada; [2]Toronto General Hospital, Toronto, ON, Canada; [3]Women's College Hospital, Toronto, ON, Canada.*

Background: Fractures are common in CKD patients. Bone mineral density (BMD) by dual x-ray absorptiometry (DXA) does not discriminate well (hip BMD AUROC: 0.56) among fractured and non-fractured dialysis patients. However, the ability of BMD by DXA and bone structure by high-resolution peripheral quantitated computed tomography (HR-pQCT) to discriminate fracture status among patients with stages 3-5 CKD (CKD:III-V) is unestablished.

Methods: Baseline data from our ongoing study in adult CKD:III-V patients,was used to determine if BMD by DXA (Hologic) at the lumbar spine (LS), total hip (TH), ultradistal radius (UDR); and/or cortical area, density & thickness, trabecular area, density & separation by HR-pQCT (XtremeCT) at the radius could discriminate fracture status (fracture=self-reported low trauma fractures occurring after 40 yrs and/or prevalent vertebral fractures identified by morphometry). Results are expressed as areas under the receiver operating characteristic curves (AUROC) with 95% confidence intervals (CI), adjusted for age and weight.

Results: Data from 128 men and 86 women showed that compared to those without fractures (n=143), those with fractures (n=70) were older (59±16 vs. 69±14 yrs, p<0.001). The mean weight was 79.9±19.8 kg, most were Caucasian (70.0%), > 40% had diabetes, and most (74%) patients were CKD stage 4 or 5. The mean duration of CKD was 8.0±10.3 yrs, and 30.2% reported a fall in the last 12 mos. BMD by DXA discriminated among those with and without fractures (AUROC for LS: 0.70 [95% CI: 0.62-0.78]; TH: 0.69 [95% CI: 0.61-0.77]; UDR: 0.71 [95% CI: 0.63-0.78]). HR-pQCT also performed well for cortical measures (AUROC for area: 0.67 [95% CI: 0.59-0.76]; density: 0.70 [95% CI: 0.63-0.78] and thickness: 0.70 [95% CI: 0.62-0.78]) and trabecular measures (AUROC for area: 0.70 [95% CI: 0.62-0.78]; density: 0.70 [95% CI: 0.63-0.78]; and separation: 0.70 [0.63-0.78]).

Conclusions: BMD by DXA and HR-pQCT can discriminate fracture status in CKD:III-V patients. Prospective studies are needed to evaluate the fracture predictive ability of BMD and HR-pQCT.

Funding: Government Support - Non-U.S.

SA-PO2278

Dialysis as a Treatment Option for Primary Tumoral Calcinosis in a Patient with a New FGF Mutation Patricia T. Goldenstein,[1] Manuel C. Castro,[1] Rodrigo B. Oliveira,[1] Hugo Abensur,[1] Claudio Luders,[1] Rosilene M. Elias,[1] Harald Jueppner,[2] Alexandre Costa Pereira,[3] Vanda Jorgetti,[1] Rosa M.A. Moyses.[1] *[1]Nephrology, Hospital das Clínicas da Faculdade de Medicina da USP, Sao Paulo, Brazil; [2]Nephrology, Massachussets General Hospital, Boston, MA; [3]Cardiology, Hospital das Clínicas da Faculdade de Medicina da USP, Sao Paulo, Brazil.*

Background: Primary tumoral calcinosis (TC) is a rare autosomal recessive metabolic disorder characterized by ectopic calcified tumoral masses and dental abnormalities, as well as soft tissue periarticular and vascular calcifications. Until now, mutations in three genes were ascribed to be responsible for the human disorder.

Methods: Here we describe a case of a 24-yr-old male with TC, with a new mutation in FGF23 and an unusual treatment option in an attempt to effectively control his phosphate and reduce his calcified lesions.

Results: The patient presented subcutaneous nodules, as well as periarticular and vascular calcifications. Biochemical analysis disclosed hyperphosphatemia (9.0 mg/dL), normocalcemia (4.8 mg/dL) with a normal renal function and FeP = 3%. PTH was suppressed (15 pg/mL), associated with a low-normal 25-OH-vitamin D (26 ng/mL). Serum intact FGF-23 was undetectable. As for FGF23, a heterozygous state was observed defined by p.Q67H (exon 1) and p.Q156stop (exon 3). Despite of four surgeries for tumoral resection and medical treatments with aluminum hydroxide, sevelamer and acetazolamide, lesions continued to progress. Due to lack of other treatment options, patient was included in a daily dialysis program, leading to a better phosphorus control. After 24 months of therapy, TC lesions decreased in size and he recovered most of his mobility. This is the first report of a new mutation in FGF gene and the first case in which dialysis was described as an effective treatment option for TC with normal renal function.

Conclusions: This patient, with his rare genetic disease, gave us the opportunity to test in vivo the role of phosphate in extra-osseous calcification and emphasizes the need of its control in the context of CKD.

SA-PO2279

The Value of DXA Versus QCT for Diagnosis of Osteoporosis in Patients with CKD Hartmut H. Malluche,[1] Hanna W. Mawad,[1] James N. Wise,[2] Stephanie Fugate,[1] Kimberly McLaughlin,[1] Anthony Wolbarst,[2] Daniel Davenport,[3] Marie-Claude M. Faugere.[1] *[1]Division of Nephrology, University of Kentucky; [2]Department of Radiology, University of Kentucky; [3]Department of Surgery, University of Kentucky.*

Background: Bone mineral density (BMD) of the lumbar spine and hip was measured by dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) in 82 patients with CKD-5D.

Methods: There were 42 men (age 52.0 ± 2.13 yrs) and 40 women (age 56.2 ± 2.18 yrs). Mean dialysis duration was 59.1 ± 6.30 mos. Thirty seven % of the patients were diabetics. Iliac crest bone biopsies were done for histomorphometry at time of BMD measurements in a subgroup of 23 patients. Diagnosis of osteoporosis by DXA or QCT was made when t-scores were < -2.5 and by histomorphometry when cancellous bone volume and/or cortical thickness were below normal and/or cortical porosity was above normal values.

Results: Osteoporosis was found in the spine by DXA in 10% and by QCT in 14%; osteoporosis in the hip was found by DXA in 26% and by QCT in 16% (p=0.008). Absolute scores of the hip obtained from DXA and QCT were not different and correlated. T-scores of the hip from DXA and QCT also correlated but were lower with DXA than QCT (-1.3 ± 0.20 vs. -0.12 ± 0.22, p <0.001). This suggests that the differences observed in recognition of osteoporosis were not due to absolute test results, but related to differences in normative databases used to calculate t-scores. Bone histology identified presence of osteoporosis in 90% of the patients.

Sensitivity, specificity, positive and negative predictive values of DXA versus QCT for osteoporosis diagnosed by histology are shown below.

	DXA Hip	QCT Hip	DXA Spine	QCT Spine
Sensitivity (%)	43	29	5	29
Specificity (%)	50	100	100	100
Positive Predictive Value (%)	90	100	100	100
Negative Predictive Value (%)	8	12	10	12

Conclusions: In conclusion, for prediction of histologically documented osteoporosis DXA and QCT are equally specific (100%) for the spine while for the hip QCT had superior specificity. Sensitivity is poor with both DXA and QCT of spine and hip. For diagnosis, specificity and sensitivity of at least 80% is required, thus neither DXA nor QCT is reliable.

Funding: NIDDK Support, Private Foundation Support

SA-PO2280

Evaluation of Bone Mineral Density in Long-Standing Type 1 Diabetic Patients with or without Diabetic Nephropathy Maria Lajer,[1] Simone Theilade,[1] Christel Joergensen,[1] Frederik I. Persson,[1] Gudbjörg Andrésdóttir,[1] Henrik Reinhard,[1] Hans-Henrik Parving,[2] Lise Tarnow,[1] Peter Rossing.[1] *[1]Steno Diabetes Center; [2]University Hospital of Copenhagen.*

Background: Patients with chronic kidney disease including diabetic nephropathy are suggested to have reduced bone mineral density (BMD).

Methods: Cross-sectional evaluation of 293 prospectively followed long-standing, type 1 diabetic patients. 115 patients with diabetic nephropathy (54% men; age [mean±SD] 54±9 years, 42±8 years of diabetes, duration of nephropathy 22±6 years) and 178 patients with persistent normoalbuminuria (49% men, age 59±10 years, 41±10 years of diabetes) were included. BMD (g/cm^2) in femoral neck, total femoral bone and lumbar spine was measured by dual energy x-ray absorptiometry (DXA). Osteopenia and osteoporosis were defined by any sex matched T-score from -2.5 to -1.0 and <-2.5, respectively. The difference between BMD and age-sex matched average defines the Z-score. GFR was measured by ^{51}Cr-EDTA clearance in patients with nephropathy.

Results: Among patients with diabetic nephropathy, 58 (50%) and 31(27%) had osteopenia and osteoporosis, respectively compared to 106 (60%) and 22 (12%) of the normoalbuminuric patients; p=0.006. Among male patients with nephropathy, the prevalence of osteoporosis was 37% *vs.* 9% with normoalbuminuria (p<0.001), whereas no difference was seen among women. Similarly, men with nephropathy had significantly lower age-sex matched spinal, femoral neck and total femoral bone Z-scores compared to normoalbuminuric patients; p≤0.002. There was no statistically difference in woman.

Finally, among patients with diabetic nephropathy, baseline GFR levels did not differ between men and women; 94(29) *vs.* 95(30), respectively but correlated positively with femoral neck and total femoral BMD (r=0.36 and r=0.38; p<0.001, respectively).

Conclusions: The risk of osteoporosis was highest among male patients with type 1 diabetes and long-standing diabetic nephropathy and femoral BMD correlated with renal function. Hence screening, prevention and treatment of osteoporosis in patients with impaired renal function should be considered.

SA-PO2281

Low-Dose Calcifediol Supplementation Augments Serum PTH Levels in Hemodialysis Patients at Risk for Adynamic Bone Disease Francesco Iannuzzella, Silvia Mattei, Fabio Carlini, Alfredo Stefani, Sonia Pasquali. *Division of Nephrology, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.*

Background: Adynamic bone disease (ABD) is a common form of renal osteodystrophy. In hemodialysis (HD) patients, the use of active vitamin D analogs may favor the development of ABD by means of the suppression of PTH secretion. By contrast, treatment of low 25-hydroxyvitamin D (calcifediol) levels may exert a stimulatory effect on bone formation. We undertook this study to assess the effect of low-dose calcifediol supplementation on mineral metabolism and serum PTH levels in long-term HD patients considered at risk for ABD because of low serum PTH levels.

Methods: This was a 3-month follow-up, prospective study of a cohort of prevalent HD patients from a single center. Eligible subjects were on HD for ≥3 months with PTH levels <150 pg/mL. All patients were included in the study, except for those who were taking cinacalcet or any active vitamin D analog and those who had undergone parathyroidectomy. Calcium based-phosphate binders dose remained unchanged during the study. Serum levels of 25-hydroxyvitamin D, PTH, Ca, P and alkaline phosphatase were measured before and after supplementation. All patients received 20 drops of oral calcifediol (1.5 mg/10 mL) once a week after the first HD session of the week for 3 months. Statistical analysis was performed using paired Wilcoxon test.

Results: The study included 18 patients: 8 men and 10 women with mean age of 57.8±19.5 years. Mean HD vintage was 24.3±32.4 months. Calcifediol serum levels were <30 ng/ml in all our patients at baseline and significantly increased at the end of the study (8.1±4.9 vs. 23.8±10.1, P<0.001). Serum PTH increased significantly (79.6±34.2 vs. 118.9±59.0, P<0.05). Serum phosphate showed a slight but significant increase with supplementation (4.3±1.3 vs. 4.8±1.0, P<0.05), whereas no change was reported for both serum calcium (9.2±0.8 vs. 8.8±0.5, P>0.05) and alkaline phosphatase levels (175.6±58.3 vs. 179.8±61.1, P>0.05).

Conclusions: The parathyroid glands of vitamin D deficient HD patients with relative hypoparathyroidism or ABD responded to a low-dose 25-OH-vitamin D supplementation with a significant increase in serum PTH levels.

Funding: Government Support - Non-U.S.

SA-PO2282

DXA and HRpQCT Detect Minimal Bone Loss after Kidney Transplantation with Early Corticosteroid Withdrawal Lucas Nikkel, Chiyuan Amy Zhang, Sumit Mohan, Bekir Tanriover, David J. Cohen, Lloyd Ratner, Geoffrey K. Dube, Stephanie Boutroy, Xiaowei Sherry Liu, Donald J. McMahon, Elizabeth Shane, Thomas L. Nickolas. *Columbia University.*

Background: With corticosteroid (CS)-based immunosuppression (CSBI), areal bone mineral density (aBMD) declines 2-10% during the first 6 months (m) after kidney transplant (KTx), particularly at trabecular (Tb) rich sites, such as the lumbar spine (LS); fracture rates are high at both spine and hip. Early corticosteroid withdrawal (ECSW) protocols, based on calcineurin inhibitors (CNI) are increasingly used after KTx. We hypothesized that KTx recipients managed with ECSW would not experience significant bone loss.

Methods: We included KTx recipients ≥18 yrs managed with ECSW. At baseline, 3m and 6m post-KTx, we measured aBMD by dual energy X-ray absorptiometry (DXA) at predominantly Tb sites: LS and ultradistal radius (UDR); and cortical (Ct) sites: total hip (TH); femoral neck (FN); 1/3 radius (1/3R). High resolution peripheral QCT (HRpQCT; Scanco Medical, AG, voxel size~82µm) was used to measure total, Ct and Tb volumetric BMD (vBMD); Ct thickness (CtTh); and Tb number (TbN), thickness (TbTh) and separation (TbSp) at the distal radius (DR) and tibia (DT). Comparisons were made using paired T-tests; results are expressed as Means±SD.

Results: Of 48 subjects enrolled, 29 and 24 completed 3m and 6m of observation respectively. Age was 51±13 years; 27% were women; 73% were white. BMI was 29±4 Kg/m2. Mean baseline T-Scores were > -2.5 at all sites. At 6m, aBMD declined significantly only at the 1/3R (-1.8%; p=0.02) and UDR (-2.7%; p=.04). By HR-pQCT at 6m, DR total vBMD decreased by -2.6% (p=0.01), Tb vBMD decreased by -2.5% (p<0.001), and there was a non-significant 1.3% decrease in DT total vBMD (p=0.08). TbN, TbTh, and TbSp were unchanged at both DR and DT at 6m.

Conclusions: After KTx, ECSW was associated with less bone loss at the LS, FN, and TH than previously reported with CSBI. Significant bone loss occurred at the 1/3R and UDR which HR-pQCT suggested is primarily Tb; the mechanism of bone loss at the DT and DR was unclear. Larger and longer studies are needed to assess ECSW effects on fracture rates after KTx.

Funding: Private Foundation Support

SA-PO2283

Does Simultaneous Pancreas-Kidney Transplantation Reduce Fracture Risk? An Analysis of the USRDS Lucas Nikkel,[1] Sumit Mohan,[1] Bekir Tanriover,[1] Lloyd Ratner,[1] Chiyuan Amy Zhang,[1] Donald J. McMahon,[1] Mary B. Leonard,[3] Christopher S. Hollenbeak,[2] Elizabeth Shane,[1] Thomas L. Nickolas.[1] *[1]Columbia University; [2]Penn State Hershey; [3]Childen's Hospital of Pennsylvania.*

Background: Simultaneous pancreas-kidney transplant (SPK) is treatment of choice for patients with Type 1 Diabetes Mellitus (DM1) and end stage renal disease (ESRD). Both DM1 and ESRD increase fracture (Fx) risk due to metabolic abnormalities that negatively impact bone quality and strength. SPK improves metabolic disarray due to both DM1 and ESRD and may decrease Fx risk. We hypothesized that in patients with DM1, SPK would reduce Fx associated with hospitalizations compared to patients with DM1 who received a kidney transplant (KTx) alone.

Methods: Adults age≥18 yrs with DM1 undergoing either SPK or KTx between January 1, 2000 and December 31, 2006, were identified from USRDS. First Fx events were identified from hospital discharge ICD9 codes after Tx. Continuous covariates (age, BMI, HLA mismatches) were compared with Students t tests and categorical covariates were compared by Chi-square. Time to first post-Tx Fx was modeled by Kaplan-Meier and Cox methods. Propensity score adjustment was used to balance differences in age, gender, race, BMI, prior dialysis, HLA mismatches, and immunosuppression between Tx groups.

Results: Of 11,237 patients with DM1, 4,987 and 6,250 underwent SPK and KTx, respectively. There were 221 and 378 Fxs in the SPK (4.4%) and KTx (6.1%) cohorts respectively (p<0.0001). In univariate analysis, SPK had 27% lower Fx risk than KTx (p<0.001). After adjustment for age, gender, race, BMI, prior dialysis, HLA mismatches, and immunosuppression by propensity score, SPK had 23% lower Fx risk (Hazard Ratio [HR]: 0.77; p=0.009) and 40% lower hip Fx risk (HR: 0.60; p=0.04) versus KTx. Older age, white race, prior dialysis, and pre-Tx Fx were also associated with increased risk.

Conclusions: SPK results in 23% reduced risk for all Fx types requiring hospitalization and 40% reduced risk for hip Fx in comparison to KTx alone in adults with DM1. Prospective studies are needed to elucidate the mechanisms that account for the differential effects of SPK and KTx on bone quality and propensity for Fx.

Funding: Private Foundation Support

SA-PO2284

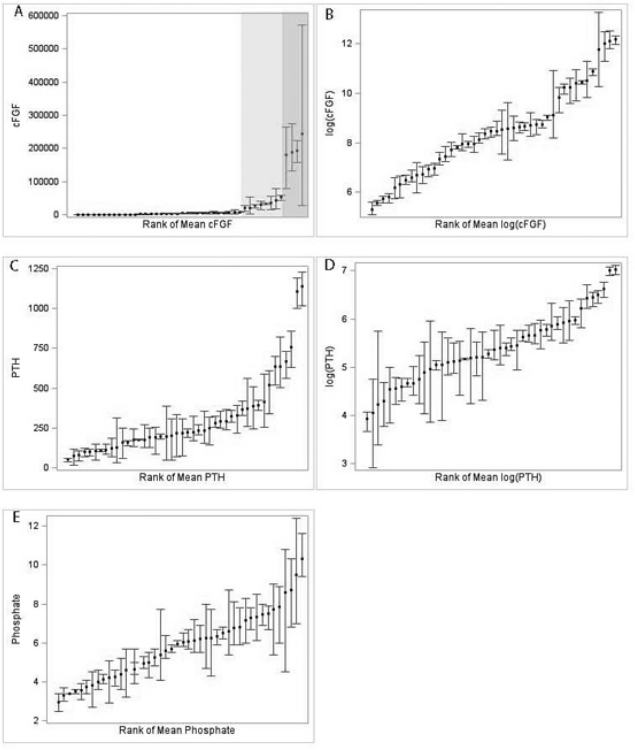
Fibroblast Growth Factor 23 in Patients Undergoing Peritoneal Dialysis Tamara Isakova,[1] Huiliang Xie,[1] Allison Barchi-Chung,[1] Gabriela S. Vargas,[1] Nicole Sowden,[1] Jessica Houston,[1] Patricia R. Wahl Pristau,[1] Andrew L. Lundquist,[2] Michael Epstein,[2] Kelsey T. Smith,[1] Luis M. Ortega,[1] Gabriel Contreras,[1] Oliver Lenz,[1] Talat Alp Ikizler,[3] Harald Jueppner,[2] Myles S. Wolf.[1] *[1]University of Miami Miller School of Medicine; [2]Massachusetts General Hospital; [3]Vanderbilt University.*

Background: Fibroblast growth factor 23 (FGF23) is an independent risk factor for mortality in patients with end-stage renal disease (ESRD). Before FGF23 testing can be integrated into clinical practice of ESRD, further understanding of its determinants is needed.

Methods: In a study of 67 adults undergoing peritoneal dialysis, we tested the hypothesis that longer dialysis vintage and lower residual renal function and renal phosphate clearance associate with higher FGF23. We also compared the monthly variability of FGF23 versus parathyroid hormone (PTH) and serum phosphate.

Results: In unadjusted analyses, FGF23 correlated with serum phosphate (r = 0.66, P <0.001), residual renal function (r = – 0.37, P = 0.002), dialysis vintage (r = 0.31, P = 0.01), and renal phosphate clearance (r = – 0.38, P = 0.008). In adjusted analyses, absence of residual renal function and greater dialysis vintage associated with higher FGF23, independent of demographics, laboratory values, peritoneal dialysis modality and adequacy, and treatment with vitamin D analogs and phosphate binders. Urinary and dialysate FGF23

clearance was minimal. In three serial monthly measurements, within-subject variability accounted for only 10% of total FGF23 variability compared with 50% for PTH and 60% for serum phosphate.



Conclusions: Increased serum phosphate, loss of residual renal function, longer dialysis vintage and lower renal phosphate clearance associate with elevated FGF23 levels in ESRD patients undergoing peritoneal dialysis. FGF23 may be a more stable marker of phosphate metabolism in ESRD than PTH or serum phosphate.

Funding: NIDDK Support

SA-PO2285

Impact of Lanthanum Carbonate on FGF23 in Chronic Kidney Disese Stages 3-5

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Background: FGF23 plays an important role in the regulation of phosphate as well as vitamin D and parathyroid hormone (PTH) metabolism. As CKD progress, phosphate retention with hyperparathyroidism develops. Serum phosphate levels are not a good early marker of phosphate retention, whereas increases in FGF23 which help maintain normal phosphate balance may be a better indicator of phosphate retention and subsequent development of hyperparathyroidism. The purpose of this study was to determine if reducing FGF23 levels with lanthanum could prevent or reverse the development of hyperparathyroidism.

Methods: A randomized double blinded placebo controlled study was performed in subjects with CKD stages 3-5 to determine if treatment with lanthanum carbonate 500 mg or placebo given with meals could reduce FGF23 and PTH. Subjects were treated for 60 days and doses increased to 1000 mg pc for phosphorous > 5.5 mg/ dl. We report interim data on first 14 patients randomized. One way ANOVA was used to assess significance

Results: Fourteen patients were randomized to placebo versus treatment. Two patients in treatment group and 1 in placebo group dropped out secondary to GI side effects. Data from 5 patients on Lanthanum and 6 patients in placebo are presented. There was no change in serum FGF23, calcium, phosphorus, 25 vitamin D and change in PTH (%) levels in patients treated for 60 days with placebo. There were significant declines in FGF23 levels and change in PTH in patients treated with lanthanum for 60 days.

Impact of Lanthanum Carbonate versus Placebo on Markers of Mineral Metabolism

	PLACEBO GROUP			LANTHANUM GROUP		
Values (mean ±SD)	Time 0	60 Days	P value	Time 0	60 Days	P value
FGF23 (RU/ml)	123.4±35	105.6±55	0.51	150.6±56	39.9±17	**0.003**
Calcium (mg/dl)	9.5±0.3	9.6±0.4	0.61	9.6±0.4	9.5±0.3	0.79
Phosphorus(mg/dl)	3.65±0.4	4±0.8	0.37	4.2±0.6	4.26±0.7	0.88
% Change PTH	100	88±41	0.49	100	57.2±40	**0.04**
25Vit D(ng/ml)	47.8±9	50.7±11	0.67	39.4±17	37.8±14	0.89

Conclusions: Lanthanum carbonate given with meals significantly reduces FGF23 and PTH levels with no changes in calcium, phosphorus and 25 D levels.

Funding: Pharmaceutical Company Support

SA-PO2286

Disordered Mineral Metabolism in the CKiD Children: Role of FGF23

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Background: FGF23 is an important regulator of phosphorus (Pi) and vitamin D metabolism. However, little is known about the prevalence and determinants of FGF23 excess across the spectrum of CKD in children.

Methods: We measured plasma C-terminal FGF23 (Immutopics 2nd gen) in 426 children, ages 1-16 yrs, with CKD stages 2-4 enrolled in the observational Chronic Kidney Disease in Children (CKiD) study. GFR was measured by plasma clearance of iohexol (n=286) or estimated using the CKiD estimating equation.

Results: Mean age of subjects was 11.5 ± 4.3 (SD) yrs. CKD was due to glomerular disease in 21% and non-glomerular disease in 79%. Mean GFR was 46 ± 18 ml/min/1.73 m2; 18% of subjects had CKD stage 2, 59% stage 3, and 21% stage 4. Overall, median serum Pi and PTH levels were within the normal range, but median FGF23 was 132 RU/ml (IQR: 88-223), 2.3-fold higher than that in healthy children. 66% of subjects met criterion for FGF23 excess (>100 RU/ml), but only 12% had hyperphosphatemia. FGF23 was strongly associated with GFR (r=-0.44), age-adjusted Pi (r=0.30), and PTH (r=0.36) (P<0.001 for each). In stage 2 CKD, mean age-adjusted Pi was below the normal mean (P<0.01), PTH was >65 pg/ml in only 15%, whereas FGF23 was >100 RU/ml in 44% of subjects. As GFR declined further, the prevalence of FGF23 excess increased (stage 3, 66%; stage 4, 91%). FGF23 was higher in children with glomerular than with non-glomerular disease (P<0.001), after adjusting for age, GFR, and Pi.

Conclusions: Plasma FGF23 concentrations are increased early in the course of CKD in children, before increases in serum Pi or PTH. Thus, increased FGF23 may be an early biomarker of disordered Pi homeostasis and an initiating mechanism of abnormal mineral metabolism in children with progressive CKD.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2287

Evaluation of the Relationship between Vitamin D Analogs and FGF23: Is There a Difference between Agents?

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Background: Fibroblast growth factor (FGF23), a key regulator of mineral metabolism, has been associated mortality in the dialysis population. Activated vitamin D administration increases FGF23 levels. Observational studies have shown that the beneficial effects of vitamin D analogs (VDAs) on survival are reduced at higher doses. The aim of this study was to explore the relationship between FGF23, VDA selection and VDA dosage among hemodialysis (HD) patients.

Methods: Adults on HD for ≥ 3 months were included in this cross-sectional study. Patient demographics, laboratory values and current medication regimens were obtained. Weekly VDA doses were converted to doxercalciferol equivalents (4.6 µg of paricalcitol = 3.1 µg doxercalciferiol) for uniformity. Pre-dialysis levels of intact FGF23 were measured via ELISA. Stepwise linear regression was used to identify factors associated with FGF23.

Results: A total of 127 patients (57% male, 46% diabetic etiology, 64% on doxercalciferol therapy) with a mean age = 62.4±14.9 years, HD vintage = 3.5±2.9 years and log FGF23 = 2.57±0.37 pg/ml were included in this analysis. Log FGF23 levels positively correlated with HD vintage (r=0.24, p=0.0065), calcium (r=0.26, p=0.0036), phosphorus (r=0.57, p < 0.0001), PTH (r=0.25, p = 0.0054), VDA dose (r=0.21, p=0.0181) and negatively correlated with age (r=-0.18, p=0.0388). Log FGF23 was lower in those on doxercalciferol compared to patients on paricalcitiol (2.50±0.33 pg/ml versus 2.70±0.42 pg/ml, p=0.002). Use of paricalcitol (β=0.15, p=0.0032), VDA dose (β=0.007, p=0.0258), calcium (β=0.21, p<0.0001), phosphorus (β =0.15, p<0.0001) and HD vintage β=0.02, p=0.028) were independently associated with log FGF23.

Conclusions: The positive independent association between VDA dose and FGF23 are indicative of a dose response relationship between VDAs and FGF23. Furthermore, paricalcitol therapy was independently associated with higher FGF23 concentrations, compared to doxercalciferol therapy. These results suggest that individual VDAs may have differential effects on FGF23 regulation in HD patients and warrants further investigation.

SA-PO2288

FGF-23 Levels Are Modified by Loop Diuretic Treatment in CKD Patients

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Background: FGF-23 is a central regulator of calcium phosphate metabolism. Similar to parathyroid hormone (iPTH), FGF-23 stimulates renal phosphate excretion. While plasma levels of both iPTH and FGF-23 increase in advanced CKD in order to combat phosphate retention, an elevation of FGF-23 may precede the decline of estimated glomerular filtration rate (eGFR) in very early CKD for unknown reasons. In CKD patients, use of loop diuretics is a known stimulus for increased renal calcium loss and consecutively increased iPTH levels. To date it is unclear whether the use of loop diuretics results in a concomitant increase of FGF-23.

Methods: We studied 343 CKD stage 2-4 patients in our ongoing CARE FOR HOMe study. In all patients we measured plasma levels of iPTH, FGF-23 and urinary fractional calcium excretion (FeCa) , and recorded the intake of diuretic medication (loop diuretics: LD; thiazide diuretics: TD). In addition, we assessed left ventricular function using echocardiography and eGFR using the MDRD study equation 4.

Results: Study participants had a mean eGFR of 44.2±15.7 ml/min/1.73 m², and were 65±12 years of age. Median FGF-23 level was 99.0 rU/ml (IQR 60.3-158. rU/ml), mean iPTH level was 70.2±57.9 pg/ml. 281 patients (81.9 %) were on diuretic medication, comprising 161 patients (46.9 %) receiving LD, and 174 patients (50.7%) receiving TD. Intake of thiazides was associated with lower FeCa (TD: 0.52±0.51%; no TD: 0.89±0.98%; p<0.001), LD were associated with higher FeCa (LD: 0.88±1.01%; no LD: 0.54±0.48%; p=0.010). Moreover, the intake of LD was associated with higher iPTH (LD: 90.6±72.2 pg/ml; no LD: 52.3±32.7 pg/ml; p<0.001) and FGF-23 levels (LD: 125.4 rU/ml [IQR 78.0-187.3 rU/ml]; no LD: 76.4 rU/ml [IQR 50.5-116.3 rU/ml]; p<0.001), while the intake of thiazides was not. This association remained significant after correction for eGFR and left ventricular function in a linear regression analysis.

Conclusions: The results from our study complements earlier data on the impact of LD on secondary hyperparathyroidism, reporting for the first time an association between choice of diuretic medication and FGF-23 levels in CKD patients.

SA-PO2289

24,25 Dihydroxyvitamin D Levels Are Elevated in Dialysis Patients and Correlate with FGF23 Levels Marta Christov,[1, 2] Nigel Clarke,[3] Julia Beth Wenger,[2] Richard E. Reitz,[3] Ravi I. Thadhani,[2] Harald Jueppner.[2] *[1]Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [2]Medicine, Massachusetts General Hospital, Boston, MA; [3]Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.*

Background: 25(OH) vitamin D (25D) deficiency is common in patients treated with dialysis and may contribute to the adverse outcomes observed in this cohort. In these patients increased FGF23 levels are thought to inhibit the renal 1-alpha hydroxylase and stimulate the 24-hydroxylase thus contributing to diminished 1,25(OH)2 vitamin D (1,25D) levels and to the development of secondary hyperparathyroidism. Recent data suggest the presence of increased amounts of the 24-hydroxylase protein in the kidneys of rodents and humans with CKD, which may contribute to both 25D deficiency (by metabolism into inactive analogs) as well as 1,25D deficiency (by "siphoning off" of the precursor 25D).

Methods: Using a novel mass spectrometry approach, we measured 24,25D levels in the circulation of 120 individuals with no known renal dysfunction and 60 patients initiating hemodialysis.

Results: In the reference group, 24,25D levels were positively correlated with 25D status. Specifically, the mean 24,25D level in individuals with 25D levels <20 ng/mL was 1.27±0.3 ng/mL (mean±SEM), while in those with 25D levels >30 ng/mL it was 6.1±0.56 ng/mL. In the dialysis population 24,25D levels were significantly elevated; patients with baseline 25D levels <20 ng/mL had a 24,25D level of 9.0±1.26 ng/mL (mean±SEM), at times rising above the absolute 25D level in individual patients. 24,25D levels correlated positively with 25D, calcium and albumin. Treatment with an active vitamin D analog did not increase 24,25D levels and neither did the dialysis procedure itself. FGF23 levels measured in a subset of patients correlated positively with 24,25D levels (N=23, R=0.42, p<0.05).

Conclusions: In summary, 24,25D levels in dialysis patients are increased compared with those in a reference population and correlate with FGF23 levels. This correlation is consistent with the FGF23-mediated increase in 24-hydroxylase expression in renal and possibly extra-renal tissues.

Funding: NIDDK Support

SA-PO2290

Association of Fibroblast Growth Factor 23 on Pulse Wave Velocity in Chronic Kidney Disease Stage 3-5 Patients with 2 Years Follow-Up Annet Bouma-de Krijger,[1] Arjan D. Van Zuilen,[2] Marc G. Vervloet,[1] Frans J. van Ittersum,[1] Pieter M. Ter Wee,[1] Jack F. Wetzels,[3] Peter J. Blankestijn.[2] *[1]Nephrology, VU University Medical Centre, Amsterdam, Netherlands; [2]Nephrology, University Medical Centre Utrecht, Utrecht, Netherlands; [3]Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: FGF23 is independently associated with cardiovascular outcome in CKD. However, the mechanism of this association is not known. To investigate the influence of FGF23 on vascular function we evaluated the association between baseline FGF23 and PWV and changes in FGF23 and PWV during two years.

Methods: We performed a post-hoc analysis of PWV in a subset of 188 CKD patients (132 male, ,mean age 56,5 years +/-12,7) included in the multicenter Masterplan-trial. Multiple logistic regression was used to evaluate the association between of FGF23 and PWV at baseline; generalized estimating equations were applied to evaluate this relationship in the entire 2 years study period.

Results: At baseline, mean arterial pressure (MAP), age and HbA1c were associated with PWV (all p < 0,05). In a model adjusted for these covariates, FGF23 was not associated with PWV. In the entire 2 years study period, FGF23 had a significant effect on PWV (p <0.001). In addition, PWV increased significantly over time (p=0.019).

table1

	Regression coefficient	p-value	Explained variance (model)
PWV baseline (m/s)			0.338
FGF23 baseline (U/ml)	0.001	0.405	
Age baseline (year)	0.103	0.000	
MAP baseline (mmHg)	0.058	0.000	
Hba1c baseline (%)	0.574	0.017	
PWV change (m/s) Baseline to 2 years			0.3980
Male gender	0,269	0.111	
Diabetes	0.396	0.335	
FGF23 (U/ml)	0.001	0.000	
Age (year)	0.110	0.000	
MAP (mmHg)	0.69	0.000	
HbA1c (%)	0.526	0.12	
Time (2 years vs baseline)	0.745	0.19	

Conclusions: Baseline FGF23 was not associated with baseline PWV. In two year follow-up FGF23 is significantly associated with PWV. This association is modest and clinical meaning remains to be determined.

SA-PO2291

Correction of Hyperphosphatemia Suppresses Cardiac Remodeling in Uremic Rats Masahide Mizobuchi,[1] Hiroaki Ogata,[2] Chiaki Kumata,[1] Ai Nakazawa,[1] Fumiko Kondo,[1] Eriko Kinugasa,[2] Tadao Akizawa.[1] *[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan; [2]Department of Internal Medicine, Showa University Northern Yokohama Hospital, Yokohama, Japan.*

Background: Hyperphosphatemia is associated with cardiovascular disease in patients with CKD. Correction of hyperphosphatemia is imperative from a perspective of the progression of cardiovascular disease. We investigated the association between phosphate metabolism and cardiac remodeling in 5/6th nephrectomized rats.

Methods: Adult Sprague-Dawley rats underwent subtotal nephrectomy (Nx) by the ligation method. Four groups were studied for 8 wk: 1) control (sham), 2) Nx rats fed a normal phosphate (0.9%) diet (Nx+NP), 3) Nx rats fed a high phosphate (2%) diet (Nx+HP), and 4) Nx rats fed a high phosphate diet containing 2% lanthanum (Nx+HP+La).

Results: Subtotal nephrectomy caused blood pressure elevation, and renal dysfunction as evidenced by a gradual increase in proteinuria and elevation in serum creatinine levels. Nx+HP rats showed a significant increase in serum phosphate and PTH levels compared with Nx+NP rats while Nx+HP+La rats showed slight decrease in these levels. Not only Nx+HP rats, but also Nx+HP+La rats showed a significant increase in serum FGF23 levels compared with Nx+NP rats. Urinary phosphate excretion showed a similar trend as serum FGF23 levels. Both Nx+NP rats and Nx+HP rats showed a significant increase in the left ventricle weight compared with sham rats while the increase was significantly suppressed in Nx+HP+La rats. Nx+HP rats showed a significant increase in matrix deposition of the cardiac tissue compared with the Nx+NP rats while the increase was significantly suppressed in Nx+HP+La rats.

Conclusions: Correction of hyperphosphatemia could suppress the cardiac remodeling, which was independent of serum FGF23 levels.

SA-PO2292

Effects of the Correction of 25-hydroxyvitamin D Deficiency on Hyperparathyroidism and Malnutrition-Inflammation Status in Hemodialysis Patients Raquel Ojeda, Sagrario Soriano, Maria Luisa Agüera, Maria Antonia Alvárez-Lara, Alejandro Martin-Malo, Pedro Aljama-Garcia. *Nephrology, Hospital Universitario Reina Sofia, Cordoba, Spain.*

Background: It has been reported that 85% of hemodialysis (HD) patients have decreased vitamin D (25-OH-D) levels (< 30 ng/ml). The aim of this study was to analyze the effect of the correction of 25-OH-D deficiency on the control of hyperparathyroidism (HPT) and the malnutrition-inflammation status in patients on HD.

Methods: Twenty seven stable patients on HD with low levels of 25-OH-D (< 20 ng/ml) were included in this study. They were 65.2 ± 16.8 years old and 48% were men. All patients were prospectively treated with calcifediol (mean dose 266 mcg/ week) for an average of 3 months. Calcium, phosphorus, alkaline phosphatase, PTH, albumin, hemoglobin, TAST, cholesterol, tryglicerides, eKt/V, URR and darbepoetin and paricalcitol doses were analyzed before and after the correction the deficiency of 25-OH-D.

Results: After treatment with calcifediol all patients normalized the levels of 25-OH-D (> 30 ng/ml), and decreased PTH with a reduction of paricalcitol requirements (before/ after correction n=11/3) and an improvement in their malnutrition-inflammation status (increased albumin and decreased CRP).

	Pre-Calcifediol	Post-Calcifediol	p
PTH (pg/ml)/LnPTH	318.78/5.26	229.61/4.72	0.001/0.000
Calcium (mg/dl)	8.72±0.61	9.2±0.56	0.000
Phosphorus (mg/dl)	3.98±0.99	4.41±1.28	0.057
Ln Alkaline phosphatase	4.83±0.56	4.54±0.5	0.000
Paricalcitol (mcg/week)	1.78±2.62	0.52±1.52	0.026
LnCRP	2.15±1.32	1.52±0.96	0.000
Albumin (g/dl)	3.2±0.38	3.43±0.42	0.000
Hemoglobin (g/dl)	11.45±0.94	11.92±0.93	0.140

Table 1. Main biochemical and pharmacological parameters determined before and after correction the deficiency of 25-OH-D.

With this protocol there were no episodes of severe hypercalcemia or hyperphosphatemia. The dose of phosphate binders remained stable along the study.

Conclusions: Our results strongly suggest that an adequate dose of calcifediol may correct the deficiency of 25-OH-D in HD patients without relevant side-effects. The normalization of 25-OH-D levels (>30 ng/ml) improved the malnutrition-inflammation status and allows a better control of HPT with a reduction of paricalcitol requirement.

SA-PO2293

Update on the PACE Study: Randomized Controlled Trial of Paricalcitol and Calcitriol Endpoints Seth Goldberg,[1] Stuart M. Sprague,[2] Mark D. Faber.[3] *[1]Washington University, St Louis, MO; [2]North Shore University Health System, Evanston, IL; [3]Henry Ford Hospital, Detroit, MI.*

Background: Exogenous activated vitamin D can reduce parathyroid hormone (PTH) levels and reverse bone disease in secondary hyperparathyroidism (SHPT). In preclinical studies, paricalcitol causes a smaller increase in serum calcium and phosphorus levels than calcitriol, possibly due to less activation of the intestinal and bone vitamin D receptors. The aim of this study is to compare the safety and efficacy of these active vitamin D analogs in patients with CKD stages 3 and 4.

Methods: Randomized, open label, active comparator, multicenter, phase 4 study of paricalcitol vs calcitriol in patients with CKD stages 3 and 4 with SHPT (PTH >120 pg/mL), albumin-corrected calcium >8.5 mg/dL and <10.0 mg/dL, and phosphorus <4.6 mg/dL. Goal enrollment is 110 patients among the four participating sites.

Results: To date, 103 patients have been randomized (72 completed, 17 early termination). Data from 53 patients have been evaluated, 27 on paricalcitol and 26 on calcitriol. Baseline lab values are shown in the table.

Baseline Values

	Paricalcitol	Calcitriol
Ca (mg/dL)	9.34 +/- 0.35	9.34 +/- 0.35
PO4 (mg/dL)	3.72 +/- 0.51	3.79 +/- 0.48
eGFR (mL/min)	26.1 +/- 6.5	26.3 +/- 9.6
iPTH (pg/mL)	172.5 (137-219)	246 (190-320)

iPTH expressed as median (interquartile range)

At least one adverse event was reported in 64% of patients. Of these, only hypercalcemia was deemed likely to have been caused by the study drug. None of the serious adverse events are classified as being likely secondary to study drug. The final data are expected in January 2012.

Conclusions: Baseline characteristics are similar between the groups in the 53 patients analyzed. The median baseline iPTH values did differ between the two groups but as drug dose titration is dependent on the percentage of reduction from baseline, this difference is not anticipated to affect the secondary efficacy endpoints. Patients reporting adverse events that were possibly or likely to be related to the study drugs were few in number.

Funding: Pharmaceutical Company Support

SA-PO2294

Normal DXA Scans Predicts Low Fracture Risk in Hemodialysis Patients Scott A. Rasgon, Richard M. Dell, John J. Sim. *Nephrology, Kaiser Permanente Los Angeles Medical Center, Los Angeles, CA.*

Background: Hip fracture rates in dialysis patients have been reported to be 4.4 times higher than the general population. KDIGO makes the following recommendations for Bone Mineral Density (BMD) testing "**3.2.2 In patients with CKD stages 3-5D with evidence of CKD-MBD, we suggest that BMD testing not be performed routinely, because BMD does not predict fracture risk as it does in the general population, and BMD does not predict the type of renal osteodystrophy (2B).**" This is based on thirteen studies with the most subjects in any of the studies was 242. Five of the studies showed no correlation with fracture rates and 8 showed some correlation with low BMD and increased fracture rate.

Methods: We reviewed the BMD data from DXA scans in our population of hemodialysis patients at Kaiser Permanente Southern California to see if there was any correlation between BMD testing and hip fracture rates in dialysis patients greater than 60 years of age. In our 60+ population there was 607,000 DXA scans done of which 1883 were done on hemodialysis patients during the time period of 01/01/1998-03/04/2011.

Results: We found the following results. 288 hemodialysis patients had a normal DXAscan and had only one hip fracture for a rate of .3 %.

Hemodialysis Hip Fracture and DXA Results

DXA score	Age 60-64	Age 65-69	Age70-74	Age 75-79	Age 80-84	Age 85 +	Total
	N (Fracture)						
Normal	40 (1)	45	75	70	35	23	288 (1)
-1 to -2	53 (1)	88 (1)	118 (2)	116 (1)	60	29	464 (5)
-2 to -2.49	32	48 (1)	82 (1)	61 (2)	51 (4)	32 (3)	306 (11)
Osteoperosis	68 (1)	114 (2)	198 (4)	193 (5)	143 (4)	107 (2)	823 (18)
No DXA	466 (1)	287 (1)	133 (2)	67 (0)	49 (2)	38	1040 (6)

Comparing a DXA score of <-2 vs >=-2 the relative risk of hip fractures was 3.22 p<.005.

Conclusions: We conclude that a normal DXA scan in a hemodialysis patient may be predictive of low fracture risk and patients with a DXA score >2 has a higher relative risk of hip fracture. We have more subjects in our study than other previous studies combined. Our study shows that in hemodialysis patients with a normal DXA scan the rate of hip fracture is very low. We should concentrate our fall reduction and treatment options for patients who are at high risk who do not have normal DXA scans.

SA-PO2295

Oral Paricalcitol Versus Oral Calcitriol for the Treatment of Secondary Hyperparathyroidism in ESRD Patients on CAPD Ema Juliaty Jamaluddin,[1] Abdul Halim Abdul Gafor,[1] Norella C.T. Kong,[1] Rizna Cader,[1] Rozita Mohd,[1] Vincent Chui Wei Wong,[1] Shamsul Azhar Shah.[2] *[1]Medical Department, University Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia; [2]Public Health Department, University Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia.*

Background: Few studies have reported on the use of oral paricalcitol for the treatment of SHPTH in ESRD patients on CAPD.

Objective: We evaluated the efficacy of oral paricalcitol versus oral calcitriol on serum iPTH and parameters of bone mineral metabolism in CAPD patients with SHPTH.

Methods: Patients randomized to receive oral paricalcitol or oral calcitriol for 12 weeks. Serum iPTH, Ca/PO_4 and ALP were measured at baseline and 3-weekly. Serum hsCRP and peritoneal membrane function tests (Kt/V_{urea} & PFT) were also recorded

Results: Results are presented in the table.

	Drug	Baseline	Week 3	Week 6	Week 9	Week 12	Intra group p-value
Serum iPTH (pmol/L)(1.5-7.6)	Paricalcitol	85.65 (46.6)	44.25 (24.4)	49.15 (24.4)	43.00 (30.3)	37.2 (30.9)	<0.001
Serum iPTH (pmol/L)(1.5-7.6)	Calcitriol	98.9 (70.5)	69.75 (105.3)	61.55 (110.0)	22.70 (16.8)	16.8 (88.6)	<0.001
Inter group p-value		0.700	0.165	0.564	1.000	0.292	
Serum calcium(mmol/L) (2.14-2.58)	Paricalcitol	2.24 (0.49)	2.36 (0.30)	2.47 (0.27)	2.48 (0.43)	2.42 (0.48)	0.004
Serum calcium(mmol/L) (2.14-2.58)	Calcitriol	2.25 (0.34)	2.20 (0.23)	2.41 (0.21)	2.41 90.21)	2.41 (0.50)	0.002
Inter group p-value		0.758	0.150	0.410	0.440	0.777	
Serum phosphorus (mmol/L)(0.71-1.36)	Paricalcitol	1.65 (0.65)	1.48 (0.55)	1.49 (0.64)	1.65 (0.73)	1.77 (0.64)	0.504
Serum phosphorus(mmol/L)(0.71-1.36)	Calcitriol	2.02 (0.71)	1.74 (0.75)	1.93 (0.97)	1.88 (0.80)	2.41 (0.50)	0.201
Inter group p-value		0.095	0.315	0.258	0.085	0.554	
CaxPO4 product (mmol2/L2)(<4.4)	Paricalcitol	3.83 (1.83)	3.61 (1.50)	3.82 91.67)	4.00 (1.82)	4.29 (1.20)	0.025
CaxPO4 product (mmol2/L2)(<4.4	Calcitriol	4.67 (1.49)	4.55 (2.06)	4.45 (2.24)	4.51 (1.68)	4.78 (1.68)	0.010
Inter group p-value		0.900	0.537	0.382	0.198	0.425	

Both vitamin D analogues were equally efficacious. The number of hypercalcemic events were similar. Pre-and post-treatment serum hsCRP and peritoneal membrane function were also not different.

Conclusions: Oral paricalcitol was as efficacious as oral calcitriol in reducing serum iPTH in CAPD patients with SHPTH.

Funding: Government Support - Non-U.S.

SA-PO2296

Ergocalciferol (ergo) Therapy in Calcidiol Deficient Hemodialysis (HD) Patients on Therapeutic Doses of Paricalcitol Does Not Decrease Inflammation Vidya M. Raj Krishnamurthy,[2] Christi M. Terry,[1,2] Tom H. Greene,[1,2] Guo Wei,[2] Alfred K. Cheung,[1,2] Srinivasan Beddhu.[1,2] *[1]VA; [2]Univ Utah.*

Background: Even though, most HD patients receive active vitamin D, it is unclear whether additional therapy with ergo in those with calcidiol deficiency would be beneficial.

Methods: We conducted a randomized double blind cross-over trial of ergo (50,000 U/wk) vs placebo in 24 HD patients with plasma 25(OH)vitamin D levels <30 ng/ml and high sensitivity C-reactive protein (hs-CRP) > 3mg/L and who are on paricalcitol with iPTH range between 150-600 pg/ml. The primary outcome was serum IL6 and secondary end points were plasma hs-CRP and TNFα as well as peripheral blood monocyte production of IL-6 and TNFα (presented as a separate abstract). The study was 80% powered to detect 40% reduction in the geometric mean IL6.

Participants were randomly assigned to ergo vs placebo for 12 wks, followed by 4 wk washout and cross-over for 12 wks. Pre-HD blood samples were collected at baseline and wks 12, 16 and 28. The samples were stored at -80°C freezer before analyses. Plasma IL6 and TNFα levels were measured using a DuoSet ELISA development system. Mixed effects model for a 2-period 2-treatment cross-over design was used.

Results: The mean age was 59 ± 13 yrs. 42% were men and 80% were white. 67% had DM. Average duration of ESRD was 3.7±4.6 yrs. Mean serum 25 OH vitamin D levels were 15.6±5.2 ng/ml and median hsCRP levels were 8.8 (IQR 4.2- 19.1) mg/L. The main effects (post vs. pre in active group vs. post vs. pre in placebo group) are summarized in Table.

Difference of plasma biomarkers in the pre and post ergo vs placebo treatment

Biomarkers	mean(95%CI)	p
Plasma 25(OH)D ng/ml	17.5(9.3-25.7)	<0.001
Serum IL6pg/ml*	0.84(0.55-1.29)	0.26
Serum TNFαpg/ml*	1.06(0.54-2.11)	0.75
Serum hs-CRP mg/L*	1.16(0.63-2.14)	0.76

*log transformation of IL-6, TNFα and hs-CRP

Even though active therapy increased serum vitamin D levels, active therapy did not decrease serum levels of IL-6, hsCRP or TNFα.

Conclusions: Ergo Rx ↑ plasma 25(OH)D levels but did not ↓ inflammation in calcidiol deficient HD patients on therapeutic doses of paricalcitol.

SA-PO2297

Effects of Paricalcitol or Cinacalcet Treatment on CKD-BMD Markers in Subjects with Secondary Hyperparathyroidism (SHPT): Results from IMPACT-SHPT

Kevin J. Martin,[1] Markus Ketteler,[2] Mario Cozzolino,[3] David J. Goldsmith,[4] Amit Sharma,[5] Michael Amdahl,[6] Samina Khan.[6] *[1]Saint Louis U; [2]Klinikum Coburg; [3]Paolo Hosp; [4]Guy's Hosp; [5]Boise Kidney and Hypertension Inst; [6]Abbott.*

Background: Managing SHPT is vital for the prevention of CKD-BMD in subjects on hemodialysis (HD). IMPACT compared SHPT treatment effects of the selective vitamin D receptor (VDR) activator, paricalcitol, with the calcimimetic, cinacalcet. Here we report effects on markers of CKD-BMD.

Methods: IMPACT was a randomized 28 wk, phase 4, international, open-label study of HD subjects receiving IV (IV Stratum) or oral paricalcitol (Oral Stratum) with supplemental cinacalcet for hypercalcemia, compared to subjects treated with cinacalcet with low dose vitamin D. Mean reductions in CKD-BMD markers from baseline to end of study were determined.

Results: In the IV Stratum, there were 62 paricalcitol and 64 cinacalcet subjects (60% male, mean age:61±12 years, mean duration of dialysis:4.1±4.0 years). For the Oral Stratum, there were 72 paricalcitol subjects and 70 cinacalcet subjects (65% male, mean age:65±13 years, mean duration of dialysis:3.9±3.2 years). In the IV Stratum, paricalcitol had significantly greater reductions in iPTH, compared to cinacalcet. In the Oral Stratum paricalcitol demonstrated numerically, but not statistically, greater reductions in iPTH. In both strata paricalcitol reduced alkaline phosphatase (AP) and bone-specific alkaline phosphatase (BAP) while cinacalcet increased AP and BAP.

Conclusions: In contrast to cinacalcet treatment, therapy with paricalcitol was associated with decreases in AP and BAP. There were minimal changes in calcium in both groups. However, long term effects on bone markers of CKD-MBD remain to be determined.

	IV Stratum		Oral Stratum	
	Paricalcitol	Cinacalcet	Paricalcitol	Cinacalcet
iPTH (pg/mL)	N=60	N=60	N=70	N=70
BL	527.6 ± 18.8	523.8 ± 18.9	500.6 ± 19.9	509.5 ± 16.6
Mean Δ	-244.2* ± 36.4	-78.4 ± 36.4	-216.3 ± 24.5	-150.2 ± 24.5
Alkaline Phosphatase (IU/L)	N=50	N=51	N=53	N=64
BL	109.2 ± 7.1	124.0 ± 6.0	95.6 ± 4.7	104.5 ± 5.7
Mean Δ	-17.5* ± 6.6	+28.9 ± 6.5	-14.4* ± 5.1	+4.3 ± 4.6
Bone-specific Alkaline Phosphatase (U/L)	N=50	N=50	N=54	N=61
BL	35.9 ± 2.3	41.9 ± 3.1	39.2 ± 3.1	47.6 ± 2.5
Mean Δ	-8.5* ± 3.6	+20.4 ± 3.6	-12.0 * ± 2.6	+0.8 ± 2.5
Calcium (mg/dL)	N=60	N=61	N=70	N=70
BL	9.0 ± 0.1	9.1 ±0.1	9.1 ± 0.1	9.1 ± 0.1
Mean Δ	+0.5* ± 0.1	-0.7 ± 0.1	+0.3* ± 0.1	-0.7 ± 0.1
Phosphorus (mg/dL)	N=60	N=60	N=70	N=70
BL	4.8 ±0.1	4.9 ± 0.1	4.7 ± 0.1	4.4 ± 0.1
Mean Δ	+0.2 ± 02	-0.2 ± 0.2	+0.7* ± 0.2	+0.2 ± 0.2

*P<0.05 between treatment groups, values are units ± SEM

Funding: Pharmaceutical Company Support

SA-PO2298

Alienating Effect of Cinacalcet on PTH Secretion from Parathyroid Growth in Patients with Secondary Hyperparathyroidism

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Background: Cinacalcet with various doses and derivatives of vitamine D is effective in controlling secondary hyperparathyroidism (SHPT) complicated with chronic kidney disease. We had conducted a clinical trial to confirm the suppressive effect of the combination therapy of cinacalcet with maxacalcitol, a vitamin D derivatives, on moderate to severe SHPT whose intact parathyroid hormones (iPTH) were ranged from 300 to 1000pg/ml for one year (UMIN 000001793). We demonstrated the combination therapy could be useful to keep the suppression of PTH secretion (43rd ASN Annual Meeting, SA-PO2164, 2010). However, little is known whether this suppression would last for long time. In this extensive study, we shows the follow-up data of SHPT and parathyroid gland size for another two years in this cohort.

Methods: 53 patients with 157 of enlarged parathyroid glands were enrolled at the initiation. The biochemical data and gland size was measured every 6 months.

Results: The average dose of cinacalcet and maxacalcitol were 39mg daily and 14μg per week at the end of the study, respectively. PTH decreased significantly from 565.5±32.0pg/ml (mean±SE) to 164.3±20.1pg/ml after one-year treatment, 134.0±14.2 after another one-year (n=29), and 178.7±22.5 after another two-year (n=18). Half of the enlarged parathyroid glands showed significant volume reduction after the one-year treatment. On the other hand, the growth rate of expanding glands even under the treatment was similar to that of maxacalcitol alone. Thus, the total volume of glands did not change throughout this study periods. The cystic change that was observed in 10% of glands at the first year could be mostly undetectable by a high-resolution color Doppler ultrasonography after that. The major causes of drop-out were intolerance, ineffectiveness, caidio-vascular event, and hemorrhagic gastric ulcer.

Conclusions: Our findings indicate that the combination of cinacalcet with maxacalcitol might have a differential effect on parathyroid growth and PTH secretion in patients with SHPT. Further study would be required to clarify the limitation of this therapy.

Funding: Private Foundation Support

SA-PO2299

Modification of Human Artery Inflammation by Paricalcitol Is Altered in CKD

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Background: Arterial inflammation in patients with CKD is associated with vascular calcification. We investigated the role of vitamin D receptor activators (VDRa), paricalcitol and calcitriol on inflammatory responses of arterial wall.

Methods: Arterial explants from CKD patients undergoing renal transplant (n=18) and healthy donors (n=7) were incubated for 48 hours with TNF (20ng/ml), calcitriol (100nM) or paricalcitol (300nM) with/without TNFα. Expression of target gene mRNA was quantified by real-time PCR.

Results: Cbfa1 and osteocalcin (osteoblast markers) were significantly higher in CKD arteries (2.5 fold, $p<0.05$) and both VDRa inhibited Cbfa1 ($p<0.05$) but not osteocalcin. Matrix metabolizing enzymes were quantified. Basal expression of MMP2 was similar, but MMP9 was significantly lower in CKD arteries. Both were unaltered by VDRa.

Components of the vascular vitamin D system (VDR, CYP27B1 and CYP24A1) were analyzed. Basal VDR expression was suppressed by 60% in CKD explants ($p<0.05$) and increased in both groups by VDRa ($p<0.05$). Basal CYP24A1 expression was similar in both groups and was induced by VDRa. The response was much greater in CKD group (20 fold in control; $p<0.05$ vs 300-900 fold in CKD; $p<0.01$). Basal CYP27B1 was 2 fold higher in CKD and was unaltered by VDRa.

To assess inflammatory responses, explants were treated with TNFα and changes in osteoblastic genes, MMPs and VDR measured. Paricalcitol attenuated TNFα induction of these genes in control group, but did not modify the muted response in CKD group. In control arteries CYP27B1 and CYP24A1 were induced by TNFα but not modified by paricalcitol. Both enzymes were induced by TNFα in CKD arteries. Interestingly TNFα attenuated paricalcitol mediated CYP24A1 upregulation, whereas paricalcitol attenuated TNFα induction of CYP27B1.

Conclusions: Our findings indicate that arteries from patients with advanced CKD have undergone phenotypic transformation with reduced VDR expression and enhanced CYP24A1 responsiveness. There was a differential VDRa effect in healthy arteries compared to CKD.

Funding: Pharmaceutical Company Support

SA-PO2300

Safe and Effective Treatment of Vitamin D Deficiency in Pediatric Dialysis Patients

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Background: We and others have shown that vitamin D deficiency (vit D def) is highly prevalent in Pediatric (ped) dialysis pts. However, no treatment (tmt) guidelines exist for ped dialysis pts. Purpose of this study was to establish an safe and effective tmt regimen for vit D def in ped dialysis pts and assess effects of tmt on biochemical mineral parameters**.**

Methods: 10 month prospective study of 58 ped pts (median age 15y; range 1-24y; 28 Hispanic, 16 Black, 13 White, 1 Asian; 31 HD/25 PD /2 home HD). Vit D def was diagnosed by serum 25-hydroxy vitamin (25vitD) <30 ng/dL. KDOQI guidelines for tmt of vit D def in adults with chronic kidney disease (CKD) were followed: if serum 25vitD level <15 ng/mL, oral ergocalciferol 50,000 units weekly x 4 followed by monthly doses x 6 or 4000 units daily x 12 weeks; if 25vitD level 16-29, 50,000 units monthly x 6 or 2000 units daily for 12 weeks. 28/31 HD pts were treated in-center with either weekly or monthly ergocalciferol. Serum 1,25-dihydroxy vit D (1,25vitD) was assessed in 55 pts pre- and end tmt.

Results: 54/58 pts (93%) had vit D def with 13/58 (23%) severe (serum 25vitD <7 ng/mL). 54/58 pts were taking IV or oral calcitriol; 4/58 received IV paracalcitol. Ergocalciferol tmt improved mean serum 25vitD from 15.5 to 30.1 ng/mL ($p=0.0001$, Table). Tmt response did not differ by modality or pt ethnicity. Serum Ca, P, PTH & 1,25vitD were not different at end of tmt compared with pre-tmt.

Effects of Oral Ergocalciferol Treatment of Vitamin D Deficiency

	25vitD(ng/ml)*	1,25vitD(pg/ml)	Ca(mg/dL)	P(mg/dL)	PTH(ng/L)
Pre-ergocalciferol	15.5(8.8)	31.3(20.4)	10.1(0.7)	5.7(1.6)	385(402.5)
End ergocalciferol	30.1(15.4)	30.6(19.1)	10.1(1)	6.2(1.9)	355(313.9)

Values expressed as mean(SD) *p=0.0001

Conclusions: 1) Vit D def can be treated safely and effectively with oral ergocalciferol in ped dialysis pts as young as 1 y old using adult CKD KDOQI guidelines. 2) If pt adherence is a concern, in-center tmt with weekly or monthly doses is safe and not associated with hypercalcemia. 3) Serum PTH was not different at end ergocalciferol tmt, so oral ergocalciferol improves vit D def in ped dialysis pts, but does not by itself correct high serum PTH.

Funding: Clinical Revenue Support

SA-PO2301

Comparison of Paricalcitol and Cinacalcet for the Treatment of Secondary Hyperparathyroidism (SHPT) in Subjects on Hemodialysis: Secondary Analyses from IMPACT Study

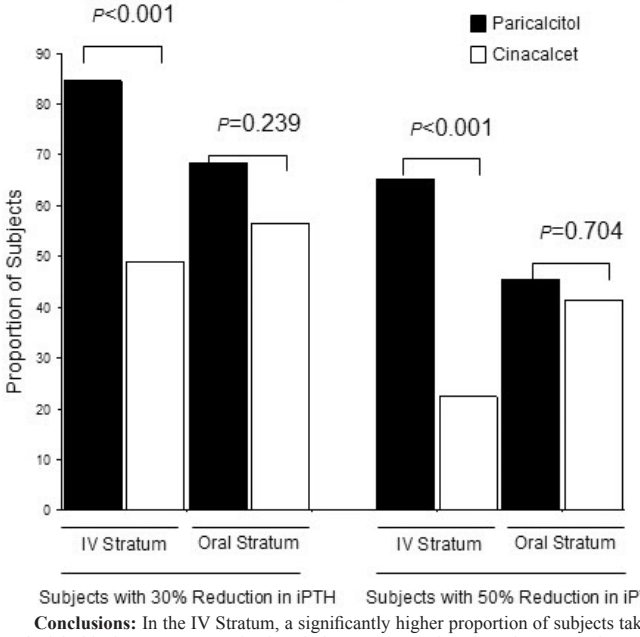
Markus Ketteler,[1] Kevin J. Martin,[2] Mario Cozzolino,[3] David J. Goldsmith,[4] Amit Sharma,[5] Michael Amdahl,[6] Samina Khan.[6] *[1]Klinikum Coburg; [2]Saint Louis U; [3]Paolo Hosp; [4]Guy's Hosp; [5]Boise Kidney and Hypertension Inst; [6]Abbott.*

Background: Optimal management of SHPT is important for the prevention of CKD-BMD in patients undergoing hemodialysis (HD). IMPACT-SHPT compared the reduction of iPTH levels in HD subjects treated with the selective vitamin D receptor (VDR) activator, paricalcitol, or the calcimimetic, cinacalcet. Here we report the secondary endpoint results on iPTH suppression.

Methods: IMPACT was a randomized 28 wk, phase 4, international, open-label study of subjects undergoing hemodialysis receiving IV (IV Stratum) or oral paricalcitol (Oral Stratum) with supplementary cinacalcet for hypercalcemia, compared with subjects receiving cinacalcet with low-dose vitamin D. The proportion of subjects with at least 30% and 50% reduction from baseline to mean iPTH during weeks 21-28 were determined as secondary analyses.

Results: In the IV Stratum there were 62 paricalcitol subjects and 64 cinacalcet subjects (60% male, mean age:61±12 years, mean duration of dialysis:4.1±4.0 years). In Oral Stratum there were 72 paricalcitol subjects and 71 cinacalcet subjects (65% male, mean age:65±13 years, mean duration of dialysis:3.9±3.2 years.

Figure: Proportion of Subjects with at Least 30% or 50% Reduction from BL to Mean iPTH During Weeks 21-28



Conclusions: In the IV Stratum, a significantly higher proportion of subjects taking paricalcitol had 30% or 50% reductions in iPTH compared to cinacalcet. In Oral Stratum subjects taking paricalcitol had numerically greater but not statistically significant reductions in iPTH compared with subjects taking cinacalcet. However, the beneficial effect of iPTH reduction on long-term outcomes from observational studies needs to be determined in clinical trials.

Funding: Pharmaceutical Company Support

SA-PO2302

Prevalence and Risk Factors for 25OH Vitamin D Deficiency in Hemodialysis Patients

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Background: Despite routine administration of 1,25OH Vit D, 25OH Vit D (25OHD) deficiency is common in dialysis patients, and the reasons for this deficiency remain poorly understood.

Methods: We performed 25OHD testing and analyzed demographic data and medication histories in 97 hemodialysis outpatients. A subset of 41 patients participated in dietary and lifestyle surveys to assess their sun exposure and average intake of Vit D containing foods.

Results: The prevalence of Vit D deficiency was 90.8% (25OHD level <30ng/mL), and 24.5% of patients had severe Vit D deficiency with level <10ng/mL. Median Vit D level was 14.8ng/mL (IQR=11.2). We found no association between Vit D level and age, gender, race, dialysis vintage, smoking status, serum albumin or URR. Vit D levels negatively correlated with BMI (Spearman's ρ=-0.271/p=0.010).

Lifestyle surveys revealed that in summer, patients spent a median number of 7 days/week outside with daily >15 min sun exposure; while in winter they spent a median number of 3 days. 26.8% of patients reported sunscreen use.

Dietary surveys showed a median weekly vitamin D intake of 1113 IU (IQR=748, Table 1), which was significantly below FDA recommendation of 2800-5600 IU/week.

Nutritional guidelines for dialysis patients suggest a limited intake of Vit D-containing dairy products because they are also phosphate-rich. Since many patients struggle to adhere to phosphate binder regimen, they reported being counseled against dairy product consumption.

Conclusions: Our study demonstrates a higher than previously reported prevalence of Vit D deficiency among hemodialysis patients despite seemingly adequate sun exposure and a low rate of sunscreen use. Based on the findings indicative of a physician-initiated bias against intake of Vit D-containing foods, we recommend oral D3 as a safe and inexpensive approach to correct this abnormality.

Table 1: Median intake of vit D-containing foods

Item	Vit D (IU) per serving	Amount per serving	Median number of servings/week (IQR)	Total vit D/week (IU)
Fish	100-654	3oz	2 (2)	754
Milk	100	8oz	0.5 (2)	50
Yogurt	80	6oz	0 (0.75)	0
Cheese	6-12	1oz	6 (5)	54
Cereal	40	1cup	2 (4)	80
Eggs	25	1 egg	5 (5)	125
				Total~1063 IU/ week

Funding: Private Foundation Support

SA-PO2303

Predictors of Vitamin D Status in Incident Chronic Kidney Disease Patients: A Cross-Sectional Analysis in a High UV Climate

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Background: Hypovitaminosis D is a significant problem in Chronic Kidney Disease (CKD) and is associated with adverse outcomes. Whether high ambient UV exposure can mitigate this problem is unknown. Our aim was to determine vitamin D status in a sub-tropical climate amongst an unselected incident CKD population; assess risks and correlates, and review whether higher 25-hydroxyvitamin D (25-OHD) can abate the decrement in 1,25-dihydroxyvitamin D (1,25-OHD) normally encountered with advancing CKD.

Methods: Prospective cross-sectional study of 593 consecutive CKD patients stage 1-5 referred to Princess Alexandra Hospital, Brisbane, Australia (27°28'S). The main outcome measure was 25-OHD sufficiency (≥30ng/mL), and bone-mineral parameters including 1,25-OHD, calcium, and phosphate.

Results: In spite of potentially higher environmental UV exposure, only 48% of patients with CKD were 25-OHD sufficient. Traditional risks for hypovitaminosis D were maintained, and sufficiency was independently predicted by testing in the Summer/Autumn period (OR 3.17, 95%CI: 2.07-4.86, p<0.001), male gender (OR 2.61, 95%CI: 1.68-4.05, p<0.001), Caucasian race (OR 2.19, 95%CI: 1.25-3.84, p<0.001), normal albumin (OR 0.46, 95%CI: 0.23-0.90, p=0.02), and normal BMI (OR 1.80, 95%CI: 1.08-3.00, p=0.02). Vitamin D sufficiency was also associated with higher corrected calcium (0.4 mg/dL increments; OR 1.42, 95%CI: 1.16-1.74, p=0.001) and higher 1,25-OHD (OR 1.01, 95%CI: 1.01-1.02, p<0.001). Whilst circulating 25-OHD concentrations were relatively maintained across the range of renal function observed, 1,25-OHD concentrations fell with advancing CKD.

Conclusions: 25-OHD insufficiency is mitigated but still highly prevalent in patients with CKD in a high ambient UV environment. Despite the maintenance of relatively higher 25-OHD concentrations with advancing CKD, substrate availability does not appear to be a major determinant of circulating 1, 25-OHD.

SA-PO2304

Thyroid Hormones Decrease the Plasma 1alpha, 25-dihydroxyvitamin D_3 Levels through Transcriptional Repression of the Renal 25-hydroxyvitamin D_3 1alpha-hydroxylase (CYP27B1) Gene

Mina Kozai, Hironori Yamamoto, Ayako Otani, Shoko Ikeda, Otoki Nakahashi, Yutaka Taketani, Eiji Takeda. *Depertment of Clinical Nutrition, University of Tokushima, Japan.*

Background: The renal 25-hydroxyvitamin $D_3$1α-hydroxylase enzyme (CYP27B1), is implicated in the control of plasma 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels. Previously, it has been demonstrated that the low levels of plasma $1,25(OH)_2D$ in hyperthyroidism patients and the decrease of $1,25(OH)_2D$ synthesis in perfused kidneys treated with 3, 5, 3'-tri-iodothyronine (T_3). However, the regulation mechanism of renal vitamin D metabolism by thyroid hormones is still not clear.

Methods: To address effects of T_3 on the plasma $1,25(OH)_2D$ levels and renal CYP27B1 gene expression, C57BL6 mice were rendered pharmacologically hypo- and hyperthyroid by administration of propyl-thiouracil or T_3. Western blots, real-time PCR and in situ hybridization analysis were used to elucidate the CYP27B1 gene expression in kidney. CYP27B1 gene promoter activity was measured using luciferase reporter assay system. DNA-protein interaction for cis-elemets of CYP27B1 gene were assessed by gel shift assay.

Results: Hyperthyroid mice showed low levels of plasma $1,25(OH)_2D$ and marked decrease in renal CYP27B1 expression levels. In addition, T_3 inhibited the renal CYP27B1 mRNA expression highly induced in mice given low-phosphorus diet or low-calcium diet, vitamin D receptor-knockout mice and alpha klotho mutant mice. The CYP27B1 promoter activity in renal proximal tubular cells were inhibited by T_3 in thyroid hormone receptor

β1 (TRβ1)-dependent manner. We also found the transrepression of CYP27B1 gene by T_3 involves a negative vitamin D response element (1αnVDRE) and VDR interacting repressor (VDIR). The gel shift analysis demonstrated that the binding of VDIR to 1αnVDRE was reduced by TRβ1/retinoid X receptor α. Furtheremore, the inhibitors of histone deacetylase and DNA metyltransferase completely abrogated T_3-dependent repression of CYP27B1 gene premoter.

Conclusions: These results suggest that thyroid hormones decrease the plasma $1,25(OH)_2D$ levels through transcriptional repression of the renal CYP27B1 gene by TRβ1.

Funding: Government Support - Non-U.S.

SA-PO2305

Web-Based Evaluation of Clinical Benefit of Cinacalcet in End-Stage Renal Disease – The WELCOME Study Jaroslav Rosenberger,[1] Piotr Mierzicki,[2] Alexander Selyutin,[3] Frantisek Svara,[4] Kinga Jedynasty.[5] *[1]FMC, SNP c.1., Kosice, Slovakia (Slovak Republic); [2]SP Wojewodzki Szpital Specjalistyczny, Chelm, Poland; [3]Regional Clinical Hospital #1 (Orenburg city), Moscow, Russian Federation; [4]Department of Medicine, Strahov General University Hospital, Prague, Czech Republic; [5]Amgen GmbH-CEE HO, Vienna, Austria.*

Background: Mimpara® (MIM) modulates calcium-sensing receptors and is approved for treatment of secondary hyperparathyroidism (sHPT) in End-Stage Renal Disease (ESRD). WELCOME is a multicenter, noninterventional observational study in patients with ESRD from Central and Eastern Europe (CEE) receiving MIM for sHPT.

Objective: Describe attainment of sHPT targets during MIM treatment, before (BGI) and after (AGI) release of KDIGO guidelines in 2009.

	BGI [2006 to Jun2009] N=704		AGI [Jul2009 to Sep2010] N=357	
Treatment Target (Completers)	Enroll	6-mo	Enroll	6-mo
PTH ≤300 pg/mL (%)	2.7	31.8	2.3	28.3
PTH (pg/mL) Mean (SD)	807 (436)	609 (465)	897 (521)	704 (521)
Ca 8.4-9.5 mg/dL (%)	46.4	60.8	47.3	55.5
P 3.5-5.5 mg/dL (%)	22.4	34.5	30.3	41.5
CaXP <55 mg2/dL2 (%)	37.5	66.6	45.7	66.7
PB use (%)	83.5	67.9	86.3	72.0
VitD use (%)	59.2	44.6	61.3	48.5

Methods: Patients on peritoneal dialysis (PD) or hemodialysis (HD) starting MIM ≤1 month prior to the study were eligible. Patient data were collected through a web-based case report form. Primary endpoint: % achieving PTH ≤300 pg/mL after 6 months. Secondary endpoints: % at target for Ca, P, or Ca´P at enrolment and 6 months; MIM dose; vitamin D sterol (VitD) and phosphate binder (PB) use; safety.

Results: 1061 patients completed ≥144 days of MIM (≥80% of planned). Initial MIM dose was 31.1±6.0 (BGI), 31.7±7.4 mg (AGI); mean daily dose at 6 months was 37.6±11.6 (BGI) and 37.5±11.3 mg/day (AGI); treatment results for completers are shown below. Among all enrolled (1230), adverse drug reactions (ADR) reported by 4.9% BGI and 4.4% AGI included gastrointestinal complaints and paresthesia. One serious ADR was reported; all-cause mortality was 2.8%.

Conclusions: MIM therapy increased % of patients meeting some therapeutic targets; mean PTH was not changed AGI. Reduced PB and VitD use was observed after 6 months of MIM therapy.

Sponsor:Amgen GmbH CEE Headquarters

Funding: Pharmaceutical Company Support

SA-PO2306

Vitamin D Deficiency in Chronic Renal Failure Patients: A Screening Survey and Clinical Feature Analysis from a Single Hemodialysis Center in China Ping Wen, Chunsun Dai, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Vitamin D deficiency has been reported to be closely associated with many clinical complications such as cardiovascular events and osteodystrophy in chronic renal failure patients. The aims of this study are to screen serum Vitamin D level in chronic renal failure patients from our hemodialysis center in china and analyze their clinical features.

Methods: In this study, 278 chronic renal failure patients were included and serum 25-hydroxyvitamin-D level was detected by using a commercial available ELISA kit. The correlation of patients' clinical features including age, gender, body mass index (BMI), blood pressure, medicine, primary kidney disease, duration of dialysis, complications and biochemical indexes with their serum 25(OH)D level were analyzed by using multiple logic regression analysis method

Results: Among the 278 patients, 175 were male. The patients' age was ranged from 23 to 89 years (average was 57.4 years) and the average duration of their dialysis was 62.3 months. Serum 25(OH)D deficiency (<15ng/ml) and insufficiency (15ng/ml≤25(OH)D<30ng/ml) were present in 55% and 37% of the dialysis patients respectively. Only 8% of the patients showed normal serum 25(OH)D level. Further analysis of their clinical features revealed that Vitamin D deficiency was more prevalent in female or patients with cardiovascular complications($P<0.05$), while less Vitamin D deficiency could be found in male or patients received RAAS inhibitors or a-blockers($P<0.05$). Diabetic patients showed a low 25(OH)D level compared to the other patients. No close correlation was found as to the other clinical features including age, BMI, serum calcium and iPTH levels with Vitamin D deficiency.

Conclusions: The prevalence of hypovitaminosis D is high in chronic renal failure patients in china. Female, cardiovascular complications and diabetes are correlated with Vitamin D deficiency and taking RAAS inhibitors or a-blockers may benefit the patients with such deficiency.

Funding: Government Support - Non-U.S.

SA-PO2307

The Cardiovascular Effects of Cinacalcet in Hemodialysis Patients with Secondary Hyperparathyroidism Sun Ryoung Choi, Hoon Suk Park, In O Sun, Byung Ha Chung, Bumsoon Choi, Chul Woo Yang, Yong-Soo Kim, Cheol Whee Park. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: Recently, it has been suggested that cinacalcet significantly improves cardiovascular survival in hemodialysis patients and up-regulation of the calcium-sensing receptor may be involved in the improved outcome. The calcium-sensing receptor has been identified in heart endocardial epithelium, microvasculature of the myocardium, aortic endothelium and the vascular smooth muscle.Thus, we evaluated the effects of cinacalcet on the cardiovascular system in hemodialysis patients with secondary hyperparathyroidism.

Methods: We studied 14 patients with ESRD who had high levels of intact PTH (iPTH,>300pg/mL) and corrected serum calcium (cCa,>9.0mg/dL) with cinacalcet over 20-week period. After treatment, we performed flow-mediated dilation (FMD), cadio-ankle vascular index (CAVI) and echocardiographic analyses.

Results: Twenty weeks cinaclacet treatment significantly decreased blood levels of iPTH (666.8±257.8 vs. 277.1±266.7pg/mL; $p<0.01$),calcium (9.4±0.50vs.8.7±0.50mg/dL; $P<0.01$),phosphorus(6.9±1.38vs.5.0±1.49mg/dL;$P<0.01$),calcium x phosphorus product (65.9±15.7vs.44.5±15.3; $P<0.01$)and 25(OH) vitamin D(8.5±2.8vs.7.6±2.7ng/mL; $P<0.05$). But the level of C-reactive protein was not affected by treatment.There were no differences in systolic(134.5±22.1vs.128.0±21.0mmHg;$P=0.2$) and diastolic blood pressures(75.2 ±14.6vs.72.7±14.2mmHg;$P=0.45$).There were notable reduction trends in the LV mass (244.6±94.0vs.212.9±63.9gm),the LV mass index (158.6±63.8vs.138.3±45.7g/m^2) and E/E'(15.2±6.8vs.13.5±5.6),although they did not achieve statistical significance.There were no significant changes in the ejection fraction and fractional shortening. In contrast, of great interest, cinacalcet significantly improved FMD(8.6±2.9vs.14.3±2.83%; $P<0.01$) and enhanced CAVI (8.8±2.3vs.7.6±2.4; $P<0.05$) as markers of endothelial-dependent arterial dilation and arterial compliance, respectively.

Conclusions: Cinacalcet treatment in hemodialysis patients with secondary hyperparathyroidism ameliorates aortic stiffness, arterial compliance and seems to improve cardiac hypertrophy

SA-PO2308

Nutritional Vitamin D Use in Chronic Kidney Disease: A Survey of Pediatric Nephrologists Lindsay M. Griffin,[1] Susan L. Furth,[1] Michelle Denburg,[1] Isidro B. Salusky,[2] Mary B. Leonard.[1] *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of California - Los Angeles, Los Angeles, CA.*

Background: Controversy abounds regarding use of vitamin D (cholecalciferol and ergocalciferol) in CKD. Our aims are to describe vitamin D supplementation by pediatric nephrologists and to identify physician and patient characteristics that influence the choice to supplement.

Methods: We surveyed 1,176 International Pediatric Nephrology Association physician members. The questionnaire included 8 vignettes comprised of potential determinants of supplementation (starred in Table). Vignettes were stratified by vitamin D level and randomly assigned to IPNA members. Nephrologists were asked if they would recommend supplementation in each case. Multivariate logistic regression was used to assess odds of supplementation.

Results: The response rate was 45% (479/1074; 50 members do not see pediatric CKD patients and 52 emails were invalid); 64% in the US (200/311) and 37% (281/763) in other countries. These preliminary data are limited to US respondents. The proportions recommending supplementation for vitamin D levels of < 10, 10-19, 20-29 and > 30 ng/mL were 96, 95, 70%, and 29%, respectively. Low vitamin D levels were the most influential factor in increasing odds of supplementation (Table) but elevated PTH, time since training, and CKD severity were also associated with supplementation.

Table. Logistic Regression Model of Recommending Supplementation Amongst US Respondents

Characteristic	Group	Adjusted Odds Ratio (95% CI)	p-value
CKD Stage*	CKD 2-3	Reference	
	CKD 4-5	1.5 (1.1 - 2.1)	<0.05
	Dialysis	1.2 (0.8 - 1.8)	0.38
PTH Level*	At target for stage	Reference	
	Above target for stage	1.4 (1.01 - 1.9)	<0.05
Skin Pigmentation*	Light	Reference	
	Dark	1.0 (0.8 - 1.4)	0.88
CKD cause*	CAKUT	Reference	
	FSGS, Normal Albumin	1.01 (0.7 - 1.4)	0.95
	FSGS, Low Albumin	0.9 (0.6 - 1.2)	0.41
Vitamin D Level (ng/mL)*	≥30	Reference	
	20-29	6.5 (4.5 - 9.4)	<0.0001
	10-19	51.8 (32.1 – 83.8)	<0.0001
	<10	60.0 (33.0 – 109.2)	<0.0001
Year Completed Training, Quartiles	2004 - 2011	Reference	
	1995 - 2003	1.8 (1.2 - 2.8)	<0.01
	1988 - 1994	1.6 (1.02 - 2.4)	<0.05
	1969 - 1987	1.7 (1.1 - 2.6)	<0.01

* Variables included in case vignettes

Conclusions: These data suggest that recommending nutritional vitamin D is common and associated with lower vitamin D levels, elevated PTH, and more time since completed training. Further analyses will assess geographical differences in supplementation. Future studies are needed to validate this questionnaire, documenting actual practices.

Funding: Private Foundation Support

SA-PO2309

Vitamin D Supplementation Is Increasing in the Chronic Renal Insufficiency Cohort (CRIC) and Is Associated with Greater Vitamin D Levels Laura H. Mariani,[1] Justine Shults,[1] Matthew T. White,[1] Cheryl A. Anderson,[2] Harold I. Feldman,[1] Mary B. Leonard.[1] *[1]University of Pennsylvania; [2]Johns Hopkins.*

Background: The recent Institute of Medicine Report on Vitamin D noted a significant increase in use of vitamin D supplements in the population.

Methods: We examined vitamin D supplement use at annual visits for the 3939 subjects in CRIC and used generalized estimating equations (GEE) to describe changes and determinants of supplementation.

Results: The proportion of subjects reporting use of a calciferol increased significantly, largely due to products containing only ergocalciferol or cholecalciferol (Figure 1). Active vitamin D sterol use was stable at 2-3%. In a multivariate logistic GEE model, supplement use was greater in older, female, non-black, married subjects with higher education and lower BMI (Table 1). Nephrology care, CKD cause, degree of proteinuria, exercise, smoking and eGFR were not independent predictors. Serum 25(OH)D levels were measured in 1529 participants at year 1. Reported use of vitamin D supplementation was associated with an 8.3 ng/mL greater (95% C.I. 6.3, 10.3, $p < 0.0001$) level adjusting for race, age, season, BMI, physical activity, proteinuria, and dietary vitamin D intake.

Conclusions: Vitamin D supplementation rates rose significantly among CRIC participants and was associated with greater levels. Studies of vitamin D deficiency and outcomes in CKD should consider changing supplementation practices.

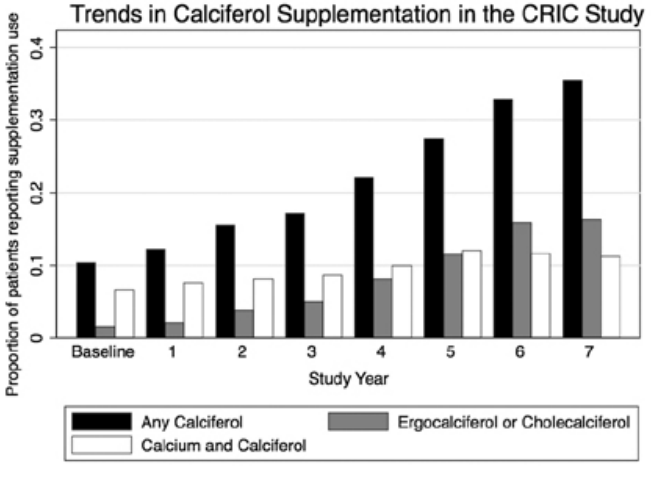

Table 1: GEE Model of Calciferol Supplementation

Predictor	OR (95% CI)
Age (per 10yr)	1.36 (1.27, 1.45)*
Black Race	0.58 (0.51, 0.67)*
Male Sex	0.29 (0.25, 0.33)*
Married	1.19 (1.04, 1.37)†
Education	
Some High School	1.0 (Reference)
High School Grad	1.67 (1.34, 2.07)*
Some College	1.77(1.44, 2.12)*
College Grad	2.00 (1.63, 2.45)*
BMI (kg/m^2)	
<25	1.0 (Reference)
25-29	0.73 (0.60, 0.88)*
30-35	0.68 (0.55, 0.82)*
>35	0.63 (0.53, 0.77)*

* p-value ≤ 0.001; † p-value =0.01

Funding: NIDDK Support

SA-PO2310

Safety of Oral 25 Hydroxy Vitamin D (cholecalciferol) in Treatment of Symptomatic Vitamin D Deficiency in Renal Transplant Recipients-Cinacalcet May Also Be Needed Nihil Chitalia, David Goldsmith. *Nephrology and Transplantation, Guy's and St Thomas' NHS foundation trust, London, United Kingdom.*

Background: Bone mineral disorders (CKD-MBD) are common post transplantation, particularly with an increasing age and longevity of transplant recipients and grafts. Not only is the treatment of CKD-MBD is less well defined in the kidney transplant population, but 'substrate' vitamin D deficiency is also common post kidney transplant in part due to avoidance of sunlight. The safety data on oral native vitamin D supplementation in transplant recipients is not available.

Methods: We present the safety data on use of oral Cholecalciferol (Dekristol®) 20,000 IU fortnightly for 6 months, up to a total of 240,000 IU, given to eight symptomatic kidney transplant recipients; symptoms ranging from muscle aches to lethargy. All patients were confirmed to be 25 hydroxy vitamin D deficient [25(OH)D<37.5 nmol/L] and other potential causes were excluded. One patient had secondary hyperparathyroidism (serum PTH=133 pmol/L) and two patients were on preceding 1α calcidol therapy.

Results: The mean age of the group was 55±16 years with eGFR 52±22 ml/min/1.73m2.

Table 1: Pre and 6 month post vitamin D supplementation biochemical parameters

Variable	Pre supplementation	Post Supplementation	P value
Serum Calcium *(mmol/L)*	2.43±0.15	2.47±0.12	0.56
Serum Phosphate *(mmol/L)*	1.07±0.32	1.12±0.26	0.58
Serum PTH *(pmol/L)*	107±80	74±35	0.26
Serum Alkaline Phosphatase *(U/L)*	156±211	98±58	0.12[¥]
eGFR ml/min/1.73m^2	52±23	50±18	0.45
Serum 25 (OH)D *(nmol/L)*	20.4±8.6	73±18.2	<0.001*

[¥] Wilcoxson Rank sum test, *P<0.05

Two patients suffered hypercalcemia (defined as atleast one serum calcium>2.60 mmol/L) whilst on vitamin D. Both of these patients were borderline hypercalcemic due to prior secondary hyperparathyroidism pre vitamin D supplementation. Both these patients required addition of Cinacalcet to allow for successful sustained oral vitamin D administration.

Conclusions: We have found oral cholecalciferol in the dose of 40,000 IU monthly to be a safe and effective therapy for symptomatic hypovitaminosis D in kidney transplant patients. Concomitant hyperparathyroidism and preexisting hypercalcemia may warrant simultaneous administration of a calcimimetic to allow for continued vitamin D administration.

Funding: Clinical Revenue Support

SA-PO2311

Low 25-hydroxyvitamin D Levels Are Associated with an Increased Risk of Community Acquired Pneumonia Anna Jeanette Jovanovich,[1] Michel B. Chonchol,[1] Shailendra Sharma,[1] Kim McFann,[1] Sidney N. Thornton,[2] Jessica B. Kendrick,[1] John R. Holmen. *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Intermountain Healthcare, Salt Lake City, UT.*

Background: 25-hydroxyvitamin D (25(OH) D) levels are inversely associated with the development of upper respiratory tract and influenza infections. However, the relationship between 25(OH) D levels and the risk of developing community-acquired pneumonia (CAP) has not been evaluated.

Methods: We performed a population-based cohort study of adult patients diagnosed with community-acquired pneumonia (CAP) who had 25(OH)D levels measured 3 to

15 months prior to admission. Vitamin D was evaluated as a continuous variable and a categorical predictor (< 15 ng/mL vs. higher). The primary outcome of interest was CAP admissions identified through ICD9 codes and confirmed with a chest X-ray. Patients with CAP were matched 1:1 with controls on age, sex, race and season. Conditional logistic regression was used to evaluate if vitamin D levels were associated with an increased risk of CAP.

Results: We identified 66 patients with CAP and 66 matched controls. The mean (SD) age of the participants was 60 ± 17 years, 71% were female and 86% were Caucasian. There was a higher prevalence of chronic kidney disease in patients with CAP compared to controls (31.8% vs. 10.6%, p=0.003). There was no significant difference in mean 25(OH) D levels between patients with CAP and controls (31.7 ± 16 and 34.9 ± 16 ng/mL, p=0.33, respectively). After adjustment for diabetes, renal disease and peripheral vascular disease, when evaluated as a categorical predictor, vitamin D levels < 15 ng/mL vs. higher (adjusted OR 2.57, 95% CI 1.08-6.08; p=0.03) were strongly associated with an increased odds of admission for CAP. When 25(OH)D levels were evaluated as a continuous variable, there was no association between increasing vitamin D levels and lower risk of CAP (adjusted OR 0.92, 95% CI 0.81-1.03; p=0.14).

Conclusions: Vitamin D deficiency (< 15 ng/mL) is associated with an increased risk of CAP. Prospective randomized studies examining the effects of vitamin D_3 supplementation on the risk of infection are needed.

SA-PO2312

Arterial Calcification and Its Association with Clinical Variables and Biochemical Markers in Incident Peritoneal Dialysis Patients

Marcela Avila,[1] Carmen Josefina Mora,[1] Carmen María del Prado,[1] Elizabeth G. Hernandez-Infante,[1] Dolores Noriega,[2] Diana Villanueva,[3] Hector Hinojosa Heredia.[4] *[1]UIMEN, CMN SXXI IMSS, Mexico; [2]CLINICA 32, IMSS, Mexico; [3]CLINICA 25, IMSS, Mexico; [4]CLINICA 47, IMSS, Mexico.*

Background: There is not sufficient information available to permit relating arterial calcification with clinical variables and biochemical markers. Our aim was to know the association of arterial calcification with biochemical markers and clinical variables in incident patients on peritoneal dialysis

Methods: We included 178 incident patients on peritoneal dialysis, of which 80% were on CAPD and 20% on APD. Demographic and clinical data were recorded. Arterial calcification (AC) was evaluated in the abdominal aorta and pelvic vessels with simple phase using 4-cut computerized multidetector tomography (MCST), Philips Mx 8000, in the abdominal aorta and pelvis. We measured total Ca, phosphorus, albumin, glucose (GLU), cholesterol, and creatinine in serum with immunoturbidimetric assay (Roche/ Hitachi 902 Germany); Osteocalcin (OT) Osteoprotegerin (OPG), iPTH and IL8 were measured by Milliplex Kit.

Results: The mean age was 49±11, BMI 24±4, 65% male, 65% with diabetic nephropathy as a cause of disease, 49% with diabetes mellitus**;** time on dialysis was 1.5 ±1.2months.

Conclusions: The patients with AC =58% were older (53±7 vs 44±13 years; p<0.05), had significantly higher mean GLU (140±74vs 106±50 mg/dL, p< 0.001); COL (200±48vs180±39mg/dL p<0.005), OPG (1374±1000vs930±600 ng/mLp<0.013), and IL8 (12±5vs9±4ng/mLp<0.013). Lower CREA (10.1±3.1 vs 13±5.3; p<0.05) and ALB(3.28±.5vs3.51±5 g/dL). Diabetes and diabetic nephropathy as a cause of disease were more frequent. At the lumbar and ilialiac levels 52 and 57% respectively of the patients showed calcification, total Score Agaston was 285 (3-9158). After controlling for potential confounding variables, there was significant interaction effect between AC and age (hazard ratio 95% CI) 1.2 (1.03- 1.2) had an increased risk of AC.

Age was the factor most associated with arterial calcification measurements through tomography in incident peritoneal dialysis patients.

Funding: Government Support - Non-U.S.

SA-PO2313

Cinacalcet Loading Test Predicts the Effect of Long-Term Cinacalcet Treatment in Hemodialysis Patients with Secondary Hyperparathyroidism

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Background: Secondary hyperparathyroidism (SHPT) is the most important complication of mineral-bone disease (MBD) in hemodialysis (HD) patients. We prospectively evaluate the effects of cinacalcet on MBD markers using the response of intact parathyroid hormone (iPTH) to cinacalcet loading test.

Methods: Fifty-three HD patients with SHPT were included based on serum iPTH≥180 pg/mL and/or adjusted total serum calcium (sCa) ≥ 10.5mg/dL. sCa, serum albumin, serum phosphate (P) and iPTH were measured before and 3 hours after oral intake of cinacalcet 25mg tablet. After loading test, 48 HD patients started on a single daily dose of 25mg of cinacalcet, and were followed serum markers (sCa, P, iPTH, bone alkaline phosphatase (BAP), intact osteocalcin (iOC) and NTx) at 12, 24 and 48 weeks after therapy. The daily dose of cinacalcet was adjusted between 25 and 75mg according to the control of SHPT.

Results: iPTH (mean +/- SD) decreased from 721 +/- 499 pg/mL to 381 +/- 280 pg/mL after loading test in 48 patients (p<0.001). Serum mean iPTH kept lower levels at 12, 24 and 48 weeks (n=46, 352 +/- 331 pg/mL, n=42, 275 +/- 210 pg/mL, n=42, 225 +/- 147 pg/mL, respectively; p<0.001). Then, mean levels of serum Ca, BAP, iOC and NTx also significantly decreased from 10.0 mg/dL, 33 U/L, 147 ng/mL, 266 nmol BCE/L to 9.6mg/dL, 20 U/L, 70 ng/mL, 140 nmol BCE/L at 48 weeks, respectively. iPTH 316 mg/d at 3hrs (odds ratio (OR) 13.4, 95%CI 2.2–81.2, p = 0.005), increased BAP (>/= 34.0µg/L in male, >/= 35.5µg/L in female; OR 11.4, 95%CI 1.7-77.7, p = 0.013), and increased NTx (>/= 102.3nmol BCE/ L; OR 0.05, 95%CI 0.003-0.908, p = 0.043) were selected by multiple logistic analysis as predictive factors for achieving iPTH </= 180 pg/mL after 48 weeks cinacalcet therapy. Finally, the cut off point of iPTH at 3hrs was 245 pg/mL (sensitivity 0.72, specificity 0.80 by ROC curve) in patients who achieved the JSDT guidelines (intact PTH </= 180 pg/mL and corrected Ca </= 10.0 mg/dL and phosphorus </= 6.0 mg/dL) at 48 weeks.

Conclusions: iPTH levels at 3hrs of cinacalcet loading test predicted the effect of long-term cinacalcet treatment for SHPT in HD patients.

SA-PO2314

Prescription Patterns and Mineral Metabolism Abnormalities in the Cinacalcet Era: Results from the MBD-5D Study

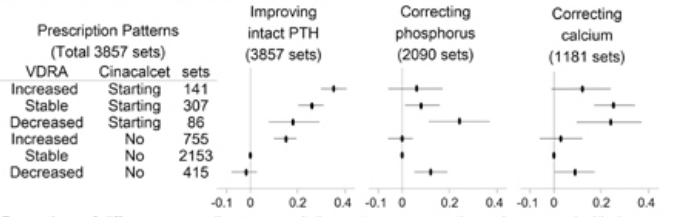
Masafumi Fukagawa,[1] Shingo Fukuma,[2] Tadao Akizawa,[3] Yoshihiro Onishi,[2] Shunichi Fukuhara,[4] Kiyoshi Kurokawa.[5] *[1]Tokai University; [2]iHope International; [3]Showa University; [4]Kyoto University; [5]National Graduate Institute for Policy Studies.*

Background: Prescription patterns for hemodialysis patients with secondary hyperparathyroidism have begun to vary widely after market introduction of cinacalcet.

Methods: Using data from the MBD-5D study in Japan (January 2008 to July 2009), we conducted a cohort study involving 1725 hemodialysis patients (3857 sets for repeated measures) with intact PTH level exceeding 180 pg/ml who used intravenous Vitamin D receptor activator (VDRA) without cinacalcet at baseline. We defined 6 prescription patterns based on combination of cinacalcet (starting or not starting) and VDRA dose (decreased, stable, or increased). The main outcome measure was proportion of difference in improving intact PTH levels by at least 1 category (<180, 180-299, 300-499, and ≥500 pg/mL) adjusting for age, gender, dialysis duration, phosphate binder, dialysate calcium concentration, Kt/V and levels of phosphorus, and intact PTH. The secondary outcome measures were proportion of difference in correcting phosphorus (3.5-6.0 mg/dL) and calcium levels (8.4-10.0 mg/dL).

Results: Adjusted proportions of difference are summarized in the figure. The patterns of "starting cinacalcet" and "increased VDRA" were independently associated with improving intact PTH levels and showed an additive effect. Among patients with high phosphorus or calcium levels, the patterns involving starting cinacalcet and decreased VDRA were independently associated with correcting phosphorus or calcium levels.

Figure| Adjusted proportion of difference for improving intact PTH, correcting phosphorus and calcium levels according to prescription patterns



Proportions of difference according to prescription patterns were estimated compared with the category of stable VDRA without cinacalcet.
Increased VDRA: >25% increased dose, Stable VDRA: between -25% to 25% dose, Decreased VDRA: >25% decreased dose

Conclusions: Our results showed association between prescription patterns of cinacalcet and VDRA and subsequent levels of intact PTH, phosphorus, and calcium. These findings may subsequently be used to determine clinically effective prescription patterns.

Funding: Pharmaceutical Company Support

SA-PO2315

Genetic Variants in the Vitamin D Receptor Gene and Individual Response to IV 1,25-Vitamin D in Hemodialysis Patients

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Background: Changes in PTH, Ca and PO4 in response to intravenous 1,25-vitamin D (IV vitD) treatment differ in individual hemodialysis (HD) patients. Genetic factors may influence response to treatment. We investigated whether HD patients with different BsmI genotypes in the vitamin D receptor (*VDR*) gene have differential responses to IV vitD.

Methods: ArMORR is a nationally representative prospective cohort study of incidental HD patients in dialysis centers operated by Fresenius in 2004-2005. 890 random patients in this cohort who had DNA available, PTH levels >150pg/ml and who received IV vitD treatment during the first 90 days of HD were defined as the treatment group. 315 patients who did not receive IV vitD during first 90 days of HD were defined as the untreated group. DNA was extracted and genotyping for the *VDR* BsmI polymorphism (rs1544410, alleles A/G) was performed using the Sequenom platform. The primary outcome was the change in PTH; secondary outcomes were changes in Ca, PO4 and CaxPO4 product after IV vit D treatment. These outcomes were compared among the three genotypes (BB *vs* Bb *vs* bb, corresponding to AA *vs* AG *vs* GG alleles) using ANOVA.

Results: In the IV vitD treatment group, there were no significant differences in PTH, Ca, PO4 and CaxPO4 product at baseline among three genotype groups (P>0.05). The dosages of IV vitD among the three genotype groups were not significantly different (P>*0.05*). The PTH percent reduction relative to baseline PTH after IV vitD treatment was significantly larger in BB homozygotes at the *VDR* BsmI polymorphism than that observed in bb homozygotes (38±38% *vs* 27±51%, P=0.03). No significant differences were observed in PTH change in the untreated group (P>0.05).

Conclusions: Carriers of the *VDR* BsmI BB genotype (A allele homozygotes) may have better response in PTH reduction than those with the bb genotype (G allele homozygotes) after IV vitD treatment. Randomized controlled trials are needed to further test the association between this variant and response to IV vitD treatment.

Funding: Other NIH Support - NIH fellowship, Private Foundation Support

SA-PO2316

A Detrimental Role for Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) in ACTN4-Associated FSGS Naomi C. Read,[1,2] Josee Coulombe,[1] Doug A. Gray,[1] Chris R. Kennedy.[1] *[1]Ottawa Hospital Research Institute, ON, Canada; [2]Cellular & Molecular Medicine, University of Ottawa.*

Background: Podocyte UCH-L1 expression increases in many glomerular diseases including FSGS. In a familial form of FSGS, mutations in the ACTN4 gene lead to podocyte α-actinin-4 aggregation and impairment of the ubiquitin-proteasome pathway (UPP). We hypothesized that UCH-L1 contributes to podocyte injury in ACTN4-associated FSGS.

Methods: UCH-L1$^{+/-}$ mice were developed and bred to mice with podocyte-specific K256E-ACTN4 overexpression (K256E-ACTN4^{pod+}) to obtain K256E-ACTN4^{pod+}/UCH-L1$^{-/-}$ mice - followed to 10 weeks of age for albuminuria (spot urine albumin:creatinine). Immunohistochemistry (IHC) determined renal expression of UCH-L1. For *in vitro* studies, podocytes were infected with adenovirus containing wild type (WT) or K256E α-actinin-4. Effect of UPP inhibition (MG132, 50μM, 16h) on WT or K256E α-actinin-4 expression was determined by western blot (WB) and apoptosis estimated by counting of DAPI stained condensed nuclei.

Results: IHC revealed elevated podocyte UCH-L1 in K256E-ACTN4^{pod+} mice. Gene deletion in UCH-L1$^{-/-}$ mice was confirmed by RT-PCR of tail snip DNA and by WB of brain lysates. UCH-L1$^{-/-}$ mice are viable but succumb to neurodegenerative effects by 15 weeks of age. ACTN4^{pod+} mice exhibited progressively elevated albuminuria at 4 and 10 weeks (1568 and 2391μg/mg) as compared to WT (153 and 186μg/mg) and UCH-L1$^{-/-}$ littermates (142 and 111μg/mg). In contrast, while K256E-ACTN4^{pod+}/UCH-L1$^{-/-}$ mice exhibited elevated albuminuria at 4 weeks (2465μg/mg), this was significantly reduced by 10 weeks (731μg/mg). Lastly, UPP inhibition induced a 2-fold increase in apoptosis in WT α-actinin-4 expressing podocytes (21% of cells), while K256E α-actinin-4 expressing cells exhibited a 3.5-fold induction (37% of cells). UPP inhibition reduced K256E α-actinin-4 levels in the triton-soluble fraction of lysates, suggesting increased levels in the triton-insoluble cytoskeletal fraction, consistent with aggregation.

Conclusions: K256E α-actinin-4 aggregates are toxic and lead to podocyte apoptosis. Deletion of UCH-L1 in mice may enhance UPP activity, reduce K256E α-actinin-4 levels and thereby reverse podocyte injury in this model of FSGS.

SA-PO2317

Activation of Multiple Signaling Pathways in Podocytes by Cardiotrophin-Like Cytokine-1 May Lead to Glomerular Injury of FSGS Virginia J. Savin,[1] Mukut Sharma,[1] Jianping Zhou,[1] Ellen T. McCarthy,[2] Shuhua Wang,[2] Ram Sharma.[1] *[1]Nephrology, Kansas City VA Medical Center, Kansas City, MO; [2]Kidney Institute, University of Kansas Medical Center, Kansas City, MO.*

Background: Focal segmental glomerulosclerosis (FSGS) is associated with circulating factor(s) that cause proteinuria, increase glomerular albumin permeability (P_{alb}) in *in vitro* assays and result in transplant recurrence and graft loss. We have used proteomics (LC-MS) to identify cardiotrophin-like cytokine-1 (CLC-1), a member of the IL-6 family, as a candidate for this activity. Podocytes express all 3 CLC-1 receptor components, gp 130, IL6R and CNTFR. CLC-1 incubation increases P_{alb} of normal glomeruli while CLC-1 injection causes proteinuria in rats and mice. We studied activation of JAK/STAT, PI3K/AKT and MAPK/ERK pathways to determine whether CLC-1 affected them.

Methods: Differentiated podocytes were incubated with CLC-1 (10 ng/mL) or control medium for 15 minutes at 37° C. Phosphorylated proteins were detected in cell lysates after electrophoresis and immunoblotting using monoclonal antibodies to phospho-JAK2, phospho-STAT 3, phospho-AKT or phospho-ERK1/2 (Cell Signaling Technology, Boston, MA).

Results: Differentiated murine podocytes expressed JAK 1, 2, 3 and STAT 1, 3, 5, 6; JAK2 and STAT3 were the predominant isoforms. Podocytes also expressed AKT, and ERK1/2. These proteins represent 3 distinct signaling pathways. Incubation with CLC-1 dramatically increased phosphorylation of JAK 2 and STAT3 and decreased phosphorylation of both AKT and ERK1/2 by more than 50%. These results confirm that, in immortalized podocytes, CLC-1 activates the JAK/STAT pathway associated with IL-6 family cytokines and also alters signaling by PI3K/AKT and MAPK/ERK pathways.

Conclusions: Results support the hypothesis that CLC-1 has direct effects on the podocyte that may alter its function and also its survival. These signals may contribute both to the early onset of proteinuria after transplantation in recurrent FSGS and to glomerular scarring that results in progression and kidney loss in both native kidneys and allografts. Further understanding of these pathways may lead to specific therapy to protect the kidney in FSGS.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2318

The Effect of Recurrent FSGS Plasma on Podocyte Cytoskeleton Is Reversed by Blocking TNFalpha Elena Torban,[1] Sima Babayeva,[1] Anil Vasudevan,[3] Martin M. Bitzan,[2] Paul R. Goodyer.[2] *[1]McGill Univeristy, Montreal, Canada; [2]Montreal Children's Hospital, McGill University, Montreal, Canada; [3]St. John's Hospital, Bangalore, India.*

Background: Patients with steroid-unresponsive FSGS, in whom the known slit diaphragm genes are normal, develop inexorable renal failure. 50% rapidly develop proteinuria in their renal allograft, reflecting a circulating factor that disrupts podocyte biology. Plasma from these patients rapidly disturbs the cytoskeleton of human podocytes *in vitro*, disperses MYH9 from actin stress fibers and nephrin from the slit diaphragm. In 2009, Leroy reported a boy with recurrent FSGS who responded to infusion of tumor necrosis factor-alpha (TNFα) antibody. We hypothesized that TNFα is responsible for the rapid cytoskeletal changes induced by FSGS plasma.

Methods: In a patient with recurrent FSGS undergoing plasmapheresis we collected plasma effluent (PPE) at the beginning and end of treatment, adding it to confluent monolayer cultures of differentiated human podocytes. In some experiments, hPod were treated with recombinant TNFα or pretreated with blocking antibodies against TNFα or TNFα receptors prior to PPE. Cytoskeletal changes were assessed by immunofluorescent microscopy and immunoblotting.

Results: Proteiuria (Uprot/creat >10g/g) was responsive (Uprot/creat <0.5g/g) to plasmapheresis. FSGS-PPE from the beginning (but not the end) of PPE, disrupted arrangement of stress fiber (phalloidin and MYH9 staining) and dispersed adhesion complexes (vimentin staining) in hPod. TNFα alone recapitulated the effect of early FSGS-PPE. Pretreatment of hPod with anti-TNFα prevented the FSGS-PPE effect in a dose-dependent manner. Likewise, pretreatment of hPod with blocking TNFα receptor I and/or II averted the FSGS-PPE-induced changes in cytoskeleton and adhesion complexes. When the patient was treated with weekly infusions of etanercept, Uprot/creat was now maintained <0.5g/g without plasmapheresis.

Conclusions: The rapid effects of FSGS plasma on the cytoskeleton of cultured podocytes are largely attributable to TNFα in this patient. Etanercept resulted in a significant sustained reduction in proteinuria.

Funding: Government Support - Non-U.S.

SA-PO2319

miR-143 Contributes to Podocyte Injury Induced by TGF-beta1 Toru Sakairi,[1] Yoshifusa Abe,[2] Keiju Hiromura,[1] Satoshi Takahashi,[1] Hiroko Hamatani,[1] Yoshihisa Nojima,[1] Jeffrey B. Kopp.[3] *[1]Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; [2]Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan; [3]Kidney Disease Section, NIDDK/NIH, Bethesda, MD.*

Background: Transforming growth factor (TGF)-beta contributes to podocyte injury in FSGS and diabetic nephropathy. Micro(mi)RNAs are small non-coding RNAs that regulate expression of target mRNAs by reducing translational efficiency. Certain miRNAs have been shown to contribute to progression of kidney diseases. We sought to identify miRNAs involved in podocyte injury induced by TGF-beta.

Methods: We used a human podocyte cell line treated with TGF-beta1 and TGF-beta1 transgenic mice that develop severe global glomerulosclerosis.

Results: For discovery, we performed miRNA profiling of the cultured human podocytes treated with TGF-beta1 at 5 ng/ml or vehicle control for 24 hours, using a miRNA microarray. Among miRNAs significantly increased by TGF-beta1 were miR-143, miR-31, miR-181a and miR-181b and these were confirmed by quantitative RT-PCR, which showed that miR-143 had the largest increase (2.93 ± 1.18 fold over control). We next assessed the function of miR-143, using lentiviral expression. Incubation of cultured podocytes with TGF-beta1 increased expression of COL1A1 mRNA (1.98 ± 0.15 fold), and decreased that of Wilms tumor suppressor gene (WT1) mRNA (0.25 ± 0.05 fold) and protein. Ectopic expression of miR-143 (without TGF-beta1 exposure) also increased expression of COL1A1 mRNA (1.65 ± 0.11 fold) and decreased expression of WT1 mRNA (0.60 ± 0.13 fold compared to control) and protein. The combination of TGF-beta1 and miR-143 had an additive effect for both genes. Finally, we found that miR-143 was up-regulated in the TGF-beta1 transgenic glomeruli compared to wild-type glomeruli (1.98 ± 0.4 fold) at three weeks of age prior to developing glomerulosclerosis.

Conclusions: In conclusion, TGF-beta1 induced miR-143 expression in cultured human podocytes and mouse glomeruli, and up-regulated miR-143 may mediate the induction of COL1A1 and the repression of WT1 by TGF-beta1. Up-regulation of miR-143 may be a biomarker for TGF-beta1 induced podocyte injury.

Funding: Government Support - Non-U.S.

SA-PO2320

Molecular Mechanism for Angiotensin II Induced Proteinuria Eva Koenigshausen, Martin Rütze, Ulf Zierhut, Sebastian Alexander Potthoff, Magdalena Woznowski, Ivo Quack, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin. *Nephrology, Heinrich Heine University, Duesseldorf, Germany.*

Background: Microalbuminuria serves as an early marker for glomerular injury in hypertensive and diabetic patients. Inhibitors of the renin-angiotensin-aldosterone system but not calcium chanel blockers reduce albuminuria in these patients. Albuminuria results from a defect in the glomerular filter and its slit diaphragms. A major component of the glomerular slit diaphragm is nephrin, that is endocytosed upon binding to the adaptor protein β-arrestin2.

Methods: Cells expressing the AT1-receptor or its mutant D125AR126L, nephrin and β-arrestin2 were stimulated with Ang II. After cell lysis, co-immunoprecipitation with subsequent western blot analysis was performed. For the inhibitor studies, cells were pretreated with the inhibitor before stimulation with Ang II. For siRNA experiments cells were transfected with Gαq siRNA and lysed three days thereafter. The effect of Ang II on the β-arrestin2 binding motif was studied by using two nephrin mutants. For the endocytosis assay, cells were stimulated with Ang II and incubated with biotin before cell lysis.

Results: Ang II stimulation increases the protein interaction between nephrin and β-arrestin2. This Ang II effect is dependent on the AT1-receptor and can be inhibited by AT1-receptor blockers. The G-protein signalling is essential for the Ang II effect, as the AT1-receptor mutant D125AR126L abolishes all G-protein signalling. SiRNA against the Gαq subunit and a phospholipase C inhibitor block the Ang II effect. Phosphorylation of T1120 and T1125 of nephrin are essential for the binding of β-arrestin2. Phosphorylation of nephrin S1146 mediates the Ang II effect. Stimulation with Ang II increases endocytosis of nephrin, which can be inhibited by AT1-receptor and PLC-blockers. The Ang II effect on nephrin-β-arrestin2 binding is also shown in isolated glomeruli.

Conclusions: Ang II weakens the integrity of the slit diaphragm through increase of nephrin endocytosis and is perceived to promote proteinuria. This previously unknown molecular effect of Ang II could help to understand the molecular mechanisms of Ang II induced proteinuria beyond hemodynamic effects.

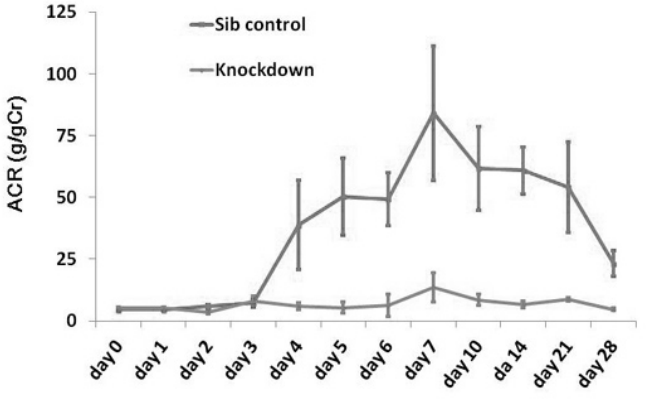
SA-PO2321

GPC5 Predispose FGF Signaling Related Podocyte Injury Koji Okamoto, Kenjiro Honda, Kent Doi, Toshiro Fujita, Eisei Noiri. *Departments of Nephrology & Endocrinology, Hemodialysis & Apheresis, University Hospital, The University of Tokyo, Japan.*

Background: Genome-wide association study for nephrotic syndrome revealed glypican 5 (*GPC5*) as risk gene (*Nature Genetics 2011*). However, the relationships between podocyte injury and GPC5 was not fully elucidated.

Methods: Podocyte specific GPC5 knock down mice and their sib control mice were studied in mice model. We created two models of heavy proteinuria; Fatal model with puromycin aminonucleoside (PAN) 300mg s.c. and bFGF 5 μg 4 days and moderate model with Fatal model with PAN 300mg s.c. and bFGF 5 μg 2 days.

Results: Massive albuminuria occurred in sibling control mice and lasted for more than 10 days to 28 days. In contrast, mild albuminuria occurred in the podocyte-specific Gpc5 knockdown mice.



Even In fatal model, the proteinuria in the podocyte-specific Gpc5 knockdown mice was relatively low compared with that of sib control mice. Electron microscopic changes was found 7 days after PAN injection, whereas light microscopic changes was found 14 days after PAN injection. Approximately 25% of glomeruli showed global sclerosis; 50% of glomeruli showed segmental sclerosis at day 28 in sibling control mice. In contrast, 90% of glomeruli showed virtually no changes in conditional knockdown mice.

Conclusions: Our in vivo data strongly suggest a role of the GPC5-FGF2 pathway in nephrotic syndrome pathogenesis.

Funding: Government Support - Non-U.S.

SA-PO2322

Heterogenous Changes in Podocyte Morphology and Function in Adriamycin Nephropathy in Mice Matthias Hackl,[1] Katalin Susztak,[2] Janos Peti-Peterdi.[1] *[1]Physiology & Biophysics, University of Southern California, Los Angeles, CA; [2]Albert Einstein School of Medicine, Yeshiva University, Bronx, NY.*

Background: Adriamycin nephropathy is a well-characterized model of focal segmental glomerulosclerosis (FSGS) in mice, however proteinuria, glomerular hyalinosis/sclerosis and tubular atrophy have been studied mainly by histology. The purpose of this study was to directly and quantitatively visualize the early changes in FSGS in vivo using advanced techniques of multiphoton microscopy (MPM).

Methods: 4 week old C57BL6 male mice were injected with 25mg/kg adriamycin iv. After 7 days proteinuria increased 20 to 50 fold and the animals were surgically instrumented for kidney MPM. The vasculature was labeled with albumin-Atto565. Injection of Lucifer Yellow (LY), a molecule which is freely filtered into the Bowman's space, identified podocytes by negative labeling. A new transgenic mouse model was developed (Pod:EGFP-tomato) featuring specific expression of the intensely green fluorescent protein EGFP in podocytes targeted to the cell membrane while other cell types express the red fluorescent protein tomato. Measurements of the glomerular sieving coefficient (GSC) for albumin were obtained to assess the local permeability of the glomerular filter.

Results: MPM of the glomeruli identified several, but not all podocytes with hypertrophy and intense LY uptake. Intense leaking of red plasma fluorescence into the Bowman's space was observed around these injured podocytes, but other glomerular regions appeared normal. GSC measurements in damaged and intact areas of the glomerular filtration barrier confirmed that the leakage of albumin was restricted to the focal regions of podocyte injury. All changes were absent in control animals injected with normal saline.

Conclusions: In vivo multiphoton imaging of the adriamycin model of FSGS in mice is technically feasible and useful for the study of the molecular players in the development of proteinuria in transgenic mice. In the early stages of adriamycin GS, podocyte damage/dysfunction appears to be limited to a few podocytes, where intense, focal leakage of albumin may be the cause of proteinuria.

Funding: NIDDK Support

SA-PO2323

Vitronectin-Binding Exogenous Plasminogen Activator Inhibitor-1 (PAI-1) Protects Podocytes Against Injury *In Vivo* and *In Vitro* Haichun Yang,[1] Jianyong Zhong,[2] Ji Ma,[2] Bridgette Corsa,[1] Agnes B. Fogo.[1] *[1]Pathology, Vanderbilt University Medical Center, Nashville, TN; [2]Pediatric Nephrology, Vanderbilt University Medical Center, Nashville, TN.*

Background: We previously found that genetic PAI-1 deficiency protects podocytes against injury both *in vivo* and *in vitro*. To determine whether this PAI-1 protective effect on podocytes is plasmin dependent or due to vitronectin (Vn) interactions, we assessed effects of mutant forms of recombinant human PAI-1 variants on podocyte function.

Methods: Uninephrectomized (UNx) mice were fed high salt diet and infused with angiotensin II (Ang II) for 8 weeks. Different human stable PAI-1 variants, including PAI-1-K (retaining protease inhibitor effects of native PAI-1 but not binding to Vn), or PAI-1-R (competitive blocker of Vn), or 14-1b (control PAI-1, retaining all known functions of native PAI-1), or PBS were injected daily. Primary cultured podocytes were injured by puromycin aminonucleoside (PAN) and various PAI-1 variants were added.

Results: Systolic blood pressure was significantly increased in mice with UNx/Ang II, and none of the PAI-1 variants changed SBP. However, the albuminuria caused by UNx/Ang II was significantly reduced by 14-1b and PAI-1-R (14-1b 1587.4±377.8, PAI-1-R 1165.8±324.16 *vs.* AngII 2348.1±920.96 ug/mg, $p<0.05$) while there was no change by PAI-1-K. Only vitronectin-binding PAI-1 variants resulted in PAI-1 deposition along glomerular basement membranes. In parallel, 14-1b and PAI-1-R maintained synaptopodin and decreased desmin on podocytes, while PAI-1-K and PBS did not. *In vitro*, Vn-binding human PAI-1, 14-1b and PAI-1-R maintained synaptopodin and α-actinin-4 expression after PAN treatment, and also decreased pro-apoprotic Bax expression in wild type podocytes (14-1b 0.69±0.03, PAI-1-R 0.56±0.03 *vs.* PAN 0.99±0.02, $p<0.05$). Previous data showed lack of protection against PAN by Vn-binding human PAI-1 in *PAI-1*$^{-/-}$ podocytes, which also expressed less uPAR compared to wild type.

Conclusions: Our data suggest that vitronectin-binding PAI-1 protects podocytes against injury. Collectively, these studies suggest the protective effect of vitronectin-binding PAI-1 may be related to uPAR.

Funding: NIDDK Support

SA-PO2324

Novel Therapeutics in Diabetic Nephropathy: Sialic Acid Precursors Reduce Proteinuria in Experimental Diabetic Nephropathy Camille E. Mace, Lionel C. Clement, Sumant S. Chugh. *Medicine/Nephrology, University of Alabama at Birmingham, AL.*

Background: We have recently shown that the expression of sialic acid deficient (hyposialylated) Angptl4 is increased in podocytes in minimal change disease (MCD) (Clement Nature Medicine 2011), and that improving sialylation with sialic acid precursors like N-acetyl D-mannosamine (ManNAc) reduces proteinuria significantly. Proteinuria in diabetic nephropathy is a complex, multidimensional process, so we investigated whether hyposialylated Angptl4 is a significant contributor towards proteinuria in this disease.

Results: Confocal imaging for Angptl4 in human kidney biopsies from patients with diabetic nephropathy reveal increased glomerular Angptl4 expression. We isolated glomeruli from hyperglycemic animals and normoglycemic controls from two rodent models of Type 2 diabetes, the db/db mouse and Zucker Diabetic Fatty (ZDF) rat. 2D gel electrophoresis and Western Blot of glomeruli revealed an overall increase in Angptl4 expression, including the high isoelectric point (pI) hyposialylated form, in both models. Treatment of proteinuric hyperglycemic ZDF rats with ManNAc in tap water (treatment group, n=5 rats/group) or plain tap water (control group) caused very significant reduction in proteinuria ($P<0.01$ on Day 3, $P<0.05$ on Day 7). Proteinuria was assessed on weekly intervals for 5 weeks. By the end of 5 weeks of therapy, fold increase in proteinuria over baseline was 2.96 ± 0.32 (control group) and 2.12 ± 0.27 (study group); $P<0.05$. Further studies with different ManNAc regimens are in progress.

Conclusions: In summary, glomerular Angptl4 expression is increased in human and rodent diabetic nephropathy, and treatment with sialic acid precursors significantly reduces proteinuria in a rat model of diabetic nephropathy.

Funding: NIDDK Support

SA-PO2325

Filtration Barrier Dysfunction in Diabetic CLIC5A Deficient Mice Yimin Zhang, Laiji Li, Abass Almomany, Barbara J. Ballermann. *Medicine, University of Alberta, Edmonton, AB, Canada.*

Background: The Chloride Intracellular Channel 5A (CLIC5A), one of two CLIC5 isoforms, is a glomerulus-predominant protein that co-localizes with Ezrin and podocalyxin in podocytes. In CLIC5 (jbg/jbg) deficient mice, podocyte Ezrin and phospho-Ezrin levels are reduced, podocyte architecture is abnormal and adriamycin-induced injury is more severe than in wild-type (+/+) mice. In human patients with diabetic nephropathy glomerular CLIC5A mRNA expression is reduced. Here, we sought to determine whether CLIC5A deficiency is a susceptibility factor for diabetic nephropathy in mice.

Methods: Heterozygous (+/jbg) mice were bred to generate jbg/jbg, +/jbg and +/+ littermates. Diabetes was induced at 8 weeks of age with intraperitoneal (50μg/g) streptozotocin for 5 consecutive days. Non-diabetic controls were similarly injected with buffer alone. The day of the first injection was defined as day 1, week 1. Mice with fasting blood glucose levels >12.6 mmol/L on day 14 were considered diabetic. Blood glucose and western blots for urine albumin were obtained every two weeks, and mice were euthanized during weeks 6 and 14 for tissue analysis.

Results: Light microscopy of PAS and H&E stained renal tissue was normal for control and diabetic mice of all genotypes at 6 and 14 weeks. There was increased deposition of extracellular matrix and mild mesangial expansion in all diabetic mice compared to non-diabetic mice, but no differences between the three genotypes were observed. By immunoblot analysis of kidney tissue, podocalyxin migrated as a single band in non-diabetic +/+ mice. An additional, more slowly migrating band suggesting reduced podocalyxin glycosylation was observed in diabetic +/+ mice and in both, diabetic and non-diabetic jbg/jbg mice. In +/+ and +/- mice, CLIC5A abundance did not change with induction of diabetes. Finally, diabetic jbg/jbg and +/jbg mice, had higher urine albumin levels than non-diabetic jbg/jbg or +/jbg controls, whereas no difference in urine albumin was observed between diabetic and non-diabetic +/+ mice.

Conclusions: The findings lead us to postulate that CLIC5A deficiency may change podocalyxin glycosylation in glomerular podocytes, increasing filtration barrier dysfunction in diabetes.

Funding: Government Support - Non-U.S.

SA-PO2326

Differential Modification of Enalapril in the Kidneys of Lean and 'Programmed' Obese Male Young Rats Hyung Eun Yim, Kee Hwan Yoo, Cheol Park, In Sun Bae, Joo Won Lee. *Pediatrics, Korea University Medical Center, Republic of Korea.*

Background: The acquired reset of the renin-angiotensin system (RAS) has been suggested to cause lifelong functional and structural alterations. We aimed to investigate the role of the RAS block in the renal pathophysiological changes in the rat model of 'programmed' obesity.

Methods: Three or 10 male pups per mother were assigned to either the small litter (Obese group) or normal litter (Lean group) rats during the first 21 days of life. With this, all pups were randomized into 4 groups, and treated with enalapril (Obese enalapril, OE; Lean enalapril, LE) or vehicle (Obese control, OC; Lean control, LC) between the ages of 2 and 4 weeks postnatally. All pups had body weight, blood pressure (BP) and renal alterations determined at 28 days of age.

Results: Pups in the OC group weighed more than rats in the LC group between 7 days and 28 days of age ($P < 0.05$). Enalapril decreased body weights in the Obese and Lean groups at 28 days ($P < 0.05$). Mean BP levels in the OC and OE groups were higher than those of the LC and LE groups ($P < 0.05$). Enalapril increased renal cell apoptosis and proliferation, glomerulosclerosis, and tubulointerstitial fibrosis compared to both Lean and Obese controls, respectively ($P < 0.05$). Pups in the OE group particularly showed the highest increases in renal cell apoptosis, glomerulosclerosis and tubulointerstitial fibrosis compared to the other groups ($P < 0.05$). Immunoblotting and immunohistochemistry showed that enalapril increased renin, angiotensin II receptor type (AT) 2, and matrix metalloproteinase (MMP)-9 and decreased AT 1, tissue inhibitor of MMP (TIMP)-1, and osteopontin expression in the kidneys of Lean group. In contrast, enalapril decreased AT2 and MMP-9 and increased TIMP-1, osteopontin, and plasminogen activator inhibitor-1 expression in the kidneys of Obese group ($P < 0.05$).

Conclusions: These data indicates that renal changes following early postnatal overfeeding may be fundamentally different to those of normal postnatal growth after exposure to the RAS inhibition. Angiotensin II can be a key player in the developmental renal programming of obesity.

SA-PO2327

Impairment of Podocyte Function in Mice with Gsα Deletion in Juxtaglomerular Cells Lanping Jiang,[1] Christoph Eisner,[2] Yuning Huang,[2] Diane Mizel,[2] Jurgen Schnermann,[2] Limeng Chen.[1] *[1]Department of Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]NIDDK, NIH, Bethesda, MD.*

Background: Mice with deletion of Gsa in renin-producing cells (RC/FF mice) have been shown to have greatly reduced renin production and responsiveness of renin secretion to acute stimuli. In addition, long-term experiments documented that renal function and pathology deteriorated progressively in RC/FF mice. In the present study we have determined a possible role of podocyte abnormalities in this low renin mouse model and the effects of ACE inhibition (ACEI).

Methods: Experiments were performed in 7-8 weeks old RC/FF mice and age-matched control mice kept on a standard diet. In addition mutant and control mice were studied after being fed a low-salt diet (0.03% NaCl w/w) for 7 days, and receiving enalapril in the drinking water (about 10 mg/kg per day). Efficacy of the prolonged treatment with ACEI was examined in anesthetized mice by determining the blood pressure (BP) response to angiotensin I. After the treatment blood were collected for plasma renin measurements and mice were sacrificed. Kidneys were fixed for histological examination. Wilms tumor protein (WT1) as a selective podocyte marker was determined by immunohistochemistry.

Results: Urinary albumin excretion was higher in RC/FF mice when adjusted by GFR. Focal segmental glomerular sclerosis was observed in older RC/FF mice. Compared with the control mice, WT1 positive podocyte counts as well as the glomerular area were significantly decreased in RC/FF mice. When stimulated by low salt and ACEI, WT1 positive podocyte area per glomerulus had markedly increased in both RC/FF and control mice. Although there were no significant changes of PRA by the chronic low salt/ACEI treatment, WT1 positive podocyte area per glomerulus was much higher in RC/FF mice than control mice.

Conclusions: Chronic Gsa deletion in JG cells leading to greatly reduced renin production may impair podocyte function resulting in increased albumin excretion and FSGS in the older age. This process may be partly improved by ACEI through currently unknown pathways.

Funding: Government Support - Non-U.S.

SA-PO2328

MicroRNA Profile Determines Progression of HIV-Associated Nephropathy Partab Rai, Dileep Kumar, Pravin C. Singhal, Ashwani Malhotra. *Medicine, NSLIJ, Great Neck, NY.*

Background: Micro RNAs with dual function to act as inducers or repressors of gene activity have been reported to be differentially expressed in various pathological and physiological states. However, the precise role of microRNAs, endogenous RNA oligonucleotides which specifically target mRNA and regulate gene expression in HIV –associated nephropathy (HIVAN) is not functionally known. Our laboratory had shown the activation of mTOR pathway in HIVAN and rapamycin, an inhibitor of mTOR pathway, which has been used to attenuate polycystic kidney disease in animal experimental models. We asked whether microRNAs regulate this process in this model.

Methods: Kidneys were harvested from FVB/N and Tg26 mice at 8wks of age (n=3). In another group,mice were given rapamycin®(5mg/kg ,ip, alternate day) for 4 weeks. In brief, total RNA from renal tissue (n=3) was isolated from FVB/N (controls), Tg26 and Tg+R by Triozol reagent (Invitrogen). A complete miRNA microarray was performed in these groups and a data analysis was generated. cDNA was synthesized using SuperScript Enzyme Mix (Invitrogen).Real-Time qPCR was performed to confirm the miRNA microarray data by using SYBR Green Universal Kit (Invitrogen) using forward primers and universal qPCR primers (Invitrogen).

Results: TG26 mice showed altered expressions of mir- 145(C=18793±2273, Tg=8454±1255, and R=13910±3232,Arbitrary Units; AU ; p<.04), mir-16(C=11594±1393, Tg=5758±1898, and R=10373±3484 AU; p<.05), mir-30c (C=22517±2908, Tg=15486±1992, and R=19537±4178 AU; p<.07), mir-466 (C=8274±1038, Tg=12856±1754, and R=9623±3237 AU; p<.09) in Tg26 mice which were reversed by rapamycin. These results were confirmed by qRT-PCR.

Conclusions: These microRNAs have been considered to play key roles in many cellular processes such as proliferation, differentiation and apoptosis by inhibiting target gene expression. Our results demonstrate that the above selective bioregulators could have a functional impact in HIVAN.

Funding: NIDDK Support

SA-PO2329

TWEAK and FN14 in Proteinuric Podocyte Injury Maria D. Sanchez-Niño,[1] Ana Belen Sanz,[1] Sergio A. Mezzano,[2] Rafael Selgas,[1] Marta Ruiz-Ortega,[3] Jesus Egido,[3] Alberto Ortiz.[3] *[1]IdiPAZ, Madrid, Spain; [2]Universidad de Valdivia, Chile; [3]IIS-Fundacion Jimenez Diaz, Madrid, Spain.*

Background: Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a TNF superfamily cytokine that activates the Fibroblast growth factor-inducible 14 (Fn14) receptor. Cultured podocytes have been shown to express Fn14. However, there is scarce information on TWEAK actions on podocytes and on in vivo Fn14 in podocytes.

Methods: Biopsies from human nephrotic syndrome were studied. Studies in mice and rats were done: TWEAK administration to mice, BSA for protein overload-induced proteinuria and puromycin aminonucleoside to rats. In vitro studies were done in murine podocytes.

Results: Kidney Fn14 mRNA and protein was increased following puromycin aminonucleoside administration to rats. The increased Fn14 at day 2 preceded the onset of proteinuria and the increment in kidney MCP-1. Total kidney Fn14 mRNA (1.5-fold) increased at day 7 in murine protein overload-induced proteinuria and was associated with increased glomerular macrophage infiltrates. Immunohistochemistry and co-staining with WT-1 localized the increased Fn14 expression to podocytes in both rat PAN nephrosis and in protein overload-induced proteinuria. In human nephrotic syndrome, Fn14-expressing podocytes displayed NFkB activation and expressed MCP-1. TWEAK administration to mice increased podocyte RelA translocation to nuclei and MCP-1 expression. In cultured podocytes stressors such as PAN and inflammatory cytokines, increased Fn14 mRNA (2.2-fold) and protein (2.2-fold) levels in a dose- and time-dependent manner with peak protein expression at 6 hours. TWEAK activated NFkB and increased MCP-1 mRNA and protein in cultured podocytes, an effect prevented by parthenolide. Indeed, anti-TWEAK neutralizing antibodies prevented PAN induction of MCP-1 mRNA in cultured podocytes.

Conclusions: In conclusion, Fn14 is upregulated in podocytes in different forms of proteinuric kidney disease and by diverse stressors. TWEAK has NFkB-mediated pro-inflammatory effects on podocytes that may be relevant for the pathogenesis of proteinuric kidney disease. Thus, TWEAK or Fn14 targeting should be explored in proteinuric diseases.

Funding: Government Support - Non-U.S.

SA-PO2330

Podocyte Specific GLUT4 Deletion Protects from the Development of Lipopolysaccharide (LPS) Induced Albuminuria Johanna Guzman,[1] Britta Sylvia Walter,[1] Cristina Muresan,[1] Mary Donnelly,[1] Kirk N. Campbell,[3] Peter H. Mundel,[4] Jochen Reiser,[1] Sandra M. Merscher-Gomez,[1] Alessia Fornoni.[1] *[1]Medicine/Nephrology, University of Miami Miller School of Medicine, FL; [2]Medicine/Nephrology, Mount Sinai Medical Center, New York, NY; [3]Medicine, Massachusetts General Hospital, Boston, MA.*

Background: Insulin resistance correlates with albuminuria in patients with diabetes as well as in normal individuals. Mice with a podocyte-specific insulin receptor deletion develop glomerular lesions resembling diabetic nephropathy (DN), suggesting an important role of insulin signaling in podocyte function.

Methods: We investigated how GLUT4 is modulated in human DN and how GLUT4 affects podocyte function.

Results: Using RT-PCR, we show that GLUT4 is downregulated in glomeruli from patients with DN when compared to controls (N=6 each, $p<0.05$). Mice with a podocyte-specific deletion of Glut4 (Glut4 KO) were generated by crossing Glut4 floxed mice with Podocin-Cre mice. Glut4 KO mice did not show an increased urinary albumin to creatinine ratio up to 19 weeks of age. However, when challenged by two daily consecutive injections of LPS (200µg each), Glut4 KO mice had an albumin/creatinine ratio of 139 ± 131 versus 1059 ± 449 µg/mg in wildtype mice ($p<0.001$). Western blot analysis of glomeruli demonstrated that Glut4 KO mice were protected from LPS induced degradation of synaptopodin and nephrin. In order to further characterize the role of GLUT4 in podocyte function, we performed GLUT4 siRNA experiments in differentiated mouse podocytes. GLUT4 siRNA podocytes showed an increased in synaptopodin and RhoA expression ($p<0.01$), associated with cell blebbing ($p<0.05$) and increased cell migration. These changes were conserved when the experiments were repeated in glucose free medium, suggesting that Glut4 may affect podocyte function in a glucose independent manner.

Conclusions: In conclusion, Glut4 is directly involved in the regulation of the podocyte actin cytoskeleton and targeting GLUT4 may represent a new strategy for certain glomerular diseases.

Funding: Private Foundation Support

SA-PO2331

The Role of the Protein Kinases MK2 and MK3 in a Mouse Model of Acute Proliferative Glomerulonephritis Rose M. Ayoob,[1] Melinda Chanley,[1] Adam J. Guess,[1] Ruma Pengal,[1] Brian Becknell,[1] Jeffrey B. Kopp,[2] Rainer Benndorf,[1] William E. Smoyer.[1] *[1]Center for Clinical and Translational Research, Research Institute at Nationwide Children's Hospital, Columbus, OH; [2]Kidney Disease Section, NIDDK, Bethesda, MD.*

Background: Signaling by various MAPK kinases, including p38 MAPK, has been implicated in both renal function and disease, although their exact role is not understood. Increased p38 MAPK activity has been observed in podocytes in patients with glomerulonephritis (GN). Two of the major substrates of p38 MAPK, MK2 and MK3, have been suspected to play a role in this injury. The goal of this study was to determine the role of both MK2 and MK3 in the development of glomerular injury using a model of acute proliferative GN in mice with disrupted MK2 and MK3 genes.

Methods: Four groups of age-and sex-matched mice (n=13/group) with different MK2 and MK3 genotypes received anti-mesangial cell serum i.v. on Days 1-4. Kidney function was assessed by serum blood urea nitrogen (BUN) and urine protein:creatinine ratios (Up:Ucr) on Days 0, 4, 8 and 12. Renal histology was analyzed at Days 8 and 16.

Results: Significant differences were found in BUN ($P<0.05$) and Up:cr ($P<0.01$) values on Day 4, and in survival to Day 28 ($P<0.01$). Survival rates for $MK2^{++}/MK3^{++}$ and $MK2^{-}/MK3^{++}$ mice were 68% vs. 52% for $MK2^{++}/MK3^{-}$ vs. 19% for $MK2^{-}/MK3^{-}$ mice. Histological analysis revealed thickening of glomerular capillary walls due to reduplication of basement membranes as well as subendothelial and mesangial deposits in each group. Glomeruli examined on Day 16 revealed 4% had crescents in $MK2^{-}/MK3^{++}$ mice vs. 15% in $MK2^{++}/MK3^{++}$ and 22% in $MK2^{-}/MK3^{-}$ mice, while crescents were absent in $MK2^{++}/MK3^{-}$ mice.

Conclusions: MK2 and MK3 both contribute to glomerular structure and function in murine proliferative GN, yet they have distinguishable effects on survival.

Funding: NIDDK Support

SA-PO2332

Galectin-9 Inhibits Cell-Mediated Immune Response in Mice Anti-GBM Glomerulonephritis Qian Zhang, Jin Yuan, Hong Luan, Yongman Lv. *Nephrology, Tongji Hospital, Tongji Medical College of HUST, wuhan, Hubei, China.*

Background: Tim-3 is a cell surface molecule expressed mostly on Th1 cells, besides Th17 cells, macrophages, DCs and NK cells. Galectin-9 identified as the ligand of Tim-3 functions in diverse biological processes, including cell adhesion, cell proliferation and apoptosis. Because Th1 and Th17 cells are the major immune factors in anti-GBM glomerulonephritis, the function of which is regulated by galectin-9 via Tim-3-galectin-9 pathway, we hypothesized that galectin-9 could regulate the immune response in mice anti-GBM glomerulonephritis, and ameliorate the pathologic damage.

Methods: For inducing anti-GBM glomerulonephritis in C57BL/6 mice, we immunized mice with rabbit IgG and complete Freund's adjuvant, 10 days later injected rabbit anti-GBM serum and then administrated galectin-9 intraperitoneally 100ug daily from day1 to day 7 and PBS as control. All mice were seperately sacrificed at day 7, day 14, day 21, day 28 and day 56. Splenic lymphocytes were isolated and employed for FACS, cytokines were detected by ELISA, and the kidneys were utilized to determine the extent of influx of $CD4^{+}$ cells and glomerular damage.

Results: Administration of galectin-9 improved histologic damage significantly, including glomerular mesangial cell and matrix proliferation, crescent formation and mouse IgG deposition. Then, we analysed the distribution of immunologic cells in immune organs and kidneys. The results showed that in spleen, the rates of $CD4^{+}CD69^{+}$ T cells, $CD8^{+}CD69^{+}$ T cells and $CD19^{+}CD69^{+}$ B cells decreased significantly from day 7, and the similar tendency was observed in Th1 and Th17 cells from day 14, but not in Th2 cells. And in kidneys, administration of galectin-9 inhibits the filtration of $CD4^{+}$T cells.The suppression of Th1 and Th17 caused the decreased secretion of specific cytokines(IL-2, IL-10, IL-12, IL-17, IFN-γ and TGF-β1) in peripheral blood. In addition, we found that the kidneys from galectin-9 treated mice expressed lower level of α-SMA, collagenIand collagen III.

Conclusions: These results demonstrate that galectin-9 inhibits Th1 and Th17 cell-mediated immune response in anti-GBM glomerulonephritis, and interferes with the progress of fibrosis.

SA-PO2333

Estrogen Receptor Alpha Knockout Mice Develop Podocyte Damage and Increased Glomerular Collagen IV Deposition Sebastian Kummer,[1] Lara Vanessa Wegerich,[1] Stefanie Jeruschke,[1] Petra Lehmann,[2] Nadezda Koleganova,[2] Marie-Luise Gross-Weissmann,[2] Jun Oh.[1,3] *[1]Dep. of General Pediatrics, University Children's Hospital, Duesseldorf, Germany; [2]Inst. of Pathology, University Hospital, Heidelberg, Germany; [3]Dep. of Pediatric Nephrology, University Medical Center, Hamburg, Germany.*

Background: Epidemiological studies have demonstrated that outcome of chronic kidney diseases can be positively influenced by female gender. This suggests critical influences of gender hormones on glomerular structure and function. We examined estrogen receptor alpha (ERα) knockout mice for podocyte/glomerular damage as well as ERα expression and protective effects of estrogens on podocytes in vitro.

Methods: ERα knock out mice were examined for podocyte and mesangial cell number, volume, expression of TGFβ1/collagen IV and glomerular sclerosis. Expression of ERα was investigated in podocytes in vitro and in vivo. Antiapoptotic action of 17β-estradiol was analyzed in puromycin-treated podocytes using Annexin-FITC flow cytometry and Hoechst nuclear staining for apoptosis detection. Destabilization of mitochondrial membrane potential, an early indicator of apoptosis, was visualized with tetramethyl rhodamine methylester staining.

Results: In ERα knockout mice, podocyte number was reduced compared to wild-type controls in female/male animals (80/86 vs. 132/135 per glomerulus, $p<0.05$). Podocyte cell volume was enhanced in ERα knockout mice (429/371µm3 vs. 264/223µm3, $p<0.05$). TGFβ1 and collagen IV expression were significantly enhanced in knockout mice, indicating glomerular damage.

ERα is expressed in human and murine podocytes on mRNA and protein level. 17β-estradiol reduced PAN-induced apoptosis in vitro by 26.5% or 56.6% (FACS and Hoechst staining, $p<0.05$). Podocyte apoptosis is associated with mitochondrial membrane potential depolarization, which could be significantly restored by 17β-estradiol.

Conclusions: ERα deficient mice develop a loss of podocytes and cellular hypertrophy, indicating cellular stress. Correspondingly, podocytes express ERα in vitro and in vivo, mediating significant protection against apoptotic stimuli. These findings may explain in part the gender differences in glomerular diseases.

Funding: Private Foundation Support

SA-PO2334

Heparan Sulfate Deficient Zebrafish Show Glomerular Malfunction and Ineffective Filtration Ramzi Khalil, Danielle Cohen, Malgorzata Wiweger, Wietske Van der Ent, Emile De Heer, Jan A. Bruijn, Pancras C.W. Hogendoorn, Hans J. Baelde. *Pathology, Leiden University Medical Center, Netherlands.*

Background: There has been a longstanding discussion on whether heparan sulfate proteoglycans (HSPG) serve as a charge selective filtration medium in the glomerular filtration barrier. A recent mouse model showed that a lack of podocyte specific HSPG does not lead to glomerular dysfunction. This project aims to investigate whether a biallelic knockout of the homologue of human EXT2 in zebrafish, leading to shortenened and functionally impaired HSPGs, results in abnormal glomerular filtration.

Methods: Zebrafish (*Danio rerio H*) *dackel* (*dak*) mutants have a premature stop codon in the *ext2* gene. The *ext1* and *ext2* gene products encoding subunits of heparan sulfate co-polymerase are essential components in the heparan sulfate chain elongation. Wild type (WT) larvae and homozygous *dak* mutants were injected intravenously with a mixture of FITC labeled lysine fixable 70kD dextran and TRITC labeled 3kD dextran at 4 days post fertilization, fixed with formalin at 5 or 60 minutes after injection, imbedded in TissueTec® for oriented sectioning and studied by immunofluorescent microscopy.

Results: Neither TRITC nor FITC fluorescent absorption droplets were found in the *dak*'s renal tubules at both timeframes. Only TRITC and no FITC droplets were found in the renal tubules of the WT zebrafish at both 5 and 60 minutes.

Conclusions: These results show that the WT glomerulus can filtrate small particles, but not the 70kD FITC. As none of the injected dyes are detected in *dak* mutants, these larvae either have an impaired tubular uptake or an inefficient glomerular filtration. Data from our previous studies have shown that homozygous *dak* mutants display structural changes of the glomerular basement membrane and podocyte foot process effacement. Therefore, a glomerular cause is most likely. Whether the impairment in glomerular filtration is caused by a developmental imperfection or a loss of function in the glomerular filtration barrier remains to be studied. Taken together, these results show that in this model, HSPGs are essential for effective glomerular filtration.

SA-PO2335

Crk1/2 Signaling Is Necessary for Foot Process Spreading in Mice and Is Activated in Human Proteinuric Kidney Disease Britta George,[1] Rakesh Verma,[2] Abdul A. Soofi,[2] Maria Pia Rastaldi,[3] Lawrence B. Holzman.[1] *[1]Renal-Electrolyte and Hypertension Division, University of Pennsylvania, Philadelphia, PA; [2]Division of Nephrology, University of Michigan, Ann Arbor, MI; [3]Renal Research Laboratory, Fondazione IRCCS Ospedale Maggiore Policlinico & Fondazione D'Amico, Milano, Italy.*

Background: The slit diaphragm protein Nephrin is necessary for establishing the morphology of the podocyte in development by transducing from the podocyte intercellular junction phosphorylation-mediated signals that regulate cytoskeletal dynamics.

Methods: Podocyte culture, podocyte-specific knockout mice and human kidney tissue immunofluorescence were employed to understand Crk biology.

Results: We found that activation of Nephrin induced lamellipodia formation in podocyte culture. Following Nephrin activation in cell culture, a FAK/Cas/Crk protein complex was recruited to Nephrin in a pI3- and Src kinase-dependent fashion. Crk1/2 and Cas were necessary for Nephrin-induced lamellipodia formation since cell spreading was attenuated in podocytes with RNAi-mediated knockdown of Crk1/2 or in Cas-null mouse embryonic fibroblasts. We found that proteins of the FAK/Cas/Crk complex were present at the intercellular junction of developing podocyte precursors *in vivo* in their activated phosphorylated state. To test the relevance of Crk1/2 signaling, mice deleted of Crk1/2 in a podocyte-specific fashion were bred and found to develop and age normally. Surprisingly, podocytes in these mice were protected from protamine sulfate (PS)-induced foot process spreading. In human proteinuric kidney disease, the FAK/Cas/Crk signaling axis was activated as p-Cas and p-FAK staining was increased in podocytes of minimal change and membranous nephropathy patients compared to healthy controls.

Conclusions: We conclude that Nephrin induces cell spreading in culture via a FAK/Cas/Crk protein complex. In mice the FAK/Cas/Crk complex is necessary for foot process spreading following podocyte injury; our additional findings in human tissue lead us to speculate that this might be true in some forms of human glomerular disease. Altogether, the Crk protein complex represents a promising therapeutic target in human podocytopathy.

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SA-PO2336

Transgenic Mice with Disrupted ABIN1 Function Develop Diffuse Proliferative Lupus Nephritis Dawn J. Caster,[1] Sambit Kumar Nanda,[2] Kenneth R. McLeish,[1,3] Philip Cohen,[2] David W. Powell.[1] *[1]Medicine, University of Louisville School of Medicine, KY; [2]Medical Research Council Protein Phosphorylation Unit, Dundee, Scotland, United Kingdom; [3]VMAC, .*

Background: Lupus nephritis (LN) occurs in 50% to 60% of patients with systemic lupus erythematosus (SLE), and 60% of these patients are classified as focal (Class III) or diffuse (Class IV) proliferative LN. Recent genome wide association studies of patients with SLE identified mutations in a number of NF-κB signal components, including ABIN1. We have recently published the characterization of a knock-in ABIN1 mouse expressing a mutated form of ABIN1 that is unable to bind to polyubiquitin chains (Nanda et al, 2011. J. Exp. Med. 20102177). These mice demonstrated enhanced NF-κB activity and developed lupus-like autoimmune disease with enlarged spleens and lymph nodes, hypergammaglobulinemia, and anti-nuclear antibodies. The present study was performed to define the renal involvement in this novel model of murine SLE.

Methods: Histology and serology analysis was performed for 3 and 5 month old ABIN1 transgenic mice.

Results: Kidneys from 3 month old transgenic mice showed mild focal or diffuse mesangial proliferation without expansion of the mesangial matrix, loss of glomerular capillaries, or interstitial changes. At 5 months transgenic mice demonstrated advanced proliferative glomerulonephritis with mesangial expansion and lobulation, occlusion of capillary lumens, thickening of capillary walls, and focal interstitial cellular infiltrates. Electron microscopy showed discrete mesangial and subendothelial electron dense deposits, and immunohistochemistry showed deposition of C3, C1q, IgG, IgM, and IgA for both ages. At 5 months transgenic mice demonstrated significantly reduced serum C3 and C4 and had elevated serum cystatin C.

Conclusions: This novel mouse model of SLE demonstrates progressive glomerulonephritis with histologic features similar to Class III and IV LN. We conclude that loss of ABIN1 regulation of NF-κB results in production of autoimmune glomerulonephritis.

Funding: NIDDK Support, Private Foundation Support

SA-PO2337

Glomerular Macrophage Heterogeneity in Murine Cryoglobulinemia-Associated Membranoproliferative Glomerulonephritis Progression Yujiro Kida, Kelly L. Hudkins, Charles E. Alpers, Jeremy S. Duffield. *Medicine & Pathology, University of Washington, Seattle, WA.*

Background: Inflammatory macrophages (Mφ) are known to play dual roles in tissue repair and regeneration or in amplification of tissue injury and scarring. Many studies in kidney diseases suggest that Mφ amplify injury and fibrosis, but recent studies in AKI show the same cells are beneficial in tissue repair.

Methods: We used mice expressing Lck-Tslp and Cd11b-DTR transgenes to explore the overall function of glomerular Mφs in cryoglobulinemia and MPGN. Lck-Tslp generates overactive B lymphocytes that produce cryoglobulins, and CD11b-DTR permits specific conditional ablation of Mφs when diphtheria toxin (DT) is injected.

Results: To study the function of inflammatory Mφs in this MPGN model we achieved glomerular Mφ ablation of 80% compared with controls, from d30-d50 with alternate day DT injections. IgG deposition was unaffected, but ablation lead to a 40% reduction in mesangial matrix expansion detected by silver stain or Coll-IV stain. Mesangial cell area was also reduced by 40% and proteinuria reduced by 60%. The overall function of Mφs is deleterious, promoting mesangial proliferation and sclerosis. To understand the phenotype of MPGN glomerular Mφs we phenotyped them using established markers of activation and differentiation. All MPGN Mφs showed evidence of activation but are highly heterogeneous, expressing both M1 (CD11a, Ly6C, CD40) & M2 (CD206, Mac2, ITGβ5) markers, suggesting that either glomerular Mφs have dual functions or that the established markers are poor discriminators of function. A minority (25%) specifically express the reparative function marker Gpnmb, and the expression of FcγRs is also heterogeneous.

Conclusions: This study indicates a key and predominately deleterious role for Mφ in the progression of kidney injury in MPGN. Glomerular Mφ activation is universal and the majority express markers of M1 & M2 functions but a minority of glomerular Mφ express markers suggestive of reparative functions.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2338

Lymphocyte Connective Tissue Growth Factor Mediates Cryoglobulin Production and Induces Cryoglobulinaemic Glomerulonephritis Alan D. Salama,[2] H. Terence Cook,[1] Charles D. Pusey,[1] Ruth M. Tarzi,[1] Ruth J. Pepper,[2] Nadia Wahab,[1] Roger M. Mason,[1] Maria Fragiadaki.[1] *[1]Renal Section, Imperial College London, London, United Kingdom; [2]UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: Cryoglobulins are immunoglobulins that precipitate at temperatures below 37 degrees and may be idiopathic, termed mixed essential cryoglobulinaemia (MEC) or associated with haematological malignancies and hepatitis C. Cryoglobulins stimulate small vessel vasculitis and renal involvement with mesangiocapillary glomerulonephritis. However, the aetiology of cryoglobulinaemia remains poorly defined and in viral associated cases therapy is often restricted by potential viral replication.

Methods: We have investigated the role of connective tissue growth factor(CTGF, CCN2)a pleiotropic growth factor upregulated in hepatocytes following hepatitis C infection and in lymphocytes during certain haematological malignancies.

We investigated CTGF expression in lymphocytes from patients with MEC, its effect on immunoglobulin production and the impact of lymphocyte overexpression of CTGF in a murine model.

Results: CTGF was significantly elevated in the serum and peripheral blood mononuclear cells (PBMC) of patients with MEC compared to controls (p=0.016). Addition of CTGF to lymphocytes stimulated lymphocyte proliferation, immunoglobulin G and M production and induced cryoglobulins. Patient's PBMC expressed altered CTGF mRNA moieties compared to controls, with excess exon 1 and 2 expression. 75% of mice that overexpressed CTGF in lymphoid cells developed a time-dependent cryoglobulinaemic glomerulonephritis, manifesting from 7 months. Bone marrow transfer from CTGF transgenic mice into irradiated wild type recipients confirmed that glomerulonephritis was transferable by haemopoetic cells.

Conclusions: Lymphocyte expression of CTGF is a critical requirement for cryoglobulin production in human cells in vitro and in an in vivo murine model which results in cryoglobulinaemic glomerulonephritis. Targeting CTGF may provide a rational non-immunosuppressive therapeutic approach for treating cryoglobulinaemia, which is most appealing in those cases associated with viral infections.

SA-PO2339

Immunostaining of Urinary Sediments for Claudin1, Neutrophil Elastase and CD68 for the Evaluation of Disease Activity of Glomerulonephritis Kojiro Yamamoto,[1] Takashi Oda,[1] Takahiro Uchida,[1] Atsushi Watanabe,[1] Taketoshi Kushiyama,[1] Keishi Higashi,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Soichiro Miura,[1] Hiroo Kumagai.[1] *[1]Department of Nephrology, National Defense Medical Collage, Tokorozawa, Saitama, Japan; [2]Department of Preventive Medicine and Public Health, National Defense Medical Collage, Tokorozawa, Saitama, Japan.*

Background: Urinary sediments of the patients who underwent kidney biopsy in our hospital were immunocytochemically analyzed in relation to renal histology.

Methods: We collected morning urine samples from 196 patients hospitalized for kidney biopsy from 2008 to 2011. Sediments of their urine were deposited onto the slide,

and indirect immunofluorescent staining was performed with anti-neutrophil elastase (NE) (neutrophil), CD68 (MØ), CD3 (T cell), synaptopodin (podocyte), cytokeratin (tubular epithelial cell), and claudin1 (CL1). CL1 is one of the tight junction proteins that are specifically expressed on epithelial cells of Bowman's capsule. The relationship between the positive cell number for various markers and the crescent formation rate (<30%: low, >30%: high) or global sclerosis rate (<10%: low, >10%: high) was evaluated.

Results: The numbers of CL1+ and NE+ cells were significantly increased in those patients with high crescent formation (p=0.04 and 0.001, respectively). However, no significant differences in CL1+ or NE+ cells were found in relation to the global sclerosis rate. We also evaluated the data from the state of crescents (cellular, fibro-cellular, fibrous, or no crescent). The numbers of CL1+ and NE+ cells were significantly increased in the patients with cellular crescent but was unchanged in those with fibro-cellular or fibrous crescents. The number of CD68+ cells was significantly increased in patients with cellular and fibro-cellular crescents but was unchanged in those with fibrous crescents. No other markers showed any significant difference in relation to crescent formation rate, glomerular sclerosis rate or state of crescents.

Conclusions: These data suggest that the immunostaining for CL1, NE and CD68 of urinary sediment is useful for the evaluation of glomerulonephritis, which may help for the decision of steroid and immunosuppressants.

SA-PO2340

Histopathological Classification of Primary Renal Vasculitis: What's New? Ana Pinho,[1] Ana Cabrita,[1] Anabela Malho,[1] André Fragoso,[1] Sandra Sampaio,[1] Elsa Morgado,[1] Ana Paula Silva,[1] Isabel Pinto,[1] Idalecio Bernardo,[1] Pedro Neves,[1] Helena Carreira.[2] *[1]Nephrology Department, Faro Hospital, Faro, Portugal; [2]Public Health Institute of Oporto Universtity, Oporto.*

Background: Current histopathological classifications for renal vasculitis are not consensual. A new classification for ANCA-associated glomerulonephritis based on chronic damage extension has been recently described (focal, crescentic, mixed and sclerotic). Our aim was to determine the predictive value for renal outcome according to chronic and active glomeruli damage extension observed on kidney biopsy samples from patients with primary vasculitis.

Methods: We performed a 25 years retrospective study of 66 patients (56% female) with clinical vasculitis (45 had Systemic Lupus Erythematosus and 21 had ANCA-associated glomerulonephritis). Demographic and laboratory data were recorded at the time of biopsy. Biopsy samples were classified as active (≥33% glomeruli combining cellular proliferation, leukocyte infiltration or fibrinoid necrosis-G1); sclerotic (≥33% sclerotic glomeruli-G2); mixed (≥33% combining crescentic and sclerotic glomeruli- G3); crescentic (≥33% glomeruli with cellular crescents-G4) and as focal (≥66% not affected glomeruli-G5). Survival analysis was used to assess differences in renal outcomes according to pathologic groups.

Results: The median of observed glomeruli was 10(49.3% of samples). At 5 years follow-up, the actuarial renal survival was 64.8% in G1, 55.6% in G2, 66.7% in G3, 36.4% in G4 and 93.5% in G5. The Cox regression revealed that hemoglobin (Hazard Ratio(HR)=0.70;p=0.004) and serum creatinine levels (HR=1.22; p=0.046) had a statistically significant effect on renal survival. Compared to G5, the HR for G1 was 5.3 fold higher (p=0.012) and the overall G2, G3 and G4 HR was 4.5 fold higher (p=0.006) .The type of vasculitis had no influence in this Cox model.

Conclusions: Extension damage in excess of one third on renal histologic analysis, in patients with primary vasculitis, emerged as an independent risk factor for progression to renal failure.

SA-PO2341

Misdiagnosed Cases of C3 Glomerulopathy among Children with Post Infectious Glomerulonephritis Badria M. AlGhaithi,[1] Paul S. Thorner,[2,3] Christoph Licht.[1,3] *[1]Nephrology, The Hospital for Sick Children; [2]Pathology, The Hospital for Sick Children; [3]U of Toronto.*

Background: C3 glomerulopathy (C3G) is a disease entity that has been recently introduced. C3G has been linked to dysregulation of the alternative complement pathway, and is characterized by isolated C3 glomerular deposits in the absence of immunoglobulins (Ig). There are a number of diseases that together with C3G form a phenotypic spectrum including dense deposit disease (DDD), atypical hemolytic uremic syndrome, and more recently, CFHR5 nephropathy. Clinically, C3G varies from mild degrees of hematuria, proteinuria, and renal impairment to end stage renal disease. We postulate that cases of C3G have been misrepresented in the past, possibly as post infectious glomerulonephritis (PIGN).

Methods: Based on this rationale, 33 patients who clinically presented with PIGN at our center from 1985 to 2010 having undergone renal biopsy were retrospectively reviewed for possible re-classification. Clinical characteristics including renal function, complements, urine profile and blood pressure data was captured from first presentation to last available follow up, which ranged from one month to 10 years.

Results: Serum C3 was low for all patients at first presentation. From re-review of the original renal biopsies, 25 patients (76%) were confirmed as PIGN based on the presence of subepithelial hump-like deposits and C3 depositions with or without Ig. Four patients (12%) were categorized as 'possible C3G' based on the absence of subepithelial humps in the presence of C3 but with Ig, while two (6%) had 'probable C3G' based on the presence of C3 only without subepithelial humps. Two patients (6%) demonstrated intermediate features suggesting both DDD and C3G. Five cases in the 'probable' and 'possible' C3G categories had a mild disease course initially resembling PIGN, however due to persistent low C3 and mild proteinuria, a renal biopsy was performed. Of these, 67% (4/6) continued to have proteinuria at last follow-up.

Conclusions: These results support our hypothesis that cases of C3G may have been categorized in the past as PIGN. Genetic testing for complement pathway abnormalities is warranted to further support this hypothesis.

SA-PO2342

Eculizumab as Life Saving Treatment in Complement Mediated MPGN Seetha Radhakrishnan, Fred G. Pluthero, Christoph Licht. *Division of Nephrology, The Hospital for Sick Children, Toronto, ON, Canada.*

Background: Membranoproliferative glomerulonephritis (MPGN) is a rare glomerulopathy with risk of progression to ESRD and post transplant recurrence. Pathogenetically, there is increasing evidence for dysregulation of the alternative complement pathway (AP). Eculizumab is a monoclonal C5 antibody that prevents terminal complement pathway activation. We present a treatment resistant case of MPGN where eculizumab served as an organ and life sustaining measure.

Methods: A 16 year old healthy female presented with nephrotic range proteinuria, peripheral edema and anemia (LDH and haptoglobin normal; occasional schistocytes). Renal biopsy showed mesangial interposition, wire loops, subendothelial and mesangial deposits and full house IF. In light of negative ANA and dsDNA a diagnosis of MPGN I was made and treatment with steroids and MMF commenced.

At 8 weeks, work up for fever and pancytopenia revealed group A strep, pseudomonas bacteremia, and CMV viremia, which was treated. Bone marrow showed signs of possible macrophage activation syndrome. MMF was held, steroid pulses and IVIG x3 were provided. Patient had persistent thrombocytopenia and anemia, increasing creatinine, and anuria eventually requiring hemodialysis (HD). Complement analysis revealed strong AP activation (undetectable C3; high C5b-9; low CH50; positive C3NeF). While no complement mutation was found, CFHR1/3 was absent on western blot. CFH antibodies were not detected.

Results: To regain complement control, plasma therapy (infusion x5 followed by pheresis x7) was commenced. While there was subtle treatment response (improvement in creatinine and C3) anuria persisted and clinical status deteriorated with respiratory compromise, GI bleeding and seizures. With eculizumab (900 mg/wk x4), treatment response was dramatic: following 1st dose neurologic complications ceased, urine output normalized and HD was discontinued. Thrombocytopenia and anemia recovered after 2nd dose.

Conclusions: Recovery of this patient from life threatening conditions in response to eculizumab strongly suggests (i) a role for AP dysregulation in MPGN pathogenesis and (ii) future AP controlling treatment strategies for MPGN – a remarkable breakthrough for this condition.

SA-PO2343

Platelets Serve as Source of Complement Factor H (CFH) Activity in a Modified Fluid-Phase Alternative Complement Pathway Cofactor Activity Assay Jiawei Zhao, Fred G. Pluthero, Antony Jeganathan, Christoph Licht. *Division of Nephrology, The Hospital for Sick Children, Toronto, ON, Canada.*

Background: We have recently shown that that the alternative complement pathway regulator complement factor H (CFH) is present in a non-granular compartment in human blood platelets and that platelets are capable of taking up and releasing CFH (Licht et al, Blood 2009). The physiological role of platelet CFH remains to be determined, given the high concentrations of this protein (up to 600 μg/ml) normally present in plasma.

Methods: In order to assess the potential physiological relevance of platelet CFH, washed normal platelets were used as the sole source of CFH in a modified fluid-phase alternative complement pathway cofactor activity assay. This assay allows for testing the potential of a protein to serve as cofactor to complement factor I (CFI) in cleaving C3b, the active form of complement C3, by observing the appearance of C3b cleavage products.

Results: Varying platelet concentrations ranging from 600 to 30/nL were incubated with 2 μg/mL C3b and 6 μg/mL CFI at 37°C for 60 min, and supernatants assayed via immunoblotting for the presence of C3b cleavage products. CFH cofactor activity was observed to increase with platelet concentration, while platelets remained in a resting state. A time course analysis with a platelet concentration of 300/nL (within the normal range of blood platelet concentration) gave results similar to those observed with plasma and purified CFH.

Conclusions: These results show that like endothelial cells, platelets can serve as a cellular source of CFH cofactor activity *in vitro*. This provides support for the emerging hypothesis that in addition to their established roles in the blood coagulation, inflammatory and immune systems, platelets may actively participate in the regulation of the complement system, particularly the constitutively-active alternative pathway that is involved in several complement-related diseases.

SA-PO2344

The Podocyte Is the Initial Target in the Renal Pathogenesis of Diarrhoea-Associated Haemolytic Uraemic Syndrome Lindsay S. Keir,[1] Gavin Iain Welsh,[1] Richard Coward,[1] Anna Richards,[2] Robert A. Spooner,[3] Moin Saleem.[1] *[1]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [2]Centre for Inflammation Research, Queens Medical Research Institute, Edinburgh, United Kingdom; [3]School of Life Sciences, University of Warwick, Coventry, United Kingdom.*

Background: D+HUS is the leading cause of pediatric acute renal failure. It occurs after infection by shigatoxin (stx) producing bacteria. Stx binds Gb3 cellular receptors. It was considered an endothelial disease until the report of an inducible podocyte-specific VEGF-A knockdown mouse that developed glomerular thrombotic microangiopathy- the hallmark of HUS. Furthermore, familial HUS is caused by dysregulation of the alternative complement pathway with mutations identified in complement regulators. Eculizumab, a drug that blocks the complement cascade, is being trialed in atypical HUS and used experimentally in D+HUS. We hypothesize that podocytes, VEGF-A and complement regulators play a co-ordinated role in the renal pathogenesis of D+HUS.

Methods: Immunofluorescence of human and mouse immortalized glomerular cell lines detected Gb3. Cellular stx1 sensitivity was determined using a radio-labeled protein synthesis assay. An ELISA measured VEGF-A produced by stx treated podocytes. Human cells were treated with VEGF-A and western blotting determined complement regulator expression. A factor H re-synthesis assay was devised to remove bound factor H. Cells were fixed or treated with serum free media +/- VEGF-A. Factor H was detected by immunofluorescence.

Results: Human glomerular cells express Gb3 and are stx1 sensitive. Human podocytes are more sensitive than endothelial cells. Mouse podocytes lack Gb3 and are stx1 insensitive. Stx stimulation of human, but not mouse, podocytes reduced VEGF-A production. VEGF-A up regulates human glomerular cells complement regulator expression. Both glomerular cells synthesize factor H in vitro and VEGF-A regulates this effect.

Conclusions: Podocytes are a sensitive stx1 target. Reduced podocyte VEGF-A down-regulates complement regulator expression and production in the glomerular endothelium, making it vulnerable to attack. This is further evidence indicating a central role for the podocyte in D+HUS.

Funding: Government Support - Non-U.S.

SA-PO2345

Small Molecule Disruptors of Interactions between Protein Kinase A and A-kinase Anchoring Proteins Inhibit AVP-Mediated water Reabsorption – Implications for Treatment of Cardiovascular Diseases Kerstin Zuehlke,[1,2] Jelena Milic,[1] Walter Rosenthal,[2] Enno Klussmann.[2] *[1]Department of Signal Transduction, Leibniz Institute for Molecular Pharmacology (FMP), Berlin, Germany; [2]Anchored Signalling, Max Delbrück Center for Molecular Medicine (MDC, Berlin, Germany.*

Background: Stimulation of the vasopressin V2 receptor by Arginine-vasopressin (AVP) is followed by an increase in cAMP and activation of protein kinase A (PKA). PKA phosphorylates the water channel aquaporin-2 (AQP2). This leads to a redistribution of AQP2 into the plasma membrane and facilitates water reabsorption from primary urine, depending on interaction of PKA with A-kinase anchoring proteins (AKAPs), which position PKA at defined cellular sites and thereby coordinate PKA signaling.

Methods: 20,064 drug-like small molecules were screened among others using surface plasmon resonance (SPR) measurements.

Live cells are incubated with compound of interest and AKAP150 was immunoprecipitated. Co-precipitated PKA was determined using the PepTag-Assay (fluorescent peptide substrate).

PKA-dependent phosphorylation of substrate proteins was measured in vivo by Western blotting.

The effects of small molecules on the development of SIADH and chronic heart failure were investigated using immunohistochemical and echocardiographic methods.

Results: Elevated secretion of AVP as it occurs in the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is the most frequent cause of hyponatremia. Moreover, elevated levels of AVP are associated with chronic heart failure. In both SIADH and chronic heart failure the elevated AVP levels cause a predominant localization of AQP2 in the plasma membrane and lead to excessive water retention. Here we present small molecules disrupting AKAP-PKA interactions in renal principal cells in culture and in animal models. The molecules prevent the AVP-induced redistribution of AQP2 suggesting that AKAP-PKA interactions are novel drug target for the treatment of diseases associated with exessive water.

Conclusions: Small molecules interrupting AKAP-PKA interactions may pave the way to new strategies for the treatment of diseases associated with excessive water, such as SIADH and chronic heart failure.

SA-PO2346

Differential Polarity of AQP2 Membrane Insertion by Vasopressin and Forskolin in a 3D MDCK Cyst Culture Model – Role of cAMP Levels William Rice, Hua Ann Jenny Lu, Dennis Brown. *Program in Membrane Biology, Massachusetts General Hospital and Harvard Medical School, Boston, MA.*

Background: In renal collecting duct principal cells Vasopressin (VP) acts through its receptor, V2R, to increase intracellular cAMP leading to the activation of PKA and subsequent phosphorylation and apical trafficking of the water channel Aquaporin 2 (AQP2). Recent studies have shown that VP modulates the phosphorylation of the AQP2 C-terminal serines, S256, S261, S264, S269 and have demonstrated that phosphorylation at S256 by PKA is the dominant signal for trafficking of AQP2 from intracellular vesicles to the apical membrane. However, the roles of the other C-terminal serines have yet to be determined.

Methods: In this study, we used AQP2 phosphorylation specific antibodies to visualize the intracellular location of phospho- AQP2 in an in vitro MDCK 3D cyst model.

Results: We found that the cyst model is well polarized, with tissue architecture that resembles that of the collecting duct in animals. As observed in vivo and in tissue slice cultures, VP, low dose forskolin (0.1μM) or (<5μM) CTP-cAMP stimulation led to apical localization of total AQP2, AQP2- pS256, pS264 and pS269. Inhibition of PKA by H89 or mPKI blocked translocation of AQP2 to the apical membrane as well as the phosphorylation at S269. Interestingly, high dose FK (50μM) or CTP-cAMP (50μM), but not VP (1μM) led to strong basolateral localization of total AQP2, AQP2-pS256 and pS269. As VP stimulation of cAMP levels is limited by V2R signaling, these data suggest that the differential polarity of AQP2 insertion is mediated by the intracellular level of cAMP.

Conclusions: This study describes the successful use of phosphorylation specific AQP2 antibodies to visualize the intracellular location of specific phosphorylation isoforms of AQP2 in an in vitro culture system. This 3D culture system will enable efficient screening of conditions that modulate AQP2 trafficking and serve as a tool for elucidating the role of AQP2 phosphorylation at each of the C-terminal serines. The relevance of our findings to the observed segment-specific apical and/or basolateral insertion of AQP2 in vivo is now under investigation.

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SA-PO2347

Endothelial Aquaporin-1 Water Channels Are Positively Regulated by Protein Kinase C and Glucose-Degradation Products Wei Zhang, Sandra Muller-Krebs, Martin G. Zeier, Vedat Schwenger. *Dept. of Nephrology, University of Heidelberg, Germany.*

Background: Aquaporin-1(AQP1) is expressed widely in microvascular endothelia including the peritoneum. AQP1, a so-called water-specific “ultrasmall pore” in the peritoneal membrane, plays a key role for ultrafiltration (UF) in peritoneal dialysis (PD).

Little has been known for the functional AQP1 regulation in endothelia. Our previous findings demonstrated that AQP1 expressed in Xenopus oocytes is strongly regulated by protein kinase C (PKC). Additionally, it was found that glucose degradation products (GDPs) generated during heat sterilization in dialysis fluids involve the activation of PKC. The aim of the study is to investigate the molecular mechanisms of the regulation of AQP1 by PKC and GDPs in endothelial cells.

Methods: AQP1 expression in HUVEC cells was studied with rt-PCR and immunocytochemistry. AQP1-regulated water permeability in Xenopus oocytes and HUVECs was measured through microscopic recordings of hypo-osmotic challenges and digital volume quantification.

Results: Aquaporin-1 is highly expressed in HUVEC cells and can be knocked down effectively with a siRNA-approach. Water permeability of HUVEC cells is strongly increased after pharmacologic activation of PKC. This effect is completely abolished after either co-application of PKC inhibitors or knock-down of AQP1.

The water permeability both in oocytes and HUVECs is markedly increased by GDPs (methylglyoxal or 3.4-dideoxyglucosone-3-ene). This effect is completely blocked in AQP1ΔPKC channels lacking PKC phosphorylation sites in oocytes, and not present in HUVECs transfected with AQP1-siRNA.

Conclusions: This is the first study to show that in endothelial cells PKC positively regulates the AQP1-mediated water permeability, and that GDPs modulate the AQP1-related water permeability via PKC. These findings may contribute insights about AQP1-mediated endothelial permeability, as well as provide new clues for AQP1 modulation in the peritoneal membrane during PD.

Funding: Government Support - Non-U.S.

SA-PO2348

Regulation of ENaC by Phosphoinositide 3-Kinase and AMP-Activated Protein Kinase: Studies with Resveratrol John P. Johnson,[1] Kelly Weixel,[2] Kenneth R. Hallows,[1] Allison L. Marciszyn,[1] Robert S. Edinger.[1] *[1]Renal-Electrolyte Division, University of Pittsburgh School of Medicine, PA; [2]Biology, Chatham University, Pittsburgh, PA.*

Background: Several studies indicate the multiple effects of resveratrol on cellular function are due to its inhibition of class 1A phosphoinositide 3-kinase (PI3K) mediated signaling pathways but it also activates (AMPK). Sodium transport in the kidney via the Epithelial Na+ Channel (ENaC) is highly sensitive to changes in phosphoinositide signaling

in the membrane and AMPK. Therefore we employed resveratrol to probe the relative effects of phosphatidylinositol species in the plasma membrane and AMPK activity and their impact on ENaC activity in a native cell culture model.

Methods: A combination of biophysical and biochemical assays were used to identify the effect of resveratrol on ENaC activity in CCD cells. Knockdown of AMPK and inhibitor studies were used to confirm the function of AMPK in mediating the effects of resveratrol on ENaC. In addition, live cell microscopy techniques were used to assess the time course and acute effects of resveratrol on phosphatidylinositol species in the plasma membrane of mpkCCDc14.

Results: We found that resveratrol acutely reduces amiloride-sensitive current in CCD cells. The time course and dose dependency of this inhibition paralleled plasma membrane depletion of a PI(3,4,5)P3 reporter in live-cell microscopy, indicating that this early inhibitory process is likely mediated by resveratrol's known effects on PI3K activity. Additionally, resveratrol induced a late inhibitory effect (3-24 hr) that appears to be mediated via AMPK activation. Resveratrol induces a significant activation of AMPK compared with vehicle controls after 3 hours which persisted through 24 hours. Knockdown of AMPK or treatment with the AMPK inhibitor, Compound C,reduced the late inhibition of ENaC but had no effect on the early inhibitory response to resveratrol.

Conclusions: Collectively, these data demonstrate that resveratrol inhibits ENaC activity by two mechanisms: an early effect seen within 5 minutes related to depletion of membrane PIP3, and a sustained late (3-18 hour) effect secondary to activation of AMPK.

Funding: NIDDK Support

SA-PO2349

Purinergic Cascade Contributes to the Activation of Ca^{2+}-Permeable TRPV4 Channels by Mechanical Forces in the Aldosterone-Sensitive Distal Nephron Mykola Mamenko, Oleg L. Zaika, Roger G. O'Neil, Oleh Pochynyuk. *Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Medical School, Houston, TX.*

Background: Epithelial cells in the aldosterone-sensitive distal nephron (ASDN) respond to changes in flow/composition of the ultrafiltrate by elevating $[Ca^{2+}]_i$. It is thought that this is critical for adaptation of water-electrolyte transport at this site. At the same time, environmental changes are known to trigger paracrine ATP release in the ASDN.

Methods: In this study, we probed a role for purinergic signaling in mediating mechano-sensitive responses by directly monitoring changes in $[Ca^{2+}]_i$ in individual cells within split-opened ASDN of mice.

Results: Purinergic stimulation caused a similar transient Ca^{2+} peak followed by a sustained plateau in both principal and intercalated cells. Genetic deletion of P2Y2 receptors compromised purinergic signaling and, importantly, attenuated Ca^{2+}-responses to hypotonic media and elevated flow. Furthermore, we show here that activation of mechano-sensitive TRPV4 channel plays a major role in the sustained $[Ca^{2+}]_i$ elevation during purinergic stimulation. Genetic deletion of TRPV4 channel disrupted ATP-induced Ca^{2+}-plateau.

Conclusions: We concluded that paracrine release of ATP in response to mechanical stimulations reciprocally modulates cellular responses by activating mechano-sensitive TRPV4 channel in ASDN cells.

Funding: Private Foundation Support

SA-PO2350

Identification of CNK3 as an Important Component of the Aldosterone-Controlled ENaC Regulatory Complex Rama Soundararajan, Tim Ziera, Jian Wang, David Pearce. *Medicine (Nephrology), University of California San Francisco, San Francisco, CA.*

Background: Members of the Connector Enhancer of KSR (CNK) family of proteins possess multiple protein interaction domains, and have been proposed to function as scaffolds possibly assisting various interactions in a multiple signaling cascade. Mammalian CNK3 is an aldosterone-induced protein that is essential for the activity of the epithelial sodium channel (ENaC). In the present study, we examine its role in ENaC stimulation.

Methods: We used biotinylation assays in transiently transfected HEK293T kidney epithelial cells to study the effect of CNK3 on ENaC surface expression. We also used these cells to test protein-protein interactions in co-immunoprecipitation assays. We used polarized mpkCCD kidney epithelial cells grown on Transwell filters to study effects on ENaC activity.

Results: We show that CNK3 is associated with ENaC at the cell surface and that it interacts with the components of the previously described ENaC regulatory complex (ERC). The PDZ domain in CNK3 appears to be crucial for its association with SGK1 and GILZ1, and for its stimulation of ENaC cell surface expression in HEK293T cells. We further demonstrate that the PDZ domain in CNK3 is required for aldosterone-controlled ENaC-mediated Na+ transport in mpkCCD cells.

Conclusions: These results strongly suggest that CNK3 is essential for the proper assembly of ENaC-regulatory factors thus facilitating appropriate aldosterone signaling to stimulate Na+ reabsorption via ENaC (Funding Sources: NIH Grants DK078679, DK056695 and DK085101).

Funding: NIDDK Support

SA-PO2351

Oxidative Stress in Regulation of Renal Na/K-ATPase Signaling and Distribution Yanling Yan,[1] Deepak K. Malhotra,[1] Zi-Jian Xie,[2,1] Nader Abraham,[2] Joseph I. Shapiro,[1,2] Jiang Liu.[1] *[1]Medicine, University of Toledo College of Medicine, OH; [2]Pharmacology and Physiology, University of Toledo College of Medicine, OH.*

Background: In Dahl salt-resistant (R) and -sensitive (S) rats, we found that impaired renal proximal tubular Na/K-ATPase/c-Src signaling contributes to salt-sensitive hypertension, but the underlying mechanism is not clear. Interestingly, the S rats have significantly higher heme oxygenase-1 (HO-1) expression level, both before and after high salt diet (2% NaCl for 7 days, compared to 0.3% NaCl), than the R rats in renal proximal tubules (n=8 per group per strain, $p<0.01$). We investigated the role of oxidative stress in regulation of renal proximal tubular Na/K-ATPase signaling and distribution.

Methods: c-Src phosphorylation, cellular fractionation.

Results: In pig LLC-PK1 cells, ouabain stimulated c-Src activation, ROS generation, protein carbonylation,and Na/K-ATPase endocytosis. These ouabain effects were significantly attenuated by pretreatment with ROS-scavenging NAC. Increases in oxidative stress by glucose oxidase (GO, 1 and 3mU/ml) also activated c-Src and stimulated Na/K-ATPase endocytosis in LLC-PK1 cells. Induction of HO-1 by CoPP or knockdown of Na/K-ATPase α1 subunit by siRNA significantly attenuated ouabain-induced c-Src activation. These data suggest that Na/K-ATPase is required in ouabain-induced ROS generation and this increase in ROS is a downstream effector of Na/K-ATPase/c-Src that is critical in ouabain-induced Na/K-ATPase endocytosis. The data also suggest that oxidative stress may affect Na/K-ATPase/c-Src signaling and redistribution.

Considering that HO-1 is one of the most potent antioxidant enzyme, we further speculate that the HO-1 is capable of neutralizing high salt diet- and/or ouabain-stimulated elevation of ROS in the S rats, either as a stimulator or as a downstream effector of Na/K-ATPase/c-Src signaling. This leads to the impairment of Na/K-ATPase/c-Src signaling and redistribution of the Na/K-ATPase and NHE3 in the S rats, thus the salt-sensitive hypertension.

Conclusions: Oxidative stress, including ouabain-induced ROS generation, is capable of regulating Na/K-ATPase/c-Src signaling and redistribution.

SA-PO2352

AS160: A New Na,K-ATPase Partner That Regulates the Trafficking of the Sodium Pump in Response to Energy Depletion Daiane Santana Alves,[1] Glen A. Farr,[1] Michael Kashgarian,[1] Johannes Loffing,[2] Michael J. Caplan.[1] *[1]Molecular and Cellular Physiology, Yale University, New Haven, CT; [2]Institute of Anatomy, University of Zurich, Switzerland.*

Background: The Na,K-ATPase is the major active transport protein found in the plasma membranes of most epithelial cell types. The Na,K-ATPase appears to be located in two different cellular pools. The major sodium pump pool is located at the plasma membrane, while the other pool resides in cytoplasmic vesicular compartments which can translocate to the plasma membrane in response to physiological stimuli. We have identified AS160 as a new Na,K-ATPase partner. AS160 is a Rab GAP that regulates the trafficking of the GLUT4 and the ENaC in response to insulin/muscle contraction and aldosterone, respectively.

Methods: We characterized the interaction between AS160 and Na,K-ATPase by co-immunoprecipitation and co-localization assays. We characterized the physiological role of the AS160 interaction with sodium pump by using RNAi techniques to knockdown AS160 expression in cultured MDCK renal epithelial cells. Finally, we examined the regulation of this interaction in mice subjected to renal ischemia followed by reperfusion.

Results: In COS cells, coexpression of AS160 and Na,K-ATPase led to the intracellular retention of the sodium pump, suggesting that AS160 may participate in mediating the accumulation of an intracellular pool of Na,K-ATPase. We also demonstrated that knockdown of AS160 increased the total cellular levels of Na,K-ATPase activity, suggesting that AS160 modulates the size of the active pool of the pump. We characterized the physiological role of the interaction between AS160 and Na,K-ATPase. We found that AS160 is required for the intracellular accumulation of the Na,K-ATPase following energy depletion in cultured epithelial cells. Finally, we performed in vivo experiments that suggest that the phosphorylation state of AS160 correlates with the subcellular distribution of the Na,K-ATPase following renal ischemia and reperfusion.

Conclusions: We conclude that AS160 is a new sodium pump partner that regulates Na,K-ATPase retention in the cytoplasm and its trafficking to the plasma membrane following energy depletion.

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SA-PO2353

Coordinated Action by Tsg101 and USP2-45 in the Negative Feedback Loop of the Mineralocorticoid Receptor Olivier Staub, Nourdine Faresse. *University of Lausanne.*

Background: In order to avoid prolonged response and consequently tissue damages, desensitization mechanisms often take place in order to downregulate stimulated pathways. In the context of aldosterone/mineralocorticoid receptor (MR) signalling, it was shown that prolonged exposure to aldosterone decreased MR expression via the proteasome, but the mechanisms of this feedback regulation remain unclear. We were interested into pathways involving MR signalling termination in response to aldosterone.

Methods: These questions were studied either in transfected M1 or in mCCD cells by applying immunoprecipitations, immunoblotting, luciferase assays and measurements of transepithelial Na transport.

Results: We first observed that MR was ubiquitylated at the basal state and that this modification was removed after aldosterone treatment. As for other nuclear receptor, we found that MR interacted with Tsg101 at the basal state and this association was disrupted after aldosterone treatment. We found that Tsg101 can stabilize MR probably by maintaining its ubiquitylation. Because USP2-45 is an aldosterone induced deubiquitylating enzyme, we wondered if USP2-45 was involved in the deubiquitylation of the receptor. We found that USP2-45 interacted with MR, removed its ubiquitylation and decreased its expression via the proteasome.

Our data imply a mechanism in which MR is ubiquitylated at the basal state and protected by Tsg101. Aldosterone treatment stimulates USP2-45 expression, which interacts with MR and deubiquitylates the receptor. The removal of the ubiquitin destabilizes the MR/Tsg101 interaction and induces MR degradation via the proteasome by a so far unknown mechanism.

Conclusions: These results reveal the existence of a functional network involving USP2-45 and Tsg101 into a negative feedback loop of the MR pathway that mediates the degradation of MR in response to aldosterone.

Funding: Government Support - Non-U.S.

SA-PO2354

Sensory Renal Innervation: Does a Kidney-Specific Expression Pattern of Voltage-Activated Sodium Channels Lead to a Specific Firing Activity and Higher Excitability? Wolfgang Freisinger, Tilmann Ditting, Sonja Heinlein, Johannes Schatz, Roland Veelken. *Medical Clinic 4, Nephrology and Hypertension, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany.*

Background: Thermal ablation of renal innervation has been effective in trating hypertensive patients. However, especially afferent renalinnervation is poorly understood. We recently found that renal afferent neurons exhibited predominantly tonic firing patterns, i.e. sustained action potential (AP) firing throughout depolarizing current injection (DCI), pointing to a higher excitability. Non-renal neurons responded mostly with a phasic response, i.e. <4 APs. We now tested the hypothesis that different voltage-gated sodium-channels, which initiate the action potential generation, are responsible for these differences.

Methods: Dorsal root ganglion neurons from Sprague Dawley rats (Th11-L2) were recorded in current clamp technique and characterized as tonic or phasic according to their response to DCI. Then, single cells were switched to voltage-clamp, potassium and calcium channel blockers were added to the external solution. The application of the sodium channel blocker Tetrodotoxin (TTX) then allowed to further characterize voltage-gated sodium currents.

Results: 66 DRG neurons were investigated (n=40 phasic, n=26 tonic). Tonically firing cells showed significantly a lower firing threshold (-21,75 ± 1,43 mV vs.-29,33 ± 1,63 mV*), a lower overshoot (46,79 mV [38,63 - 54,75] vs. 56,74 mV [53,6 – 60,96]) and longer action potential duration (4,61 ms[4,15 – 5,85] vs. 3,35 ms[2,12 – 5,67]). Tonic cells showed a significantly smaller fraction of TTX-sensitive Na+- currents (60,45 ± 12,94pA/pF vs. 140,44 ± 30,88pA/pF), whereas activation did not reveal a significant change (*p<0,05).

Conclusions: We could show for the first time that a smaller TTX-sensitive channel expression is very likely linked to electrophysiological differences observed between renal versus non-renal petidergic afferent nerve fibers. This finding points to an organ-specific expression pattern of sodium channels concerning renal innervation. Sodium channel expression and consequently excitability is likely to be altered in hypertension and inflammation.

SA-PO2355

Identification of Two Novel Renal Olfactory Receptors Ryan J. Protzko, Jennifer L. Pluznick. *Physiology, Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: ORs are chemosensory GPCRs which comprise the largest gene family in the mammalian genome. We previously found that the OR signaling pathway plays a functional role in the kidney (Pluznick, PNAS 2009) and identified six renal ORs. In this study, we identify two additional novel renal ORs and examine their tissue distribution and ligand specificity.

Methods: An RT-PCR screen using primers to ORs with known or predicted ligands was performed on murine kidney cDNA. Primers for ORs found in kidney were also tested on cortex, medulla, and 8 non-renal tissue cDNAs. Trafficking to the cell surface was assayed by immunofluorescence (IF); one OR was screened for responses to various ligands using a dual-luciferase ligand screen.

Results: An RT-PCR screen for 27 ORs with known ligands revealed 2 novel renal ORs: Olfr691 (cortex and medulla, 2/8 non-renal tissues) and Olfr545 (medulla, 6/8 non-renal tissues). Olfr691 and Olfr545 were cloned and expressed in HEK293T cells. Because the luciferase ligand screen requires surface expression, surface delivery was assayed by IF. Olfr691 reaches the cell surface when co-expressed with RTP1L (receptor transporting protein 1, long); Olfr545 does not reach the cell surface under any condition tested. Olfr691 was reported to respond to valeric and isovaleric acid (Saito, Cell 2004); we screened Olfr691 for responses to chemically related compounds, and also performed an unbiased screen against a chemical library. Olfr691 responds only to short and medium chain fatty acids (FAs; C3-8, including isomers): chiefly propionoic, isobutryic, butyric, isovaleric and valeric acids.

Conclusions: We have previously shown that the OR signaling pathway plays important functional roles in the kidney. Here, we identify two additional renal ORs. Olfr691, found in the cortex and medulla, responds to short- and medium-chain FAs. The primary biological source of short chain FAs is intestinal bacterial metabolism, indicating that Olfr691 may signal in response to gut flora activity. Further studies will focus on localizing Olfr545 and Olfr691, identifying Olfr545 ligands, and examining the potential roles of these receptors in vivo.

Funding: NIDDK Support

SA-PO2356

Advanced Glycation End-Products, Glomerular Endothelial Cells, Tight junction, Renin Angiotensin System Zengchun Ye, Canming Li, Cheng Wang, Tan-Qi Lou. *Department of Nephrology, Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.*

Background: To investigate whether advanced glycation end-products (AGEs) influence the permeability and tight junction proteins of cultured rat glomerular endothelial cells (rGEnCs) *in vitro*, and the underlying role of renin angiotensin system activation in this process.

Methods: The GEnCs permeability was detected by measuring the transendothelial electrical resistance and flux of FITC-BSA across the GEnCs monolayers. Angiotensin II was detected by ELISA, and ZO-1, Occludin , Claudin-5 and JAM-A were detected by immunofluorescence and Western blotting.

Results: The results show that AGEs increased the permeability of glomerular endothelial cells monolayers in a concentration- and time-dependent manner. This effect of AGEs was accompanied by a redistribution of tight junction proteins, including ZO-1, occludin, JAM-A and claudin-5, from the cell–cell borders of glomerular endothelial cells. AGEs also down-regulated the expression of the tight junction proteins, occludin, JAM-A and claudin-5. In addition, a significant finding in this study was that AGEs could increase the permeability of rGEnC monolayers by activating the intracellular renin angiotesin system (RAS) in a RAGE–dependent signaling pathway. This was supported by the finding that AGEs were able to stimulate the activity of angiotensin converting enzyme (ACE), and up-regulate the expression of angiotensin II and angiotensin II type 1 receptor (AT1R) in rGEnCs. This activation was blocked by a neutralizing antibody to RAGE, and by pharmacologic inhibitors of RAS.

Conclusions: The results collectively indicate that the induction of increased permeability of rGEnCs by AGEs may be predominantly via a RAGE-angiotensin II-AT1 pathway. This effect on glomerular endothelial cells illustrates a novel action of ACEI/ARB for use in diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2357

Diabetic Nephropathy-Associated IHG-1 Is a Novel Regulator of Mitochondrial Fusion Fionnuala B. Hickey, James B. Corcoran, Brenda Griffin, Una Bhreathnach, Catherine Godson, Madeline Murphy. *Conway Institute, University College Dublin, Dublin, Ireland.*

Background: *Induced in high glucose-1* (IHG-1) is a highly conserved, glucose-regulated transcript associated with diabetic kidney disease. In addition, we have detected increased IHG-1 expression in a murine model of renal fibrosis [rat unilateral ureteral obstruction]. A mitochondrial localization sequence is the only predicted functional domain identified in IHG-1. We have confirmed the mitochondrial localization of IHG-1 and further investigated a potential role in mitochondrial dynamics. Mitochondria are plastic organelles that frequently change shape and size and these dynamic fusion and fission events are important for the bioenergetic function of mitochondria.

Methods: The subcellular localization of IHG-1 has been determined by immunofluorescence, electron microscopy and biochemical methods. Mitochondrial morphology has been examined by live cell imaging following labelling of mitochondria with GFP. Mitochondrial fusion/fission was analysed using fluorescence recovery after photobleaching (FRAP) and other live cell imaging techniques. Binding of IHG-1 to mediators of mitochondrial fission and fusion has been examined by co-immunoprecipitation.

Results: IHG-1 is predominantly localised to the mitochondrial matrix and is associated with the inner mitochondrial membrane. To date there is little information on mitochondrial dynamics in kidney. This study demonstrates that inhibition of endogenous IHG-1 expression results in fragmented mitochondrial morphology and leads to decreased mitochondrial fusion. Conversely, overexpression of IHG-1 leads to increased mitochondrial fusion. The effect of IHG-1 on mitochondrial dynamics is dependent on its mitochondrial localization. We have also demonstrated that IHG-1 binds to known mediators of mitochondrial fusion – mitofusin 1(Mfn1) and Mfn2. This provides a possible mechanism of action through which IHG-1 affects mitochondrial dynamics.

Conclusions: Mitochondrial dysfunction is a major contributor to hyperglycemic-induced kidney damage and also has a well documented role in fibrosis. Therefore, IHG-1 may have a critical function in both diabetic nephropathy and other fibrotic conditions.

Funding: Government Support - Non-U.S.

SA-PO2358

The Role of Toll-Like Receptor Proteins (TLR) 2/4 in Human Proximal Tubular Cells In Vitro – A Potential Mediator of Diabetic Nephropathy?
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Background: Inflammatory responses are crucial in the pathogenesis of diabetic nephropathy (DN). TLRs are ligand activated membrane-bound receptors which activate nuclear factor-kappaB (NF-κB). TLR 2/4 are present in proximal tubular cells (PTC) and are activated by ligands pathological in DN (i.e. HMGB1, fibronectin and heat shock proteins). We have previously shown that 30mM glucose increases HMGB1 expression in PTCs. Here, we explored the effect of varying glucose levels and HMGB1 on TLR 2/4 signalling to help understand the inflammatory pathway activation in DN.

Methods: HK2 cells (a human kidney PTC line) were exposed to control (5mM), 30mM glucose, fluctuating glucose (5mM /30mM) and 11.25mM glucose for 72h. Cells were harvested for nuclear extract and protein. TLR2/4 expression and NF-κB binding were measured. The role of HMGB1 in TLR2/4-regulated NF-κB activation was assessed with silencing TLR2/4. The effect of glucose on TLR2/4 expression was examined by restricting glucose uptake with a sodium-glucose co-transporter2 (SGLT2) inhibitor.

Results: TLR2/4 expression and downstream NF-κB binding increased drastically with 11.25mM glucose compared to 30mM glucose and fluctuating glucose. Blocking glucose uptake with an SGLT2 inhibitor prevented any increase in TLR2/4 expression. Recombinant HMGB1 increased NF-κB binding at 2h and this was prevented by TLR2 silencing.

Conclusions: Increase in TLR2/4 expression stimulated by 11.25mM glucose may occur through intracellular hyperglycemia within PTCs as this effect was blocked by SGLT2 inhibitor. NF-κB activation was also induced at high glucose levels, and by recombinant HMGB1, suggesting a role for TLR2/4. Furthermore, TLR2 may function as the predominant receptor in mediating HMGB1-induced NF-κB activation.

Funding: Government Support - Non-U.S.

SA-PO2359

Transcription Regulation of Cell Matrix Proteins in Proximal Tubular Cells Treated with High Glucose
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Background: The rate of deterioration of kidney function correlates best with the degree of tubular fibrosis and accumulation of cell matrix proteins. The mechanisms of renal cell matrix expansion as well as the signal transduction pathways activated in the diabetic milieu are incompletely characterized.

Methods: Phosphorylation of two effector molecules of downstream target of mTOR, p70 ribosomal protein S6 kinase (S6K) and 4E-BP1 was measured in proximal tubular cells treated with high glucose. Cell matrix proteins (Collagen IV and fibronectin) expression was also measured in cells treated with high glucose (25mM) for 0-24hrs. Transcription factors expression of cAMP-responsive element binding protein (CREB) and yin-yang 1 (YY1) was evaluated in cells treated with high glucose.

Results: Exposure of cells to high glucose (0-24hrs) resulted in increase mTOR activity through increasing the phosphorylation 4E-BP1 and p70S6K that lead to increased the protein expression of fibronectin and collagen IV. Pretreatment the cells with rapamycin blocked fibronectin and collagen IV accumulation induced by high glucose at 12 and 24hrs. In addition, blocking mTOR by rapmaycin leads to decreased the expression of CREB that involve in transcription regulation of fibronectin and collagen IV. Our data also show that rapamycin treatment resulted in increase the cytoplasmic expression of YY1 and lead to deceased the accumulation of fibronectin and collagen IV.

Conclusions: Our data show that treatment with rapamycin resulted in decease the phosphorylation of 4E-BP1 and p70S6K and lead to decreased the accumulation of cell matrix proteins through CREB in cells treated with high glucose. These data provide a novel role of TOR inhibitor as therapeutic agent that transcriptionally regulates the cell matrix proteins in diabetes.

SA-PO2360

mTORC2 Knockdown Slows the Progression of Cysts in Type I MDCK Cells
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Background: mTOR exists in association with two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 consists of mTOR and Raptor while mTORC2 consists of mTOR and Rictor. Recent studies on rapalogues (rapamycin or everolimus) in humans with ADPKD were very unimpressive. While there are multiple reasons for the unimpressive effect of rapalogues in human PKD, one possible reason is that while rapamycin inhibits mTORC1 it does not have an effect on pro-proliferative mTORC2.

Methods: The downstream substrates of mTORC2 (pAktSer^{473}, pPKC-alpha Ser^{657}, pSGK Ser^{422}) were analyzed by immunoblot in 8 wk old Han:SPRD (Cy/+) rats. The effect of specific mTORC2 knockdown on cyst formation was determined in an *in vitro* model of Type 1 MDCK cells that form cysts in collagen. Rictor, the functional component of mTORC2 was silenced using a plasmid-based shRNA vector system (pTRIPZ) expressing RNAs under the Tet-On inducible promoter. pAktSer^{473} expression was 90% reduced in Rictor knockdown cells (MDCK-puro+dox) compared to puromycin-treated controls (MDCK-puro). Cysts diameters were measured using Image J software.

Results: On immunoblot the downstream targets of mTORC2 (pAktSer^{473}, pPKC-alpha Ser^{657}, pSGK Ser^{422}) were upregulated in Cy/+ rats compared to wild type littermate controls (+/+). In Cy/+ compared to +/+, pAktSer^{473} was three fold increased (P<0.001,N=4), pPKC-alpha Ser^{657} was twofold increased (P<0.05, N=3) and pSGK Ser^{422} was twofold increased (P<0.05, N=3). The diameter of the cysts was significantly decreased in Rictor knockdown MDCK-puro+dox cells.

Diameter of cysts in microns

EXPERIMENTAL GROUPS	Day4	Day8	Day12	Day16
MDCK Control	57.36±0.46	106.7± 0.50	239.7 ±1.32	393.3 ±1.76
MDCK-puro	51.37± 0.37	104.6± 0.65	239 ± 2.09	360.4 ±5.43
MDCK-puro+dox	45.63± 0.60*	81.56± 0.96*	189.6 ±2.45*	240.3 ±2.90*

*P<0.05 vs MDCK Control and MDCK-puro

Conclusions: 1) There is increased mTORC2 signaling in 8wk old Cy/+ kidneys with PKD, 2) Silencing of mTORC2 in an *in vitro* model reduces cyst size. In conclusion, mTORC2 merits further study in PKD.

Funding: Other NIH Support - AARA

SA-PO2361

Hyperactive TORC1 and TORC2 Launch Hif1alpha-Dependent Expression of PTEN To Regulate Phosphorylation of Akt in Tuberous Sclerosis Complex (TSC)
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Background: TSC results from mutations in one of two genes, TSC1 and TSC2. TSC2 mutations contribute more to the pathology of the disease, including renal angiomyolipomas. TSC2 acts as a signaling hub in the PI 3 kinase/Akt/mTOR node. Of the two mTOR complexes, TORC1 and TORC2, TSC2 deficiency results in constitutive activation of TORC1.We have reported recently that human renal angiomyolipomas and TSC2 null murine embryonic fibroblasts (MEFs) display high levels of the tumor suppressor protein PTEN with reduced phosphorylation of Akt. The mechanism of PTEN upregulation is not known. Use of rapamycin and constitutively active mTOR (engineered to block TORC1 only) in TSC2 null MEFs and in 293 cells showed that TORC1 increases PTEN mRNA and protein levels. Also, kinase dead mutant of mTOR (mTOR KD), which blocks both TORC1 and TORC2, inhibited expression of PTEN via transcriptional mechanism in TSC2 null MEFs and in 293 cells. Moreover, mTOR KD enhanced and suppressed phosphorylation of Akt at the catalytic loop site Thr-308 and hydrophobic motif site Ser-473, respectively. Furthermore, the inhibition of deregulated mTOR in TSC2 null MEFs and in 293 cells by shRNA-mediated downregulation of raptor, an exclusive component of TORC1, inhibited expression of Hif1alpha, resulting in attenuation of PTEN protein and mRNA expression and transcription, concomitant with enhanced phosphorylation of Akt at Thr-308 and Ser-473. Knockdown of rictor, a sole component of TORC2, reduced expression of Hif1alpha and PTEN but significantly decreased the Akt phosphorylation at both sites. Importantly, rescue of Hif1alpha prevented rictor-downregulated PTEN expression. In contrast, overexpression of rictor increased Hif1alpha and PTEN protein levels. Thus we provide evidence for a novel cell autonomous function of both TORC1 and TORC2 in Hif1alpha-mediated upregulation of PTEN, thereby decreasing phosphorylation of Akt and contributing to the nonmalignant nature of tumors in TSC patients.

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SA-PO2362

Ca^{2+} Influx through Reverse-Mode Na^+/Ca^{2+} Exchange Is Critical for Vascular Endothelial Growth Factor (VEGF) Mediated – ERK1/2 Activation and Angiogenic Functions of Human Endothelial Cells
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Background: Cardiovascular complications are a major cause of morbidity/mortality in CKD. Deregulated Ca^{2+} levels are universal in CKD patients. However, the effect(s) of altered Ca^{2+} signalling on the vascular endothelium remains largely unknown. Here, by using VEGF, as a paradigm, we investigated the effect of extracellular Ca^{2+} on endothelial cell signalling.

Methods: Human umbilical vein endothelial cells (HUVECs) were serum starved in a physiological buffer and preincubated with reverse-mode (Ca^{2+} in - Na^+ out) Na^+/Ca^{2+} exchanger (NCX). ERK1/2 activation was determined by western blot. PKC and B-Raf activities were determined by scintilation counting. $[Ca^{2+}]_i$ was measured in HUVECs loaded with the fluorescent Ca^{2+} indicator Fluo-4NW.

Results: Here, we report that extracellular Ca^{2+} is required for VEGF-induced ERK1/2 activation and that release of Ca^{2+} from intracellular stores alone, in the absence of extracellular Ca^{2+}, is not sufficient to activate ERK1/2. Furthermore, inhibitors or reverse-mode NCX suppressed the VEGF-induced activation of ERK1/2 in a time- and dose-dependent manner and attenuated VEGF-induced Ca^{2+} transients. Knock-down of NCX1 (the main NCX isoform in HUVECs) by siRNA confirmed the pharmacological data. A panel of NCX inhibitors also significantly reduced VEGF-induced B-Raf activity

and inhibited $PKC_{[sup]\alpha[/sup]}$ translocation to the plasma membrane and total PKC activity *in situ*. Finally, application of NCX inhibitors reduced VEGF-induced HUVEC proliferation, migration and tubular differentiation in surrogate angiogenesis functional assays *in vitro*.

Conclusions: We propose that Ca^{2+} influx through reverse-mode NCX is required for the activation and the targeting of PKCα to the plasma membrane, an essential step for VEGF-induced ERK1/2 phosphorylation and downstream EC functions. Thus, in CKD, deregulated Ca^{2+} may affect endothelial signalling and potentially contribute to endothelial dysfunction.

SA-PO2363

FTI-277 – A Novel Inhibitor of Vascular Calcification Arvind Ponnusamy,[1,2,3] Smeeta Sinha,[1,2] Gareth D. Hyde,[2] Philip A. Kalra,[1] Ann E. Canfield.[2,3] *[1]Renal, Salford Royal NHS Trust Foundation; [2]University of Manchester; [3]NIHR BRC Manchester, United Kingdom.*

Background: Patients with chronic kidney disease have poor cardiovascular outcomes due to increased vascular calcification and atherosclerosis. Vascular calcification is a highly-regulated process involving the loss of calcification-inhibitory molecules, osteogenic differentiation of VSMC, and VSMC apoptosis. This study aims to determine whether vascular calcification is regulated by protein farnesylation

Methods: Bovine and human vascular smooth muscle cells were used in mineralisation and apoptosis experiments. Mineralisation and apoptosis were induced using 5 mM β-glycerophosphate or 2.6 mM phosphate in serum-free medium, respectively. Akt phosphorylation and active caspase 3 were assessed by western blotting. Gene expression was analysed using qRT-PCR.

Results: The farnesyl transferase inhibitor, FTI-277, significantly inhibits calcification of VSMC in a dose-dependent manner ($p<0.001$). Pre-incubation of VSMCs with FTI-277 (10 μM) inhibits Ras activation and markedly enhances serum-induced Akt phosphorylation. Wortmannin inhibits PI3K/Akt signalling. Therefore, VSMCs were induced to mineralise in the presence of wortmannin (100 nM), FTI-277, wortmannin and FTI-277, or vehicle. Wortmannin significantly promotes VSMC mineral deposition ($p<0.001$). Some mineralisation was detected in cells incubated in the presence of both reagents, demonstrating that the effects of FTI-277 can be partially negated by preventing downstream PI3K signalling. FTI-277 also reduces caspase 3 activation and significantly inhibits phosphate-induced VSMC apoptosis ($p<0.05$). To determine whether FTI-277 also regulates the osteogenic differentiation of VSMC, RNA was collected from cells cultured +/- FTI-277 for 9-10 days. Results show that FTI-277 inhibits Runx2, Msx 2 and alkaline phosphatase mRNA expression ($p<0.05$), promotes matrix Gla protein mRNA expression ($P<0.05$), and maintains α-smooth muscle actin expression.

Conclusions: These studies demonstrate that FTI-277 inhibits VSMC mineralisation by activating downstream PI3K/Akt signalling and preventing apoptosis, and by regulating the osteogenic differentiation of VSMC.

SA-PO2364

Microstructured Platform Systems To Study *Tunneling Nanotube* (TNT) Related Processes in Human Peritoneal Mesothelial Cells (HPMCs) Julia Ranzinger,[1] Amin Rustom,[2] Marcus Abel,[2] Julia Leyh,[1] Lars Kihm,[1] Martin G. Zeier,[1] Vedat Schwenger.[1] *[1]Nephrology, University of Heidelberg, Germany; [2]New Materials and Biosystems, Max Planck Institute for Intelligent Systems, Stuttgart, Germany.*

Background: Efficacy of peritoneal dialysis (PD) depends on the integrity of the peritoneal membrane. Anew discovered membrane channels, TNTs, mediate intercellular communication between cells of different cellular systems and therefore attain physiological and pathological relevance. Due to the sensitivity of TNTs and the dependence on density of respective cells, quantitative investigations of TNT-related processes are difficult. We developed micropatterned gold dot surfaces as platform systems to study cell-cell interactions in HPMCs.

Methods: Microstructured surfaces were prepared by means of photolithography. The size and the distance of the gold dots were set to match with the average diameter of HPMCs and to prevent cell clustering. For biofunctionalization, IKVAV peptide representing the cell-binding domain of the α-chain of laminin was coupled covalently on the gold dots. Mesothelial cells from i) omentum of healthy donors and ii) effluents in dialysis fluid from patients undergoing PD were isolated and cultured on the platforms. 3D fluorescence and scanning electron microscopy were applied to detect TNTs.

Results: HPMCs selectively adhere to the functionalized gold dots when cultured on the platforms. No unspecific association to the glass on the surfaces occurred. When we investigated the number of TNTs connecting HPMCs cultured on these platform systems, we found that TNT- formation was hindered almost completely. Compared to the number of TNTs detected between cells plated on glass cover slides, only a negligible number of TNTs could be visualized when cells were cultured on biofunctionalized microstructures.

Conclusions: The existence of TNTs connecting HPMCs *in vitro* points to a distinctive communication among these cells at least under cell-culture conditions and is presumably crucial for the integrity of the peritoneal membrane. We show that the spatial separation of HPMCs by culturing the cells on biofunctionalized microstructures leads to an inhibition of the TNT-formation.

SA-PO2365

Renal Kinome Scan in Lupus Nephritis Chun Xie,[1,2] Tianfu Wu,[2] Chandra Mohan.[2] *[1]Division of Nephrology/Internal Medicine, UT Southwestern Medical Center, Dallas, TX; [2]Division of Rheumatology/Internal Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: Protein kinases are key regulators of a wide variety of biological processes. Several protein kinases have been elucidated to play important roles in the pathogenesis of systemic lupus erythematosus (SLE) and glomerulonephritis. However, almost all of these investigations focused only on single molecules or pathways. The human genome contains more than 500 protein kinase genes. Most of these kinases have not been investigated in regard to their roles in SLE or glomerulonephritis. In the current study, we performed a systemic kinome scan in kidneys of spontaneous lupus mouse strains

Methods: Protein extracts from renal cortices of 6-8 months old NZB/NZW F1, and MRL.lpr mice as well as C57BL/6 controls were subjected to kinome profiling, using Kinex Antibody Microarray. Some of the differentially expressed kinases were then validated by Western blot or immunohistochemistry.

Results: We were able to detect 193 protein kinases, 24 protein phosphatases and 150 regulatory subunits of these enzymes by using this platform. 87 of these molecules were found to be differentially expressed in lupus kidneys, compared to the controls. Using the Ingenuity Pathway Analysis (IPA), we identified several biological networks, functions and pathways, including cell growth and proliferation, gene expression, posttranslational modification, immune response, and inflammation, etc., that are involved in lupus nephritis. In addition, some of these molecules have been validated by Western blot or immunohistochemistry. The role of tyrosine hydroxylase, one of the molecules that are highly expressed in lupus kidneys, in the pathogenesis of lupus nephritis, is being tested in animal models.

Conclusions: We have identified several molecules that have altered expression in lupus nephritis, by systemic kinome profiling. We also revealed several disease pathways that are involved in lupus nephritis. The functional studies of these molecules and pathways are in progress.

SA-PO2366

Uremia Decreases the IL-6 Response to Exercise of Skeletal Muscle: Alteration in JAK2/STAT3 and AMPK Signaling Natalia H. Dünner, Fabiola Venegas, Juan P. Peña, Francisco G. Coronado, Enrique Jaimovich, Luis F. Michea. *Facultad de Medicina, Universidad de Chile, Santiago, Chile.*

Background: Chronic renal failure (CRF) induces muscular weakness and decreased exercise endurance. Interleukin-6 (IL-6) is a cytokine upregulated by exercise in skeletal muscle, with autocrine effects on skeletal muscle metabolism. The AMP-activated protein kinase (AMPK) is a fuel sensing enzyme activated by changes in the energy state of the cell, and recent findings indicate that IL-6 can activate AMPK *in vivo*. Several studies show that IL-6 mRNA is altered in CRF, but it is unknown if CRF affects the response to exercise. We hypothesized that the regulation of muscular IL-6 expression and AMPK in response to exercise is impaired in CRF.

Methods: Male Sprague-Dawley rats were given 5/6 nephrectomy (NPX) or sham surgery and pair-fed. The response to exercise was measured in *extensor digitorum longus* (EDL) fatigued by *in situ* electrical stimulation through the sciatic nerve, or after swimming. At the end of the exercise protocols, EDL was dissected for mRNA and protein evaluation.

Results: We found no significant differences in the abundance of IL-6 mRNA of EDL at rest. After *in situ* stimulation we observed 85±8 fold induction of IL-6 mRNA in the EDL of Sham rats; however, IL-6 mRNA in EDL from NPX rats increased only 22±7 fold ($n=6$, $P<0.01$). Since IL-6 exerts a positive feedback in muscle, we analyzed IL-6 signaling pathway. NPX did not affect mRNA of IL-6 receptors, IL-6Ra and gp130. The Janus Kinase 2 (JAK2) protein decreased to 63±4.5% of control levels in EDL of NPX rats ($n=5$, $P<0.01$). We observed reduced activation of Stat3 and reduced induction of Suppressor of cytokine signalling-3 (SOCS-3) mRNA in EDL of NPX rats after exercise ($n=3$-4, $P<0.05$). Basal P-AMPK levels were reduced in NPX (54±14% of control, $n=6$, $P<0.05$) and this difference was maintained after exercise (61±10%, $n=6$, $P<0.05$).

Conclusions: The data show reduced activation of IL-6 pathway in EDL of NPX rat in response to exercise. The decrease of AMPK phosphorylation could contribute to low endurance to exercise in CRF.

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SA-PO2367

Stimulation of RANKL and Bone Resorption by Metabolic Acidosis Requires Gβγ Signaling Nancy Krieger, Christopher D. Culbertson, David A. Bushinsky. *Medicine, University of Rochester, NY.*

Background: Chronic metabolic acidosis (Met) stimulates cell-mediated net calcium (Ca) efflux from bone, mediated primarily through increased osteoblastic prostaglandin E_2-induced stimulation of RANKL and osteoclastic bone resorption. Osteoblasts express OGR1, a proton (H^+)- sensing G-protein coupled receptor (GPCR), and we demonstrated that H^+ activation of OGR1 increases intracellular Ca in osteoblasts and the OGR1 inhibitor, $CuCl_2$, blocks H^+-induced bone resorption. Our results suggest that OGR1, coupled to Gq, is the sensor that detects increased [H^+] during Met and initiates osteoblastic signaling leading to increased osteoclastic bone resorption.

Methods: To explore regulation of G-protein coupled signaling by Met, neonatal mouse calvariae were incubated for 48 h in physiologically neutral (Ntl, pH=7.50, Pco_2=34 mmHg, $[HCO_3^-]$=28 mM) or acid (Met, pH=7.17, Pco_2=33 mmHg, $[HCO_3^-]$=13 mM) medium ± 20 μM gallein (Gal), a selective inhibitor of Gβγ signaling, and net Ca efflux measured. To determine RANKL mRNA expression, primary osteoblasts were isolated from calvariae and incubated in Ntl (pH=7.43, Pco_2=39 mmHg, $[HCO_3^-]$=25 mM,) or Met (pH=7.10, Pco_2=39 mmHg, $[HCO_3^-]$=15 mM) medium ± 20 μM gal for 48h. Cells were collected and RANKL mRNA levels measured by real time PCR.

Results: Compared to incubation in Ntl (442±80 nmol/bone/24h), Met induced net Ca efflux (1339±92, $p<0.05$); Gal did not alter Ca efflux in Ntl (218±62) but blocked the increase with Met (264±138, $p<0.05$ vs Met alone) (n=8/group, mean ± SE). Compared to incubation of primary osteoblasts in Ntl (0.85±0.11), Met induced an increase in RANKL mRNA (1.29±11, $p<0.05$); Gal did not alter RANKL in Ntl (0.69±0.06) but blocked the increase with Met (0.91±0.05, $p<0.05$ vs Met alone) (n=6/group). OPG mRNA levels were not altered by Met either in the absence or presence of Gal.

Conclusions: Thus gallein inhibited Met stimulation of osteoblastic RANKL production and acid-induced bone resorption. These results, together with our previous finding of acid inhibition of RGS16, which can limit G protein signaling, suggest that regulation of Gβγ is important for modulating the OGR1-mediated response of the osteoblast to metabolic acidosis.

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SA-PO2368

Exposure to Uremic Serum Induces a Pro-Calcific Phenotype in Human Mesenchymal Stem Cells Rafael Kramann,[1] Rebekka K. Schneider,[2] Uta Kunter,[1] Jurgen Floege.[1] *[1]Department of Nephrology and Clinical Immunology, RWTH University, Aachen, Germany; [2]Institute of Pathology, RWTH University, Aachen, Germany.*

Background: Arterial media calcification in chronic kidney disease (CKD) patients involves osteoblast-induced calcification of the collagen extracellular matrix (ECM) resulting in intramural ossification. The current study is based on the hypothesis that mesenchymal stem cells (MSC) constitute critical cells for pro-calcific ECM remodelling in CKD patients.

Methods: Human MSC were cultured in media supplemented with pooled sera from either healthy or uremic patients (20%). Results were compared to MSC cultured in growth medium as well as osteogenic differentiation medium. Calcification and matrix remodelling were further analysed in a three-dimensional in-vitro collagen scaffold recapitulating the vascular collagen I/III environment.

Results: In two-dimensional culture, exposure to uremic serum enhanced the proliferation of MSC (cell counting, BrdU incorporation), whereas apoptosis and necrosis were not affected (annexin V and 7-AAD staining). Uremic serum-exposed MSC recapitulated osteogenesis by calcification of their ECM (von Kossa staining, calcium quantification) and expression of bone-related genes (BMP-2 receptor, ALP, osteopontin, Runx2, collagen I) over a period of 35 days. The uremic serum-induced osteogenesis was completely blocked by a BMP-2/4 neutralizing antibody. In the three-dimensional system, uremic serum-induced calcification was shown to occur along collagen fibers (SEM, energy-dispersive X-ray spectroscopy) and was accompanied by extensive matrix remodelling. Uremic serum exposed MSC acquired a myofibroblast phenotype as shown by a significant contraction of the ECM and by *de novo* expression of α-smooth muscle actin. The procalcific ECM remodelling was accompanied by increased expression of collagen I, osteopontin, laminin and fibronectin (immunohistochemistry) and exhibited parallels to the ECM remodelling observed in arteries of CKD-patients (n=8) (with arteries from children serving as control).

Conclusions: Uremic serum induced in a BMP-2/4-dependent manner an osteoblast-like phenotype in MSC accompanied by matrix remodelling and calcification.

SA-PO2369

Hypoxia-Inducible Factors in Glomerular Endothelial Cells Alexander Weidemann, Susanne Olbrich, Margarete Goppelt-Struebe, Kai-Uwe Eckardt. *Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Hypoxia-inducible factors (HIFs) regulate genes which are central for the adaptation to low oxygen conditions. In normoxia HIF is degraded and inactive. Pharmacological activation of HIF in normoxia is protective in kidney injury. The cellular compartment which exerts the protective effects upon HIF activation is still elusive but endothelial HIF might be one of the effectors. To dissect the molecular mechanisms of how HIFs affect endothelial cell function we established an in vitro model of glomerular endothelial cells (glEnd) with stable HIF-knockdown.

Methods: glEnds with stable knockdown of both HIFα isoforms were generated by lentiviral transfection with shRNA producing constructs. Molecular analyses were performed by real-time PCR and westernblotting. HIF was stabilized by DMOG which inhibits HIF-prolyl hydroxlases (PHD). Proliferation was analysed by MTT assays.

Results: glEnds express both HIFα proteins, however HIF-2α levels is much lower. Deletion of HIF-1α does not affect expression of HIF-2α and vice versa. Of the taget genes investigated, most of them are regulated by HIF-1α only. HIF-2α only contributes to a limited extend (e.g. to VEGF regulation). HIFα deficiency does not affect cell proliferation under normoxic conditions. However, HIF-1α deletion significantly impares proliferation in hypoxia, whereas deletion of HIF-2α has no effect. Treatment with the PHD-inhibitor DMOG significantly slows proliferation in normoxia. This antiproliferative effect was rescued by deletion of HIF-1α.

Conclusions: Surprisingly the functional relevance of HIF-2α in glEnd cells in vitro is limited whereas HIF-1α is the dominant HIF-isoform. Hypoxic proliferation is critically dependent on HIF-1 for metabolic adaptation. In contrast, reduction of proliferation by PHD-inhibition is also mediated by HIF-1. This indicates that although most target genes are regulated similarly by both stimuli, the functional consequences of HIFα stabilization are diverse. Given the increasing interest in using HIF-activation as therapeutical approach to renal disease, these cells are a valuable tool to investigate the functional roles of HIFα in the renal endothelium in vitro.

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SA-PO2370

Reduction of Na/K-ATPase in Marinobufagenin-Induced Cardiac Myocyte Apoptosis Changxuan Liu,[1,2] Yan Bai,[2] Yu Wang,[1] Lijun Liu,[2] Deepak K. Malhotra,[3] Christopher J. Cooper,[3] Joseph I. Shapiro,[2,3] Zi-Jian Xie,[2,3] Jiang Tian.[3] *[1]Renal Division and Institute of Nephrology, Peking University First Hospital, Beijing, China; [2]Department of Physiology and Pharmacology, University of Toledo, OH; [3]Medicine, University of Toledo, OH.*

Background: Na/K-ATPase functions as a receptor for cardiotonic steroids (CTS) such as ouabain, marinobufagenin (MBG), and digoxin. Decreases in cardiac Na/K-ATPase have been documented in patients with heart failure and other disease states. Our previous studies demonstrate that reduction of Na/K-ATPase attenuates CTS-induced Src signaling, resulting in increased cell death in renal epithelial cells.

Methods: We employed a Na/K-ATPase α1 heterozygote knockout mouse model (α1+/-), in which, the expression of heart tissue Na/K-ATPase α1subunit is reduced by about 30% in comparison to its wild type (a1+/+) littermate. Experimental mice were subject to marinobufagenin (MBG) infusion using osmotic mini pumps and the echocardiography was performed. Cardiac myocytes were also isolated from these mice for in vitro experiments.

Results: The results showed that MBG infusion increases myocyte apoptosis and induces left ventricle dilation in the heterozygote mice, but not in the wild type mice. Moreover, MBG significantly reduced the contractile function in these heterozygote mice. Mechanistically, we demonstrated that MBG activates Akt and the mammalian target of rapamycin (mTOR) in wild type mice. Activation of mTOR further increases the phosphorylation of ribosome S6 kinase and Bcl-2-associated death promoter (BAD) and protects cells from apoptosis. However, this survival signaling induced by MBG was abolished in the heterozygote mice. Instead, MBG activates caspase 9 in heterozygote mice, which may account for the cardiac myocyte apoptosis. Using rat neonatal cardiac myocytes, we further demonstrate that directly inhibiting the mTOR pathway by rapamycin also enable MBG to activate caspase 9 and induce myocyte apoptosis.

Conclusions: Reduction of cardiac Na/K-ATPase attenuates MBG-induced activation of survival signaling while making cardiac myocytes susceptible to MBG-induced apoptosis.

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SA-PO2371

Albumin Overload Leads to Low Molecular Weight Proteinuria in Normal Mice, While a Compensatory Proteolytic Pathway Develops in Limp-2 Null Mice Darren H.K. Lee,[1] Scott Andrew Fraser,[1] Marina Katerelos,[1] Kurt Gleich,[1] Paul Saftig,[2] David A. Power.[1] *[1]Depts of Nephrology and Medicine, Austin Health, VIC, Australia; [2]Institut für Biochemie, Christian-Albrechts-Universität zu Kiel, Kiel, Germany.*

Background: Mutations of the lysosomal protein Limp-2 cause low molecular weight (LMW) proteinuria and albuminuria due to reduced proteolysis in the proximal convoluted tubule (PCT).

Methods: To determine how Limp-2 affects the response to protein overload, WT and Limp-$2^{-/-}$ mice received daily bovine serum albumin (BSA) injections i.p. for 10 days.

Results: Urinary albumin/creatinine ratio did not increase significantly in BSA-treated Limp-$2^{-/-}$ mice (Day 0: 134±104; Day 10: 211±210 mg/mmol), whereas levels in WT mice increased to those seen in Limp-$2^{-/-}$ mice (Day 0: 30±52; Day 10: 246±299 mg/mmol; $p<0.05$). BSA treated Limp-$2^{-/-}$ mice showed no increase in LMW proteinuria (retinol-binding protein (RBP), vitamin D-binding protein and α1-microglobulin), while WT mice developed LMW proteinuria. There was no difference in megalin or cubilin mRNA expression.

Quantitative immunofluorescence microscopy showed that RBP and LAMP-1 labelled vesicles in the PCT were distributed more basally in the untreated Limp$2^{-/-}$ mice compared with WT ($p<0.001$), confirming delayed proteolysis. Surprisingly, RBP was distributed more basally in BSA-treated mice in both groups ($p<0.01$), also suggesting delayed protein degradation. Western blots showed that cathepsin B and L expression was increased in BSA-treated Limp-$2^{-/-}$ mice ($p<0.05$) while cathepsin L but not cathepsin B was up-regulated in BSA-treated WT mice ($p<0.001$). Similarly, the proportion of RBP vesicles co-localising with cathepsin B increased in BSA-treated Limp-$2^{-/-}$ mice (Mander's coefficients: 0.18±0.07 vs 0.13±0.09; $p<0.05$) whereas it did not change in BSA-treated WT mice (0.30±0.10 vs 0.30±0.09).

Conclusions: In response to BSA overload, WT mice develop tubular proteinuria and delayed proteolysis of reabsorbed proteins. In contrast, Limp-$2^{-/-}$ mice show no significant increase in pre-existing tubular proteinuria, apparently due to the presence of increased cathepsin B. This data suggests considerable plasticity in the response of the PCT to protein overload.

Funding: Government Support - Non-U.S.

SA-PO2372

TGFβ Modulates Mitochondrial Bioenergetics and Morphology in Podocytes Gabriella Casalena, Ilse S. Daehn, Erwin P. Bottinger. *Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: TGFβ regulates differentiation, growth, and apoptosis of podocytes and mediates podocyte depletion in glomerulosclerosis. In patients with progressive glomerular diseases the increased expression of TGFβ in podocytes can be one of the causes of damage induction and perpetuation in glomerular tufts. Mitochondrial dysfunction and oxidative stress emerged recently as potential therapeutic targets in glomerular injury. Whether TGFβ regulates mitochondrial dysfunction/oxidative stress in podocytes is not known.

Methods: Mitochondrial function in WT, CD2AP-deficient and Smad2-Smad3-deficient murine immortalized podocytes grown in non-permissive conditions was analyzed upon treatment with 5 ng/ml of TGFβ for 6, 24 and 48 hr.

Results: TGFβ treatment induced a significant Smad-dependent increase of podocyte oxygen consumption rate starting at 24 hr. Increased oxygen consumption was associated with mitochondrial membrane depolarization, mitochondrial network fragmentation, and increased cellular reactive oxygen species (ROS). TGFβ-induced ROS production was reverted by NADPH oxidase inhibitor apocynin. In contrast, TGFβ did not alter mitochondrial superoxide level (MitoSox). ATP content was not different from untreated podocytes and increased respiration was not associated with increase mitochondrial mass as shown by citrate synthase activity. At early time points, TGFβ treatment partially inhibited the expression of nuclear and mitochondrial genes encoding subunits of Complex I of respiratory chain. In contrast, expression of SOD2 increased with TGFβ treatment.

Conclusions: Our results suggest that TGFβ-Smad activation increases OCR as part of a secondary metabolic response to preserve cellular energy (ATP) homeostasis during TGFβ-induced cellular phenotype responses. Compensatory stimulation of anti-oxidant mitochondrial gene expression (SOD2) may prevent increase of mitochondrial superoxide levels. In a context of oxygen deprivation, often observed in chronic and acute kidney diseases, the lack of adaptive changes in mitochondria could promote apoptosis commitment in podocytes. We conclude that canonical TGFβ-Smad pathways regulate adaptive modifications of mitochondrial metabolism in podocytes.

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SA-PO2373

Glomerulotubular Disconnection Is Rescued by the Antiapoptotic Effect of Ouabain in Rats with Passive Heymann Nephritis Ulla B. Holtback,[1] Xiao Liu,[1] Ann-Christine Eklof,[1] Agnes B. Fogo,[2] Anita Aperia.[1] *[1]Women´s and Children´s Health, Karolinska Institutet, Stockholm, Sweden; [2]Pathology, Vanderbilt University.*

Background: Glomerulotubular disconnection and formation of atubular glomeruli, which is a common feature in both tubular and glomerular disorders, contribute to the progression of chronic renal disease. Apoptosis of proximal tubular cells is considered as a main cause of the disconnection. Our group has shown that a cardiotonic steroid, ouabain, protects kidney cells from apoptosis by triggering a Ca^{2+}-NF-kB signal (JASN 2006, Nature Com 2010).

Here we have examined whether ouabain may protect from apoptosis and glomerulotubular disconnections in rats with passive Heymann nephritis (PHN), a model of human membranous nephropathy.

Methods: PHN and control rats were followed for 4 months. One PHN group (n=7) received ouabain (15 mg/kg/day) and the other received vehicle via intraperitonal mini pumps.

Results: S-Cr was significantly increased in PHN rats compared to control and PHN/ouabain rats. Significant proteinuria was recorded in both PHN groups, but was somewhat less pronounced in PHN/ouabain rats. Morphometric analysis of the kidneys revealed glomerular fibrosis and basement membrane damage in both PHN groups, but significantly less pronounced in PHN/ouabain rats. For assessment of apoptosis, kidney sections were TUNEL stained and counterstained with hematoxylin. The number of apoptotic cells at the glomerulotubular disconnection was significantly, 3.2 fold, higher in PHN than in PHN/ouabain rats. To assess the level of glomerulotubular disconnections, kidneys were serially sectioned and an average of 75 sections/kidney were analyzed. In control rats 95% of glomeruli were normally connected. In PHN 64% were normally connected, 19 % were connected to atrophic tubules and 17% to atubular glomeruli. In PHN/ouabain rats 84% of glomeruli were normally connected, 10 % were connected to atrophic tubules and 6 % to atubular glomeruli

Conclusions: The results support the notion that glomerulotubular disconnection is triggered by local apoptosis and imply that formation of atubular glomeruli can be alleviated by the antiapoptotic effects of ouabain.

SA-PO2374

Identifying P2X7 Receptor as a Key Regulator of Deleterious Renal Epithelial-Fibroblast Cross Talk Murugavel Ponnusamy,[1] Shougang Zhuang.[1,2] *[1]Department of Medicine, Alpert Medical School, Brown University, Providence, RI; [2]Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China.*

Background: P2X7 is an extracellular ATP dependent membrane receptor, which can acts as a mediator of cell death. Despite P2X7 receptor protein is barely expressed or not detectable in normal kidney, its expression is increased in some kidney disease models or human kidney diseases. Currently, the potential pathological role of P2X7 receptor and the signaling cascades leading to its expression in the kidney are largely understood. As a reduced number of peritubular fibroblast is observed in the interstitium adjacent to damaged tubular epithelium in the early phase of AKI, we examined whether damaged renal epithelial cells would directly induce renal interstitial fibroblast death via activation of purinergic signaling in vitro.

Methods: Renal proximal tublular cells (RPTC) and rat renal interstitial fibroblast cell line (NRK-49F) were used.

Results: Exposure of cultured NRK-49F cells to necrotic RPTC lysate or supernatant induced expression of $P2X_7$ receptor and cell death in NRK-49F. Inhibition of P2X7 with A438079, a highly selective $P2X_7$ receptor inhibitor, or knockdown of $P2X_7$ receptor with siRNA blocked the deleterious effect of necrotic RPTC supernatant. In response to necrotic RPTC, multiple signaling pathways including ERK1/2, p38, JNKs and AKT are activated in NRK-49F, pharmacological inhibition of ERK1/2, but not p38, JNK and AKT pathways blocked RPTC supernatant-induced P2X7 expression and cell death. Similar results were also obtained in NRK-49F with knockdown of ERK1/2 or MEK1, a direct upstream activator of ERK1/2. Conversely, over-expression of MEK1 enhanced these responses. Further, siRNA mediated knockdown of Elk1, a transcriptional factor targeted by ERK1/2, also reduced necrotic RPTC-induced P2X7 expression and renal fibroblast death.

Conclusions: These data indicate that necrotic RPTC supernatant can directly induce death of renal interstitial fibroblasts via up-regulation of P2X7 receptor through an ERK signaling-dependant mechanism.

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SA-PO2375

Apoptotic Cells Alter Proximal Tubular Cell (PTC) Viability Via Multiple Complex Signaling Pathways Vimal Patel,[1] Lanfei Feng,[1] Donald Massenburg,[1] Angelika Antoni,[2] Joyce Rauch,[3] Wilfred Lieberthal,[4] Jerrold S. Levine.[1] *[1]Medicine, University of Illinois at Chicago, IL; [2]Medicine, Kutztown University, Kutztown, PA; [3]Medicine, McGill University, Montreal, QC, Canada; [4]Medicine, SUNY, Stoney Brook, NY.*

Background: Cells undergoing apoptosis acquire new activities that modulate the fate and function of neighboring live cells. We have previously shown that apoptotic target cells decrease the viability of live kidney PTC responder cells. Here, we elucidate the signaling pathways responsible for decreased viability of PTC responders following their receptor-mediated interaction with apoptotic targets.

Methods: We used BU.MPT cells, a conditionally immortalized PTC line, as responder cells. BU.MPT cells, induced to undergo apoptosis in several ways, were used as apoptotic targets.

Results: Apoptotic targets induced apoptotic death in ~80% live PTC responders by 48 hrs. Modulation of PTC viability occurred through at least 3 distinct pathways. First, inhibition of Akt promoted apoptosis via activation of Bad and decreased cellular abundance of β-catenin. However, a constitutively active Akt construct (mAkt) reduced target-induced PTC apoptosis to ~40%, indicating a role for Akt-independent pathways. Second, combined use of mAkt and the caspase-8 inhibitor Z-IETD-FMK reduced PTC apoptosis to <20%, indicating that activation of caspase-8 contributes to target-induced PTC death. Finally, activation of p38 served a counter-regulatory role and protected PTC from target-induced death by increasing β-catenin abundance. Inhibition of p38 by SB203580 increased target-induced PTC death to ~100% by 48 hrs.

Conclusions: Together, these data emphasize the complexity and robustness of the signaling events induced in viable PTC responders following interaction with apoptotic targets. By acting as sentinels of environmental change, apoptotic targets may allow neighboring viable cells, especially non-migratory epithelial cells, to monitor and potentially adapt to local stresses.

Funding: NIDDK Support

SA-PO2376

Attenuating Effect of Angiotensin-(1-7) on Angiotensin II-Mediated Reactive Oxygen Species Induced Apoptosis through Regulation of Mitochondrial NOX4 Su-Mi Kim,[1] Yang Gyun Kim,[1] Sul-Ra Lee,[2] Jay-Sung Gim,[1] Kyung-Hwan Jeong,[2] Sang-Ho Lee,[1] Eun Young Kim,[1] Tae Won Lee,[2] Chun-Gyoo Ihm,[2] Ju-Young Moon.[1] *[1]Internal Medicine, Kyung Hee University Hospital, Seoul, Sangil-Dong, Gangdong-Gu, Korea; [2]Internal Medicine, Kyung Hee University Medical Center, Seoul, Hoegi-dong, Dongdaemun-gu, Korea.*

Background: Angiotensin II (Ang II)-mediated reactive oxygen species (ROS) are important second messengers for the transcriptional effects of Ang II, and NOX4 is the central enzyme of Ang II-induced ROS. Recent evidence suggests mitochondrial NOX4 has a remarkable role. In this study, we examined the hypothesis that angiotensin-(1-7) (Ang-(1-7)) attenuates Ang II-induced mitochondrial NOX4 mediated ROS injury in proximal tubular epithelial cells.

Methods: The normal rat kidney tubular epithelial cells (NRK-52E) were cultured, and then stimulated with Ang II (10^{-6}M) with or without pre-incubation with 10^{-6}M of Ang-(1-7). The mitochondrial NOX4 activation was determined to isolation of subcellular fraction by Western blotting. Intracellular ROS generation was measured using DCF-DA and MitoSOX. Mitochondrial membrane potential ($\Delta\Psi$) was detected using JC-1 by flow cytometry and confocal microscopy. Apoptosis was measured using a TUNEL assay and FITC-Annexin V staining.

Results: The mitochondrial and membrane NOX4 were activated in response to Ang II stimuli for 24 hours, however, pre-incubation of Ang-(1-7) inhibited both activation of NOX4. Pre-incubation with Ang-(1-7) in addition to Ang II significantly inhibited the Ang II-induced ROS production as the level of control. Ang-(1-7) attenuated the Ang II induced depolarization of mitochondrial membrane potential, and release of AIF and

cytochrome C from mitochondria to cytosol. Ang II -induced apoptotic cell death was attenuated by Ang-(1-7).

Conclusions: Ang-(1-7) attenuated the Ang II-stimulated activation of NOX4 in both mitochondria and membrane. These findings ware related to improved mitochondrial dysfunction and apoptosis in response to Ang II and suggest that Ang-(1-7) may attenuate Ang II-stimulated ROS-mediated apoptosis NRK-52E cells.

SA-PO2377

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

Background: To maintain adequate peritoneal function is important for long-term peritoneal dialysis (PD) therapy. Peritoneal dialysate, containing non-physiological materials such as glucose, is implicated in long-term damage of the peritoneal membrane. Endoplasmic reticulum (ER) stress is associated with the progression of Diabetes mellitus, hyperglycemia-associated atherosclerosis and kidney diseases. However, the involvement of ER stress in PD has yet to be elucidated. We investigated whether high glucose levels induce apoptosis through ER stress pathway in rat peritoneal mesothelial cells (PMCs).

Results: The primary PMCs were obtained from the peritoneal parietal wall of Wistar rats. Serum-starved PMCs were incubated with 2% glucose in culture medium for 0 - 72 hours. High glucose concentrations significantly increased both the expression and phosphorylation levels of eIF2α, a key signaling molecule that attenuates general protein translation and induces apoptosis in the PERK-eIF2α pathway, in a time-dependent manner. The ratio of phosphorylation to total eIF2α was also increased in the same manner. After a 24 hour incubation of PMCs with 0.1% - 4% glucose, the relative phosphorylation levels of eIF2α were increased in a dose-dependent manner. At concentrations greater than 3%, glucose suppressed cell viability examined by WST-1 assay and increased DNA fragmentation examined by DNA ladder formation assay. These results demonstrate that high glucose concentrations promote eIF2α-mediated ER stress and apoptosis in PMCs.

Conclusions: In PMCs, it was assumed the high concentration of glucose induced the cell death by apoptosis through the ER stress pathway. Thus, we consider at present that the ER stress is likely to be involved in peritoneal damage in patients under PD therapy.

SA-PO2378

Advanced Oxidation Protein Products Induce Podocyte Apoptosis Via a RAGE-Mediated Signaling Pathway Li Li Zhou, Jing Nie, Fan Fan Hou. *Nephrology, Nanfang Hospital, Guangzhou, Guangdong, China.*

Background: The accumulating of advanced oxidation protein products (AOPPs) was found in diabetes, metabolism syndrome and chronic renal disease. We previously reported that accumulated AOPPs could induce the apoptosis and depletion of podocytes through activation of NADPH activity. The aim of the present study is to identify the receptor on the surface of podocyte which mediated the effect of AOPPs on podocytes.

Methods: The binding activity of AOPPs-MSA to RAGE in cultured podocytes was determined by co-immunoprecipitation and co-immunolocalization fluorescence analysis. Assessment of podocyte apoptosis was determined by Annexin V-labeling and TUNEL Assay. Expression of apoptosis related molecules and activation of NADPH oxidase were analyzed by western blotting, immunoprecipitation and lucigenin-enhanced chemiluminescence. The sequences of custom small interfering RNA (siRNA) duplex for mouse RAGE and scrambled siRNA were also used for detection the aboved objectives .

Results: By immunoprecipitaion assay, we found that AOPPs interacted with RAGE receptor in conditionally immortalized podocyte cells. Immunofluorescence staining demonstrated that AOPPs colocalized with RAGE at the cell membrane. The apoptosis of podocyte induced by AOPPs was inhibited by a RAGE neutralizing antibody in a dose-dependent manner. However, the neutralizing antibodies against other scavenger receptors such as CD36, SR-A, LOX1, AGE-R3 and inflammation related receptor TLR4 had no effect. Knockdown RAGE expression by siRNA interference significantly inhibited AOPPs-induced the upregulation of apoptotic proteins such as p53, Bax, caspase 3 and PARP-1, and the activation of NADPH oxidase, in podocyte.

Conclusions: These data suggest AOPPs induce podocyte apoptosis and activation of NADPH oxidase-dependent p53-Bax apoptotic pathway via a RAGE-mediated signaling pathway.

SA-PO2379

Aldehyde-Mediated Accumulation of ROS Accounts for Cellular Apoptosis in HK-2 Cells Eun Hui Bae,[1] Sunghee Cho,[2] Soo Yeon Joo,[2] Seong Kwon Ma,[1] Suhn Hee Kim,[3] Jongun Lee,[2] Soo Wan Kim.[1] *[1]Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea; [2]Department of Physiology, Chonnam National University Medical School, Gwangju, Korea; [3]Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea.*

Background: Aldehyde products of lipid peroxidation such as 4-hydroxy-2-hexenal (HHE) may in part be responsible for the pathogenesis of various kidney injuries. The present study was aimed at investigating the effects of HHE on cellular apoptosis in renal tubular epithelial cells.

Methods: Human proximal tubular epithelial (HK-2) cells were treated with HHE. Cell viability was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The fluorescent probe 2',7'-dichlorofluorescein diacetate was used to measure intracellular levels of reactive oxygen species (ROS). The expression of NF-kB, mitogen activated protein kinase (MAPK), pro-apoptoic Bax, and anti-apoptotic Bcl-2 proteins was determined by semiquantitative immunoblotting. Apoptosis was assessed by flow-cytometry after the cells were stained by fluorescein isothiocyanate-conjugated annexin V protein and propidium iodine.

Results: HHE caused dose-dependent decreases of cell viability along with increases of ROS. Flow-cytometry confirmed HHE-induced apoptosis. HHE increased the expression of p38 MAPK, extracellular signal regulated kinase (ERK), and c-Jun N-terminal kinase (JNK). HHE also induced an activation of NF-kB and degradation of IkB-a. The NF-kB activation was prevented by inhibitors of ERK (PD98059) or JNK (SP600125), but was not affected by inhibitors of p38 MAPK (SB203580). HHE decreased the expression of Bcl-2, while it increased that of Bax; of which magnitudes were attenuated by NF-kB inhibitors (Bay 117082). The inhibition of NF-kB also prevented HHE-induced apoptosis.

Conclusions: HHE-induced tubular cell apoptosis is mediated by ROS generation and modulation of Bax and Bcl-2 in HK-2 cells; in which ROS may play a role inducing redox-sensitive transcription factors, NF-kB, through activation of ERK and JNK.

SA-PO2380

Role of Extracellular Matrix Renal Tubulo-Interstitial Nephritis Antigen in Cell Survival Ping Xie,[1] Lin Sun,[2] Yashpal S. Kanwar.[1] *[1]Pathology, Northwestern University, FSM, Chicago, IL; [2]Department of Nephrology, Central South University, Changsha, Hunan, China; [3]Pathology, Northwestern University, FSM, Chicago, IL.*

Background: Tubulo-interstitial nephritis antigen (TINag) is an ECM protein expressed in tubular basement membranes (TBMs). Since some of the ECM proteins are known to modulate cell survival, studies were initiated in Lewis rats lacking TINag expression to see if they are relatively susceptible to cisplatin induced-injury. Cisplatin administration caused relatively high degree of tubular cell damage and apoptosis, exclusively in regions where TINag is normally expressed in the parental Wistar strain. This was accompanied with an accentuated increase in serum creatinine and renal Kim-1 RNA, and protein expression of Bax, p53 and of its phosphorylated form and nuclear accumulation, high molecular weight mitochondrial DNA fragmentation, and a reciprocal decrease of Bcl-2. Inclusion of cisplatin in the culture medium led to a fulminant apoptosis of HK-2 cells with increased Caspase activity, mtDNA fragmentation and reduced cell survival. These adverse effects were partially reversed in cells maintained on a TINag substratum. Far-Western assays established TINag binding with integrin receptors αvβ3 and α3β1. Gene disruption with αv-siRNA accentuated cisplatin-induced apoptosis, translocation of mitochondrial cytochrome-C, cytoplasmic Bax and reduced cell survival, while having a marginal effect with α3-siRNA. Gene disruption of αv also decreased the expression of integrin recruited focal adhesion kinase (FAK) with marked dephosphorylation, while there was an increased p53 expression and phosphorylation. These adverse effects were reduced when cells were grown on TINag substratum. In vivo as well a higher degree of decrease in the expression and phosphorylation of FAK was observed in Lewis rats following cisplatin treatment. These studies demonstrate an essential contribution of TINag towards cell survival to maintain tubular homeostasis via interacting with αvβ3, as a result the FAK is recruited to suppress p53 and events that lead to apoptosis.

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SA-PO2381

Receptor Interacting Protein 1-Mediated Necroptosis Essentially Contributes to Renal Ischemia/Reperfusion Injury Andreas Linkermann,[1] Jan H. Bräsen,[2] Nina Himmerkus,[3] Ulrich Kunzendorf,[1] Stefan Krautwald.[1] *[1]Clinic for Nephrology and Hypertension, Christian-Albrechts-University, Kiel, Germany; [2]Institute for Pathology, Christian-Albrechts-University, Kiel, Germany; [3]Institute for Physiology, Christian-Albrechts-University, Kiel, Germany.*

Background: Loss of kidney function in renal ischemia/reperfusion injury (IRI) is caused by programmed cell death (PCD) but the contribution of necroptosis, a recently discovered form of programmed necrosis, has not been investigated.

Methods: We employ murine renal tubular cells and freshly isolated proximal tubules for analysis of the sensitivity to undergo Fas- or TNF-induced tubular cell PCD. By the addition of caspase-inhibitors or the specific RIP1-inhibitor Necrostatin-1 (Nec-1) we inhibit apoptosis and necroptosis, respectively. In vivo, both sublethal and lethal renal ischemia/

reperfusion injury (IRI) was performed in the presence and absence of PCD-inhibitors. Electron microscopy, immunohistochemistry, conventional PAS-staining, western blotting and FACS analysis were employed to quantify apoptosis and necroptosis ex vivo.

Results: We identify death receptor-mediated caspase-independent cell death in murine tubular cells and characterize it as necroptosis by addition of Nec-1. The necroptotic key players RIP1 and RIP3 were detected in whole kidney lysates and freshly isolated murine proximal tubules. In vivo, Nec-1 reduces organ damage and renal failure, even if administrated after reperfusion and resulted in a significant survival benefit in a model of lethal renal IRI. We functionally compared these results with the contribution of apoptosis to renal IRI. Unexpectedly, the specific blockade of apoptosis by zVAD neither prevented organ damage nor the increase of retention parameters in renal IRI.

Conclusions: Our results demonstrate the presence and functional relevance of necroptosis in the pathophysiologic course of ischemic kidney injury and a functional predominance of necroptosis over apoptosis in this setting. Above that we identify the therapeutical potential of Nec-1 as a drug for the prevention and treatment of renal IRI.

Funding: Private Foundation Support, Clinical Revenue Support

SA-PO2382

Renin Induces Podocyte Apoptosis through Renin Receptor and p38 MAPK Pathway Independent of Ang II Generation Hai Yuan, Zhilong Ren, Fengqi Hu, Wei Liang, Guohua Ding. *Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China.*

Background: Podocyte plays an important role in the pathogenesis and progression of glomerulosclerosis. Various elements of renin-angiotensin-aldosterone system (RAAS), such as angiotensin II and aldosterone, can induce podocyte apoptosis. However, little is known about the direct effect of renin on podocytes through renin receptor. In the present study, we evaluated the effect of renin on cultured podocyte apoptosis and the role of p38 MAPK pathway.

Methods: Expression of renin receptor was detected by fluorescent staining and RT-PCR. Podocytes were incubated in media containing either buffer or renin for variable time periods. The cells were also treated with either inhibitor of p38 MAPK or buffer. At the end of the incubation period, apoptosis was evaluated by cell nucleus staining, and caspase 3, p38, phospho-p38 MAPK were measured by Western blotting.

Results: We demonstrated that both renin receptor mRNA and protein were expressed in cultured podocytes. Exposure of podocytes to renin induced podocyte apoptosis in a time-dependent manner, which was accompanied by up-regulation of active caspase-3 and increased expression of p38MAPK. Renin-induced podocyte apoptosis and p38 MAPK phosphorylation were inhibited when the cells were pretreated with p38 MAPK inhibitor. Transfection of renin receptor siRNA ameliorated the above changes induced by renin. Furthermore, these effects of renin were not altered by blocking of angiotensin II using either enalapril or losartan.

Conclusions: These data suggest that renin induces podocyte apoptosis, which is mediated through renin receptor and p38 MAPK pathway independent of Ang II generation.

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SA-PO2383

Connexin43 Contributes to Puromycin-Elicited Podocyte Injury Qiaojing Yan, Yuan Chi, Ying Zhu, Masanori Kitamura, Jian Yao. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Background: Gap junctions (GJs), formed by specific protein termed connexin (Cx), play important roles in regulation of cell phenotype and in the control of cell survival. Podocyte injury is one of the major factors contributing to the initiation and development of proteinuria and glomerulosclerosis. In our previous study, we demonstrated that puromycin-induced podocyte injury *in vivo* is preceded by a markedly increased Cx43 level *(Am J Pathol, 2002, 161:1597-1606).* However, the mechanisms involved in the elevation of Cx43 and the functional roles of Cx43 in podocyte injury have not been elucidated. The purpose of this study was to tackle these questions.

Methods: Podocyte viability was evaluated by MTT assay, caspase-3 cleavage and Hoechst staining. The levels of Cx43, P38 and caspase-3 were evaluated by Western blot analysis. Cx43 distribution and function were assayed by immunofluorescent staining and dye-transfer assay, respectively.

Results: 1) Exposure of cultured podocytes to puromycin caused a time- and concentration-dependent podocyte injury. This effect could be significantly blocked by antioxidants, indicating a critical role of oxidative stress in cell injury. 2) Puromycin-induced podocyte injury was preceded by an obviously increased Cx43 protein level. Pretreatment of cells with antioxidants also largely abolished the Cx-43 elevating effect, indicating an involvement of oxidative stress in induction of Cx43. 3) Consistent with a critical role of oxidative stress in Cx43 expression and podocyte injury, agents that induce intracellular superoxide anion formation were found to be able to mimic the effect of puromycin on Cx43 levels and cell injury. In addition, puromycin, indeed, elevated intracellular superoxide. 4) Suppression or inhibition of GJs with the GJ inhibitors significantly abrogated the cytotoxic effect of puromycin and inhibited activation of P38 MAP kinase and cleavage of caspase 3.

Conclusions: Our study thus revealed an important role of GJ protein Cx43 in mediation of oxidative stress-induced podocyte injury. Cx43 could be used as a marker of oxidative stress in podocytes and a therapeutic target for prevention and treatment of podocyte injury.

Funding: Government Support - Non-U.S.

SA-PO2384

SSeCKS Sequesters Cyclin D1 in Glomerular Parietal Epithelial Cells Bettina Burnworth, Jeffrey W. Pippin, Ron D. Krofft, Kelly L. Hudkins, Charles E. Alpers, Kelly D. Smith, Stuart J. Shankland, Peter J. Nelson. *University of Washington, Seattle, WA.*

Background: Glomerular parietal epithelial cells (PECs) are precursors for podocytes in mature glomeruli, however, as progenitors, the distinct mechanisms that allow for repeated periods of cell-cycle arrest and re-entry of PECs after glomerulogenesis are unknown. Here, we show that the Src-suppressed protein kinase C substrate, SSeCKS, a multivalent scaffolding A kinase anchoring protein, sequesters cyclin D1 in the cytoplasm of quiescent PECs.

Methods: The expression and interaction of SSeCKS and cyclin D1 was studied in PECs in tissue culture, during glomerulogenesis, and in post-natal glomeruli of $SSeCKS^{+/+}$ and $SSeCKS^{-/-}$ mice.

Results: SSeCKS expression is induced in embryonic PECs but not in embryonic podocytes starting at the S-phase of glomerulogenesis, and is constituitively expressed post-natally by PECs, but not podocytes, in normal glomeruli. Cyclin D1 immunoprecipitated with SSeCKS from capsulated glomeruli containing PECs, whereas decapsulated glomeruli without PECs lacked SSeCKS and cyclin D1. Cell-cell contact inhibition of proliferation in cultured PECs induced SSeCKS expression and binding of cyclin D1 by SSeCKS in the cytoplasm, whereas phosphorylation of SSeCKS by activated PKC disrupted binding, resulting in nuclear translocation of cyclin D1. $SSeCKS^{-/-}$ mice showed hyperplasia of PECs in otherwise normal glomeruli and developed significantly worse proteinuric glomerular disease, marked by increased PEC proliferation and expression of nuclear cyclin D1, from nephrotoxic nephritis.

Conclusions: These results suggest that SSeCKS controls the localization and activity of cyclin D1 in PECs.

Funding: NIDDK Support

SA-PO2385

HIV-Nef Induces Detachment, Migration and Proliferation of Human Podocytes by Compromising Cytoskeletal Integrity Hitesh Patni, Divya Salhan, Ashwani Malhotra, Mohammad Husain, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: HIV-1 gene Nef has been considered to play an important role in the development of HIV-1 associated nephropathy (HIVAN). We hypothesized that Nef decreases adhesion and enhances detachment, migration, and proliferation of podocytes by compromising their cytoskeletal integrity.

Methods: We developed stable colonies of conditionally immortalized human podocytes expressing either Nef (Nef/CIHP) or empty vector (EV/CIHP). To identify Nef-linked proteins, GST pull down assay followed by mass spectrometry was carried on EV/CIHP and Nef/CIHP. To identify Nef interacting proteins, yeast two hybrid assay was carried out. To determine mRNA expression of relevant proteins, microarray array analysis of RNA from EV/CIHP and Nef/CIHP was carried out. To identify co-localization of Nef with interacting proteins, imaging studies were performed. To determine alteration in Nef-induced functionality of podocytes, adhesion, detachment, migration, proliferative and apoptotic studies were done.

Results: GST pull down assay in Nef/CIHPs displayed a band at 45 kD, which was identified to be actin by mass spectrometry; whereas, yeast two hybrid assay identified the following nef-interacting proteins: syntrophin, filamin B, syntaxin, translational elongation factor 1, and zyxin. Microarray analysis of RNAs from Nef/CIHP revealed enhanced expression of Rac1 and syndecan-4 and attenuated expression of syndecan-3 and syntenin when compared with EV/CIHPs. Imaging studies displayed co-localization of Nef with actin and zyxin in Nef/CIHPs. Nef /CIHP displayed scant number of actin filaments and enhanced number of lamellipodia. Since Nef/CIHPs displayed enhanced expression of Rac1, it appears that Nef-induced Rac1 expression may be contributing to increased number of lamellipodia in Nef/CIHPs.Nef/CIHP displayed decreased adhesion, enhanced detachment and migration. Moreover, Nef/CIHPS displayed proliferative phenotype.

Conclusions: We conclude that interaction of Nef with actin compromises cytoskelatel integrity of human podocytes and make them prone to detach, migrate and proliferate.

Funding: NIDDK Support

SA-PO2386

A Novel, Dual Role of CCN3 in Experimental Glomerulonephritis: Pro-Angiogenic and Anti-Mesangioproliferative Effects Claudia R.C. van Roeyen,[1] Peter Boor,[1] Uta Kunter,[1] Ina V. Martin,[1] Ana Kaitovic,[1] Erawan Borkham-Kamphorst,[2] Bernard Perbal,[3] Ralf Weiskirchen,[2] Tammo Ostendorf,[1] Jurgen Floege.[1] *[1]Nephrology, RWTH Aachen University, Aachen, Germany; [2]Clinical Chemistry and Pathobiochemistry, RWTH Aachen University, Aachen, Germany; [3]L´oreal R&D, Clark, NJ.*

Background: In contrast to factors promoting mesangial cell proliferation, little is known about their endogenous regulators. During experimental mesangioproliferative nephritis glomerular CCN3 (nephroblastoma overexpressed gene) expression is reduced prior to the proliferative phase and overexpressed both in glomeruli and serum when mesangial cell proliferation subsides.

Methods: To further elucidate its role in mesangioproliferative glomerulonephritis (GN), CCN3 was systemically overexpressed by muscle electroporation in healthy or nephritic rats. This increased CCN3 serum concentrations more than 3-fold for up to 35 days after electroporation.

Results: At day 5 after induction of mesangioproliferative GN, CCN3 transfected rats exhibited an increase in glomerular endothelial area and in glomerular mRNA levels of the pro-angiogenic factors VEGF and PDGF-C. *In vitro* CCN3 induced proliferation of glomerular endothelial cells. In the mesangioproliferative phase (day 7), CCN3 overexpression decreased albuminuria, the α-smooth-muscle actin (αSMA) positive mesangial area, the number of proliferating mesangial cells and glomerular accumulation of fibronectin and type IV collagen. In progressive nephritis at day 56, systemic overexpression of CCN3 resulted in decreased albuminuria, glomerulosclerosis and reduced cortical collagen type I accumulation. In healthy rat kidneys, overexpression of CCN3 induced no morphological changes but regulated glomerular gene transcripts (reduced transcription of PDGF-B, PDGF-D, PDGFR-ß, fibronectin, and αSMA and increased PDGFR-α and PDGF-C).

Conclusions: Our data identify a dual role of CCN3 in experimental glomerulonephritis with both pro-angiogenic and anti-mesangioproliferative effects, both of which serve to reconstitute the normal glomerular architecture. Manipulation of CCN3 could represent a novel approach to help repair glomerular endothelial damage and mesangioproliferative changes.

Funding: Government Support - Non-U.S.

SA-PO2387

Profiling of Inflammatory Mediators Produced by Primary Human Mesangial Cells *In Vitro*

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Background: The study of fibrotic changes occurring in the development of chronic kidney disease has shown the importance of inflammatory products in initiating and perpetuating the local environment in the diseased kidney during the progression towards end stage renal disease.

Methods: Normal primary human mesangial cells were stimulated with AngII, IL-1β, IL-6 or TGFβ1, factors known to be dysregulated in renal disease. To determine which cytokines or chemokines were produced, an initial screen of supernatants was performed using multi-analyte arrays. A 12 cytokine array including TNFα, IL1β, IL6, IL12, IL17A, IL8, MCP-1, RANTES, MIP-1a, MIP-1b, MDC and Eotaxin was selected based on preliminary experiments. Subsequent determinations for dose and time studies were done using single analyte ELISA kits.

Results: Production of inflammatory mediators IL6, IL8 and MCP-1 was induced by AngII, IL1β, IL6 and TGFβ1. IL1β stimulation also increased production of RANTES and MIP-1a. TGFβ1, an important modulator in kidney disease, was quantified in separate experiments. AngII, IL1β and IL6 all increased production of TGFβ1. Stimulation of cells with TGFβ1 induced an autocrine increase in TGFβ1 production. The production of IL6, IL8, MCP-1 and TGFβ1 was found to be both dose and time dependent. IL1β was the only factor to induce GM-CSF and ICAM-1 surface expression whereas IL1β and IL6 induced a modest increase in TSP-1. Fibronectin and collagen I deposition were detected 4-7 days after stimulation.

Conclusions: This data provides an *in vitro* kidney specific cellular assay with the potential to triage inhibitors of inflammatory pathways participating in kidney disease.

Funding: Pharmaceutical Company Support

SA-PO2388

Up4A, Elevated in Patients with End-Stage Renal Disease, Induced a Phenotype Switch of Vascular Smooth Muscle Cells Via P2Y Activation

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Background: Vascular mineralization is a major risk factor in patients with chronic kidney disease (CKD) and contributes to the increased cardiovascular mortality. Vascular calcification is an actively induced process Smooth muscle cells differentiate twoard osteoblast-like phenotype. The dinucleoside polyphosphate uridine adenosine tetraphosphate (Up4A) has been previously shown to be a potent calcification inducing substance. Now we searched for the involved mechanism.

Methods: In vitro and ex vivo calcification in rat VSMCs and aortic rings was measured. Calcium deposition was monitored. Expression of cbfa1 was measured in rat VSMCs after stimulation with Up4A.

Results: Up4A induced calcification of rat VSMCs in vitro and ex vivo in rat aortic rings, visualized by Alizarin Red Staining and quantified. The non-selective P2Y inhibitors suramin, PPADS, and RB-2 as well as MRS2578, which antagonizes P2Y6, have a significant inhibitory effect on the Up4A-induced mineralization in VSMCs. For mineralization of VSMCs a trans-differentiation of VSMCs in osteoblast-like cells could be detected. Investigating the gene expression in rat VSMCs Up4A is able to induce the initial transcription factor cbfa1. To proof the antagonistic potential of the mentioned antagonists, these were also investigated on their inhibitory potential on Up4A-induced cbfa1 expression. Suramin, PPADS, RB-2 and MRS2578 co-incubation is able to significantly diminish the Up4A-induced cbfa1 expression. P2Y2/4/6 receptor activation might be responsible for this effect. ATPγS, a selective P2Y2 agonist could mimic the effect to induce the cbfa1 expression. To further verify the P2Y2 activation, we used P2Y2-/- mice for ex vivo mineralization assay. In P2Y2-/- mice, Up4A is not able to potentiate the effect of CM, compared to wild-type mice.

Conclusions: Up4A-mediated mineralization depends on the activation of P2Y2 to stimulate trans-differentiation in VSMCs. Up4A has influence on vascular mineralization and therefore, the purinergic signaling might be involved in arteriosclerotic processes.

SA-PO2389

4-Phenylbutyrate Inhibits Transforming Growth Factor-β1-Induced Apoptosis Via a Caspase-Mediated Mechanism in Human Renal Proximal Tubule Epithelial Cells

Elise Brimble, Richard Austin, Jeffrey G. Dickhout. *Nephrology, McMaster University and St. Joseph's Healthcare Hamilton, Canada.*

Background: TGF-β1 signaling is an important inducer of apoptosis in tubular epithelium during renal injury, however, the precise mechanism is unknown. Smad3 may mediate TGF-β1-induced renal tubular epithelial cell (RTEC) apoptosis, as shown by a reduction in its incidence in the $Smad3^{-/-}$ mouse in a model of obstructive nephropathy. Histone deacetylase (HDAC) inhibition reduced TGF-β1-induced apoptosis in cultured RTECs. However, it is unclear how HDAC inhibitors interfere with this apoptotic response. We hypothesize that HDAC inhibitors reduce RTEC apoptosis by disrupting TGF-β1 signaling.

Methods: We used human primary RTECs and HK-2 cells treated with human recombinant TGF-β1 to determine the effects of pan-HDAC inhibitors, 4-Phenylbutyrate (4-PBA) and vorinostat, on the incidence of apoptosis. Tauroursodeoxycholic acid (TUDCA), a low molecular weight chemical chaperone, was used as a control, as 4-PBA has chemical chaperone properties. LDH release and TUNEL assays were used to assess cytotoxicity and apoptosis. Western blotting was used to monitor TGF-β1 signaling and the endoplasmic reticulum (ER) stress response. Z-VAD FMK, a pan-caspase inhibitor, was used to determine the dependence of TGF-β1-induced apoptosis on caspase activity.

Results: We found that TGF-β1 treatment induced cytotoxicity and apoptosis in RTECs. This effect was significantly inhibited by treatment with 4-PBA and vorinostat, but not with TUDCA. TGF-β1 was not found to induce apoptosis through the ER stress-inducible protein CHOP/GADD153; however, 4-PBA induced XBP1 splicing, a cytoprotective response to ER stress. TGF-β1-induced apoptosis was found to be dependent on caspase activity, as was the ability of 4-PBA to inhibit cell death. 4-PBA, vorinostat, and TUDCA were not found to have an effect on TGF-β1-induced Smad3 phosphorylation or expression.

Conclusions: TGF-β1-induced apoptosis contributes to the deterioration of tubule function in chronic kidney disease. Thus, HDAC inhibitors may be an effective treatment to maintain renal function by preserving tubule integrity via inhibition of caspase mediated TGF-β1-induced apoptosis.

Funding: Government Support - Non-U.S.

SA-PO2390

Loss of the β Isoform of Calcineurin A (CAβ) Reduces Hypertrophy, Suppresses Protein Synthesis, and Activates AMP Dependent Kinase (AMPK)

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Background: Transgenic mice with CAβ knocked out (CAβ-/-) have normal renal development and function, but do not exhibit renal hypertrophy when diabetic (Reddy RN et. al. 2009). Because calcineurin regulates protein synthesis and breakdown in cardiac hypertrophy, we examined its role in protein metabolism and in phosphatidylinositol 3 kinase (PI3K) mitogen associated kinase (MAPK), and AMPK signaling in proximal tubule cells.

Methods: C^{14} phenylalanine pulse/chase was used for protein synthesis and breakdown in NRK-52E renal epithelial cells and SV40 immortalized cell lines created from CAβ-/- and wildtype litter mate proximal tubules. Signaling intermediates were examined by Western blotting comparing the ratio of phospho-specific to whole protein antibodies. Inhibitors of CA (8 μM cyclosporine A (CYA) or 200 nM tacrolimus), of PI3K (25 μM LY249002) and of MAPK (50 μM U0126) were used. All results are $p<0.05\%$.

Results: CYA decreased protein content/well (48h) by 14±2% and protein synthesis (20h) 51±2% of control in NRK-52E cells. In contrast, protein degradation (0h-28h) was decreased by 10±1%. Tacrolimus gve similar results on protein content and degradation. Inhibitors of MAPK or PI3K pathways reduced protein synthesis by 48±3% or 56±2% in these cells. However, CYA or tacrolimus increased both basal and EGF-induced MAPK and AKT (downstream of PI3K) phosphorylation (1h) by 50% to 3-fold. Downstream of MAPK and AKT, AMPK (which acts to inhibit the mammalian target of rapamycin) phosphorylation was ~6 fold higher with CYA compared to control. CAβ-/- tubular cells had lower protein per well (48h, 20±2%) and protein synthesis (20h, 16±1) than CAβ+/+ cells. In CAβ -/- cells, CYA treatment (20h) did not decrease protein synthesis further, while it reduced it 20±2% in CAβ +/+ cells. AMPK phosphorylation was >10 fold higher in CAβ -/- compared with CAβ+/+ cells.

Conclusions: The inhibition of calcineurin or knockout of CAβ reduces protein accumulation in renal tubular cells via reduced protein synthesis possibly through activation of AMPK. Activation of AMPK may explain decreased renal hypertrophy in CAβ mice.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2391

Consideration of Protein Expression Changing with Pitavastatin or Edaravone Therapy in Chronic Puromaycin Aminonucreoside Nephropaty

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Background: In puromaycin aminonucreoside nephrosis rats which is one of human nephritic syndrome animal model. Excessive oxidants, especially oxidative LDL play roles in the pathogenesis of glomerular injury. To clarify the protective effects of anti-

oxidative therapy, we investigated changes in chemical markers of lipid peroxidation and renal histology, and protein array in rats with chronic puromycin aminonucleoside (PAN) nephrosis (C-PAN).

Methods: C-PAN was induced by intraperitoneal injections of PAN (130 mg/kg on day 1 and 60 mg/kg on day 14). Rats administered normal saline served as controls (n=5). C- PAN rats were divided into four groups (each group: n=4, positive control, negative control, pitavastatin treatment , edaravone treatment). Blood and urinary samples were collected every week. Animals were sacrificed at the end of experiment for histological and protein array analysis.

Results: In C-PAN+Pit and C-PAN+Eda rats, urinary excretion of albumin and 8-isoPGF2α and thiobarbituric acid-reactive substances (TBARS) were significantly decreased compared with that in C-PAN rats ($p<0.01$). On histological examination, glomerular injury score, 4-HNE positive area, Infiltration of macrophages and tunnel positive cell counts were significantly lower in both groups than in C-PAN rats ($p<0.05$). All of these results shows their features. In protein microarray, Plk-2 (Polo-like protein kinase) and IkBa (Inhibitor of NF-kappa-B alpha) were upregulated [Plk-2; Pit/Eda: 118 /114 folds, IkBa; Pit/Eda: 39 /122 folds]. And edaravone treatment group showed Mn-SOD upregulation (Eda: 32 folds).

Conclusions: These findings showed anti-oxidative treatment to C-PAN ameriolated glomerular injury. And these effects correlated with anti-apoptotic protein and cell signaling protein upregulation. In edaravone treatment, anti-oxidant protein also upregulated. But both treatment changing thses molecules, finding from other results, pitavastatin has not only protective effects but regeneration of constituent cells. Because edaravone changed anti-oxidative protein, this effects would useful for renal protection as an anti-oxidative treatement.

SA-PO2392

Overexpression of GFP-GABARAP Causes Deterioration of Proteinuria and Glomeruloscrelosis in Adriamycin-Induced Nephropathy Mice Katsuhiko Asanuma,[1] Miyuki Takagi,[1] Kanae Nonaka,[1] Etsuko Asanuma,[1] Takashi Ueno,[4] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo, Japan; [3]Research Institute for Diseases of Old Age, Juntendo University Faculty of Mediine, Tokyo, Japan; [4]Department of Biochemistry, Juntendo University Faculty of Mediine, Tokyo, Japan.*

Background: We previously reported that LC3, a promising marker of autophagy, played an important role in recovery from podocyte damage in an experimental nephrosis model. GABARAP is a γ-aminobutyric acid A receptor associated protein and has recently been characterized as another homolog of LC3, although its precise role in autophagy remains unclear.

Methods: We recently generated GFP-GABARAP transgenic mice, in which GFP-GABARAP is abundantly expressed in glomerular podocytes.

Results: We observed that aged transgenic mice showed slight albuminuria and showed low levels of foot-process effacement. Surprisingly, a single injection of adriamycin caused a more significant increase in proteinuria and sclerotic glomeruli in transgenic mice compared with wild type mice. Under these conditions, neither GFP-GABARAP nor endogenous GABARAP appeared to be recruited by autophagosomes and remained in the cytosol. Moreover, cytosolic GFP-GABARAP was significantly colocalized with p62 to form aggregates.

Conclusions: It appears that the GFP-GABARAP/p62 complex is responsible for impairment of glomerular function, and that it retards recovery from the effects of adriamycin.

SA-PO2393

Anti-Proliferative Effect of Asymmetric Dimethylarginine (ADMA) – A Clue to Renal Fibrosis? James Alexander Tomlinson,[1] Ben Caplin,[2] Jill T. Norman,[2] David C. Wheeler,[2] James M. Leiper.[1] *[1]Nitric Oxide Signalling Group, MRC Clinical Sciences Centre, London, United Kingdom; [2]Renal Department, UCL/ Royal Free, London, United Kingdom.*

Background: The endogenous nitric oxide synthase (NOS) inhibitor, asymmetric dimethylarginine (ADMA), has been implicated in a wide variety of diseases including chronic kidney disease (CKD) although a true cause and effect remains to be established. CKD is characterised by progressive functional decline histologically manifest as progressive tubulointerstitial fibrosis. Renal tubular epithelial cells play a key role in fibrosis and are known to express components of the NO system. We hypothesised that ADMA may contribute to fibrosis by impairing the ability of renal epithelial cells to repair following injury.

Methods: Confluent conditionally-immortalised human proximal tubular epithelial cells were made quiescent and a wound was scratched across the monolayer. Cells were incubated in the presence or absence of 10% foetal calf serum (FCS) and ADMA [3 and 10uM] for 24 hours. The extent of repair (in-filling) of the wound was measured. Proliferation was distinguished from migration using the cytostatic drug Mitomycin C and subsequently confirmed using a BrdU incorporation assay to measure DNA synthesis.

Results: FCS (10%) markedly stimulated epithelial repair of the scratch (48% ±7 vs 11% ±2; $p<0.0001$). Mitomycin C significantly retarded repair (18% ±0 vs 48% ±7; $p<0.0001$), suggesting that wound repair was largely due to epithelial cell proliferation. ADMA significantly inhibited serum-induced wound repair (32% ± 3 vs 48% ±7; $p<0.05$) and BrdU incorporation (0.96 ±0.23 vs 1.67 ±0.45 arbitrary units (AU); $p<0.0001$) when compared with FCS treatment alone, suggesting a potent anti-proliferative effect. Inhibition of NOS with two other inhibitors, PBITU and L-NAME also suppressed cell proliferation (0.36 ±0.11 vs 1.67 ±0.45 AU; $p<0.0001$ and 1.14 ±0.32 vs 1.67 ±0.45 AU; $p<0.05$ respectively).

Conclusions: ADMA exerts an anti-proliferative effect on renal epithelial cells, most likely due to reduced NO availability. This suggests a potential mechanism for impaired renal repair and fibrosis.

SA-PO2394

Fucosylation Influences Extracellular Matrix Accumulation in TGF-β1-Stimulated HK-2 Cells Hong Li Lin, Zheng Mei Jie, Fang Ming, Ke Ping Wang. *Department of Nephrology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China.*

Background: TGF-β1 plays an key role in the development of renal fibrosis.Our previous work found that core fucosylation which catalized by its specific transferase Fut8 was essential for TGF-β receptors to fulfill their functions, however, it remains unclear whether fucosylation exerts effects on ECM accumulation and tubular epithelial cell apoptosis.

Methods: A fibrosis HK-2 cell model was established with 10ng/ml TGF-β1. Fut8 siRNA was transfected into HK-2 cells with Lipo2000 at the concentration of 30nM. The changes of Fut8 expression were examined by western blot, and the apoptosis were determined by flow cytometry. Also, expression levels of protein MMP-2,3,9 and TIMP-1 were detected by western blot. Additionally, ECM proteins, including collagen type I,III,IV, were examined by immunofluorescence. Besides, fibronectin and laminin were examined by Real time-PCR.

Results: The expression of Fut8 protein increased after stimulated by TGF-β1, and Fut8-siRNA transfection reversed the significant up-regulation of Fut8 protein and inhibited the apoptosis of HK-2 cells. Moreover, TGF-β1 enhanced the expression of MMP-2, 3 as well as TIMP-1, but had no effect on MMP-9; Furthermore, Fut8-siRNA up-regulated the expression of MMP-2,3,9 , and decreased the expression of TIMP-1. Collagen typeI, III, IV, fibronectin and laminin,were excessive synthesis after stimulated by TGF-β1, while they had no obvious change when treated by TGF-β1 together with Fut8-siRNA.

Conclusions: Bolocking fucosylation may suppress ECM accumulation and apoptosis in HK-2 cells stimulated by TGF-β1, then suppress interstitial fibrosis.

SA-PO2395

Aristolochic Acid Enhances Invasion and Migration of Human Urothelial Cancer TSGH Cells In Vitro and In Vivo Horng-Rong Chang,[1,2] Hui-Pei Huang,[3] Jen-Pi Tsai,[1] Jong-Da Lian,[2] Chau-Jong Wang.[3] *[1]Chung Shan Medical University, Institute of Medicine, Taichung, Taiwan; [2]Division of Nephrology, Department of Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan; [3]Chung Shan Medical University, Institute of Biochemistry and Biotechnology, Taichung, Taiwan.*

Background: Aristolochic acid I (AAI) had been implicated in urothelial carcinoma (UC) in humans. However, whether AAI promotes invasion/migration of UC has not been established.

Methods: A study of human UC TSGH cells cultured with AAI was conducted. Cell viability, the effects of AAI on the activity of MMP-9, the abilities of invasion/migration and the migration-related proteins (Ras, RhoA, ROCK1, PI-3K, p-Akt and NF-κB) of the TSGH cells were assessed. The TSGH cells were subcategorized to one-day or 30-day AAI exposure. An *in vivo* study using a nude mice xenograft model was employed to test the anti-tumor effects of Rho kinase inhibitor or Y27632.

Results: A time- and dose-dependent increase in both activity and mRNA level of MMP-9 were demonstrated. The mRNA level of uPA was increased and of TIMP1 was decreased in the cells with 30-day but not one-day AAI exposure. A dose-dependent enhancement in wound healing rate and cell migration was demonstrated, especially in the 30-day AAI-exposed cells. Expressions of Ras/RhoA and other migration-related proteins were increased after AAI treatment which could be inhibited by Y-27632. The *in vivo* results demonstrated that Y27632 was able to attenuate the speed of growth of the inoculated tumors in nude mice.

Conclusions: Our results provided *in vitro* and *in vivo* evidence that prolonged AAI exposure enhances invasion and migration of human TSGH cells.

SA-PO2396

Runx3 Mediates Suppression of Tumor Growth and Metastasis of Human CCRCC by Regulating Cyclin Related Proteins and TIMP-1 Hanmin Wang, Shiren Sun, Lijie He.

Background: Although our previous studies indicated that Runx3 has been implicated in tumor suppression in several tumors, the precise molecular mechanisms in renal cancer remained unclear.

Methods: Here we tested Runx3 in clear cell renal cell carcinoma (CCRCC) in vivo and in vitro.

Results: We presented that the expression of Runx3 was absent or significantly decreased in 75 cases of CCRCC tissues ($p<0.05$, table1,figure1,a is cancer, b is non-cancerous). Enforced Runx3 expression mediated 786-O CCRCC-derived cells to exhibit significant inhibition of growth, G1 cell cycle arrest and metastasis in vitro, and to lost 786-O tumorigenicity in nude mouse model. Runx3-induced growth suppression was found

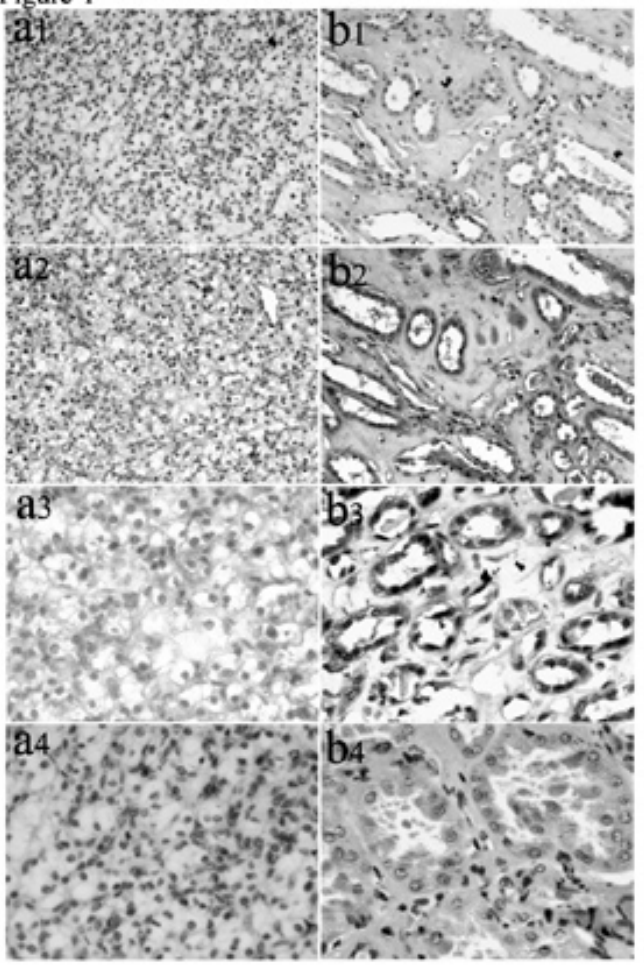
partially to regulate various proteins, including inhibition of cyclinD1, cyclinE, cdk2, cdk4 and p-Rb, but up-regulation of $p27^{Kip1}$ and Rb. Simultaneously, overexpression of Runx3 led to significant induction of TIMP-1 expression.

Clinicopathological associations of Runx3 expression in CCRCC patients

Category	Total number of cases	Runx3 expression -	Runx3 expression +	Runx3 expression ++	Positive case	P value
Adjacent tissue of CCRCC	77	12(15.58%)	16 (20.78%)	49 (63.64%)	65 (84.42%)	
CCRCC tissues	75	52 (69.33%)	12 (16%)	11 (14.67%)	23 (30.67%)	<0.001**

Runx3 staining was graded as negative (-; score: 0–1), weak (+; score:2–4), and strong (++; score:5–8). Pearson's X2 Test, **P<0.001 vs non-cancerous tissues.

Figure-1



Conclusions: Therefore, Runx3 has the proliferative and metastatic abilities of CCRCC cells, which was mediated, at least partially by regulating cyclins and TIMP1.

Funding: Government Support - Non-U.S.

SA-PO2397

Age Associated Loss in Tubular Epithelial Proliferation Reserve Is Intrinsically Determined Nathan D. Susnik, Inna V. Kuznetsova, Birgit Berkenkamp, Christoph Jacobi, Inga Soerensen, Hermann G. Haller, Anette Melk, Roland Schmitt. *Department of Nephrology, Hannover Medical School, Hannover, Germany.*

Background: Data from kidney injury models suggests that the loss of renal repair capacity with aging is largely due to a decreased tubular epithelial proliferative reserve. This concept has been challenged because age dependent factors might lead to different damage loads in experimental kidney injury, this may cause changes in the reparative proliferation response.

Methods: In order to test for age-dependent changes in the renal epithelial proliferative reserve without inducing cellular injury, we used lead acetate, a potent direct mitogen, to stimulate tubular cell proliferation. Lead acetate was used in old (22 months) and young (4 months) mice in vivo and in old and young primary tubular epithelial cells (PTEC). Differences in cell cycling, cell damage and markers of senescence were quantified by histology, immunoblot and qPCR.

Results: Lead acetate significantly increased the rate of tubular epithelial proliferation without causing cell damage as shown by unchanged levels of injury markers Ngal and Kim-1. The increase in proliferation was significantly smaller in old mice. Cyclin D1 positive tubular cells increased only in young kidneys, but not in old kidneys. Kidneys from old mice expressed significantly more of the senescence markers p16INK4a, senescence associated β-galactocidase, and phospho-γH2AX, regardless of lead acetate stimulation. Only young isolated PTEC showed increased proliferation after lead acetate stimulation but to a lesser extent than in vivo. This may have been due to a dramatic increase of p16INK4a and p21 in both age groups, resulting in almost identical levels of senescence markers in PTEC from young and old mice after 6 days of culture.

Conclusions: These data indicate that the aged kidney has an intrinsically reduced proliferative capacity. This is in agreement with a higher load of senescence markers from both the stress- and telomere-induced pathway. Age-dependent differences in tubular cell proliferation quickly diminish in vitro because cultured PTEC from young kidneys undergo accelerated aging with rapid induction of cellular senescence.

SA-PO2398

Inactivation of Mxi1 Regulates IFT20 Expression in Polycystic Kidney Jong Hoon Park, Je Yeong Ko, Kyung Hyun Ryu. *Department of Biological Science, Sookmyung Women's University, Seoul, Republic of Korea.*

Background: Most cell types have primary cilium, which consists of protruding structures that sense mechanical and chemical signals from the extracellular environment. Cilia are assembled via a process known as intraflagellar transport (IFT) and a variety molecules, including IFT20, IFT88 and Kif3a, participate in the assembly of cilia. It is critical that the size control system of cilia be elucidated to enable a through understanding of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects in cilia formation, maintenance or function.

Methods: To confirm the effect of Mxi1 on cilia, we performed immuno assay in Mxi1-deficient MEFs and Mxi1-deficient mice. Also, IFT genes were screened *in vitro* and *in vivo* using real-time PCR to select candidate gene. Furthermore, we performed promoter assay to check the effect of Mxi1 on candidate gene regulation.

Results: Previously, multiple tubular cysts were observed in the kidneys of Mxi1-deficient mice aged six months or more. Here, we clarify the relationship between inactivated Mxi1-induced cyst formation and cilia assembly. In Mxi1-deficient MEFs, the length of structurally normal cilium decreased, but we still confirmed the presence of cilia in cysts of Mxi1-deficient mice. To elucidate the cilia regulatory mechanism related to Mxi1, IFT genes are validated in Mxi1 MEFs and Mxi1 mice using real-time PCR. IFT20, IFT88, Kif3a genes are decreased in Mxi1-deficient model and IFT20 is selected for candidate gene. We observed that cilia length and IFT20 expression are regulated by Mxi1 level *in vitro*. Also IFT20 promoter activities are regulated by Mxi1 level in mIMCD.

Conclusions: These results indicate that inactivation of Mxi1 plays an important role in the down-regulation of IFT20 expression in polycystic kidneys.

Funding: Government Support - Non-U.S.

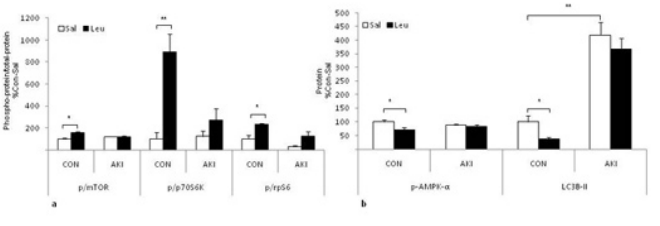
SA-PO2399

Resistance to Leucine Induced Signal Transduction and Regulation of Autophagy in Acute Kidney Injury Kevin L. McIntire,[1,2] Yu Chen,[1,2] Sumita Sood,[1,2] Ralph Rabkin.[1,2] *[1]Research Services, VA Palo Alto HCS, Palo Alto, CA; [2]Medicine/Renal Division, Stanford University, Palo Alto, CA.*

Background: Adequate nutrient intake in acute kidney injury (AKI) is a key part of patient management. Of particular interest are the branched chain amino acids(BCAA) especially leucine(LEU). LEU serves as a substrate, stimulates insulin release and directly activates the mTOR anabolic signaling pathway, stimulating protein synthesis via phosphorylation(p-) of p70 ribosomal S6 kinase-1(p70S6K), S6 ribosomal protein(rpS6) and eukaryotic 4E-binding protein1(4E-BP1). LEU also inhibits proteolysis in part by suppressing autophagy which is increased in AKI. Of note, LEU activation of mTOR is regulated in part by p-AMPK which inhibits mTOR activation. Since LEU resistance develops in other acute catabolic conditions and signaling defects are common in uremia, we set out to determine whether LEU is effective in stimulating skeletal muscle mTOR anabolic signaling and inhibiting autophagy, in AKI.

Methods: Rats with post-renal AKI(ureters ligated)and sham operated controls(CON) were gavaged with LEU or saline(Sal)and sacrificed 60 mins later.

Results: BCAA levels post-LEU were similar.Western blots show that LEU significantly stimulated p-mTOR, p-P70S6K and p-rpS6 in CON but not AKI rats(Fig 1a).LEU suppressed LC3B-II protein levels(an autophagy marker) in CON, but had no effect on the elevated levels in AKI and also suppressed p-AMPK in CON, but not AKI rats(Fig 1b).



Conclusions: Together, failure of LEU to stimulate phosphorylation of the mTOR anabolic pathway, and failure to suppress both p-AMPK and autophagy strikingly demonstrates that AKI induces a leucine resistant state. We suggest that this AA resistance may contribute to protein energy wasting that often persists in AKI patients despite nutritional supplementation.

Funding: Veterans Administration Support

SA-PO2400

Snail Destabilizes Cell Surface Localization of the Apical Polarity Protein Crumbs3a Jennifer L. Harder, Eileen L. Whiteman, Jay Pieczynski, Benjamin L. Margolis. *Internal Medicine, University of Michigan, Ann Arbor.*

Background: During Epithelial to Mesenchymal Transition (EMT), cells modulate expression of proteins resulting in loss of apical-basal polarity. Mechanisms involved in this switch target the polarity protein Crumbs3, a small transmembrane protein that is essential for generation of the apical membrane and tight junctions of epithelial cells. The Crumbs3 gene is a direct target of transcriptional regulation by Snail, a potent inducer of EMT. However, Snail has also been shown to have multiple non-transcriptional roles, including regulation of cell adhesion and movement as well as cell proliferation and survival. In this set of experiments, we defined the normal kinetics of Crumbs3a in polarized epithelial cells and then explored Snail's post-translational effects on Crumbs3a (Crb3a).

Methods: We used MDCKs, a tissue culture model, stably expressing epitope-tagged versions of Crb3a and Snail in an inducible or constitutive manner to study the dynamics of Crb3a.

Results: Using fluorescently-tagged Crb3a in combination with fluorescence recovery after photobleaching (FRAP), we found that Crb3a normally has a half-life of under 6 hours and is highly mobile at the cell surface. We then used covalently modifiable SNAP epitope-tagged Crb3a to examine kinetics of Crb3a at the cell surface. We showed that Crb3a is rapidly turned-over in the presence of Snail, decreasing the half-life of Crb3a on the cell surface from 2 to 1 hour. In contrast, cell surface stability of Crb3a is unaffected by disruption of cell polarity following dissolution of cell-cell contacts under low calcium conditions. We further observed that Crb3a's mobility on gel electrophoresis is altered in the presence of Snail, in part due to alterations in both N- and O-glycosylation.

Conclusions: Taken together, these results suggest that Snail induces post-translational modifications that alter Crb3a's trafficking and stability at the epithelial cell surface. Moreover, our results support the concept of discernable transcriptional and post-translational effects of Snail on cell polarity and that these post-translational effects involve post-translational modulation of the polarity protein Crb3a.

Funding: NIDDK Support, Private Foundation Support

SA-PO2401

Analysis of Williams-Beuren Syndrome-Related Genes; by Using a Caenorhabditis Elegans as a Model Organism Tomoko Uehara, Eriko Kage-Nakadai, Shohei Mitani. *Physiology, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: Williams-Beuren syndrome (WBS) is a contiguous gene syndrome with an autosomal dominant inheritance pattern and that is caused by microdeletion at 7q11.23 and haploinsufficiency of this region. Approximately 50% of patients with WBS have morphological or functional anomalies of kidney or urinary tract. There is insufficient knowledge of candidate genes of these anomalies. In this study, we analyzed genes that are located on the deletion area by use of a model organism, Caenorhabditis elegans.

Methods: At least twelve genes in WBS deletion region are expressed in kidney. Firstly, we searched six C. elegans gene homologs with the human genomic DNA database. Secondly, we identified that each gene expression pattern using its own promoter mediated expression of green fluorescent protein (GFP). We also analyzed phenotypes using RNA interference (RNAi). Furthermore, we isolated a deletion mutant of the C28H8.1 gene, the homologue of human BCL7B gene, because of showing the significant effect of RNAi and assayed the phenotype more extensively.

Results: Expression patterns of these six genes were as follows. C28H8.1, a BCL7B gene homologue, was expressed ubiquitously. C27F2.4, a WBSCR22 gene homologue and the gene product may have metylation-associated function, was expressed in motor neurons and intestine cells. dnj-16, F46C5.9 and F32B4.6, a homologue of a DNAJC30 gene, a TBL2 gene and an ABHD11 gene, respectively, were expressed in intestine cells particularly during in larvae stage. Downregulation of C28H8.1 function by feeding RNAi induced reproductive defect and morphological deformation. The phenotype of C28H8.1 deletion mutant was also the failure of reproduction and developmental anomaly.

Conclusions: To summarize this study, we analyzed six gene homologs of human genes associated with WBS and expressed in kidney. C28H8.1, the homolog of BCL7B, appears to be involved in early embryonic development, in particular, cell cycle system. The other five of them did not show any specific expression-pattern or specific phenotype. We are planning to analyze these genes in more detail.

SA-PO2402

Molecular Mediators of Endoplasmic Reticulum Stress-Induced Cytotoxicity in Human Proximal Tubular Epithelial Cells Rachel Carlisle, Elise Brimble, Alana Heffernan, Richard Austin, Jeffrey G. Dickhout. *Nephrology, St Joseph's Healthcare Hamilton and McMaster University, Hamilton, ON, Canada.*

Background: Various drugs, including gentamicin, cisplatin, the acetaminophen metabolite p-aminophenol, and cyclosporine A, induce endoplasmic reticulum (ER) stress causing acute renal injury in proximal tubular epithelial cells (PTEC). This injury may be mediated by the expression of CHOP/GADD153, an ER stress-inducible proapoptotic gene. However, direct evidence of CHOP/GADD153-induced PTEC cell death is lacking.

Methods: In this study, cytotoxicity was measured by LDH release assay and apoptosis was measured by TUNEL assay. CHOP/GADD153 and TDAG51 protein levels were measured by Western blotting. CHOP/GADD153 knockdown was achieved using siRNA transfection, while CHOP/GADD153 and TDAG51 overexpression was performed by transient transfection.

Results: Using HK-2 cells, we demonstrated that the nucleoside antibiotic tunicamycin (Tm, 1 g/mL) induces ER stress and causes substantial upregulation of CHOP/GADD153 in PTEC at 18 h, followed by a significant increase in apoptotic cell death at 48 h. This effect was inhibited by siRNA-mediated CHOP knockdown. The SERCA inhibitor thapsigargin (Tg, 200 nM) induces ER stress, but unlike Tm, produced little or no CHOP/GADD153 expression at 18 h. However, Tg did induce expression of the novel proapoptotic gene TDAG51 at 18 h leading to similar cytotoxicity as Tm at 48 h. To directly determine if CHOP/GADD153 overexpression induced apoptosis in PTEC, overexpression of CHOP/GADD153 was achieved and increased the incidence of apoptosis to 26.6+8.6% (from 3.8+0.9% in pcDNA3.1 transfected controls). Transient transfection of TDAG51 in an eGFP vector resulted in increased apoptosis in the HK-2 model of PTEC (vector, 0.5+0.4% vs. TDAG51, 26.0+3.4%) and in primary human PTEC (vector, 5.2+1.3% vs. TDAG51, 30.0+4.6%).

Conclusions: ER stress is a common pathological pathway that leads to acute renal injury from numerous drugs; however, the molecular mediators of PTEC death appear to differ depending on the mechanism of action of the specific ER stress inducer, as shown, in this case, by Tm and Tg treatment.

Funding: Government Support - Non-U.S.

SA-PO2403

Phagocytosis of Apoptotic HIV-1- Infected CD4+/PD-1+ T Cells Is Critical for Establishment of HIV-1 Reservoirs in Tubular Cells Hersh Goel,[1] Mohammad Husain,[1] Nirupama Chandel,[1] Joanna Mikulak,[2] Ashwani Malhotra,[1] Helena Schmidtmayerova,[1] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Immunology, Istituto Clinico Humanitas, Rozzano (Milano), Italy.*

Background: HIV-1 infection of Kidney cells has been suggested to be a key factor which contributed to the pathogenesis of HIV-associated nephropathy (HIVAN). In the present study, we evaluated the potential of tubular cells to serve as an HIV-1 reservoir.

Methods: Human renal proximal tubular cells (HRPTECs and HK2s) were pulsed with HIV-1 (X4 HIV-$1_{92HT599}$) and then evaluated for HIV infection. To rescue HIV from tubular cells, HK2s were intubated with lymhocytes (LY) and followed by evaluation of LY for HIV replication. To determine viral transfer from HIV infected lymphocytes (HIV-LY) to tubualr cells, HIV-LYs were co-cultivated with HIK2/HRPTECs. To determine the role of apopotosis, LYs and HIV-LYs were pre-treated with anti-PDL-1 antibody and then evaluated for apoptosis by FACS analysis. To determine the role of apoptosis and phagocytosis of LYs in HIV transmission to HK2s, HIV-LYs were pretreated with either anti-PDL-1 antibody, cyotchalasin B, or a caspase-3 inhibitor followed by co-culitvation with HK2s for 24 hours and then evaluation of tubular cells for HIV expression.

Results: Both, HK2s and HRPTECs endocytosed viral particles; however, it was non-productive infection; nonetheless, HK2s transmitted viral particles to T cells. Co-cultivation of HIV-LYs with HK2s/HRPTECs triggered apoptosis of CD4+/T cells via PD-1/ PD-L1 interactions. Subsequent phagocytosis of PD-1+/CD4 T cells by HK2s facilitated activation of tubular cells and HIV expression. Both anti-PD-L1 antibody and cytochalasin-B inhibited uptake of HIV-LY by HK2s, their activation, and HIV expression. These findings identify a novel pathway that enables tubular cells to serve as an HIV-1 reservoir.

Conclusions: Our results indicate that tubular cells not only facilitated apoptosis of HIV-1 infected T cells but also showed capability of phagocytosing them. It appears that phagocytosed apoptosed T cells provided a suitable milieu for productive HIV-1 infection in tubular cells.

Funding: NIDDK Support

SA-PO2404

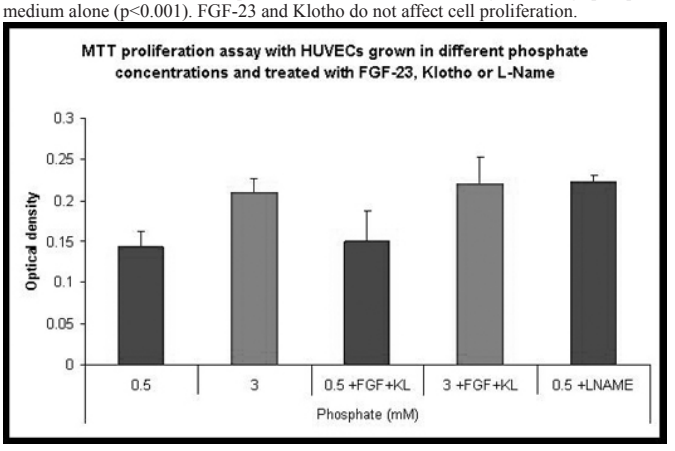
Hyperphosphataemia Increases Endothelial Cell Size, Granularity and Rate of Proliferation Kathryn K. Stevens, William A. Sands, Rajan Kantilal Patel, Christian Delles, Alan G. Jardine. *Renal Research Group, ICAMS, University of Glasgow, United Kingdom.*

Background: The mechanism of action of elevated serum phosphate as a risk factor for cardiovascular disease is unclear. We studied the effect of phosphate on the growth and proliferation of human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were cultured in standard (0.5mM) or high phosphate media (3mM). Cells were counted and then photographed and cell length and area measured. Cell proliferation was measured using the MTT assay in the presence of standard and high phosphate media alone or in combination with FGF-23, Klotho and L-NAME (eNOS

inhibitor). Western blot was performed for phospho and total eNOS. FACscan was used to assess granularity of the cells. Differences between groups were calculated in SPSS using a Student's t test or ANOVA with post hoc analysis as appropriate.

Results: HUVECs grown in high phosphate medium are more granular ($p=0.007$) and proliferate more rapidly than cells in standard medium ($p<0.001$). The morphology of the cells is different with a bigger cell area. MTT assays in cells grown in standard phosphate medium, treated with L-NAME proliferate at a similar rate to cells grown in high phosphate medium alone ($p<0.001$). FGF-23 and Klotho do not affect cell proliferation.



Expression of Phospho and total eNOS is reduced in HUVECs grown in high phosphate medium ($p=0.037$).

Conclusions: Nitric oxide inhibits cell growth. HUVECS, grown in high phosphate medium, are bigger, more granular and proliferate more rapidly. This may be secondary to increased oxidative stress with reduced nitric oxide production and thus removal of growth inhibition. Western blot confirms reduced eNOS expression in cells grown in high phosphate medium. These experiments mimic a uraemic state and may offer an explanation for elevated serum phosphate as a cardiovascular risk factor.

SA-PO2405

Renal Epithelial Cells Repress Calreticulin Expression To Increase Free Calcium and Adaptation to Osmotic Stress Asima Bibi, Gry Helene Dihazi, Marwa Youssef Eltoweissy, Gerhard A. Mueller, Hassan Dihazi. *Georg-August-University, University Medical Centre Goettingen.*

Background: ER resident calcium binding proteins play an important role in different stress balance mechanisms. The thick ascending limb of Henle's loop (TALH) is normally exposed to variable and often very high osmotic stress and involves different mechanisms to counteract this stress. The alteration of ER stress proteins is a part of TALH cells reaction to osmotic stress. The aim of the present study was to investigate the role of calreticulin (CALR) in adaptation and survival of TALH epithelial cells under osmotic stress.

Methods: Two-dimensional difference in-gel electrophoresis (2D-DIGE) combined with mass spectrometry, Western blot and RT-PCR analyses were done to analyze the protein expression in TALH cell line exposed to hyperosmotic stress. MTT assay was performed to check the percent viability of cells. Free intracellular calcium concentration was monitored with fura 2/AM fluorescent dye.

Results: 2D-DIGE and Western blot analyses demonstrated that TALH cells showed a significant down-regulation of CALR as a part of their reaction to variable osmotic stress. However, primary renal inner medullary collecting duct cells and interstitial cells showed no significant changes in CALR expression. Furthermore, RT PCR analysis of TALH cells exposed to osmotic stress showed a time dependent downregulation of CALR accompanied with continuous change in the level of free intracellular calcium. Inhibition of the calcium release by the IP3R antagonist, prevented CALR expression alteration under hyperosmotic stress, whereas the cell viability was significantly impaired. Overexpression of wild type CALR in TALH cells resulted in significant decrease in cell viability under hyperosmotic stress. In contrast, the hyperosmotic stress did not have any effect on cells overexpressing the CALR mutant, lacking the calcium-binding domain. MTT cell viability test showed that silencing CALR with siRNA significantly improved the cell survival under osmotic stress conditions.

Conclusions: Thus we conclude that CALR due to its calcium binding property plays a crucial role in osmotic stress adaptation and survival of TALH cells.

SA-PO2406

Discovery of a Splice Variant and Endogenous Inhibitor of Chk1 That Regulates Cell Cycle and DNA Damage Checkpoints Navjotsingh P. Pabla, Zheng Dong. *Georgia Health Sciences University and Charlie Norwood VA Medical Center.*

Background: The DNA damage response (DDR) and cell cycle checkpoints are essential regulatory mechanism for maintaining genomic stability. Checkpoint Kinase 1 (Chk1) is a key regulator of checkpoint signaling in both unperturbed cell cycle and DNA damage response. Under these conditions, Chk1 becomes active to prevent CDK1 activation and mitotic entry till DNA is properly replicated or repaired. It is unclear how Chk1 activity is controlled in unperturbed cell cycle. During DNA damage, Chk1 is activated by ATR mediated phosphorylation; however it is not entirely clear how the phosphorylation activates Chk1.

Methods: Here we report an N-terminally truncated alternative splice variant of Chk1, Chk1-S.

Results: This splice variant lacks the ATP binding domain and hence is kinase-inactive. Its expression is regulated in a cell-cycle dependent manner with highest expression seen in G2 phase. Importantly, we show that Chk1-S is an endogenous repressor and regulator of Chk1. In unperturbed cell cycle, Chk1-S interacts with and antagonizes Chk1 to promote S to G2/M phase transition. Overexpression of Chk1-S induces premature mitotic entry, resulting in mitotic catastrophe. In DNA damage, Chk1 is phosphorylated and the phosphorylation disrupts the Chk1/Chk1-S interaction, resulting in free, active Chk1 to arrest cell cycle to facilitate DNA repair. Chk1-S is widely expressed in multiple cell types with higher expression observed in fetal and cancer tissues than normal tissues. In tumor xenografts, forced overexpression of Chk1-S induces mitotic catastrophe and reduces tumor growth.

Conclusions: The identification of Chk1-S as a novel splice variant and key regulator of Chk1 provides new insights into cell cycle regulation, DNA damage response, and cancer therapy.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2407

Na+-Sensitive Chronic Kidney Disease in Mice Lacking Renal Principal Cell Histone H3 K79 Methyltransferase Dot1 Hongyu Wu,[1] Lihe Chen,[1] Qiaoling Zhou,[2] Stefan Berger,[3] Günther Schütz,[3] Yang Xia,[1] Wenzheng Zhang.[1] *[1]University of Texas Medical School at Houston; [2]Central South University; [3]German Cancer Research Center.*

Background: Little is known regarding the epigenetic programs regulating the pathogenesis of chronic kidney disease (CKD), possibly due to a lack of rodent models in which epigenetic modifiers are specifically disrupted. We have previously reported that histone H3 K79 methyltransferase Dot1 is highly expressed in mouse kidney and regulates ENaC-mediated Na+ transport in IMCD3 cells.

Methods: To identify new functions of Dot1 in kidney, we used LoxP-Cre system to inactivate Dot1 in AQP2-expressing cells (Dot1f/f AQP2Cre or mutants) and applied histological, immunochemical, TUNEL and metabolic analyses to characterize the phenotype.

Results: We found that on a normal Na+ diet, the mutants decreased principal cells (PC) by 20% and increased urine volume by 40% (2011 ASN abstract 21278). Here we report that the CKD phenotype of the mutants after chronic Na+ loading (3.1% dietary Na+ for 32 days). The control mice were capable of handling the chronic Na+ loading to maintain the body weight (BW), renal physiology, blood pressure and apparent normal cortex and medullar structure. The mutants progressively became very sick, and eventually lost BW by 12.4% vs. the initial BW, and had over 4-fold higher urine volume, 56% lower urine osmolarity, and 20 mmHg lower systolic BP vs. control. Multifocal cortex-like lesions, inflammatory infiltration, protein casts, dilated distal tubes with detached cells, fibrosis, apoptosis and epithelial-to-mesenchymal transition (EMT) as evidenced by loss of epithelial marker E-cadherin expression and gain of myofibroblast marker α- smooth muscle actin expression was also readily detected in the mutants, but hardly found in controls. These changes were accompanied by ~40-58% decrease in PC, 23-30% increase in IC and 13-28% increase in cells lacking expression of both PC and IC markers vs. controls.

Conclusions: In summary, Dot1f/f AQP2Cre mice may represent a new mouse CKD model, linking dietary Na+ intake and H3 K79 methylation to cell differentiation and pathogenesis of the disease.

Funding: NIDDK Support, Private Foundation Support

SA-PO2408

Aldosterone Promotes Proliferation of Cultured Renal Fibroblasts Via Activation of Mineralocorticoid/PDGF/EGF Receptors and MAPK/PI3K Signaling David J. Nikolic-Paterson,[1,2] Louis L. Huang,[1,2] Frank Yuanfang Ma,[1,2] Gregory H. Tesch.[1,2] *[1]Nephrology, Monash Medical Center, Clayton, Victoria, Australia; [2]Medicine, Monash University, Clayton, Victoria, Australia.*

Background: The development of tubulo-interstitial fibrosis (TIF) is critical for the progression of renal injury to end-stage renal disease. The severity of TIF is dependent on the accumulation of interstitial fibroblasts, which results from increased fibroblast recruitment and proliferation. Aldosterone, a mineralocorticoid hormone, has pro-fibrotic properties and can induce renal or cardiac fibrosis. However it is not known whether aldosterone can stimulate renal fibroblast proliferation. Therefore, we examined the effects of aldosterone on the proliferation of cultured kidney fibroblasts and identified the intracellular signaling mechanisms involved.

Methods: Uptake of ^{3}H-Thymidine was used to determine the dose-dependent effects of aldosterone on the proliferation of rat renal fibroblasts (NRK49F cells) cultured in serum-free media. Specific kinase inhibitors were used to identify the importance of growth factor receptor and mitogenic signaling pathways in aldosterone-induced proliferation.

Results: Aldosterone at physiologic concentrations (1-10nM) increased NRK49F proliferation by 2-fold after 24 hours ($p<0.0001$). This effect was inhibited by pretreatment with mineralocorticoid receptor (MR) antagonist eplerenone. Further characterization identified that the proliferative effects of aldosterone could also be blocked by inhibition of PDGFR (STI-571), EGFR (AG1478), ERK (UO126), JNK (SP600125), and PI3-K (LY294002) at doses that were not cytotoxic. In comparison, treatment with inhibitors of TGF-βR1/ALK5 (SB431542) and p38MAPK (SB202190) had no effect on the aldosterone-induced proliferation of NRK49F cells.

Conclusions: Aldosterone induces renal fibroblast proliferation via ligation of MR and subsequent activation of growth factor receptors (PDGFR/EGFR) and specific intracellular signaling pathways (ERK, JNK, PI3-K). These findings suggest that increased levels of aldosterone in diseased kidneys may promote renal fibrosis by inducing proliferation of kidney fibroblasts.

Funding: Government Support - Non-U.S.

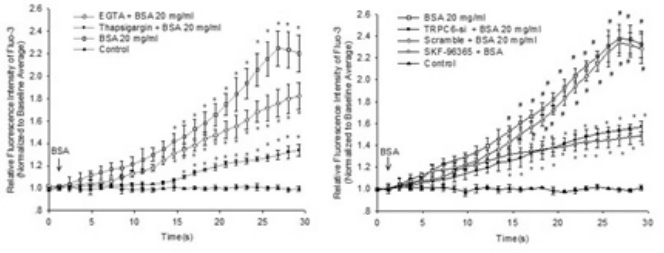
SA-PO2409

Calcium Entry Via TRPC6 Mediates Albumin Overload-Induced Endoplasmic Reticulum Stress and Apoptosis in Podocytes Shan Chen, Hui Wang, Chun Zhang. *Department of Nephrology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.*

Background: Albumin, the most abundant component of urine proteins, exerts injurious effects on podocytes. In the current study, we tested the hypothesis that albumin overload may induce functional and structural changes in podocytes via TRPC6-mediated Ca^{2+} entry.

Methods: Cultured podocytes were divided into several groups: control; vehicle; EGTA (extracellular Ca^{2+} chelater), Tapsigargin (intracellular Ca^{2+} store inhibitor), scramble siRNA; TRPC6 siRNA; SKF-96365 (TRPC channel blocker). Fluo 3-AM was used as a calcium indicator and the calcium-dependent fluorescence was monitored using confocal microscope upon albumin stimuli. Western blot was used to detect TRPC6, endoplasmic reticulum (ER) stress proteins GRP 78 and caspase-12. Rhodamine-labeled phalloidin was used to stain the F-actin. Annexin V-FITC and PI double staining was used to detect the apoptosis rate.

Results: High concentration of albumin (BSA 20 mg/ml) triggered intracellular calcium ($[Ca^{2+}]_i$) increase through mechanisms involving the intracellular calcium store release and extracellular calcium influx in podocytes. Albumin-induced increase in $[Ca^{2+}]_i$ was blocked by TRPC6 siRNA or SKF-96365.



Long-term albumin exposure caused an up-regulation of TRPC6, which was inhibited by TRPC6 siRNA. Additionally, the inhibition of TRPC6 prevented the F-actin cytoskeleton disruption that is induced by albumin overload. Moreover, albumin overload induced expression of GRP78, led to caspase-12 activation and ultimately podocyte apoptosis, all of which were abolished by the knockdown of TRPC6 using siRNA.

Conclusions: The amelioration of podocyte injury in albumin-overloaded podocytes by TRPC6 blockade further strengthened the critical role of this calcium channel. TRPC6 may be an therapeutic target in treating proteinuric kidney diseases.

Funding: Government Support - Non-U.S.

SA-PO2410

Protein Kinase B: A Multifaceted Protein in Albumin Endocytosis Elif Erkan. *Pediatrics, University of Pittsburgh, PA.*

Background: Orchestrated efforts of clathrin and clathrin associated proteins (CLASPs) regulate albumin endocytosis in the proximal tubule. In glomerulonephritis high concentrations of albumin passing through the altered glomerular filtration barrier results in proximal tubule cell apoptosis. The purpose of this study is to investigate a possible link between albumin endocytosis and survival pathways.

Methods: Co-immunoprecipitation and GST pull-down experiments were utilized to examine protein-protein interactions. Genetic manipulations were accomplished by transfection of human kidney proximal tubule cells with plasmid DNA and small interfering RNA. Albumin endocytosis was evaluated by FITC labeled albumin uptake.

Results: Co-imunoprecipitation showed an interaction between protein kinase B (Akt) and clathrin heavy chain, Ap2μ2 and adaptor protein disabled-2 (Dab2). GST pull down experiments utilizing Dab2 PTB domain (1-206), 1-368, 335-610 (M15) and proline rich domain (PRD, 600-730) identified PRD as the Akt interacting domain. In order to narrow down the interaction site, shorter constructs of PRD were created. Disabled-2 600-638 was found to be the binding domain of Akt. Overexpression of Akt by constitutively active and wild type plasmids increased albumin uptake where as dominant negative Akt caused a decrease in albumin internalization. Silencing Dab2 abolished Akt induced albumin uptake demonstrating physiological relevance of Akt-Dab2 interaction. Albumin overload resulted in attenuation of this interaction as a result of downregulation of both Dab2 and Akt expression in association with apoptosis.

Conclusions: We concluded that Akt is involved in albumin endocytosis via its interactions with CLASPs. We postulate that inhibition of Akt and Dab2 and attenuation Akt-Dab2 interaction with albumin overload may represent a defense mechanism of proximal tubule to limit albumin endocytosis. This mechanism may induce apoptosis by release of mitochondrial proapoptotic proteins that stay in phosphorylated form under the control of Akt in physiological conditions. Further delineation of interactions of Akt with upstream proteins and receptors will enhance our understanding of protein-protein interactions that regulate albumin endocytosis.

Funding: NIDDK Support

SA-PO2411

Growth Arrest Specific Protein 1 (GAS1) Is a Novel Secreted, Endogenous, and PDGF-Regulated Inhibitor of Mesangial Cell Proliferation Claudia R.C. van Roeyen,[1] Stephanie Zok,[1] Peter Boor,[1] Jessica Pruessmeyer,[2] Tammo Ostendorf,[1] Andreas Ludwig,[2] Jurgen Floege.[1] *[1]Nephrology, RWTH Aachen, Aachen, Germany; [2]Pharmacology&Toxicology, RWTH Aachen, Aachen, Germany.*

Background: GAS1 is a glycosyl-phosphatidyl-inositol (GPI)-anchored protein which is highly expressed in embryonal mouse fibroblasts (NIH3T3) and inhibits their proliferation.

Methods: In a cDNA-array analysis we identified GAS1 as one of the most potently suppressed genes following PDGF-BB or –DD stimulation of mesangial cells (MC).

Results: *In vitro* MC released soluble GAS1-protein into the cell culture supernatant. Growth-arrest led to GAS1 overexpression and an increased release. The secretion process was also enhanced by stimulation with phorbolester or ionomycin and involved the activity of the disintegrins and metalloproteinases (ADAM) 10 and 17 as indicated by inhibition experiments. In addition recombinant soluble GAS1 protein markedly inhibited the growth of proliferating MC. *Vice versa* induction of MC proliferation by PDGF-BB or -DD led to downregulation of GAS1 mRNA while specific ligands of the PDGF α-receptor PDGF-AA and –CC had no effect. In healthy rat kidneys we localized GAS1 protein to podocytes. During mesangioproliferative glomerulonephritis (GN) in rats glomerular GAS1 mRNA expression decreased prior to the onset of MC proliferation and increased at later stages when the glomeruli recovered.

A soluble GAS1 tagged with an Fc-fragment (GAS1/Fc) was systemically overexpressed by muscle electroporation in rats with mesangioproliferative GN at day 3 after induction. This resulted in 3-fold increased GAS1 serum concentrations. At day 9 overexpressed Gas1/Fc ameliorated renal damage as indicated by decreased albuminuria and serum-creatinine. GAS1/Fc-transfected rats exhibited a reduction of the glomerular α-smooth-muscle actin positive mesangial area and glomerular mRNA expression and a decreased number of proliferating glomerular cells. The number of infiltrating glomerular $ED1^+$-macrophages and mRNA expression of CCL2 were also reduced.

Conclusions: In summary we identify GAS1 as a novel endogenous growth inhibitor of MC. Gas1 presents a potentially novel therapeutic target in mesangioproliferative glomerular diseases.

Funding: Government Support - Non-U.S.

SA-PO2412

Identification & Characterization of a Novel Oncogenic HIF-1 Target Involved in Renal Cell Carcinoma Kumi Shoji,[1] Imari Mimura,[1,2] Takamoto Ohse,[1] Takashi Murayama,[3] Reiko Inagi,[1] Takehiko Wada,[1] Tetsuhiro Tanaka,[1] Haruki Kume,[4] Akiteru Goto,[5] Toshiro Fujita,[1] Hiroyuki Aburatani,[2] Tatsuhiko Kodama,[2] Masaomi Nangaku.[1] *[1]Nephrol Endocrinol, Univ Tokyo Sch Med, Tokyo, Japan; [2]Lab Syst Biol Medi, RCAST, Univ of Tokyo, Tokyo, Japan; [3]Pharmacology, Juntendo Univ Sch Med, Tokyo, Japan; [4]Urol, Univ Tokyo Sch Med, Tokyo, Japan; [5]Pathol, Univ of Tokyo, Tokyo, Japan.*

Background: Hypoxia inducible factor (HIF) is upregulated under hypoxia and regulates coordinated transcriptional response, playing a key role in many pathological conditions such as tumorigenesis.

Methods: We performed genome-wide analysis of HIF targets utilizing microarray analysis in combination with ChIP-Sequencing. We focused one of the new targets, sperm associated antigen 4 (SPAG4), which is expressed in a limited number of normal tissues.

In order to elucidate a role of SPAG4 in renal cell carcinoma (RCC), we analyzed SPAG4 expression by immunohistochemistry on tissue microarray (TMA) containing 208 RCC specimens.

We also performed cell biological studies of cells with overexpression or knockdown of SPAG4, utilizing immunofluorescence, immunoprecipitation, and live cell imaging using SPAG4-EGFP, dynein-mCherry, and calnexin-mCherry.

Results: We identified 26 new targets of HIF-1, and SPAG4 was selected for a further analysis, of which function remains unknown. In cultured cells, SPAG4 was up-regulated under hypoxia, and luciferase assays demonstrated that hypoxia response element in the intron of SPAG4 is essential for the response.

RCC TMA analysis showed expression of SPAG4 in more than 80% RCC. High expression of SPAG4 correlated with poor prognosis in early stage cancer and increased risk for metastasis. The intensity of SPAG4 expression was associated with vein invasion and nuclear grading, suggesting a role of SPAG4 in tumor development and metastasis.

We localized SPAG4 in the nuclear membrane and the cytoplasm. SPAG4 was co-localized with dynein and was involved in cytokinesis.

Conclusions: In conclusion, we identified a novel HIF target, SPAG4, by the genome-wide system biological method. Our functional analysis showed a potential role of SPAG4 in cytokinesis and RCC.

Funding: Government Support - Non-U.S.

SA-PO2413

Perspectives of an Interaction between Caveolae and Notch Signalling on the Pathogenesis of Renal Fibrosis Yuliya Sharkovska,[1] Carsten Dittmayer,[1] Markus Schuelke,[2] Michael S. Goligorsky,[3] Sebastian C. Bachmann.[1] *[1]Institut für Vegetative Anatomie, Charité Universitätsmedizin, Berlin, Germany; [2]Department of Neuropediatrics, Charité Universitätsmedizin, Berlin, Germany; [3]Departments of Medicine, Pharmacology, and Pathology, Renal Research Institute, New York Medical College, Valhalla.*

Background: As a common pathological alteration in progressive chronic kidney disease, renal fibrosis is associated with tubular atrophy and deposition of extracellular matrix. TGFβ is considered as a central mediator in fibrosis. The mechanistic roles of caveolae and Notch signalling have further been implicated in its pathogenesis, and caveolin-1 (Cav-1) may affect TGFβ signalling and as well as gamma-secretase activity. The molecular detail of this hypothetical cascade and its impact on renal fibrosis is not yet clear.

Methods: To elucidate this, we have used Cav-1-deficient mice (Cav-1 -/-) and human fibroblasts from patients with congenital generalized lipodystrophy (CGL4), lacking caveolae due to mutations of polymerase I and transcript release factor (PTRF), an essential protein for caveolae biogenesis (Rajab A, PLoS Genet 6/1/2010).

Results: Ultrastructural analysis demonstrated the near-complete absence of caveolae in both models. In Cav-1 -/- mice, the degree of fibrosis upon induction of tubulointerstitial scarring was substantially enhanced as compared to wildtype mice (Park HC, AJP 298/F357/2010). Human fibroblasts displayed enhanced Notch signalling in the absence of PTRF as revealed by expressional analysis and immunohistochemistry of its components, Jagg-1, Notch-1, Notch intracellular domain, and HES1. Increases in Notch signalling were more pronounced in control, than in PTRF-deficient fibroblasts upon stimulation with TGFß. Changes in Notch were paralleled by alpha-smooth muscle actin and collagen I expression.

Conclusions: These data indicate that caveolae and the components of Notch signalling are involved in the pathogenesis of fibrosis. We further conclude that the profibrotic effect of caveolar malformation is likely to be mediated by Notch activation.

SA-PO2414

VEGF Receptor 2 Direct Interaction with Nephrin Links VEGF-A Signals to Actin in Kidney Podocytes Claudia A. Bertuccio,[1] Delma Veron,[1] Pardeep Kumar Aggarwal,[1] Lawrence B. Holzman,[2] Alda Tufro.[1] *[1]Yale University School of Medicine, New Haven, CT; [2]University of Pennsylvania, Philadelphia, PA.*

Background: The transmembrane protein nephrin is an essential component of slit-diaphragms, the specialized cell junctions that link podocyte foot processes. Podocytes are epithelial cells that surround the glomerular capillaries in the kidney and are necessary for the organ filtering function. Nephrin signaling complex transduces extracellular cues to the podocyte cytoskeleton and regulates podocyte shape and function. Vascular endothelial growth factor A (VEGF-A) is a required growth factor produced and secreted by podocytes. Accumulating evidence suggests a crosstalk between VEGF-A and nephrin signaling pathways. Our previous studies have shown that nephrin associates with VEGF receptor-2 (VEGFR2) tyrosine kinase, the signaling receptor for VEGF-A in vivo.

Methods: In the present study we characterized the interaction between nephrin and VEGFR2 in vitro and in cultured cells (podocytes and COS cells transfected with the corresponding expression plasmids), using mass spectrometry, co-immunoprecipitation, GST-binding assays and blot overlay experiments.

Results: We demonstrate that nephrin-VEGFR2 interaction is direct. This interaction occurs through VEGFR2 and nephrin cytoplasmic domains. Nephrin-VEGFR2 interaction is modulated by tyrosine phosphorylation of both cytoplasmic domains. Furthermore, our results indicate that the nephrin-VEGFR2 complex involves Nck and actin. We provide evidence that this multi-protein interaction occurs in cultured podocytes.

Conclusions: We propose that nephrin-VEGFR2 complex acts as a key mediator to transduce local VEGF-A signals to the podocyte actin cytoskeleton, thereby regulating the normal foot process structure and glomerular filter integrity.

Funding: NIDDK Support

SA-PO2415

Simvastatin Inhibits Angiopoietin-2 Release and Production – A Novel Pleiotropic Effect? Sascha David,[1,2] Chandra C. Ghosh,[1] Aditi Mukherjee,[1] Stephen G. John,[3] Chris W. McIntyre,[3] Samir M. Parikh.[1] *[1]Division of Medicine and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [2]Nephrology, Medical School Hannover; [3]University of Nottingham.*

Background: We recently found that circulating levels of Angiopoietin-2 (Anpt-2) increase with the extent of chronic kidney disease (CKD) and correlate with prevalent atherosclerotic burden. Angpt-2 is a growth factor, stored in endothelial cells (ECs), that sensitizes ECs to diverse inflammatory stimuli thereby potentially contributing to the accelerated atherogenesis in CKD. Anti-inflammatory effects of statins are well-known, yet the mechanisms of these pleiotropic properties remain incompletely understood. We hypothesized that statins may directly inhibit Angpt-2 action and test in vivo and in vitro.

Methods: Angpt-2 was measured in EC media, lysates, as well as sera from 128 CKD patients by commercial ELISA (R&D Systems). Different EC lines were treated with simvastatin (10-100 μM) or vehicle. Immunofluorescence (IF) was performed using antibodies against 'Angpt-2' and 'von Willebrand Factor' (vWF). Total RNA was amplified with a commercial TaqMan qPCR assay (Applied Biosystems).

Results: CKD stage 4-5 patients on statins (n=48) had significantly lower circulating levels of Angpt-2 compared to CKD patients without statin treatment (n=80) (statin: 1.87 ± 0.68 vs. no statin: 2.43 ± 1.18 ng/mL; p=0.0002).

In vitro, a high amount of Angpt-2 accumulated in the media of all three EC types. Simvastatin dose-dependently reduced not only the release of Angpt-2 (64% reduction at a dose of 100 μM after 24 h, $p<0.05$), but also the intracellular Angpt-2 protein concentration (66% reduction, $p<0.05$) and transcript abundance (10-fold decrease, $p<0.05$). Furthermore, IF experiments demonstrated that other contents of endothelial Weibel-Palade bodies (e.g. vWF) were virtually unaffected by simvastatin.

Conclusions: CKD patients on maintenance statin therapy had lower circulating Angpt-2 levels than their CKD controls. Simvastatin markedly reduced Angpt-2 release and production by ECs in vitro. The mechanism for this effect and its downstream consequences merit further investigation.

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SA-PO2416

In Vivo Erythropoietic Activity of Peginesatide Correlates with Erythropoietin Receptor Residence Time and Plasma Half Life Jennifer M. Green, Karen Leu, Qing Fan, Brian T. Frederick, Richard B. Mortensen, Peter R. Young, Peter J. Schatz, Kathryn W. Woodburn, Christopher P. Holmes. *Affymax, Inc., Palo Alto, CA.*

Background: Peginesatide is a PEGylated, investigational, peptide-based erythropoiesis stimulating agent that was designed and engineered to stimulate the erythropoietin receptor (EPOR). Peginesatide has a unique structure that consists of a synthetic peptide dimer (with no sequence similarity to erythropoietin) conjugated to a 40 kDa PEG moiety.

Methods: To determine the effects of PEGylation on the biological activity of peginesatide, a series of compounds were synthesized where the peginesatide peptide dimer was conjugated to PEG moieties ranging in molecular weight from 2 kDa up to 60 kDa. Each of these compounds was evaluated for kinetics of binding to the EPOR, the ability to stimulate the proliferation and differentiation of $CD34^+$ bone marrow cells into erythroid precursors, and the ability to stimulate erythropoiesis in vivo.

Results: As PEG size increased, progressively slower association and dissociation rates were observed resulting in an overall lower affinity for EPOR (KD = 24 to 2400 pM, from 2 kDa to 60 kDa, respectively) and reduced potency for stimulating differentiation of $CD34^+$ cells into erythroid precursors (EC_{50} = 22 to 781 pM, respectively). Interestingly, the progressively slower dissociation rates resulted in an increase in EPOR occupancy time ($t_{1/2}$ = 260 to 1300 min, respectively), which contributed to an extended activation of EPOR signaling. This extended EPOR activation, in combination with a prolonged plasma half-life due to increased PEG size, led to the opposite trend in vivo with peptides conjugated to larger PEGs exhibiting increased erythropoietic potency. Peptides conjugated to PEG moieties larger than 20 kDa induced a robust production of red blood cells whereas those linked to PEGs smaller than 10 kDa were not efficient in driving erythropoiesis.

Conclusions: These results suggest that the extended erythropoietic activity of peginesatide in vivo results from both prolonged peptide-receptor residence time and plasma half life which may contribute to maintenance of hemoglobin levels with monthly dosing.

Funding: Pharmaceutical Company Support

SA-PO2417

Peginesatide Stimulation Allows for a Longer Lifespan of the Activated Erythropoietin Receptor Jennifer M. Green,[1] Karen Leu,[1] Peter J. Schatz,[1] Don Wojchowski.[2] *[1]Affymax, Inc., Palo Alto, CA; [2]Maine Medical Center Research Institute, Scarborough, ME.*

Background: Peginesatide (Hematide™) is a PEGylated, investigational, peptide-based erythropoiesis stimulating agent (ESA) that is designed to specifically stimulate the erythropoietin receptor (EPOR).

Methods: To investigate the regulation of EPOR trafficking after peginesatide vs. recombinant human erythropoietin (rHuEPO, epoetin alfa) dosing, we have developed a novel panel of specific rabbit monoclonal anti-EPOR antibodies, and have used these tools together with Western blotting and flow cytometry to study EPOR cell surface expression, internalization, and degradation.

Results: In exponentially growing erythropoietin-dependent UT-7/EPO cells maintained in rHuEPO, cell surface EPORs were detected at low levels, and EPOR turnover/degradation appeared to predominate. In contrast, cell surface EPOR levels were increased significantly (~ 5.9 fold) in cells maintained in functionally equivalent levels of peginesatide, suggesting that EPOR turnover was lessened. After culture of cells for 18 hours in the absence of EPOR stimulation, subsequent treatment with rHuEPO led to the rapid tyrosine-phosphorylation of the EPOR, subsequent receptor internalization, and the rapid appearance of the major 42 kDa and 30 kDa EPOR degradation fragments observable within 15 minutes of treatment. In contrast, EPOR phosphorylation occurred at reduced magnitude following peginesatide treatment, but was sustained and correlated with an observed 30 minute delay in EPOR fragmentation. Since the ubiquitin/proteasome system plays a major role in EPOR internalization and down-modulation, we investigated EPOR ubiquitination after peginesatide vs. rHuEPO treatment. Each ESA induced EPOR ubiquitination, but peginesatide did so at significantly lower levels (~ 50% less).

Conclusions: These studies provide new mechanistic insights into the molecular basis for the extended erythropoietic activity of peginesatide.

Funding: Pharmaceutical Company Support

SA-PO2418

Blockade of p38 MAPK Pathway Ameliorates Aldosterone-Induced Renal Injury in Guanylyl Cyclase-A Deficient Mice Yukiko Kato, Masashi Mukoyama, Hideki Yokoi, Yoshihisa Ogawa, Kiyoshi Mori, Masato Kasahara, Takashige Kuwabara, Hirotaka Imamaki, Tomoko Kawanishi, Akira Ishii, Kenichi Koga, Keita P. Mori, Akira Sugawara, Kazuwa Nakao. *Department of Medicine and Clinical Scinence, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: Recent clinical and experimental studies have shown that aldosterone plays an important role in the pathogenesis of renal injury. We have already reported that natriuretic peptide/guanylyl cyclase-A (GC-A) signaling exerts renoprotective effects in 5/6 nephrectomized, anti-GBM glomerulonephritis and streptozotocin-induced diabetic mice. Furthermore, we demonstrated that uninephrectomized GC-A knockout (KO) mice with four-week administration of aldosterone and sodium overload exhibited accelerated hypertension with marked nephrotic-range proteinuria (~300-fold urinary albumin excretion from baseline) compared to wild-type mice. Administration of aldosterone also increased phosphorylation of ERK and p38 MAP kinase (MAPK) mainly in podocytes of GC-A KO mice.

Methods: To determine the interaction between p38 MAPK and GC-A, we examined the effect of p38 MAPK inhibitor on renal findings. Two weeks after uninephrectomy, mice were administered with aldosterone (0.2 μg/kg/min) subcutaneously using an osmotic minipump, with 6% sodium diet and with hydralazine or p38 MAPK inhibitor (FR 167653, 33mg/kg/day) by drinking water. We examined systolic blood pressure, urinary albumin to creatinine ratio, and histological findings.

Results: Although a hydralazine treatment significantly reduced systolic blood pressure, urinary albumin excretion did not change compared with aldosterone-infused GC-A KO mice. Interestingly, urinary albumin excretion was dramatically decreased by 90% in aldosterone-infused GC-A KO mice with FR treatment. Glomerular hypertrophy and mesangial expansion was decreased in FR-treated aldosterone-given GC-A KO mice.

Conclusions: These results suggest that p38 MAPK acts as an important molecule connecting aldosterone and GC-A pathways, and could be a potential target against aldosterone-induced glomerular injury.

Funding: Government Support - Non-U.S.

SA-PO2419

Mutation of Endocytic Motifs in the Cytoplasmic Loop of AT_{1a} Receptors Impairs Angiotensin II Uptake and Activation of MAP Kinases ERK1/2 and Sodium and Hydrogen Exchanger-3 in Proximal Tubule Cells Xiao C. Li,[1] Ulrich Hopfer,[2] Jia L. Zhuo.[1] *[1]Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS; [2]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.*

Background: The present study tested the hypothesis that the motif Leu^{316} to Tyr^{319} in the cytoplasmic loop of the AT_{1a} receptor is required for AT_{1a}-mediated Ang II uptake and activation of ERK1/2 (p-ERK1/2) and the sodium and hydrogen exchanger-3 (p-NHE-3) in mouse proximal tubule cells.

Methods: GFP-tagged wild-type (AT_{1a}R/GFP) or mutant AT_{1a} receptors with deletion of the motif Leu^{316} to Tyr^{319} in the cytoplasmic loop (AT_{1a}R/GFP[ΔL316-Y319]) were expressed in AT_{1a}-knockout (AT_{1a}-KO) mouse proximal tubule cells. The transfected cells were stimulated with Ang II (10 nM) in the presence or absence of the AT_1 antagonist losartan (10 μM) or the MEK1/MEK2 inhibitor UO126 (10 μM).

Results: AT_{1a}R/GFP was endocytosed into the cytoplasm and nuclei in a time-dependent manner. Ang II-induced AT_{1a}R/GFP endocytosis was associated with a twofold increase in p-ERK1/2 (control: 0.25 ± 0.03 vs. Ang II: 0.56 ± 0.06 p-ERK1/2/t-ERK1/2 ratio, $p<0.01$) and a 70% increase in p-NHE-3 proteins (control: 0.22 ± 0.02 vs. Ang II: 0.38 ± 0.03 p-NHE-3/actin ratio, $p<0.01$). Co-expression of AT_{1a}R/GFP with an intracellularly expressed cyan fluorescent Ang II fusion protein (ECFP/AII) in AT_{1a}-KO cells also induced similar activation of ERK1/2 (control: 0.26 ± 0.03 vs. ECFP/AII: 0.48 ± 0.07 p-ERK1/2/t-ERK1/2 ratio, $p<0.01$) and NHE-3 (control: 0.23 ± 0.02 vs. ECFP/AII: 0.46 ± 0.03 p-NHE-3/actin ratio, $p<0.01$). Losartan and UO126 blocked the p-ERK1/2 and p-NHE-3 responses to Ang II or ECFP/AII. By comparison, the endocytosis of the mutant AT_{1a}R/GFP(ΔL316-Y319) receptor was impaired, while Ang II- or ECFP/AII-induced activation of p-ERK1/2 and p-NHE-3 was markedly attenuated.

Conclusions: We conclude that the endocytic motif Leu^{316} to Tyr^{319} in the cytoplasmic loop of the AT_{1a} receptor may play an important role in AT_{1a} receptor-mediated Ang II uptake, and in extracellular or intracellular Ang II-induced activation of ERK1/2 and NHE-3 in mouse proximal tubule cells.

Funding: NIDDK Support

SA-PO2420

Angiotensin-Converting Enzyme 2 (ACE2) Is Shed from Proximal Tubular Cells Via ADAM-17 Joe A. Zimpelmann, Fengxia Xiao, Renisha Padmini Nadarajah, Chris R. Kennedy, Kevin D. Burns. *Kidney Research Centre, Division of Nephrology, Dept. of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada.*

Background: Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the proximal tubule where it converts angiotensin (Ang) II to Ang-(1-7). As an integral membrane protein, ACE2 may cleaved by a disintegrin and metalloproteinase (ADAM)-17, resulting in extracellular shedding. We determined if ACE2 is shed from proximal tubule, and studied potential stimuli for shedding.

Methods: In primary cultures of mouse proximal tubular cells and in urine samples, ACE2 activity was measured using a fluorogenic ACE2 substrate.

Results: A time-dependent and significant increase in ACE2 activity was observed in the media from cultured proximal tubular cells. After 6 days in culture, ACE2 activity was 10-fold higher in media than in the membrane fraction (media: 3,103 ± 219 vs. membrane: 266 ± 29 ng-eq/dish; n=8, $p<0.001$). Incubation of cells in high D-glucose (25 mM), but not L-glucose, for 72 h significantly increased ACE2 activity in the media, an effect blocked by the ADAM-17 antagonist, TAPI-1 ($p<0.001$, n=11). Similarly, incubation of cells for 48-72 h with Ang II (10^{-7} M) enhanced ACE2 activity in the media ($p<0.001$, n=11), an effect that was blocked by the AT_1 receptor antagonist losartan, or TAPI-1. In cells transiently transfected with a human ACE2 cDNA expression plasmid, ACE2 activity was significantly increased in supernatant fractions ($p<0.001$ vs cells transfected with empty vector; n=3). In streptozotocin (STZ)-diabetic mice, and in mice infused with Ang II (1000 ng/kg/d) and fed a high salt diet, a progressive increase in urinary ACE2 activity occurred, compared to baseline levels (STZ-diabetes: $p<0.001$ vs non-diabetic mice; n=15). Renal tubular immunostaining for ADAM-17 was increased in diabetic mice and mice infused with Ang II.

Conclusions: These data suggest that ACE2 is shed from proximal tubular cells into the urinary space. Shedding is stimulated by high glucose or Ang II, via an ADAM-17-mediated pathway. Loss of membrane-bound ACE2 might alter the peritubular levels of Ang II and Ang-(1-7) and thereby affect kidney disease progression.

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SA-PO2421

Regulation of AngII-Induced Pro-TGFalpha Cleavage in CKD Progression Andreas Herrlich. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Low-level Angiotensin II (AngII)-stimulation in mice induces CKD lesions similar to human CKD in normotensive ACE/ARB treated patients with partially suppressed AngII; this depends on AngII-mediated cleavage of pro-TGF-alpha in tubular cells. Lesions were strongly reduced with metalloprotease inhibition, in pro-TGFalpha knockout mice, or mice expressing a defective EGF receptor in tubular cells. We have identified regulators of pro-TGFalpha cleavage: PKCalpha and the PKC-regulated protein phosphatase 1 (PP1) inhibitor PPP1R14D.

We provide evidence that the AngII-TGFalpha signaling pathway is relevant in humans and show that AngII-induced TGFalpha cleavage in kidney cells depends on the action of PKCalpha and PPP1R14D.

Methods: Kidney microarray data
Western blot and FACS
shRNA knockdown
Immunoprecipitation

Results: pro-EGF expression in diabetic nephropathy is highest in CKD stage I-II and decreases strongly with disease progression. pro-TGFalpha levels are very low in CKD I + IV-V, but significantly elevated in the earlier stages, CKD stage II-III. This observation mirrors the situation in the AngII-TGFalpha mouse model. Genetic CKD susceptibility loci in mice, rats and humans contain the pro-TGFalpha gene.

In kidneys of hypertensive mice, PKCalpha and PPP1R14D are significantly upregulated as compared to normotensive mice.

AngII- and TPA-induced TGFalpha cleavage in human embryonic kidney cells can be blocked with a PKC inhibitor (BIM1) or specific knockdown of PKCalpha or PPP1R14D.

Only TPA-treated PPP1R14D samples co-precipitate other proteins not present in the control (silver gel). PP1alpha co-immunoprecipitates only with AngII- or TPA-induced PPP1R14D-signaling complexes (western blot).

Conclusions: 1. TGFalpha expression in human kidney correlates with disease activity in diabetic nephropathy and is contained in several genetic loci associated with CKD progression shared between human, mouse and rat.

3. PKCalpha and PPP1R14D are
a) upregulated in the kidneys of hypertensive mice.
b) required for AngII-induced TGFalpha cleavage in kidney cells.

6. PP1alpha specifically regulates AngII-induced TGFalpha cleavage.

5. Targeting TGFalpha cleavage downstream of the receptor could provide new drug targets for human CKD progression.

Funding: NIDDK Support

SA-PO2422

Activation of the Retinoid X Receptor Modulates Angiotensin II Induced Inflammation and Hypertrophy in Vascular Smooth Muscle Cells Seth B. Furgeson, Vicki J. Van Putten, Peter Simpson, Mary C.M. Weiser-Evans, Raphael A. Nemenoff. *Medicine/Division of Renal Diseases and Hypertension, University of Colorado-Anschutz Medical Campus, Aurora, CO.*

Background: PPARγ agonists have been shown to interfere with the biological effects of angiotensin II (AII) and decrease blood pressure in animal models. PPARγ forms a heterodimer with the retinoid X receptor (RXR) to activate target genes. Rexinoids represent a class of RXR agonists that have been shown to activate a subset of RXR heterodimers, including PPARγ, PPARα, PPARδ, LXR, and FXR. In vascular smooth muscle cells (VSMCs), AII increases expression of pro-inflammatory cytokines and smooth muscle contractile proteins and induces cellular hypertrophy. The purpose of this study was to examine the effects of the rexinoid bexarotene (Bex) on VSMC responses to AII.

Results: In cultured rat aortic VSMCs, using a PPAR-RE luciferase construct, Bex (1 μM) increased PPARγ activity by 80% while pioglitazone (Pio; 10 μM), a PPARγ agonist, led to a 60% increase. Bex also increased LXR activity 3 fold while Pio had little effect. AII (1 μM) significantly increased IL-6 and MCP-1 mRNA and protein. Both Bex and Pio blocked cytokine induction by AII, but Bex was more effective (decrease in IL-6 mRNA: 96% vs. 68%, decrease in IL-6 protein secreted: 77% vs. 54%; decrease in MCP-1 mRNA: 94% vs. 57%, and decrease in MCP-1 protein: 71% vs. 43%). AII also significantly increased calponin (a smooth muscle cell marker) as measured by both immunoblotting and qRT-PCR; Bex, but not Pio, prevented this increase. Bex completely inhibited the pro-hypertrophic effects of AII, as determined by 3H-leucine incorporation into protein, while Pio had no effect. Effects on signaling pathways were also examined. Bex inhibited activity of p38, ERK, and JNK at 15 and 30 minutes following AII stimulation.

Conclusions: Bexarotene, an RXR agonist, inhibits pathological effects of AII in the vasculature. Given the stronger responses to bexarotene compared to pioglitazone, the effects of bexarotene likely involve other nuclear receptors. Rexinoids may be useful agents in treating vascular disease.

Funding: Other NIH Support - NHLBI K08

SA-PO2423

Influence of Angiotensin II (ANG II) on Morg1 Expression in Renal Cells

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Background: The mitogen-activated protein kinase organizer 1 (Morg1) serves as a scaffold molecule for a number of proteins involved in the MAPK signalling complex. In addition it is involved in the regulation of the HIF-1α activity via PHD3 stabilization. Morg1 is expressed in kidney. The purpose of this study was to analyze the influence of ANG II on Morg1 expression and its correlation with PHD3 activity and HIF-1 alpha promoter activity in MMCs, MTCs and differentiated podocytes.

Methods: Morg1 expression was investigated in MMC (mouse mesangial cells), MTC (mouse tubular cells) and in differentiated podocytes by real-time PCR. HIF-1α transcriptional activity was tested using Luciferase reporter-gene assay. PHD3 activity was tested based on a HIF-1α peptide assay.

Results: MMCs, MTCs and differentiated podocytes were stimulated with ANG II for different time points. We observed a biphasic effect of ANG II on Morg1 mRNA expression which was time dependent and differentially regulated by the ANG II-receptor (ATR) 2 subtype. While 9 h ANG II incubation inhibited Morg1 expression in MMCs via AT1 and AT2 subtypes based on ATR2 blockers studies, it enhanced Morg1 mRNA expression after 24 h mainly through ATR1 subtype. In MTCs 9 h stimulatory effect of ANG II on Morg1 expression was reduced to basal at the presence of PD 123319, an AT2 blocker, while the suppression of Morg1 after 24 h ANG II was reversed by addition of losartan, an AT1 inhibitor. Podocytes demonstrated an elevated Morg1 expression after 3 h ANG II treatment, which was partially suppressed by both PD 123319 and losartan. On the other hand, 24 h ANG II treatment inhibited Morg1 expression in differentiated podocytes through AT2 receptor subtype. We also found that ANG II effects on Morg1 mRNA expression in renal cells are independent of MAPK activity. In correlation with Morg1 expression pattern was the measured PHD3 activity. Reporter-gene assays have shown possible negative regulation of HIF-1α activity via Morg1 and PHD3 in MTCs.

Conclusions: Our study indicates that ANG II regulates Morg1 expression and HIF-1α transcriptional activation through cell type specific complex mechanisms in renal cells.

Funding: Government Support - Non-U.S.

SA-PO2424

Interactions between Adiponectin and Angiotensin II in Human Renal Tubular Cells

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Background: Chronic kidney disease (CKD) is a major risk factor for all-cause mortality, and its incidence is on the rise. The mechanism responsible for the progression of CKD is not completely understood, and epidemiology studies suggest that obesity can contribute to this process. One way that obesity may exacerbate CKD is by dysregulation of the adipokines. Adiponectin is a 30kDa hormone secreted by adipose tissue, and its circulating level decreases with obesity. Previous studies indicated that adiponectin could protect the heart and vasculature from various insults, which may be partially due to its inhibitory effect on the generation of reactive oxygen species. However the effect of adiponectin on kidney has not been fully elucidated. We hypothesized that adiponectin also plays a protective role in the kidney. To test our hypothesis, we studied the interaction of adiponectin and angiotensin II (AngII), which is well known to contribute to kidney injury, in human renal tubular cells.

Methods: Primary human renal proximal tubule epithelial cells (RPTECs) of passage 4 -7 were used for all experiments. NADPH oxidase activity was measured by lucigenin-enhanced chemiluminescence assay. NFκB activity was measured with commercial kits.

Results: Both the AdipoR1 and AdipoR2 were expressed in RPTEC and were present on cell membrane. AngII treatment didn't alter the expression level of neither the receptors. Adiponectin inhibited the activation of NADPH oxidase by AngII in RPTECs, which can be mimicked by the AMPK agonist AICAR and stable cAMP analogues pCPT-cAMP and db-cAMP. The effect of adiponectin was blocked by the AMPK antagonist compound C, adenylate cyclase inhibitor SQ22536 and PKA inhibitor H89. The AngII-induced increase in NFκB activity was also reduced by adiponectin.

Conclusions: Adiponectin attenuated the AngII-induced superoxide generation by NADPH oxidase in RPTEC, which was dependent on AMPK and cAMP/PKA pathways. NFκB activation caused by AngII in renal tubular cells was also attenuated by adiponectin. Our results suggest that decreasing adiponectin levels associated with obesity may be one of the mechanisms by which obesity contributes to the progression of CKD.

Funding: Government Support - Non-U.S.

SA-PO2425

FXR or TGR5 Agonists Prevent High Glucose Mediated Glucolipotoxicity in Human Podocytes

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Background: Diabetic nephropathy has been associated with abnormal lipid metabolism and lipotoxicity. Accumulation of lipid droplets (LDs) in podocytes is a frequently observed phenotype in the diabetic kidney. Bile acid activated nuclear hormone receptor the farnesoid X receptor (FXR) and the G protein-coupled receptor (TGR5) regulates bile acid, glucose, and lipid metabolism and inflammation. Activation of FXR by synthetic ligands GW4064 or INT-747 attenuates renal fibrosis in animal models of diabetic nephropathy. The aim of this work was to study the mechanism of the adipogenic program and LD accumulation in human podocytes and the effect of FXR and TGR5 agonists.

Methods: Podocytes were treated with high glucose to induce de-novo lipogenesis. Lipid content was assessed by Oil Red O as well as Nile Red staining. Expression of mRNA and protein of molecules controlling lipid homeostasis was examined by real-time quantitative PCR, LDs were determined using FACS method and by confocal microscopy.

Results: Administration of high glucose increased the number of LDs and the LD-associated protein adipocyte differentiation-related protein (ADRP). Selective FXR agonist (GW4064) and TGR5 agonist (INT-777) significantly inhibited high glucose induced increase of lipogenic genes including sterol regulatory element binding protein-1 (SREBP-1), which is a transcription factor regulating the synthesis of fatty acid and triglycerides, and its target gene an acetyl-CoA carboxylase (ACC) . We also found that GW4064 attenuates podocyte lipid accumulation.

Conclusions: FXR or TGR5 selective agonists may attenuate glucose induced podocyte glucolipotoxicity by inhibiting podocyte lipid droplet accumulation and transdifferentiation into adipocyte-like cells.

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SA-PO2426

MCP-1/CCR2 System Is Involved in Epithelial-Mesenchymal Transition of Peritoneal Mesothelial Cells

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Background: MCP-1 and its receptor, CCR2, have been known to be directly involved in extracellular matrix synthesis. In addition, the MCP-1/CCR2 system was present in peritoneal mesothelial cells(PMCs), and MCP-1 production was increased in PMCs under high glucose(HG) conditions. Therefore, we presumed that the MCP-1/CCR2 system may play an important role in the pathogenesis of peritoneal fibrosis(PF) and conducted this study to investigate the functional role of the MCP-1/CCR2 system in epithelial-mesenchymal transition(EMT) of PMCs and PF.

Methods: In vitro, human PMCs(HPMCs) were incubated in M199 media containing 5.6 mM glucose(NG), NG+MCP-1, or 100 mM glucose(HG) with or without mutant MCP-1(mMCP-1) for 72 hours. In vivo, peritoneal catheter was inserted into Sprague-Dawley rats, and saline(control, C) or 4.25% PD solution(PD) was infused. In the PD group, rats were treated either with empty lentivirus vector or lentivirus vector containing mMCP-1 intraperitoneally, once a week. After 4 weeks, peritoneum was removed. Western blot analysis was performed to evaluate fibronectin(FN), E-cadherin, and α-smooth muscle actin(α-SMA) protein expression. PF was determined by Masson's trichrome(MT) staining.

Results: FN and α-SMA protein expression were significantly increased, and E-cadherin protein expression was significantly decreased in HPMCs exposed to NG+MCP-1 and HG compared to NG cells($p<0.05$). These changes in FN, α-SMA, and E-cadherin expression in NG+MCP-1 and HG cells were significantly abrogated by mMCP-1($p<0.05$). In rats infused with PD solution, the expression of FN protein and the ratios of α-SMA/E-cadherin protein expression of peritoneum were significantly higher compared to C rats($p<0.05$). In addition, the thickness of PMC layer and the intensity of MT staining in the peritoneum of PD rats were significantly increased compared to C rats($p<0.05$). These changes in PD rats were significantly ameliorated by the treatment with lentivirus containing mMCP-1.

Conclusions: These findings suggest that the MCP-1/CCR2 system is involved in peritoneal EMT and its inhibition may be a potential therapeutic target for PF.

SA-PO2427

Adenosine 1 Receptor-Mediated Effects on Energy Balance and Body Weight Lingli Li,[1] Robert Faulhaber-Walter,[2] Christoph Eisner,[3] Yuning George Huang,[1] Jurgen B. Schnermann.[1] *[1]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD; [2]üGMP, Standort Rinteln, Germany; [3]Department of Anesthesiology, UMM, Mannheim, Germany.*

Background: Adenosine 1 receptors (A1AR) presumably by activation of the tubuloglomerular feedback pathway exert an important role in limiting glomerular hyperfiltration in the Akita mouse model of type I diabetes mellitus. In addition, A1AR-/- mice develop insulin resistance suggesting that A1AR mediate direct anti-diabetogenic effects.

Methods: Body weight, food intake, urinary osmolarity and Cl- concentration were measured by standard methods. The body fat fraction was determined by Echo MRI 3-in-1™ . Plasma adenosine was measured by HPLC. Serum leptin was measured by ELISA (Tatyana Chanturiya, NIDDK, NIH, Bethesda, MD).

Results: A1AR-/- mice have a 13% greater body weight than age matched wild type (WT) mice. Body fat fraction was 15±4% in A1AR-/-, significantly higher than 10.8±2% in WT. Food intake measured over 10 days (g/mouse/day) averaged 3.85 ± 0.1 in WT (n=10) and 4.12 ± 0.06 in A1AR-/- (n=12; $p<0.05$). Water intake was also significantly higher in A1AR-/- than WT (+12.5%) accompanied by a decrease in urinary osmolarity (-29.5%) and Cl- concentration (-30.8%). Despite the higher fat content serum leptin levels (ng/ml) were significantly lower in A1AR-/- than WT mice (2.14±0.05 vs. 3.75±0.36; $p<0.01$). Mice with a deletion of the equilibrative nucleoside transporter (ENT1-/-) have an elevated plasma adenosine concentration (1173±78 vs. 178±24 nM; $p<0.01$). Body weight (g) of 14 wk old ENT1-/- averaged 24.4±0.37 (n=5), significantly less than that of age-matched WT mice (28.5±0.76; n=5; $p<0.05$), and this was associated with a decreased food intake (g/day) in ENT1-/- mice compared to WT (3.6±0.1 vs. 4.14±0.19; $p<0.05$).

Conclusions: We conclude that adenosine through tonic activation of A1AR in fat tissue stimulates the release of leptin and that this suppresses food intake and prevents the development of insulin resistance. A1AR-mediated effects of adenosine contribute to the maintenance of a normal energy balance and to the prevention of obesity.

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SA-PO2428

Prostaglandin E2 (PGE2), a Mediator of Proliferation and Cl- Secretion in Polycystin-1 Deficient Cells Yu Liu,[1] Madhumitha Rajagopal,[3,4] Daniel Armando Flores,[1] Lorenzo Battini,[1] G. Luca Gusella,[1] Alan C. Pao,[3,4] Rajeev Rohatgi.[1,2] *[1]Medicine, Mount Sinai School of Medicine, New York, NY; [2]Medicine, James J. Peters VAMC, Bronx, NY; [3]Medicine, Stanford University, Palo Alto, CA; [4]Medicine, VA Palo Alto Health Care System, Palo Alto, CA.*

Background: PGE2 and calcium activated Cl- channels (CaCCs), specifically bestrophin-1, are implicated in proliferation of epithelial cells. Thus, we hypothesized that PGE2 mediated signaling may activate dysregulated CaCCs which contribute to the proliferative and secretory phenotype of ADPKD epithelia.

Methods: Utilizing wildtype IMDC3, control transfected (siLuc) IMCD3, and IMCD3 cells transfected with siRNA against PKD1 (siPKD1), we measured PGE2 mediated proliferation (cyquant[Invitrogen]), PGE2 induced Cl- secretion, and mRNA abundance of CaCCs.

Results: Proliferation was presented as a ratio of the fluorescence at each time point (18-96 hrs) to the fluorescence measured at 18 hrs. siPKD1 cells proliferated faster than wildtype and siLuc cells (n=6 wells for each group, $p<0.05$) at 72 and 96 hrs after plating. Next, cyclooxygenase(COX)-1 (SC560), COX-2 (CAY10404) and COX-1/2 (indomethacin) inhibitors were incubated with siPKD1 cells, and proliferation compared to untreated siPKD1(n=6 for each group) cells. COX inhibition reduced proliferation at all time points compared to untreated cells. On the other hand, incubation with 50 nM PGE2 enhanced siPKD1 cellular proliferation (n=6, $p<0.05$), but reduced wildtype cellular proliferation(n=6, $p<0.05$), suggesting that PGE2 has dichotomous effects. Moreover, PGE2 induced Cl- secretion was 5 fold greater in siPKD1 ($p<0.05$) than in wildtype cells. 45% of the Cl- current was inhibited by a CFTR inhibitor ($P<0.05$) and ~30-40% was inhibited by FFA ($P<0.05$), a non-selective CaCC inhibitor. Quantitative RT-PCR showed ~3 and ~6 fold increase in TMEM16a and bestrophin-1 (CaCCs), respectively, compared to control cells.

Conclusions: PGE2 induces proliferation and CFTR-, CaCC-dependent Cl- secretion in siPKD1 cells. We speculate that the over-expression of CaCCs, contribute to PGE2 mediated Cl- secretion and proliferation seen in siPKD1 cells.

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SA-PO2429

Rosuvastatin Reduces Pressure-Induced Fibrotic Signals through PGI_2-PPARγ Pathway in Renal Tubular Cells Tso Hsiao Chen, Cheng-Hsien Chen. *Department of Nephrology, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan.*

Background: Statins have been reported to alleviate renal fibrosis in the animal model with unilateral ureteral obstruction. However, the molecular mechanism of this anti-fibrotic effect is still a mist. Pressure force is an important mechanisms contributing to the induction and progression of tubulo-interstitial fibrogenesis in ureteric obstruction.

Methods: We established an *in vitro* pressure culture system to study the influence of statins on renal tubular cell fibrosis. Fibrosis-associated molecules, such as COX-2, connective tissue growth factor (CTGF), fibronectin, were monitored by Western blotting. TGF-b, PGE2 and PGI2 generated from pressure-treated cells were identified using ELISA kits. We also used PGIS siRNA transfection to block the expression of PGI2 in rat renal tubular cell NRK-52E.

Results: When NRK-52E cells were cultured in the pressure culture system, 60-mmHg pressure induced the expression of connective tissue growth factor (CTGF), transforming growth factor beta (TGF-b), fibronectin and phospho-Smad3. These pressure-induced fibrotic signals were reduced by rosuvastatin in a dose-dependent manner. Rosuvastatin also reduced the TGF-b-induced expression of fibronectin and CTGF in NRK-52E cells. The pretreatment of rosuvastatin significantly induced prostacyclin (PGI_2) generation, but reduced pressure-induced prostaglandin E2 (PGE_2). The trasfection of PGI_2 synthase siRNA decreased the inhibitory effect of rosuvastatin on pressure-induced CTGF, TGF-β and fibronectin. On the other hand, the specific inhibitor for cyclooxygenase-2, NS398, diminished pressure-induced PGE_2 generation, and partially reduced pressure-induced fibrotic signals. Additional PGE_2 decreased the anti-fibrotic effect of rosuvastatin. When we monitored the influence of the nuclear receptors for PGI_2 on the anti-fibrotic effect of rosuvastatin using siRNA transfection, the blockage of PPARγ reduced the inhibitory effect of rosuvastatin on pressure-induced fibrotic signals.

Conclusions: We conclude that rosuvastatin prevents renal tubular cells from pressure-induced fibrosis by enhancing PGI_2-PPARγ pathway and reducing PGE_2 generation.

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SA-PO2430

Effects of Wnt-7a on Aristolchic Acid Induced Renal Tubular Epithelial-Mesenchymal Transition Guo-Qin Wang, Hong-Liang Rui, Yan-Yan Wang, Hong Cheng, Yi-Pu Chen. *Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China.*

Background: The Wnt family controls both ureteric bud development and signaling, but it also serves as inductive factor to regulate nephrogenesis in the mesenchymal. It was reported that Wnt protein is expressed in the nephron precursor cells, bing important for their development to nephrons. This study was to observe whether Wnt7 reverses epithelial-to-mesenchymal transition (EMT) and induces mesenchymal-epihtelial transition (MET) on aristolchic acid-induced renal tubular epithelial cell.

Methods: Human proximal renal tubular epithelial cell line HK-2 cells were cultured in different conditions: (1) serum free as control, (2) cultured with AA (30mg/l) for 24h, (3) treated with Wnt7a (20ng/ml) for 48h after stimulated with AA (30mg/l), (4) treated with Wnt7a(20ng/ml) plus LiCl (Wnt's blocker, 1ug/ml) for 48h after stimulated with AA(30mg/l). Expression of cytokeratin, E-cadherin, a-smooth muscle actin (a-SMA) and vimentin was assessed by immunofluorescence and western-blot.

Results: After incubation with AA, Levels of a-SMA (698.1±20.7 vs 66.5±9.8, $P<0.05$) and vimentin (496.3±19.7 vs 187.3±20.5, $P<0.05$) expressions increased significantly, but levels of cytokeratin (211.3±16.1 vs 624.7±19.4, $P<0.05$) and E-cadherin (168.7±11.3 vs 426.5±21.9, $P<0.05$) expressions decreased in comparison to control group. Treatment of HK2 cells stimulated by AA with Wnt7 resulted in down-regulation of a-SMA (151.5±14.3 vs 698.1±20.7, $P<0.05$) and vimentin (214.5±20.2 vs 496.3±19.7, $P<0.05$) expression, but up-regulation of cytokeratin (516.8±14.2 vs 211.3±16.1, $P<0.05$) and E-cadherin (310.7±10.5 vs 168.7±11.3, $P<0.05$) expressions. Compared to HK2 cells treatment with Wnt7a, the expression of a-SMA (411.2±18.5 vs 151.5±14.3, $P<0.05$) and vimentin (472.7±23.1 vs 214.5±20.2, $P<0.05$) enhanced while cytokeration (239.6±13.2 vs 516.8±14.2, $P<0.05$) and E-cadherin (199.7±17.1 vs 310.7±10.5, $P<0.05$) expression reduced markedly on HK2 cells treatment with Wnt7a and Licl.

Conclusions: Wnt7a is able to reverse EMT induced by AA, which represents a potential therapeutic approach to aristolchic acid nephropathy prevention.

Funding: Government Support - Non-U.S.

SA-PO2431

Reciprocal Expression of microRNA-214 (miR-214) and Insulin-Like Growth Factor-1 Receptor (IGF-1R) Dictates Renal Carcinoma Cell (RCC) Proliferation Via Akt/TORC1 Circuit Nirmalya Dey,[1] Falguni Das,[1] Nandini Ghosh-Choudhury,[2] B. S. Kasinath,[1] Hanna E. Abboud,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA, San Antonio, TX.*

Background: IGF-1R signal transduction significantly contributes to development of the renal cancer irrespective of the VHL status of the tumors. We found increased levels of IGF-1R in the VHL-deficient 786-O and in VHL positive ACHN RCCs as compared to normal human proximal tubular epithelial cells (HK2). Rate of proliferation of the RCCs and mitogenic effect of IGF-1 were significantly elevated. The mechanism by which the expression of IGF-1R in these RCCs is increased is not known. We considered a miRNA-mediated posttranscriptional mechanism. We detected decreased expression of mature, pre- and pri-miR-214 in the ACHN and 786-O RCCs compared to HK2 cells. Molecular cloning of the human promoter for miR-214 and reporter assay showed a significant transcriptional silencing of miR-214 in the RCCs compared to HK2 cells. We identified miR-214 recognition elements in the 3'UTR of IGF-1R mRNA and established their responsiveness to miR-214. miR-214 inhibited expression of IGF-1R, resulting in attenuation of IGF-1-stimulated Akt phosphorylation in RCCs. Furthermore, IGF-1 increased PRAS40 phosphorylation at Thr-246, which was necessary for RCC proliferation. Moreover, the inactivating phosphorylation of PRAS40 by IGF-1R stimulation enhanced TORC1 activity, resulting in S6 kinase and 4EBP-1 phosphorylation. Phosphorylation-deficient mutant of 4EBP-1 inhibited IGF-1R-provoked proliferation of RCC. Expression of miR-214 significantly inhibited IGF-1R-forced phosphorylation of PRAS40, S6 kinase and 4EBP-1. Finally, miR-214 prevented proliferation of RCCs in response to IGF-1 but

without causing apoptosis of the cells. Together our results identify a reciprocity between miR-214 and IGF-1R levels in RCCs independent of the VHL status of the cells. Our data provide evidence for a previously unrecognized mechanism of RCC proliferation involving miR-214 and TORC1.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

SA-PO2432

Target Genes of Endogenous Retinoic Acid and Retinoic Acid Receptors in Collecting Duct Cells: A Pan-Genomic View Qihe Xu,[1] Yuen Fei Wong,[1] Patricia D. Wilson,[2] Robert J. Unwin,[2] Jill T. Norman,[2] Bruce M. Hendry.[1] *[1]Department of Renal Medicine, King's College London, London, United Kingdom; [2]Centre for Nephrology, University College London, London, United Kingdom.*

Background: Gestational vitamin A deficiency (VAD) impairs renal development and post-natal VAD causes polyuria, urolithiasis, renal inflammation and fibrosis. Vitamin A is converted into retinoic acid (RA) to activate RA receptors (RAR). We previously showed that endogenous RA/RAR signaling was confined to the ureteric bud (UB)/collecting duct (CD) cell lineage in mouse kidneys, including the mIMCD-3 mouse inner medullary cell line. We hypothesized that endogenous RA/RAR interactions in CD cells regulate genes that may mediate VAD-associated renal anomalies.

Methods: Affymetrix GeneChip Mouse Gene 1.0 ST Arrays and RT-qPCR were used to shortlist and validate RA/RAR target genes in mIMCD-3 cells treated for 24h with 2 distinct RA/RAR inhibitors, 1 μM AGN193109 (RAR pan-antagonist) or 25 μM DEAB (RA synthesis inhibitor), ± 0.01-0.2 μM RA (n=3). Target genes of endogenous RA/RAR were defined as those: (i) significantly regulated by both inhibitors and reversed by RA ($P \leq 0.05$); (ii) changed ≥2-fold by either inhibitor.

Results: Microarray analysis showed that inhibitors induced 22 and suppressed 105 genes. While changes of all induced genes were <2-fold and not further investigated, RT-qPCR validated 18 of 19 genes suppressed ≥2-fold as target genes of endogenous RA/RAR. Dhrs3, which encodes an enzyme converting RA precursors to vitamin A, was most highly suppressed (25-100-fold) by inhibitors. Ten other validated genes have potential roles in kidney injury/repair (Tns1, Lcn2, Itga2, Muc20, Galns), renal/epithelial development (Sprr1a, Ppbp, Muc20) and water/solute homeostasis (Npr3, Clca4, Slc37a1). Seven remaining validated genes have unknown functions in the kidney (9930023K05Rik, Cpm, Klhdc7a, Sorcs2, 2310007B03Rik, Upk3b, Ebf1).

Conclusions: Endogenous RA/RAR in CD cells regulate a broad profile of genes involved in renal development, repair and homoestasis. Further investigation should provide valuable insights into the complex role of RA/RAR in normal and diseased kidneys, including the pathogenesis of VAD-associated anomalies.

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SA-PO2433

ATP Secretion by Rat Loops of Henle *In Vivo* Sara Damiano,[1] Robert J. Unwin,[2] David G. Shirley.[2] *[1]Chair of Nephrology, Department of Internal Medicine, Seconda Universita degli Studi di Napoli, Napoli, Italy; [2]Centre for Nephrology, University College London, London, United Kingdom.*

Background: Recent evidence from *in vitro* studies indicates that the cells of the thick ascending limb of the loop of Henle can secrete nucleotides into the lumen and that this secretion is stimulated by increases in intraluminal pressure (Praetorius & Leipziger 2010, Ann Rev Physiol 72: 377). Further *in vitro* studies suggest that ATP inhibits sodium reabsorption in the thick ascending limb (Garvin et al 2011, Ann Rev Physiol 73: 359). The purpose of the present investigation was to assess, *in vivo*, whether significant amounts of ATP are secreted by the loop of Henle and whether this is affected by flow rate.

Methods: Rats were anaesthetised and prepared surgically for micropuncture studies. Loops of Henle of superficial nephrons were functionally isolated by the introduction of intraluminal oil columns and perfused from late proximal convoluted tubules for 5-7 min using artificial proximal tubular fluid containing no ATP; the fluid emerging from the loop was collected at the early distal tubule. Each loop was perfused twice: with and without the ATPase inhibitor ARL 67156, or at 20 *vs* 40 nl/min. The collectates from each category of perfusion were pooled (n = 8-13 loops) and the ATP concentration of the pooled sample was measured using the luciferin-luciferase reaction.

Results: When loops were perfused at 20 nl/min, in the absence or presence of intraluminal ARL 67156 (100 μM), the ATP concentrations in the collected fluid were 162 ± 22 nmol/l and 275 ± 41 nmol/l, respectively (n = 5 pairs of pooled collections, P<0.05, paired t). When loops were perfused at 20 nl/min or 40 nl/min (in the absence of ARL 67156), the ATP concentrations in the collected fluid were 177 ± 42 nmol/l and 334 ± 92 nmol/l, respectively (n = 5 pairs of pooled collections, P<0.05, paired t); in the latter experiments, the rates of ATP collection were 2.1 ± 0.5 fmol/min and 5.8 ± 1.4 fmol/min, respectively (P<0.01).

Conclusions: These data indicate that physiologically significant amounts of ATP are secreted by loops of Henle in *vivo*. Furthermore, the amount secreted is profoundly affected by changes in tubular flow rate.

Funding: Pharmaceutical Company Support

SA-PO2434

Nephron Segment-Specific Gene Deletion Using a Cre-Adenovirus Pablo D. Cabral, Marcela Herrera, Pablo A. Ortiz, Jeffrey L. Garvin. *Hypertension and Vascular Research, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.*

Background: Most pharmacological tools used to study renal physiology have limited specificity. This has led to the development of methods to manipulate gene expression to study protein function. However, current methods to delete genes of interest are limited. Non-selective knockout from birth may result in compensation by other genes and eliminate the ability to study the function of a specific protein in a specific tissue. Development of transgenic mice using a Cre/loxP strategy requires two strains, being expensive and time-consuming. We have previously shown that injection of adenoviral vectors in the renal outer medulla resulted in efficient and specific transduction of thick ascending limbs. The development of the Cre/loxP system selectively allows gene disruption. We hypothesized that in vivo injection of adenoviruses expressing Cre recombinase under the control of the promoter for the NaK2Cl cotransporter (NKCC2) in the renal outer medulla results in selective transduction of medullary thick ascending limbs.

Methods: A double fluorescent Cre reporter mouse strain that expresses td tomato, a red fluorescent protein, before Cre-mediated excision and green fluorescent protein (GFP) after excision was used. Adenoviruses containing Cre recombinase driven by the NKCC2 promoter (Ad-NKCC2Cre), which is specific for thick ascending limbs, were injected in the renal outer medulla.

Results: Western blots showed GFP expression in outer medullary lysates 7 days after Ad-NKCC2Cre injections. Immunofluorescence of kidney sections transduced with Ad-NKCC2Cre showed expression of GFP in outer medullary thick ascending limbs, identified by positive Tamm-Horsfall labeling. Neither renal cortex nor inner medulla were positive for GFP. Immunofluorescence microscopy showed that 82 ± 5 % and 82 ± 1 % of the outer medullary thick ascending limbs expressed GFP at 7 and 14 days respectively.

Conclusions: We concluded that combining Cre/loxP technology and efficient adenovirus-mediated transduction of thick ascending limbs could be a useful tool to study gene function in adult mice preventing compensation.

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SA-PO2435

Relaxin Signals through the Nitric Oxide Pathway To Promote Matrix Metalloproteinase Activity in the Kidney Bryna Chow,[1] Timothy Hewitson,[2] Chrishan S. Samuel.[1] *[1]Neuropeptides/Relaxin Division, Howard Florey Institute, Parkville, Victoria, Australia; [2]Nephrology, Royal Melbourne Hospital, Parkville, Victoria, Australia.*

Background: The inevitable consequence of end-stage kidney disease is the excessive accumulation of extracellular matrix, primarily collagen which drives the pathological setting of renal fibrosis. Pre-clinical and signal transduction studies have shown that the anti-fibrotic hormone relaxin can inhibit TGF-β1 activity by binding to Relaxin Family Peptide Receptor 1 (RXFP1) and activating a neuronal nitric oxide (NO) synthase (nNOS)-NO-cyclic guanosine monophosphate (cGMP)-dependent pathway to abrogate Smad2 phosphorylation; thereby inhibiting TGF-β1-induced myofibroblast differentiation and collagen synthesis. Thus, this study sought to determine if relaxin's well-documented additional ability to induce collagen degradation by matrix metalloproteinases (MMPs) was by a similar mechanism.

Methods: Primary myofibroblasts (propagated from rat kidneys 3 days after unilateral ureteric obstruction) were treated with relaxin (100ng/ml) in the absence or presence of the general NO inhibitor L-NAME (75 μM), the nNOS inhibitor NPLA (2 μM), the inducible NOS (iNOS) inhibitor 1400W (0.5 μM) or cGMP inhibitor ODQ (5 μM) over a 72 hour culture period. Media samples were collected and assessed for changes in MMP-2 and MMP-9 activity (by gelatin zymography), while cell layer protein was assessed for changes in MMP-13 levels (by Western blotting).

Results: Relaxin significantly up-regulated MMP-2, MMP-9 and MMP-13 expression and activity when administered over a 72 hour culture period to renal myofibroblasts by 128%, 115% and 91%, respectively (all p<0.01 vs untreated cells). This relaxin-induced up-regulation of MMPs was significantly blocked by L-NAME, NPLA, 1400W and cGMP (by ~50–90%) (all p<0.05 vs relaxin alone-treated levels), while neither of these inhibitors affected basal MMP levels.

Conclusions: These findings suggest that relaxin signals through a RXFP1-nNOS/iNOS-NO-cGMP pathway to promote renal MMP activity; and that the NO-cGMP pathway is central to relaxin's anti-fibrotic actions.

Funding: Government Support - Non-U.S.

SA-PO2436

Attenuation of Systolic Hypertension and Angiotensinogen Gene Expression in Diabetic Akita Transgenic Mice Overexpressing Heterogenous Nuclear Ribonucleoprotein F in the Kidney Chao-Sheng Lo,[1] Shiao-Ying Chang,[1] Yixuan Shi,[1] Isabelle Chenier,[1] Shao-Ling Zhang,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hospital, Montreal, QC, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hospital, Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp, Boston, MA.*

Background: Heterogeneous nuclear ribonucleoprotein F (hnRNP F) binds to the insulin-responsive element in the rat angiotensinogen (Agt) gene promoter and inhibits Agt gene transcription *in vitro*. We now investigate whether overexpression of hnRNP F

can modulate renal Agt gene expression and subsequently attenuate systolic hypertension (sHTN) in Akita mice with type 1 diabetes.

Methods: Akita transgenic (Tg) mice specifically overexpressing hnRNP F solely in their renal proximal tubular cells (RPTCs) were created by cross-breeding of Akita mice with hnRNP F-Tg mice that specifically overexpress hnRNP F in their RPTCs by employing kidney specific androgen-regulated protein promoter. Non-Akita littermates served as controls. Blood glucose, systolic blood pressure (SBP) and albuminuria were monitored weekly from 10 to 20 weeks of age. Kidneys were processed for histology studies. Renal proximal tubular Agt mRNA and protein expression were quantified by respective real time-qPCR and Western blotting. Urinary angiotensin II (Ang II) levels were quantified by ELISA.

Results: Our results demonstrate that Akita mice develop sHTN (~136 ± 3.8 mm Hg) and display renal hypertrophy and hydronephrosis as compared to non-Akita controls (~108 ± 0.7 mm Hg). HnRNP F overexpression markedly decreased sHTN, kidney/body weight ratio and renal hypertrophy without affecting blood glucose level and the urinary albumin/creatinine ratio in Akita hnRNP-F Tg mice as compared to Akita mice. Furthermore, Agt mRNA and protein expression as well as urinary Ang II levels are significantly increased in Akita mice but normalized in Akita hnRNP F-Tg mice.

Conclusions: Our data suggest that hnRNP F plays a protective role by attenuating sHTN and preventing RPTC injury in diabetes, and indicate that these actions are mediated, at least in part, through attenuating intrarenal Agt gene expression and RAS activation *in vivo*.

Funding: Government Support - Non-U.S.

SA-PO2437

Stimulatory Effect of Insulin on Renal Proximal Na Transport Is Preserved in Obesity-Induced Insulin Resistant Rats Motonobu Nakamura, Osamu Yamazaki, Hideomi Yamada, Masashi Suzuki, Shoko Horita, George Seki, Toshiro Fujita. *Internal Medicine, Tokyo University, Tokyo, Japan.*

Background: The role of hyperinsulinemia in the pathogenesis of obesity-induced hypertension remains to be established. We have shown that the stimulatory effect of insulin on renal proximal tubule transport is preserved in the insulin resistant IRS2 KO mice (JASN 16:2288,2005).

Methods: In the present study, we tried to examine whether the renal tubular actions of insulin are preserved in the more generalized forms of insulin resistance. We determined the activity of Na-HCO_3 cotransporter NBCe1 in isolated renal proximal tubules by cell pH measurements with a fluorescence dye BCECF. Glucose uptake into adipocytes obtained from periepididymal fat tissues was also measured to estimate the degree of insulin resistance. Akt phosphorylation was analyzed by Western blotting.

Results: We first confirmed that a PI3-kinase inhibitor wortmannin completely inhibited the stimulatory effect of insulin on NBCe1 activity in Wistar rats. We next compared the effects of insulin in the hyperphagic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and the control LETO rats. By 22 weeks of age, OLETF rats developed obesity (Body weight: 534 ± 13 vs 426 ± 9g), hyperinsulinemia (3.5 ± 0.2 vs 1.7 ± 0.3 ng/ml), and insulin resistance as evidenced by the increased homeostasis model assessment of insulin resistance (28 ± 1 vs 10 ± 2). The stimulation of glucose uptake into adipocytes by 10^{-8} M insulin was severely reduced in OLETF rats (57 ± 27%) compared to that in LETO rats (269 ± 26%). By sharp contrast, the stimulation of NBCe1 activity by 10^{-8} M insulin was comparable in OLETF (70 ± 11%) and LETO rats (74 ± 15%). In OLETF rats, the insulin-induced Akt phosphorylation was preserved in renal cortex tissues, but severely blunted in adipocytes.

Conclusions: These results indicate that the stimulatory effect of insulin on NBCe1 activity in proximal tubules was dependent on PI3-kinase/Akt pathway, and that this stimulation was completely preserved in obesity-induced insulin resistance. Hyperinsulinemia associated with obesity may contribute to the occurrence of hypertension by facilitating renal Na absorption.

Funding: Government Support - Non-U.S.

SA-PO2438

TIF2/SRC2 Expression in Blood Leukocytes Is a Determinant of Steroid Response in Pediatric Idiopathic Nephrotic Syndrome: A Midwest Pediatric Nephrology Consortium Study Danica Petrovic-Djergovic,[1] Seetharamaiah Chittiprol,[1] Phylip Chen,[1] Tad Eichler,[1] Richard F. Ransom,[1] The MWPNC.[2] *[1]Nationwide Children's Hospital; [2]Midwest Pediatric Nephrology Consortium.*

Background: Resistance to glucocorticoid (GC) therapy in nephrotic syndrome (NS) is considered a poor prognostic sign, since the 8-10% of steroid-resistant NS (SR) account for ~15% of all end-stage renal diseases in children. Defects in the anti-inflammatory effect of GC, rather than its effects on metabolic or endocrine pathways, are thought to be the basis of GC insensitivity in NS. TIF-2 (GRIP1/SRC2) is a member of the mammalian p160 nuclear cofactor family with a critical role in the anti-inflammatory effects of GC. We hypothesized that TIF2 (encoded by NCOA2) is essential for the therapeutic action of GC in NS.

Methods: Samples were obtained from steroid-sensitive (SS) and SR pediatric patients at both presentation (PS) and after the first 6-8 week course of GC therapy (TS). Relative mRNA expression was measured in peripheral blood RNA by qRT-PCR, and flow cytometry (geometric means) was used to measure relative protein expression.

Results: The expression of NCOA2 but not the two other p160-family members (NCOA1,3) increased after GC therapy in SS ($P<0.01$) but not SR patients. NCOA2 expression correlated, as expected, with DUSP1 expression ($P<0.0001$), since TIF2 is known to be essential for GC induction of DUSP1 mRNA. Flow cytometry confirmed that more TIF2 protein was present in the CD19+ leukocyte sub-population ($P<0.05$) in TS samples from SS vs. SR patients. There was also an increase in the amount of TIF2 (ratio of TS:PS) in CD4+ leukocytes from SS patients but not from SR patients ($P<0.05$). Finally, we found that GC treatment of cultured PS leukocytes from SS but not SR patients resulted in significant increases in TIF2 protein in CD4+ ($P<0.05$), CD8+ ($P<0.01$), and CD19+ ($P<0.05$) but not CD16+ sub-populations.

Conclusions: Together these results suggest that GC induction of the TIF2 nuclear receptor cofactor is essential for proper steroid responsiveness in NS, and that GC-induced TIF2 expression in leukocytes is a biomarker of patient steroid response in NS.

Funding: NIDDK Support

SA-PO2439

Temporal Effects of Calcimimetics on Parathyroid Gland Gene Expression in Rats with Early Secondary Hyperparathyroidism Victoria Shalhoub,[1] Edward Shatzen,[1] Sabrina Ward,[1] Michael J. Boedigheimer,[1] Mara Campbell,[1] Michael A. Damore,[1] Zheng Pan,[2] James R. Davis,[1] Charles M. Henley III,[1] William G. Richards.[1] *[1]Amgen, Inc, Thousand Oaks, CA; [2]Amgen, Inc, San Francisco, CA.*

Background: Secondary hyperparathyroidism (SHPT) is a complication of chronic kidney disease (CKD). Calcimimetics (CM), modulators of the calcium-sensing receptor (CaSR), lower serum PTH and calcium (Ca) in CKD patients and rodent models. Although much is known about CaSR signaling pathways, the precise events are not well defined. Here, changes in gene expression were examined after CM treatment, in thyroparathyroid glands (TPG) of a rat model of CKD with SHPT.

Methods: Two-month-old 5/6 nephrectomized (Nx) SD rats, fed a standard diet (1.17%Ca/1.0%Pi), developed renal insufficiency and SHPT over 6 weeks. Rats (13/group) were then dosed p.o. daily with vehicle (veh) or the research CM R-641 (10 mg/kg). Blood and TPG were harvested at 4, 48, 72 and 96 hours.

Results: Six weeks post 5/6Nx, rats had higher serum PTH (1153±124 vs 435±30pg/ml, mean±SEM), BUN (50±3 vs 18±0mg/dL) and creatinine (0.65±0.02 vs 0.24±0mg/dL) than sham-veh rats. Gene profiles and the number of dividing cells (Ki67+) in PG, were no different comparing sham and 5/6Nx rats, reflecting the slow progression of PG cell hypertrophy/hyperplasia in rats fed a standard diet. Calcimimetic treated 5/6Nx rats had lower serum PTH levels than veh-treated 5/6Nx rats (52±8 vs 1153±124pg/ml). Gene expression changes occurred in pathways that included CaSR, proliferation, apoptosis and lipid (fold change <-1.5 or >1.5; $P<0.001$). Two major categories of gene expression changes were observed: Those that occurred early (by 4 hours) included cyclin dependent kinases and inhibitors, G proteins, FOS, TNF and other apoptosis family members; and, those that occurred after 48-hours of treatment, which included genes involved in G protein, prostaglandin, arachidonate, lipoxygenase and cytoskeletal pathways.

Conclusions: This study extends knowledge of molecular events elicited in TPG within hours to one week after a single CM dose in an early rat CKD model. Confirmation of these findings in PGs may lead to novel therapeutic targets for SHPT.

Funding: Pharmaceutical Company Support

SA-PO2440

Parathyroid Hormone Related Protein Dissociates Increased cAMP from Renin Secretion in Mouse Juxtaglomerular Cells Douglas K. Atchison,[1,2] Pamela Harding,[2] William H. Beierwaltes.[1,2] *[1]Department of Physiology, Wayne State University School of Medicine, Detroit, MI; [2]Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Health System, Detroit, MI.*

Background: cAMP is the stimulatory second messenger for renin secretion. Primary signals that stimulate renin do so by increasing the production of cAMP via the calcium-inhibitable adenylyl cyclase-V (AC-V) in the juxtaglomerular (JG) cell. Parathyroid hormone related protein (PTHrP) stimulates renin secretion in the isolated perfused kidney, but it is unknown if this is due to a direct effect on the JG cell. We hypothesized that JG cells would express the receptor for PTHrP (PTH1R), and that PTHrP would increase cAMP and renin secretion.

Methods: Experiments used primary cultures of isolated mouse JG cells.

Results: RT-PCR showed that JG cells express PTH1R. 10 nM and 1 μM PTHrP increased JG cell cAMP levels from 1.60±0.27 to 7.14±0.87 and 23.0±11.5 pmol/mg protein, respectively ($p<0.05$). However, neither 10 nM nor 1 μM PTHrP increased renin release from a basal level of 301.0±56.0 ng Ang I/ml/hr mg protein (10 nM PTHrP: 314.3±62.9; 1 μM PTHrP: 342.5±65.1 ng Ang I/ml/hr/mg protein, respectively). Since renin secretion is enhanced by low extracellular Ca, we repeated the experiments in either low or high calcium media with 1 μM PTHrP. PTHrP increased cAMP in the low-calcium medium from 4.76±2.17 to 20.51±11.93 pmol/mg protein ($p<0.05$) and in the high-calcium medium from 5.14±2.57 to 14.85±8.33 pmol/mg protein ($p<0.05$). However, basal renin release did not increase in either low- or high-calcium media with the addition of PTHrP (low-calcium basal: 432.0±127.5; low-calcium PTHrP: 497.2±165.4; high-calcium basal: 214.5±81.3; high-calcium PTHrP: 209.4±53.6 ng Ang I/ml/hr/mg protein).

Conclusions: Our data demonstrate that PTHrP increases cAMP by acting on PTH1R without stimulating renin release in primary cultures of mouse juxtaglomerular cells. The implication of this is that PTHrP dissociates cAMP formation from renin secretion in the JG cell by stimulating an adenylyl cyclase besides AC-V, or that adenylyl cyclase signaling in the JG cell may be compartmentalized.

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SA-PO2441

Relationships between Physical Activity and Nutrition with Quality of Life in CKD Patients Robert G. Fassett,[1,3] Iain Robertson,[2] Madeline J. Ball,[2] Dominic P. Geraghty,[2] Jeff S. Coombes.[3] *[1]Renal Medicine, University of Queensland, Brisbane, Queensland, Australia; [2]Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; [3]Human Movement Studies, University of Queensland, Brisbane, Queensland, Australia.*

Background: The Lipid lowering and Onset of Renal Disease (LORD) trial was a three-year randomised, double-blind, placebo-controlled trial investigating the effects of atorvastatin on kidney function in CKD patients. The study design included measures of physical activity and nutrition every nine months. The aim of this sub-study was to investigate the relationships between physical activity and nutrition with quality of life (QoL).

Methods: 132 patients with serum creatinine levels >120µmol/l, not taking lipid-lowering therapy and at all levels of proteinuria and serum cholesterol were enrolled. For this sub-study data was available for 120 patients and they were followed for a mean of 2.9 years. Every nine months physical activity, nutrition and QoL were assessed using the Active Australia questionnaire, 4-day diet diaries analysed with Foodworks software and the SF-36 questionnaire respectively. The association (Odds Ratio) between a number of predictors and 10 SF-36 QoL measurement scales were estimated using repeated measures ordinal logistic regression. An OR >1.00 indicates a positive association, and an OR <1.00 indicates a negative association.

Results: Weekly physical activity (METmins) was strongly associated with all measures on the SF-36 including the mental component score (MCS), (OR 1.3, 95% CI 1.1-1.6, P=0.01) and the physical component score (PCS), (OR 1.6, 95% CI 1.1-2.2, P=0.008). Dietary phosphate intake was strongly positively associated with improved PCS (OR 1.6, 95% CI 1.1-2.2, P=0.02) and dietary zinc was associated in a milder but consistent manner with poorer physical QoL and a lower MCS (OR 0.7, 95% CI 0.4-1.0, P=0.048). Dietary calcium and iron were both associated with poorer physical quality of life and better mental QoL.

Conclusions: Greater levels of physical activity are strongly associated with improved QoL in CKD patients. Micronutrients such as dietary phosphate, zinc, calcium and iron are also related to QoL.

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-PO2442

High Levels of Adiponectin in End Stage Renal Disease May Be Due to Increased Production in Visceral Fat Maria P. Martinez Cantarin,[3] Cataldo Doria,[2] Adam Mathias Frank,[2] Warren R. Maley,[2] Carlo B. Ramirez,[2] Bonita E. Falkner.[1] *[1]Medicine, Thomas Jefferson University Hospital; [2]Surgery, Thomas Jefferson University Hospital; [3]Pharmacology, Thomas Jefferson University.*

Background: Plasma adiponectin is elevated in End Stage Renal Disease (ESRD) but the cause of abnormally increased adiponectin is unclear. Possible mechanisms include augmented production secondary to chronic inflammation, decreased clearance from the failed kidneys, or increased adiponectin resistance. The purpose of this study was to determine if there is increased adipose tissue production of adiponectin in ESRD. We compared plasma levels of adipokines and expression of adiponectin and adiponectin receptors in fat and muscle of ESRD patients and controls.

Methods: The study sample included 16 ESRD patients and 9 kidney donors (controls). Blood and tissue samples were obtained at the time of kidney transplantation and kidney donation. Adiponectin, hrCRP, IL-6, TNF-α and PAI-1 were measured in plasma samples by ELISA. Adiponectin protein and adiponectin receptor-1/2 mRNA expression analysis (by TaqMan Assays) was performed in subcutaneous, visceral fat and muscle.

Results: Mean plasma adiponectin in ESRD was 14 9.1 µg/ml and 85.4 µg/ml in controls (p=0.14). Plasma TNF-α (13 ± 5 pg/ml) and CRP (4.2 ±3.1 mg/L) values were significantly higher in ESRD compared to controls (8 ± 0.9 pg/ml; and 0.9 ± 0.9 mg/L; p<0.05). There were no differences in plasma levels of IL-6 and PAI-1 between the groups. Adiponectin protein expression in visceral fat was 1.5 fold higher in ESRD than controls (p<0.001). Adiponectin receptor 1 was 1.4 times higher in muscle and 1.2 times higher in visceral fat of ESRD compared to controls (p<0.05). There were no significant group differences in the expression of adiponectin protein or adiponecin receptor in subcutaneous fat.

Conclusions: In ESRD there is greater expression of adiponectin protein and adiponectin receptor 1 in visceral adipose tissue compared with controls. These results represent the first evidence of increased adiponectin expression in human adipose tissue in ESRD; and could explain at least one of the mechanisms by which this hormone is elevated in kidney disease.

SA-PO2443

Executive Functioning May Vary with GFR in Children with Mild to Moderate CKD Susan R. Mendley,[1] Stephen R. Hooper,[2] Matthew Matheson,[3] S. Shinnar,[4] Arlene C. Gerson,[3] Alison G. Abraham,[3] Marjolaine M. Limbos,[5] Robert W. Butler,[6] Marc Lande,[7] Susan L. Furth,[8] Bradley A. Warady.[9] *[1]U Maryland; [2]U North Carolina; [3]Johns Hopkins U; [4]Albert Einstein Coll of Med; [5]British Columbia Children's Hosp; [6]Ohio State U; [7]U Rochester; [8]U Pennsylvania; [9]Children's Mercy Hosp.*

Background: Neurocognitive dysfunction, with selective defects in executive function (EF), complicates advanced CKD and ESRD. In children, performance improves with renal transplantation. It is unknown whether EF is also impaired by moderate CKD; this would inform educational intervention during rapid neurocognitive development in childhood.

Methods: Tests of problem solving, inhibitory control and working memory were incorporated into the prospective, observational NIH-sponsored CKiD cohort study of children with mild to moderate CKD. Cross-sectional analysis of baseline test performance was by linear regression, stratified by estimated GFR: <30, 30 to<40, 40 to<50, ≥50 ml/min/1.73 m^2; blood pressure; renal diagnosis; and duration of CKD.

Results: 340 subjects, age 10-17 y (median 13) were tested. 26% had BP >90th %ile for age and height; 18% had glomerular disease; CKD duration was 6-13 y (median 10).

Performance on the Delis-Kaplan Tower Test, an EF task of planning and problem solving, was lowest in the group with GFR<30 and was progressively less affected in each higher GFR strata when compared to those >50. Mean values all fell within normal ranges and between-GFR differences did not reach significance in this cross-sectional comparison. By contrast, neither working memory (Wechsler Intelligence Scale Reverse Digit Span) nor inhibitory control (Errors of Commission subscale of the Connors Continuous Performance Test) varied with GFR at baseline. Likewise, neither high BP nor cause or duration of CKD affected performance of any test.

Conclusions: Cross-sectional analysis suggests tests of planning and problem solving may vary inversely with GFR in children with mild-moderate CKD. Large inter-individual variation in these measures of subtle cognitive impairment will require repeated observations and within-subject comparisons over time to discern an effect of progressive CKD.

Funding: NIDDK Support

SA-PO2444

Effects of Low-Protein Diet Supplemented with Ketoacid on Decreasing the Proteinuria in Primary Glomerular Diseases: An Additive Renoprotection Apart from Glucocorticoid and Immunosuppressant Hong Li Lin,[1] Wei Sun,[2] Hua Xie,[3] Yan Ling Sun.[4] *[1]Department of Nephrology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China; [2]Department of Nephrology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China; [3]Department of Nephrology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China; [4]Department of Nephrology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China.*

Background: We designed a prospective, randomized and controlled study to evaluate the effects of low-protein diet (LPD) supplemented with ketoacid on decreasing proteinuria and protecting renal function in primary glomerular disease patients.

Methods: 60 early-stage primary glomerular disease patients (eGFR≥60ml·min^{-1}·1.73m^{-2}) with predominant proteinuria(≥2g/24h)were randomly enrolled to LPD group(diet protein intake 0.6g·kg^{-1}·d^{-1} and ketoacid 1890mg·d^{-1})or normal-protien diet (NPD) group (diet protein intake 1.0g·kg^{-1}·d^{-1}) . All patients received the same treating protocols of glucocorticoid and immunosuppressant and were followed up for 12 months. Urinary excretion of urea nitrogen, proteinuria, creatinine, eGFR, serum prealbumin, albumin, anthropometric parameters, and blood pressure were measured or calculated monthly. The compliance and therapeutic effects were evaluated.

Results: 22 patients in LPD group and 19 in NPD group achieved the 12-month follow-up. Proteinuria decreased significantly in LPD group compared with NPD group from the 3rd to 12th month(p<0.05). Serum creatinine decreased and eGFR increased in LPD group(p<0.05). Serum albumin and prealbumin increased obviously in LPD group, and a significant difference was observed between two groups(p<0.05). After follow-up, there were 10 and 9 patients achieving complete remission and partial remission in LPD group, respectively, while 4 and 7 in NPD group.

Conclusions: LPD supplemented with ketoacid has beneficial effects on decreasing proteinuria, protecting renal function and maintains nutritional status in early-stage primary glomerular disease patients. The additive renoprotective effects exist apart from glucocorticoid and immunosuppressant.

SA-PO2445

A Cross-Sectional Study of Serum Potassium Abnormalities Associated with Prescribed Medication in a CKD Population Darren Green, David I. New, Philip A. Kalra. *Vascular Research Group, Manchester Academic Health Sciences Centre, University of Manchester, Salford Royal Hospital, United Kingdom.*

Background: Hyper- and hypokalemia are associated with cardiac arrhythmia. They may be caused by drugs such as anti-hypertensives which are prescribed with particular frequency in renal clinics. We sought to understand which drugs were most frequently associated with abnormal serum potassium in this setting.

Methods: We undertook a cross-sectional analysis of the serum potassium and concomitant medication history of 1208 patients visiting a tertiary renal out-patient clinic over 5 years. We examined the effect of drugs which are known to alter potassium distribution and excretion, whilst accounting for co-morbidities and age.

Results: The mean age was 57.9 years, eGFR 32.4mL/min/1.73m2, 63% were male, 37% diabetic. 10.5% of serum potassium results were >5.5mmol/L, 5.0% were ≥6.0mmol/L and 1.1% were <3.5mmol/L. ACE inhibitors, angiotensin II receptor blockers and beta-blockers were associated with hyperkalemia, but digoxin and spironolactone were not. This latter effect may be because 92% of spironolactone users (45 of 49) were also on a loop or thiazide diuretic. These two diuretics were associated with a lower mean serum potassium but prednisolone, nebulised β2 agonists and insulin were not. Multivariate logistic regression showed that only ACE inhibitors (odds ratio 2.1), eGFR (k+ ≥6.0 vs. k+ <6.0 = 22.3 vs. 32.9 mL/min/1.73m2), and male gender (OR 1.8) were independent predictors of a serum potassium ≥6.0mmol/L. Only thiazide use was an independent predictor of potassium <3.5mmol/L (OR 5.7). Of the 60 episodes of k+ ≥6.0mmol/L, 10 blood samples were repeated within 12 hours. In 4 serum potassium remained elevated. These were all CKD stage 5, and only 1 was taking an ACE inhibitor. The mean eGFR of those with a repeat potassium <6.0mmol/L was 23.6mL/min/1.73m2.

Conclusions: Beta-blockers should not be ignored as a potential cause of hyperkalemia. However, ACE inhibitors were the only drug predictive of potassium ≥6.0mmol/L. The effect was less significant than that of eGFR, and unrelated to episodes of persistent hyperkalemia. Hypokalaemia was almost exclusively an association of thiazide diuretics.

SA-PO2446

Endogenous Testosterone as a Determinant of Muscle Mass and Strength in Nondialyzed Men with Chronic Kidney Disease Secundino Cigarran,[1] Guillermina Barril,[2] Francisco Coronel,[3] Juan J. Carrero.[4] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Hospital Universitario de la Princesa, Madrid, Spain; [3]Nephrology, Hospital Clinico Universitario San Carlos, Madrid, Spain; [4]Division of Renal Medicine, Karolinska Institutet, Stockholm, Stockholm, Sweden.*

Background: Testosterone deficiency is a common finding in men with chronic kidney disease (CKD). Testosterone also plays an important anabolic role in muscle synthesis. Muscle wasting is an important and deleterious feature of protein-energy wasting (PEW) in CKD. It is currently unknown if endogenous testosterone associates with features of muscle wasting in men with CKD.

Methods: We cross-sectionally studied 267 nondialyzed men (mean±SD age 67±13 years) with manifest CKD (stages 1-5, eGFR 42.9 [interquartile range 30.2-56.7] ml/min/1.73m²). Endogenous testosterone and markers of PEW such as albumin, prealbumin, CRP or nPNA were analyzed. Muscle mass was estimated by whole tetrapolar bioelectrical vectorial impedance (BIVA) and muscle strength by handgrip dynamometry.

Results: Across decreasing tertiles of testosterone distribution, patients were incrementally older and CRP levels rose significantly. Prealbumin, hemoglobin, nPNA, handgrip strength (both dominant and nondominant hands) and BIVA-estimated surrogates of muscle mass (lean body mass, body cell mass and phase angle) were progressively reduced (P<0.05 for all). In hypothesis-driven multivariate regression analyses, testosterone significantly and independently contributed to explain the variance of handgrip strength and lean body mass. Through stepwise forward exclusion, the variance of endogenous testosterone in this patient population was best explained by handgrip strength, lean body mass, hemoglobin, eGFR, CRP and cardiovascular comorbidity (P<0.05 for all).

Conclusions: Endogenous testosterone is an independent and important determinant of both muscle mass and strength in nondialyzed men with CKD. Because preceding evidence indicates that these relationships are likely causal, we speculate that by targeting changes in circulating testosterone we may improve muscle mass and function in these patients.

Funding: Other NIH Support - SERGAS

SA-PO2447

Impact of Subclinical Bacterial Infection on Epigenetic DNA Methylation in Japanese CKD Stage 5 Patients Sawako Kato,[1] Yoshinari Yasuda,[1] Bengt Lindholm,[2] Peter Stenvinkel,[2] Karin Luttropp,[5] Tomas J. Ekström,[6] Yukio Yuzawa,[3] Yoshinari Tsuruta,[4] Shoichi Maruyama,[1] Seiichi Matsuo.[1] *[1]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden; [3]Fujita Health University school of Medicine, Aichi, Japan; [4]Meiyo Clinic, Aichi, Japan; [5]Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [6]Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden.*

Background: Inflammation is mortality risk factor in chronic kidney disease (CKD) stage 5 pts. Although a report from 2007 showed that inflamed uremic pts had signs of global DNA hyper-methylation, the impact of subclinical bacterial infections on systemic inflammation and on DNA methylation status is not known.

Methods: In 44 consecutive incident dialysis patients (27 males, mean age 59±12 years) without clinical signs of infections, global DNA methylation in peripheral blood DNA was evaluated using HpaII/MspI ratio by the luminometric methylation assay method. Lower ratio of HpaII/MspI indicates global DNA hypermethylation. Procalcitonin (PCT), a marker of inflammation and bacterial infections, was measured using immuno-chromatography assay.

Results: HpaII/MspI ratio was 0.31 (0.26-0.61) and sPCT was 0.08 (0-0.87) ng/mL. The pts were divided into hyper- and lower-methylation groups by the median value. The pts in hyper-methylation group had higher ferritin levels (201.6(15.0-879.0) vs. 92.9(20.0-346.0) ng/mL; p=0.047), but there was no significant difference in age, gender, prevalence of diabetes, smoking habit, anemia or serum albumin levels between the two groups. However, HpaII/MspI ratio showed significant negative correlations to PCT (ρ=-0.32 P=0.035), and ferritin (ρ=-0.33 P=0.027), and CRP correlated to ferritin (ρ=0.37 P=0.014). In a multiple linear regression analysis, PCT and ferritin, but not CRP, were associated with lower HpaII/MspI ratio (R2=0.24, p=0.0131).

Conclusions: Global DNA hyper-methylation was associated with elevated PCT and ferritin, which could suggest that subclinical bacterial infection leads to DNA hyper-methylation.

Funding: Government Support - Non-U.S.

SA-PO2448

Gender Differences in Body Composition in Chronic Kidney Disease Patients at the Different Functional Stages Carlo Donadio. *Internal Medicine, Nephrology, University of Pisa, Italy.*

Background: Malnutrition is frequent in end-stage renal disease patients treated by dialysis. Few data are available on the evaluation of nutritional status in chronic kidney disease (CKD) patients at different renal functional stage. The measurement of body electrical impedance (BIA) is a simple, inexpensive, and validated method to analyze body composition and measure body fluids in CKD patients.

The aim of this study was to evaluate the effect of the decreased renal function on body composition of CKD patients.

Methods: One thousand two hundred and eighty-nine and sixteen adult patients (617 f, 672 m), aged 15-85 years, mean 51.3; body weight 36.6-160.0 kg, mean 72.4; BMI 15.8-67.5 kg/m2, mean 26.4, affected by different kidney disease with different degree of functional impairment (serum creatinine 0.4-14.4, mean 1.77) participated in this study.

Glomerular filtration rate (GFR) was measured as the renal clearance of 99mTc-DTPA. Body composition was evaluated by means of single-frequency bioimpedance analysis. Values of fat mass (FM), fat-free mass (FFM), body cell mass (BCM) and extra-cellular water (ECW) were indexed to squared height of patients, similarly to BMI.

Results: BMI e FMI significantly increased with the reduction of GFR in females, while were unchanged in males. FFMI was unmodified independently from the gender. BCMI significantly decreased only in males, while was unmodified in females. Finally, ECWI significantly increased in all patients. These results indicate significant differences between male and female CKD patients, and in particular a different effect of decreased GFR on body mass and body composition in male and female patients.

Conclusions: Traits of malnutrition are present in chronic kidney disease patients, before starting renal replacement therapy. Significant differences in body composition have been found according to gender. In particular, the decrease in GFR was accompanied by the reduction in body cell mass only in male CKD patients. It is possible that the higher fat mass plays a protective role in females.

Funding: Government Support - Non-U.S.

SA-PO2449

Plasma Adiponectin Concentration and Estimated Glomerular Filtration Rate (eGFR) in the Elderly. Polish Population Study "PolSenior" Marcin Adamczak, Magdalena Szotowska, Jerzy Chudek, Andrzej Wiecek. *Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland.*

Background: The consequence of impairment kidney function and aging on the endocrine function of adipose tissue is not fully elucidated. Adiponectin is an adipokine with antiatherosclerotic, cardioprotective and antidiabetic properties. The aim of present study was to estimate plasma adiponectin concentration and their relationship with estimated glomerular filtration rate (eGFR) in representative samples of the elderly population in Poland.

Methods: The study was carried out as a part of the nationwide PolSenior project in the population of randomly selected 4979 (2412 females and 2567 males) aged 65-101 years. In 3692 subjects (1762 females and 1930 males) plasma adiponectin concentration was assessed by an ELISA method and eGFR was estimated using the CKD-EPI formula.

Results: If compared with males, females aged 65-90 years were characterized by significantly higher plasma adiponectin concentration (11.0±5.9 vs 13.3±6.8; p<0.001). Analysis of variance, between groups stratified according to eGFR with respect of plasma adiponectin concentration showed significant (p<0.001) differences with increase with the degree of the impairment kidney function [in subject with eGFR 90-120 ml/min (n=304) – 11.2±6.3; eGFR 60-90 ml/min (n=2273) – 11.9±6.3; eGFR 45-60 ml/min (n=723) – 12.4±6.6; eGFR < 45 ml/min (n=392) – 13.1±6.6 μg/ml]. A significant negative correlations were found between plasma adiponectin concentration and eGFR (r=-0.082, p<0.001). Multiple regression analysis with gender, age and eGFR as independent factors showed that only age has significant influence on plasma adiponectin concentration in the elderly Polish population (β=-0.08; p=0.035).

Conclusions: 1/ Among the subjects older than 65 years, plasma adiponectin concentration was increased with the degree of the kidney function impairment. 2/ Such an increase of plasma adiponectin concentrations may be secondary to the age-related decline of the kidney function.

Funding: Government Support - Non-U.S.

SA-PO2450

Insulin Resistance in Children with Primary Nephrotic Syndrome and Normal Renal Function Aihua Zhang, Songming Huang, Guixia Ding, Yanggang Yuan, Chunhua Zhu. *Nephrology, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, China.*

Background: Clinical research has demonstrated that children with ESRD on hemodialysis have IR and hyperinsulinism. The present study was to investigate whether the children with **early stage primary nephritic syndrome (PNS)** have IR, and IR in this group is associated with renal pathology, therapeutic response to glucocorticoids, and clinical outcome.

Methods: One-hundred and nineteen PNS patients with normal renal function and 125 normal controls were studied. Fasting blood glucose (FBG), fasting serum insulin (FISN) and fasting serum C-peptide (FCP) were measured. The Homa index of insulin resistance (HOMA-IR), islet B cell function (HOMA-islet) and insulin sensitive index (ISI) were calculated. The correlations were assessed between HOMA-IR, FCP, blood pressure (BP), blood lipids, renal function, blood coagulation, clinical disease type, pathology, and the early therapeutic effectiveness of high-dose glucocorticoid.

Results: There was no evidence of IR in the early stage of PNS. Although levels of FBG, FISN and FCP were all within the normal range, FCP was significantly higher than in the control group. Spearman's correlation analysis revealed a significant correlation between FCP and age, BP, SCr, TG and FISN. FCP was positively correlated with BMI and negatively with GFR. Pearson's correlation analysis showed that $\log_{10}$ (FCP) positively correlated with age, SCr, TG, FBG and FISN. There was a positive correlation between $\log_{10}$ (FCP) and BMI and a negative correlation with GFR and FBG. Furthermore, multivariate stepwise regression analysis revealed TG as an independent risk factor for $\log_{10}$ (FCP).

Conclusions: Although IR was not detected, a significant increase in BP, uric acid (UA), blood lipids and blood coagulability was observed in the PNS group. A correlation observed between HOMA-IR, age, BP, serum creatinine (SCr) and TG may suggest that insulin sensitivity will emerge as renal disease progresses. FCP levels were increased in the PNS group, suggesting that FCP may be a protective factor.

Funding: Government Support - Non-U.S.

SA-PO2451

Validation of Predictive Equations for Resting Energy Expenditure in Chronic Kidney Disease Enric Vilar,[1,2] Ashwini Machado,[1] Andrew Garrett,[2] David Wellsted,[2] Ken Farrington.[1,2] *[1]Lister Renal Unit, Stevenage, United Kingdom; [2]University of Hertfordshire, Hatfield, United Kingdom.*

Background: The effect of the uremia on metabolic rate is poorly understood and knowledge of metabolic rate in CKD is important for provision of accurate dietary advice. Validated predictive equations for energy expenditure are needed in CKD.We studied the effect of physical activity and GFR on resting energy expenditure(REE) in CKD. We evaluated algorithms for REE, including one recently developed at our unit.

Methods: We performed a metabolic analysis of subjects on dialysis and with CKD. All subjects had a metabolic analysis including measurement of body size parameters and REE by indirect calorimetry. Physical activity was measured with Metabolic Equivalent of Task (MET) using the Stanford 7 day recall questionnaire. Physical activity and REE were compared between the dialysis and CKD groups. Predictive equations for REE were evaluated using the Bland-Altman method.

Results: 400 subjects were recruited, 200 on dialysis and 200 with CKD. Weight, serum albumin and body mass index was lower in patients on HD ($p<0.001$ for each). Physical activity was lower in patients on dialysis compared to those with CKD (mean MET 1.44 v 1.50 for males, 1.42 v 1.56 for females, overall $p<0.001$).There was no significant difference in REE between subjects with CKD and those on dialysis ($p=0.14$ for females, $p=0.26$ for males). In a regression model, eGFR did not predict REE after correcting confounding variables. In a separate model, eGFR significantly predicted estimated Total Energy Expenditure (REE*Mean MET). Equations for REE (Schofield, Mifflin-St Jeor and Harris-Benedict) underestimated REE in the CKD group (bias +138 to +193kCal/day). A novel equation developed at our unit had lower bias (-36kCal/day). All equations had similar limits of agreement(424-484kCal/day).

Conclusions: Patients on dialysis are less active than those with CKD, but REE does not differ. Equations for REE derived in normal individuals tend to underestimate in CKD. A recently described equation improves bias with similar level of agreement. Considerable variance in REE is unexplained by such equations and results should be interpreted with caution.

SA-PO2452

Metabolomic Profiling of Uremic Solutes in CKD Patients Takehiro Suzuki,[1] Yasutoshi Akiyama,[1] Takafumi Toyohara,[1] Hiroshi Sato,[2] Yoichi Takeuchi,[1] Sadayoshi Ito,[1] Tomoyoshi Soga,[3] Takaaki Abe.[1] *[1]Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Graduate School of pharmaceutical Sciences, Tohoku University, Sendai, Japan; [3]Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan.*

Background: Chronic kidney disease (CKD) is strongly associated with cardiovascular events and prognosis. Early detection and accurate monitoring of patients with CKD is likely to improve care and decrease the risk of cardiovascular and cerebrovascular disease. As a new diagnostic tool, we examined the retention of uremic solutes as a simpler, more accurate method to assess renal function.

Methods: Comprehensive metabolome analysis of 41 CKD patients by capillary electrophoresis mass spectrometry (CE-MS) of blood samples were performed (Toyohara T. JASN 2009).

Results: By CE-MS, we found 22 cations and 30 anions that accumulated significantly as the estimated GFR decreased. These compounds included 9 cations and 27 anions that were newly identified in our study. In contrast, we also found 7 cations (2 new) and 5 anions (all new),that decrease significantly as eGFR declines. We next evaluated each substance for its suitability to detect early CKD stage. Compounds highly correlated with eGFR and whose plasma concentration changed in a manner approximated by the first-degree equation are excellent candidates for detecting CKD and identifying uremic toxins that might aggravate kidney function in the early stage of CKD compared with 2nd -degree model such as creatinine (1-methyladenosine rs= -0.772, N-acetylglucosamine rs= -0.751, γ-butyrobetaine rs= -0.734, sebacate rs= -0.751, cis-aconitate rs= -0.719, and homovanillate rs= -0.711).

Conclusions: These results identify a number of uremic compounds. Many of them are novel, and increase in a manner approximated by the first-degree equation, that predict worsening renal function in early stage of CKD. Thus these compounds provide early diagnostic information. In addition CE-MS should be useful tools for exploring the uremic toxins (Toyohara T. Hypertens Res 2010).

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SA-PO2453

Analysis of Urinary Proteomic Profiles between Progressive and Non-Progressive IgA Nephropathy by 2D-DIGE and MALDI -TOF-MS Ling Wang,[1] Zhaohui Ni,[1] Liou Cao,[1] Fuquan Yang,[2] Qin Wang,[1] Shan Mou,[1] Minfang Zhang.[1] *[1]Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; [2]Institute of Biophysics, China Academy of Science, Beijing, China.*

Background: IgA nephropathy (IgAN) is the most common glomerular disease in adults. Searching for prognostic markers which could identify those who will progress to renal failure is highly necessary.

Methods: Urine samples were obtained from non-progressive and progressive IgAN patients.2D-DIGE and MALDI/TOF/MS was used. Biological variations of the protein expression level among gels were analyzed with DeCyder software and evaluated for statistical significance. The deferentially regulated spots were picked out and were identified by MALDI/TOF MS or LC MS.

Results: 36 spots were significantly differentially expressed. We had identified 23 proteins significantly expressed with 19 up-regulated and 4 down-regulated.

No	Protein	Up or Down
1	zinc-alpha-2-glycoprotein, chain B	down
2	plasma retinol-biding protein; RBP precursor	down
3	histone H2B	down
4	carbamoyl-phosphate synthetase 1, mitochondrial	down
5	zinc-alpha-2-glycoprotein chain A	up
6	alpha-1-microglobulin/bikunin precursor	up
7	Transthyretin Chain A	up
8	ATP synthase, H+ transporting, mitochondrial F1 complex, beta subunit	up
9	T-cell antigen receptor VJ junction beta chain	up
10	immunoglobulin heavy chain	up
11	lectin, galactoside-binding, soluble, 2	up
12	Interferon-induced protein with tetratricopeptide repeats 2	up
13	T-cell receptor beta chain	up
14	serum transferin n-terminal lobe,chain A	up
15	Chain A, Solution Structure Of Domain 3 From Human Serum Albumin Complexed To An Anti-Apoptotic Ligand Directed Against Bcl- Xl And Bcl-2	up
16	transferrin	up
17	RNA polymerase II subunit	up
18	ubiquitin-conjugating enzyme E2 variant 1	up
19	suppression of tumorigenicity 7	up
20	MAP3K12 binding inhibitory protein 1,	up
21	transcription factor HFK3	up
22	T cell receptor beta chain	up
23	hypothetical protein	up

Conclusions: We have established a proteomic method to discover urinary biomarkers of IgAN and identified several urinary proteins or peptide which could be further studied. These findings could be helpful in early diagnosis of the progressive IgAN.

SA-PO2454

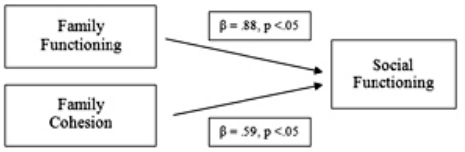
The Disability-Distress-Coping Mode – One Way To Conceptualize Adjustment in Adolescents with Chronic Kidney Disease Nicole M. Fenton,[1] Kristi Bickford,[2] Maria E. Ferris.[2] *[1]Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Studies suggest that among individuals with CKD, illness severity may not adequately predict an adolescent's adjustment. Rather, psychosocial factors may be one key to understanding adjustment. The current study utilized the Disability-Distress-Coping Model. Our aims were to:Examine if 1) family functioning and 2) overall coping efficacy significantly influences adjustment (social functioning, depressive symptoms, and ER visits).

Methods: Participants were recruited from the UNC Kidney Center's outpatient clinic and included 50 adolescents age 13-18 with a diagnosis of CKD stage ≥2. De-identified web-based measures were administered including: the FRI, KidCope, PedsQL, and the CDI.

Results: Data analysis consisted of a series of regressions where age and disease severity were controlled for. Results indicated that family functioning and family cohesion were significant positive predictors of the adolescent's social functioning.

Figure 1: Significant Findings - Aim 1:
Family functioning and family cohesion are both significant predictors of the adolescent's social functioning



Overall coping efficacy was not a significant predictor of the adolescent's social or psychosocial functioning, however; the efficacies of two specific coping strategies were: emotion regulation and seeking social support. Finally, neither family functioning nor coping efficacy were significant predictors of depressive symptoms or ER visits.

Conclusions: These findings suggest that adolescents with CKD are able to adjust well to having a chronic illness and that family functioning and the coping efficacy strategies of emotion regulation and seeking social support play an important role in facilitating this positive adjustment.

SA-PO2455

Clinical Presentation of Sleep Apnea in Patients with Chronic Kidney Disease David Donald McTavish Nicholl, Sofia B. Ahmed, Andrea H. Loewen, Brenda Hemmelgarn, Darlene Y. Sola, Jamie M. Beecroft, Tanvir Chowdhury Turin, Patrick Hanly. *Medicine, University of Calgary, Calgary, AB, Canada.*

Background: Sleep apnea is an important and common co-morbidity in patients with chronic kidney disease (CKD). However, few studies have addressed how sleep apnea presents in this patient population and whether it is clinically apparent. The objective of this study was to compare the prevalence and severity of sleep-related symptoms in CKD patients with and without sleep apnea.

Methods: One hundred twenty-four patients were recruited from out-patient nephrology clinics. All patients completed a questionnaire examining symptoms of sleep apnea (snoring, witnessed apneas, nocturnal choking), the Epworth Sleepiness Scale (ESS>10=daytime sleepiness), Pittsburgh Sleep Quality Index (PSQI>5=poor sleep quality), and overnight cardio-pulmonary monitoring for determination of sleep apnea (respiratory disturbance index >15). Patients with sleep apnea (n=51) were compared to patients without apnea (n=73).

Results: CKD patients with sleep apnea did not differ from those without sleep apnea in the prevalence of snoring (76% vs. 74%, p=0.8), witnessed apneas (35% vs. 19%, p=0.060), and nocturnal choking (33% vs. 23%, p=0.2). There was a higher prevalence of daytime sleepiness in CKD patients with sleep apnea compared to non-apneic patients (39% vs. 20%, p=0.024) and there was a non-significant increase in ESS scores in CKD patients with apnea (median (range), 9 (2-24) vs. 6 (0-17), p=0.055). There were no differences in the prevalence of poor sleep quality between apneic and non-apneic patients (47% vs. 43%, p=0.7; median (range), 5 (0-15) vs. 5 (0-16), p=0.6).

Conclusions: Sleep apnea is highly prevalent in patients with CKD but is not clinically apparent. Consequently, objective cardio-pulmonary monitoring during sleep is required to reliably identify this co-morbidity.

SA-PO2456

Validity of the Berlin Questionnaire and Adjusted Neck Circumference in Identifying Sleep Apnea in Patients with Chronic Kidney Disease David Donald McTavish Nicholl, Sofia B. Ahmed, Andrea H. Loewen, Brenda Hemmelgarn, Darlene Y. Sola, Jamie M. Beecroft, Tanvir Chowdhury Turin, Patrick Hanly. *Medicine, University of Calgary, Calgary, AB, Canada.*

Background: Given the reported high prevalence of sleep apnea in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD), a clinical instrument to assess the prevalence of sleep apnea in these populations would be useful. The Berlin Questionnaire (BQ) and Adjusted Neck Circumference (ANC) are 2 such instruments that have been validated in patients with normal kidney function. The objective of this study was to determine the validity of the BQ and ANC in patients with CKD and ESRD, using overnight cardio-pulmonary monitoring to diagnose sleep apnea.

Methods: Two hundred fifty-four patients were recruited from nephrology clinics and hemodialysis units. All patients completed the BQ, ANC, and overnight cardio-pulmonary monitoring for diagnosis of sleep apnea (respiratory disturbance index (RDI)>15). Patients were stratified into 3 groups based on estimated glomerular filtration rate (eGFR): eGFR≥60 mL/min/1.73m^2 (n=55); CKD (eGFR<60 mL/min/1.73m^2, n=124); and ESRD (on hemodialysis, n=75). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the BQ and the ANC (Table 1) and the association between the BQ and ANC and a diagnosis of sleep apnea (RDI>15) was determined using logistic regression (Table 2).

Results:

Table 1		eGFR>60	CKD	ESRD		
BQ	Sensitivity	86.7%	78.7%	60.5%		
	Specificity	60.0%	37.7%	43.8%		
	PPV	44.8%	46.3%	59.1%		
	NPV	92.3%	72.2%	45.2%		
ANC	Sensitivity	73.3%	69.4%	54.8%		
	Specificity	84.2%	67.1%	71.0%		
	PPV	64.7%	58.6%	71.9%		
	NPV	88.9%	76.6%	53.7%		
Table 2		Univariate			Multivariate	
eGFR>60	OR	95% CI	P-value	OR	95% CI	P-value
BQ	9.75	1.93-49.15	0.006	5.40	0.92-31.68	0.062
ANC	14.67	3.48-61.82	<0.001	9.46	2.08-43.04	0.004
CKD						
BQ	2.24	0.96-5.24	0.062	1.55	0.60-4.03	0.369
ANC	4.63	2.12-10.09	<0.001	3.89	1.68-8.99	0.001
ESRD						
BQ	1.19	0.47-3.01	0.714	0.87	0.32-2.35	0.784
ANC	2.96	1.11-7.93	0.031	3.04	1.11-8.28	0.030

Conclusions: The BQ and the ANC were less accurate in patients with CKD and ESRD than in patients with eGFR>60. However, the ANC was consistently better than the BQ.

SA-PO2457

Intestinal Microbial Flora Is Altered by Uremia Nosratola D. Vaziri,[1] Jakk Wong,[2] Madeleine V. Pahl,[1] Jun Yuan,[1] Todd Z. Desantis,[2] Yvette M. Piceno,[2] Gary L. Andersen.[2] *[1]Med/Neph, UC Irvine, Irvine, CA; [2]Molecular Microbial Ecology Group, LBNL, Berkeley, CA.*

Background: The community of microbes residing in the intestinal tract (microbiome) constitutes a symbiotic ecosystem which confers trophic and protective functions and contributes to micronutrient homeostasis. Alteration in microbiome contributes to diverse illnesses including inflammatory bowel disease, diabetes, cancer, cardiovascular disease, obesity, etc. Selection pressures on part of the host and microbes shape the structure-function of microbiome. We believe that influx of urea (and its hydrolysis to ammonia), urate and oxalate, dietary restrictions, phosphate binders and antibiotics change the milieu of intestine in ESRD. This can, in turn, alter the microbiome and cause production of toxic, pro-inflammatory and pro-oxidant metabolites. We tested the hypothesis that uremia may change the composition and function of microbiome.

Methods: Microbial DNA was isolated from the stools of 24 ESRD patients and 12 controls. A phylogenetic microarray was used for comprehensive identification of microbial populations.

Results: While large inter-individual variations were observed in the microbiome composition, significant differences were found in the abundance of 183 bacterial taxonomic units between the ESRD patients and the control groups. Microbial families showing the largest increase in ESRD patients were Brachybacterium, Catenibacterium, Enterobacteriaceae, Halomonadaceae, Micromonosporaceae, Moraxellaceae, Nesterenkonia, Polyangiaceae, Pseudomonadaecea, and Thiothrix including several families possessing *urease* and *uricase* genes (P<0.015). Overall, a number of species from Clostridia, Bacteroidetes, and Betaproteobacteria were less abundant while species from Actinobacteria and Gammaproteobacteria were most abundant in ESRD patients compared to controls.

Conclusions: ESRD significantly alters the abundance of several microbial families which appears to be in part driven by overabundance of urea and urate in the intestine. Studies are planned to focus on individual members of these families and their contribution to complications of ESRD.

SA-PO2458

Asymmetric Dimethylarginine, Oxidative Stress, and Tubular Dysfunction in Patients with Chronic Kidney Disease Stages 3 and 4 – A One Year Follow-Up Study Jaromir Eiselt,[1] Daniel Rajdl,[2] Kamila Rulcova,[1] Jan Wirth,[1] Jaroslav Racek.[2] *[1]Internal Dept. 1, Charles Univ., Plzen, Czech Republic; [2]Dept. of Biochemistry, Charles Univ., Plzen, Czech Republic.*

Background: Asymmetric dimethylarginine (ADMA) is a mediator of endothelial dysfunction and a prognostic factor in patients with chronic kidney disease (CKD). Production and eliminaton of ADMA may be affected by oxidative stress and metabolic function of the kidneys.

Results: In a one year follow-up study (examinations in months 0, 6 and 12) we measured plasma levels of ADMA, markers of oxidative stress (advanced oxidation protein products (AOPP), advanced glycation end-products (AGE)) and tubular dysfunction (the ratio of cystatin-C to creatinine in urine, U_CysC/Cr) in 95 patients with CKD stages 3 and 4. All of the patients measured parameters were compared with 41 healthy controls.

Methods: During the one year follow-up, we observed a gradual decrease of glomerular filtration rate (GFR) and an increase of AGE and AOPP. We did not document changes of ADMA and U_CysC/Cr. All tested markers were clearly elevated in CKD patients as compared to healthy controls. Results are summarized in the table.

	Controls (n=41)	Pts (n=95) Mo 0	Pts Mo 6	Pts Mo 12	ANOVA on Ranks
GFR (mL/min)	111.3 (90.7-130.5)[a]	26.7 (20.3-37.6)	25.3 (19-38.7)	21.1 (14.1-38)[b]	p=0.021
ADMA (µmol/L)	0.78 (0.68-0.86)[a]	0.86(0.76-0.93)	0.80(0.70-0.93)	0.87(0.73-1.01)	p=0.064
AOPP (µmol/L)	109 (101-121)[a]	142 (121-171)	137 (121-153)	146 (126-174) [c]	p=0.037
AGE (FU/g of protein)	1.39 (1.3-1,52)[a]	1.55 (1.38-1.84)	1.72 (1.52-2.2)	1.76 (1.52-2.18)[b]	p<0.001
U_CysC/Cr (µg/mmol)	9.7(8.2-16)[a]	21.7 (9-87.8)	20.3 (10-153)	13.0(5.8-93.4)	p=0.095

Data is median (interq. range); comparisons using Mann-Whitney test or Kruskal-Wallis ANOVA on Ranks followed by Tukey or Dunn's Method as appropriate; [a]p<0.01 vs. Mo 0, [b]p<0.01 vs. Mo 0, [c]p<0.05 vs. Mo 6

Conclusions: Despite a decline in GFR and increase of markers of oxidative stress, the levels of ADMA remained unchanged during the one year follow-up. Stable tubular function documented by U_CysC/Cr supports the hypothesis that metabolic function of the kidneys in CKD stages 3 or 4 can partially compensate the efect of oxidative stress and prevent the increase of ADMA.

Funding: Government Support - Non-U.S.

SA-PO2459

Treatment Needs of Patients with Chronic Kidney Disease Stage 3 in Primary Care Natasha J. McIntyre,[1] Richard J. Fluck,[1] Chris W. McIntyre,[1,2] Maarten W. Taal.[1] *[1]Department of Renal Medicine, Royal Derby Hospital, Derby, Derbyshire, United Kingdom; [2]Department of Vascular Medicine, University of Nottingham, United Kingdom.*

Background: Since 2006, English GP practices have been required to keep a register of patients with CKD stage 3-5. NICE guidelines recommend regular follow-up, but these patients are often perceived to be low risk, not requiring active management. We aimed to assess treatment needs of CKD stage 3 patients in primary care as well as their level of awareness of CKD.

Methods: We studied 1741 patients on GP registers with CKD stage 3. Each subject underwent clinical assessment as well as urine and blood tests. At screening, participants were asked if they had been aware of their CKD diagnosis. Results were reviewed by a Nephrologist and a letter recommending a treatment plan in accordance with NICE guidelines was sent to the GP.

Results: The mean age was 73±9yrs and 60% (n=1052) were female. Diabetes mellitus was present in 17%. 41% were unaware of their CKD diagnosis. Multivariable logistic regression analysis identified subjects with fewer educational qualifications, aged <75yrs, eGFR 30-44mL/min/1.73m^2 and significantly albuminuric as more likely to be aware of their diagnosis. Advice given are shown in the table.

Table 1. Advice given

	n	%
Continue routine follow up	576	33.1
Specific advice regarding BP control	576	33.1
Refer to Nephrology	103	5.9
Investigations for anaemia	142	8.2
Statin therapy for dyslipidaemia	69	4.0
Advice to stop potentially nephrotoxic drugs	120	7.5

Reasons for recommending nephrology referral include GFR decline>10mL/min/1.73m^2 over 5yrs (67%), GFR decline >5mL/min/1.73m^2 in 1yr (15%), proteinuria (13%), compications of CKD (1%), progression to CKD 4/5 (4%). Of subjects with a GFR decline of >10mL/min/1.73m^2 over 5yrs, 8% had significant proteinuria with 8% having progressed to stage 4.

Conclusions: A large proportion of patients were unaware of their CKD diagnosis. Our data indicate increasing engagement with patients to become active partners in their management will require improved communication targeted at specific groups. Our data support the use of primary care CKD registers to identify patients with CKD stage 3 to facilitate evaluation and follow up.

Funding: Other NIH Support - Kidney Research UK and the British Renal Society

SA-PO2460

Current Practice in Diagnosis and Treatment of Anemia in Stage 3 or 4 Chronic Kidney Disease Francesco Locatelli,[1] Peter F. Barany,[2] Carlo A. Gaillard,[3] Walter Hoerl,[5] Daniell Mitchell,[4] Claudio Mori.[4] *[1]Ospedale A. Manzoni, Lecco, Italy; [2]Karolinska Institutet, Stockholm, Sweden; [3]VU University Medical Center, Amsterdam, Netherlands; [4]Vifor Pharma, Glattbrugg, Switzerland; [5]Medical University Vienna, Vienna, Austria.*

Background: In chronic kidney disease (CKD), anemia is often associated with iron deficiency (ID). Thus, repletion of iron stores is recommended before treatment with erythropoiesis-stimulating agents (ESAs). This study evaluated current practice in diagnosis and treatment of CKD-associated anemia and ID in non-dialysis (ND) patients.

Methods: From Aug-Nov 2010, randomly selected nephrologists in Austria, Italy, Netherlands and Sweden spending >50% of working time on patient-care and managing >10 CKD patients monthly were surveyed for patient demographics, tests for anemia and ID, and therapies of 5 CKD patients (stage 3-4) most recently treated for anemia within 6 months prior to the survey. Randomly selected questionnaires (10%) were returned to physicians for validation. Results are presented as median [range] amongst countries.

Results: 125 physicians (119 hospital-, 6 office based) reported 623 cases (57% [54-71] male; 40% [29-60] >70 years old). Tests performed by nephrologists to confirm anemia and ID were hemoglobin (Hb) in 85% [59-87] and/or hematocrit in 89% [83-94] of patients and ferritin (63% [52-69]) and/or transferrin saturation (TSAT, 28% [16-54]). Median Hb at diagnosis was 97g/L [96-102]; 54% [44-60] presented with Hb <100g/L and 3% [0-16] with Hb <80g/L. Median ferritin and TSAT at diagnosis were 91µg/L [40-121] and 20% [14-23]. Absolute ID (defined as ferritin <100µg/L) was found in 53% [34-67] and TSAT was <20% in 53% [13-58] of those tested. 54% [15-66] received ESA treatment combined with iron, 41% [24-54] ESA alone and 9% [5-31] iron alone. Except for Sweden (46%), only a small minority of iron-treated patients received intravenous (IV) iron (7-11%).

Conclusions: High rates of severe anemia and ID indicate suboptimal monitoring and treatment of anemia in ND-CKD patients. Awareness of the prevalence of ID in ND-CKD patients and the importance of an integrated (iron in combination with ESA) treatment approach, needs to be increased.

Funding: Pharmaceutical Company Support

SA-PO2461

Relationship between Kidney Damage and Mortality among a Community-Based Chinese Population Fukun Niu,[1,2] Luxia Zhang,[1] Xingyu Wang,[3] Lisheng Liu,[3] Haiyan Wang.[1] *[1]Institute of Nephrology and Division of Nephrology, Peking University First Hospital, Beijing, China; [2]Nephrology and Rheumatology, First Affiliated Hospital of Zhengzhou University, Zheng Zhou, Henan, China; [3]Beijing Hypertension League, Beijing, China.*

Background: Previous studies indicate that indicators of kidney damage are associated with adverse outcome, while studies among community-based population are limited, especially among developing countries.

Methods: This prospective cohort study included 1189 community-based participants in Beijing, China. Estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR) were assessed at baseline, and all participants had eGFR above 30mL/min/1.73m2. All deaths were confirmed by medical record review. Multivariable Logistic regression models were used to explore the association between kidney damage and mortality.

Results: The average age was 60.1±9.4 years; and male accounted for 49.5%. During a median of 6 year's follow-up, 87 participants(7.3%) died, among whom 38 died of cardiovascular disease (3.2%) and 29 died of cancer (2.4%). After adjusting for potential confounders including eGFR, albuminuria was independently associated with increased risk of cardiovascular mortality and all-cause mortality. For every 10 mg/g increase of ACR, the odds ratio(OR) of cardiovascular mortality and all-cause mortality was 1.05 [95%CI, 1.01–1.10]and 1.06[95%CI,1.02-1.10]. eGFR were not independently associated with mortality, and both baseline albuminuria and eGFR were not significantly associated with cancer mortality . Receiver operating characteristic (ROC) curve showed the cut-off values with both maximal sensitivity and specificity of ACR for the prediction of cardiovascular and all-cause mortality was 3.89 mg/g creatinine and 3.76mg/g creatinine.

Conclusions: Increased urinary ACR is independently associated with increased risk of both cardiovascular mortality and all-cause mortality, even at levels markedly lower than the current definition of "albuminuria".

SA-PO2462

Safety, Immunogenicity, and Efficacy of Subcutaneous Biosimilar Epoetin Alfa (HX575) in Non-Dialysis Patients with Renal Anemia: A Multi-Center, Randomized, Double-Blind Study Karsten Roth,[1] Kai-Uwe Eckardt,[2] Simon D. Roger.[3] *[1]Sandoz Biopharmaceuticals, Holzkirchen, Germany; [2]Universitätsklinikum Erlangen, Erlangen, Germany; [3]Renal Research, Gosford Hospital, Gosford, New South Wales, Australia.*

Background: HX575 is a biosimilar version of epoetin alfa that is approved in Europe and Australia for the treatment of anemia associated with chronic kidney disease (CKD) using the intravenous route of administration. HX575 is not authorized for use in the USA. Here we report data from a study of anemic pre-dialysis patients to assess the safety, immunogenicity and efficacy of subcutaneous (SC) administration of HX575 versus Erypo®/Eprex®.

Methods: This was a randomized, double-blind study in adult patients (n=337) with stage III–V CKD and a hemoglobin (Hb) level of 7.5–11.0 g/dL. Eligible patients were randomized to 52 weeks of treatment with HX575 or Erypo®/Eprex® at a starting dose of 25 IU/kg body weight three times weekly or 75 IU/kg body weight once weekly during weeks 1 to 5. This could be adjusted after 5 weeks to maintain Hb levels between 10 and 12 g/dL. The primary objective was to assess the safety and immunogenicity of HX575 compared with Erypo®/Eprex®. Efficacy endpoints were mean absolute change in Hb from baseline to end of week 13 and mean weekly epoetin dosage in weeks 11–13.

Results: SC HX575 was comparable to Erypo®/Eprex® in terms of maintaining Hb levels and epoetin dose requirements. Two patients in the HX575 group developed neutralizing antibodies (NAbs) to erythropoietin, which resulted in the study being terminated prematurely. Aside from these two events, reported adverse events were as expected for patients with stage III–V CKD and similar in both treatment groups.

Conclusions: In this study, SC HX575 demonstrated efficacy and comparability with the reference epoetin alfa, but two patients developed NAbs during treatment with SC HX575 in this study. Results of a thorough root-cause analysis reported elsewhere indicate that increased tungsten exposure in pre-filled syringes precipitated immunogenic reactions.

Funding: Pharmaceutical Company Support

SA-PO2463

Chronic Kidney Disease Prevention and Management in Community Health Centers Cynthia R. Christiano, Paul Bolin, Melanie I. Hames, Karen R. Parker, Rachel Ward, Courtland Winborne, Lacy Dean. *East Carolina University, Greenville, NC.*

Background: Chronic Kidney Disease (CKD) leads to high rates of morbidity and mortality. Early detection and management may prevent progression to kidney failure. Primary care providers (PCPs) in Community Health Centers (CHCs) are strategic agents for detection of early CKD.CHCs primarily serve low-income and AA patients who are at high risk for CKD. However, PCP management of CKD is sub-optimal. We aimed to improve CKD detection, prevention and management among PCPs in CHCs. We anticipate that an educational collaboration with nephrologists and PCPs working in a CHC will result in improved clinical practices related to CKD care. We hypothesized that decreases in BP and improved anti-hypertensive filling practices would result from the intervention.

Methods: Nephrologists conducted lectures based on KDOQI and JNC-7 guidelines with the CHCs' providers. Site visits occurred weekly for 6 months (intervention) then gradually decreased. Electronic pharmacy records were queried for data on antihypertensive prescriptions pre-and post-intervention in the initial clinic. A random sample of hypertensive, adult patients were stratified by their respective PCPs. Provider management of BP was evaluated by comparing multiple BP data points from the patient sample pre-and post-intervention. Changes were assessed using paired sample t-tests. This program was repeated in an additional CHC where BPs were analyzed.

Results: The initial clinic demonstrated a statistically significant increase (27%) in prescriptions for antihypertensives pre-and post-intervention. When comparing individual provider management pre-and post- intervention, significant decreases in SBP were observed for all three providers ($p<.05$). Decreases in DBP were significant for two providers ($p<.05$). Interim data from an additional CHC is currently insignificant.

Conclusions: Intervention in the initial CHC demonstrated improved provider practices and patient outcomes. Although when repeated, in a different CHC, similar success was not achieved. The recent growth of and unique challenges in CHCs may require flexible interventions to be effective.

Funding: Private Foundation Support

SA-PO2464

Imbalance of Growth Factors Favouring Anti-Angiogenesis in Children with Chronic Kidney Disease David A. Long,[1] Karen Price,[1] Maria Kolatsi-Joannou,[1] Richard J. Johnson,[2] Adrian S. Woolf,[3] Lesley Rees,[1] Rukshana C. Shroff.[1] *[1]UCL Institute of Child Health, United Kingdom; [2]University of Colorado; [3]University of Manchester, United Kingdom.*

Background: Cardiovascular disease (CVD) is a cause of morbidity and mortality in childhood chronic kidney disease (CKD). Endothelial damage and dysfunction is one of the earliest events in CVD development and may result from disturbances in vascular growth factors. Angiopoietin (Ang)-1 promotes endothelial survival and stabilisation, whereas Ang-2 is an endogenous Ang-1 antagonist causing endothelial death and vessel regression when ambient VEGF-A is low, pro-inflammatory responses and is induced by acute stimulation with uric acid.

Methods: We measured circulating Ang levels in pre-dialysis CKD 4-5 and dialysis children and correlated these with clinical, biochemical and vascular measures. We also examined local Ang and VEGF expression in arterial biopsy samples.

Results: Ang-2 levels were markedly elevated in dialysis compared to pre-dialysis CKD4-5 patients (10.5±1.3 vs 2.6±0.2 ng/ml, $p<0.001$). Using paired samples Ang-2 was not cleared by haemodialysis itself. There was no correlation between time in pre-dialysis CKD 4-5 and Ang-2, but time on dialysis showed a significant positive correlation ($p=0.0023$, $r=0.54$). Amongst dialysis patients Ang-2 positvely correlated with mean time-averaged systolic blood pressure ($p=0.0003$, $r=0.64$), serum urate levels ($p=0.0038$, $r=0.52$), carotid intima media thickness ($p= 0.0053$, $r=0.58$) and aortic augmentation index ($p=0.04$, $r=0.47$). On multiple regression analysis, strongest relationships were found between Ang-2 and systolic blood pressure ($\beta=2.73$, $p<0.001$) and serum urate ($\beta=0.12$, $p=0.03$). Ang-1 and VEGF-A were downregulated in patients' peripheral arterial smooth muscle, whereas Ang-2 was unchanged.

Conclusions: Children on dialysis have an anti-angiogenic and inflammatory milieu evidenced by depletion of Ang-1 and VEGF-A in their vessels and increased circulating Ang-2 which may lead to elevated blood pressure through changes in serum urate. Vascular growth factors could constitute important biomarkers and possible future therapeutic targets in children with CKD.

SA-PO2465

Effectiveness and Safety of MIRCERA® for Anemia Treatment in Chronic Kidney Disease (3-5) Patients on Hemodialysis or Not on Dialysis, in Routine Clinical Practice – The Atenea Study J.M. Portolés,[1] N.J. Vega,[2] G. Fernández-Fresnedo,[3] Alejandro Pérez,[4] S. Bea,[5] M.J. Camba.[6] *[1]Hospital Puerta de Hierro, Spain; [2]Hospital Doctor Negrin, Spain; [3]Hospital Marqués de Valdecilla, Spain; [4]Clinica Souto Boo, Spain; [5]Centro de Diálisis Gamapal, Spain; [6]Complejo Hospitalario de Ourense, Spain.*

Background: MIRCERA® has strongly demonstrated its efficacy and safety in anemia control of adult CKD patients, in clinical trials.

Methods: This observational cross-sectional study involving 20 Spanish centers examined these benefits when administered up to 6 months for correction (naïve patients) or maintenance (converted patients) in CKD patients in Hemodialysis (HD) or not on dialysis (ND), in a real clinical setting. Final results are presented.

Results: Of the 201 patients evaluated, 6.5% were naïve-ND, 25.4% converted-ND and 68.2% converted-HD. Overall baseline data: Mean age: 66.9±14.3 years. Male: 66.2%. CKD 3/4/5: 8%/19.4%/72.6%. Predominant etiologies: 22% vascular and 21% diabetes. The most frequent previous ESAs were darbepoetin alfa in 51% of ND patients and epoetin beta in 58.4% of HD. Hb levels significantly increased from 10.2±0.7g/dL at the start of MIRCERA® to 11.6±1.3g/dL at month 6 ($p<0.005$) in naïve-ND patients. Those converted showed stable Hb values after 6 months of MIRCERA® in both ND (12.1±1.3 g/dL *vs* 12.3±1.2 g/dL) and HD group (11.6±1.3 g/dL *vs* 11.4±1.2 g/dL). Starting doses of MIRCERA® resulted lower than recommended on SPC: from darbepoetin alfa<40µg/w and epoetin<8,000IU/w to MIRCERA® 94.6±35.7µg/m (n=120); from darbepoetin alfa 40-80µg/w and epoetin 8,000-16,000IU/w to MIRCERA® 138.2±34µg/m (n=51); from darbepoetin alfa >80µg/w and epoetin>16,000IU/w to MIRCERA® 301.5±129.4µg/m (n=17). Throughout the study, mean MIRCERA® dose adjustments were 1.1±0.4 in naïve-ND, 1.5±0.7 in converted-ND and 1.7±0.9 in converted-HD, and no dose adjustment was required in 38.5%, 54.9% and 23.4%, respectively. No MIRCERA®-related AEs were reported.

Conclusions: MIRCERA® successfully provides anemia control with doses bellow to those described in the SPC and few dose adjustments. MIRCERA® is safe and simplifies anemia treatment in CKD in routine clinical practice.

SA-PO2466

Understanding Transition Readiness: How To Facilitate High Transition Readiness and What Are the Implications of Low Transition Readiness? Nicole M. Fenton,[2] Mary Hunter Benton,[1] Stephen R. Hooper,[2] Kristi Bickford,[1] William A. Primack,[1] Maria E. Ferris.[1] *[1]School of Medicine - Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: The transition from pediatric to adult-focused health care is a critical time yet, little is known about how to facilitate transition readiness or the implications of low transition readiness. Utilizing the Disability-Distress-Coping Model we examined psychosocial function and transition readiness. Our aims were to:

1) Examine if psychosocial functioning (family functioning, coping efficacy, and quality of life) and disease characteristics (disease severity/burden and age at diagnosis) would predict transition readiness.

2) Determine if transition readiness would be a predictor of emergency room (ER) visits and non-adherence.

Methods: Thirty adolescents age 13-18 with a diagnosis of CKD stage ≥2 were included. De-identified web-based measures were administered including: the Family Relationship Index, KidCope, PedsQL, and the UNC TR$_x$ANSITION Scale. Adherence and ER visits were documented based on chart review and self-report.

Results: Hierarchical regressions were performed. Family cohesion was a significant positive predictor of both overall transition readiness and adherence ($p<.05$). However, none of the other variables (quality of life, coping efficacy, and disease characteristics) were significant predictors of transition readiness. Higher transition readiness was a significant predictor of higher medication adherence and fewer ER visits ($p<.05$).

Conclusions: In our cohort, family cohesion appears to facilitate transition readiness. Healthcare providers may want to encourage the family unit to provide support when adolescents are preparing for transition. Additionally, this study also suggests that low transition readiness may predict non-adherence to medications and ER utilization. This highlights the importance of preparing adolescents for the transition from pediatric to adult-focused health services and suggests a significant influence on low adherence on healthcare utilization.

SA-PO2467

Serum Vitamin B_{12} and Folic Acid Concentrations and Estimated Glomerular Filtration Rate (eGFR) in the Elderly. Preliminary Results of the Polish Population Study "PolSenior" Andrzej Wiecek, Magdalena Szotowska, Marcin Adamczak, Jerzy Chudek. *Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland.*

Background: During the last years increasing prevalence of vitamin B_{12} and folic acid deficiency is observed in different groups of patients. Taking into consideration important role of vitamin B_{12} and folate in hematopoesis, skin and brain function, such deficiency seems to be one of the crucial issues of the public health. The aim of this study was to examine serum vitamin B_{12} and folic acid concentrations and their relationship with eGFR in representative samples of the elderly population in Poland.

Methods: In 1193 subjects (609 males; 584 females) aged over 65 years (mean age 79±9) from the "Polsenior" study the relationship between vitamin B_{12} or folate serum concentrations and eGFR was calculated. Serum vitamin B_{12} and folate concentration was measured by RIA method. eGFR was estimated using the CKD-EPI formula.

Results: Analysis of variance, between groups stratified according to eGFR with respect of both vitamin B_{12} and folic acid, have showed significant ($p<0.05$) differences [in subject with eGFR 90-120 ml/min (n=199) - 334±769 and 5.63±7.44; eGFR 60-90 ml/min (n=659) - 312±220 and 4.90±4.34; eGFR < 60 ml/min (n=278) - 285±142 pmol/l and 4.59±4.70 nmol/l, respectively]. However no significant correlation was found between age or eGFR and serum vitamin B_{12} or folate concentrations.

Conclusions: 1. Both vitamin B_{12} and folate concentrations decrease with worsening of kidney function in elderly patients. 2. These results suggest a particular need for increased vigilance for deficiency of vitamin B_{12} and folic acid in elderly patients with chronic kidney disease.

Funding: Government Support - Non-U.S.

SA-PO2468

Clinical Characteristics of Chronic Kidney Disease Patients with Hyperkalemia Due to Angiotensin II Receptor Blocker

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Background: Angiotensin II receptor blocker (ARB) is often used in the clinical settings. Most patients with chronic kidney disease (CKD) take ARB for renal and cardiovascular protection. Though hyperkalemia is a life-threatening complication resulting from use of ARB especially in CKD patients, an available way of predicting hyperkalemia remains unknown.

The purpose of our study is to elucidate the characteristics of CKD patients with hyperkalemia due to ARB.

Methods: Twelve consecutive CKD patients with hyperkalemia (≥5.5mEq/l) due to ARB were included in this study. Patients with rennin angiotensin system inhibitor, loop diuretics, thiazide, spironolactone or eprelenone were excluded from the present study. Patients with rapidly progressive glomerulonephritis, acute kidney injury, congestive heart failure and acute coronary syndrome were also excluded. We measured serum potassium levels, serum creatinine levels, blood urea nitrate levels, urinary protein excretion, urinary β_2-microglobrin (U-β_2MG) levels, and urinary N-acetylglucosamine (U-NAG) levels before and after taking ARB. In addition, we calculated fractional excretion of potassium (FEK) and transtublar potassium gradient (TTKG) before taking ARB. In 3 of the 12 patients, we investigated the change of serum potassium levels, FEK and TTKG depending on the dose of ARB. Case-note review enabled us to assess other clinical characteristics of the study patients.

Results: Averaged serum creatinine levels were 2.59±1.37 mg/dl and averaged maximum serum potassium levels were 6.94±1.04 mEq/l. The presence of diabetes and hypertension was 33% and 100%, respectively. Regardless of renal function, U-β_2MG and U-NAG were high in most study patients. Furthermore, FEK and TTKG were relatively low in these patients. They were lowering with increasing the dose of ARB.

Conclusions: In CKD patients with hyperkalemia, TTKG and FEK were low and urinary tubular injury markers were elevated before taking ARB. It is suggested that TTKG and FEK are available as a predictive marker of hyperkalemia due to ARB in CKD patients.

SA-PO2469

Characteristics of Patients with CKD and Anemia Treated with Darbopoetin Who Failed To Maintain Hemoglobin Levels at 4-Week Dosing Intervals

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Background: Resistance to erythropoietin-stimulating agents (ESA) is associated with increased CV risk in renal anemia patients. Darbopoetin alpha therapy may be dosed at 2- or 4-week (wk) intervals based on convenience and other factors. Some patients require a higher monthly dose at 4-wk intervals. We investigated whether characteristics differ between anemic CKD patients requiring ESA dosing at 2- vs. 4-wk intervals.

Methods: Patients who initiated darbopoetin therapy in our renal anemia clinic at 2-wk intervals then switched to 4-wk intervals after Hb stabilized in target range between Sep 2007 and Nov 2009 were included. Patients were considered a failure of 4-wk therapy if their Hb dropped or monthly ESA requirement increased after the switch and were returned to 2-wk dosing. Patients who failed 4-wk dosing were compared to those who did not. We evaluated factors associated with time to failure of 4-wk dosing using Kaplan-Meier survival estimates and Log-rank tests for categorical variables and Cox proportional hazards model for continuous variables.

Results: 186 patients started ESA therapy at 2-wk intervals during the study period. 81 (44%) switched to a 4-wk interval and had continued follow-up (median 5 months). 28 (35%) switched back to 2-wk intervals because of failure to maintain their Hb. At 6 months, fewer kidney transplant patients (25% vs. 76%, p=0.03) and patients on corticosteroids (36% vs. 77%, p=0.01) were at 4-wk intervals. Higher Hb (p=0.003) and eGFR (p=0.003) at switch to 4-wk intervals were associated with lower risk of failing 4-wk interval therapy.

Conclusions: Lower Hb level and eGFR at the time of switch to 4-wk intervals, kidney transplant status and corticosteroid use were significantly associated with failing 4-wk interval ESA therapy. Patients at risk for failure on 4-wk interval therapy should be monitored and considered for dosing interval change if failure occurs.

Funding: Clinical Revenue Support

SA-PO2470

Iron Replacement Therapy for CKD Patients Not on Dialysis

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Background: Standardized protocols have been developed for iron replacement therapy for patients with chronic kidney disease patients (CKDP) maintained on dialysis; the vast majority of hemodialysis patients (pts) receive intravenous (IV) iron. The iron utilization requirements for CKDP-not on dialysis (NOD) have not been well defined. The present study was designed to examine the percent of CKDP-NOD who require iron replacement to maintain their ferritin levels > 100 ng/ml and TSat > 20%.

Methods: 1065 CKDP-NOD who were followed in our clinic for 12 months between 1/08 and 5/11 and had hemoglobin levels and iron studies checked were included. The 12 month follow-up included the first 12 months after 1/08 or the initial 12 mths after initiation of ESA therapy or referral to the CKD clinic. 567 of these pts received ESAs; these pts had Hgb levels checked monthly and iron studies checked every 3 months; iron was prescribed if TSats were below 20% and/or ferritin levels were below 100 ng/ml at any time during the study period. The decision to give IV or po iron was left up to the discretion of the nephrologist but IV iron was prescribed if pts could not tolerate po iron or if the degree of iron deficiency was felt to be severe (TSat < 15% and/or ferritin <70 ng/ml). The 498 pts not receiving ESAs had iron studies checked if Hgb levels fell below 10 gm%

Results: 14.3% of pts who were receiving ESAs were prescribed IV iron. 63% of pts were prescribed oral iron. Of pts not receiving ESAs, 9.1% received IV iron and 22.2% were prescribed oral iron.

Conclusions: In conclusion, iron supplementation is frequently needed in CKDP-NOD receiving ESAs to maintain adequate iron stores. Those pts not receiving ESAs require iron supplementation less often to maintain iron stores and Hgb levels > 10 gm%.

SA-PO2471

Functional Health Literacy in the Chronic Renal Insufficiency Cohort Study

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Background: Low health literacy is associated with increased risk of death, yet the health literacy of individuals with chronic kidney disease (CKD) is rarely assessed.

Methods: We evaluated the prevalence and correlates of low health literacy in a cross-section of 2872 CRIC Study participants. Low health literacy was defined as a Short Test of Functional Health Literacy in Adults (STOFHLA) score ≤16.

Results: Table 1 shows significant differences in demographic and clinical characteristics between participants with low v. adequate health literacy (p<0.05).

Table 1. Demographic and Clinical Characteristics*

	Health Literacy	
	Low	Adequate
	n=640 (22)	n=2232 (77)
Age, yrs, Mean (SD)	64.2 (9)	61 (11)
Male gender	384 (60)	1193 (53)
Income <$20,000/year	393 (61)	451 (20)
Education ≤6th grade	163 (26)	23 (1)
Hispanic Ethnicity	245 (38)	185 (8)
eGFR, ml/min/1.73m2, Mean (SD)	34.4 (16.4)	42.1 (17.7)
Urine Protein g/24 hr, Median (IQR)	0.8 (0.2-2.6)	0.3 (0.1-1.3)
Diabetes	460 (72)	1012 (45)
Self-reported CVD	318 (50)	805 (36)
Left Ventricular Hypertrophy**	171 (75)	181 (47)
Blood Pressure >130/80	358 (56)	949 (43)
Hemoglobin A1C (HbA1C) >7%	202 (33)	377 (18)

*Results are presented as n (%), unless otherwise noted. **Echocardiogram obtained within one year of the STOFHLA. SD, standard deviation; IQR, interquartile range; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease.

In adjusted analyses, low health literacy was a significant predictor of lower eGFR (β coefficient, 2.01 ml/min/1.73m2), HbA1C >7% [odds ratio (OR) 1.6, 95% confidence interval (CI) 1.2-2.2], and higher prevalence of self-reported CVD (OR 1.4, 95% CI 1.1-1.8). Significant predictors of low health literacy were: older age, male gender, Hispanic ethnicity, low educational attainment, and diabetes (p<0.05).

Conclusions: In the CRIC cohort, low health literacy was prevalent and associated with a greater burden of chronic illness. Health literacy interventions may have the potential to improve clinical outcomes in patients with CKD.

Funding: NIDDK Support

SA-PO2472

The Validity of Current CKD Staging Paradigms Revisited and Disputed: A 2-Year Snap Shot of Stage IV CKD Patients in a Mayo Clinic Laboratory Database: A Call for Process Reengineering in Nephrology Practice

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Background: It is generally believed that there is a predictable, linear and time-dependent progressive decline in eGFR, ultimately in some patients, ending inexorably in ESRD and the need for RRT. It is on this premise that the current CKD staging protocols

popularized by the NKF are based. Furthermore, several attempts have been made to fabricate mathematical formulas to accurately predict CKD progression. This author's hypothesis is that all these assumptions remain unproven and factually flawed, and that indeed eGFR in CKD patients, is mostly stable and that often, CKD progression to ESRD follows a staccato and unpredictable pattern precipitated and triggered by acute kidney injury (AKI).

We set out to prove this by analyzing a two-year snap shot of patients with eGFR 15.0-29.9 ml/min/1.73 sq m BSA in a Mayo Clinic electronic laboratory database.

Methods: We pulled by IT reporting, all patients with eGFR in the 15.0-29.9 ml/min/1.73 sq m BSA range in a Mayo Clinic electronic laboratory database, reported between April 19, 2009 and April 19, 2011. All patients who had received RRT for AKI or ESRD were excluded from analysis. We included for analysis, all patients with at least three recorded eGFR values, and with a minimum of 6 months between the first and the last reported eGFR estimations.

Results: After excluding 62 ESRD patients, and all who received RRT for AKI, 241 patients qualified for this analysis. There were 102 males and 139 females. In over 95% of the patients, eGFR remained very stable and did not vary by as much as 5 eGFR points (<25% from baseline) over the two-year study period.

Conclusions: We submit that eGFR in the majority of CKD stage IV patients remains stable even after two years. Current CKD staging paradigms are flawed. Formulas attempting to estimate CKD progression by assuming a steady state decline in eGFR in ml/min/year are likely misguided. Further studies are warranted to properly inform and educate both the medical profession and the general population on these very critical knowledge deficiencies.

SA-PO2473

Effect of Conversion from Other ESAs to CERA Once Monthly for Maintaining Hemoglobin (Hb) Concentration in Pre-Dialysis CKD Patients Ji-Young Choi,[1] Chul Woo Yang,[2] Yeong Hoon Kim,[3] Kwon Wook Joo,[4] Tae-Hyun Yoo,[5] Kang Wook Lee,[6] Sang-Ho Lee,[7] Sug Kyun Shin,[8] Wooseong Huh,[9] Sun-Hee Park,[1] Chan-Duck Kim,[1] Yong-Lim Kim.[1] *[1]Kyungpook National University; [2]Catholic University of Korea; [3]Inje University; [4]Seoul National University; [5]Yonsei University; [6]Chungnam National University; [7]Kyung Hee University; [8]National Health Insurance Corporation Ilsan Hospital; [9]Sungkyunkwan University.*

Background: The purpose of this study was to identify whether Hb concentrations can be maintained stably when switching from other ESAs to CERA.

Methods: Pre-dialysis CKD patients (n=191) maintained Hb level of 10.0-12.0 g/dL through epoetin-α, β or darbepoetin-α were enrolled. Conversion ratio from other ESAs to CERA was represented in Table 1.

Table 1. Conversion ratio from other ESAs to CERA

Previous weekly dose of ESA		Starting dose
Epoetin (IU)	Darbepoetin-α (μg)	CERA (μg/month)
<8,000	<40	120
8,000~16,000	40~80	200
>16,000	>80	360

The dose of CERA was titrated to maintain the Hb within a range of ± 1.0 g/dL of the reference Hb and target Hb levels of 10.0-12.0 g/dL. Hb level and the proportion of patients maintaining average Hb concentration were assessed.

Results: The mean Hb level was 10.86 ± 0.71, 11.87 ± 0.93 and 11.16 ± 0.94 g/dL at baseline, 3 and 6 month, respectively. One hundred eight patients (74.5%) maintained target Hb levels (10.0-12.0 g/dL). The mean monthly dose of CERA was 121.2 ± 9.8, 72.7 ± 46.6, and 66.2 ± 47.1 μg at 1st, 4th, and 6th month, respectively. Patients who received decreased dose of CERA because of higher Hb level than 12.0 g/dL were 119 (82.1%), whereas only 6 patients (4.1%) received increased dose because of Hb values < 10.0 g/dL. Patients with Hb overshoot (Hb > 13.0 g/dL and had to stop CERA until Hb level decreased to < 12.0 g/dL) were 59 (40.7%).

Conclusions: Conversion from other ESAs to CERA in pre-dialysis CKD patients can efficaciously maintain Hb concentration and the dose requirement of CERA had significantly decreased compared with those at conversion. It may be needed to adjust the conversion ratio than recommendation for switching from other ESAs to CERA.

Funding: Pharmaceutical Company Support

SA-PO2474

Pedometer Determined Physical Activity in Children and Adolescents with Chronic Kidney Disease, End Stage Renal Disease and Kidney Transplant Recipients and Association with Physical Performance and Physical Functioning Aalia Akber,[1] Kirsten L. Johansen,[2] Anthony A. Portale.[1] *[1]Pediatrics, UCSF; [2]VAMC, San Francisco, CA.*

Background: Data and recommendations on physical activity (PA) are limited in children with CKD. The objectives of this study were to: 1) measure the level of PA in children with all stages of CKD and compare these with the recommendations for healthy children; 2) to determine patient characteristics associated with PA; and; 3) to determine the association of PA with physical performance (PP)and physical functioning (PF).

Methods: Participants were enrolled from the Pediatric Nephrology and Transplant Clinics at UCSF. PA was measured for 7 days using the Yamax Digi-Walker SW-200 pedometer and expressed as daily step counts; PP was measured by the 6 minute walk test (6MWD), and PF with the self-report PedsQL 4.0. Univariate and multivariable linear regression analyses were used.

Results: We studied 44 participants aged 7-20 years with CKD Stages 1-4 (n=12), with ESRD on hemodialysis or peritoneal dialysis (n=7), and kidney transplant recipients (n=25). As a group, participants were very sedentary, walking 6218 (3637, 9828) steps/d and thus falling short of levels recommended for healthy children, 15,000 steps/d for boys and 12,000 steps/d for girls. There was no difference in PA between the 3 groups. Girls were less active than boys ($p < 0.01$) and PA was 44% lower among young adults (18-20 years) compared with younger age groups ($p<0.05$). PA was associated positively with maternal education and hemoglobin concentration but inversely with BMI. PP in boys and girls were -2 and -4 standard deviations below the expected 6MWD, respectively, with worse performance in older subjects. Low levels of PA were associated with poor PP and PF, after adjusting for age, sex, and BMI.

Conclusions: In most participants with CKD, PA levels were considerably below levels recommended for healthy children, without apparent improvement after transplantation. The associations of decreased PA with BMI, decreased PP, and PF suggest negative consequences to the limited activity of these children. Studies to determine if interventions to increase PA can improve PP and PF are needed.

Funding: Other NIH Support - T-32 National Research Service Award

SA-PO2475

Polymorphisms of UCP2 Gene Are Associated with Carbohydrate and Lipid Metabolic Disorders in Chinese Peritoneal Dialysis Patient Tongying Zhu, Lihua Zhang, Yun Li, Lin Tang, Chuan-Ming Hao. *Department of Nephrology, Huashan Hospital of Fudan University, Shanghai, China.*

Background: We investigated the association of three functional variants of UCP2 polymorphism with metabolic disorder and low-grade inflammation in prevalent Chinese peritoneal dialysis patients.

Methods: 116 prevalent PD patients were enrolled, who were genotyped for the UCP2 variants -866G>A, Ala55Val and 45bp ins/del. HOMA-IR, plasma lipid profile, CRP, ferritin, peritoneal glucose absorption, and adipocytokines were examined and compared in different genotypes.

Results: The patients with GG genotype in -866 G/A gene had a higher serum triglyceride than those with A allele(P=0.011).Patients with VV genotype in Ala55Val gene, have a higher level of serum triglyceride (P=0.005), HOMA-IR (P=0.028), ferritin (P=0.045) and peritoneal glucose absorption (P=0.007), and a lower adiponectin level (P=0.014). GG and VV genotype were also found to be significant predictors of hypertriglyceridemia even after adjusted for glucose absorption (GG:OR=2.441, P=0.031, VV: OR=2.40, P=0.03) by logistic regression analysis. While DD genotype in 45bp ins/del polymorphism had higher triglyceride (P =0.021) and HOMA-IR (P =0.001) than DI/II genotype.

Compared to those with only one or none above inferior genotypes, patients who carrying GG- VV-DD simultaneously showed a significant higher degree of de novo diabetes (6/27 vs. 2/45, P=0.02), higher HOMA-IR (3.93 vs. 2.43,P=0.00) , triglyceride (2.68 vs.1.4mmol/L,P=0.00), cholesterol (5.36 vs. 4.65mmol/L,P=0.01), CRP(5.77 vs1.72mg/L, P=0.018), ferritin (270 vs. 209ug/L, P=0.019), leptin (2.85 vs. 2.05ng/ml, P=0.011), and higher glucose absorption (108.99 vs. 83.67g/day, P=0.00). Logistical regression analysis showed that GG- VV- DD genotype had a significant tendency to become hypertriglyceridemia (OR=5.97, P=0.002) and insulin resistance(OR=3.084, P=0.031) even adjusted for glucose absorption.

Conclusions: PD patients with UCP2 -866G/G, Ala55Val V/V, and 45bp DD genotype demonstrated a significant critical metabolic disorder , suggesting a possible role of UCP2 gene polymorphism in the liability of metabolic disturbance under a high glucose environment.

SA-PO2476

Predictors of Total Sleep Time in Advanced Chronic Kidney Disease Patients Nadeem R. Kolia, Bahar Laderian, Cynthia D. Grady, Mahlet Nega, Enyinna L. Nwachuku, Sarah Ramer, Mark L. Unruh. *University of Pittsburgh School of Medicine.*

Background: Sleep is an important daily activity that aids the body in maintaining homeostatic balance. Sleep deprivation has been linked to increased risk of cardiovascular disease as well as an overall diminished quality of life. This study examined the total sleep time of advanced chronic kidney disease (CKD) and end-stage renal disease (ESRD) patients to determine if modifiable risk factors are associated with reduced total sleep time.

Methods: 88 patients with advanced CKD (stages 4-5) and 93 patients with ESRD on dialysis were compared with 224 controls form the Sleep Strategies-Concentrating on Risk Evaluations study of sleep and cardiovascular risk. All participants underwent home polysomnography. Patient demographics; blood pressure; hypertension, diabetes, and depression status; and anti-hypertensive and anti-depressant medications were also assessed.

Results: The sample (median age 58 years) was 56.3% male and 62.1% white. Total sleep time in minutes differed significantly between controls (365.80±83.19), CKD patients (362.93±100.1), and ESRD patients (318.98±117.46), p=0.001. The percent of the sample with less than 5 hours of sleep also differed significantly between controls (20.1%), CKD patients (27.3%), and ESRD patients (43.2%), p<0.001. In a multivariable linear regression model adjusted for race and hypertension, a 1-year increase in age (-1.44, 95% CI -2.29 to -0.62), female sex (20.75, 95% CI 1.40 to 40.1), presence of sleep-disordered breathing (-0.62, 95% CI -1.08 to -0.16), periodic limb movements index (-2.88, 95% CI -4.52 to -1.23), and ESRD (vs. control) (-45.96, 95% CI -69.7 to -22.3) were all independently associated with total sleep time. The presence of CKD (vs. control) (-10.95, 95% CI -36.4 to 14.5) was not independently associated with total sleep time.

Conclusions: ESRD patients sleep significantly less (on average 46 minutes) compared to controls, possibly contributing to increased risk of cardiovascular disease and reduced quality of life. Addressing sleep-disordered breathing and restless legs, common complaints in ESRD, might lead to increases in total sleep time for these patients.

Funding: NIDDK Support

SA-PO2477

Increased Intrarenal Angiotensin II Activity and Risk of Chronic Kidney Disease Katherine T. Mills, Hiroyuki Kobori, L. Lee Hamm, Arnold B. Alper, Islam Enver Khan, Md Mahfuzur Rahman, Myra A. Kleinpeter, L. Gabriel Navar, Grace Browne, Chung-Shiuan Chen, Yanxi Liu, Ji Hua Xu, Nishant B. Jalandhara, Kanwaljit K. Chouhan, Vecihi Batuman, Jiang He, Jing Chen. *Tulane University.*

Background: Urinary angiotensinogen (UAGT) is a stable biomarker and strongly associated with intrarenal angiotensin II activity. However, less is known about UAGT levels and risk of chronic kidney disease (CKD).

Methods: We investigated urinary and plasma angiotensinogen (PAGT) with risk of CKD in 201 CKD cases and 201 controls without CKD. CKD was defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m² or presence of albuminuria.

Results: Compared to controls, median 24 hour UAGT (44.4 vs. 7.4 ug/24 hr, p<0.0001) and UAGT/creatinine ratio (UCr) (26.3 vs. 4.4 ug/g, p<0.0001) were significantly higher in CKD cases. PAGT was similar between cases and controls (23.3 vs. 22.4 ug/mL, p=0.14). The 24 hour UAGT per 1.73 m² body surface area was highly correlated with the UAGT/UCr ratio (r=0.90, p<0.0001) but not PAGT. Both 24-hour UAGT and the UAGT/UCr ratio were significantly correlated with eGFR and 24-hour urine albumin (p<0.0001). After adjusting for age, gender, race, smoking, drinking, education, physical activity, systolic blood pressure, glucose, LDL cholesterol, and body mass index, the odds ratio (OR) for CKD comparing the highest to the lowest tertile of UAGT was 5.8 (95% CI, 3.0, 11.4). Similar results were seen for tertiles of the UAGT/UCR ratio (OR: 5.8, 95% CI, 3.1, 10.7). Weaker associations were observed for PAGT (ug/ml) with an odds ratio of 3.3 (95% CI, 1.7, 6.3) for the highest compared to the lowest tertile. The results did not change substantially after further adjustment for history of CVD, diabetes, hypertension, and use of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker medications.

Conclusions: These data indicate that UAGT level is associated with an increased risk of CKD and may be a useful biomarker for the prediction of CKD risk. The UAGR/UCr ratio could be used to evaluate 24 hour UAGT. The predictive value of UAGT for the risk and progression of CKD should be examined in a prospective cohort study.

Funding: Other NIH Support - the National Center for Research Resources

SA-PO2478

NGAL and Progression of ADPKD Patients in Stage II-III CKD Grazia Maria Virzì,[1,3] Valentina Corradi,[1,3] Fiorella Gastaldon,[1] Massimo de Cal,[1] Dinna N. Cruz,[1,3] Maurizio Clementi,[2] Claudio Ronco.[1,3] *[1]Nephrology, S Bortolo Hosp; [2]Pediatrics-Clinical Genetics, Padua; [3]IRRIV, .*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous inherited condition with a variable rate of progression.

In addition to NKF-KDOQI guidelines regarding routine screening, recent studies suggest a possible role for NGAL in assessing CKD progression; it could be a good marker also for ADPKD progression.The aim of the study was to evaluate whether NGAL could predict loss of function in ADPKD patients in stage II-IIICKD.

Methods: Pts with ADPKD, based on ultrasound criteria, were enrolled and followed prospectively. Creat and NGAL values were measured at baseline and followed up.

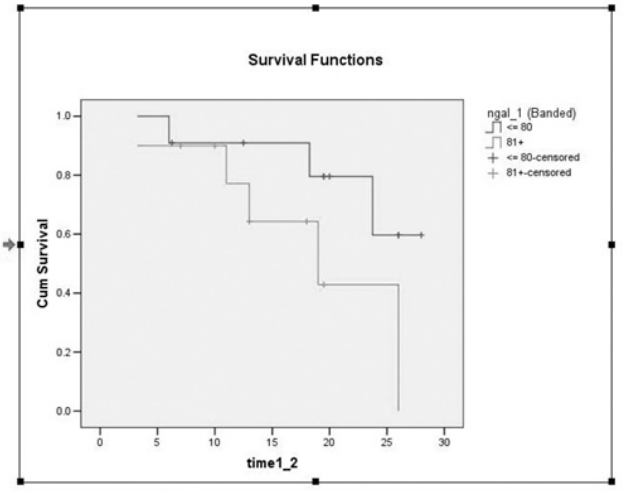
eGFR was calculated with 4-variable standardized-MDRD equations. NGAL was measured in plasma by the Triage®NGALDevice.

Survival data were analyzed by the Kaplan-Meier curve using the median NGAL (80pg/ml) as a cut-off value.

Results: We enrolled 21 ADPKD pts (12M/9F; mean age39±9yrs); mean creat was 1.3±0.7mg/dl and mean eGFR was 67.8±23.3mL/min/1.73m². After a median follow-up of 16.5mo, 8pts (38%) progressed to a worse stage of CKD and 62% were stable.

Kaplan-Meier curves are presented in Fig1.

Fig.1: Kaplan-Meier survival curves in patients with NGAL level above and below the cut-off of 80 pg/ml (median values)



NGAL values inversely correlated with eGFR; eGFR was higher in pts with NGAL values<80pg/ml.

Although, no statistically significant relationship between higher NGAL and decreasing eGFR was observed, a positive trend was evident.

Pts with NGAL values<80pg/ml were followed for a longer period of time-18.7mo versus 14mo for NGAL above the cut off.

Conclusions: We observed a positive trend, but no significant relationship between NGAL and progression of CKD in ADPKD patients in stage II-IIICKD. The present study has limitations: it was a single-center study and the cohort of patients was small. It is necessary to increase the sample size of ADPKD pts enrolled to validate our hypothesis.

SA-PO2479

Urinary Neutrophil Gelatinase-Associated Lipocalin Does Not Correlate with Renal Dysfunction in Polycystic Kidney Disease Graham D. Smith,[1] Caroline M. Robinson,[1] Keith A. Burling,[2] Anthony G. Norden,[2] Richard N. Sandford,[1] Fiona E. Karet.[1,3] *[1]Medical Genetics, University of Cambridge, United Kingdom; [2]Core Biomedical Assay Lab, NIHR Cambridge Medical Research Centre, United Kingdom; [3]Renal Medicine, University of Cambridge, United Kingdom.*

Background: Neutrophil gelatinase-associated lipocalin (NGAL) was first identified as a component of neutrophil granules and has since been found in the majority of tissues. It is implicated in a variety of cellular processes e.g. inflammation and cell survival. It is a 25kDa protein that exists in mono-, homo- and heterodi-meric forms, the latter in association with matrix metalloproteinase 9 (MMP-9).

NGAL has been implicated in pathological conditions and its presence in urine is proposed as a biomarker for various kidney diseases, including Polycystic Kidney Disease (PKD) (1).

Methods: We used commercially available ELISA kits to assess levels of urinary NGAL (uNGAL) or uMMP9/NGAL in adult PKD patients (n=41, mean age 43±10.5 y); creatinine 70-627 µmol/l) compared to matched healthy controls with normal renal function.

Results: We found no differences in uNGAL levels between PKD patients and controls: median 12.5 ng/ml (range 0-60) vs 4.16 ng/ml (0.2-60), p=0.12. In contrast, a cohort of AKI patients had median 234 ng/ml (138-310). Correction of uNGAL for urinary creatinine resulted in a statistically but clinically insignificant difference between PKD and control groups: median 2.1 (0.1-26) and 1.1 (0.02-11) ng/ml, (p=0.013), with AKI patients' median being 20.3 (2.7-51.7) ng/ml.

For uMMP9/NGAL, only negligible amounts were detectable in either patients or controls, with median 0 ng/ml for both groups, range 0-0.203 (patients) and 0-0.395 (controls), p=0.31. We saw no correlation between levels of adjusted uNGAL (r^2=-0.27) or uMMP9/NGAL (r^2=0.13) and degree of kidney dysfunction in the patient population.

Conclusions: The similar ranges of uNGAL or uNGAL/MMP-9 found in patient and normal urine samples, and the lack of change with altered renal function, indicate that neither would be a reliable biomarker for disease progression in PKD, and their utility in a clinic setting are not supportable.

(1) Boligano et al. 27:373-378, 2007

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SA-PO2480

Angiotensinogen Is Filtered through the Glomerulus and Reabsorbed by the Proximal Tubule in Both Mice and Humans Fumio Niimura,[1] Taiji Matsusaka,[2] Takeshi Matsuyama,[3] Shojiro Okamoto,[1] Masuhiro Shimoda,[5] Tomohiro Udagawa,[6] Tae Omori,[7] Iekuni Ichikawa.[4] *[1]Pediatrics, Tokai University School of Medicine, Kanagawa, Japan; [2]Nephrology, Tokai University School of Medicine, Kanagawa, Japan; [3]Pediatrics, Fussa Hospital, Tokyo, Japan; [4]Pediatric Nephrology, Vanderbilt University, Nashville, TN; [5]Pediatrics, Musashino Red Cross Hospital, Tokyo, Japan; [6]Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan; [7]Pediatrics, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan.*

Background: Recently, in liver- or kidney-specific Angiotensinogen (Agt) knockout mice, we demonstrated that circulating Agt of liver origin is filtered through the glomerulus, and reabsorbed by the proximal tubule. This is in contrast to the notion that Agt cannot be filtered through the glomerulus and that Agt in the urine is produced by the proximal tubule (PT).

Methods: To clarify whether Agt is reabsorbed by PT in human subjects, urinary Agt was measured in 12 patients with Dent disease, Lowe syndrome, and idiopathic low molecular weight proteinuria, using human Agt ELISA kit (IBL), developed by Kobori et al. These patients are characterized by inability to reabsorb filtered low molecular weight proteins, resulting in massive urinary excretion of β2 microglobulin (MG) and α1 MG.

Results: In these patients, urinary Agt was markedly elevated, ranging from 1,446 to 33,410 μg/gCr (median, 11,312). These values are far above the published values. Urinary β2 MG in these patients was 18,600 to 173,000 μg /L. Urinary Agt correlated well with urinary β2 MG (r=0.82), α1 MG (r=0.98). In control subjects without proteinuria, urinary Agt, measured concurrently using the same kit, ranged from 11.0 to 108.7 μg/gCr (median, 25.9), values comparable to previously published values, verifying that the assay method was appropriate.

Conclusions: Observed markedly augmented urinary excretion of Agt in patients with PT dysfunction indicates that, in human subjects, Agt is filtered through the glomerular filtration barrier, and reabsorbed by the proximal tubule. Thus, abnormal elevation in urinary Agt excretion reflects either or both disrupted glomerular sieving function and/or defective tubule reabsorptive capacity.

Funding: Government Support - Non-U.S.

SA-PO2481

Renal Uptake of Technetium-99m DMSA Is Mediated by the Megalin/Cubilin Receptor Complex Kathrin Weyer,[1] Rikke Nielsen,[1] Michael Rehling,[2] Henrik Birn.[1] *[1]Department of Anatomy, Aarhus University, Aarhus, Denmark; [2]Department of Clinical Physiology and Nuclear Medicine, Aarhus University Hospital Skejby, Aarhus, Denmark.*

Background: Dimercaptosuccinic acid (DMSA) labeled with technetium-99m (Tc-99m) is the major renal imaging agent used in diagnosis of renal parenchymal disorders. Tc-99m DMSA is highly accumulated in the kidney cortex, but despite the extensive clinical use, the mechanism for renal targeting of the tracer remains elusive. In this study we have tested the role of the proximal tubule endocytic receptor complex megalin/cubilin in the uptake of Tc-99m DMSA.

Methods: Control mice or conditional megalin/cubilin-deficient mice were i.v. injected with the tracers Tc-99m DMSA or Tc-99m MAG3 (mercaptoacetyltriglycine). Six hours post-injection, samples of plasma, urine, and kidneys were collected and analysed using a gamma counter. Whole-body autoradiographies of the mice were made with a gamma-camera.

Results: The renal uptake of Tc-99m DMSA was reduced to 11.4% (+/- 2.5%, n=7) in the megalin/cubilin-deficient mice compared to controls, and a corresponding increase in the urinary excretion of Tc-99m DMSA could be detected. This was further confirmed by whole-body autoradiographies of the mice, where no Tc-99m DMSA activity could be detected in the kidneys of the megalin/cubilin-deficient mice. In contrast, urinary excretion of Tc-99m MAG3 by the megalin/cubilin-deficient mice was similar to control mice, suggesting that the basolateral uptake of the proximal tubule cells is not disturbed by the megalin/cubilin-deficiency.

Conclusions: Our data show that Tc-99m DMSA is taken up by tubular luminal reabsorption mediated by the megalin/cubilin receptor complex and further demonstrate that the tracer distribution is an index of renal proximal tubule endocytic function.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2482

Complement (C) Activation in Renal Ischemia/Reperfusion (I/R) Injury Is Mediated by Pentraxin 3 (PTX3): A Species-Specific Difference G. Castellano,[1] C. Divella,[1] Antonia Loverre,[1] Claudia Curci,[1] Alessandra Stasi,[1] M. Rossini,[1] P. Ditonno,[1] M. Battaglia,[1] Mohamed R. Daha,[2] Pieter van der Pol,[2] Cees van Kooten,[2] G. Pertosa,[1] Loreto Gesualdo,[1] G. Grandaliano,[3] Francesco Paolo Schena.[1] *[1]Dept. of Emergency and Organ Transplantation, Univ. of Bari, Italy; [2]Dept. of Nephrology, Univ. of Leiden, Netherlands; [3]Dept. of Biomedical Sciences, Univ. of Foggia, Italy.*

Background: Next to the short pentraxin CRP and SAP, the long pentraxin PTX3 is characterized by a different gene organization and cellular source. PTX3 has been recently implicated in the priming of vascular inflammation via C activation. Aim of this work was to investigate the possible involvement of PTX3 in renal I/R injury.

Methods: Two experimental models of I/R were used: 6 rats and 5 pigs underwent 45' (rat) and 30' (pig) of renal warm I by clamping renal artery. Renal tissues were analyzed at different time points.

Results: In the pig model, confocal microscopy demonstrated PTX3 deposits already at 15' of R, localized at peritubular (T0 .1±.05, T15 7.7±1.1; p=.005) and glomerular (T0 .5±.2, T15 8.2±2.5; p=.01) capillary levels, showing a specific co-localization with CD31, an endothelial cell marker. We observed a significant increase in infiltrating interstitial leucocytes, including $CD163^{+}/PTX3^{+}$monocyte-macrophages (T0 .01±.02, T15 6.2±2.1; p=.01) and $SWC3a^{+}/PTX3^{+}$dendritic cells (T0 .5±.3, T15 3.7±.5 p=.04). Finally, we identified interstitial $FSP1^{+}/PTX3^{+}$myofibroblast (T0 .3±.2, T15 4.1±1.3; p=.03). Co-localization between C5b-9 and PTX3 on renal endothelial cells clearly demonstrated the C activation in the presence of PTX3 deposits. On the contrary, the analysis of rat kidneys showed that PTX3 was not modulated by I/R injury. Rat PTX3 specifically co-localized only with alpha-SMA positive cells at the vascular level without any association with infiltrating leukocytes and C activation

Conclusions: This study first demonstrates a significant difference in PTX3-mediated C activation between pig and rat kidney. Since PTX3 can activate the Classical and Lectin pathways of C via specific binding with C1q and MBL, we hypothesize that PTX3 might be a new therapeutic target to prevent C-induced renal I/R injury

Funding: Government Support - Non-U.S.

SA-PO2483

Coagulation and Complement Cascade Priming Induces NOX4 Activation in Renal Ischemia-Reperfusion (I/R) Injury S. Simone,[1] F. Rascio,[1] Antonia Loverre,[1] G. Castellano,[1] C. Cosola,[1] P. Ditonno,[1] Francesco Paolo Schena,[1] Loreto Gesualdo,[1] G. Pertosa,[1] G. Grandaliano.[2] *[1]Dept of Emergency and Organ Transplantation, University of Bari, Bari, Italy; [2]Dept of Biomedical Sciences, University of Foggia, Italy.*

Background: Renal I/R plays a key role in the pathogenesis of delayed graft function after renal transplantation and is characterized by a cascade of inflammatory events, including an increased reactive oxygen species generation. The complement and coagulation cascades has been suggested to play a pathogenic role in I/R-induced renal damage. Aim of the study was to investigate the activation of NADPH oxidases in a pig model of renal I/R injury focusing on the coagulation and complement systems.

Methods: Renal I/R was induced in 5 pigs by arterial clamping. The NADPH oxidase activity was assessed by chemiluminescence on renal tissue taken before ischemia (T0) and a different time after reperfusion (T15', 30', 60'). NOX4 protein expression and fibrin deposition were evaluated by immunohistochemistry. In vitro, NOX4 protein expression was assessed by immunoblotting in human proximal tubular epithelial cells (HK2) treated with thrombin (5*10-3U/μl) or C3a (5*10-7M) for different time periods.

Results: NADPH-oxidase activity was significantly increased during reperfusion in a time-dependent manner with a peak at T60 (T0 1.3±.4, T60 5.3±2.0 ΔURL/Dt, p=.03). We observed a significant increase in tubular NOX-4 expression (T0 5.1±.8, T30' 18.6±.5, pixels/total area, p=.03) and interstitial fibrin (T0 2.2±.7, T30' 10.3±.3 pixels/total area, p=.03) and C5b9 deposition (p=.03), all with a peak at T30'. NOX4 expression and fibrin or C5b9 deposits co-localized at the different time points. In addition we observed a significant and direct correlation between fibrin or C5b9 deposition and NOX4 protein expression (r=.86, p<.001; r=.58,p=.01). In vitro, thrombin and C3a induced NOX4 expression in HK2 cells in a time-dependent manner with a peak at T15'(p=.02).

Conclusions: NOX4 is activated during I/R; coagulation and complement cascades may play a role in NOX4 activation. Coagulation, complement and NADPH oxidase may represent a pharmacological target to prevent oxidative damage during I/R injury.

Funding: Government Support - Non-U.S.

SA-PO2484

Role of the Uremic Solute Indoxyl Sulfate on Tissue Factor Production Via Aryl Hydrocarbon Receptor Pathway Bertrand Gondouin,[1,2] Claire Cerini,[1] Laetitia Dou,[1] Ariane Duval-Sabatier,[1,2] Philippe Brunet,[1,2] Stephane Burtey.[1,2] *[1]INSERM U608, Université Aix-Marseille - UFR Pharmacie, Marseille, France; [2]Centre de Nephrologie et Transplantation Renale, Hôpital La Conception, Marseille, France.*

Background: Endothelial dysfunction is implicated in the high cardiovascular mortality seen in CKD patients. The uremic solute indoxyl sulfate (IS) is particularly deleterious for the endothelium and IS levels are correlated with cardiovascular mortality. Tissue factor (TF) is a key initiator of coagulation and an actor in atherogenesis. TF plasma levels are elevated in CKD patients, but the mechanisms involved are unknown. Therefore, we have performed a clinical and in vitro study to assess the potential role of IS on TF production and the cellular pathways involved notably the AHR pathway.

Methods: Plasma IS and soluble TF (sTF) levels were measured in 72 hemodialysis (HD) patients, 50 undialyzed CKD patients (CKD) and 37 control subjects (controls).

We studied in vitro the effect of IS at uremic concentrations on TF production and procoagulant activity in cultured endothelial cells (HUVEC) and leukocytes (PBMC). Finally, we studied the involvement of aryl hydrocarbon receptor (AHR) in this TF production using siRNA experiments.

Results: sTF levels were respectively 142±48 pg/mL in HD, 77±66 pg/mL in CKD and 36±14 pg/mL in controls, with significant differences between all groups. In CKD patients, sTF levels were negatively correlated with eGFR (r= - 0,333, p < 0,05) and positively correlated with IS (r= - 0,333, p < 0,05).

In vitro, IS increased TF protein levels and TF-dependent procoagulant activity in HUVEC and PBMC.

This increased TF production is preceded by increased mRNA levels suggesting an elevated transcriptional activity. siRNA directed against AHR abolished the increase in TF induced by IS.

Conclusions: The increase in sTF in CKD patients is related to renal function and levels of IS. In vitro, IS induces TF production in HUVEC and PBMC, which involves the transcription factor AhR.

As AHR and TF have a demonstrated role in atherogenesis, IS could thereby contribute to the high cardiovascular risk observed in uremic patients.

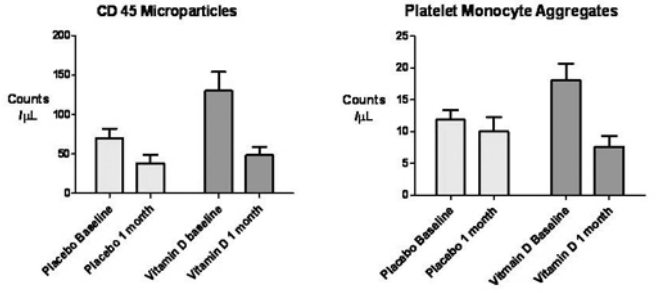
SA-PO2485

Ergocalciferol Significantly Reduces Markers of Vascular Inflammation in Patients with Chronic Kidney Disease Stage 3-4 Gavin Dreyer,[1] Marion Macey,[2] Martin J. Raftery,[1] Magdi Yaqoob.[1] *[1]Renal Unit, Royal London Hospital, United Kingdom; [2]Haemtology Unit, Royal London Hospital, United Kingdom.*

Background: Chronic kidney disease (CKD) causes vascular inflammation and endothelial dysfunction leading to cardiovascular disease (CVD). Newer vitamin D analogues may reduce the burden of CVD in CKD but are expensive and the mechanism of action is incompletely understood. We conducted a pilot, randomised, placebo controlled trial evaluating the effect of ergocalciferol, an inexpensive vitamin D compound, on known markers of vascular inflammation in CKD 3-4.

Methods: Patients with CKD stage 3-4 (n=13) were enrolled. Baseline samples for flow cytometry quantified platelet monocyte aggregates (PMA) and CD 45 microparticles (CD45MP), both markers of vascular inflammation. Patients were randomised to ergocalciferol (n=7) 50,000iu weekly for 4 weeks or placebo (n=6). Patients with diabetes mellitus were excluded to remove the confounding effect of diabetes on the endothelium. Samples were taken at baseline and repeated after 1 month.

Results: The mean age, eGFR, blood pressure, ethnicity and tobacco use did not differ between treatment groups with CKD. PMA and CD45MP were similar between placebo and ergocalciferol treated CKD patients at baseline (PMA - p=0.056, CD45MP - p=0.41). At 1 month, PMA and CD45MP were significantly lower in the ergocalciferol group (p=0.002 and p=0.007) but unchanged in the placebo group.



Conclusions: Ergocalciferol reduces markers of vascular inflammation in CKD 3-4. These findings may explain the reduction in CVS disease in previous studies of patients with CKD treated with vitamin D compounds but could also reflect the unique effect of ergocalciferol itself. Multi centre clinical studies are required to further evaluate the role of ergocalciferol therapy to reduce CVD in CKD and to better understand its precise mechanism of action.

SA-PO2486

The Correlation between Albumin to Creatinine Ratio and Total Protein to Creatinine Ratio in Patients with Chronic Kidney Disease Chun Soo Lim,[1, 2] Yon Su Kim.[2] *[1]Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Republic of Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background: The quantification of urinary protein excretion is important for monitoring of chronic kidney disease (CKD). Random urine total protein-to-creatinine ratio and albumin-to-creatinine ratio have been supposed as alternatives to 24 h urine measurements.

Methods: A total of 808 patients were prospectively enrolled from renal out-patient clinics. We measured albumin-to-creatinine ratio, protein-to-creatinine ratio and urine dipstick test simultaneously in random urines, and we investigated the relation between albuminuria and proteinuria in random urine samples in CKD patients.

Results: Albuminuria was well correlated with proteinuria [$\beta = 1.114$ (95% CI 1.061 to 1.166), $P < 0.001$, $R2 = 73.6\%$]. The correlation between albuminuria and proteinuria was significantly better in patients with dipstick albumin positive than those with negative ($P < 0.001$ for interaction), in patients with urine creatinine level ≥ 60 mg/dL than those with < 60 mg/dL ($P = 0.024$ for interaction), and in patients with estimated GFR < 60 mL/min/1.73m2 than those with ≥ 60 mL/min/1.73m2 ($P = 0.040$ for interaction). However, the correlation between albuminuria and proteinuria was not different according to the sex ($P = 0.941$), status of diabetes mellitus ($P = 0.820$), and age (≥ 60 years vs < 60 years) ($P = 0.706$).

Conclusions: The determination of proteinuria or albuminuria in random urine samples, when properly interpreted by taking into consideration the urine concentration, the amount of proteinuria and renal function, could be acceptable for the measure of monitoring of proteinuria or albuminuria.

SA-PO2487

Polyclonal Immunoglobulin Free Light Chains and hs-CRP Kinetics in Inflammatory and Renal Scenarios Anne Bevins,[1] Lakhvir Assi,[1] Paul Cockwell,[2] Richard Hughes,[1] Colin A. Hutchison.[2] *[1]The Binding Site Group Ltd, Birmingham, United Kingdom; [2]Renal Unit, University Hospital Birmingham, United Kingdom.*

Background: Chronic kidney disease (CKD) patients are at increased risk of infection & cardiovascular disease (CVD). Currently patients can be risk stratified with high sensitivity C-reactive protein (CRP), a robust marker of innate immunity. We hypothesized that if a marker of adaptive immunity was also studied, further prognostic information could be gained. The purpose of this study was to determine the relationship between CRP and a potential marker of the adaptive immune response (polyclonal free light chains (FLC)).

Methods: Four cross-sectional renal cohorts were studied; CKD (n=871) diabetes (n=736), vasculitis (n=68) and renal transplant recipients (n=399). Serum CRP levels were correlated to polyclonal FLC levels (κ+λ, Freelite™) and FLC production rates ([κ+λ]/GFR), these compared to a healthy control group (n=108). This was further explored longitudinally in an intensive care case series (n=11).

Results: The correlations between CRP and both total FLC and FLC production rates were weak across all renal cohorts: CKD 0.328 & 0.299; diabetes 0.185 & 0.162; vasculitis 0.273 & 0.260; and renal transplant recipients 0.152 & 0.151, respectively. These correlations were weaker in the diabetics and transplant recipients than the CKD population (all p<0.01). There were no significant differences in correlation of CRP with total FLCs between the control population and all renal cohorts, however correlation with FLC production was significantly stronger in the CKD population (p=0.016). Serial samples collected from ICU patients displayed elevated CRP levels at presentation, and a delayed increase in FLC concentration (8/11 patients). The three patients that did not display increased FLC were admitted to ICU for non-inflammatory diagnoses, including respiratory failure, asthma, and acute kidney injury.

Conclusions: These results demonstrate clear differences in response kinetics of CRP and FLCs to inflammatory insult and disease. Potentially use of CRP and FLCs, as markers of innate and adaptive immunity, can provide a novel insight into inflammation and allow risk stratification.

SA-PO2488

Immunohistochemical Analysis of Vasohibin-1 Using Renal Biopsy Specimens of Patients with Renal Disorders Norikazu Hinamoto,[1] Yohei Maeshima,[1] Daisuke Saito,[1] Hiroko Yamasaki,[1] Hiroyuki Watatani,[1] Haruyo Ujike,[1] Shinji Kitamura,[1] Hitoshi Sugiyama,[1] Naoki Kanomata,[2] Hikaru Sonoda,[3] Yasufumi Sato,[4] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ. Graduate School of Medicine, Okayama, Japan; [2]Pathology, Kawasaki Medical School, Kurashiki, Okayama, Japan; [3]Discovery Research Laboratories, Shionogi, Settsu, Osaka, Japan; [4]Vascular Biology, Tohoku Univ., Sendai, Miyagi, Japan.*

Background: Glomerulosclerosis and tubulointerstitial injuries are involved in the progression of renal disorders. Vasohibin (VASH)-1 serves as a negative feedback regulator of angiogenesis and we recently reported the therapeutic efficacy of VASH-1 in mouse diabetic nephropathy models (Diabetes, 2009, AmJPhys-Renal, 2011). The aim of the current study is to examine the renal levels of VASH-1 in patients with renal disorders and to evaluate its clinical usefulness.

Methods: We performed immunohistochemistry for VASH-1 and CD31 using renal biopsy sections obtained from 54 patients (glomerulonephritis, glomerulosclerosis, diabetic nephropathy etc.) and 6 renal sections obtained from normal portions of surgically nephrectomized kidneys (renal cell carcinoma). Correlation between histological parameters and the number of VASH-1$^+$ cells was examined.

Results: VASH-1 was observed in renal endothelial cells of normal subjects and additionally in mesangial cells, crescent, and in interstitial inflammatory cells in subjects with renal diseases. Significant positive correlation was observed between the number of VASH-1$^+$ cells in each renal compartment and plasma levels of VASH-1. Significant positive correlation was observed between 1) crescent formation and the number of VASH-1$^+$ cells in glomerulus (r=0.48, P=0.001) or cortex (r=0.64, P<0.0001), and 2) interstitial cell infiltration and the number of VASH-1$^+$ cells in cortex (r=0.34, P=0.02). The number of VASH-1$^+$ cells in glomerulus did not correlate with glomerular CD31$^+$ endothelial area.

Conclusions: These results suggest that Vasohibin-1 may be associated with crescentic lesion and interstitial inflammatory cell infiltration in renal disorders, thus implicating its potential to serve as a novel renal biomarker.

SA-PO2489

Clinical Utility of Tests for Hepatitis C Surveillance David B. Van Wyck, Nancy Culkin, Marc Eckhert, Eileen J. Peacock, Kathy Holland, Timothy Sherrill. *DaVita Inc, Denver, CO.*

Background: KDIGO, KDOQI and CDC recommendations specify retesting seronegative patients every 6-12 months for the surveillance of hepatitis C (HCV) infection in hemodialysis (HD) facilities. However, the clinical utility of recommended tests has not been examined in large populations.

Methods: We initiated baseline and annual surveillance in 104,129 in-center HD patients for the presence of antibodies to HCV using a screening chemiluminescent immunoassay. Consistent with guidelines, we considered patient specimen signal/cutoff ratios of <0.8 to be nonreactive (presumed negative), ratios ≥11 to be reactive without recommendation for supplemental testing (presumed positive), and ratios of 0.8 to < 11.0 equivocal (0.8 to <1.0) or reactive (1.0 to <11) with a recommendation for supplemental testing using recombinant immunoblot assay (RIBA) for confirmation of HCV antibody. RIBA results were reported as positive (confirmatory), negative or indeterminate. From 11/2009 to 4/2011, we tested new patients and those previously found to be HCV negative.

Results: Overall HCV prevalence by screening was 7.4%. Among patients previously nonreactive for HCV, 0.099% showed reactive results ≥11 on follow-up surveillance testing. 1.4% underwent supplemental testing for RIBA: of these, 22.2% were confirmed positive; 41.7% were found to be negative; and, 36.1% returned indeterminate results. No previously nonreactive patient was found to be positive by RIBA supplemental testing. Among patients previously reactive ≥11, 0.078% were nonreactive on follow-up, and 0.223% showed results in the 1.0 to <11 range.

Conclusions: Among HCV-positive patients, we found evidence that reactivity in HCV screening antibody assays wanes or fluctuates. Since the resulting pre-test probability of new-negative results in previously seropositive patients approaches that of new-positive results in previously seronegative patients, our findings suggest that HCV surveillance in seronegative patients should be conducted no more frequently than annually, and that new positive results in HD patients should be interpreted with caution and case-by-case clinical adjudication.

Funding: Clinical Revenue Support

SA-PO2490

Correlation between the Renal Resistive Index and Histology Steven Grange,[1] Fabien Soulis,[1] Dominique Guerrot,[1] Arnaud Francois,[2] Caroline Freguin,[1] Michel R. Godin,[1] Bruno Legallicier.[1] *[1]Nephrology, Rouen University Hospital, Rouen, France; [2]Pathology, Rouen University Hospital, Rouen, France.*

Background: Renal interstitial fibrosis (IF) and arteriosclerosis are determinants of CKD progression. Intra-renal arterial resistance index (RI), a simple non-invasive and reproductive ultrasonographic tool, has become increasingly used in CKD patients. Studies evaluating associations of RI with robust histological variables and renal prognosis are lacking. The objective of this prospective study was to analyze correlations between RI and renal histomorphological measurements, and predictive value for CKD progression.

Methods: Patients hospitalized for renal investigation (n = 47) underwent RI measurement (3.5 MHz) in interlobar arteries and own kidney biopsy. Arteriosclerosis, interstitial surface and IF were assessed by standardized morphometric analyses of blinded Sirius Red (SR) stained slides, under normal and polarized light. MDRD eGFR was measured at M0, M12, M24 and M36. CKD progression combined endpoint was defined as ≥ 10% decrease in eGFR, dialysis requirement or death.

Results: Mean RI was 0.65 ± 0.08. RI was positively correlated with interstitial surface and negatively with eGFR at M0, M12, M24, and M36 (all p<0.05). Interlobar RI was moderately associated with interlobular arteriosclerosis (p<0.09), suggesting that thickening of distal arteries may influence RI. At M36, 14 patients reached the progression endpoint (eGFR decrease: n=5, dialysis: n=5, death: n=4). Compared to stable patients, subjects with CKD progression presented significantly higher RI and interstitial surface but reduced IF (all p<0.05), suggesting that increased RI could be related in this population to interstitial infiltration and active extracellular matrix production (type IV collagen, proteoglycans and laminins), and not to chronic accumulation of type I and III collagen (SR +).

Conclusions: In CKD patients, resistance index is associated with renal interstitial and arterial lesions. Active interstitial lesions rather than chronic interstitial fibrosis, seems to be a major determinant of high RI in patients with CKD progression, and further suggest the RI predictive potential in this setting.

SA-PO2491

Correlation between the Renal Resistive Index and Graft Histology Steven Grange,[1] Fabien Soulis,[1] Dominique Guerrot,[1] Arnaud Francois,[2] Caroline Freguin,[1] Michel R. Godin,[1] Bruno Legallicier.[1] *[1]Nephrology, Rouen University Hospital, Rouen, France; [2]Pathology, Rouen University Hospital, Rouen, France.*

Background: The progressive loss of renal allograft function is related to interstitial fibrosis (IF) and arteriosclerosis. Intra-renal arterial resistance index (RI) has become increasingly used in kidney transplantation. Large studies evaluating associations of RI with robust histological variables and the evolution of graft function are lacking. The objective of this prospective study was to analyze correlations between RI and renal histomorphological measurements, and their predictive value for graft function.

Methods: At inclusion 105 kidney transplant patients underwent RI measurement (3.5 MHz) in interlobar arteries, and renal graft biopsy. Arteriosclerosis, interstitial surface and IF were assessed by standardized morphometric analyses of blinded Sirius Red (SR) stained slides, under normal and polarized light respectively. MDRD eGFR was measured at M0, M12, M24 and M36. Progression combined endpoint was defined as ≥ 10% or ≥ 20% decrease in eGFR at M12 or M24 respectively, dialysis requirement or death.

Results: Patients were included 23±36 months post-transplantation. Mean RI was 0.68±0.08. RI was negatively correlated with eGFR at M0, M12, M24, and M36 (all p<0.05). At M24, 30 patients reached the progression endpoint (eGFR decrease: n=10, dialysis: n=18, death: n=2). After exclusion of patients with acute graft failure and/or rejection (conditions associated with interstitial edema), RI was positively correlated with interlobular arteriosclerosis, and subjects with progressive loss of graft function presented higher RI and interstitial surface (including cells and type IV collagen, laminins and proteoglycans) but reduced IF evaluated by the only detection of type I and III collagens (SR+) (all p<0.05).

Conclusions: In kidney transplant patients, resistance index is associated with interstitial and arterial lesions and predicts progressive loss of allograft function. Our results suggest that interstitial infiltration and active extracellular matrix production, rather than chronic interstitial deposition of fibrillar collagens, are a major determinant of high RI in this setting.

SA-PO2492

Vascular Sclerosis in Tumor Nephrectomy Specimens Has Prognostic Significance Steven P. Salvatore,[1] Eugene K. Cha,[2] James S. Rosoff,[2] Surya V. Seshan.[1] *[1]Pathology, Weill Cornell Medical College, New York City; [2]Urology, Weill Cornell Medical College, New York City.*

Background: Evaluating non-tumor portions of tumor nephrectomies is useful to diagnose non-neoplastic renal disease. The purpose of this study is to determine the medical renal disease frequency and to assess the prognostic significance of the degree of vascular sclerosis in long term follow-up of tumor nephrectomy patients.

Methods: We reviewed non-neoplastic kidney sections of 465 cases from 1998 to 2008. Sixty-four cases were excluded (18 tumor compression, 25 no non-neoplastic, 21 embolized). PAS staining, immunofluorescence, and/or electron microscopy was performed where appropriate. Vascular sclerosis was scored from mild to severe. Follow-up of at least 1 year was evaluated in 178 cases. The degree of vascular sclerosis was compared to the change in pre-op creatinine to follow-up.

Results: Of 401 cases, 59 had additional medical renal disease (15%): diabetic nephropathy 29, hypertensive nephropathy 13, FSGS 7, collapsing glomerulopathy 2, granulomatous tubulointerstitial nephritis 2, acute pyelonephritis, TMA, atheroembolic disease, reflux nephropathy, proliferative glomerulonephritis, and membranous glomerulonephritis 1 each. 80% of the cases with additional non-neoplastic diagnoses and follow-up showed severe arteriolosclerosis. Higher corresponding global glomerulosclerosis (GS) was seen in the more affected vascular cases, mean 3.6% GS for mild versus 15% GS for severe. Three patients progressed to end stage renal disease from 1 to 4 years after nephrectomy, all with diabetic nephropathy.

Degree of vascular sclerosis compared with mean change in creatinine

Arterioles	N	mean Cr difference (mg/dL)	p value	mean f/u (yrs)
mild	89	0.2	0.002	3.6
moderate	55	0.4	0.019	3.7
severe	34	0.8	n/a	3.9
Arteries				
mild	88	0.3	0.04	4.0
moderate	60	0.4	0.09	3.1
severe	30	0.7	n/a	3.6

Conclusions: Medical renal disease was identified in 15% of tumor nephrectomy specimens in our center. The degree of vascular sclerosis is highly predictive of elevated creatinine levels and decline in renal function in post-nephrectomy patients. The prognostic implications of the non-tumor pathology are therefore important in patients with reduced renal mass.

SA-PO2493

Plasma Cell-Rich Acute Rejection Has a Higher Graft Failure Rate Compared to Antibody Mediated Rejection of Renal Allografts Sharad Sathyan, Thangamani Muthukumar, Marie Matignon, Manikkam Suthanthiran, Surya V. Seshan.

Background: Acute rejection (AR) is classified as T cell or antibody mediated. Rarely, interstitial infiltrates during AR are enriched with plasma cells. Graft outcome following an episode of such plasma cell-rich acute rejection (PCR-AR) has not been well defined.

Methods: We reviewed charts of kidney recipients with biopsies classified as PCR-AR (plasma cells >20% of the interstitial infiltrates), from 1999-2010. Primary outcome was graft loss. Patients who did not reach the primary outcome were censored at their last follow up. We compared their graft survival with a cohort of kidney recipients with acute antibody mediated rejection (AMR).

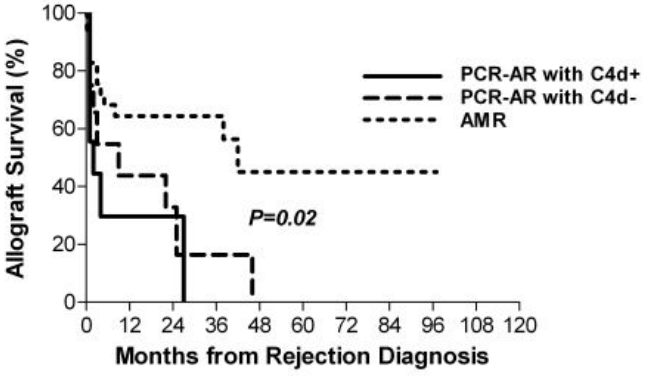
Results: 26 kidney recipients had PCR-AR (mean age: 41±16 years, 14 females, 13 Blacks, 10 deceased donors). Immunosuppression was CNI/steroid based in 14, steroid free in 9, and sirolimus based in 3. None had BKV or CMV infection. 11 (42%) had prior rejections.

PCR-AR occurred 39 months median (range: 1-144) after transplant. At rejection, creatinine was 4.21 (2.0-35.6) and Prograf trough was 3.4 (2.0-16.6). Prograf was 5.2 (2.0-9.5) 3-months prior.

Biopsies showed both lymphocytic and plasma cell tubulitis (moderate-10 (38%) and severe-13 (50%)). Interstitial fibrosis was mild in 6 (23%), moderate in 13 (50%) and severe in 5 (19%). C4d was available in 21 (81%) of which 9 (43%) were positive.

Ten (38%) were treated with steroids alone, 11 (42%) with IVIG/plasmapheresis, and 11 (42%) with antibody based therapy. 2 did not receive specific therapy.

21 lost their graft. Median graft survival was 5 months after PCR-AR.



Compared to a cohort of 29 kidney recipients with AMR (all C4d+ and matched for time to rejection), allograft survival in PCR-AR was significantly worse whether they were C4d positive or not.

Conclusions: Plasma cell rich AR has an inferior allograft outcome compared to AMR.

SA-PO2494

Potential Role of Alternatively Activated Macrophages in Chronic Allograft Nephropathy Yohei Ikezumi,[1] Toshiaki Suzuki,[1] Tamaki Karasawa,[1] Hiroya Hasegawa,[1] Masanori Hara,[2] Toshio Yanagihara,[2] David J. Nikolic-Paterson,[3] Makoto Uchiyama.[1] *[1]Department of Pediatrics, Niigata University Medical and Dental Hopital; [2]Department of Pediatrics, Yoshida Hospital, Niigata-City, Japan; [3]Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.*

Background: Interstitial fibrosis is an important mechanism in chronic allograft nephropathy (CAN). Macrophages with an alternatively activated phenotype (M2) have been implicated in promoting fibrosis in non-inflammatory kidney diseases, including cyclosporine toxicity. This study examined the possible involvement of M2-type macrophages in CAN.

Methods: A total of 30 biopsy sections from 14 children who underwent kidney transplantation were stained for α-smooth muscle actin (αSMA), CD68 (total macrophages), CD163 (M2 marker) and CD3 (T cells). Urinary CD163 excretion was assessed by ELISA.

Results: None of the patients examined had a history of clinical symptoms of rejection. At the time of biopsy, all patients were on maintenance immunosuppression therapy, including methylprednisolone, mycophenolate mofetil, cyclosporine and/or tacrolimus. Immunostaining identified a significant increase in interstitial fibrosis with accumulation of CD68+ macrophages. Dual immunofluorescence staining showed that most interstitial CD68+ macrophages also expressed CD163, indicating an M2 phenotype. CD163+ cells were frequently localized to areas of interstitial fibrosis and there was a significant correlation between the number of interstitial CD163+ cells and the degree of interstitial fibrosis ($r=0.71$, $p<0.0001$), and between interstitial fibrosis and estimated GFR ($r=-0.79$, $p<0.0001$). There was also a significant correlation between interstitial fibrosis and the time from transplantation to biopsy. Although CD3+ T cell infiltration was also observed in some patients, there was no relationship with macrophage accumulation or fibrosis. Urine CD163 levels correlated with the number of interstitial CD163+ cells ($r=0.51$, $p<0.01$).

Conclusions: Our findings identify a major population of M2-type macrophages in patients with CAN, and suggest that these M2-type macrophages may promote the development of interstitial fibrosis in CAN.

Funding: Government Support - Non-U.S.

SA-PO2495

Peritubular Capillaritis in Renal Biopsies of Early Acute Rejection Episodes Hanneke De Kort,[1] Marian C. Van Groningen,[1] Marko J. Mallat,[1] Natascha Goemaere,[2] Johan W. De Fijter,[1] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Leiden University Medical Center; [2]Pathan Foundation Rotterdam.*

Background: A consensus for the ptc-score was reached at the Banff Conference in '07, although it was emphasized that it did not equate with any specific diagnosis, and ongoing reproducibility and diagnostic studies were required. The ptc-score involves the scoring of cortical peritubular capillaries for capillaritis (defined as luminal inflammation by neutrophils, monocytes/macrophages, and lymphocytes). We assessed clinical relevance and inter-observer agreement of the ptc-score.

Methods: We reviewed all 723 patients who received a renal transplant in our center from '95-'06. 122 patients who had a histologically proven first acute rejection episode within 6 mo after transplantation according to the Banff '08 working proposal for classification were included. Mean follow-up was 7.3 yrs (+/-3.9). In Kaplan-Meier survival analyses, time to graft failure and patient death were assessed for the combined ptc grades 0&1 versus 2&3 and for mononuclear versus mixed ptc-infiltrate. All Banff components were scored by 2 pathologists. Ptc-score was according to the Banff '07 report and scored by 2 pathologists independently.

Results: The inter-observer agreement on several aspects of the ptc-score gave poor to fair κ results. The dichotomized ptc-score gave a fair κ of 0.231 ($p=0.004$). Difficulties in identifying peritubular capillaries, especially in areas with vast infiltrates, probably lie at the basis of the inter-observer discrepancy. Ptc-grading was associated with interstitial infiltrate (OR 2.9 [CI 1.1-7.6], $p=0.029$) and the total inflammation-score (OR 7.5 [CI 2.7-20.3], $p=0.001$). Ptc grading did not correlate with C4d staining (performed on frozen tissue samples). Ptc grading did not predict for graft outcome. However, both ptc grades 2&3 and a mixed composition of the infiltrate were associated with worse patient survival.

Conclusions: In this study, ptc correlates with interstitial and total inflammation scores, and in itself, correlates with worse patient survival. To improve the applicability in clinical pathological analyses, we propose a reduction in the components of the ptc grading scheme.

SA-PO2496

Morphometric Quantitation of Interstitial Fibrosis and Peritubular Capillary Density in Renal Allograft Biopsies with Transplant Glomerulopathy Kuang-Yu Jen, Sindhu Chandran, Flavio G. Vincenti, Zoltan G. Laszik. *University of California San Francisco, San Francisco, CA.*

Background: Loss of peritubular capillaries (PTCs) is considered to play an important role in chronic antibody-mediated rejection (AMR) of renal allografts. However, the precise relationship between the severity of interstitial fibrosis (IF) and the degree of PTC loss has not been well-established. The objective of our study is to evaluate the relationship of PTC density and the degree of IF in C4d-positive and C4d-negative renal allograft biopsies with chronic AMR in the form of transplant glomerulopathy (TxGP).

Methods: C4d-positive (n=12) and C4d-negative (n=21) renal allograft biopsies with TxGP were examined in this study. Paraffin sections were stained with a mixture of anti-CD34 and anti-collagen type III primary antibodies followed by incubation with a mixture of fluorescein isothiocyanate (FITC)-labeled and Texas Red-labeled secondary antibodies. Digital images of multiple cortical fields were taken from each double-stained slide at 10x magnification under immunofluorescence microscopy. The images were processed using the ImageJ software to calculate the cortical fractional area of collagen and CD34 positivity. Glomeruli and large vessels were excluded from the analysis.

Results: Strong and specific staining for CD34-positive PTCs and interstitial collagen was achieved. Comparing cortical fractional area of collagen positivity to CD34 positivity for C4d-negative and C4d-positive cases indicated a correlation coefficient of -0.84 and -0.67, linear regression slope of -0.58 and -0.39, and R-squared values of 0.70 and 0.45, respectively. A few C4d-positive biopsies show disproportionately low PTC density, which contributed to a less steep slope by linear regression and a lower correlation coefficient and R-squared value.

Conclusions: Morphometric analysis using immunofluorescence staining for collagen type III and CD34 provides precise quantitative measurements of IF and PTC density, respectively. In allograft biopsies with TxGP, there is an inverse relationship between IF and PTC density independent of C4d status.

SA-PO2497

Histologic Assessment of Renal Transplant Biopsies Using Whole Slide Digital Images Kuang-Yu Jen,[1] Jean L. Olson,[1] Tibor Nadasdy,[2] Zoltan G. Laszik.[1] *[1]Pathology, University of California San Francisco, San Francisco, CA; [2]Pathology, Ohio State University, Columbus, OH.*

Background: Whole slide digital image technology allows histologic evaluation of pathologic specimens on digitally scanned slides for diagnostic purposes. However, there are no data documenting the feasibility of such technology in the field of renal transplant (Tx) pathology. The purpose of our study was to assess the diagnostic accuracy of evaluating digital whole slide scans of kidney Tx biopsies as compared to conventional microscopic evaluation of glass slides.

Methods: Twenty-five kidney Tx biopsies demonstrating a spectrum of pathologic changes were evaluated by four well-trained renal pathologists. Each case included one H&E and one PAS-stained section, which were scanned using the Aperio ScanScope Scanner. Each pathologist evaluated each case twice, on separate occasions: once using a microscope to view the glass slides and once using the digital scans viewed on a computer monitor. The cases were coded to minimize bias. Thirty-one pre-specified features, most of which are Banff scores, were categorically scored by each pathologist for both the conventional slides and the digital scans. Intra-observer reliability was assessed using linearly-weighted Cohen kappa coefficient. Inter-observer reliability for conventional glass slides was evaluated using linearly-weighted Fleiss kappa coefficient.

Results: Mean intra-observer kappa coefficients (range from 0.59 to 0.94) were generally higher than inter-observer kappa coefficients (range from 0.16 to 0.66) for thirty features evaluated. This finding indicates that intra-observer reliability in scoring morphologic features is higher than inter-observer reliability. Detection of oxalate crystals was suboptimal on digital slides. The average time spent on examining digital scans was approximately 1.5 times greater than that spent on conventional glass slides.

Conclusions: Digital scans of histologic sections for the evaluation of renal Tx biopsies appear to be a reliable approach for diagnostic purposes. One significant drawback of digital scans is that they are more time-consuming for pathologists to fully evaluate.

SA-PO2498

Pediatric Kidney Transplantation into Adults; a Biopsy Review Hae Yoon Grace Choung,[1] Suzanne Meleg-Smith.[2] *[1]Debakey Scholars Program, Tulane University School of Medicine, New Orleans, LA; [2]Pathology, Tulane University School of Medicine, New Orleans, LA.*

Background: Although the good clinical outcome of single pediatric-donor-graft (PedDG) renal transplantation has been shown at several institutions, including Tulane (*Zhang et al, Clin J Am Soc Nephrol, 2009*), there are only scant descriptions of the post-transplantation graft biopsy (bx) in these recipients.

Methods: During a period of 11 years (1998-2009), single kidneys from donors aged 1 to 9 years were transplanted into 105 recipients.

Results: To investigate the graft pathology in PedDG , we studied the 54 PedDG recipients who had 101 "for cause" graft bx , obtained between 3 and 5840 post transplant days. As control, we randomly selected 50 adult-donor graft (ADG) recipients with 79 "for cause" bx during the same period. Immunohistochemistry (IF) had been performed in 39 PedDG and in 47 ADG bx.

Results:

Table 1 - Diagnosis	PedDG bx n=101		ADG bx n=79	
Acute tubular necrosis	12	12%	15	19%
Acute T-cell mediated rejection	32	32%	19	24%
Transplant glomerulopathy	7	7%	4	5%
Calcineurin inhibitor toxicity	5	5%	2	2.5%
TMA	10	10%	3	4%
Infectious interstitial nephritis	8	8%	5	6%
BKV nephritis	2	2%	3	4%
Vascular event	1	1%	2	2.5%

Table 2 – IF	PedDG recipients		ADG recipients	
Acute antibody mediated rejection	5/30	17%	10/47	21%
Immune complex (IC) mediated GN *	9/39	23%	1/32	3%
* p value is < 0.05				

Table 3 - Post tx interstitial fibrosis	< 3 months		3 to12 months		> 12 months	
	PedDG	ADG	PedDG	ADG	PedDG	ADG
Mild: < 25%	1/36	4/31	2/31	4/24	4/43	8/25
Moderate: 25-49%	0	1/31	3/31	8/24	7/43	3/25
Severe: >50%	0	1/31	3/31	3/24	9/43	11/25

Table 4 - Diagnosis	PedDG recipients n=54		ADG recipients n=50	
Focal Segmental Glomerulosclerosis	12	22%	12	24%
Diabetic nephropathy	2	4%	1	2%

Conclusions: Detailed study of a series of 180 "for cause" post transplantation renal bx shows a statistically significant increased incidence of IC mediated GN in recipients of single PedDG as compared to ADG.

SA-PO2499

Extracapillary Proliferation as an Independent Predictive Factor in IgAN Konstantinos Giannakakis,[1] Rosaria Polci,[2] Ilaria Serriello,[3] Antonietta Gigante,[3] Margherita Rosa,[5] Sandro Feriozzi,[2] Marco Galliani,[4] Massimo Morosetti,[6] Francesco Pugliese,[3] Tommasangelo Petitti,[8] Andrea Onetti-Muda.[7] *[1]Pathology, S, Rome, Italy; [2]Nephrology, Bellcole Hospital, Virerbo, Italy; [3]Clinical Medicine, Rome, Italy; [4]Nephrology, Pertini Hospital, Rome, Italy; [5]Nephrology, San Camillo Hospital, Rome, Italy; [6]Nephrology, G.B. Grassi Hospital, Ostia, Italy; [7]Pathology, Campus Biomedico University, Rome, Italy; [8]Data Analysis, Campus Biomedico University, Rome, Italy.*

Background: The predictive value of the Oxford calssification histological lesions in IgAN has been validated; attention has been placed on its predictive value of the decline of renal function. The aim of our work was to correlate active glomerular lesions at biopsy and progression of renal damage.

Methods: We have studied 473 renal biopsies with diagnosis of IgAN; of these, 184 had availability of clinical data at follow-up (sCr and eGFR by CKD-EPI formula) up to a maximum of 25 years. The median age at diagnosis was 36.7 years; 70% of patients were males. Histological parameters were from the Oxford classification (mesangial and endocapillary proliferation, segmental glomerulosclerosis, tubular atrophy, extracapillary proliferation, interstitial fibrosis) and in addition glomerular fibrinoid necrosis. Data were analyzed by univariate and multivariate investigation, according to linear regression of longitudinal data, taking into account the distance between time of biopsy and time points of acquisition of clinical data.

Results: Statistical analysis showed a correlation between progression of renal damage (eGFR) and segmental glomerulosclerosis (p=0.001), cellular crescents (p=0.01), fibrous crescents (p=0.02), fibrinoid necrosis (p=0.04) and interstitial fibrosis (p=0.03); no correlation was evident with fibrocellular crescents and endocapillary proliferation.

Conclusions: Our preliminary results suggest that active glomerular lesions, such as cellular crescents and fibrinoid necrosis, correlate with decline of renal function, differently from the Oxford classification. These histological parameters should therefore be taken into account to classify histologically cases of IgAN, and for the appropriate treatment.

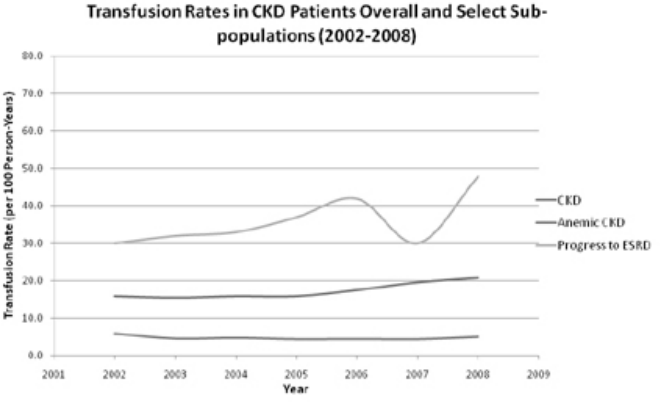
SA-PO2500

Red Blood Cell (RBC) Transfusion Rates among Young, Commercially Insured Chronic Kidney Disease (CKD) Patients Karminder S. Gill,[1] Jeffrey Petersen,[1] Paul Muntner,[2] Richard A. Lafayette,[3] Sai Ram Reddy Keithi Reddy,[1] Jeffrey C. Fink,[4] David T. Gilbertson,[5] Brian D. Bradbury.[1] *[1]Amgen, Inc., Thousand Oaks, CA; [2]Dept of Epidemiology, U. of Alabama, Birmingham, AL; [3]Div of Nephrology, Stanford U. School of Medicine, Palo Alto, CA; [4]Dept of Medicine, U Maryland, Baltimore, MD; [5]Chronic Disease Research Group, Minneapolis, MN.*

Background: The rate of RBC transfusions among older CKD patients not on dialysis has been estimated at 15-25%, but there is scant data among younger CKD patients.

Methods: We used data from the Ingenix medical claims database, a large, geographically diverse database of ~40 million commercially insured US individuals (2002-2008). We identified yearly cohorts of patients 18-64 years of age diagnosed with CKD; we required patients to have 1 yr of data available before diagnosis to characterize each cohort. We followed each cohort for 1 yr to estimate the RBC transfusion rates, using Poisson regression.

Results: We identified 144,100 CKD patients (range: 15,565 in 2002 up to 28,013 in 2008); 57% were 50-64 years of age; 46% were female. Comorbidities were common: hypertension (54%), diabetes (33%), hyperlipidemia (31%), and coronary artery disease (18%), and 23% were diagnosed with anemia. Overall, the transfusion rate was 4.0/100 person-years (PYs) (95% CI: 3.9-4.1). Rates were noticeably higher among those with diagnosed anemia (14.1/ 100 PYs[95% CI: 13.6-14.5]) and were highest among those who progressed to end stage renal disease (ESRD) (30/100 PYs [95% CI: 26-35]).



Conclusions: RBC transfusions are not uncommon among young, commercially insured anemic CKD patients, particularly those transitioning to ESRD. The risk of allosensitization and its effects on renal transplantation needs to be considered in patients who are likely to transition to ESRD.

Funding: Pharmaceutical Company Support

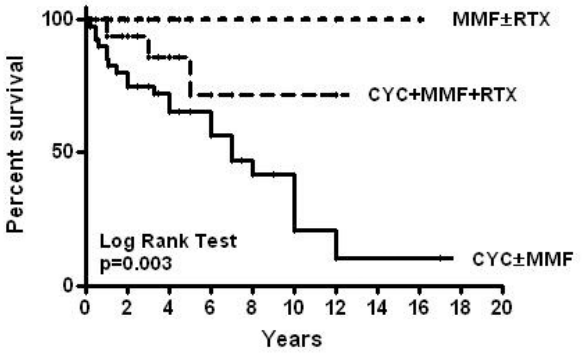
SA-PO2501

Rituximab Use in Childhood Onset Lupus Nephritis: "Early Versus Late" Tanya E. Pereira, Carolyn L. Abitbol, Chryso P. Katsoufis, Wacharee Seeherunvong, Jayanthi Chandar, Michael Freundlich, Gaston E. Zilleruelo. *Pediatric Nephrology, University of Miami/Holtz Children's Hospital, FL.*

Background: Rituximab, a monoclonal anti-B cell antibody, is increasingly used as adjuvant treatment of rheumatoid arthritis and sporadic cases of systemic lupus nephritis (LN). No large experience in children has been reported. The aim of our study was to examine renal survival in a cohort of patients with childhood onset LN treated with sequential treatment regimens of intravenous Cyclophosphamide (CYC), mycophenolate mofetil (MMF) and Rituximab (RTX).

Methods: A retrospective analysis was performed on 78 patients with childhood onset severe LN (≥WHO Class 3) over 25 years. This largely non-white (56% African American, 29% Hispanic) cohort was stratified into 3 "Eras" according to the introduction of RTX as a new adjuvant medication: Baseline Era1 (N=41): CYC±MMF; Era2 (N=16): CYC+MMF+RTX; Era3 (N=21): MMF±RTX. Histological biopsy classification was similar across all groups.

Results: Average period of follow-up was 6±4 years. Median renal survival for the cohort was 10 years. In Era1, the median renal survival was 7 years. Renal survival improved with the addition of RTX in Era2 as "salvage therapy" in 16 refractory patients. Era3 marked the early use of RTX with MMF as induction therapy. None of these patients has experienced renal demise with a median follow-up of 3.3 years (range 1 to 16 years). Three patients in the entire cohort expired, 1 in Era1 and 2 in Era2. The overall five year patient survival was 96% for the entire cohort. One patient who received RTX expired but her death was not attributed to the medication.



Conclusions: Our data suggest that the early use of RTX as adjuvant induction therapy may improve short term renal survival. Confirmation of its efficacy and safety in children will require controlled treatment trials.

SA-PO2502

Clinical Impact of LDL-C to HDL-C Ratio in Association with CKD Yoshinari Yasuda,[1] Kiyoshi Shibata,[1] Sadao Suzuki,[2] Sawako Kato,[1] Shoichi Maruyama,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]Nephrology/CKD Initiatives, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Nagoya City University, Nagoya, Japan.*

Background: Chronic kidney disease (CKD) has been increasingly highlighted as a serious risk factor for cardiovascular diseases (CVD). Dyslipidemia plays important role in both CKD and CVD onset and progression through atherogenesis. Low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol cholesterol (HDL-C) ratio (LHR) was reported as a promising predictor for CVD and mortality, but it has not been fully elucidated in association with CKD. In this study, we analyzed the relationship between the LHR and CKD.

Methods: The study subjects were 3,983 men and 3,742 women, who underwent health check in Kasugai City Medical Care Center in 2008. Patients under treatment for dyslipidemia were excluded from the study. Estimated GFR (eGFR) was calculated by the Japanese eGFR equation, and cases with eGFR less than 60 mL/min/1.73m² and/or with proteinuria were diagnosed as CKD. LDL-C and HDL-C were measured directly by enzymatic method and LDL-C was divided by HDL-C for LHR. Multivariate odds ratios of LHR (> 1.5), LDL-C (≥ 140 mg/dL) and HDL-C (≤ 40 mg/dL) were calculated for CKD using logistic regression adjusted for age and sex. Average eGFR values were compared in the quartile for LHR, LDL-C and HDL-C.

Results: Among CKD subjects, LHR and LDL-C were significantly higher and HDL-C was significantly lower compared to non-CKD. Odds ratios for CKD increased significantly as LHR category advanced.

Odds ratio for CKD

LHR	OR	95% CI
1.5≥	1.00	reference
1.51 - 2.50	1.42	1.21 - 1.68
2.51 - 3.50	1.64	1.36 - 1.97
3.51≤	2.15	1.67 - 3.05

Multivariate analysis revealed that LHR but LDL-C or HDL-C was a significant risk factor for CKD. In quartile analysis, average eGFR values (mL/min/1.73m²) decreased in order in LHR (Q1: 76.83, Q2: 73.56, Q3: 72.58, Q4: 70.94), LDL-C (Q1: 76.01, Q2: 73.78, Q3: 72.43, Q4: 71.79) and HDL-C (Q1: 74.85, Q2: 74.76, Q3: 73.20, Q4: 71.17), but the best difference was observed in LHR.

Conclusions: LHR revealed stronger association with CKD than LDL-C or HDL-C, suggesting the importance of well-balance of LDL-C and HDL-C to prevent arteriosclerosis.

Funding: Private Foundation Support

SA-PO2503

Increased Risk of Kidney Cancer in Young Men with Moderately Impaired Renal Function after a Long Follow-Up in a Large, Population-Based Cohort Anders G. Christensson. *Nephrology and Transplantation, Clinical Sciences, Malmö, Sweden.*

Background: There are reports that impaired renal function increases the risk of cancer. We evaluated whether moderately impaired renal function influenced risk of developing cancer in a large, population-based cohort.

Methods: We used data from Malmoe Preventative Medicine Project, a large, population-based cohort (74% participation) containing demographic data and blood samples collected from 33,346 persons aged 33-50 during 1974-86 in Malmoe, Sweden. Incident cancers were identified from the Swedish Cancer Registry, updated to 12/31/2006. Median follow-up time was 28 years. Glomerular filtration rate (GFR) was estimated using the Modified Diet and Renal Disease formula. Patients were classified as having normal kidney function and mild kidney disease (GFR >90 and ≥60-90 mL/min/1.73m2) or moderate kidney dysfunction (GFR<60 mL/min/1.73m2). Males and women were divided into older men (age 60), younger men (age 40-52), older women (age 47-57) and younger women (age 35-43). We calculated the risk of developing cancer using the competing risks regression to account for the competing risk of death from other causes after a follow-up of 20 years. Adjustment for age was conducted. We also evaluated the risk of cardiovascular events.

Results: The representativeness of the study population was seen in the significantly increased risk of cardiovascular events among persons with GFR <60 mL/min/1.73m2 for both males and females. 6,595 participants were diagnosed with cancer. The median age at baseline was 47 (IQR 40, 49). 22,183 (69%) participants were male; prostate cancer was the most common cancer diagnosis (n=1,579). Most subjects had mild kidney disease (n=22,336; 70%), 27% (n=8,621) had normal renal function and 1242 subjects GFR <60 mL/min/1.73m2 . We found no significant increase in cancer incidence among those with GFR <60 vs ≥60 mL/min/1.73m2. There was an increased risk for kidney cancer in young men with <60 vs ≥60 mL/min/1.73m2 (SHR: 2.38; 95% CI: 1.05, 5.44; p=0.039).

Conclusions: Among young men we found an increased risk of kidney cancer in those with moderately impaired renal function after a long follow-up.

SA-PO2504

Cost-Effectiveness of Statins for Primary Cardiovascular Prevention in Chronic Kidney Disease Kevin F. Erickson,[1,2] Sohan Japa,[2] Arjun S. Adhikari,[2] Joshua Glucoft,[2] Glenn M. Chertow,[1] Alan M. Garber.[2] *[1]Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Center for Primary Care and Outcomes Research, Stanford University, Stanford, CA.*

Background: Patients with chronic kidney disease (CKD) have a high risk of myocardial infarction (MI) and stroke compared to the general population. Although statins are effective at preventing cardiovascular (CV) disease in patients with CKD, guidelines give conflicting recommendations about statin use in CKD. This study assesses the cost-effectiveness of statins for primary prevention of cardiovascular disease in patients with hypertension and CKD.

Methods: A decision analytic model was constructed to evaluate the costs and benefits of statin use in preventing MI and stroke in patients with CKD, without CKD, and with varying assumptions about CKD progression. An increased absolute risk reduction from statins in CKD was modeled along with higher costs, increased morbidity and mortality, increased incidence of adverse events, and varying treatment effects associated with CKD. A separate micro-simulation model determined progression between CKD stages. The primary outcome was the incremental cost-effectiveness ratio (ICER) associated with statin therapy.

Results: In the base case, a 65 year old male with hypertension and stage 3 CKD, statin therapy increased costs by about $5,000 and led to a gain of 0.10 QALYs. The ICER from statins was about $50,000/QALY in the base case, similar in magnitude to the cost-effectiveness estimated from an identical cohort without CKD. Statins were more cost-effective in patients whose CKD did not increase in severity.

Conclusions: Statins can lead to large absolute reductions in CV disease in CKD patients, due to their high underlying risk of cardiac events. These gains are partially offset by an elevated risk of adverse events and decreased length and quality of life CKD patients can expect, independent of CV disease. The cost-effectiveness of Statins in CKD is sensitive to CKD progression, suggesting Statins would be cost-effective at a willingness to pay threshold of $50,000/QALY in a wide range of patients with stable (i.e., non-progressive) CKD.

Funding: Other NIH Support - Agency for Healthcare Research and Quality

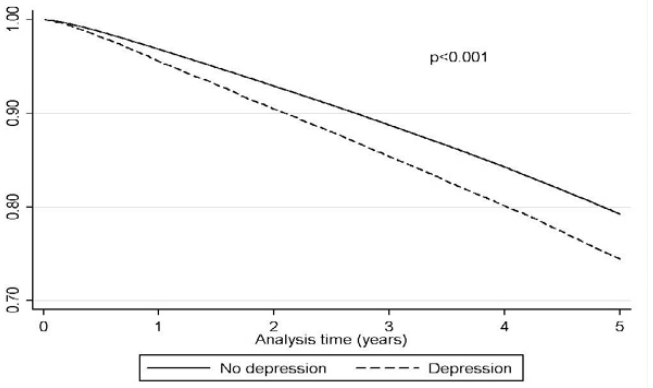
SA-PO2505

Association of Depression, Severity of Non-Dialysis Dependent CKD and Mortality in US Veterans Rasheed A. Balogun,[1] Emaad M. Abdel-Rahman,[1] Seki A. Balogun,[1] Evan H. Lott,[3] Jun Ling Lu,[3] Sandra M. Malakauskas,[2] Jennie Z. Ma,[1] Mark D. Okusa,[1] Kamyar Kalantar-Zadeh,[4] Csaba P. Kovesdy.[1,2] *[1]U of Virginia; [2]Salem VAMC; [3]VA Inf&Com; [4]Harbour-UCLA.*

Background: Depression (D) is known to be associated with higher mortality in ESRD patients. Less information is available in earlier stages of CKD

Methods: We examined association of D with all-cause mortality in 657,614 US veterans with CKD stages 1-5. Associations of D with all-cause mortality and stratified by CKD stages were examined with Kaplan Meier method,in fixed effect and time-dependent Cox models. Models were adjusted for sociodemographics, comorbidities, blood pressure and laboratory variables

Results: Patients were 73.9±9.8 yrs, 97% male and 71% white. There were 37,032 patients (5.6%) with D (67.1±11.3 years, 93% males and 71% white, with eGFR 54±17 ml/min) in 2005-2006. D was more common in patients with CKD 1 (13%) than in patients with CKD 2 (9%), 3A (6%), 3B (5%), 4 (4%) and 5 (4%). Patients with D were younger, more likely to be female, not married, lack insurance and had higher prevalence of comorbidities. During a median follow-up of 4.8 yrs, 189,561 patients died (mortality rate, 95% CI: 67.3/1000 patient yrs, 67.0-67.6). D was associated with higher age-adjusted mortality (Haz.ratio (95%CI): 1.31 (1.30-1.32) p<0.001, which was, however, attenuated after multivariable adjustments (1.00, 0.97-1.03 p=0.9).



Associations between D and mortality were similar in patients with different stages of CKD except those with CKD 1, in whom D remained significantly associated with mortality after multivariable adjustments (1.18, 1.02-1.36; p=0.02)

Conclusions: Patients with CKD 1 had higher prevalence of depression and showed a significant association with mortality than those with more advanced CKD. Screening, diagnosing and treating depression may have the maximum impact if done in earlier stages of CKD.

SA-PO2506

Abnormal Nitric Oxide Metabolism, Prothrombic State, Impaired Angiogenesis and Risk of Chronic Kidney Disease Jing Chen, L. Lee Hamm, Myra A. Kleinpeter, Fred E. Husserl, Islam Enver Khan, Federico Teran, Grace Browne, Chung-Shiuan Chen, Yanxi Liu, Katherine T. Mills, Eva Lustigova, Ji Hua Xu, Stephanie E. Rogers, Joseph Tarsia, Kamal Ramesh Shah, Chuan He, Arnold B. Alper, Eric E. Simon, Jiang He. *Tulane University.*

Background: Endothelial dysfunction is frequently observed in patients with chronic kidney disease (CKD). The underlying mechanism is not fully understood.

Methods: We investigated the association of endothelial dysfunction biomarkers in the nitric oxide pathway [asymmetric dimethylarginine (ADMA), and L-arginine], thrombosis [von Willebrand factor (vWF)], and angiogenesis (endostatin) with the risk of CKD in 201 CKD patients and 201 controls without CKD.

Results: Compared to those without CKD, patients with CKD were older (56 vs. 53 yrs), more likely to be male (55% vs. 45%), less likely to have high school education (59% vs. 82%), or consume alcohol (28% vs. 59%). Race and cigarette smoking were comparable between CKD patients and controls. Mean systolic blood pressure (132 vs. 122 mmHg), body mass index (32 vs. 29 kg/m^2), fasting glucose (120 vs. 103 mg/dL), history of hypertension (88% vs. 24%), history of diabetes (49% vs. 6%), and CVD (44% vs. 7%) were higher while LDL cholesterol (102 vs.118 mg/dL) was lower in CKD patients than in controls. After adjustment for above risk factors, the median [inter-quartile range(IQR)] of ADMA [0.58 µmol/L (IQR, 0.55-0.62) vs. 0.27 µmol/L (IRQ, 0.27-0.30), p<0.0001], L-arginine [71.39 µmol/L (IQR, 67.06-75.73) vs. 33.08 µmol/L (IRQ, 28.77-37.38), p<0.0001], vWF [1321.29 mU/mL (IQR, 1244.02-1398.57) vs. 1157.54 mU/mL (IRQ, 1080.53-1234.55), p=0.01], and endostatin [277.15 ng/mL (IQR, 259.55-294.76) vs. 142.18 ng/mL (IRQ, 124.63-159.73), p<0.0001] were higher in CKD patients than in controls.

Conclusions: These data indicate that abnormal nitric oxide metabolism, prothrombic state, and impaired angiogenesis are associated with risk of CKD.

Funding: Other NIH Support - The National Center for Research Resources

SA-PO2507

Untreated Bacteriuria, Cardiovascular Events, and Death among Female Patients with CKD Susmitha Dhanyamraju,[1] Michael Foltzer,[2] Xiaoqin Tang,[3] H. Lester Kirchner,[3] Robert M. Perkins.[1,3] *[1]Nephrology, Geisinger Medical Center; [2]Infectious Diseases, Geisinger Medical Center; [3]Center for Health Research, Geisinger Medical Center.*

Background: Untreated bacteriuria may trigger local and systemic inflammatory responses. This study examined the relationship between untreated UTI and *de novo* CV events and death among female patients with CKD.

Methods: A retrospective cohort of female patients receiving care between 2004 and 2010 at a tertiary health care system in Central Pennsylvania and with stages 1-4 CKD was established. Patients with a prior history of MI, CHF, or stroke were excluded. Study exposure was a positive urine culture (bacterial growth of ≥ 100,000 CFU/ml) without an antibiotic prescription within 90 days of culture order. Adjusted Cox models were developed to estimate the association between infection (treated and untreated) and time to first CV event (composite of MI, CHF, and stroke), and time to death. Models account for cumulative episodes of UTI during follow up.

Results: 6807 patients (81.8% without UTI, 7.9% with only treated UTI(s), 7.6% with only untreated UTI(s), and 2.7% with both treated and untreated UTIs) met criteria. Median (IQR) follow up was 5.2 (3.4, 5.9) years. Mortality rates were 22.8, 29.9, and 44.3 deaths/1000 PY in the no-UTI, treated-UTI, and untreated-UTI groups, respectively.

Cox PH models for time to death and first CV event, by treatment status

Death			
		HR (95% CI)*	**p-value**
Unadjusted	Treated UTI(s)	1.31 (1.03, 1.66)	0.03
	Untreated UTI(s)	1.94 (1.57, 2.38)	<0.001
Fully Adjusted**	Treated UTI(s)	0.81 (0.63, 1.05)	0.1
	Untreated UTI(s)	1.42 (1.15, 1.76)	<0.001
CV Event (MI, CHF, Stroke)			
		HR (95% CI)*	**p-value**
Unadjusted	Treated UTI(s)	3.68 (2.95, 4.59)	<0.001
	Untreated UTI(s)	3.56 (2.53, 4.78)	<0.001
Fully Adjusted**	Treated UTI(s)	2.11 (1.66, 2.69)	<0.001
	Untreated UTI(s)	2.01 (1.58, 2.57)	<0.001

*Reference group: no UTI (n = 5559). **Model adjusted for age, race, CKD stage at baseline, Charlson score, hyperlipidemia, diabetes, hypertension, peripheral vascular disease, history of cancer, COPD, connective tissue disease, and hospitalization during follow up

Conclusions: Untreated UTIs among female patients with CKD may increase the risk of CV events and death.

SA-PO2508

Serum Creatinine Is Associated with Carotid Intima-Media Thickness in Males: Results of a Population-Based Study (SHIP) Sylvia Stracke,[1] Friedlinde Ernst,[1] Daniel Robinson,[2] Christian Schwahn,[3] Ulrich John,[3] Stephan Felix,[2] Henry Voelzke.[3] *[1]University Medicine Greifswald, Nephrology, Germany; [2]University Medicine Greifswald, Cardiology, Germany; [3]University Medicine Greifswald, Institute of Epidemiology and Social Medicine, Germany.*

Background: Chronic kidney disease (CKD) remains asymptomatic until its late stage. There is only little information on the association of renal function with subclinical atherosclerosis as depicted by carotid intima media thickness (cIMT). The aim of the present study was to examine the association of cIMT with various serum markers of renal function in a large population based study.

Methods: The Study of Health in Pomerania (SHIP) is a cross-sectional, population based study in the North East of Germany. Data from 2367 individuals (1199 males) aged 45 years or older were available for analysis. Creatinine clearance was calculated by the Cockroft-Gault, Jelliffe, Wright and the abbreviated Modification of Diet in Renal Disease formulae. Women and men were analyzed separately.

Results: 240 males (20.0%) and 244 females (20.9%) showed serum creatinine values above the normal range. Males and females with high serum creatinine had higher serum HbA_1C levels, pulse pressure, BMI, and serum uric acid levels. After adjusting for confounding factors, multivariable analysis revealed that in males, raised serum creatinine levels were independently associated with carotid IMT (non-standardised ß-coefficient 0.0274, 95% CI 0.005-0.050, p= 0.018). However, we could not find an association for raised serum creatinine levels in women nor for reduced values of calculated creatinine clearances in either sex.

Conclusions: We found a gender-specific relation between impaired renal function measured by serum creatinine and cIMT. With rising serum creatinine, the exponential relationship between creatinine and eGFR leads to relatively higher increases in serum creatinine for each mL loss of GFR and thus creatinine in already established renal failure may be more sensitive biomarker for renal function than eGFR.

SA-PO2509

The Association of Stage of CKD and Hemoglobin A1C in a Diabetic Primary Care Population Bessie A. Young,[1,2,3] Margaret K. Yu,[2] Courtney R. Lyles.[3] *[1]Division of Nephrology, VA Puget Sound Health Care System, Seattle, WA; [2]Division of Nephrology, University of Washington, Seattle, WA; [3]Health Services, University of Washington, Seattle, WA; [4]Kidney Research Institute, University of Washington, Seattle, WA.*

Background: Hemoglobin A1C (A1C) levels decrease as estimated glomerular filtration rate (eGFR) declines. Few studies have detailed the decline in glycemic control as a function of chronic kidney disease (CKD) stage. We determined differences in baseline A1C values as a function of CKD stage.

Methods: The Pathways Study is a prospective cohort study of diabetic patients at a large managed care health maintenance organization. Baseline laboratory data were extracted from linked automated records. EGFR was determined using the modified equation by Levy. Stage of CKD as defined by the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines. Cross-sectional associations of A1C and stage of CKD were determined by student t-test and linear regression. Association of A1C <6.5% with stage of CKD was determined by multivariable logistic regression.

Results: Of the 4842 subjects enrolled in the study, 3999 had baseline laboratory data sufficient to be included. By stage of CKD, 14% were stage 1, 35.7% stage 2, 20.0% stage 3, 10.9% stage 4, and 1.5% were stage 5. Those with late stage CKD were more likely to be older, married, African American, and to have been hospitalized in the prior year. Mean A1C levels were 7.6±1.6, 7.9±1.6, 7.8±1.5, 7.8±1.5, 7.5±1.4, and 6.4±1.4, for stages 0, 1, 2, 3, 4, and 5 respectively. Differences were significant between stages 0 to 1, 1 to 2, and 4 to 5 (p<0.02). A1C levels increased in stages 1 (β=0.31, 95% CI=0.17, 0.45) and 2 (β=0.27, 95% CI=0.10, 0.42), while they decreased in stages 4 (β=-0.40, 95% CI=0-.78, -0.02) and 5 (β=-1.80, 95% CI=-2.57, -1.03). Stage 5 CKD was associated with a 17-fold odds of A1C <6.5 (OR=17.74, 95% CI=5.54-56.76).

Conclusions: Among subjects with diabetes, A1C decreased after stage 3 CKD and was significantly below recommended guidelines by stage 5 CKD. CKD influences level of glycemic control and may increase the risk of hypoglycemic reactions among diabetic patients with CKD.

Funding: NIDDK Support, Other NIH Support - NIMH, Veterans Administration Support

SA-PO2510

Accuracy of Race Imputation Using the BISG Method and Applicability for CKD Stephen F. Derose, Richard Contreras. *Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA.*

Background: The use of existing health plan data for studies on racial and ethnic differences is often limited by missing race data. Methods of imputation using have recently improved: the Bayesian Improved Surname Geocoding (BISG) uses US Census block group and surname to predict race, and can be modified for use of additional inputs. We tested the accuracy of BISG imputation against existing administrative health plan data and compared actual and imputed race for predicting differences in serum creatinine, which is useful for estimating GFR in CKD epidemiology.

Methods: Participants were adult members of Southern California Kaiser Permanente with an outpatient serum creatinine test from 1998-2006 (N = 1,585,983). Race data was available in 70.6% of participants. The accuracy of individual race imputation was tested using AROC. Racial differences in the first available serum creatinine were assessed using linear regression on log(serum creatinine) with adjustment for age and gender (Table 1).

Results: Race group AROC (prevalence %) were: White 0.90 (34); Hispanic 0.93 (22); Black 0.94 (9); Asian & Pac. Isl. 0.90 (5.5); Native Amer. 0.6 (0.1); Multiple 0.62 (0.1). Regression results showed very similar estimates of racial differences in serum creatinine (discrepancies ranged from 1-16%), although 95% CIs rarely overlapped.

Percent Difference in Serum Creatine vs. Whites (95% CI)

Race	Actual (Administrative)	BISG
Black	8.25 (8.13, 8.37)	8.86 (8.71-9.00)
Hispanic	-8.41 (-8.50, -8.32)	-8.49 (-8.58, -8.40)
Asian & Pac. Isl.	-7.32 (-7.47, -7.17)	-8.49 (-8.66, -8.32)
Native Amer.	-3.42 (-4.65, -2.18)	-3.30 (-5.69, -0.90)
Multiple	-1.40 (-2.46, -0.34)	-4.20 (-5.16, -3.24)
Other	-1.29 (-1.95, -0.62)	-
Unknown	-2.40 (-2.48, -2.31)	-

Conclusions: The BISG method was accurate for higher prevalence race groups. The imputation of black race was very good in our settings, which is especially helpful for estimating GFR. Missing data can be eliminated, which helps determine complete population disparities. While not a substitute for measured race, BISG is a promising method for obtaining information on racial differences in CKD from existing health plan datasets.

Funding: Clinical Revenue Support

SA-PO2511

Obesity Is a Risk Factor for End-Stage Renal Disease and Mortality in Pre-Dialysis Patients Ellen K. Hoogeveen,[1] Nynke Halbesma,[2] Moniek C.M. de Goeij,[2] Elisabeth W. Boeschoten,[3] Friedo W. Dekker.[2] *[1]Nephrology, Jeroen Bosch Hospital, Den Bosch, Netherlands; [2]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; [3]Hans Mak Institute, Naarden, Netherlands.*

Background: Obesity is associated with increased mortality and decline in renal function in the general population. In contrast, obese dialysis patients may have an improved survival, the so called "obesity paradox". The aim of this study was to assess whether obesity is a risk factor for end-stage renal disease (ESRD) and mortality in chronic kidney disease stage 4-5 pre-dialysis patients with an estimated GFR <30 ml/min/1.73 m^2 not yet on dialysis.

Methods: In a prospective multi-center cohort study incident pre-dialysis patients (>18y) were included when referred to a specialized pre-dialysis outpatient clinic between 2004 and 2011 and followed until start of dialysis, transplantation, death or January 2011. We used body mass index (BMI) measured at baseline as an index of adiposity. Patients were stratified into four categories of baseline BMI: <20, 20-25 (reference), 25-30, and ≥30 (obesity) kg/m^2.

Results: A total of 473 patients were included (mean (SD): age 65y (14), men 68%, BMI 27 (6) kg/m^2, median follow-up time 1.15 years, 365 patients completed the study: 9% died and 64% started dialysis). The combined endpoint was defined as ESRD for which dialysis was needed or death on pre-dialysis care. The combined endpoint rates (95%-CI)/100py per BMI category were: 29.6 (17.4-45.4), 29.6 (24.41-35.3), 42.6 (64.6-76.2) and 46.4 (38.8-54.3). The combined endpoint rate was 1.6 times higher in patients with obesity than with a normal BMI, which corresponded with an excess rate of 16.8 ESRD-deaths/100 py. After adjustment for age, sex, smoking and history of cardiovascular disease the hazard ratios (95%-CI) for the combined endpoint by category of increasing BMI were 1.13 (0.61-2.08), 1 (reference), 1.46 (1.08-1.97), 1.52 (1.10-2.10).

Conclusions: In conclusion, pre-dialysis patients with obesity compared with patients with a normal BMI, have a 50% higher risk of ESRD or death on pre-dialysis care.

SA-PO2512

Predictive Role of Serum Cystatin C on Survival in Cardio-Surgery Patients Roberto Palumbo,[1] Annalisa Noce,[2] Michele Ferrannini,[1] Mariarita Dessì,[3] Simone Manca di Villahermosa,[2] Olga Durante,[2] Mariapaola Canale,[2] Nicola Di Daniele.[2] *[1]Nephrology and Dialysis, S. Eugenio Hospital, Rome, Italy; [2]Department of Internal Medicine, University of Tor Vergata, Rome, Italy; [3]Department of Laboratory Medicine, University Tor Vergata, Rome, Italy.*

Background: Pre-operative renal dysfunction is a known risk factor for morbidity and mortality in cardio-surgery patients. Serum Cystatin C (sCysC) is a well recognized marker of early renal dysfunction, and previous studies suggest a sCysC predictive role for cardiovascular events in the general population. To assess predictive value of pre-operative sCysC on long-term mortality in adult cardio-surgery patients.

Methods: Between November 2005 and March 2007 421 consecutive patients aged 67,72±10,76 years were admitted in our cardio-surgery department. (217 for coronary artery bypass graft, 150 for valvular prosthetic surgery, 54 for other surgical procedures). We conducted a prospective observational study evaluating all causes of long-term mortality till December 2009. At admission sCys C was dosed in all patients (normal 0.5-0.92mg/L). Patients were stratified in quartiles according to sCysC: Q1 sCys C<0,81mg/L (29 patients), Q2 sCysC 0,81-0,92mg/L (81), Q3 sCysC 0,93-1.10mg/L (29) and Q4 sCys C>1.10mg/L (282 patients). Kaplan–Meier cumulative survival curves were plotted for sCysC quartiles. Patients' cardiovascular mortality was the primary endpoint of the study.

Results: One-hundred twenty four patients (29,4%) reached the study end-point. Q3 and Q4 patients (67 and 66% 2-year actuarial survival) showed a significantly higher cumulative mortality compared to Q1 and Q2 patients (100 and 85%, respectively) (p=0,0007).

Conclusions: Serum Cystatin C may be considered a good predictor of long-term cardiovascular mortality in cardio-surgery patients.

SA-PO2513

Estimated GFR, Albuminuria, and Risk of Fracture Hospitalization in the Atherosclerosis Risk in Communities (ARIC) Study Emma K. Williams, Yingying Sang, Kunihiro Matsushita, Elizabeth Selvin, Brad C. Astor, Josef Coresh. *Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Background: Few studies have examined the association between the full range of kidney function and bone fractures. We examined the relationship of estimated glomerular filtration rate (eGFR) based on plasma cystatin C (eGFRCys) and albuminuria with incident fracture hospitalizations in the ARIC Study. The eGFR estimate based on cystatin C was used because it has a linear relationship with other adverse outcomes.

Methods: Plasma cystatin C and urine albumin:creatinine ratio (ACR) were measured in samples collected during 1996-98 (ARIC visit 4). Hospital record surveillance ascertained incident fracture hospitalizations through 2008. Cox proportional hazards models were used to estimate the linear associations of eGFRCys and ACR with fracture hospitalization.

Results: The median ACR was 3.73 (IQR 5-95 percentile 0.59-63.9) mg/g and the mean eGFRCys was 80 mL/min/1.73m2 (SD 20). During a median of 11 years of follow-up, 572 fracture hospitalizations occurred among 11,138 study participants who were aged 53-75 at visit 4. Each eightfold higher ACR was associated with a hazard ratio (HR) of 1.30 (95% CI 1.16-1.46), after adjusting for age, sex, race, cholesterol level, diabetes, history of cardiovascular disease, smoking status and systolic blood pressure.

	HR	95% CI
Higher ACR, 8-fold	1.30***	1.15 - 1.46
Lower eGFR, 30 ml/min/1.73m2	1.25***	1.09 - 1.43
Age, 10 years	2.10***	1.79 - 2.47
Female Sex	1.64***	1.36 - 1.97
Black Race	0.73*	0.58 - 0.93
Total cholesterol level, mmol/L	1.02	0.94 - 1.11
Diabetes	1.10	0.88 - 1.38
History of CVD	1.24	0.98 - 1.55
Smoking status	1.18	0.99-1.40
Systolic blood pressure	1.00	1.00-1.00

*** p<0.001, ** p<0.01, * p<0.05

In the same model, every 30 mL/min/1.73m2 lower eGFRCys was associated with an HR of 1.25 (95% CI 1.09-1.44). Both risk factors showed a linear association with no clear threshold.

Conclusions: Kidney damage, as reflected by microalbuminuria or moderately decreased GFR, is associated with increased risk of fracture in this community-based cohort.

Funding: Other NIH Support - National Heart, Lung, and Blood Institute contracts

SA-PO2514

Baseline Characteristics and Therapeutic Projects of Patients Older Than 75 Years with MDRD below 20. PSPA Investigator Group Olivier Moranne,[1] Cécile Couchoud,[2] Vincent L.M. Esnault.[1,3,4,5] *[1]Nephrology & Public Health, Hospital, Nice, France, Metropolitan; [2]REIN Registry, Biomedecine Agency, Saint Denis La Plaine, France; [3]University of Nice Sophia-Antipolis, France; [4]VIGNEAU C Service de néphrologie, CHU Rennes, France; [5]CNRS/UMR 6061Université Université de Rennes 1, France.*

Background: The Incidence of End Stage Renal Disease (ESRD) is growing in patients older than 75 years. Pre-ESRD clinical characteristics, care pathways nor outcome are well described in this population. Therefore, we set up a cohort study in this population and report the baseline characteristics.

Methods: PSPA is a multicenter prospective cohort study including patients older than 75 years with MDRD below 20 without renal replacement therapy (RRT) followed by nephrologists. 586 patients were included in 23 nephrology departments in France. Socio-demographic data, comorbidities, mobility, laboratory tests, medical treatments and therapeutic projects were collected for each patient.

Results: Baseline characteristics were as follow: mean age 82 ± 5 y/o, MDRD: 13 ± 4 mL/min/1.73m^2, 58% men, 36% diabetes, BMI 26 ± 5 kg/m^2, SBP 145 ± 22, DBP 74 ± 11 mmHg, prot/creatUratio 1 [0.1-2.0] g/g. Other comorbidities were: 31% congestive heart failure, 22% peripheral vascular disease, 10% active malignancy and 5% dementia. Most of the population was home resident (85%) and 80% could walk without help. Treatments included a median of 11 oral daily drug intakes including ACE inhibitors (44%), ESA (32%). Mean hemoglobin level was 12 ± 7 g/dL. The therapeutic projects were distributed as follows: 17% were under discussion, 43% had no planned RRT because of stable kidney function, 24% had planned RRT and 16% were not considered for RRT (half of the time by the nephrologist). The most frequent criteria for no indication for RRT by the nephrologist were dementia or cognitive dysfunction, active malignancy or denutrition and for patients older age and women.

Conclusions: In this population few patients are planned for RRT and many are considered as having a stable kidney function. Patient characteristics are different when RRT is not considered by patients or nephrologists. This cohort will be followed for 4 years.

Funding: Biomedecine agency, France, Pharmaceutical Company Support

SA-PO2515

Evaluation of a Public Health Campaign To Increase Kidney Health Awareness in a Canadian Province Krista S. Ryz,[1] Mauro Verrelli,[1] Jan Schneider,[2] Amie C. Lesyk,[2] Manish M. Sood,[1] Claudio Rigatto,[1] Paul Komenda.[1] *[1]Dept . of Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]Manitoba Renal Program, Winnipeg, MB, Canada.*

Background: To evaluate the effectiveness of a Canadian province wide public health campaign in raising awareness of risk factors that can lead to kidney disease and the ability of the campaign to encourage behaviours that lead to early detection.

Methods: A multi-faceted public health campaign was undertaken in urban and rural/remote Manitoba, Canada in March, 2011. The March 2011 campaign was built on a similar advertising platform that had been executed the previous year. A variety of media were employed to reach the target audience including radio, television, newspaper, web based efforts, postcards with prescriptions, and advertising on the side of buses. We performed a pre and post campaign telephone interview omnibus survey of Manitobans 18 years of age and over in February and April 2011 respectively. Respondents were selected by random digit dialling and each respondent was asked a question to determine awareness of the campaign, and if aware, their understanding of the message. Weighting was applied to data correcting for differences between the demographics of the sample and the 2006 census data on Manitoba's population.

Results: In pre-campaign sampling, there were a total of 3,751 co-operative contacts, of which 804 completed interviews. In post-campaign sampling a total of 3,231 co-operative contacts were established of which 802 completed interviews. Overall, percentage of respondents that were familiar with the campaign increased from 7% to 25%. 30% of urban respondents were aware of the message versus 18% of those in rural/remote Manitoba. In those that recalled the campaign, there was no difference in the pre and post campaign surveys in knowledge gain including: awareness of kidney disease, risk factors that lead to kidney disease or the need for follow-up to have kidney function evaluated.

Conclusions: A public health awareness campaign increased familiarity but not knowledge gain regarding kidney disease. Further research will focus on improving knowledge gain in the general population regarding the importance of kidney health.

SA-PO2516

High Dietary Salt Intake Is Associated with a Lower Risk of Chronic Kidney Disease in US Adults Shailendra Sharma,[1] Anna Jeanette Jovanovich,[1] Kim McFann,[1] Michel B. Chonchol,[1] Jessica B. Kendrick.[1,2] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Denver Health Medical Center, Denver, CO.*

Background: Extrapolations from observational studies and intervention trials suggest that population-wide moderation of salt intake might reduce cardiovascular events, however, it is unclear whether dietary sodium intake is associated with chronic kidney disease (CKD).

Methods: The cross-sectional association between sodium intake and CKD was examined in 13,917 adults 18 years of age or older who participated in the National Health and Nutrition Examination Survey (NHANES 2001-2006). Dietary sodium intake was calculated from 24-hour dietary recall obtained by trained interviewers. Dietary sodium intake was evaluated in quartiles 1 through 4 (≤ 2116, 2117-3061, 3062-4267, > 4267 mg/day). The primary outcome was chronic kidney disease (CKD) defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2 or eGFR > 60 mL/min with albuminuria (>30 mg/g). Multivariate logistic regression analysis was used to examine the association between dietary sodium intake and CKD.

Results: The mean (SE) age of the participants was 45(0.4) years, 73% were white, 28% had a history of elevated blood pressure and 7% had a history of diabetes. The mean (SE) dietary sodium intake and mean eGFR were 3520 ± 26 mg/day and 88.0 ± 0.60 mL/min/1.73m^2, respectively. In this cohort, 14.2% had CKD (7.3% had an eGFR <60 mL/min and 8.4% had albuminuria > 30 mg/g). After adjustment for age, sex, race, diabetes, hypertension and diuretic usage there was a significant association between higher quartiles of sodium intake and decrease odds of CKD, with adjusted odds ratios of 1.0, 0.85 (95% confidence interval [CI], 0.71 to 1.01;p=0.06), 0.77 (95% CI, 0.64 to 0.91; p=0.003), and 0.66 (95% CI, 0.54 to 0.82; p=0.0001) for quartiles 1 through 4, respectively.

Conclusions: In this population-based cohort study, higher dietary salt intake is associated with a lower risk of CKD. Clinical trials are needed to evaluate if dietary salt intake is a modifiable risk factor for CKD or kidney disease progression.

SA-PO2517

Low Dietary Potassium Intake Is Associated with an Increased Risk of Chronic Kidney Disease in US Adults Shailendra Sharma,[1] Kim McFann,[1] Anna Jeanette Jovanovich,[1] Michel B. Chonchol,[1] Jessica B. Kendrick.[1,2] *[1]Division of Renal Disease and Hypertension, University of Colorado Denver, Aurora, CO; [2]Denver Health Medical Center, Denver, CO.*

Background: Potassium intake is inversely related to blood pressure levels but the relationship between potassium intake and chronic kidney disease (CKD) has not been examined.

Methods: We performed a cross-sectional study using the National Health and Nutrition Examination Survey (2001-2006). 13,917 adult participants with dietary data were included in the analysis. Dietary potassium intake was calculated from 24-hour dietary recall. Potassium intake was examined in clinically significant categories [Low <2000, normal: 2000-4000 and high >4000 mg/day) and in quartiles 1-4 (≤ 1737, 1738-2455, 2456-3341, > 3342 mg/day). The primary outcome was CKD defined as a eGFR <60 mL/min or eGFR >60mL/min with albuminuria (>30mg/g). Multivariate logistic regression models were used to examine the association between potassium intake and CKD.

Results: The mean age and eGFR (SE) of participants was 45 ± (0.4) years and 88.00 ± 0.56 mL/min/1.73m^2. The mean (SE) potassium intake was 2760 ± 22 mg/day. After adjustment for age, sex, race, diabetes and hypertension, low potassium intake (<2000 mg/day) was associated with an increased risk of CKD (OR= 1.35,95% 1.14-1.59; p=0.0005) when compared to normal intake group. High potassium intake (>4000 mg/day) was nearly significantly inversely related to CKD (OR = 0.78, 95% 0.61-1.00, p = 0.05). When potassium intake was evaluated in quartiles, subjects in the first quartile had a 55% increased odds of CKD compared to subjects in the 4th quartile (OR= 1.55, 95% CI 1.25-1.93; p<0.0001). We also examined the relationship of combinations of potassium and sodium intake with CKD. Median intake of potassium and sodium were used to determine high and low potassium and sodium intake. Subjects with high potassium and high sodium intake had a decreased odds of CKD compared to subjects with low potassium and low sodium intake (OR=0.66, 95% CI 0.57-0.78; p<0.0001).

Conclusions: Low dietary potassium intake is associated with an increased risk of CKD. Studies are needed to examine effects of potassium on kidney disease.

SA-PO2518

Association of Common GFR-Associated SNPs with Albuminuria in Individuals of European Descent Conall M. O'Seaghdha,[1] Ming-Huei Chen,[2] Meredith C. Foster,[1] Carsten A. Böger,[3] Ian H. de Boer,[4] Anna Kottgen,[5] Afshin Parsa,[6] Murielle Bochud,[7] Wen Hong Linda Kao,[8] Caroline S. Fox.[1] *[1]NHLBI's Framingham Heart Study; [2]Boston University; [3]Regensburg University Medical Center, Germany; [4]University of Washington, USA; [5]Freiburg University, Germany; [6]University of Maryland; [7]Centre Hospitalier Vaudois, Switzerland; [8]Johns Hopkins University.*

Background: Albuminuria is an important marker of prognosis in chronic kidney disease (CKD). The presence of albuminuria predicts CKD progression and the development of end-stage renal disease. We hypothesized that single nucleotide polymorphisms (SNPs) recently identified as associated with estimated glomerular filtration rate (eGFR) and CKD might also be associated with albuminuria.

Methods: We investigated 16 eGFR/CKD-associated SNPs in a cohort of 32,324 individuals of European ancestry for their association with the urinary albumin-to-creatinine ratio (UACR) using regression analyses, adjusting for age and sex. We performed meta-analysis of results from 13 contributing cohorts using an inverse variance weighted method. We accounted for multiple testing using a false discovery rate adjustment.

Results: The minor allele of rs17319721 in the SHROOM3 gene, which is associated with lower GFR, was associated with lower UACR levels (β-coefficient for UACR -0.034, p-value 0.0002). While several additional eGFR/CKD SNPs demonstrated nominal statistical association with UACR at DAB2, UBE2Q2, STC1 and GCKR, none were significantly associated after false discovery rate adjustment (q-value range for association with UACR 0.06 – 0.30). Furthermore, alleles associated with lower eGFR tended to be associated with lower UACR levels (DAB2, UBE2Q2, and STC1) and an allele associated with higher GFR was nominally associated with higher UACR levels (GCKR).

Conclusions: Apart from the SHROOM3 locus, we observed no robust associations between eGFR-associated SNPs and UACR. These findings suggest distinct genetic components to these traits.

Funding: Other NIH Support - NHLBI

SA-PO2519

Health Literacy and Blood Pressure Control among Hispanic Americans with Chronic Kidney Disease: A Report from the Paso del Norte Kidney Disease Study (PNKDS) Jimena A. Blandon, Jinyi Ling, Tarek Alhamad, German T. Hernandez. *Divisions of Nephrology & Hypertension and Biostatistics & Epidemiology, Paul L Foster School of Medicine, Texas Tech University Health Sciences Center at El Paso, TX.*

Background: Health literacy (HL) refers to a patient's ability to comprehend medical instructions. Adequate HL may be important in achieving blood pressure control in CKD patients. We assessed HL levels among Hispanic patients with CKD stages 2-4. We evaluated the cross-sectional relationship between adequate levels of HL and BP control.

Methods: We enrolled patients with CKD stages 2-4 attending our nephrology clinic in El Paso, TX. Patients underwent an interview & exam. The patients' HL was measured using the short-form Test of Functional Health Literacy in Adults (s-TOFHLA) in either English or Spanish. HL was classified as inadequate for s-TOFHLA scores 0-16, marginal for 17-22, and adequate for 23-36. BP control was defined as <130/80 mmHg.

We performed logistic regression with BP control as the outcome, comparing subjects with adequate HL to subjects with inadequate/marginal HL. Analyses were stratified by gender & diabetic status and were adjusted for age, language, health insurance, income & eGFR.

Results: 245 patients were enrolled, 51% female, 91% self-identified as Hispanic, 73% had an annual income <$20,000, 61% had diabetes, the mean eGFR was 44 ml/min, the mean BP was 135/75 mmHg. 36% had a BP <130/80 mmHg. The mean HL score was 22; 52% had adequate HL & 46% had inadequate/marginal HL (8% missing).

After adjusting for age, language, insurance, income & eGFR, there was no relationship between HL adequacy & BP control among diabetic men, non-diabetic men, and non-diabetic women. Among women with diabetes (n=69), the adjusted odds ratio for BP control was 3.33 (95% CI: 1.01-11.01; p=0.048) for women with adequate HL compared to those with marginal/inadequate HL.

Conclusions: Among Hispanics with CKD, there is a high prevalence of inadequate or marginal HL and poor BP control. Among diabetic females with CKD, adequate HL is associated with a higher odds of BP control. Treating providers should be aware of patients with poor HL as they may be at risk for uncontrolled BP.

Funding: Private Foundation Support

SA-PO2520

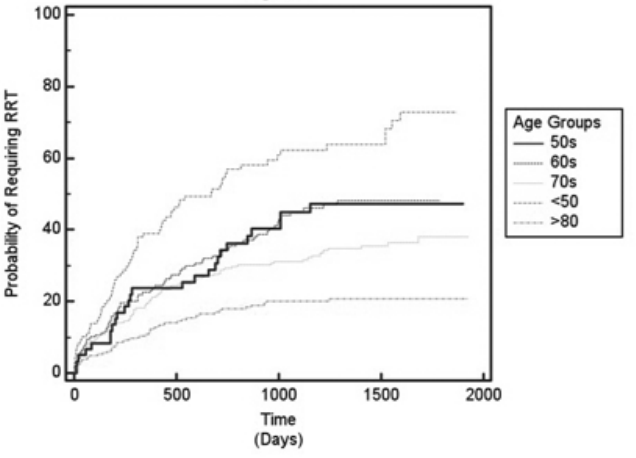
Outcome of Patients Attending Low Clearance Clinics in Northern Ireland Anil Kumar Xavier, Ying C. Kuan. *Renal Unit, Altnagelvin Hospital, Londonderry, United Kingdom.*

Background: Low clearance clinics (LCC) are designed to focus multidisciplinary care on patients with advanced renal disease, particularly in preparation for renal replacement therapy (RRT). Although studies have highlighted the high mortality in this cohort, few studies have specifically looked at the outcomes of patients entered into LCC. This retrospective study looks at the outcome of patients, specifically at RRT or death, in LCC in Northern Ireland.

Methods: Patients who entered LCC between 2006-10 were included, and followed up until 1st April 2011. Duration for RRT commencement or death was calculated. Analysis of likelihood of either outcome, depending on age category was then analysed using Kaplan-Meier method and Cox proportional hazards model.

Results: Data of 910 patients (478 males, 432 females) were analysed. The age range was 18-98 years (median age - 75). At the end of the follow up period, 324 (36%) patients had commenced RRT and 170 (18%) patients had died without receiving RRT. Using Coxs hazard analysis, the risk of death increased with age (p<0.0001), and conversely, likelihood of RRT requirement correlated negatively with age (p<0.0001). Kaplan Meier survival chart of outcome related to probability of requiring RRT is as below.

Figure 1



Conclusions: 1. The risk of dialysis requirement was highest amongst those in the youngest age group, and decreased with advancing age.

2. Conversely, the risk of death without initiation of RRT is highest amongst those in the oldest age group.

3. LCC's are beneficial for overall care of this vulnerable population group, and identified many patients at high risk of requiring RRT. Further studies in this population may allow for better targeting of therapy and resources to optimise outcome.

SA-PO2521

Fluid Balance and Patient Outcomes in AKI: Analysis of the Renal Study Participants Martin P. Gallagher,[1] Rinaldo Bellomo,[2] Alan Cass,[1] Louise Cole,[3] Joanne Y. Lee,[1] Serigne N. Lo,[1] Shay McGuinness,[4] John A. Myburgh.[1,5] *[1]The George Institute for Global Health, Sydney, Australia; [2]Austin Hospital, Heidelberg, Australia; [3]University of Sydney, Kingswood, Australia; [4]Auckland City Hospital, Auckland, New Zealand; [5]St George Hospital, Kogarah, Australia.*

Background: Fluid resuscitation is considered beneficial in critically ill patients with AKI, but there is little evidence to support it. Some evidence from observational studies suggests liberal fluid administration may be harmful. Using data from the RENAL Study we sought to examine the relationship between fluid balance (FB) and clinical outcomes in critically ill patients with acute kidney injury (AKI) requiring dialysis.

Methods: Fluid balance and clinical outcomes data from all participants in the study, a randomised trial of dialysis dose intensity in the setting of AKI in intensive care patients, was analysed using multivariable logistic regression, Cox proportional hazards, time dependent analysis and repeated measure analysis models.

Results: Data on 1464 participants was analysed. During study treatment, mean daily FB among survivors was -201 ml/day compared with +476 ml/day among non-survivors (P<0.001). Mean cumulative FB over the same period was -1294 vs. 311 ml (p<0.001). A negative mean daily FB during study treatment was independently associated with a decreased risk of death at 90 days (OR: 0.32, 95%CI: 0.24-0.43; p<0.001) and with increased survival time (P<0.001). In addition, a negative mean daily FB was associated with significantly increased renal replacement (RRT) free days (p=0.002), ICU free days (p<0.001) and hospital free days (p=0.01). These findings included adjustment for co-morbidities and patient acuity, and were unaltered after the application of different statistical models.

Conclusions: In RENAL Study participants, a negative fluid balance was consistently associated with improved clinical outcomes. Future interventional studies targeting fluid replacement may be one means of improving the poor outcomes of patients with AKI.

Funding: Government Support - Non-U.S.

SA-PO2522

Distribution of CKD and Albuminuria in Shropshire, U.K R. Diwakar,[1] Katherine Richmond,[2] Kevin Eardley.[1] *[1]Department of Nephrology, Royal Shrewsbury Hospital, Shrewsbury, England, United Kingdom; [2]Department of Biochemistry, Royal Shrewsbury Hospital, Shrewsbury, England, United Kingdom.*

Background: In the elderly (> 65 years) non-diabetic population there is on going debate about using eGFR as a screening tool for diagnosing Chronic Kidney Disease (CKD). CKD in Shropshire involve predominantly this age group. The Department of Health Quality and Outcomes Framework (QOF) recommends annual assessment including testing of Urine Albumin Creatinine ratio (UACR) to be performed. This is an expensive strategy the benefits of which is unclear in elderly non-diabetics with CKD Stage 3A without albuminuria. Hence we decided to analyse what proportion of nondiabetic CKD patients in Shropshire is above 65, has CKD stage 3A with no albuminuria.

Methods: Shropshire is a county of about 500, 000 people. All samples sent by general practitioners are analysed in one of 2 laboratories. Data was collected retrospectively from the computer systems of these laboratories of nondiabetic adult patients (age> 18) who had eGFR of < 60 ml/min (Stage 3 CKD or above) between October 2009 to September 2010.

Results: 9333 patients were found to have a GFR of < 60 ml/min (Stage 3a 6582, Stage 3b-2256, Stage 4- 454 and stage 5- 41). Among these 9333 patients UCR data was missing in 142 patients. Data from the remaining 9191 patients (1372 < /= 65, 7819 > 65 years of age) were analysed further. ACR analysis revealed normalbuminuria (UACR < 3 mg/mmol) in 7131 patients (5984 in the over 65), microalbuminuria (UACR 3-30) in 1753 patients (1581 > 65 years), overt proteinuria (UACR > 30) in 307 patients (254 > 65 years). In those > 65 years 4377 patients had CKD stage 3a and normalbuminuria, 1409 patients had stage 3b CKD and normalbuminuria.

Conclusions: Of the 9191 patients with CKD stage 3 - 5 in Shropshire (in whom UACR data was available), 47.6 % were elderly patients with CKD Stage 3A with no albuminuria. Yearly assessment including UACR in this group is probably not beneficial as it neither predicts progression of renal failure nor is it predictive of increased cardiac risk compared to general population. Recognising this in future QOF guidelines to General Practitioners in the UK could represent considerable savings.

SA-PO2523

Effects of Age and Case Definitions of Periodontitis on Associations with Albuminuria and CKD Afshin Parsa,[1] Nawi Ng,[2] Shabnam Salimi.[1] *[1]Nephrology, University of Maryland School of Medicine, Baltimore; [2]Umeå University, Sweden.*

Background: A wide variety of definitions of periodontitis have been employed, many focusing on acute periodontal disease activity. Since the development of renal disease is more likely due to chronic periodontal disease, we hypothesized that markers of chronic periodontal disease burden, including edentulous state, would be favorably associated with renal dysfunction. We also explore whether age, which can be correlated with both periodontal disease duration and susceptibility to chronic kidney disease (CKD), modifies these associations.

Methods: A total of 13,958 adult participants from NHANES III were included in this study. We used an adapted CDC definition to define periodontitis severity and also look at the effect of case definitions on our renal measures. The association between periodontitis and edentulous state with macroalbuminuria (ACR ≥ 300 mg/g), glomerular filtration rate (GFR) and CKD (GFR ≤ 60 ml/min/1.73.m2) was analyzed using multivariate logistic regression to adjust for related clinical and socioeconomic factors. We also looked for differential association between periodontal disease and renal function measures by age groups (age ≥ or < 40years).

Results: We found strong associations between severe periodontitis and both macroalbuminuria and CKD (OR: 1.89, CI:1.18-3.03; OR: 1.60, CI:1.28-2.00, respectively). There was also a strong association between edentulous state and macroalbuminuria (OR: 1.96, CI 1.21-3.16) and CKD (OR: 1.77, CI 1.38-.2.28). Further adjustment for inflammatory markers only slightly attenuated these associations. Edentulous state was a more robust predictor of CKD than periodontitis in the younger group, while periodontitis was a better predictor in those older than 40. We also find novel significant additive interactions between age, periodontitis and CKD (p<0.001).

Conclusions: In addition to case definition, age group strongly affects the association between periodontal disease and renal function measures. These findings not only further argue for the standardization of periodontal case definitions, but also suggest that age is a critical determinator of the association between periodontal and renal disease.

Funding: Other NIH Support - K12 MCRCDP

SA-PO2524

Is There a Lower Competing Risk of Death Than Risk of ESRD To Account for the High Incidence of ESRD in Taiwan Wu-Chang Yang,[1] Yee-Yung Ng,[1] Chih-Ching Lin,[1] Shang-Jyh Hwang.[2] *[1]Division of Nephrology, Taipei Veterans General Hospital and National Yang-Ming U., Taipei, Taiwan; [2]KMU, Kaohsiung, Taiwan.*

Background: Chronic kidney disease (CKD) is a disease of medical progress. A better healthcare for cardiovascular disease (CVD) prolong the life span of diabetic and elderly patients, who are very high risk group of CKD. Thus, the longer these patients survive, the higher chance they will develop CKD and the consequent end-stage renal disease (ESRD). A recent study shows that Asian CKD patients had faster CKD progression but lower mortality rate than Caucasian patients. Moreover, the incidence of of CVD in general population is much less in Asians than Caucasians. It is therefore reasonable to hypothesize that a lower risk of death competing for risk of ESRD is one of rationales to account for the high incidence of ESRD in Taiwan.

Methods: Two groups of study cohort were collected for comparison. One is hospital-based CKD registry which include 8,404 patients from 89 medical centers, collected from Jan 2007 to Dec 2009 and folllowed up to Jun 2010. The second one is a population-based sero-epidemiologic survey including 6,600 cases conducted for TW3H (Hypertension, Hyperglycemia, and Hyperlipidemia) Project in year 2002. A post hoc survival analysis was conducted in 2008.

Results: In a 2.5 years follow-up, the hospital-based CKD registry does show a 5.4 times lower competing risk of death than risk of ESRD in CKD patients. However, The population-based TW3H project showed a 18.4 times higher competing risk of death during 7 years' follow-up. The ratio of risk of death vs. risk of ESRD were 49.7, 11.4, 4.5 and 1.0 for patients with eGFR 60~89, 30~59, 15~29, and ≤15 ml/min, respectively. Subjects with lower eGFR have a higher CVD, cerebrovascular, and all-cause mortality.

Conclusions: The risk of death in CKD patients is much higher than risk of ESRD in stage 2-4 CKD patients. Thus, a lower competing risk of death is unlikely a rationale for the high incidence of ESRD in Taiwan. However, a better optimized CKD care in medical centers can reduce the metabolic burden of CKD and leads to a lower competing risk of death, further emphasizing the importance of multi-disciplined renal care.

SA-PO2525

Aortic Valve Calcification Predicts Coronary Artery Disease in Patients with Chronic Kidney Disease Soo Bong Lee, Il Young Kim, Bo Kyung Choi, Dong Won Lee, Harin Rhee, Eun Young Seong, Sang Heon Song, Ihm Soo Kwak. *Department of Internal Medicine, Pusan National University School of Medicine, Yangsan, Republic of Korea.*

Background: Aortic valve calcification (AVC) is common in chronic kidney disease (CKD) patients and the prevalence of coronary artery disease (CAD) in CKD patients is higher than other disease populations. But little is known about the association between AVC and CAD in CKD patients

Methods: We retrospectively reviewed the medical records of 408 patients who underwent both transthoracic echocardiography (TTE) and coronary angiography in our university hospitals. The patients were divided into 2 groups according to their estimated glomerular filtration rate (eGFR), CKD group (n=131, eGFR 15-59 mL/min/1.73m^2) and non-CKD group (n=277, eGFR ≥ 60 mL/min1.73m^2). TTE reports were reviewed to evaluate the AVC and the Gensini score was used to evaluate the severity of CAD.

Results: The CKD group showed higher prevalence of AVC (32% vs. 11%, p<0.001), CAD (61% vs. 24%, p<0.001) and more severe CAD (Gensini score, 25.86 ± 30.76 vs 15.83 ± 27.39, P<0.001) than non-CKD group. In multivariate logistic regression analysis, AVC was the predictor of CAD only in CKD group (OR=11.079, P<0.001), whereas there was no statistical significance in non-CKD group (OR=1.658, P=0.228).

Conclusions: The severity and prevalence of CAD were higher in CKD group compared to non-CKD group. AVC seemed to predict the CAD only in CKD group. Identifying the presence of AVC by non-invasive TTE could be useful in predicting the CAD in patients with CKD.

SA-PO2526

Prevalence and Predictors of Fatigue in CKD and ESRD Manisha Jhamb,[1] Kelly V. Liang,[1] Khaleelah Glover,[2] Nirav A. Shah,[1] Mark L. Unruh.[1] *[1]Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, PA; [2]Department of Psychiatry, University of Pittsburgh, PA.*

Background: Fatigue is a common debilitating symptom in patients with advanced chronic kidney disease (CKD), but there are no studies comparing the severity of fatigue using validated measures among non-dialysis dependent CKD and end-stage renal disease (ESRD) patients. The Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) is a 13-item fatigue scale, which has been shown to be reliable and responsive to interventions. The aim of this study is to examine the prevalence and severity of fatigue; and identify predictors of fatigue in a cross-sectional survey of 173 CKD and ESRD patients.

Methods: We examined fatigue in 87 CKD stage 4-5 patients and 86 ESRD patients using the FACIT-F and SF-36 scales. The participants were divided into two groups (FACIT-F scores >24 versus <=24) based on the mean score among anemic cancer patients. Predictors of worse fatigue at baseline were determined using linear regression model.

Results: The mean FACIT-F score among all participants was 34.5±11.0 and was similar among the CKD and ESRD groups (p=0.73). The FACIT-F score correlated closely with the SF-36 vitality score (Pearson correlation coefficient 0.81). After adjusting for age, race, gender, group (CKD versus ESRD), and medication use (benzodiazepine, antidepressant), the presence of cardiovascular disease, low serum albumin, depression, poor subjective sleep quality, and excessive daytime sleepiness were found to be independent predictors of worse fatigue at baseline.

Characteristic	Beta	Standard Error	p-value
Cardiovascular disease	-4.75	2.19	0.032
Albumin	24.78	7.68	0.002
Depression	-1.63	0.16	<0.0001
Sleep quality	-0.67	0.22	0.003
Daytime sleepiness	-4.57	1.10	<0.0001

Conclusions: Patients with CKD stage 4-5 and ESRD experience profound fatigue. Interestingly, the severity of fatigue is similar in these two groups. The FACIT-F is a useful scale to assess the severity of fatigue in this population and correlates highly with the SF-36 vitality scores. Depression, poor sleep and low albumin levels may be targets for interventions to improve fatigue in patients with advanced CKD.

SA-PO2527

A Comparison between Patients Referred and Not Referred to an Inner:city Nephrology Clinic Anjali Acharya,[1] Sophie Kwok,[1] Venu Gopal Kankani,[1] Rakesh Malhotra.[1] *[1]Nephrology, Jacobi Medical Center, Bronx, NY; [2]Scarsdale, NY; [3]Scarsdale, NY.*

Background: The prevalence of chronic kidney disease (CKD) is increasing. Ethnic minorities are at high risk, constituting a majority of the population in Bronx inner-city hospitals. There is a high prevalence of diabetes mellitus (DM) and hypertension in this population, which places them at a high risk for CKD. Early detection of CKD and renoprotection are crucial in this group.

Objective: To compare the baseline characteristics, comorbid conditions and stage of CKD of patients at referral to a nephrology clinic to those not referred.

Methods: We conducted a retrospective chart review of all patients seen in the primary care clinic from July 1, 2009 to December 31, 2009 who had a serum creatinine (SCr) of 1.5mg/dl or greater. Data was collected on demographics, comorbid conditions and stage of CKD for those referred to nephrology as well as for those not referred.

Results

	Nephrology Consults n (%)	No – Consults n (%)	P value
Number	71	253	
Demographics			
Gender			
Male	42 (59.2)	161 (63.6)	.490
Female	29 (40.8)	92 (36.4)	
Age	59.77±10.72	60.71±11.74	
BMI	30.1±6.5	31.4±10.3	
Smoker, n(%)	13 (18.3)	79 (31.3)	.09
Insurance, n (%)	68 (95.8)	245 (97.2)	.17
Comorbidity			
DM, n (%)	34 (47.9)	149 (58.9)	.002
Hypertension, n (%)	60 (84.5)	212 (83.8)	.885
CAD*, n (%)	34 (47.9)	74 (29.2)	.0001
CHF*, n (%)	21 (29.6)	43 (17)	.009
HIV*, n (%)	9 (12.7)	34 (13.4)	.855
Hepatitis C, n (%)	5 (7)	17 (6.7)	
CKD			
Stage 3	52 (80)	222 (87.7)	
Stage 4	10 (15.4)	27 (10.7)	
Stage 5	3 (4.6)	1 (0.4)	

*Coronary artery disease (CAD); Congestive heart failure (CHF); Human immunodeficiency virus (HIV)

Results: Only 21% (71 of 324) of patients with an SCr of greater than 1.5mg/dl were referred to the renal clinic.

58.9% of those with an SCr greater than 1.5mg/dl and not referred for nephrology consultation had DM.

Despite early stage of CKD in 80% of those referred, CAD was present in almost 48% of patients.

10.7% of the patients in the non-referral group were in stage 4 CKD.

Conclusions: Education of primary care physicians for early nephrology referral of a high-risk population for progression of CKD should be a priority.

SA-PO2528

Effect of Imputing Non-Ignorable Missing Data on Serial Measures of Renal Function: The Strong Heart Study Nawar M. Shara,[1,2] Hong Wang,[1,2] Barbara V. Howard,[1,2] Jason G. Umans.[1,2] *[1]MedStar Health Res Inst; [2]Georgetown-Howard Universities Clin & Translational Sci Ctr.*

Background: Kidney and cardiovascular disease (CVD) co-progress over time. However, it is challenging to simultaneously study interactions between progressive CKD and CVD in longitudinal studies because their morbidity and mortality lead to missing data which are likely not missing at random (NMAR). We have previously assessed the choice of imputation methods to best account for MAR renal function data in prediction of CVD (Kidney Int, 2007). We now explore approaches for cases in which data are NMAR and when exclusion of the missing data would likely bias relationships.

Methods: A cohort of 4549 American Indians from the Strong Heart Study with complete serial measures of serum creatinine data was considered. This cohort has a high burden of obesity, DM, CKD, and CVD. We used 4 different approaches to derive renal data subsets with MAR or NMAR data using the following algorithms: 1. missing completely at random 2, autoregressive MAR data 3. autoregressive MAR augmented with age and sex 4. and empirical non-ignorable missing data. These four subsets with missing data were used to examine the performance of five imputation methods: 1. listwise deletion (LD) 2. mean of serial measures 3. adjacent value (AV) 4. multiple imputation (MI) and 5. pattern-mixture (PM). One-hundred bootstrap samples were obtained with replacement to ascertain the accuracy of the point estimates.

Results: The hazard ratios (HRs) from each of the imputed sets were contrasted with HRs obtained from complete data set and compared across the different models. HRs generated by the PM method were closest to those using complete data. For NMAR data, the mean Scr imputed by PM differed less from that in the full dataset than did mean Scr using any of the of the imputation methods. The PM and AV imputation methods provided HRs for CVD risk which were similar to those using the complete data. By contrast, the PM method overestimated the mean of Scr and underestimated the hazard ratio for CVD risk when data were MAR.

Conclusions: Pattern-mixture imputation best accounted for non-random missing renal function data in a large study of progressive CKD and CVD.

Funding: Private Foundation Support

SA-PO2529

Lack of Specialised Renal Care in Patients with Chronic Kidney Disease: The Implicate Study Patrick Saudan, Marangon Nicola, Chantal Martinez, Catherine Stoermann, Belen Ponte, Sophie M. De Seigneux, Pierre-Yves F. Martin. *Nephrology, Geneva University Hospitals, Geneva City, Geneva, Switzerland.*

Background: We undertook a prospective randomised trial to determine the impact of specialised care by nephrologists compared to guidelines-directed management by primary care physicians (PCP) on prognosis, planning of RRT and patient satisfaction in CKD patients.

Methods: Single center prospective randomised study. *Inclusion criteria:* CKD patients with an eGFR< 45 ml/min, aged 18-80 years old and enrolled during a hospitalization. *Exclusion criteria:* AKI or ESRD, estimated life expectancy < 2 yrs, refusal or inability to sign writing consent and patients previously known by nephrologists. The primary composite endpoint is death and/or hospitalisation during the 24 months after inclusion. The secondary endpoints are initiation of urgent RRT, decline of renal function and quality of life. *Study design:* Patients are randomised in two arms: -Combined management PCP and nephrologists (4 nephrology visits/year) -Management by PCPs with the help of written instructions and consultations being provided by our unit if requested by PCPs. Quality of life will be assessed every year with

Results: At the end of May 2011, 289 patients have been eligible, of whom 69 patients refused to participate and 70 (24%) were already followed by a nephrologist. One hundred and fifty patients have been randomised. Mean age is 66 ± 9 yrs, mean baseline eGFR is 31 ± 9 ml/min and mean Charlson comorbidity score is 4.7. Out of the 289 patients, 219 had either diabetes or nephrosclerosis, 19 had chronic glomerulonephritis (CGN) and 51 other diagnoses of CKD. Follow-up for patients with CGN rises to 89% and falls to 17% in patients with CKD due to diabetes or nephrosclerosis.

Conclusions: These preliminary results indicate that there is a lack of renal care for patients with CKD, especially for those whose renal impairment is due to diabetes or nephrosclerosis. The Implicate study may demonstrate whether regular renal care in these patients brings substantial benefits in terms of survival, hospitalisation rate and decline of renal function. ClinicalTrials.gov**:** NCT00929760

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-PO2530

Chronic Kidney Disease Is a Risk Factor for Complications of Peripheral Artery Disease Ojas A. Naik, Jose Jesus Perez, Sreedhar A. Mandayam, Venkataraman Ramanathan. *Department of Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Peripheral artery disease (PAD) coexists in patients with chronic kidney disease (CKD) and is often associated with significant morbidity and mortality. During the 1-year follow up period after diagnosis of PAD, we identified risk factors associated with composite end point of gangrene, death, or re-hospitalization for PAD.

Methods: In this retrospective study, we obtained data from veterans who were evaluated for PAD and who had abnormal ankle-brachial index (0.9> ABI > 1.3), digital pressure (<50) or abnormal qualitative waveform. Veterans who had prior history of PAD were excluded. MDRD GFR was estimated. Veterans were followed for one year after index vascular evaluation. Primary end point was composite of gangrene, ischemic ulcer, death or re-hospitalization for PAD symptoms.

Results: A total of 597 veterans underwent vascular evaluation and after excluding normal studies and veterans with prior history of PAD, our study group included 346 veterans. During the 1-yr follow up, 27 veterans (group A) reached composite endpoint and 319 did not (group B). On comparing the two groups, patient characteristics including history of diabetes (56% vs. 50%), hypertension (89% vs 92%), smoking (70% vs 67%), hyperlipidemia (70% vs 80%, ischemic heart disease (30% vs 39%) were statistically similar.

Risk Factor	Group A (n=27)	Group B (n=319)	P value
Age	70 +/- 10	66 +/-10	0.03
Vascular Imaging Study	12 (46%)	146 (46%)	> 0.05
Severe PAD ((ABI < 0.7)	20 (73%)	153 (48%)	0.013
Revascularization	3 (11%)	56 (18%)	> 0.05
CKD (eGFR < 60ml/ min)	14 (52%)	90 (28%)	0.01

As shown, risk factors for veterans reaching composite endpoint include older age, severe PAD and stage III or worse CKD.

Conclusions: Chronic kidney disease appears to be associated with an increased risk of PAD-related complications and death. Further study is needed to see if earlier screening for PAD in patients with CKD can lead to earlier diagnosis and management and reduce these complications.

SA-PO2531

Effect of Angiotensin Receptor Blockers on Serum Phosphate and Calcium and Its Association with Renal and CV Outcomes Paul Smink,[1] Stephan J.L. Bakker,[1] William F. Keane,[2] John N. Harvey,[3] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Kidney Center, University Medical Center, Groningen, Netherlands; [2]Dept. Medicine, University of Minnesota, Minneapolis; [3]Dept of Endocrinology, Maelor Hospital, Wrexham, United Kingdom.*

Background: Previous studies have shown that high serum phosphate and low calcium are associated with increased risk of renal and cardiovascular outcomes. Angiotensin Receptor Blockers (ARB) delay the progression of renal disease and provide cardiovascular (CV) protection. We assessed the effects of ARB treatment on phosphate and calcium and investigated to what extent short-term effects of ARBs on these parameters translate into long-term renal and CV outcomes.

Methods: In a combined analysis of the Reduction of Endpoints in non insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy (IDNT) trials, we first determined the effect of ARB therapy on phosphate and calcium by testing the one year change versus placebo, secondly determined the association of these changes with long-term outcomes and finally adjusted the effect of ARB treatment on long-term outcomes for change in calcium and phosphate in Cox regression analyses.

Results: Compared to placebo, ARB treatment increased calcium levels with 0.07 mg/dL (95%CI 0.03 to 0.11; p<0.001) at year 1, but did not change phosphate (-0.01 mg/dL (95%CI -0.05 to 0.08; p=0.64). Each 0.1 mg/dL increment in calcium in the first 12 months resulted in a reduction of long term renal risk of 7% (95%CI 5 to 8; p=<0.001). No association was observed between the one year change in calcium and cardiovascular outcome. Adjustment of the renoprotective effect of ARBs for the observed one year change in calcium attenuated the effect of ARB treatment from 21% (95%CI 9 to 31) to 7% (95%CI -7 to 19), suggesting that part of the renoprotective effect is attributable to its effect on serum calcium. Essentially similar results were obtained when the analyses were repeated in the trials separately.

Conclusions: ARB treatment induces an increase in serum calcium, which explains part of its long-term renoprotective effect in diabetic patients with nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2532

Patterns of Progression in Chronic Kidney Disease (PoPe) Study: Baseline Data Craig L. Nelson,[1,4] Robert G. Fassett,[2,4] Neil Boudville,[3,4] Anna Kemp,[3] Eugenia Pedagogos,[4] Helen G. Healy,[4] George Jack Mangos,[4] Henry R. Moody,[4] Geoffrey S. Kirkland,[4] Troy D. Kay,[4] David A. Waugh.[4] *[1]Nephrology, Western Health, Melbourne, Victoria, Australia; [2]University of Queensland, Australia; [3]University of Western Australia, Australia; [4]electronic Kidney Disease National Audit Alliance, Australia (eKiDNAA), Australia.*

Background: The factors influencing the rate of CKD progression remain unclear. eKiDNAA is a National collaborative CKD research group assessing CKD patients referred to nephrology practices in Australia. We present baseline data from the first 3105 patients entered into the study. We aim to assess referred Australian CKD patients longitudinally for rates of progression of CKD, and to explore factors that may influence it such as demographics, biochemistry, therapies and co-morbidities.

Methods: This is an observational cohort study. Data were collected on referred CKD patients attending nephrology practices in Australia using Audit 4 software. The data collected included demographics, diagnosis and stage of CKD.

Results: The data on the first 3105 patients seen and entered into the Audit 4 database are presented. The study is ongoing. The average age of the cohort was (mean/SD) (68/14.9) years. CKD Stages were represented as follows: 1-2: 11%, 3: 39%, 4:21%, 5: 27%. Males had greater representation at all stages of CKD most marked in Stage 5, M:F 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 27%, Glomerulonephritis 14%, APCKD 4%, Reflux Nephropathy 3%, Other 25%. Hypertension may be over represented as it is recorded as a co-morbidity and may not be the primary cause of the CKD.

Conclusions: We have initiated a CKD research group of Audit 4 users investigating CKD longitudinally in Australia. Baseline data shows a large number of stage 3 CKD patients with mainly hypertension and diabetic nephropathy. Longitudinal data will enable assessment of factors determining the rate of CKD progression, adequacy of CKD therapies, models of CKD care and resource utilization.

SA-PO2533

Use of Coenzyme Q10-Depleting Medications by Chronic Kidney Disease Status in the U.S. Vanessa Grubbs,[1,2] Laura C. Plantinga,[1] Delphine S. Tuot,[1] Neil R. Powe.[1,2] *[1]University of California, San Francisco; [2]San Francisco General Hospital.*

Background: Many commonly used medications deplete body stores of coenzyme Q10 (CoQ), which is believed to have antioxidant effects. This depletion may contribute to oxidative stress and subsequent renal dysfunction. The use of CoQ-depleting medications in the U.S. by CKD status is unknown.

Methods: Using 1999-2008 National Health and Nutrition Examination Survey data, we examined reported use of CoQ-depleting medications among 21,169 non-pregnant adults (age 20+ years). CoQ-depleting medications included: beta blockers, clonidine, gemfibrozil, statins, hydralazine, thiazide diuretics, sulfonylureas, tricyclic antidepressants, and phenothiazine derivatives. CKD stage 1/2 was defined by urinary albumin:creatinine ratio of ≥30 mg/g with eGFR ≥60 ml/min/1.73 m2 and CKD stage 3/4 by eGFR 15-59 ml/min/1.73 m2. The prevalence and odds of taking a CoQ-depleting medication by CKD status were estimated via multivariable logistic regression, weighted to the U.S. population.

Results: Overall, an estimated 20.7% of participants reported taking at least one CoQ-depleting medication, but prevalent use was significantly higher among those with greater CKD severity: 16.3%, 33.2%, and 53.0% of those with no CKD, CKD stage 1/2, and CKD stage 3/4, respectively (p<0.001). Among 10,388 participants without diabetes (self-reported or glycosylated hemoglobin >6.5) or hypertension (self-reported or blood pressure >140/90), conditions for which most CoQ-depleting medications are indicated, prevalent use followed a similar pattern: 5.4%, 8.4%, and 24.8% of those with no CKD, CKD stage 1/2, and CKD stage 3/4, respectively (p<0.001). After adjustment for demographics, co-morbid disease, and healthcare visits, those with advanced CKD were 1.5-fold more likely to be taking a CoQ-depleting medication than those without CKD (stage 1/2: OR 1.10, 0.93-1.29; stage 3/4: OR 1.53, 1.30-1.80, vs. no CKD).

Conclusions: Greater CKD severity is associated with higher use of CoQ-depleting medications. The extent to which CoQ–depleting medications contribute to CKD development and progression and whether CoQ dietary supplementation may ameliorate this effect is worthy of investigation.

Funding: NIDDK Support

SA-PO2534

Referral Patterns for CKD to Nephrology; the Implications in the Management of the Renal Patient in an Academic Internal Medicine Practice Alejandro Solano Bayardo, Tiffanie M. Lowy, Leela M. Mathew. *Internal Medicine Department, Unity Health System, Rochester, NY.*

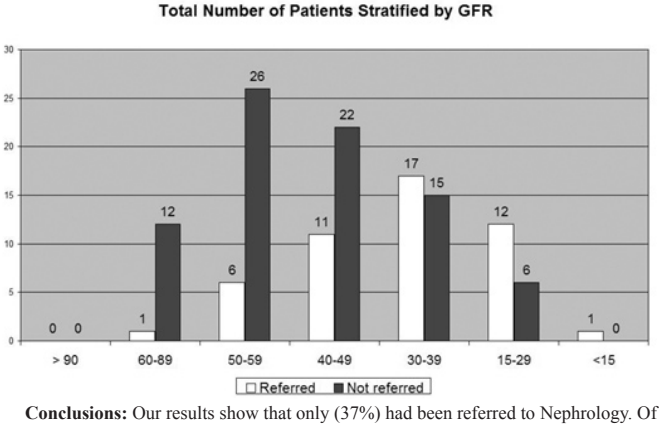
Background: Our objective was to determine the rate of referral and primary care physician patterns of referral to nephrologists in patients with CKD.

Methods: We conducted a retrospective review through NextGen EMR and Unity Faculty Partners, an academic ambulatory Internal Medicine clinic in Rochester, NY. We analyzed all patients with an ICD-9 code for CKD from 2007-2010, encompassing a total of 166. Out of this, 37 were excluded based on ESRD, those who had expired or lost to follow-up. Data was collected from the remaining 129 patients and graphed based on GFR, age and attending physician.

Results:

CKD Referral to Nephrology

	CKD I	CKD II	CKD III	CKD IV	CKD V	Total
Referred	0	1	34	12	1	48
Not Referred	0	12	63	6	0	81
			Mean Age			
Referred	0	62	63	79	82	67.1
Not Referred	0	68	76	86	0	75.2



Conclusions: Our results show that only (37%) had been referred to Nephrology. Of those with stage IV, most had been referred (66%). However, those with stage III, only (35%) had been referred. Stage III showed a wide variation, thus we further subdivided GFR into 3 groups from 30-39, 40-49 and 50-59. We were able to demonstrate an inverse relationship between a decline in GFR and an increase in referral with the majority of them being referred when the GFR fell to < 40.

Many of the patients that were not referred had advanced age, reflecting the decline of GFR to be age-related. Also some of them had shortened life expectancy from other causes. Additionally, we determined the patterns of referral according to attending physicians and found variable results demonstrating a non referral rate from 29-79%.

Acknowledging the current state of increasing co-morbidities in the U.S., both hypertension and diabetes mellitus are on the incline creating enormous potential for renal complications, optimal management of CKD is fundamental.

SA-PO2535

Analysis of Cause of Death and Level of Active Medical Management in a Conservative Care Programme for End Stage Renal Disease Linda H. Bisset,[1] Maria Fish,[1] Vicky Hinton,[1] Linda Evans,[1] Christine Porter,[1] Mark A.J. Devonald.[1,2] *[1]Renal and Transplant Unit, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [2]School of Clinical Sciences, University of Nottingham, United Kingdom.*

Background: In end stage renal disease (ESRD), renal replacement therapy (RRT) does not always improve quality or duration of life. Nottingham Renal Unit has an established conservative care (CC) programme which advises on symptom control and management of complications of CKD. We analyzed our 10 year prospective database to determine impact of the CC team on patients who have died. We determined the proportion of deaths attributed to ESRD and the proportion where bone disease or anaemia were being actively managed.

Methods: We analyzed data from patients who died on the CC programme, assessing demographics, CKD stage, changes in eGFR and laboratory parameters from start of CC to death. Aetiology of CKD and cause of death were coded by EDTA-ERA classification.

Results: At analysis 269 patients had died on the programme. 53% male, 93% Caucasian and median age 81. 37% had diabetes mellitus. Aetiology of CKD was uncertain in 42%. 43% were CKD stage 5 at start compared with 70% at death. Median number of days to death was 315. Table 1 illustrates median age, eGFR and laboratory parameters. Serum albumin and bicarbonate were lower at death. At start 7% pts were prescribed erythropoietin (EPO) therapy compared with 54% at death. Most common documented cause of death was therapy ceased at 38%, respiratory 17% and cardiac disease in 16%.

	Age	eGFR	Hb	Ca	P	Bic	Alb
Baseline	81	16	10.6	2.4	1.3	24	35
Death	82	11	10.2	2.4	1.4	21	29

Conclusions: Classification of 'therapy ceased' in this cohort is likely to mean death from ESRD, though there might be some exceptions to this (currently under analysis). The majority of patients did not die of renal failure. Median eGFR at start of programme is higher than expected from published data due to the inclusion of CKD stage 3 and 4. The CC programme at Nottingham Renal Unit offers patients an important alternative to RRT. Active medical management from the CC team appears to be important, regardless of whether patients subsequently die of renal failure or another pathology.

SA-PO2536

Evaluation of Chronic Kidney Disease Detection among High Risk Populations Using Automated Estimated Glomerular Filtration Rate Reporting M. Ahinee Amamoo,[2] Maria E. Ferris,[1] Keisha L. Gibson,[1] M. Alan Brookhart,[2] Abhijit V. Kshirsagar.[1] *[1]UNC Kidney Center, University of NC- Chapel Hill, Chapel Hill, NC; [2]Epidemiology, University of NC- Chapel Hill, Chapel Hill, NC.*

Background: Automatic eGFR reporting occurs in several US healthcare organizations but its impact remains debated. In April 2005, the University of North Carolina Healthcare System(UNCHS) voluntarily implemented automatic reporting of eGFR, calculated using the Modification of Diet in Renal Disease(MDRD) equation on all serum creatinine(SCr) tests. This study evaluates whether automatic eGFR reporting results in higher CKD detection among high-risk populations.

Methods: We compared the detection of CKD using ICD9 codes in the periods before and after the implementation of eGFR reporting, using linear risk regression. Hypertensive and diabetic adults, age 18-70 seen in the UNCHS between January 1, 2004 and August 31, 2009 with two SCr measurements, at least six months apart were included in the study.

Results: We identified 2077 hypertensive and 1042 diabetic patients. Patient characteristics were comparable before and after eGFR reporting. Mean age was 58±9 years, 60% females, and 55% Caucasian. The one year cumulative incidence of CKD detection was 0.09 for hypertensive patients and 0.12 for diabetics. Hypertensive and diabetic patients seen after eGFR reporting had a higher 1-year risk of CKD detection than before eGFR reporting,(Relative risk(RR:1.66, 95% confidence interval (CI):1.01,2.73) and (RR=1.2;95%CI:0.75,2.16), respectively. After adjusting for age, race, and gender, the 1-year risk of CKD detection among those who were seen after automatic eGFR reporting was 77% higher among hypertensive patients (RR:1.77;95%CI:1.03,2.77) and 25% higher among diabetics (RR=1.25;95%CI 0.75,2.10), than the 1-year risk of CKD detection among those seen before automatic eGFR reporting.

Conclusions: Increased detection of CKD occurred after eGFR reporting among high risk populations. These data suggest that automatic eGFR reporting promotes earlier detection of CKD among high risk populations. Future studies should examine whether earlier detection leads to changes in management of CKD.

Funding: NIDDK Support

SA-PO2537

Comparing eGFR Equations in the Elderly Natalie R. Ebert,[1] Olga Jakob,[2] Jens Gaedeke,[1] Martin K. Kuhlmann,[3] Peter Martus,[2] Markus van der Giet,[1] Elke Schaeffner.[1] *[1]Nephrology, Charité, Berlin, Germany; [2]Clinical Epidemiology and Biostatistics, Charité, Berlin, Germany; [3]Nephrology, Krankenhaus Friedrichshain, Berlin, Germany.*

Background: Although chronic kidney disease (CKD) is disproportionately affecting the elderly, these are underrepresented in most studies. We used the Berlin Initiative Study (BIS) population to compare present eGFR formulas.

Methods: The BIS is an ongoing prospective population-based cohort studying CKD in people ≥70. Sampling was done stratified for age and gender. Confirmatory analysis was done with t-test and chi^2-test. Agreement between formulas was assessed using Bland Altman and kappa statistics.

Results: The characteristics of the study population and subpopulations ($eGFR_{CKD\text{-}Epi}$ ≥ and <60 ml/min/1.73m^2) are shown in Table 1.

Table 1

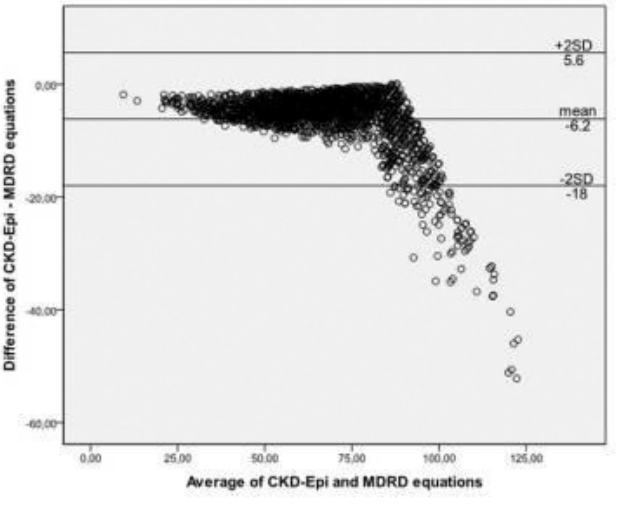
Characteristics	Total	$eGFR_{CKD\text{-}Epi}$≥60	$eGFR_{CKD\text{-}Epi}$<60	p-value
n	1967	1240	727	
Age (yrs)[1]	80	78	83	<0.001
Women[2]	52	53	50	n.s.
BMI[1]	27	26	28	n.s.
Art. Hypertension[2]	77	71	88	<0.001
Diabetes mel.[2]	23	21	27	0.004
MI[2]	14	11	19	<0.001
Stroke[2]	8	7	10	0.02
Cancer[2]	23	22	24	n.s.
Creatinine (mg/dl)[1]	1.0	0.8	1.3	<0.001
Cystatin C (mg/l)[1]	1.22	1.04	1.54	<0.001
$eGFR_{CKD\text{-}Epi}$(ml/min/1.73m^2) [1]	65	76	46	<0.001
ACR >30[2]	26	20	36	<0.001

Data are presented as [1] mean and [2] percentage

Paired t-test of eGFR equations showed sign. difference (p<0.001) of eGFR calculated with MDRD (71 ml/min/1.73m^2) or Cystatin C (60 ml/min/1.73m^2) compared to CKD-Epi for the total cohort.

Agreement between CKD-Epi and Cystatin C equations as well as CKD-Epi and MDRD for diagnosis of CKD with weighted kappa statistic was 0.58 and 0.85, respectively.

Bland Altman analysis between CKD-Epi and MDRD showed better agreement in lower but not in higher eGFR levels corresponding to the structure of the CKD-Epi formula (Figure 1).



Conclusions: Significant discrepancies of eGFR can be observed when using different estimating equations in the elderly. Comparison to GFR measurement is needed in people ≥ 70.

Funding: Private Foundation Support

SA-PO2538

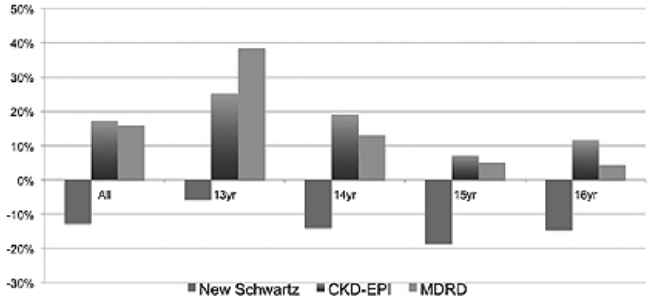
Which Is the Best Estimating Equation for GFR in Adolescents? John Brandt,[1] Craig S. Wong,[1] Antonia M. Harford,[1] Amy Staples.[1] *[1]Department of Pediatrics, University of New Mexico, Albuquerque, NM; [2]Albuquerque, NM; [3]Albuquerque, NM.*

Background: In 2009, Schwartz, et al. via the CKiD study revised a commonly used equation (eqn) to estimate GFR (eGFR) for children. The CKiD bedside (bCKiD) eqn tends to underestimate measured GFR (mGFR) in children over the age of 4 years(yrs) especially in adolescents (adol). Adol patients(pts) may be closer to adult body size and physiology than to younger pts. We investigate whether the adult derived eGFR eqns or the new bCKiD eqn are more accurate to estimate mGFR in adol pts.

Methods: Retrospective analysis of 140 pts 13 to 17 yrs with mGFR by iothalamate steady-state methodology at a tertiary care pediatric hospital. Diagnoses (Dx) include solid tumor pts both pre and post chemotherapy (chemo), children with chronic kidney disease (CKD) and with renal transplants(txp). Serum creatinine (sCr) was determined by enzymatic assay.

Results: Age range 13 to 17 yrs, mean age 14.9 (SD1.1). 49% male. The mean sCr for all pts was 0.76 mg/dl (SD 0.4). By Dx group, 15% were done pre-chemo, 56% were post-chemo, 16% had CKD and 13% were txp pts.

The bCKiD eqn underestimated mGFR by 13%, while CKDEPI overestimates by 17% and MDRD by 16%. The bCKiD eqn was most accurate in the youngest subjects and increasingly underestimated mGFR as age increased.



All three equations where more accurate for girls compared to boys.

Conclusions: The bCKiD eqn is reliable in younger pts, but less reliable in older adols, particularly boys. The MDRD and CKDEPI eqns accuracy are similar to bCKiD eqn, but overestimating mGFR in the opposite direction. Stratifying by age and gender, both CKDEPI and MDRD have an advantage over the bCKiD eqn in pts over 14 yrs of age. Overall, the bCKiD eqn may benefit from an adjustment in adols for gender, similar to what is seen in the adult-based eqs. and the original Schwartz eqn.

SA-PO2539

Comparison of Creatinine-Based and Cystatin C-Based GFR Estimating Equations in Chinese Children Xuemei Li,[1] Mengchun Gong,[2] Xuewang Li,[1] Hongmei Song,[3] Yan Qin,[1] Ke Zheng.[1] *[1]Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]Internal Medicine, Peking Union Medical College Hospital, Beijing, China; [3]Pediatrics, Peking Union Medical College Hospital, Beijing, China.*

Background: There is no data about glomerular filtration rate, measured through plasma or renal clearance of the exogenous markers, in the Chinese children. The objective of this study is to measure GFR and validate the GFR estimating equations in the Chinese children, aiming at selecting the equations with best performance in GFR estimation and CKD staging.

Methods: 85 children were enrolled in the study. Single-compartment plasma clearance of ^{99m}Tc-DTPA was measured using the radioactive counts of the two plasmas sampled after a bolus injection and transformed into two-compartment plasma clearance (mGFR). mGFR was normalized (nGFR) by body surface area. eGFR was calculated using 9 different equations, based on Creatinine (3), Cystatin C (3) or their combination (3). Creatinine and cystatin C were measured using kinetic Jaffe method and immunoturbidimetry, respectively. Correlation and agreement between eGFR and nGFR and the accuracy of GFR estimation was compared to validate and selet GFR estimating equations.

Results: Compared with the other six equations, original Schwartz equation, Filler equation and CKiD equation produced the eGFR with better correlation with nGFR ($\gamma_{Pearson}$ was 0.78, 0.73 and 0.75, respectively), stronger explanation capacity of variance in nGFR (R^2 was 0.612, 0.716 and 0.730), smaller bias (bias was 0.2, -4.2 and 3.5), narrower 95% LOA ([-61,61], [-50,42] and [-37,44]), better performance in Bland-Altman analysis , higher intraclass correlation coefficient (0.594, 0.724 and 0.741), higher CCC (0.592, 0.722 and 0.735), higher ratio of eGFR within nGFR±10% (30.6%, 33.8% and 37.7%) and within nGFR±30% (81.2%, 76.6% and 77.9%), higher ratio of correct CKD staging (65.9%, 68.8% and 75.3%) and better agreement in CKD staging between eGFR and nGFR (Kappa value was 0.60, 0.61 and 0.65).

Conclusions: The selected equations used to estimate GFR in Chinese children were original Schwartz equation, Filler equation and CKiD equation, while CKiD equation has the best performance.

Funding: Government Support - Non-U.S.

SA-PO2540

Estimating GFR in Renal Transplantation: A Multicentric Evaluation of Cystatin C Performance Ingrid Masson,[1] Nicolas Maillard,[1] Eric Alamartine,[1] Etienne Cavalier,[3] Pierre Delanaye,[2] Christopher R. Mariat.[1] *[1]Nephrology, University Hospital of St-Etienne, St-Etienne, France, Metropolitan; [2]Nephrology, University Hospital of Liege, Liege, Belgium; [3]Biology, University of Liege, Belgium.*

Background: Serum cystatin C (SCy) might be a better GFR marker than serum creatinine (SCr) in renal transplant patients. The aim of our study was 1- to confirm or not the superiority of SCy in estimating renal graft function and 2- to determine the influence of confounding bio-clinical variables susceptible to explain SCy concentration independently of GFR in renal transplant patients.

Methods: 826 renal inulin clearances (true GFR) performed in 423 renal transplant patients from 3 different European transplant centres were analysed. SCy and SCr were measured by nephelemetry calibrated on IFCC material and enzymatic method traceable to IDMS , respectively. GFR was estimated according to SCr-based equations (MDRD study equation and CKD-EPI(SCr) equation), SCy-based equation (CKD-EPI(SCy) equation) and both SCr and SCy-based equation (CKD-EPI(mix) equation). Primary endpoint was defined as the accuracy 30% (P30). The following co-variables were assessed : Weight, Gender, Age, Time post-tx, Stages of CKD, Diabetic status, Proteinuria, IS regimen, usCRP and Albuminemia.

Results: Mean (+/-SD) inulin clearance, SCr and SCy were 50.6 (+/-19) mL/min/1.73m2, 129 (+/-52) µmol/l and 1.64 (+/-0.61) mg/l, respectively. P30 was 75%, 77%, 81% and 84% for the CKD-EPI(SCr) equation, the MDRD Study equation, the CKD-EPI(Scy) equation and the CKD-EPI(mix) equation, respectively ($p<0.05$ for the comparison between SCr-based and SCy-based equations). Aera under ROC for a GFR threshold of 60 ml/min/1.73m2 was 0.832 and 0.901 for SCr and SCy, respectively ($p<0.0001$). After adjustments for the different non-GFR determinants tested, SCy remained significantly superior to SCr in predicting renal graft function.

Conclusions: Our data, obtained in a large population of transplant patients and with a standardized method of SCy dosage, confirm the superiority of SCy over SCr. Whether this better predictive performance may clinically translate into a better management of the transplant patient remains to be determined.

Funding: Government Support - Non-U.S.

SA-PO2541

GFR Estimation in the Morbidly Obese Pre and Post Bariatric Surgery: One Size Does Not Fit All Samra Abouchacra,[1] Ahmed Chaaban,[1] Nicole Gebran,[1] Qutaiba Abdulatif Daoud,[1] Bassam O. Bernieh,[1] Hanan Luay Al Omary,[2] Mohamed Ahmed,[1] Said Abuhasna.[1] *[1]Tawam Hospital, United Arab Emirates; [2]Baghdad University, Iraq.*

Background: Hyperfiltration with increased glomerular filtration rate (GFR) is commonly associated with obesity. This is expected to improve post bariatric surgery. However formula-based GFR estimation in the obese is limited by body size confounders necessitating use of modified equations, the reliability of which remains uncertain.

Our aim was to compare GFR- estimating formulae in morbidly obese patients at baseline & post bariatric surgery.

Methods: Retrospective review of 220 patients (145 females), mean age 34.7± 10 yrs with post-op followup over 6 months.

GFR was calculated using: MDRD4, CKD-epi: Usual & Body Surface Area (BSA)-adjusted; Cockroft Gault (CG) & Lean Body Weight-corrected (CG-LBW) formulae.

Results: Significant reduction in weight & BP was achieved with data as shown.

	Baseline	Postop
Weight (kg)	128.6±26	97.6±22*
BMI (Kg/m2)	47.0 ± 9	36.12±7*
SBP (mmHg)	132.75±12	121.64±11*
DBP (mmHg)	78.7±9	71.31±9*
Serum Creatinine (µmol/L)	63.05±14	58.01±13*
GFR estimation (ml/min):		
CG	227.05±76	172±65*
CG-LBW	128.67±44	109.6±43*
MDRD4	107.8 ±36	110.7±47†
CKD-Epi	108.7 ±27	107.1±37†
Adjusted MDRD	139.49 ±66	112.41±73*
Adjusted CKD-Epi	127.28 ±59	110.59 ±64*

* p <0.05 vs baseline † p NS

GFR estimation by different formulae showed marked variation. The expected supranormal GFR was apparent in estimators which took into account body size descriptors. This was grossly overestimated by CG but attenuated with adjustment for LBW. Conversely, MDRD and CKD-epi underestimated GFR which increased with BSA –correction. Post-operatively, significant decrease in GFR was seen in adjusted BSA- versions and CG-LBW. This became apparent when BMI decreased by 4.35 kg/m2.

Conclusions: Though clinicians must be critical in application of GFR estimates to patient care, CG-LBW appears to be a practical solution in the obese. This is because LBW can be more reliably assessed compared to BSA. However since adjusted MDRD & CKD-epi correlated well with it, these can be alternatives given their less cumbersome calculation. Future comparisons are needed with a "gold standard" for GFR.

SA-PO2542

Renal Effects of Bariatric Surgery in Patients with Chronic Kidney Disease Samra Abouchacra,[1] Ahmed Chaaban,[1] Nicole Gebran,[1] Qutaiba Abdulatif Daoud,[1] Hanan Luay Al Omary.[2] *[1]Tawam Hospital, Al-Ain, United Arab Emirates; [2]Baghdad University, Iraq.*

Background: Obesity is known to be associated with hyperfilteration state which improves with weight reduction. However, the impact of bariatric surgery on glomerular filtration rate (GFR) of patients with already compromised renal function is not yet established.

Methods: Patients with kidney disease (CKD I-II) who underwent bariatric surgery were retrospectively reviewed. Their eGFR was calculated using Cockroft Gault- lean body weight adjusted formula (GC-LBW). This was also estimated by both CKD-Epi and BSA- adjusted version pre and post operatively.

Results: From 220 patients, 41 were found to have pre- op e*GFR* 60- 90ml/min. M:F 1: 40, mean age 45.15± 8 yrs. 7 patients had diabetes and another 7 were hypertensive. After mean follow up of 7.2 ±3 months, significant decrease in weight, BMI and BP was achieved. Baseline and post op data are shown.

	Pre	Post
Weight (kg)	110.54±18	86.33±17*
BMI (kg/m2)	43.83±8.1	34.04±6.7*
sBP (mmHg)	132.55±13	120.8±11*
dBP (mmHg)	78.71±9.2	72.15±8*
Serum Creatinine (µmol/l)	80.32±14	69.6±13*
eGFR CG-LBW (ml/min)	76.26±9	86.63±18*
eGFR CKD-Epi (ml/min)	80.99±18	103.6±19*
eGFR adjusted CKD-Epi (ml/min/BSA in m2)	102.25±50	98.02±45
BSA (m2)	1.87±0.8	1.65±0.7

* p value < 0.05

Conclusions: Despite the small sample size and short follow up, using CG-LBW formula, a significant increase in GFR was seen in obese patients with early CKD after bariatric surgery. This was in contrast to the decrease reported previously in those with hyperfilteration preoperatively. Improvement in BP and overall metabolic profile may be partly responsible.

Though similar changes were seen with CKD-Epi eGFR, this is clearly unreliable without BSA correction. When adjusted, an overestimation of GFR was observed pre, with no sig change post op.

In the absence of gold standard for eGFR measurement, CG-LBW appears to be a more reliable estimator with possible role in CKD staging.

Whether the renal benefits from bariatric surgery are sustained or seen in more advanced CKD stages is uncertain. Long term studies are needed to explore this further.

SA-PO2543

Relationship between Glomerular Filtration Rate, Body Surface Area and Gender Belen Redal-Baigorri,[1] James G. Heaf,[2] Knud Rasmussen.[1] *[1]Nephrology, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark; [2]Nephrology, Copenhagen University Hospital at Herlev, Herlev, Denmark.*

Background: Normalisation of GFR with BSA with extreme body sizes might be misleading and this can have consequences in drug dosing and living donor kidney assessment.

Methods: Cross sectional study of 895 cancer patients, to investigate whether GFR indexed for BSA is reliable in extreme body sizes and whether this relationship is influenced by gender. GFR was investigated with (^{51}Cr-EDTA), the results were expressed as absolute values (GFR ml/min) or normalised for BSA by Du Bois and Du Bois formula (GFR ml/min per 1.73m^2,GFR bsa), and extracellular volume, ECV (GFRecv).

Results: A useful normalization index (NI) should have a high correlation to GFR, no residual correlation after correction, give similar values for both genders, and be robust over the entire range of values. The overall correlation between GFR and BSA was high (r=0.52), but was low for BSA <1.6 kg/m^2 (0.13) in both sexes. In males BSA was correlated to GFRbsa if BSA >1.8 (r=0.28). GFR correlation to ECV was high (0.49), and robust over the entire range of ECV values. However GFR correlated to GFRecv in women (r=-0.15). There were no significant differences between genders for GFRbsa or GFRecv.

BSA and ECV correlation with GFR

BSA correlation to GFR and GFR ml/min per 1.73m² . R values						
		Men		Women		
BSA	n	GFR ml/min	GFR ml/min per 1.73m²	n	GFR ml/min	GFR ml/min per 1.73m²
All	421	0,51 ***	0,22***	474	0,43***	0,07
<1,6	18	-0,18	-0,32	150	0,17*	-0,04
>1,6	403	0,48***	0,20***	324	0,28***	-0,02
<1,8	112	0,17	-0,01	370	0,34***	0,09
>1,8	309	0,50***	0,28***	104	0,25**	0,01
<2,0	293	0,30***	0,07	451	0,41***	0,10*
>2,0	128	0,44***	0,25**	23	0,32	0,09
ECV correlation to GFR and GFR corrected for ECV. R values.						
	Men			Women		
ECV	n	GFR	GFRecv	n	GFR	GFRecv
All	418	0,45***	0,02	469	0,41***	-0,15***
<12	22	0,31	0,04	101	0,23*	-0,07
>12	393	0,42***	0,05	327	0,29***	-0,13*
<14	101	0,23*	-0,07	331	0,33***	-0,08
>14	311	0,40***	0,09	131	0,19*	-0,15
<16	275	0,30***	-0,05	430	0,40***	-0,08
>16	140	0,30***	0,04	35	0,27	0

*:p<0.05; **p<0,01; ***:p<0,001;

Conclusions: Neither NI performed optimally. BSA was inappropriate when BSA<1.6 for both sexes. ECV performed better particularly in males.

SA-PO2544

Cystatin C- vs Creatinine-Based Equations Compared to 99m TcDTPA Scintigraphy for the Assessment of Glomerular Filtration Rate in Chronic Kidney Disease Hernan Trimarchi. *Nephrology, Hospital Britanico, Buenos Aires, Argentina.*

Background: In Chronic kidney disease (CKD) accurate estimation of the glomerular filtration rate (GFR) is essential. Gold standards are expensive and labourious. Different equations based on creatinine have been proposed to estimate GFR. However,creatinine levels are frequently far from reflecting real GFR. We compared creatinine- with cystatin C-based formulae, using ^{99m}TcDTPA scintigraphy as gold standard, and propose the best equation for each stage of CKD.

Methods: Prospective, cross-sectional, observational enrolling 300 subjects in 1 year. CKD and stages were defined as to K/DOQI guidelines and stratified into its 5 stages using ^{99m}TCDTPA. Group (G) 1n=26; G2n=52; G3n=90; G4n=37; G5n=60; Control Gn=35. Creatinine equations: Creatinine clearance, Crockoft-Gault, MDRD-4, CKDEPI. Cystatin C equations: Larsson, Larsson modified, Grubb, Hoek.

Results: Age and body mass index were different among groups; proteinuria, hypertension, diabetes and primary glomerulopathies significantly increased as kidney disease worsened. In the global assessment, CKDEPI and Hoek gave the highest significantly correlations with DTPA: ρ=0.826,p<0.001 and ρ=0.704,p<0.001. Lineal regression analyses: CKDEPI vs DTPA, r0.826, r^{2}0.682; Hoek vs DTPA, r0.704, r^{2}0.496; CKD EPI vs Hoek: r0.811; r^{2}0.658. Hoek kappa constant: 0.585, p<0.001; CKDEPI kappa constant: 0.505, p<0.001. However, important differences emerged when each group was studied separately. Most significant correlations with ^{99m}TcDTPA: Control G, creatinine clearance ρ=0.421, p=0.012; G1, Crockoft-Gault ρ=0.588, p=0.003; CKDEPI ρ=0.460, p<0.05; G2, CKDEPI ρ=0.462, p< 0.05; G3, CKDEPI ρ=0.508, p<0.001; MDRD-4 ρ=0.506, p<0.001; Hoek ρ=0.475, p<0.001; G4, Hoek ρ =0.618, p<0.001; creatinine clearance ρ =0.507, p=0.04; CKDEPI ρ=0.463, p=0.02; G5, CKDEPI ρ=0.604, p<0.001; Hoek ρ=0.592, p<0.001

Conclusions: If GFR<60 ml/min (stages 3 and 4), CKDEPI and Hoek equations appear to best correlate with ^{99m}TcDTPA. In stage 5, all formulae significantly coincide. However, in controls and in early stages of CKD creatinine-based methods or equations may correlate better with ^{99m}TcDTPA.

Funding: NONE

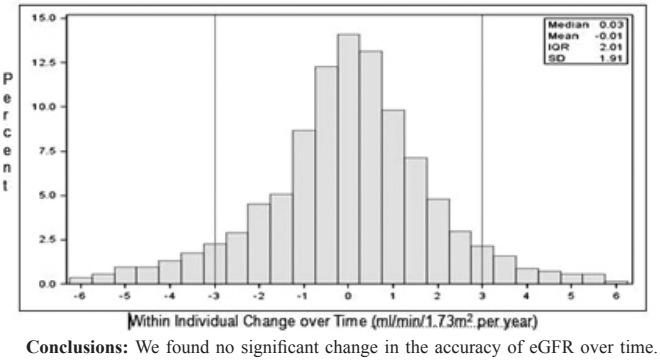
SA-PO2545

Accuracy of a Glomerular Filtration Rate Estimating Equation over Time in People with a Wide Range of Kidney Function Smita Padala,[1] Hocine Tighiouart,[1] Lesley Stevens Inker,[1] Gabriel Contreras,[2] Julia Lewis,[3] Michael Steffes,[4] Roger A. Rodby,[5] Christopher H. Schmid,[1] Andrew S. Levey.[1] *[1]Tufts Medical Center; [2]University of Miami; [3]Vanderbilt University Medical Center; [4]University of Minnesota; [5]Rush University Medical Center.*

Background: The accuracy of GFR estimated from serum creatinine (Scr) over time is not well known. We examined the rate of change in measured GFR (mGFR), estimated (eGFR) and the difference between mGFR and eGFR (error) over time in a large dataset with a wide range of kidney function.

Methods: GFR was measured using urinary clearance of ^{125}I-iothalamate (reference test) in subjects with and without kidney disease and diabetes. GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation (index test). The change over time was modeled using mixed models. We considered a change in error larger than ±3 ml/min/1.73 m^2 per year as clinically significant.

Results: There were 13,708 GFR measurements in 3635 subjects over a mean follow up of 3.6 years. The mean mGFR, eGFR and error at baseline were 76, 76, and -0.3 ml/min/1.73 m^2. The mean (standard error) change in mGFR, eGFR and error were -2.3 (0.12), -2.2 (0.09) and -0.1 (0.10) ml/min/1.73 m^2 per year (P <.0001, <.0001 and 0.6 respectively). The variability (SD) among subjects in changes in mGFR, eGFR and error was 2.24, 1.59, and 1.91 ml/min/1.73m^2 per year, respectively. Only 16% of subjects had changes in error larger than ± 3 ml/min/1.73 m^2 per year (Figure 1).



Conclusions: We found no significant change in the accuracy of eGFR over time. Changes in eGFR over time in most individuals were due to changes in mGFR rather than changes in non-GFR determinants of Scr. In the absence of recognized changes in non-GFR determinants of Scr, clinicians should interpret changes in eGFR as reflecting changes in mGFR.

SA-PO2546

Simple Cystatin C Formula Compared to Sophisticated CKD-EPI Creatinine & Cistatin C-Based Formula for Estimation of Glomerular Filtration Rate in Elderly Patients with Mild to Moderate Impaired Kidney Function Sebastjan Bevc, Radovan Hojs, Robert Ekart, Maksimiljan Gorenjak, Ludvik Puklavec. *Dept. of Nephrology, Nuclear Medicine, Clinical Chemistry, UKC Maribor, Slovenia.*

Background: Serum creatinine (Screa) concentration and Screa-based formulas are the most commonly used markers to estimate glomerular filtration rate (GFR). Recently, serum cystatin C (Scys)-based formulas and the newer creatinine & cystatin C-based formula (The Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI creatinine & cystatin formula)) were proposed as new GFR markers. The aim of our study

was to compare CKD-EPI creatinine & cystatin formula and simple Scys formula (100/Scys) against 51CrEDTA clearance in eledrly patients with mild to moderate (GFR 89-30 ml/min/1.73m2)impaired kidney function.

Methods: 106 adult Caucasians patients older than 65 years (58 women, 48 men; mean age 72.5 years) were included. In each patient 51CrEDTA clearance, Screa (Jaffe – IDMS traceable method) and Scys (immunonephelometric method) were determined. GFR was calculated using the CKD-EPI creatinine & cystatin formula and simple Scys formula.

Results: The mean 51CrEDTA clearance was 52.2 ± 15.9 ml/min/1.73m2, mean Screa 1.6 ± 0.47 mg/dl, mean Scys 1.79 ± 0.6 mg/l. Statistically significant correlations between 51CrEDTA clearance and both formulas were found (P<0.0001). In the ROC curve analysis (cut-off for GFR 60 ml/min/1.73m2) no significant difference of diagnostic accuracy between CKD-EPI creatinine & cystatin formula and simple Scys formula was found (P=0.395). Bland and Altman analysis for the same cut-off value showed that CKD-EPI creatinine & cystatin formula (bias: -21.2 ml/min/1.73m2) underestimated and simple Scys formula (bias: 4.1 ml/min/1.73m2) overestimated measured GFR. All equations lacked precision. It was 8.7 ml/min/1.73m2 for CKD-EPI creatinine & cystatin formula and 9.8 ml/min/1.73m2 for simple Scys formula.

Conclusions: Our results indicate that simple Scys formula which requires just one variable (Scys concentration) is reliable marker of GFR in elderly patients with mild to moderate impaired kidney function and comparable to sophisticated CKD-EPI creatinine & cystatin formula.

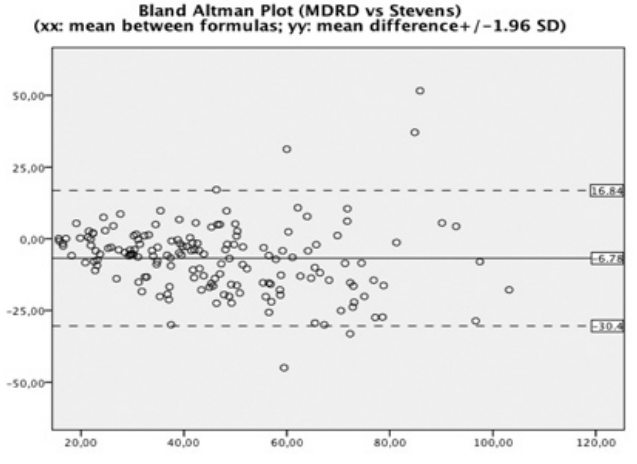
SA-PO2547

Creatinine and Cystatin C: Different Performance in Staging Chronic Kidney Disease in the Elderly Ana Rocha, Jorge Malheiro, Pedro V.A. Aguiar, António Manuel Nunes Cabrita. *Centro Hospitalar do Porto, Portugal.*

Background: An older population with chronic kidney disease (CKD) has frequently a high cardiovascular (CV) comorbidity and a pro-inflammatory status. Several clinical and analytical variables in this context may interfere with serum creatinine (sCr) and cystatin C (sCys).

Methods: We proposed to analyse in a stage 1-4 CKD and over 60 years population the agreement between glomerular filtration rate (GFR) formulas based on sCr (MDRD) and sCys (Stevens) through Kappa statistics and Bland-Altman plot. The degree of difference between formulas and their association with clinical variables was analysed by spearman correlation. A multivariate linear regression model was used to determine the influence on sCr and sCys of prevalent clinical and analytic variables.

Results: We studied 163 subjects, mean age 74 years, 53.4% females and with 2 or more CV risk factors (hypertension, diabetes mellitus (DM), dyslipidemia) in over 80%. The prevalence of CKD stages 3-4 was 81% by MDRD and 70% by Stevens formulas with a poor agreement between them (K=0.55).



The mean difference was -6.78 ml/min/1.73 m2, being significantly higher in younger age, male gender, milder CKD, in proteinuric and hyperuricemic patients. A multivariate adjusted model for age, gender, log-body mass index, DM, high ferritin status, albumin and HDL-cholesterol revealed a higher correlation with log-sCys (r=0.53) than log-sCr (r=0.38). DM and high ferritin were significant predictors of increased log-sCys and log-sCr. Higher log-sCys was also independently predicted by older age (p=0.005), female gender (p=0.02), lower albumin (p=0.005) and HDL-cholesterol (p=0.001).

Conclusions: In the elderly, a stronger association of sCys with nonrenal factors may limit its accuracy as determinant of GFR, but a higher correlation with these prevalent clinical and analytical variables can add clinical pertinence to it.

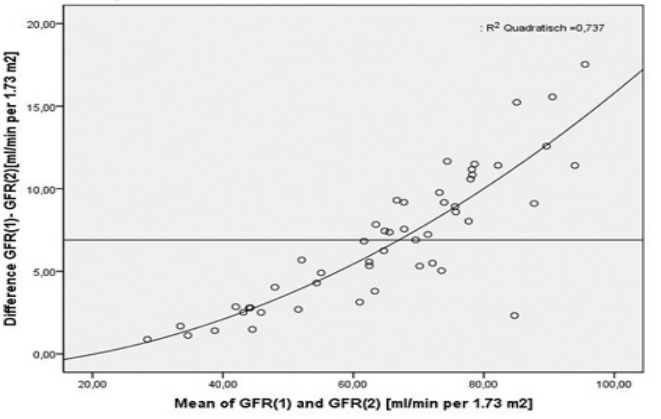
SA-PO2548

Glomerular Filtration Rate Via Plasma Iohexol in the Elderly – A Comparison of Slow and Fast Component Peter Martus,[1] Markus van der Giet,[2] Natalie R. Ebert,[3] Jens Gaedeke,[3] Olga Jakob,[1] Martin K. Kuhlmann,[4] Elke Schaeffner.[3] *[1]Dpt. Biostatistics and Clinical Epidemiology, Charité, Berlin, Germany; [2]Dpt. of Nephrology, CBF, Charité, Berlin, Germany; [3]Dpt. of Nephrology, CVK, Charité; [4]Vivantes, Berlin, Germany.*

Background: GFR measurement can be done using the clearance of Iohexol. We compare the modeling of Iohexol clearance using one or two components in a half logarithmic model.

Methods: We use a subsample (n=50) of the Berlin Initiative Study (BIS) with 10 measurement points of Iohexol concentration in the plasma at 10-300 min after infusion. The analysis of the slow and fast component of Iohexol clearance follows Schwarz et al.(2006); fast component: 10 to 90 min, slow component: 120 to 300 min. GFR was calculated from the area under the curve using the slow (GFR(1)) or both components (GFR(2)). We assess bias and agreement using the method of Bland and Altman.

Results: Median age was 76 ys (IQR 73-83 ys), 27 males, 23 females. Median body surface area was 1,81 m2(IQR 1,71-1,94). The area of the slow component (median 44,2 mg min/ml; IQR: 37,6 to 57,9) did not correlate with the additional area of the fast (median 4,9; IQR 3,7-5,9; r = -0.16). GFR (1) (median 71,4; 54,4-82,4) was about 10-15 % larger as compared to GFR (2) (median 62,6; 50,0-72,7). The correlation between GFR (1) and GFR (2) was 0.992. Bland Altman Plot shows moderate agreement (average difference 6.9 ml/min +- 8.1 (2SD) per 1.73 m2) and larger differences for larger GFR values. After removal of systematic differences we could predict GFR(2) from GFR(1) with a precision of +- 3.7 ml/min per 1.73 m2.



Conclusions: In elderly subjects agreement between both approaches (slow vs. slow+fast component) was less compared to Schwarz et al (2006) probably due to smaller Iohexol measurements at 10 and 20 min after infusion in our sample. After including a bias correction the slow component was sufficient.

SA-PO2549

Glomerular Filtration Rates in a Healthy, Multi-Ethnic Asian Population Boon Wee Teo, Evan J.C. Lee. *Medicine, Yong Loo Liin School of Medicine, National University of Singapore, Singapore.*

Background: Measured glomerular filtration rates (GFR) in healthy Asians without chronic kidney disease (CKD) (Indians, Nephron physiol 2005 Barai et al; Chinese, AJKD 2010 Zuo et al) have reportedly been lower than in European populations. We assess the normal distribution of GFR in a multi-ethnic Asian population without kidney disease and the GFR estimated with the CKD-EPI equation.

Methods: We prospectively recruited 103 healthy participants (49.5% male, Chinese 34%, Malay 24.3%, Indian 23.2%, and others 19.4 %). We measured Scr by an enzymatic method, and measured GFR (all GFR; in mL/min/1.73m^2) using 3-sample plasma clearance of ^{99m}Tc-DTPA, normalized to body surface area (du Bois). We use linear regression to assess the association of demography with mGFR, and to develop a GFR prediction equation. We use demographic data (mean age) from previously published studies to predict the GFR of those populations. We estimate GFR (eGFR) using the CKD-EPI equation, and assess its performance using the bias (median difference of eGFR-mGFR), precision (IQR), root mean square error (RMSE), and the percentage accuracy of eGFR to within 15%, 30% and 50% of mGFR.

Results: Population means: age 42.5±14.3years, body mass index 24.9±4kg/m^2, body surface area 1.7±0.2m^2, serum creatinine 0.8±0.2 mg/dL, mGFR 101±15.8, and eGFR 104±15.2. By linear regression, predicted GFR = 124.6 – 0.565(Age). In the full multivariate model (age, gender, race), GFR was lower in males (-2.58, P=0.04) and in Indians (-9.3, P<0.001). Predicting mean GFR using data from previously published studies, the GFR for Chinese was similar: GFR 99 (male, mean age 41.6); GFR 98 (female, mean age 46.4). But is higher in our Indians: predicted GFR 97 (mean age 31.2). For eGFR, the bias is 4, precision 17.7, RMSE 14.8. Percentage accuracy of eGFR to within 15%, 30% and 50% of mGFR are 70.9, 93.2, and 99, respectively.

Conclusions: Healthy Indians have significantly lower GFR compared to other ethnic groups. When adjusted for age, the mean measured GFR in Chinese participants was similar to Chinese in China, but our Indians had higher GFR than those in India. The CKD-EPI equation estimates GFR accurately in a multi-ethnic Asian population without CKD.

Funding: Government Support - Non-U.S.

SA-PO2550

The Significance of Cystatin C in Different Stages of Chronic Kidney Disease Zilong Li, Yanhui Lv, Juan Wang, Hua Zhou, Lining Wang, Jun Wang. *Department of Nephrology, First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China.*

Background: To analyze the sensibility of cystatin C in reflecting glomerular filtration rate (GFR) of chronic kidney diseases (CKD), estimate the scope of cystatin C in different CKD stages and different total GFR (TGFR) groups, assess the relevance of cystatin C with proteinuria and anemia.

Methods: 70 CKD patients were divided into five stages based on estimated GFR (eGFR), and four groups based on TGFR. 20 healthy persons were selected as control group. Scr, cystatin C, the quantity of 24-hours urinary protein, hemoglobin were measured, and TGFR was measured by kidney ECT, eGFR was calculated through Cockroff-Gault formula.

Results: There is statistical difference in the levels of serum cystatin C between the patients in CKD2-5 stages and control group ($P < 0.05$), as well as in different stages of the CKD2-5patients.The concentration range of cystatin C in five stages of CKD are as follows: stage 1 is ≤1.07 mg/L, stage 2 is between 1.07 and 1.62 mg/L, stage 3 between 1.62 and 2.31 mg/L, stage 4 between 2.31 and 3.58 mg/L, and stage 5 is over 3.58 mg/L. The range of cystatin C in four TGFR groups are: in normal group cystatin C ≤1.11 mg/L, in slight injury group between 1.11~1.52mg/L, in moderate injury group 1.52~2.58 mg/L, and in severe injury group >2.58 mg/L. Significant correlation coefficient exist in the cystatin C and the quantity of urinary protein in CKD 3 stage ($R = 0.481$, $P < 0.05$).There is correlation coefficient between cystatin C and the hemoglobin of 33 patients with anemia(R=–0.688, P<0.01).

Conclusions: Cystatin C is more sensitive than serum creatinine in reflecting renal function.The quantity of urine protein have positive correlation with the level of cystatin C. And there is correlation between the concentration of cystatin C and the degree of anemia.

SA-PO2551

SCreening for Occult REnal Disease (SCORED) Is Useful tool To Identify Individuals at High-Risk for Chronic Kidney Disease Itágores Hoffman II Coutinho,[1] Manuel C. Castro,[2] José Gerley Diaz Castro,[1] Joao Egidio Romao.[2] *[1]Department of Medicine, Federal University of Tocantins, Palmas, Tocantins, Brazil; [2]Nephrology Division, Hospital das Clínicas – São Paulo University Medical School, São Paulo, Brazil.*

Background: The health burden of renal disease is high for patients and health services worldwide, and screening for chronic kidney disease (CKD) has been increasingly advocated. Population-based studies relating to the prevalence of CKD in the community are limited. We prospective studied whether stratification by SCORED values could be useful to identify subjects who are at high-risk for having CKD in a general population-based sampling.

Methods: The frequency of individuals at high-risk for CKD was determined using a cross-sectional study of 873 adult households in Palmas, Brazil, randomly selected using a stratified, cluster method. Age, gender, and race were similar to the entire Palmas´ urban population.

Results: An estimated GFR <60 ml/min/1.73 m2 was present in 46 (5.3%) of participants studied, and the risk for having CKD was greater in women than in men, and it increased with age from 2.7% in the 18 to 44 yr age group to 19.0% in those 65 yr of age older. The frequencies of CKD Stage 3, 4 and 5 were 4.8%, 0.5% and 0%, respectively. SCORED values included 224 (25.7%) patients with high SCORED values (≥4), and 649 (74.3%) subjects with low SCORED values. Subjects with higher SCORED values were at a significantly higher risk of having CKD compared with those who had lower SCORED values (12.9% vs 2.6%, $\chi 2 = 35.58$; $p < 0.001$). The sensivity for predicting CKD by SCORED model was 63% and the specificity was 76%; the positive predictive value was 13%, whereas the negative predictive value was 76%.

Conclusions: High SCORED values were associated with a higher risk for having CKD in a general population-based sampling. This simple screening tool was a useful tool to identify individuals at high-risk for CKD.

Funding: Government Support - Non-U.S.

SA-PO2552

Comparison of Anticancer Drugs Dosing Recommendations for Chronic Kidney Disease Patients Based on Three Methods for Assessing Kidney Function Elerson Costalonga,[1] Veronica T. Costa e Silva,[1] Henrique Palomba,[1] James Hung,[1] Emmanuel A. Burdmann,[1,2] Luis Yu.[1,2] *[1]Nephrology Department, Cancer Institute of Sao Paulo, Brazil; [2]Nephrology Department, University of Sao Paulo School of Medicine, Brazil.*

Background: The aim of this study was to determine whether a difference exists when making anticancer drug dosage adjustments in patients with CKD and cancer based on estimation of GFR using the Chronic Kidney Disease Epidemiology Collaboration, Modification of Diet in Renal Disease(MDRD) simplified and Cockcroft-Gault(CG) equations.

Methods: eGFR was calculated using CG, MDRD simplified and CKD-EPI equation for 131 adult outpatients with cancer submitted to nephrology consultation. Equations were compared using Bland-Altman methodology. Dosage discordance rates of the antineoplasics were determined on the basis of manufacturer renal dose recommendations.

Results: On avarege patients had 66 ± 12 years and 68% were male. Median (IQR) eGFRs (ml/min) for all patients using the CG, MDRD and CKD-EPI equations were 37.7 (5 - 53), 38.8 (24 - 51) and 39.4 (24 - 52) respectively (pns). Comparisons between the three equations are shown on Table 1. A significant discordance rate of 3 - 16% ($p < 0.01$) was demonstrated among the recommended dosing adjustments of the selected anticancer drugs except for fludarabine, etoposide and bleomycin when CKD EPI was compared to MDRD. Sixty two patients (47.3%) had at least one dosage discordance.

eGFR results and anticancer drugs discordance rates

	CCG vs. MDRD	CCG vs. CKD EPI	MDRD vs. CKD EPI	
Correlation Coefficient	0.93	0.95	0.99	p < 0.01
Bland-Altman plot limits of agreement	-14.7 and 16.3	-15.1 and 15.7	-8.6 and 7.6	
Discordance Rates				
Cisplatin	16.0%	16.0%	6.1%	
Etoposide	10.6%	9.1%	3.0%	
Methotrexate	15.9%	15.3%	6.9%	
Topotecan	16.0%	13.7%	6.1%	
Fludarabine	10.7%	9.9%	3.8%	
Bleomycin	10.6%	9.1%	3.0%	

Conclusions: This analysis demonstrated significant different dosing anticancer recommendations for CKD patients according to CG, MDRD and CKD EPI equations. These results have important potential clinical implications since different outcomes may occur as a result of the discordant doses.

SA-PO2553

Influence of Thyroid Function on Different Kidney Function Tests Martin Kimmel, Niko Braun, Mark Dominik Alscher. *Department of Internal Medicine, Division of General Internal Medicine and Nephrology, Robert-Bosch Hospital, Stuttgart, Germany.*

Background: The commonly used kidney function tests (KFT) have limitations, especially in thyroid dysfunction. Therefore we studied the most commonly used KFT in patients with hypo- and hyperthyroidism and after reaching euthyroidism.

Methods: Prospective case series in 16 patients with thyroid dysfunction. Serum creatinine, 24-hour creatinine-clearance, calculated GFR by Cockroft-Gault, estimated GFR (eGFR) by CKD-EPI equation, serum cystatin C (CysC), eGFR based on CysC, eGFR based on a combined (CysC and creatinine) formula and plasma NGAL were measured in hypo- and hyperthyroidism and after gaining euthyroidism.

Results: When fT4 normalized in hypothyroid patients, creatinine decreased significantly (P<0,001) from 1,2±0,2 to 0,9±0,1 mg/dl and creatinine based eGFR increased significantly: 24 hour creatinine clearance (P<0,001), Cockroft-Gault (P<0,001) and CKD-EPI equation (P<0,001). The combined (CysC and creatinine) GFR formula increased significantly (P=0,01). In contrast CysC increased significantly (P=0,02) and eGFR based on CysC decreased significantly (P=0,02) . There was no significant change in NGAL levels (P=0,9).

When fT4 normalized in patients with hyperthyroidism creatinine increased significantly (P<0,001) from 0,63±0,10 to 0,77±0,13 mg/dl and creatinine based eGFR decreased significantly: 24 hour creatinine clearance (P=0,03), Cockroft-Gault (P=0,01) and CKD-EPI equation (P=0,02). There was no significant change (P=0,76) for the combined (CysC and creatinine) GFR equation. In contrast CysC decreased significantly (P=0,01) and CysC based GFR increased significantly (P<0,001). There was no significant change in NGAL levels (P=0,61).

Conclusions: Thyroid function has a major influence on the vast majority of KFTs. CysC is strongly influenced by the thyroid function and should be avoided in thyroid disorders, but there was no effect of the thyroid function on the low NGAL levels.

The recommended KFT in thyroid dysfunction is a creatinine based GFR estimation. Furthermore kidney and thyroid function should always be used together to avoid misleading interpretations.

SA-PO2554

Vitamin D Insufficiency and Incident Chronic Kidney Disease in Australia: The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study Matthew J. Damasiewicz,[1] Dianna J. Magliano,[2] Robin M. Daly,[3] Claudia Gagnon,[4] Zhong X. Lu,[5] Peter R. Ebeling,[6] Steven J. Chadban,[7] Robert C. Atkins,[2] Peter G. Kerr,[1] Jonathan E. Shaw,[2] Kevan R. Polkinghorne.[1] *[1]Monash University, Australia; [2]Baker IDI, Australia; [3]Deakin University, Australia; [4]Laval University, Canada; [5]Melbourne Pathology, Australia; [6]Melbourne University, Australia; [7]Sydney University, Australia.*

Background: 25-OH vitamin D (25D) insufficiency has been associated with CKD (albuminuria and impaired GFR) in cross-sectional studies, however this association is less clear in prospective studies. We aim to measure the 5-yr incidence of CKD in subjects with 25D insufficiency and assess 25D insufficiency as a risk factor for CKD.

Methods: The prospective AusDiab Study followed subjects over 5 years. Incident CKD was defined as subjects negative at baseline but positive after 5 years for (1) eGFR <60 ml/min/1.72m2 using the CKD-EPI equation (impaired GFR), n=6285; or (2) spot urine

albumin to creatinine ratio of ≥2.5 mg/mmol for males and ≥3.5 for females (albuminuria), n=6096. 25D levels of <50nmol/L were considered insufficient. The incidence of CKD in 25D insufficient subjects was standardised to the 1998 Australian population. Logistic regression was used to assess 25D insufficiency as a risk factor for CKD.

Results: The annual age-standardized incidence of an eGFR<60 in subjects with and without 25D insufficiency was 0.86% (95% CI 0.65-1.07) and 0.47% (0.38–0.55), respectively; for albuminuria the incidence was 1.14% (0.89–1.38) and 0.61% (0.50–0.71), respectively. 25D insufficiency was not associated with development of an eGFR<60 (univariate OR 1.35, p=0.076, multivariate OR 0.93, p=0.725). 25D insufficiency was significantly associated with an increased likelihood of new albuminuria in both the univariate (OR 1.55,p=0.0002) and multivariate models (OR 1.42,p=0.02).

Conclusions: 25D insufficiency was significantly associated with the 5-year incidence of albuminuria, but not impaired GFR. Given this association and potential mechanistic links between 25D insufficiency and kidney damage, including modification of inflammation, cell proliferation and apoptosis, further research in this area is warranted.

Funding: Government Support - Non-U.S.

SA-PO2555

Vitamin D Insufficiency and Chronic Kidney Disease in Australia: AusDiab Study Matthew J. Damasiewicz,[1] Dianna J. Magliano,[2] Robin M. Daly,[3] Claudia Gagnon,[4] Zhong X. Lu,[5] Peter R. Ebeling,[6] Steven J. Chadban,[7] Robert C. Atkins,[2] Peter G. Kerr,[1] Jonathan E. Shaw,[2] Kevan R. Polkinghorne.[1] *[1]Monash University, Australia; [2]BakerIDI, Australia; [3]Deakin University, Australia; [4]Laval University, Canada; [5]Melbourne Pathology, Australia; [6]Melbourne University, Australia; [7]Sydney University, Australia.*

Background: Low 25D levels have been associated with albuminuria, however the association with GFR is less clear. We aim to determine the associations between 25-hydroxy vitamin D levels (25D), and albuminuria and an impaired glomerular filtration rate (GFR) in a population-representative cohort of Australian adults.

Methods: The study population was the baseline Australian Diabetes, Obesity and Lifestyle (AusDiab) Study cohort, surveyed from 1999–2000 (n=10732). Estimated GFR (eGFR) was calculated using the CKD-EPI equation using enzymatic creatinine measurements, with CKD defined as <60ml/min/1.73 m2. Albuminuria was defined as a spot urine albumin to creatinine ratio of ≥2.5 mg/mmol for males and ≥3.5 for females. 25D insufficiency was defined as levels <50 nmol/L. Logistic regression models accounting for survey design were used to assess the effect of 25D insufficiency on albuminuria and CKD, adjusted for gender and age (model 1), and adjusted for multiple variables including age, gender, diabetic status, body mass index, cholesterol and triglyceride levels, smoking status, time of blood test (season), ethnicity, cardiovascular disease and systolic blood pressure, as well as eGFR or albuminuria, respectively (model 2).

Results: 30.6% of the study population had 25D level <50 nmol/L (95% CI 25.6-35.8). 25D insufficiency was significantly associated with albuminuria in the unadjusted model (OR 2.05, p<0.0001), model 1 (OR 1.78, p<0.0001), and model 2 (OR 1.56, p=0.003). While 25D insufficiency was significantly associated with an eGFR<60 in the unadjusted model (OR 1.52, p=0.02), the association was not significant in the adjusted models (model 1: OR 0.95, p=0.78; model 2: OR 0.85, p=0.31).

Conclusions: 25D insufficiency was common in this population and levels of <50 nmol/L were independently associated with albuminuria, but not with impaired eGFR.

SA-PO2556

Uric Acid Is Associated with Chronic Kidney Disease and Hypertension in the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) Study Stacey Jolly,[1] Mihriye Mete,[2] Hong Wang,[2] Jianhui Zhu,[2] Sven O.E. Ebbesson,[3] V. Saroja Voruganti,[4] Anthony Comuzzie,[4] Jason G. Umans,[2,5] Barbara V. Howard.[2,5] *[1]Cleveland Clinic; [2]MedStar Health Research Institute; [3]Norton Sound Health Corporation; [4]Texas Biomedical Research Institute; [5]Georgetown-Howard Universities Center for Clinical and Translational Science.*

Background: Uric acid has been associated with both prevalent and incident HTN and CKD, perhaps contributing to cardiovascular disease (CVD). No studies have explored these associations in Alaska Eskimos, a population with high rates of CVD but low prevalence of DM and CKD.

Methods: Cross-sectional analysis of 89% (n=1078) of GOCADAN study participants with available lab data at baseline (2000-2004). CKD was defined by an eGFR <60ml/min/1.73m^2. Albuminuria was defined as a urine albumin/creatinine ratio 30 mg/g. Using logistic regression models, we sequentially adjusted for age, sex, smoking, BMI, DM, trigylcerides, systolic BP (or eGFR), and hsCRP to separately determine independent associations of uric acid with CKD and with HTN.

Results: The 7% (n=75) of GOCADAN participants with prevalent CKD were more likely to be older (63 v 41 y), have DM (9% v 3%), albuminuria (20% v 6%), or HTN (63% v 18%). Likewise, the 21% (n=230) with prevalent HTN were more likely to be older (57 v 39y), have DM (13% v 1%), or albuminuria (17% v 4%) (All p<0.05). Uric acid was independently associated with prevalent CKD and with prevalent HTN (Table).

Table. Associations of Uric Acid and CKD in GOCADAN

	CKD (n=1078)	HTN (n=1077)
Models	Odds Ratio (95% CI)	Odds Ratio (95% CI)
1-univariate	1.8 (1.6-2.1)	1.7 (1.5-1.9)
2-age, sex, smoking	1.8 (1.5-2.1)	1.5 (1.3-1.8)
3-above + BMI, diabetes, trigylcerides	2.0 (1.6-2.5)	1.3 (1.1-1.5)
4-above + systolic BP (or eGFR for the HTN model)	2.0 (1.6-2.5)	1.2 (1.1-1.5)
5-above + hsCRP	2.0 (1.6-2.6)	1.2 (1.1-1.5)

Conclusions: Among Alaska Eskimos, uric acid is independently associated both with prevalent CKD and with prevalent HTN. Future studies are needed to determine whether uric acid contributes to increasing CVD in this population.

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SA-PO2557

Gout Risk Factors and Treatment among Chronic Kidney Disease Patients: A Hospital Based Cross Sectional Study Erdal Sarac. *Internal Medicine, Saint Elizabeth Health Center, Youngstown, OH.*

Background: Gout has doubled in prevalence in the United States. Recent studies have proposed hyperuricemia and gout as a risk factor for progression of chronic kidney disease (CKD). This study investigated the prevalence gout in patients with CKD and compared the current practice patterns to recommendations and guidelines put forward by European League Against Rheumatism (EULAR).

Methods: A total of 1,827 patients admitted between 2009 and 2010 at St. Elizabeth Health Center with a diagnosis of chronic kidney disease were included by database search using ICD-9 codes. Patients were divided into two groups: CKD with gout and CKD without gout. Data collection included age, sex, and comorbid diagnoses. A subset of 50 patients was randomly selected to compare the treatment they received with EULAR guidelines.

Results: The prevalence of gout in the hospitalized patients with CKD was 251/1827 (13.7%: 95% CI 12-15%). Males exhibited higher rates of gout: 150/814 (18.4%) vs. 101/762 (13.3%), respectively (X^2=5.40, p=0.02). Gout prevalence increased with age - highest prevalence was observed among patients age 71 to 80 years of age, with elderly males exhibiting even higher rates of disorder (17% in female and 26% in male). CKD patients with and without gout has a prevalence rate of CAD of 41.8% vs. 36.3% (X^2=2.62, p=0.11). Diabetes mellitus was higher in the CKD patients without gout, 29.8% versus 36.3% (X^2=3.99, p=0.04). Both hyperlipidemia and hypertension were higher in the CKD patients with gout: 46.2% versus 38.7% (X^2=3.99, p=0.004), and 82.5% versus 78.1%, respectively (X^2=2.20, p=0.14). Only 65% of CKD patients with gout were treated following EULAR guidelines.

Conclusions: This study reveals a high prevalence of gout in patients with CKD. Male sex, advanced age, CAD, hypertension, and hyperlipidemia were significantly associated with gout among CKD patients. In contrast, the prevalence of diabetes was higher in CKD patients without gout. Treatment for gout was sub-optimal in the subset of examined CKD patients. Newer treatment modalities for gout may change these findings in the future. Greater awareness is needed to improve the management strategies for gout in CKD patients

SA-PO2558

Association of Simple Renal Cysts by Computed Tomography (CT) with Kidney Function and Chronic Kidney Disease (CKD) Risk Factors Mira T. Keddis,[1] Terri J. Vrtiska,[2] Vicente E. Torres,[1] Andrew D. Rule.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Radiology, Mayo Clinic, Rochester, MN.*

Background: Simple renal cysts are common and can be better detected by CT scan than ultrasound, but the clinical relevance is not clear. The objective of this study was to determine if kidney function and CKD risk factors associate with cysts.

Methods: Contrast enhanced CT images of potential kidney donors between 2000 and 2008 at Mayo Clinic were reviewed to characterize renal cysts. Clinical and laboratory data was abstracted from the medical record. Hypertension was defined by use of anti-hypertensive medications or blood pressure (BP) >140/90 mmHg. The association of kidney function and CKD risk factors with renal cysts was assessed with nominal logistic, ordinal logistic, and linear regression adjusting for age and sex.

Results: There were 1946 potential kidney donors, of which, 28% had at least one renal cyst ≥ 5 mm in diameter, 42% were male, and 15% had hypertension. Mean ± SD age was 43 ± 12 years, body surface area (BSA) was 1.98 ± 0.25 m², diastolic BP 74 ± 10 mmHg, 24-h urine albumin excretion was 8.2 ± 18 mg, measured glomerular filtration rate (GFR) was 102 ± 19 ml/min/1.73 m², and serum creatinine was 0.87 ± 0.17 mg/dL.

Odds ratios or % difference for cyst findings

Kidney function or CKD risk factor	Any cyst ≥5mm	Number of cysts ≥ 5mm among persons with any cyst ≥5mm	Any cyst ≥10mm	Any cyst ≥20mm	Any cortical cyst ≥5mm	Largest cortical cyst diameter (% difference)
Age per 10 years	1.6*	1.6*	1.8*	2.3*	1.9*	11*
Male	1.5*	2.1*	2.5*	3.1*	1.9*	26*
BSA per 0.25 m²	1.0	1.2	1.3*	1.5*	1.1	12*
Hypertension	1.0	1.7*	1.4*	1.2	1.1	18
Diastolic BP per 10mmHg	1.0	1.2*	1.1	1.2	1.0	7.1*
24-h urine albumin excretion per doubling	1.1*	1.2*	1.2*	1.2*	1.1*	4.9*
Measured GFR per 19 ml/min/1.73 m²	1.1*	1.1	1.1	1.0	1.1	-0.8
Serum creatinine per 0.17 mg/dL	0.9	0.7*	0.8*	1.2	0.9	0.0

*P<0.05

Conclusions: Increased number and size of renal cysts showed some association with older age, male gender, larger BSA, diastolic BP, hypertension, increased albumin excretion, and hyperfiltration. These findings suggest simple cysts may be a marker of early kidney injury in relatively healthy adults.

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SA-PO2559

Diabetic Nephropathy: Prevalence and Progression (DNP²) Study Ping Tyug Loh,[1] Matthias Paul Han Sim Toh,[2] Christine Xia Wu,[2] Valerie Ma,[1] Vathsala Anantharaman.[1] *[1]National University of Singapore, Singapore; [2]National Healthcare Group, Singapore.*

Background: Diabetes is the leading cause of End Stage Renal Failure (ESRF) worldwide with Asia contributing the largest burden; nevertheless, there is little data on prevalence and progression of Diabetic Nephropathy (DN) from Asian countries. In Singapore, population prevalence of diabetes of 11.3% and incidence of ESRF due to DN of 134 per million population are both very high. As majority of diabetics are managed in primary care setting, this retrospective study aimed to assess the burden of DN in a community healthcare cluster (National Health Care Group; NHG) by describing its prevalence and progression.

Methods: From the Chronic Disease Management Registry 2006-2009, 76,641 prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% Hypertensive, 75% on Angiotensin-Converting-Enzyme-Inhibitor (ACEI) or Angiotensin-Receptor-Blocker (ARB)] were identified. These patients, were studied for prevalence of normo-(NA), micro-(MI) and macro-albuminuria (MA) as defined by ADA and for renal impairment as defined by eGFR< 60 ml/min (estimated Glomerular Filtration Rate with MDRD equation). Annualized progression from NA to MI, MI to MA and MA to renal impairment was calculated.

Results: As there were 49.2% with NA and no renal impairment, the prevalence of DN was as 50.8%; 19.1% had MI, 4.6% had MA while 27.1% had renal impairment. Progression from NA to MI occurred at 9.9% per year, from MI to MA at 7.5% per year, and from MA to renal impairment at 10.2% per year. Those who progressed were older, hypertensive with longer duration of diagnosis. HbA1c was not observed to be significantly associated with DN progression. Of note, only 13.5% of those with MI at baseline were on maximal dose of ACEI/ARB.

Conclusions: The observed rates of progression from stage to stage were very high despite relatively good glycemic control. While hypertension control could not be evaluated here, the majority of patients were on less than maximal doses of ACEI/ARB. Strategies to optimize ACEI/ARB dosage could potentially reduce the rates of DN progression.

SA-PO2560

Progression in Diabetic Nephropathy: Is Ethnicity an Independent Risk Factor? Syed Atif Mohiuddin, Rohini Mathur, Omer H. Ali, Gavin Dreyer, Sally Hull, Magdi Yaqoob. *Barts and the London NHS Trust, United Kingdom.*

Background: Higher prevalence of ESRD in south Asian and Black populations compared to Caucasians is not fully accounted for by higher prevalence of diabetes mellitus (DM). Our renal unit serves an ethnically diverse population and we analysed the progression of diabetic nephropathy (DN) in these subsets of population.

Methods: In this prospective study we analysed the progression of DN in ethnic subgroups. All patients with biopsy proven or clinical diagnosis of DN attending nephrology outpatient clinics were included in the study. 4 variable MDRD equation was used to estimate GFR (eGFR).

Results: A total of 329 patients (105 Caucasians, 75 Black and 149 south Asians, mean age 60 years ± 11.9, 121 women and 208 men) were analysed. Mean duration of follow up was over 6 years and similar in all 3 cohorts. Baseline eGFR was significantly higher in south Asians (44±21 ml/min) compared with Caucasians (38±19 ml/min, p value 0.02) but was similar to Blacks. Baseline systolic BP was significantly higher in Black (158±26mmHg) and Caucasians (146±23 mmHg) compared with south Asians (136±24 mmHg p value<0.001). Baseline diastolic BP was also higher in Black (83±15 mmHg) compared with Caucasians (76±11mmHg) and south Asians (75±11mmHg p value 0.002). ACE inhibitor or ARB use, baseline HBA1C and baseline proteinuria was not statistically different. Unadjusted analysis showed significantly higher rate of GFR decline in Black (5.38 ± SEM 0.77 ml/min/year) and south Asians (4.42±SEM 0.43) compared with Caucasians population (2.67±SEM 0.33, p 0.002 and 0.01 respectively). After adjustment for BP, glycaemic control, medication and proteinuria, there was no difference in progression between Black and Caucasians but difference persisted in south Asians (p 0.04). Systolic BP (p value <0.001) and baseline proteinuria (p value 0.02) were the other predictors of DN progression. Mortality or incidence of ESRD was not different.

Conclusions: Black and south Asians have higher rate of DN progression. This is explained by higher BP in Black but south Asians have higher rate of progression despite low BP. Further studies are needed to determine optimal target BP in this ethnic group.

SA-PO2561

Role of Asymmetric Dimethyl Arginine and Endothelial Dysfunction Associated Gene Polymorphisms in Early Stage CKD Development in Obese Patients Marat G. Gallyamov,[1] Evgeniya Saginova,[1] Maria M. Severova,[2] Marietta Lianidis.[1] *[1]Faculty of Fundamental Medicine, Lomonosov Moscow State University, Moscow, Russian Federation; [2]First Moscow State Medical University, Moscow, Russian Federation.*

Background: Endothelial dysfunction associated with obesity is one of the main mechanisms in damage such target organs as heart, vessels and kidney. Asymmetric dimethyl arginine (ADMA) is an endogenous inhibitor all types of NO-synthases (NOS) and novel marker. ADMA is tightly associated with methylen tetrahydrofolate reductase (MTHFR) and NADPH-oxidize. The aim of our study was to define role of ADMA and endothelial NOS (eNOS), MTHFR, and NADPH-oxidize gene polymorphism in early stage CKD development in obese patients.

Methods: We investigated 66 patients (17f) with BMI over 25 (mean 33.9+/-6.8) and age 18-60 yrs (44+/-11). They did not have eGFR less 45 ml/min/1.73 m^2, hematuria, albuminuria over 2.0 g/l, proved kidney diseases, coronary heart disease, brain insult. All patients were tested on leptin, insulin, C-peptide, ADMA. We detected polymorphism of C242T allele p22 subunit NADPH-oxidize gene (p22-NADPH), G894T allele eNOS gene, and C677T allele MTHFR gene. Intima-media thickness (IMT) of common carotid artery was assessed by duplex ultrasonography.

Results: 36% patients had CKD. Leptin, insulin, C-peptide levels, HOMA-index and waist circumference were higher in patients with CKD. CKD was associated with higher prevalence of increased IMT (52% vs. 30%). In CKD ADMA was significantly correlated with IMT and leptinemia. Although prevalence of pathological alleles in the genes was comparable in patients with and without CKD, homozygous p22-NADPH and MTHFR mutation carriers had the highest level of insulinemia (19.1+/-5.8 uUnits/ml) and HOMA-index (4.7+/-1.3). Combined C242T- and C677T- carriage aggravated insulin resistance and leptinemia. Patients with homozygous G894T-eNOS had lower eGFR (73+/-11).

Conclusions: In obese subjects development of CKD was tightly associated with vascular remodeling. We suggested that ADMA and C242T- p22-NADPH and C677T-MTHFR gene polymorphism can cause early stage CKD mainly via aggravation of insulin resistance and leptinemia.

Funding: Government Support - Non-U.S.

SA-PO2562

Urinary F2-Isoprostanes Fail To Predict Cardiovascular Disease in a Population with High Prevalence of Diabetes, Obesity and CKD: The Strong Heart Study Nawar M. Shara,[1,2] Hong Wang,[1] Mihriye Mete,[1,2] Ginger Milne,[3] Barbara V. Howard,[1,2] Jason G. Umans.[1,2] *[1]MedStar Health Research Inst; [2]Georgetown-Howard Universities Ctr for Clin & Translational Sci; [3]Vanderbilt U.*

Background: Oxidative stress (OS) may contribute to progressive atherosclerosis and to CVD risk. Among OS biomarkers, urinary F2 isoprostanes, including 8-iso-prostaglandin F2α (8-iso), nonenzymatic products of arachidonate peroxidation, appear to provide superior and stable estimates of integrated OS, responsive to known oxidants and to antioxidants. We tested the association between urinary 8-iso and subsequent CVD events in a population with high prevalence of DM, obesity and CKD.

Methods: A nested case-control study within the Strong Heart Study (SHS) of 4,549 American Indians, aged 45-75y at baseline, with high prevalence of DM (44.8%), obesity (50.7%), and CKD (11.3%). During a median of 15y follow-up from 1989 to 2007, there were 1329 total fatal and non-fatal incident CVD events. From this population, 336 cases of incident CVD and 343 controls free from CVD at last follow-up were randomly selected and included in this analysis. Urinary 8-iso was measured by GC/MS and indexed to urinary creatinine. Samples had been stored at -80C.

Results: Compared with controls, cases developing CVD were older; had higher baseline SBP, DBP, LDL-C, fasting plasma glucose, HbA1c; had lower HDL-C; and had higher prevalence of hypertension, diabetes, microalbuminuria, and macroalbuminuria (all p<.05). Sex and BMI did not differ significantly between groups (all P>.05). Urinary 8-iso was not elevated in cases with incident CVD (p=.16). The multivariate-adjusted odds ratios (ORs) (95% confidence interval [CI]) of incident CVD across the increasing quartiles of urinary 8-iso/creatinine were 1(referent), 0.59(0.31, 1.12), 0.59(0.31, 1.14), and 0.57(0.30, 1.09); the OR (95% CI) for log (8-iso/creatinine) was 0.88(0.65, 1.19). Similar results were observed after excluding those with 8-iso >10 ng/mg creatinine.

Conclusions: There was no prospective association between baseline urinary 8-iso PGF2α and incident CVD events over a median 15y follow-up in a population with high prevalence of DM, obesity and CKD and high risk of CVD.

Funding: Private Foundation Support

SA-PO2563

Factors Associated with Serum NGAL (neutrophil Gelatinase-Associated Lipocalin) in Patients with Chronic Kidney Disease Hidekazu Moriya, Kunihiro Ishioka, Machiko Oka, Sumi Hidaka, Takayasu Ohtake, Shuzo Kobayashi. *Department of Nephrology, Immunology and Vascular Medicine, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan.*

Background: It is well known that serum or urinary levels of NGAL increases earlier than that of serum creatinine (Cr) in acute kidney injury (AKI), and that NGAL has been identified to be a useful biomarker of AKI. In addition, it has been reported that NGAL also increases in chronic kidney disease (CKD) with the property of inhibition of human erythropoiesis by stopping differentiation or apoptosis of erythroid precursors. The present study investigated the factors associated with serum levels of NGAL in patients with CKD.

Methods: We measured serum NGAL, using a ELIZA Kit (Triage® NGAL, Biosite Incorporated, USA), in 103 patients with CKD stage 1-4 who are stable for more than three months. We also measured hemoglobin, hematocrit, serum creatinine and blood pressure. We also investigated the existence of comorbidity (hypertension, chronic heart failure, urinary protein, diabetes mellitus).

Results: Thirty three patients showed that serum NGAL levels were below 60ng/ml, which was upper limit of measurement. Mean levels of remaining seventy patients were 148.4±62.7ng/m. NGAL levels are significantly positively correlated with age and negatively correlated with hemoglobin and 1/Cr, respectively. In patients with chronic heart failure, Serum levels of NGAL were significantly increased, compared with those without it (200.4±63.4 vs 138.8±58.1ng/ml, respectively). Serum levels of Cr are only an independent factor related with NGAL in a multivariate analysis.

Conclusions: NGAL increases in correlation with residual renal function in CKD patients. Moreover, chronic heart failure is an important factor to increase serum levels of NGAL.

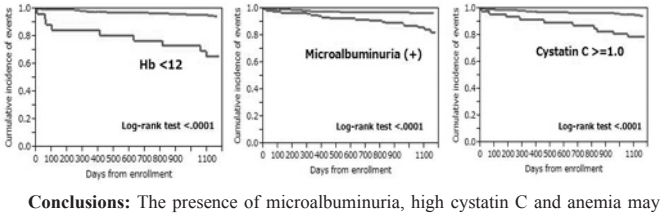
SA-PO2564

Cumulative Impact of Anemia, Albuminuria and Cystatin C on Adverse Prognosis in HIV-Infected Men Minoru Ando,[1] Naoki Yanagisawa,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Background: The heightened risk for cancer and cardiovascular disease (CVD) in well-treated HIV-infected patients may be due to residual immunodeficiency and inflammation. The presence of anemia and chronic kidney disease (CKD) likely accelerates such ill linkage. We examined cumulative impact of anemia and CKD on prognosis in HIV-infected subjects.

Methods: A 3-year prospective cohort study was conducted in 520 HIV-infected men with undetectable HIV-RNA level. All-cause death and incident cancer and CVD were considered critical events. Cumulative incidence of such events was analyzed by the Kaplan Meier method, stratified by presence or absence of microalbuminuria (ACR ≥30mg/g), high cystatin C (serum level ≥1.0 mg/L) or anemia (Hb <12 g/dL). Multivariate Cox proportional hazards analysis was used to calculate the HR of developing critical events for the combined impact of 3 variables. 'Score 1' was assigned to each variable, and the maximum score is a total of 3 points. The model was adjusted for age, CD4 counts and presence of comorbidites including viral hepatitis, hypertension and diabetes at baseline.

Results: The prevalence of microalbuminuria, high cystatin C, and anemia at baseline was 23.7%, 9.0%, and 5.0%, respectively. During the follow-up period, critical events developed in 34 subjects (6.5%). The cumulative incidence of events was significantly higher in the group with microalbuminuria, high cystatin C, or anemia than in each opposite. The HR for critical events increased 2.0-fold per one-point increase in the number of risk factors (95% CI; 1.72-3.25, P=0.0002).



Conclusions: The presence of microalbuminuria, high cystatin C and anemia may have a great synergistic impact on the incidence of critical events in well-treated HIV-infected men.

SA-PO2565

Renal Endogenous Angiotensin II as a Principal Source of Urinary Angiotensin II in Chronic Kidney Disease Patients Xiaoyan Zhang, Xiaoqiang Ding, Jie Teng, Yi Fang, Jianzhou Zou. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: It has been demonstrated that there was a significant relationship between UAGT and kidney Ang II content and renal Ang II immunostaining intensities. These results provide further evidence that UAGT may be a useful index of renal endogenous Ang II activity. This study analyzed the relationship of UAng II with circulating and intrarenal RAS activity in chronic kidney disease (CKD) patients to investigate the origin of UAng II in human.

Methods: 128 CKD patients who had not received ACEI or ARB during last 2 months were included in the study. Urinary and plasma renin activity, AGT, Ang II, aldosterone and type IV collagen were measured by RIA or ELISA. Urinary AGT, Ang II, aldosterone, and type IV collagen levels were expressed per 1 mg of urinary creatinine (mg Cr). Common logarithmic transformation for RAS components and urinary type IV collagen were performed because these variables did not exhibit normal distribution.

Results: Average UAng II in 128 CKD patients was 4.92 +/- 0.70 pg/(mg Cr). 54 (42.2%) patients were male, which was negatively correlated with UAng II (r =-0.36, P<0.01). Average proteinuria was 2.03 +/- 2.66 g/24h, which was negatively correlated with UAng II (r=-0.20, P<0.05). Average plasma renin activity(PRA) was 162.10+/-81.94, 6.80+/- 1.30 pg/ml/h , which was negatively correlated with UAng II(g=-0.20, P<0.05). Average urinary angiotensinogen (UAGT) was 4.64 +/- 1.27 ng/(mg Cr), which was positively correlated with UAng II (g=0.29, P<0.01). Average urinary type IV collagen(UCol IV) was 5.19 +/- 1.39 ng/(mg Cr) , which was positively correlated with UAng II (g=0.31, P<0.01). Multiple regression analysis indicated that male (P<0.01), low proteinuria (P<0.01) and high UAGT (P<0.01) were correlated significantly with high UAng II. There was no correlation of UAng II with plasma angiotensin II.

Conclusions: Renal endogenous angiotensin II maybe the principal source of urinary angiotensin II and plasma angiotensin II doesn't play an important role in the formation of urinary angiotensin II in chronic kidney disease patients.

SA-PO2566

Body Mass Index Predictive Performance of Estimated Glomerular Filtration Rate: A cystatinC- Versus Creatinine-Based Formulas Comparison Jorge Malheiro, Isabel Fonseca, Maria Joao Carvalho Azevedo Rocha, Josefina Santos Lascasas, António Manuel Nunes Cabrita. *Nephrology Unit, Centro Hospitalar do Porto, Porto, Portugal.*

Background: Higher than normal body mass index (BMI) is a risk factor for incident chronic kidney disease (CKD). Serum creatinine and cystatin C are the two main endogenous markers of kidney function. The relations of high BMI (≥25Kg/m^2) with CKD as estimated by creatinine- and cystatinC-based estimating equations were analyzed.

Methods: Stage 1-3 CKD patients from our ambulatory clinic with stable kidney function and simultaneous measurement of serum creatinine and cystatin C were randomly selected (n=179). Skewed data was log_transformed. CKD was defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m^2, using creatinine- or cystatinC- based formulas. Regression models evaluated the relationship between high BMI, CKD status (logistic) and eGFR (linear), adjusted for age, gender and cardiovascular risk factors (diabetes, hypertension, dyslipidemia).

Results: Patients sampled were mainly old (median age, 65 years), with a high prevalence of CKD (by creatinine- and cystatinC-based formulas 59.8% and 51.4% respectively) and cardiovascular risk factors.

Clinical comparison between patients with normal versus high BMI

	Normal BMI (n=67)	High BMI (n= 112)
Age in years, median*	58	67.5
Male gender, %	67.2	55.4
Diabetes, %*	19.4	41.1
Hypertension, %*	74.1	89.3
Dyslipidemia, %*	59.7	75
CKD prevalence by creatinine-based eGFR, %	50.7	65.2
CKD prevalence by cystatin-based eGFR, %*	34.3	61.6

* P <0.05

High BMI was not associated with CKD by creatinine-based eGFR (*P*=0.48). In contrast, high BMI was associated with CKD when defined using cystatinC-based equation in a multivariate-adjusted model (OR=2.20, *P*=0.03). In linear regression, BMI was also a predictor of eGFR by cystatinC with an estimated decrease of 0.9 ml/min/1.73m^2 per 1 Kg/m^2 increase in BMI (*P*=0.04). Linear regression between BMI and eGFR by creatinine was not significant (*P*=0.23).

Conclusions: High BMI status was associated with CKD when cystatinC-based eGFR was used, but not with creatinine-based eGFR. Influences of adiposity associated factors on serum cystatin C merit further investigation.

SA-PO2567

Iron Chelation with Deferiprone for Diabetic Nephropathy in the db/db Mouse Ahmad Bilal Malik,[1] Robert Camferdam,[1] R. Stafford Justus,[2] Songthip Ounpraseuth.[1] *[1]University of Arkansas for Medical Sciences; [2]Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: Catalytic iron (CatFe), by participating in redox cycling generates powerful reactive oxidant species and chelation may reduce progression of diabetic nephropathy (DN) and stabilize clinical parameters.

Methods: We tested the efficacy of the oral iron chelator deferiprone in a prototypical mouse model of human diabetes mellitus type 2 ($Lepr^{db}$/ $Lepr^{db}$). Using male mice from two batches of 6 animals for the control and treatment groups each, the latter received deferiprone dissolved in drinking water (125mg/kg body weight) starting at age 4.5 weeks till euthanasia at 28 weeks.

Results: Compared with baseline (25.92 ± 0.49g vs. 30.75 ± 0.53g), weight was better preserved in the treatment group (59.2 ± 2.29g vs. 54.15 ± 8.81g) at euthanasia. Mean blood glucose deteriorated with age in both groups (160.56 ± 10.74 vs. 345.69 ± 31.98mg/dL at 6 weeks) with lower final values in the treatment group (569.38 ± 98.49 vs. 685.04 ± 31.31mg/dL at euthanasia). The linear trend in the mean 24-h urinary CatFe excretion (CatFe/Cr ratio) values was evaluated using a repeated measures analysis of variance (ANOVA) as a function of treatment, batch, weeks, and their two-way interactions. Chelation was significantly better at week 6 (p= 0.0163) and despite a considerable reduction in CatFe excretion by the end of the study in the treatment group, this did not reach statistical significance with least squares means (LSM) giving values of 50.10 ± 233.25 vs. 942.75 ± 233.01µmol/mmol in the treatment and control groups respectively. 24-h urinary protein excretion (albumin/cr ratio) at weeks 6 and 24, using repeated measures ANOVA and determining the effect of treatment with deferiprone using LSM for the main treatment effect gave values of 529.02 ± 259.7 vs. 942.08 ± 257.38µg/mg, a statistically significant reduction (p= 0.0316). Serum Creatinine was slightly better, albeit not significantly different in the treatment group (0.541 ± 0.03 vs. 0.556 ± 0.03mg/dL by LSM).

Conclusions: CatFe chelation using deferiprone in a non-iron overload prototypical DN mouse model resulted in a significant reduction in proteinuria over a 6 month period.

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SA-PO2568

The Effects of Antagonism of Toll-Like Receptors by GIT27 on Lpid Metabolism and Diabetic Nephropathy in High Fat-Fed Mice Jin Joo Cha,[1] Hyunwook Kim,[2] Mihwa Lee,[1] Hye Kyung Song,[1] Deok Hwa Nam,[1] Jung Eun Kim,[1] Young Youl Hyun,[1] Ji Eun Lee,[2] Sang Youb Han,[3] Kum Hyun Han,[3] Young Sun Kang,[1] Dae R. Cha.[1] *[1]Division of Nephrology, Department of Internal Medicine, Korea University College of Medicine Ansan Hospital, Ansan-si, Gyeonggi-do, Korea; [2]Division of Nephrology, Department of Internal Medicine, Wonkwang University College of Medicine Sanbon Hospital, Gunpo-si, Gyeonggi-do, Korea; [3]Division of Nephrology, Department of Internal Medicine, Inje University College of Medicine Ilsan Baik Hospital, Goyang-si, Gyeonggi-do, Korea.*

Background: Numerous studies have demonstrated that obesity is a state of a systemic inflammatory condition. However, the underlying mechanism for linking metabolic and inflammatory pathway remains elucidated. Toll-like receptors (TLRs) have been recognized as proinflammatory and pattern-recognition receptors that play a key role in the innate immune system mainly against microbial pathogens. But recently it has been demonstrated that TLRs are also involved in sterile inflammation such as atherosclerosis and insulin resistance. Therefore, we asked whether GIT27, an immunomodulatory agent interfering TLR 4 and TLR2/6 signaling pathway, has beneficial effects on lipid metabolism and progression of kidney disease in obese high fat-fed mice.

Methods: Eight-week old male C57BL/6 mice were used and divided into 3 groups. Mice on normal chow, mice on high fat diet, and mice on high fat diet administered with 12-week intraperitoneal GIT27 treatment.

Results: With 3-month treatment of GIT 27, treated animals showed significantly improved serum cholesterol and triglyceride levels as well as gained less cholesterol and triglyceride accumulation in both kidney and liver. In addition, lipid hydroperoxide levels in the kidney, adipose tissue, and liver were significantly suppressed and urinary levels of inflammatory cytokines such as IL-2 and TNF-α and 8-isoprostane were also reduced. Moreover, treatment with GIT27 markedly decreased urinary albumin excretion and improved renal histologic changes.

Conclusions: Collectively, TLR antagonism by GIT27 might protect against obesity-related kidney disease by anti-inflammatory properties mainly affecting lipid metabolism.

SA-PO2569

Losartan Reverts Glomerular Sclerosis Induced by Type 2 Diabetes by Reducing Mesangial Cell Transdifferentiation Mirian A. Boim, Carine Prisco Arnoni, Edgar Maquigussa, Luciana G. Pereira. *Medicine - Renal Division, Federal University of Sao Paulo, Sao Paulo, Brazil.*

Background: Myofibroblastic transition (MFT) contributes to the pathogenesis of the diabetic nephropathy (DN). Ang II may have a role in this phenomenon. The present study evaluated the presence of MFT markers in a model of type 2 diabetes in rats and the capacity of the AT1 receptor inhibition by losartan to induce the regression of MFT-induced glomerulosclerosis. The MFT induction and reversion was also evaluated in cultured mesangial cells (MC), stimulated with high glucose concentration and with AngII.

Methods: Type 2 diabetes was induced by a high fat diet (55%) and low dose of streptozotocin (30mg/kg). After 12 weeks animals were divided into two groups untreated and treated with losartan (50mg/Kg/day,p.o.) for another 8 weeks. Results were compared with an age-control group (CT). Immortalized mouse MC were stimulated with 30mM glucose or AngII (10^{-7}M) during 4 days. Then cells were treated with growing doses of losartan (10^{-4}M-10^{-6}M) for 48 hr. The expression levels of fibronectin, type 4 collagen, TGF-β1 and the transdifferentiation markers including α-smooth muscle actin (α-SMA), desmin and fibroblast-specific protein 1 (FSP1) were estimated by real time PCR, Western blot and immunohistochemistry. MC transdifferentiation was confirmed by migration assay.

Results: Diabetic animals presented hypertension, hypercholesterolemia, hyperglicemia and severe proteinuria characterizing type 2 DN. Collagen accumulation, macrophages infiltration, expression of MFT markers and TGF-β1 were observed in 12 weeks diabetic kidneys. MC presented high migration capacity (more than 100% vs control cells), and increased expression levels of α-SMA according to the miofibroblast phenotype. Losartan treatment initiated after the nephropathy onset was able to significantly reduce these pre existent fibrotic manifestations and reversed the MFT displayed by MC.

Conclusions: Losartan can be a potential strategy, not only to minimaze the progression of the diabetic nephropathy, but also to reverse the fibrogenic processes by bringing cells back to their original phenotype .

Funding: Government Support - Non-U.S.

SA-PO2570

Insulin and Angiotensin Converting Enzyme 2 in Podocytes: A Protective Mechanism Against Albuminuria in Diabetic Nephropathy? Eva Marquez, Marta Riera, Judit Rigol-Giner, Julio Pascual, Maria Jose Soler. *Kidney Disease Research Group, Nephrology Department, Hospital del Mar - IMIM, Barcelona, Spain.*

Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in our environment. Renin-Angiotensin System (RAS) blockade has been shown to delay the progression of chronic kidney disease. Angiotensin-converting enzyme 2 (ACE2) is an element of RAS that cleaves angiotensin II to angiotensin 1-7, a vasodilator and anti-proliferative peptide. ACE2 is present in glomerulus, mainly in the podocytes. ACE2 inhibition in experimental DN increases albuminuria and worsens glomerular injury. The podocyte is a key cell involved in the development of albuminuria since early stages of DN and presents a functionally active local RAS and insulin receptors in its cell surface. It is known that insulin resistance is related with the severity of albuminuria.Our hypothesis is that insulin increases ACE2 expression in podocytes as a renoprotective mechanism against albuminuria.

Methods: A conditionally immortalized mouse podocyte cell line proliferated in permissive conditions with mouse γ-interferon at 32ºC. After that cells were induced to differentiate in nonpermissive conditions at 37ºC without γ-interferon for 14 days. Cells were then incubated without or with insulin (200nM) for 24 hours and collected for gene expression studies (qPCR), immunofluorescence staining, and protein studies (WB).

Results: ACE2 protein expression was significantly increased in podocytes incubated with insulin (n=6) as compared to controls (n=6) (1.27±0.2 vs 0.67±0.1 ACE2/β-actin p=0.03). In concordance, ACE2 gene expression was significantly increased in podocytes incubated with insulin (n=6) as compared to controls (n=6) (1.83±0.21 vs 1.16±0.03 ACE2/GAPDH, p=0.004). By immunoflurescence, ACE2 staining was increased in podocytes incubated with insulin as compared to controls.

Conclusions: In the podocyte, a cell that expresses a functional intrinsic RAS, insulin incubation increases ACE2 expression. This finding may suggest a specific role of insulin in maintaining intraglomerular RAS balance and protect against the progression of diabetic kidney disease.

SA-PO2571

A2B Adenosine Receptor as Novel Therapeutic Target during Diabetic Nephropathy Douglas Ridyard, Alexander Badulak, Holger Eltzschig, Almut Grenz. *Dept. of Anesthesiology, UC Denver, Denver, CO.*

Background: More than half of patients suffering from juvenile diabetes go on to develop diabetic nephropathy (DN). Only limited therapeutic approaches are available, including dialysis or kidney transplantation. Therefore, novel therapeutic approaches to prevent or at least treat DN are presently an area of intense investigation. Adenosine represents an endogenous signaling molecule to balance inflammatory reactions under different pathophysiological conditions. Extracellular adenosine is derived mainly via phosphohydrolysis of adenosine 5'-monophosphate (AMP) by the ecto-5'-nucleotidase (CD73) and can activate four individual adenosine receptors. At present, the role of adenosine generation and signaling in DN is unknown.

Methods: At 8 weeks of age, mice received daily streptozotocin injections intraperitoneally (50 mg/kg in 0.1 M citrate buffer, pH 4.5) for 5 consecutive days. The mice were considered diabetic if the blood glucose concentration (BGC) was above 500 mg/dl.

Results: Sixteen weeks after induction of STZ-induced diabetes histological evaluation by PAS staining showed significantly more glomerular sclerosis in CD73-/- compared to WT controls. Furthermore we could show a selective induction of the renal A2B adenosine receptor transcript and protein 16 weeks following STZ-induced diabetes. After 4 months of diabetes A2BAR-/- mice showed more apoptosis in the glomeruli compared to wild type STZ-induced diabetic mice. Treatment with an A2BAR agonist (BAY60-6583)

via Alzet pumps in wild type STZ-induced mice ameliorated the severity of apoptosis thereby indicating a protective role of extracellular adenosine by stimulating the A2BAR. Interestingly we could show an increase of the A2BAR in renal vessels during STZ-induced diabetes mellitus in an A2BAR reporter mouse by utilizing XGal staining.

Conclusions: In summary we show that adenosine generation by CD73 and stimulation of the endothelial A2BAR ameliorates renal damage occurring during diabetic nephropathy. We hope these studies will lay the groundwork for novel and specific therapeutic approaches in the treatment of DN, which are urgently needed for improving the outcome of Type 1 diabetic patients.

SA-PO2572

Defective Autophagy Promotes Podocyte Injury and Diabetic Nephropathy Li Fang, Chunsun Dai, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Podocyte injury is one of the prominent features in the progression of chronic kidney diseases as well as in the condition of diabetic nephropathy. As terminally differentiated cell, the podocyte has the ability to maintain homeostasis under certain pathophysiological stress. Autophagy, a highly regulated lysosomal pathway in the recycling of cytosol portions and the removal of superfluous or damaged organelles, is essential for cell homeostasis. However, the role and mechanisms of autophagy in podocyte dysfunction under diabetic state remain largely unknown.

Results: In this study, we found that a progressive albuminuria and podocyte injury was accompanied by defective autophagy manifested as decreased volume and numerical densities of autophagosome, along with, the downregulated expression of autophagy-related proteins including beclin-1, Atg12-Atg5, and LC3II/LC3I in kidneys from STZ-induced diabetic mice. Autophagy could be triggered in conditionally immortalized mouse podocytes in vitro with high glucose, advanced glycation end-products (AGEs), TGF-b1, AngII or bovine serum albumin (BSA) respectively. Blockade of autophagy with 3-Methylamphetamine (3-MA) or Beclin-1 siRNA promoted podocyte injury under both basal and autophagy-stimulating conditions. To investigate the correlation of ER stress with podocyte autophagy, we detected the abundance of CHOP, a sensor of severe ER stress, in podocytes treated with 3-MA or Beclin-1 siRNA. CHOP abundance was remarkably increased. Downregulation of CHOP with siRNA transfection protected podocytes from 3-MA or Beclin-1 siRNA-induced damage.

Conclusions: Taken together, these observations indicate that autophagy protects podocytes against damage through interfering ER stress and the defective autophagy may promote podocyte injury and proteinuria in diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2573

Nicorandil Ameliorates Podocyte Injury through the Activation of ATP-Dependent K^+ Channel in Diabetic Nephropathy Katsuyuki Tanabe, Miguel A. Lanaspa, Wataru Kitagawa, Christopher J. Rivard, Richard J. Johnson, Takahiko Nakagawa. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: Diabetic nephropathy is the leading cause for ESRD and it is urgent to find a therapeutic strategy. Unfortunately, RAS blockades are occasionally unsatisfactory, especially in patients with endothelial dysfunction. In the last ASN meeting, we reported that nicorandil ameliorated podocyte injury and reduced oxidative stress in diabetic endothelial nitric oxide synthase (eNOS) knockout (KO) mice. However, precise mechanism remains unclear. This reagent has dual actions; one is to release NO and the other is to open ATP-dependent K^+ channel. Here, we further examined a mechanism by which nicorandil prevented podocyte injury.

Methods: Podocyte injury was morphologically examined in eight week-old male eNOS-KO mice in which diabetes was induced by 50mg/kg of streptozotocin (ip) for 5 consecutive days, and nicorandil (30mg/kg/day) was administered for 8 weeks. To study direct effects of nicorandil on the differentiated podocytes, cells were stimulated by normal or high glucose with/without 10^{-5}M nicorandil for 72 hours.

Results: In diabetic eNOSKO mice, an increase in urinary microalbuminuria and urinary 8-OHdG excretion, both of which caused by diabetic condition, was significantly reduced by nicorandil. In the podocyte, podocin expression as well as number of WT-1 positive cells was less while oxidative stress was higher. However, nicorandil significantly blocked such podocyte injury. Interestingly, we found the expression of sulfonylurea receptor-2 (SUR-2), which is the component of ATP-dependent K^+ channel and importantly a binding site for nicorandil, in glomeruli, especially in podocytes. These data suggest that nicorandil could directly protect podocyte. Likewise, this assumption was proven by our in vitro evidence that high glucose caused an increase in ROS, as evidenced by the conversion from H_2DCFDA to DCF, in cultured human podocytes whereas nicorandil significantly reduced ROS production.

Conclusions: These results suggest that nicorandil has protective effects by reducing ROS on podocytes through opening ATP-dependent K^+ channel.

Funding: Pharmaceutical Company Support

SA-PO2574

Serum and Urinary ACE2 Is Increased in NOD Diabetic Mice Marta Riera,[1] Eva Marquez,[1] Heleia Roca-Ho,[1] Daniel Batlle,[2] Julio Pascual,[1] Maria Jose Soler.[1] [1]*Kidney Disease Research Group, Nephrology Department, Hospital del Mar- IMIM, Barcelona, Spain;* [2]*Nephrology, Northwestern University Feinberg School of Medicine, Chicago, IL.*

Background: ACE2 is a homologue of ACE that plays a counter-regulatory role. Renal cortex ACE2 activity is increased in some diabetic mouse models. However, little is known about ACE2 and the effect of insulin in NOD(non-obese diabetic).

Methods: Serum, urinary and renal cortical ACE2 was studied in NOD mice at 20 and 40 days after onset diabetes and compared with NOR controls and with insulin-treated NOD.

Results: Blood glucose and UAE increased in NOD as compared to NOR (21d; BG:571.1±20.3 vs 112.0±4.9mg/dL. UAE: 91.4±27.1 vs 8.6±3.0µgAlb/mgCrea/40d; BG; 550.6±32.7 vs 138.7±4.3. UAE: 492.2±310.9 vs 22.0±4.4, $p<0.05$). Insulin administration decreased BG and UAE (21d; BG: 113,4±5,4. UAE: 20.5±4.6 / 40d: BG: 88,4±12,2. UAE: 50.4±15.7, $p<0.05$).

Serum and urine ACE2 enzyme activity was increased in NOD mice compared to NOR.Insulin administration decreased serum and urine ACE2 activity.

	Serum ACE2 activity(RFU/µl/hr)	Urine ACE2 activity(RFU/µgCrea/hr)
NOR21d	71.5±4.3	1.6±0.1
NOD21d	249.8±39.0*	7.8±1.6*
NODins21d	126.7±14.8#	3.7±1.3
NOR40d	60.9±2.5	2.4±0.7
NOD40d	543.5±145.0*	37.5±10.3*
NODins40d	106.6±15.6#	4.2±1.7#
p	p<0,05(NOD vs*NOR or#NODins)	p<0,05(NOD vs*NOR or#NODins)

In tubules from NOD, ACE2 protein expression by WB was increased compared to NOR mice at 40 days of diabetes (21d; 0.38±0.1 vs 0.3±0.1 ACE2/β-actin / 40d; 1.2±0.2 vs 0.4±0.1, $p<0.05$). Insulin administration increased protein expression in 40d NOD mice(2.3±0.6, $p<0.05$).

Renal cortex ACE2 activity in NOD at 40 days increased compared with NOR (ACE2 activity: 2552.1±730.6 vs 1576.0±576.6 RFU/µg/hr, $p<0.05$). Insulin administration restored control values (1785.0±519.6, $p<0.05$).

Correlations: serum and urinary ACE2 activity with BG ($r=0.57, p<0.05$; $r=0.76, p<0.05$) and serum and cortical ACE2 activity with UAE ($r=0.83, p<0.05$; $r=0.54, p<0.05$).

Conclusions: NOD mice show increased serum and urinary ACE2 activity in an early stage of diabetic kidney disease and it remains elevated after 40 days of diabetes. Serum and urinary ACE2 may be an early marker of diabetic kidney disease.

SA-PO2575

The Change of β-Catenin in Experimental Diabetic Nephropathy Tae-Sun Ha. *Pediatrics, Chungbuk National University College of Medicine, Cheongju-si, Chungbuk, Korea.*

Background: Proteinuria is a cardinal feature of glomerular disease including diabetic nephropathy, and glomerular filtration barrier is considered as a filter restricting protein excretion in urine. We tested whether the expression of β-catenin, a molecule known to be located at the silt diaphragm, would be altered by high glucose in the cultured podocyte *in vitro* and by diabetes *in vivo*.

Methods: To investigate whether diabetic conditions including high glucose and advanced glycosylation endproducts (AGE) induce podocyte β-catenin changes, we observed renal tissues of diabetic rat obtained at 48 hrs, 4 weeks and 10 weeks after the induction of diabetes. And, we cultured rat glomerular epithelial cells (GEpC) and mouse podocytes under normal (5 mM) or high glucose (30 mM, HG) and AGE- or BSA-added conditions and examined the distribution of β-catenin by confocal microscope and measured the change of β-catenin expression by Western blotting and RT-PCR.

Results: Immunofluorescence of rat tissues stained with α-actinin and β-catenin showed their co-localization and decrease in intensities at podocyte around capillary loops. We also found that β-catenin relocalized from peripheral cytoplasm to inner actin filaments and its decrease in intensities by both AGE-added and HG condition in GEpC and mouse podocytes. In Western blotting, HG or AGE-added condition decreased β-catenin expression by 20.5% ($p<0.05$) and 16.0% ($p<0.05$), respectively, compared to the normal glucose condition. HG plus AGE-added condition further decreased β-catenin protein expression to statistically significant level (31.5%, $p<0.05$). No significant change was seen in osmotic control. In RT-PCR, HG plus AGE-added condition significantly decreased the expression of β-catenin mRNA by 14% ($p<0.05$) compared to normal glucose condition.

Conclusions: These results suggested that the exposure of podocytes to HG and AGE *in vitro* and diabetes *in vivo* reduced β-catenin mRNA and protein expression. These findings suggest that the decrease in β-catenin expression is connected to the early changes of diabetic nephropathy and thus may contribute to the development of proteinuria.

Funding: Government Support - Non-U.S.

SA-PO2576

Short Duration of Diabetes Alters Renal Endothelial Cell Function Christine M. Sorenson, Cathy Grutzmacher. *Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI.*

Background: Diabetic nephropathy is the most common cause of end stage renal disease and is a major risk factor for cardiovascular disease. In the United States, microvascular complications during diabetic nephropathy contribute to high morbidity and mortality rates. However, the identity of the underlying molecular and cellular mechanisms remains elusive.

Methods: Male Akita/+ (autosomal dominant mutation in the insulin II gene (Ins2)) mice, which reproducibly develop diabetes by 4 weeks of age were used. The impact of short duration of diabetes on renal endothelial cell (EC) function, as well as ex vivo aortic sprouting, was determined. Capillary morphogenesis of renal EC was evaluated in Matrigel. The migration of EC was evaluated using a transwell and scratch wound assay. The changes in eNOS expression and phosphorylation, as well as production of various extracellular matrix proteins, were determined by Western blotting. The rate of EC proliferation and apoptosis was determined using Clik-it EdU and TUNEL-labeling, respectively.

Results: Renal EC from Akita/+ mice following 4 weeks of diabetes demonstrated decreased migration and capillary morphogenesis. These studies were consistent with our aortic *ex vivo* angiogenesis assays, which showed a significant decrease in the number of sprouting outgrowths in aortas from Akita/+ mice. We also observed increased total and phosphorylated eNOS expression in renal EC from diabetic mice, which in turn corresponded with a 4-fold increase in NO level. In contrast, to renal tubule cells during diabetes, renal EC demonstrated decreased osteopontin expression along with increased fibronectin expression, potentially contributing to EC dysfunction. The levels of proliferation and apoptosis were similar.

Conclusions: These studies demonstrate that aberrant EC function with short duration of diabetes may set the stage for vascular dysfunction and rarefaction at later stages of diabetes.

Funding: Private Foundation Support

SA-PO2577

Progressive Renal Failure, Proteinuria and Glomerulosclerosis in a Murine Model of Diabetic Nephropathy Is Associated with Mitochondrial Oxidative Stress Sharma S. Prabhakar. *Medicine, Texas Tech Medical Center, Lubbock, TX.*

Background: Animal models of diabetic nephropathy (DN) hitherto described do not confirm to human disease since they do not exhibit the progression of proteinuria or renal failure characteristic of human disease. We previously characterized and published the phenotypic features of ZSF1 rats at 20 weeks which develop histological and functional changes that mimic early human DN (*Prabhakar et al JASN 2007*). We studied ZSF rats for much longer period with the goal of examining the progression of nephropathy and seeking correlations with human DN.

Methods: ZSF rats were obtained at 8 weeks of age and fed on high calorie diet till 40th week to maintain hyperglycemia. The body weights and blood pressures were monitored weekly while blood samples were obtained at the 8th, 12th 24th and 40th week. A 24 hour urine sample was obtained at the same time points for the measurement of creatinine clearance and protein excretion rates. Since 8-OHdG (8- hydroxy-deoxyguanosine), a measure of mitochondrial oxidative stress correlates better with total body oxidant stress and urinary excretion of 8-OHdG parallels the renal oxidative stress, urinary 8-OHdG levels were measured at the same time points. At 40 weeks the rats were euthenized and kidneys harvested to examine the histopathology.

Results: ZSF rats developed obesity hypertension, hyperlipidemia by 12th week as well as nephropathy with progressive proteinuria and renal failure that reached end stage by 40th week. Renal histology at this stage showed advanced diffuse glomerulosclerosis. The results of the functional parameters measured are shown below.

Functional characteristics of ZSF rats at different ages

Age in weeks	8	12	24	40
Uprot (mg/G creat)	165±37	459±84	1254±148	3658±542*
CCr (L/Kg/BW/d)	5.3 ± 1.2	4.2 ± 0.9	3.1 ± 0.7	2.1±0.6*
Ur 8-OHdG (ng/ Kg/BW)	387±89	2468 ±307	5429± 453	7658±566*

n=6, *p<0.001 vs. at 8 weeks age.

Conclusions: We conclude ZSF1 rats develop obesity, diabetes and nephropathy which progresses to advanced renal failure along with nephrotic syndrome, profound mitochondrial oxidative stress and diffuse severe glomerulosclerosis. Thus ZSF rats represent an ideal murine model to study human DN.

Funding: Private Foundation Support

SA-PO2578

The Absence of Collagen Type VIII Leads to Inhibition of Epithelial-Mesenchymal Transition (EMT) in Induced Diabetic Nephropathy Ivonne Loeffler, Gunter B. Wolf. *Internal Medicine III, Universiyt of Jena, Jena, Germany.*

Background: The epithelial-mesenchymal transition (EMT) is a mechanism of renal tubulointerstitial fibrosis in diabetic nephropathy (DN) that leads to an accumulation of smooth muscle α-actin (α-SMA)-positive myofibroblasts. The process of the EMT is characterized by the progressive loss of E-Cadherin, zonula occludens 1 (ZO1) and cytokeratine (CK) of tubular epithelial cells, which is associated with increased expression of mesenchymal markers (α-SMA, fibroblast-specific protein 1 FSP1 and vimentin). It has been reported, that up to 36 % of matrix-producing cells in the tubulointerstitium are of epithelial origin and may develop from tubular epithelial cells by EMT. We have previously found that the non-fibrillar short-chain collagen VIII (genes: COL8A1 and COL8A2) expression is enhanced in renal cells of diabetic mice and in biopsies of patients with DN, COL8A1 and COL8A2 expression was also inducible in mesangial cells by stimulation with TGF-β1 in vitro. Furthermore, we have shown that the characteristic features of DN in STZ-induced diabetic Col8A1/A2 knockout (KO) mice were significantly lower compared to the wildtype. The present study investigates whether EMT plays a role in these effects.

Methods: Kidneys from healthy and STZ-induced diabetic wildtype and Col8A1/A2-KO mice were harvested and analyzed. We investigated the expression of miscellaneous EMT markers with immunohistological as well as PCR array technology.

Results: In contrast to diabetic wildtyp mice, showing Col8A1/A2 expression, no evidence on development of EMT was found in diabetic Col8A1/A2-KO mice. Both, the significant increase of renal interstitial myofibroblasts and the inhibition of the expression of epithelial markers respectively the enhanced expression of typical mesenchymal markers in tubular cells were missing in kidneys of diabetic Col8A1/A2-KO mice.

Conclusions: The results suggest that either collagen type VIII may be one of the major inducer of EMT in kidneys of diabetic wildtyp mice or at least the lack of Col8A1/A2 disrupts the process of TGF-β1-induced EMT.

Funding: Government Support - Non-U.S.

SA-PO2579

Phenotypical Changes of Podocyte CD2AP in Experimental Diabetic Nephropathy Via PI3-K/Akt Signaling Tae-Sun Ha. *Pediatrics, Chungbuk National University College of Medicine, Cheongju-si, Chungbuk, Korea.*

Background: Proteinuric conditions demonstrate ultrastructural changes in podocytes with retraction and effacement of the highly specialized interdigitating foot processes, resulting in hyperpermeability.

Methods: To investigate podocyte phenotypical changes, including quantitative and distributional changes of CD2AP protein in diabetic conditions, we prepared diabetic renal tissues and cultured podocytes in diabetic conditions. According to diabetic duration, density of CD2AP in renal tissue of experimental diabetic nephropathy became conglomerulated and diminished. To investigate how high-glucose (HG) and advanced glycosylation end products (AGE) induce podocyte phenotypical changes, including quantitative and distributional changes of CD2AP protein and search for the signaling mechanisms, we cultured rat glomerular epithelial cells (GEpC) and mouse podocytes under: (1) normal glucose (5mM, = control); (2) HG (30 mM); (3) AGE-added; or (4) HG plus AGE-added conditions. We also examined the *in vivo* changes of CD2AP in streptozotocin-induced diabetic tissues.

Results: HG plus HG and AGE induced the re-localization of CD2AP into inner actin filaments complexes by confocal imaging. In Western blotting, administration of high glucose or AGE decreased the CD2AP productions by 36.9% ($p<0.05$) and 16.0% ($p<0.05$), respectively. Furthermore, both high glucose and AGE decreased the amount of CD2AP more significantly by 64.6% compared to those of control ($p<0.01$). In RT-PCR, administration of high glucose, AGE or both high glucose and AGE decreased the expression of CD2AP mRNA by 44.9%, 27.9%, and 29.3% ($p<0.05$), respectively, compared to that of control. In addition, LY294002, a PI3-K inhibitor, could prevent the quantitative and distributional changes of CD2AP induced by HG and AGE.

Conclusions: These findings suggest that diabetic conditions induce the phenotypical changes of podocyte CD2AP via PI3-K/Akt signaling.

Funding: Government Support - Non-U.S.

SA-PO2580

The Role of MORG1 in Early-Stage Diabetic Nephropathy (DN) Carola Ruhe, Gunter B. Wolf. *Internal Medicine III, University of Jena, Germany.*

Background: DN is a progressive kidney disease resulting and is characterized by excessive deposition of extracellular matrix proteins, increasing intrarenal inflammation and chronic hypoxia. The mitogen-activated protein kinase organizer 1 (MORG1) has been identified as a novel WD-repeat protein that interact with the prolyl hydroxylase 3 (PHD3), an important enzyme involved in the regulation of the hypoxia-inducible factor (Hif). The purpose of this study was to assess the role of MORG1 in the development of early-stage diabetic nephropathy in diabetic heterozygous MORG1+/- mice.

Methods: Diabetes mellitus was induced in wildtype MORG1+/+ and heterozygous MORG1+/- mice with an intraperitonal injection of streptozotocin (STZ, 5 x 50mg/kg). Immunohistochemistry and molecular biology techniques were used to estimate the expression of extracellular matrix proteins (collagen I, collagen IV and fibronectin), proinflammatory mediators (e.g. MCP-1 – monocyte chemotactic protein-1) and the transcription factors Hif1α and Hif2α in the kidney of non-diabetic and diabetic mice.

Results: The expression of collagen I in the glomeruli of diabetic heterozygous MORG1+/- mice was significantly less compared with the diabetic wildtype MORG1+/+ mice. On the other hand, downregulation of MORG1 did not show a significant impact on glomerular deposition of both collagen IV and fibronectin The proinflammatory mediator MCP-1 was significantly increased in the diabetic group compared with the non-diabetic group, but there were no significant differences in the expression of MCP-1 between diabetic wildtype and diabetic heterozygous mice.

Conclusions: Downregulation of MORG1 reduces the deposition of extracellular matrix proteins and attenuates the progression of DN. However, the expression of proinflammatory markers seems to be independent from MORG1.

Funding: Other U.S. Government Support

SA-PO2581

The Inflammatory Genotype of Diabetic Nephropathy Katherine J. Kelly,[1] Jesus H. Dominguez.[1,2] *[1]Nephrology, Indiana U, Indianapolis, IN; [2]VAMC, Indianapolis, IN.*

Background: Obese, diabetic ZS (F1 hybrids of Zucker and SHHR) rats develop progressive renal fibrosis and inflammation on a high fat diet. Nephropathy is markedly accelerated after a single episode of renal ischemia.

Methods: Whole transcriptosome RNA sequencing was performed to fully characterize transcript expression in obese-diabetic/postischemia (OI) as compared to obese-diabetic/sham surgery (OS) and lean (L) rat kidneys.

Results: WBC (cells/hpf) are markedly increased in OI rats: 0.3±0.2 in L, 8±1 in OS, 12±1 in OI, p<0.01, with the majority being macrophages (MP). MP were also prominent in biopsies of diabetic nephropathy (30±2 vs 10±3 in membranous or hypertensive renal disease). A wide variety of genes encoding pro-inflammatory mediators were markedly upregulated (for example, 83 and 19 fold increase in IL24; 5.0 and 3.3 fold increase in the chemokine Ccl6 in OI and OS vs L). Upregulated transcripts included MP antigens (for example CD 68 was increased 2.5 and 2.2 fold in OI and OS vs L). mRNAs for chemokines associated with pro-inflammatory M1 MP polarization (Ccl19, 4.3 and 2.9 fold; Ccl7, 7.8 and 3.1 fold; Cxcl11, 2.2 and 1.7 fold) were significantly increased. Although mRNAs for M2 polarization antigens (mannose receptor, 6.0 and 3.1 fold) were also increased, the M2 genotype was not fully expressed. IL4 and IL13, mediators of M2 activation, were undetectable in OI and OS kidneys. mRNAs for MP scavenger receptors (CD163 increased 20 and 16 fold; MSR1 4.3 and 2.8 fold), MP elastase (8.3 and 5.3 fold) and Trem2 (triggering receptor expressed on macrophages; 23 and 15 fold) were among the transcripts most markedly altered in the obese-diabetic/postichemic kidneys. The increases in pro-inflammatory mediator gene expression are consistent with recruitment of multiple types of inflammatory cells to the kidney, amplification of the inflammatory response and tissue injury.

Conclusions: RNAseq data on mRNA encoded proteins are tremendously valuable for they offer a comprehensive and integrated view of the massive inflammatory response in diabetic nephropathy. This analysis shows a broad immunological dysfunction in the diabetic kidney and at the same time reveals a multitude of potential targets for therapy.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

SA-PO2582

High Glucose Upregulates Nucleoporin62 (Nup62) in Human Mesangial Cells Rekha Singh. *Research, Hines VA Medical Center, Hines, IL.*

Background: Malfunction of nuclear pore complex (NPC) proteins such as nucleoporin62 (Nup62) could alter nuclear import of transcription factors resulting in abnormal gene transcription. Our preliminary studies in Streptozotocin (STZ)-induced diabetic rats (type 1 diabetes) and Zucker diabetic fatty rats (ZDF, type 2 diabetes) showed increased protein expression of Nup62 in glomerular extracts suggesting that high glucose milieu in diabetes might affect NPC functions. In this study, we further examined the effect of high glucose on Nup62 in glomerular mesangial cells.

Methods: Primary human mesangial cells (HMC; ScienCell, CA) were cultured and incubated with media (without serum and growth factors) containing 5 mM glucose (NG) or 25 mM glucose (HG) for a period of 1-10 days. At termination of experiments, cytoplasmic and nuclear extracts were prepared and protein expression of Nup62 was determined by Western blotting. Also, HMC were seeded in Lab-Tek cell culture slides and Nup62 localization was examined by immunofluorescence. Since high glucose-induced stimulation of mesangial matrix is mediated by angiotensin II (Ang II), we tested the effect of Ang II (1 μM) on Nup62 protein expression and localization in HMC. Additionally, HMC treated with HG or Ang II were also analyzed for protein expression and localization of nuclear transport factor2 (NTF2) which is functionally associated with Nup62.

Results: Exposure of HMC to high glucose caused upregulation of Nup62 protein expression in nuclear extracts and an increase in Nup62 localization on the nuclear membrane. In addition, protein expression and nuclear accumulation of NTF2 was found to be greater in HG-treated cells compared to NG-treated cells. Incubation of HMC with Ang II resulted in increased Nup62 and NTF2 protein expression accompanied by increased co-localization of AT1 receptor with Nup62 in the nucleus. Similar to Ang II, HG also enhanced nuclear co-localization of AT1 receptor with Nup62 in HMC.

Conclusions: These results suggest that both high glucose and Ang II increase protein expression and nuclear localization of Nup62 and NTF2 in HMC which could affect nuclear import of transcription factors and gene transcription of matrix proteins.

Funding: Veterans Administration Support

SA-PO2583

Nephrinuria vs. Podocyturia: Which Is a Better Early Marker for Diabetic Nephropathy? Shuchita Sharma,[1] Andi Qipo,[1] Kwanghee Kim,[2] Pu Zong,[1] Swati Mehta,[1] Dennis Michael Bonal,[3] Belinda Bun Jim.[1] *[1]Jacobi Med Ctr, NY; [2]Trinity Health Ctr, ND; [3]Columbia Med Ctr, NY.*

Background: There is interest in using urinary podocyte (podocyturia) as a marker to assess glomerular disease activity in diabetic nephropathy (DN), as its pathogenesis is related to podocyte loss. However, technical issues and lack of validation still prevent its clinical application. Nephrin, a podocyte protein, is also found in the urine of diabetic patients (nephrinuria). With the advent of an enzyme-linked immunosorbent assay (ELISA) for nephrin (Exocell, Inc. Philadelphia, PA), we are able to accurately measure nephrinuria. Which marker offers superior correlation with clinical disease is unclear.

Methods: We compared these two markers by quantifying podocyturia and nephrinuria in 24 diabetic patients and 10 healthy controls and correlated with clinical parameters. For podocyturia, a cell pellet was obtained via centrifugation from fresh urine and fixed for immunofluorescence. Podocytes were identified by colocalization of podocyte markers podocin and synaptopodin and counted by 2 investigators. For nephrinuria, ELISA was performed on urine supernatant. The Wilcoxon rank test compared differences between study and control groups, while the Spearman rank correlation coefficients associated clinical parameters with urine podocyte-to-creatinine ratio (UPodCR) and urine nephrin-to-creatinine (UNCR).

Results: UPodCr and UNCR were both significantly higher in the study than control groups (p=0.005 and p=0.01 respectively). UNCR correlated significantly with urine albumin-to-creatinine ratio (UACR) (rho=0.92,p<0.0001), BUN (rho=0.47, p=0.02), serum creatinine (rho=0.48, p=0.01), systolic BP (rho=.75, p<.0001), while UPodCR did not: UACR (rho= -0.46,p=0.02), BUN (rho= -0.34, p=0.09), serum creatinine (rho= -0.50, p=0.01), systolic BP (rho= -0.45,p=0.02). Other variables such as serum albumin, presence of diabetic retinopathy, HB_{A1C}, use of ACEI/ARB did not show significant correlation with either method.

Conclusions: As nephrinuria correlates better than podocyturia with most clinical parameters in DN, we conclude that nephrinuria is a superior marker and avoids the technical pitfalls of counting podocytes.

SA-PO2584

High Glucose Promotes Renal Tubular CTGF Expression Via the Activation of Protease-Activated Receptors Wai Han Yiu,[1] Joseph C.K. Leung,[1] Loretta Y.Y. Chan,[1] Hui Y. Lan,[2] Kar Neng Lai,[1] Sydney C.W. Tang.[1] *[1]Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong; [2]Department of Medicine and Therapeutics, and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong.*

Background: Protease-Activated Receptors (PARs) are potent mediators of inflammation and fibrosis after being cleaved and activated by serine proteases. Overexpression of PARs in several kidney diseases suggests a possible role in the progression of kidney damage. Based on our previous study in which high glucose stimulates pro-inflammatory and pro-fibrotic signals in cultured human proximal tubular epithelial cells (PTEC), we here investigate the role of PARs in high glucose induced expression of connective tissue growth factor (CTGF), the pro-fibrotic mediator for the accumulation of extracellular matrix protein and the pathogenesis of diabetic nephropathy.

Methods: Human PTEC were cultured in medium with normal glucose (5mM), high glucose (30mM) or high glucose with specific PAR antagonist (SCH79797 for PAR-1, ENMD-1068 for PAR-2 and trans-Cinnamoyl-YPGKF-NH2 for PAR-4) added 1h before stimulation. RNA were isolated after 6h incubation for gene expression analysis by quantitative real time PCR and culture supernatant were collected after 24h incubation for protein expression analysis by ELISA.

Results: We demonstrated the expression of PARs in PTEC. PAR-2 was the most abundant receptor in PTEC compared to PAR-1 and PAR-4, whereas PAR-4 had the lowest expression among the receptor family. However, high glucose (30mM) selectively enhanced PAR-4, but not PAR-1 and PAR-2 mRNA expression in PTEC. In addition, high glucose stimulated a time-dependent increase in CTGF mRNA and protein levels, and the expression was significantly inhibited by pre-incubation with PAR-1, PAR-2 and PAR-4 antagonists.

Conclusions: These results suggest that PARs mediate the modulation of CTGF expression in renal tubular cells and the up-regulation of PAR-4 may further contribute to the progression of fibrosis in the diabetic kidney.

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SA-PO2585

The Role of FXR in Renal Diseases and Associated Metabolic Disorders Affecting Offspring Impacted by Maternal Obesity Sonia Saad,[1] Hui Chen,[2] Katherine Jane Pegg,[1] Carol A. Pollock,[1] Muh Geot Wong.[1] *[1]Medicine, Kolling, Sydney, NSW, Australia; [2]Science, UTS, Sydney, NSW, Australia.*

Background: Children of obese mothers have increased risk of developing obesity, dyslipidemia, glucose intolerance and chronic kidney disease. The link between dysregulated lipid metabolism and pathogenesis of nephropathy is increasing. The underlying mechanisms are unclear. Obesity leads to increased plasma free fatty acids. The nuclear receptor farnesoid X receptor (FXR) regulates fatty acid metabolism and abnormal FXR expression or function plays an important role in nephropathy associated with substrate metabolic disorders. The aim of this study is to investigate if disordered FXR contributes to the link between lipid metabolic disorders in obese mother and the adverse renal outcomes in their offspring in vivo.

Methods: Renal proximal tubule epithelial cells (HK2) were exposed to high glucose (HG) for 24 and 72 hrs. FXR and SREBP-1c mRNA expression was determined by real time PCR. siRNA was used to silence FXR. Collagen IV (CIV) and Fibronectin (FN) levels were determined by Western blot. FXR levels in the kidney of patients with diabetic nephropathy (DN) vs non-diabetic patients was determined by immunohistochemistry. Female Sprague Dawley rats were fed either HFD (20kJ/g) or chow (14kJ/g) before mating and for the experimental period (9 weeks). Abdominal fat, plasma and liver triglyceride, blood glucose change during glucose tolerance test in chow and HFD-fed offspring from obese mothers were measured. Renal structure and FXR expression were determined by immunohistochemistry.

Results: HK2 cells exposed to HG have reduced FXR but increased SREBP-1c mRNA expression. FXR silencing augments high glucose induced FN and CIV expression in

these cells. FXR is expressed in renal human tubule and its level is reduced in patients with DN. Renal structural changes were present in offspring from obese mothers with features of dysregulated glucose and lipid metabolism in association with reduced FXR expression in the tubule.

Conclusions: Our results suggest a role for FXR in adverse renal outcomes in offsprings from obese rats with metabolic derangement which may be of considerable therapeutic value.

Funding: Government Support - Non-U.S.

SA-PO2586

Clinical and Molecular-Pathological Effects of Chronic Hypoxia on Diabetic Nephropathy in db/db Mice Naoki Takahashi,[1] Hideki Kimura,[1] Kazuko Kamiyama,[1] Tomomi Kurose,[2] Hidehiro Sugimoto,[2] Toshio Imura,[2] Daisuke Mikami,[1] Kenji Kasuno,[1] Haruyoshi Yoshida.[3] *[1]Division of Nephrology, University of Fukui, Japan; [2]Division of Clinical Laboratories, Fukui University Hospital, Fukui, Japan; [3]Department of Internal Medicine, Sugita Genpaku Memorial Obama Municipal Hospital, Obama, Japan.*

Background: Hypertension, angiogenetic cytokines and eNOS-VEGF imbalance, which may be affected by chronic hypoxia, reportedly accelerate progression of diabetic nephropathy. However, no detailed information has been known about the clinical and molecular-pathological effects of chronic hypoxia on diabetic nephropathy in db/db mice.

Methods: 8 w.o. male db/db mice were bred in a normobaric hypoxic chamber (12%O_2). The hypoxic group (H-group) was kept in this chamber for 16 weeks (n=11) and the control group (N-group) was bred in room air (n=12). Mice were sacrificed at 12 and 24 w.o. in order to evaluate histological changes and mRNA amounts (VEGF-A, PAI-1, MCP-1, TGF-β1, CTGF, eNOS, CD34 and Angiopoetin1 and 2) extracted from whole kidney.

Results: H-group showed severer erythrocytosis, greater albuminuria and higher serum LDL levels than N-group, while the two groups did not differ in levels of blood pressure, serum Cr, Ccr or urinary VEGF-A antigen. As for histological aspects, H-group had greater glomerular swelling (1.3-fold), lower number of podocyte and CD34-positive endothelium per glomerular area and higher rates of microaneurysm formation, glomerulosclerosis and glomerular macrophage infiltration than N-group. Glomerular VEGF-A positive area levels were, however, similar in H and N-groups. Concerning molecular analyses, in H-group, mRNA levels of MCP-1 and PAI-1 were significantly increased by 1.6-fold at 4W and1.9-fold at 16W and by 2.1-fold at 16W, respectively as compared with N-group, whereas mRNA levels of VEGF-A, TGF-β1, CTGF, Angiopoetin1 and 2, CD34 and eNOS were very similar.

Conclusions: Hypoxia-specific glomerular injury may result from the decreased number of endothelium and podocyte per glomerular area and the increased macrophage infiltration via MCP-1 and PAI-1 induction, but not from hypertension or hyperfiltration.

Funding: Government Support - Non-U.S.

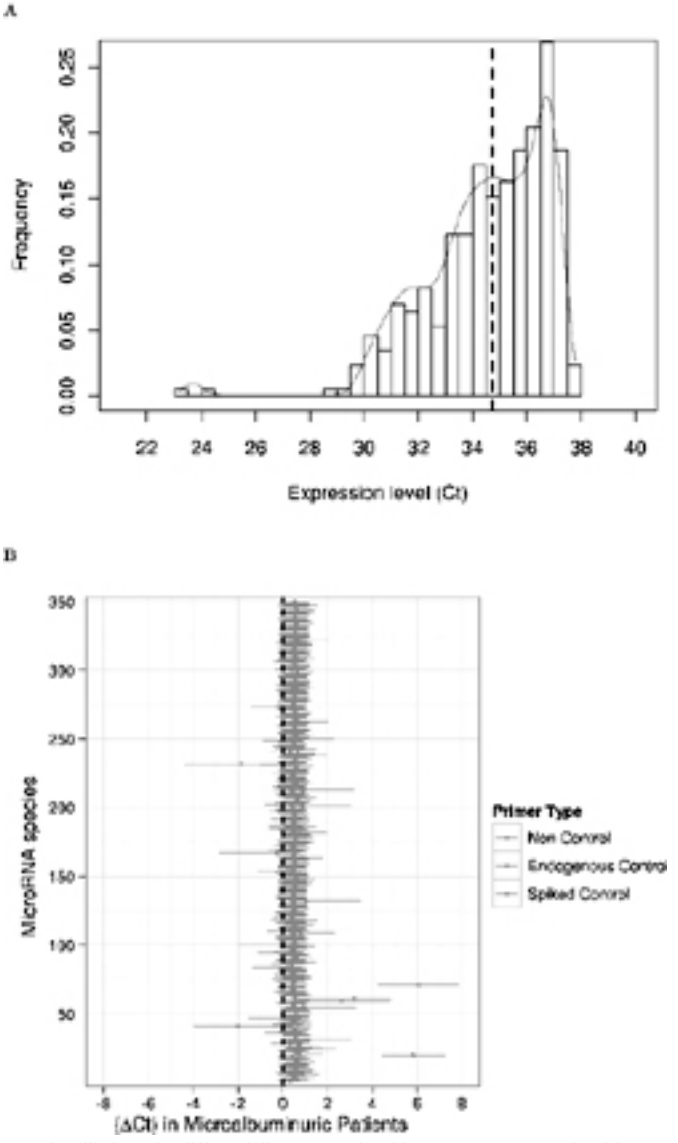
SA-PO2587

Urine microRNA Profiling in Nephropathy of Type 1 Diabetes Christos Argyropoulos,[1] Kai Wang,[2] Jose F. Bernardo,[1] David Galas,[2] John P. Johnson.[1] *[1]University of Pittsburgh, PA; [2]Institute for Systems Biology, Seattle, WA.*

Background: Diabetic Nephropathy (DN) is the leading cause of Chronic Kidney Disease (CKD) in the US, affecting 1/4 of patients with Type 1 Diabetes (T1D). We examined urine profiles of microRNA (miR), endogenous RNA negative regulators of gene expression, as biomarkers for the development of DN.

Methods: Patients (pts) with T1D from the Pittsburgh Epidemiology of Diabetes Complications (EDC),a historical prospective cohort, free from DN (N,n=10), or with microalbuminuria (MA,n=20) during follow up (f/u). We profiled miR spectrum in urine from the last f/u visit (N) or the last non-albuminuric visit (MA) pts, with qPCR panels. The PCR cycle (Ct) at which fluorescence exceeded the background was used to quantify miR abundance. We analyzed Cts with a Bayesian mixed model under a Dirichlet Process Mixture prior. This model groups miRs in clusters of similar expression, while controlling the number of false discoveries. Model estimates for the differences between MA and N (ΔCt), corresponding to log-fold changes in specific miR levels, were sequentially normalized to endogenous and spiked-in controls.

Results: Patient characteristics were similar though MA patients tended to be younger (28.4±5.7 vs 42.9±5.1). Un-normalized Cts of the N group clustered in 4 peaks (FigA,histogram/red line), around the mean (dashed line).The majority of un-normalized ΔCt clustered with the controls indicating no difference in expression between N and MA pts (FigB).



miRs likely to be differentially expressed (odds > 99:1): Downregulated in MA (miR-495: 131 fold, miR-548o: 162 fold), upregulated in MA (miR-122: 20 fold, miR-589: 16 fold).

Conclusions: Urine miR expression differs in T1D pts who develop MA compared to pts who never develop DN. Future studies should verify the utility of these miRs as early biomarkers of DN.

SA-PO2588

Podocyte-Specific JAK2 Overexpression Augments Albuminuria and Glomerular Size but Not Mesangial Matrix Expansion in 129S6 Mice Hongyu Zhang, Frank C. Brosius. *Internal Medicine/Nephrology and Physiology, University of Michigan.*

Background: JAK2 has been implicated in the pathogenesis of diabetic nephropathy (DN). Humans with type 2 diabetes express higher JAK2 levels in podocytes early in DN and in proximal tubular epithelia in progressive DN (Berthier, et. al. Diabetes, 2009;58:469).

Methods: In order to test the role of increased JAK2 expression in podocytes in early DN we knocked in a STOP/FLOX JAK2 construct to the ROSA26 locus in 129S6 mice and generated podocyte-specific enhanced JAK2 expression by crossing the mice with tissue specific Nphs2 Cre 129S6 mice allowing *JAK2* transcription driven by the ubiquitously expressed ROSA26 promoter. We subsequently crossed in the Akita insulin 2 mutation which produces type 1 diabetes in mice to test the effects of podocyte specific JAK2 overexpression in type 1 diabetes.

Results: The podocyte specific JAK2 heterozygote mice have a ~80% increase in total glomerular JAK2 mRNA similar to the increase seen in humans with progressive DN. With this relatively modest increase in JAK2 expression, there were no differences in nodiabetic JAK2 animals compared to nondiabetic animals with normal JAK2 levels, but there was a 66% increase in albuminuria in diabetic podocyte JAK2 mice at 12 wks of age ($p<0.01$) compared to diabetic littermates with normal JAK2 levels. However, the difference in albuminuria disappeared by 24 weeks and was also not present at 32 weeks. Mesangial

expansion was also not greater in the podocyte JAK2 diabetic mice than in the diabetic mice with normal JAK2 levels at 32 weeks of age compared to nondiabetic controls. However, there was a greater increase in glomerular volume in the diabetic JAK2 mice compared with the diabetics with normal JAK2 levels (2.52-fold vs. 1.67-fold; $p<0.05$).

Conclusions: These data suggest that increased expression of podocyte JAK2 may play a role in early DN by increasing glomerular growth more than podocyte foot process changes or in glomerular fibrosis.

Funding: NIDDK Support

SA-PO2589

Aberrant Activation of the Canonical Wnt Pathway in the Renal Tubular Cells in Diabetic Nephropathy Jian-Xing Ma, Ti Zhou, Rui Cheng, Xuemin He, Ying Chen. *Physiology, Univerwsity of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: The Wnt pathway mediates multiple physiological and pathological processes such as inflammation, angiogenesis and fibrosis. The present study is to investigate whether the canonical Wnt signaling plays a role in the pathogenesis of diabetic nephropathy (DN).

Methods: Expression of Wnt ligands and Frizzled receptors in the canonical Wnt pathway in the kidney was compared at the mRNA levels using real-time RT-PCR between Akita mice, streptozotocin (STZ)-induced diabetic rats and db/db mice and their respective non-diabetic controls. Renal function was evaluated by measuring the urine albumin excretion. Primary human renal proximal tubular epithelial cells were treated with high glucose and 4-hydroxynonenal (HNE). Levels of β-catenin, connective tissue growth factor and fibronectin were determined by Western blot analysis.

Results: Some of the Wnt ligands and Frizzled receptors showed increased mRNA levels in the kidneys of Akita mice, STZ-induced diabetic rats and db/db mice, compared to their non-diabetic controls. Renal levels of β-catenin and Wnt proteins were up-regulated in these diabetic models. Lowering the blood glucose levels by insulin attenuated the activation of Wnt singling in the kidney of Akita mice. In cultured primary human renal proximal tubular cells, high glucose and HNE both activated Wnt signaling. Inhibition of Wnt signaling with a monoclonal antibody blocking low-density lipoprotein receptor-related protein 6 (LRP6) ameliorated renal inflammation, fibrosis and reduced proteinuria.

Conclusions: The Wnt pathway is activated by hyperglycemia in the kidney of both types 1 and 2 diabetic models. The Wnt pathway dysregulation in diabetes represents a new pathogenic mechanism of DN and renders a new therapeutic target.

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SA-PO2590

Relationships between Serum MCP-1 and Subclinical Kidney Disease: African American-Diabetes Heart Study Mariana Murea,[1] Thomas Costin Register,[7] Jasmin Divers,[2] Donald W. Bowden,[3] John Jeffrey Carr,[6] Richarlette Caresse Hightower,[6] Susan Carrie Smith,[3] Keith A. Hruska,[5] Carl D. Langefeld,[2] Barry I. Freedman.[1] *[1]Nephrology, Wake Forest Baptist Health, Winston-Salem, NC; [2]Public Health Sciences, Wake Forest Baptist Health, Winston-Salem, NC; [3]Biochemistry, Wake Forest Baptist Health, Winston-Salem, NC; [4]Center for Diabetes Research, Wake Forest Baptist Health, Winston-Salem, NC; [5]Pediatric Nephrology, Washington University in St. Louis, St Louis, MO; [6]Radiology, Wake Forest Baptist Health, Winston-Salem, NC; [7]Pathology, Wake Forest Baptist Health, Winston-Salem, NC.*

Background: Monocyte chemoattractant protein-1 (MCP-1) plays key roles in nephropathy and atherogenesis. Relationships between serum MCP-1 concentration and kidney and cardiovascular disease (CVD) were assessed in African American (AA) population.

Methods: Serum MCP-1 was measured in 479 AAs with type 2 diabetes (T2D). Urine albumin: creatinine ratio (ACR), eGFR, and calcified atherosclerotic plaque (CP) in the coronaries, carotid arteries, and infra-renal aorta were measured. Generalized linear models were fitted to test for associations between MCP-1 and ACR, eGFR, and CP.

Results: Participants were 57% female; mean (SD) median age 55.6 (9.5) 55.0 years, diabetes duration 10.3 (8.2) 8.0 years, urine ACR 151 (569) 14.0 mg/g, MDRD eGFR 95.2 (27.2) 93.3 ml/min/1.73m^2, MCP-1 262.9 (239.1) 224.4 pg/ml, coronary artery CP 280 (634) 13.5, carotid artery CP 47 (132.9) 0, and aorta CP 1616 (2864) 319. Adjusting for age, sex, smoking, BMI, blood pressure, diabetes duration, hemoglobin A_{1c}, cholesterol, and medications, serum MCP-1 levels had direct correlation with ACR (parameter estimate 0.002, p=0.043) and inverse correlation with eGFR (parameter estimate -0.0005, p=0.007). Significant associations were not detected with the extent of CP in any vascular bed.

Conclusions: Serum MCP-1 levels are significantly associated with cross-sectional measures of kidney function and albuminuria in AAs with T2D. In this racial group, MCP-1 levels are not associated with the vascular CP, surrogate of subclinical CVD.

SA-PO2591

Altered Wnt/β-catenin Pathway and Association of β-catenin with CHOP in Tubules of Diabetic Mice Michelle T. Barati, Susan M. Isaacs, David W. Powell, Michael Merchant, Jon B. Klein. *Nephrology, University of Louisville, KY.*

Background: The presence of endoplasmic reticulum (ER) stress response and apoptosis in diabetic nephropathy (DN) is evident, yet the role of ER stress in tubule cell dysfunction during DN is unclear. The goal of this study was to characterize novel effectors of ER stress response signaling in renal tubules that may play a role in DN pathogenesis.

Methods: We first identified proteins co-immunoprecipitating with ER stress-induced pro-apoptotic transcription factor CHOP in tubules of mice, using mass spectrometry (MS). Cell extract from isolated cortical tubules of OVE26 diabetic and FVB control mice were used. Immunoprecipitated proteins were trypsinized, subjected to LC-MALDI-TOF/TOF MS and LC-ESI-MS/MS. Identified proteins were analyzed for network associations using Ingenuity Pathways Analysis (IPA). Association of MS-identified proteins in complex with CHOP was validated by immunoprecipitation of CHOP from tubule extract followed by immunoblot analysis for specific proteins.

Results: IPA identified a protein network related to Wnt/β-catenin signaling including, Frizzled related protein, Wnt, casein kinase1γ, APC, dikkopf1 homolog, zo-1, jagged1, polycystin1, myosin7A, tp73, and Jade1. CHOP/β-catenin association was increased in tubules of 3 month old diabetic mice, and decreased in 7 month old diabetic mice. Casein kinase1γ and Jade1 expression (proteins that regulate β-catenin) was increased in tubules of diabetic mice. CHOP/β-catenin interaction was studied in proximal tubule cells (HK-2) during Thapsigargin (TG)-induced ER stress conditions, by confocal microscopy. TG increased total CHOP expression, decreased membrane β-catenin localization, and increased nuclear co-localization of both proteins. Additionally, exposure of HK-2 cells to high albumin concentrations increased β-catenin nuclear localization.

Conclusions: Together, these findings demonstrate altered interaction of β-catenin and CHOP in a protein complex during the course of diabetes in renal tubules. An alternate role for CHOP action and a novel molecular mechanism of ER stress response signaling in tubule cell pathogenesis during diabetes are suggested by these findings.

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SA-PO2592

The Study of Inhibitory Effects by Aprotinin on the Mannan-Binding Lectin Pathway Activation in the Kidney of Diabetic Rats Xiaoyong Yan,[1] Songmin Huang,[1] Yuxi Yang.[1] *[1]Department of Nephrology, The West China Hospital of Sichuan University.*

Background: Our previous work has proved that the levels of Mannan-binding lectin (MBL-A and MBL-C),C3 and Membrane-attack complex(MAC) expression were increased in the kidney of diabetic Rats.The lectin pathway may contribute to the development of early stage DN.It can be beneficial for kidney protection of a therapy of MBL complement pathway inhibitors.Aprotinin can inhibit the lectin pathway through its ability to inactivate irreversibly the MBL-associated serine protease (MASP).In the present studies,we would evaluate the inhibitory effects of aprotinin on the lectin pathway activation in the kidney of diabetic Rats.

Background: SD male rats were randomly divided into three groups: normal control group(group N),DN group (group DN) and DN treated with aprotinin(group DN-A).Rats of the group DN were treated by streptozotocin(50mg/kg).After modeling,Rats of the group DN-A were injected intraperitoneally with aprotinin(40000 KIU/kg.d,Bayer,German) for four weeks (3-week to 7-week).The 6 rats of the three groups were sacrificed at week 4 and 8 respectively,meanwhile blood sugar, serum creatinine(Scr),body weight, kidney weight and 24-hours urinary protein were monitored.The expression of MBL-A,MBL-C,C3 and MAC in renal tissue were detected by immunohisochemistry and western blot.

Results: In the group DN-A,a significant reduction of urinary protein,body weight /kidney weight(KI),glomerular tuft volume(GV),mesangial matrix expansion index (MMEI) and plasma glucose levels(not to normal levels) were observed as compared with group DN at 4-week and 8-week.However the levels of Scr was not markedly increased. In group DN-A, the renal expression of MBL-A,MBL-C,C3 and MAC were obviously decreased as compared with group DN at 4-week and 8-week by immunohisochemistry and western blot.

Conclusions: The present studies suggest that a beneficial effect of aprotinin treatment on the process of glomerular damage in the diabetic Rats.Its effects may attribute to inhibition of the lectin pathway activation.

Funding: Government Support - Non-U.S.

SA-PO2593

Urinary Biomarkers Are Increased in Experimental Diabetes Franklin E. Fuenmayor-Cardozo, Ganesan Ramesh, David M. Pollock, John White. *Medicine, Georgia Health Sciences University, Augusta, GA.*

Background: Recent studies suggest an increased role of tubulointerstial injury in diabetic kidney disease. However, there are no validated biomarkers of tubulointerstitial injury in diabetes. We hypothesized that tubular injury biomarkers useful for studying acute kidney injury would be elevated early in experimental diabetes.

Methods: SD rats were made diabetic using i.v. streptozotocin (STZ) (n = 7). Controls (CTL) received normal saline (n = 9). Rats were placed in metabolic cages at baseline, 4 weeks, and 10 weeks. Urinary kidney injury molecule-1 (KIM-1) and N-acetyl-b-D-glucosaminidase (NAG) were measured by ELISA. For a positive control known to develop tubulointerstitial injury, we used the DOCA salt model. SD rats underwent uninephrectomy and were implanted with a 200 mg time-released DOCA pellet and given normal saline as drinking water (n = 7) x 4 weeks.

Results: As expected, blood glucoses were increased in STZ rats (485 ± 22 mg/dL, $P < 0.05$ vs. CTL, DOCA) and blood pressures were markedly increased in DOCA rats (190 ± 4 mmHg, $P < 0.05$ vs CTL, STZ). Urinary protein excretion was not different between STZ and CTL at 4 wks, but was increased in DOCA rats (29 ± 7.6 vs. 25.1 ± 7.7 vs. 315.3 ± 51.8 mg/24h, $P < 0.05$) suggesting worse damage in the DOCA group. By 10 weeks, urinary protein had increased in STZ vs. CTL (77.5 ± 13.4 vs. 32.3 ± 6.4 mg/24h, $P < 0.05$). At 4 wks, urinary KIM-1 excretion was increased in DOCA rats, but not in STZ compared to CTL (90.6 ± 8.8 vs. 15.8 ± 2.3 vs. 14.2 ± 8.1 ng/24h, $P < 0.05$). At 10 wks KIM-1

excretion was increased in STZ (17.9 ± 1.7 vs. 12.9 ± 0.3 ng/24h, $P < 0.05$). Conversely, NAG excretion was increased in STZ and DOCA compared to CTL at 4 wks (3167 ± 473 and 2298 ± 210 vs. 337 ± 25 IU/24h, $P < 0.05$). Likewise, NAG excretion was increased at 10 wks in STZ vs. CTL (4042 ± 353 vs. 366 ± 27 IU/24h, $P < 0.05$).

Conclusions: Urinary tubular biomarkers are increased early in experimental diabetes. In the case of NAG, the increase occurs prior to the development of significant proteinuria. Our data suggests these biomarkers may be useful surrogates for therapies targeting tubulointerstitial injury in diabetes and chronic kidney diseases.

Funding: Clinical Revenue Support

SA-PO2594

N-Acetylcysteine and Oxidative Stress in the Kidney of Uninephrectomized Rats with Diabetes Mellitus Elisa M.S Higa,[1,2] Guilherme Baia Nogueira,[1] Adelson Marçal Rodrigues,[1] Giovana Rita Punaro,[1] Margaret Gori Mouro,[1,2] Fabiane Maciel.[1] *[1]Medicine - Nephrology Division, UNIFESP; [2]Medicine - Emergency Division, UNIFESP, Sao Paulo, Brazil.*

Background: Diabetes mellitus (DM) induces intra and extracellular changes, with a substantial increase in reactive oxygen species (ROS). ROS cause damage in systemic and renal microvasculature, which could be one of the mechanisms involved in the pathophysiology of diabetic nephropathy. ROS also modulate other substances, like the nitric oxide (NO), a powerful vasodilator with important role on kidney function. N-acetylcysteine (NAC) is an antioxidant largely used to prevent contrast induced renal lesion.

The aim of this study was to evaluate the effect of NAC and oxidative stress in the kidney of uninephrectomized rats with DM.

Methods: Adult male Wistar rats were unilaterally nephrectomized (UNx). Control groups (CTL) were sham operated. DM was induced with streptozotocin (60mg/kg, iv), in half of UNx animals (DM+UNx) and the others received its vehicle (CTL+UNx). Half of the CTL+UNx and DM+UNx animals received NAC (600mg/L in water, ad libitum). Groups (N=4 each): CTL, CTL+UNx, CTL+UNx+NAC, DM+UNx and DM+UNx+NAC. Before and after 8 weeks with NAC, we collected the 24 hour urine and a blood sample. Data = mean±SEM, analyzed by one-way ANOVA, with Tukey post-test; significant for P<0.05.

Results: DM+UNx compared with CTL+UNx showed increased levels of glycemia (427±31 vs 189±25) and altered renal function, with increased plasmatic creatinine (2.0±.1 vs 1.2±.1), urea (71±5 vs 38±8) and proteinuria (40±10 vs 15±1). DM+UNx presented increased TBARS (8.9±1.3 vs 3.2±.1 and 517±44 vs 95±6) and reduced NO (55±9 vs 62±4 and 7.5±.2 vs 10.5±1.6), both in plasma and urine respectively. NAC in DM rats reduced proteinuria (16±4), plasmatic creatinine (1.5±.1) and urea (60.7±2.3) and attenuated TBARS's levels, in plasma and urine (3±.4 and 352±27), as well as increased plasma NO (82.6±9.2), all P<0.05.

Conclusions: In DM+UNx rats, NAC protected against kidney injury, probably due to the control of oxidative stress and/or increase of NO bioavailability, suggesting that NAC could be useful in the treatment of diabetic patients.

Funding: Government Support - Non-U.S.

SA-PO2595

The Role of Chymase in the Renal Lesion of the Diabetic Rats Mei Zhang, Jing Bai, Xiaodong Nie, Wen Huang. *Nephrology, Beijing Tongren Hospital, Capital Medical University, Beijing, China.*

Background: Diabetic nephropathy is a leading cause of end-stage renal disease, which characterized by renal fibrosis.The objective of the study is to investigate the role of chymase in the development of renal fibrosis of diabetic rats

Methods: 24 male SD rats were randomly divided into three groups including control , DM and DM+ Chy-Inhibitor (Chy-I)groups . Diabetes was induced by intraperitoneal injection of Streptozotocin (60mg/kg).The rats in the Chy-I group were injected with chymase inhibitor $(Oph)_2$ 1mg/kg/d for 12 weeks.Kidney weight, level of blood glucose, 24-hour urinary protein, creatinine clearance, serum lipid level, and blood pressure were measured. The renal pathologic lesion were observed by light microscope and electron microscrope, the expression of fibronectin, type IV Collagen, α-SMA and TGF-β1 were observed by immunohistochemistry and RT-PCR

Results: Diabetic rats were presented with characteristics of increasing creatinine clearance, KW/BW, level of serum cholesterol, 24-hour urinary albumin and urinary albumin /creatinin (P<0.05, P<0.01, P<0.01, P<0.05 and P<0.01), and decreasing level of serum albumin and total protein (P<0.05) respectively. Compared with DM group, level of serum cholesterol and urinary albumin /creatinin were decreased for15.58%and 75.40% in Chy-I group(P<0.05). There were no significant difference with DM group and Chy-I group in KW/BW, 24-hour urinary albumin, blood pressure, level of serum lipid, albumin and protein and creatinine clearance.

Diabetic rats had increasing deposition of extracellular matrix in glomerular area followed by elevated expression of FN, ColIV, alpha-smooth muscle actin (α-SMA) and TGF-β1. Compared with model group, the admission of chymase inhibitor could decrease expression of FN, ColIV , alpha-smooth muscle actin (α-SMA) and TGF-β1 for 25.35% ,17.24%, 26.30% and 19.98%.(p<0.05,). The lesion of foot processes of podocytes was ameliorated in Chy-I group

Conclusions: In diabetic rats, chymase promoted the secretion of urinary protein, the injury of foot processes of podocyte and deposition of extracellular matrix. Inhibition of chymase could preserve the progression of renal lesion.

SA-PO2596

Involvement of Adiponectin and Adiponectin Receptor 1 in the Albuminuria Appearance of Diabetic Nephropathy Koichi Kanozawa, Juko Asakura, Hajime Hasegawa, Taisuke Shimizu, Kaori Takayanagi, Takatsugu Iwashita, Yosuke Tayama, Tokushi Nakajima, Tetsuya Mitarai. *Nephrology and Hypertension, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan.*

Background: We reported that a decline of microalbuminuria(MA) was related to increase of adiponectin(APN) by administrating various kinds of PPARγ agonists for human diabetic nephropathy(DN). Recently, it has been reported that APN-knockout mice exhibited increased albuminuria and fusion of podocyte foot processes. On the other hand, among ligands of APN, it is revealed that APN receptor(AdipoR) 1 and T cadherin(TCd) express in the kidney. The aim of this study is to clarify participation in the appearance of MA and the formation of DN with the APN/AdiopR/TCd system.

Methods: The male Otsuka Long Evans Tokushima Fatty(OLETF) rats which were known as type 2 diabetes, and Long Evans Tokushima Otsuka(LETO) rats which were control, were used. These rats were derived into three groups, such as the OLETF received low-dose (2.5 mg/kg) pioglitazone for six weeks (PGZ-OLT), the OLETF not received PGZ (OLT), and LETO (LET). To 34 weeks of age, APN, creatinine clearance, and urinary albumin excretion rate(UAE) were measured, and the kidneys were extracted. We examined local existence of the APN, AdiopR1, and TCd in the kidney tissue of each group, using indirect immunofluorescent method.

Results: APN in 34 weeks decreased to 1.3±0.2 μg/ml in the OLT, and rose with 2.6±0.3 μg/ml in the PGZ-OLT, compared with 1.8±0.1 μg/ml in the LET. On the other hand, UAE rose significantly with 3720.5±1394.3 mg/mgCr in OLT whereas it was 8.8±5.1mg/mgCr in LET, and was controlled in 2221.7±1155.4 mg/mgCr in the PGZ-OLT. In the kidney histologic examination, APN accorded for local existence of AdiopR1 in the glomerulus, it was dyed by podocytes, mesangial cells, and endothelial cells, but APN did not accord with the local existence of TCd. Interestingly, the stainability of APN aggravated it in OLT in comparison with the LET, and improved it in PGZ-OLT, and these ware the relations that were reverse to serum APN each.

Conclusions: It was suggested that APN/AdiopR1 system in the glomerulus involved in an appearance of the MA in DN.

SA-PO2597

Involvement of Renal Cytochromes P450 and Arachidonic Acid Metabolites in Diabetic Nephropathy Assaad Antoine Eid,[1,4] Rita Maalouf,[3,4] Linda Roman,[5] Fuad N. Ziyadeh.[2] *[1]Anatomy, Cell Biology and Physiology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon; [2]Internal Medicine, Faculty of Medicine, American University of Beirut, Beirut, Lebanon; [3]Chemistry, Faculty of Sciences, Lebanese University, Beirut, Lebanon; [4]Medicine, University of Texas Health Science Center at San Antonio, TX; [5]Biochemistry, University of Texas Health Science Center at San Antonio, TX.*

Background: Diabetic nephropathy is characterized by early hyperfiltration, hypertrophy, extracellular matrix accumulation, and later by proteinuria, fibrosis and loss of renal function. In hypertrophy, tubules increase in cell size and accumulate extracellular matrix and are associated with alterations in renal sodium handling as well as hypertension; both are processes linked by involvement of the arachidonic acid (AA) metabolites 20-HETE and EETs. It is known that AA is metabolized by several cytochrome 450 (CYP) isoforms to produce 20-HETE and EETs.

Methods: The levels of CYPs 2B, 2C, and 4F were assessed in a rat model of type1 diabetes.

Results: There was significant induction of expression and activity of these CYPs in the cortex of the diabetic animals, and this was greatly prevented by insulin therapy. Immunoblot analysis of microsomes derived from cultured rat proximal tubular cells, grown in normal or high glucose (HG), confirmed this observation. As a functional measure of cellular hypertrophy, the incorporation of S^{35}-methionine into a primary culture of rat renal cortical cells was monitored. Growing cells in HG resulted in methionine incorporation increase. When cultured in HG and in the presence of 0016HETE, a specific inhibitor of 20-HETE production, methionine incorporation is decreased to control levels. Thus, inhibition of CYPs in these renal cells attenuates the cellular hypertrophy caused by HG.

Conclusions: Our results indicate that hyperglycemia in diabetes has a significant effect on the expression level of AA-metabolizing CYPs, which is manifest as increased AA metabolism, and might thus be expected to alter kidney function through alteration of type and amount of AA metabolites produced.

SA-PO2598

Sphingosine Kinase 1 (SK1) Inhibition Plays a Role in TGFbeta-Induced Epithelial-Mesenchymal Transition (EMT) and Inflammation In-Vitro and In-Vivo Model of Diabetic Nephropathy Dania Yaghobian,[1] Sonia Saad,[1] Xinming Chen,[1] Anthony Don,[2] Carol A. Pollock.[1] *[1]Medicine, Kolling Institute, Sydney, NSW, Australia; [2]Medicine, UNSW, Sydney, NSW, Australia.*

Background: Sphingosine 1- phosphate (S1P), a pleiotropic lipid mediator, is produced by phosphrylation of sphingosine by SK1 and SK2. EMT is a major contributor to the development of renal fibrosis as about 35% active fibroblasts are derived from EMT in kidney disease, contributing to the production of extracellular matrix (ECM), cytokines,

chemokines and growth factors, resulting in enhanced inflammatory and fibrotic responses. We aim to determine the role of SK1 on TGFβ induced EMT, inflammation and fibrosis an in-vitro and in-vivo model of diabetic nephropathy

Methods: HK2 cells were incubated with SKI-II (2μM) an inhibitor of SKs for 3 hours then exposed to TGFβ-1, -2 or-3 isoforms (2ng/mL) for 72 hours. Morphological changes of cells were monitored, mRNA and protein expression of SK1, E-cadherin, TNFα and MCP-1 were determined by PCR, western blot and ELISA. C57BL/6 wild-type and SK1$^{-/-}$ knockout mice were rendered diabetic with Streptozotocin for 16 weeks. Body weight, blood pressure, blood glucose, creatinine clearance and albuminuria were recorded. Levels of SK1, SK2, S1P, ceramide as well as MCP-1, E-cadherine and FN were determined in the kidney using RT-PCR and western blotting. Serum changes in SK1, SK2, S1P and ceramide is determined using mass spectroscopy.

Results: TGFβ-1, -2 and -3 isoforms equally induced phenotypic changes of EMT in HK2 cells. This was reversed with SKI-II. SK1 mRNA was significantly increased by exposure to TGFβ-1, -2 and -3 isoforms (p=<0.01). This was also reversed to basal levels with SKI-II. E-cadherin mRNA expression was suppressed by TGFβ-1, -2 and -3 compared to control (p<0.05) and partially reversed by SKI-II (P<0.05). mRNA and protein expression of MCP-1 was significantly induced by TGFβ-1, -2 and -3 and TNFα (p<0.01) and reduced to almost undetectable levels in the presence of SKI-II. In-vivo work is currently in progress and will be reported.

Conclusions: SK1 plays an important role in TGFβ-induced EMT and inflammatory responses in fibrosis and may direct future therapeutic strategies

Funding: Government Support - Non-U.S.

SA-PO2599

Early Microvascular Damage in Pigs with Diabetic Nephropathy Coincides with Angiopoetin Dysbalance in the Kidney Meriem Khairoun,[1] Mieke Vd Heuvel,[3] Bernard Van Den Berg,[1] Oana Sorop,[3] Rients De Boer,[1] Ingeborg M. Bajema,[4] Ton J. Rabelink,[1,2] Marlies E.J. Reinders,[1] Wim J. Van der Giessen,[3] Joris I. Rotmans.[1,2] *[1]Department of Nephrology, LUMC; [2]Einthoven Laboratory for Experimental Vascular Research, LUMC; [3]Experimental Cardiology, EMC Rotterdam; [4]Department of Pathology, LUMC.*

Background: Diabetes mellitus (DM) is associated with a range of microvascular complications including diabetic nephropathy (DN). Microvascular abnormalities in the kidneys are common histopathologic findings in DN. These abnormalities may represent one manifestation of ongoing systemic microvascular damage. Recently, Sidestream dark-field (SDF) imaging has emerged as a noninvasive tool to visualize the microcirculation. In this study, we investigated the early effects of diabetes on renal and systemic microvasculature in pigs.

Methods: DM was induced by streptozotocin (80 mg/kg body weight). Mean capillary density (MCD) and microvascular morphology were visualized using SDF imaging and compared among pigs with DN on atherogenic diet (N=8), nondiabetic pigs (AT) on atherogenic diet (N=7) and control pigs (N=7). Both AT and DN pigs were on a restricted atherogenic diet for 14 months. Kidneys were harvested and sections were stained with markers for microvascular integrity, Angiopoietin-1 (Ang-1), and Angiopoietin-2 (Ang-2).

Results: Kidney biopsies showed marked glomerular lesions consisting of mesangial expansion and podocyte lesions as well as tubular changes with foamy cytoplasm and hyalin droplets, which coincided with hyperfiltration. Analysis of capillaroscopic images revealed more capillary tortuosity in DN pigs (mean 2.31 ± 0.17 SEM compared to the control groups (0.89 ± 0.08 and AT 1.55± 0.11, p<0.05). Immunohistochemistry of kidney biopsies showed a disturbed Ang-2/ Ang-1 balance, with increased expression of Ang-2 in DN pigs as compared to controls.

Conclusions: Diabetic pigs showed rapid development of diabetic nephropathy, which is associated with an increase in capillary tortuosity and a dysbalance in angiopoietins. Thus, systemic microvascular damage induced by diabetes might be an important contributor to local perturbations in vital organs such as the kidney.

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SA-PO2600

FGF-23 and Vitamin D Levels: New Risk Factors of Left Ventricular Hypertrophy in Diabetic Patients Ana Paula Silva, Ana Pinho, Ana Cabrita, André Fragoso, Nelson Almeida Tavares, Nélio Santos, Marilia Faisca, Pedro Neves. *Serviço de Nefrologia, Hospital Faro, Faro, Portugal.*

Background: Left ventricular hypertrophy is a well known independent risk factor of morbidity and mortality in both the general population and in those with chronic kidney disease (CKD).

Several experimental studies showed that vitamin D and FGF23 play a role in normal physiology of cardiomyocytes and vasculature. Furthermore, low vitamin D and higher FGF 323 levels are associated with several CVD outcomes such as myocardial infarction, congestive heart failure, and cardiovascular mortality.The aim of this study was to evaluate in a group of type 2 diabetic CKD (stages 3 and 4) patients the risk factors of left ventricular hypertrophy (LVH).

Methods: We evaluated 50 type 2 diabetic patients (f = 18, m = 32), with a mean age of 60.35 years and a mean estimated GFR (MDRD) of 50 ml / min.

Exclusion criteria were uncontrolled hypertension, prior known cardiovascular disease, neoplasic or infectious disease, anti-inflammatory, phosphorus binders and vitamin D therapies. We analyzed several biological and laboratorial parameters, including those related with inflammation and the mineral metabolism. The left ventricular mass index (LVMI) was calculated using the Penn convention criteria.

Results: In a multiple regression model, we found that Interleukin-6 (r=0,265 p=0,004), FGF23 (r=0,230 p=0,004), PTH (r=0,361 p=0,016) and t25(OH) D (r= - 0,263 p=0,005) levels independently influenced the LVMI.

Conclusions: In a small but very homogeneous group of type 2 CKD diabetic patients, we found that inflammation and the mineral metabolism disturbances are related with left ventricular hypertrophy. Further studies are needed to ascertain if the correction of these parameters (including vitamin D and FGF-23) are associated with better outcomes of our kidney patients.

Funding: NIDDK Support

SA-PO2601

Identification of Novel Glomerular Molecules Implicated in Albuminuria David A. Long,[1] Karen Price,[1] Maria Kolatsi-Joannou,[1] Cecile Dessapt-Baradez,[2] Jennifer L. Huang,[1] Jenny Papakrivopoulou,[1] Ron Korstanje,[3] Luigi Gnudi,[2] Adrian S. Woolf.[4] *[1]Nephro-Urology Unit, UCL Institute of Child Health, United Kingdom; [2]King's College London, United Kingdom; [3]The Jackson Laboratory; [4]University of Manchester, United Kingdom.*

Background: Albuminuria is an early warning sign for several chronic glomerular diseases. In diabetes mellitus, small increases of albumin excretion precede, and may predict progression to, overt nephropathy. Therefore, the discovery of molecules deregulated in "leaky" glomeruli may suggest novel biomarkers and therapeutic targets in early kidney disease.

Methods: To identify such genes, we utilised male and female mice of two widely-used wild-type (i.e. "normal") strains of laboratory mice, C57Bl/6 and FVB. We isolated whole glomerular tufts using Dynabeads in 18 week old animals and performed RNA microarray analyses to examine all glomerular transcripts. These results were compared to studies pinpointing genetic loci associated with albuminuria in mouse strains.

Results: Overtly healthy animals at 13 and 18 weeks of age demonstrated increasing albumin excretion as follows: C57Bl/6 females < C57Bl/6 males < FVB females < FVB males providing a unique model to track down albuminuria genes. Thirty nine genes in isolated glomeruli were significantly altered by both strain and gender, with expression levels following the degree of albuminuria (34 genes upregulated and five downregulated with increasing albuminuria). Comparing this expression data with genetic loci associated with albuminuria in mice implicated *Cyp4a12a* (a member of the cytochrome P450 family), *Hsd3b2* (essential for steroid production) and *Acy3* (aspartoacylase-3) as strong candidates for further study. We found that all three genes were expressed by isolated mouse podocytes induced to differentiate in culture.

Conclusions: Deregulated glomerular expression of *Cyp4a12a*, *Hsd3b2* and *Acy3* is associated with albuminuria between genders and different strains of mice. Further studies are required to unravel the precise glomerular roles of these genes, and we suggest that polymorphisms of these genes may determine the susceptibility of an individual to progressive renal disease.

SA-PO2602

Heart Rate Variability Changes Induced by Reduction of Fluid Overload Manuela Ferrario,[1] Ulrich Moissl,[2] Francesco Garzotto,[3] Maria Gabriella Signorini,[1] Dinna N. Cruz,[3] Alessandra Brendolan,[3] Anna Clementi,[3] Matteo Floris,[3] Ciro Tetta,[2] Sergio Cerutti,[1] Claudio Ronco.[3] *[1]Politecnico di Milano, Milano, Italy; [2]Fresenius Medical Care, Bad Homburg, Germany; [3]San Bortolo Hospital, Vicenza, Italy.*

Background: Fluid overload (FO) is an important and independent predictor of mortality in chronic hemodialysis (HD) patients (pts). We showed previously differences in heart rate variability (HRV), a measure of the autonomic nervous system (ANS), among fluid overloaded and normohydrated HD pts. The aim of this pilot study was to investigate whether reduction of FO can modify HRV in HD patients.

Methods: We studied 5chronic HD patients with a pre-HD FO consistently >3 L. FO was estimated before every HD using a spectroscopy device (BCM, Fresenius Medical Care, Germany). Fluid removal during HD was adjusted during every HD to obtain a target average FO (average of pre- & post-HD FO) of 0.8L within 1 month. ECG Holter recordings were performed (starting before the HD session) at baseline (BL) and after 1month (1M). HRV was analyzed with parameters, such as mean HR (bpm), pNN50%, i.e. the percentage of adjacent NN intervals differing by more than 50ms. Spectral analysis was performed; we computed power in the low (LF), high frequency bands (HF), LF/HF ratio, LF% and HF% . LF relates to sympathetic activity, and HF to parasympathetic ANS activity and were calculated for different HD period.

Results: Pre-HD FO was successfully reduced within 1 month (BL: 3.45±0.99 L; 1M: 2.48±0.48L). In 4/5 pts, we observed a decrease of pNN50%, LF and LF%, indicating a decrease in sympathetic overactivity.

Table 1- Median values (interquantile ranges) of HRV indices

		HD	first30'HD	last30'HD	1hr after HD
pNN50%	BL	10.7(6.9,26.1)	12.7(5.8,25.0)	8.5(2.5,27.7)	5.7(3.4,12.3)
	1M	2.7(1.4,20.5)	16.6(2.2,19.4)	0.8(0.7,24.9)	0.9(0.8, 8.6)
LF(ms^2)	BL	955(115,1510)	931(267,1513)	1026(534,2276)	306(162,462)
	1M	271(207,743)	235(134,710)	250(157,922)	157(102,318)
LF%	BL	51(22,59)	53(31,68)	57(49,68)	27(18,36)
	1M	44(39,49)	40(31,48)	41(36,52)	27(14,31)
HF%	BL	8(1,21)	10(3,27)	15(9,32)	14(8,27)
	1M	17(7,27)	18(6,31)	18(16,27)	12(4,26)
LF/HF	BL	2(1,17)	3(2,36)	4(2,16)	2(1, 2)
	1M	3(2,7)	2(1,5)	3(1,4)	1(1,2)

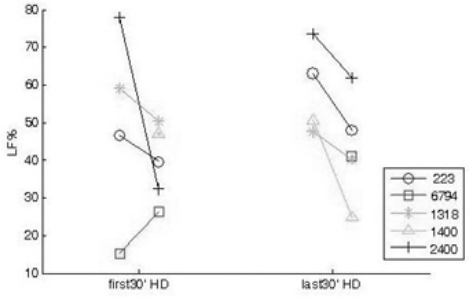


Figure 1: LF% values for each patients enrolled in the study

Conclusions: In this pilot study, we demonstrate for the first time that reduction of FO has an effect on sympathetic ANS activity and HRV. Further studies in a larger population are needed to verify this finding and to determine whether these changes persist in the long-term.

SA-PO2603

Relationship between Visceral Fat Area, Percentage of Body Fat and Prevalence of Cardiovascular Disease in Chronic Dialysis Patients Yukie Kitajima,[1] Yuzuru Sato.[2] *[1]Tokyo Healthcare University, Tokyo, Japan; [2]Sato Junkanki Hospital, Matsuyama, Ehime, Japan.*

Background: The risk of cardiovascular disease is substantially high in hemodialysis patients. The risk factors for cardiovascular disease in hemodialysis patients include age, duration of hemodialysis, diabetes mellitus and hyperphosphatemia. However it is not clear cardiovascular disease is associated with visceral fat area and percentage of body fat in hemodialysis patients. We investigated the relationship among visceral fat area, percentage of body fat, several clinical and biochemical parameters and anamnesis of cardiovascular complication in chronic hemodialysis patients.

Methods: Area of visceral fat was measured using computed tomographic scanning (CT) in 84 non-diabetic patients. Using a body composition analyzer, we measured total body fat,and percentage of body fat after completion of dialysis on the last day of the week. Patients were divided into two groups according to visceral fat area; those with visceral fat area < $100cm^2$ (Group A) and with > $100cm^2$ (Group B). Blood chemistry of lipid and complication of cardiovascular disease were evaluated in these patients. Body mass index (BMI) was calculated with body weight and height.

Results: Compared to Group A, Group B showed, significantly higher serum levels of triglyceride ($p<0.001$) and significantly lower, high-density lipoprotein-cholesterol levels ($p<0.001$). Group B also showed higher rate of cardiovascular complication, especially ischemic heart disease ($p<0.01$). Visceral fat area correlated with BMI ($R^2=0.5233$), total body fat ($R^2=0.544$), percentage of body fat ($R^2=0.283$). BMI correlate with total body fat ($R^2=0.792$), percentage of body fat ($R^2=0.491$). Furthermore, Patients with ischemic heart disease showed higher percentage of body fat ($p<0.05$).

Conclusions: In this study, higher visceral fat area was found to have the high rate of cardiovascular disease. Higher visceral fat area can be a contributing risk factor to cardiovascular disease, especially ischemic heart disease in chronic dialysis patients. In addition, BMI and percentage of body fat can be measured more easily than CT. Those assesments will evade the risk of cardiovascular disease.

SA-PO2604

Osteoprotegerin as a Prognostic Marker of Mortality in Hemodialysis Patients with Cardiovascular Disease Simon Winther,[1] Kaj A. Jørgensen,[1] Erik Berg Schmidt,[2] My Svensson.[1] *[1]Department of Nephrology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Cardiology, Aalborg Hospital, Aalborg, Denmark.*

Background: Patients with end-stage renal disease, treated with hemodialysis (HD) have an increased mortality, mainly caused by cardiovascular disease (CVD). Osteoprotegerin (OPG) is a glycoprotein involved in the regulation of the vascular calcification proces. Previous studies have demonstrated that OPG is incorporated in atherosclerotic plaques and that elevated OPG in plasma is related both to severity and progression of vascular calcification. Furthermore, elevated OPG has been shown as a prognostic marker of mortality, in several high-risk populations. The aim of this study was to investigate if OPG was a prognostic marker of mortality in patients with end-stage renal disease and previously documented CVD.

Methods: We prospectively followed 206 HD patients with documented CVD. CVD was defined as previously documented myocardial infarction, angina pectoris, angiographically documented coronary arteriosclerosis, stroke, transient ischemic attack, or peripheral vascular disease. Plasma levels of OPG were measured at baseline and the patients were followed for 2 years or until reaching the primary endpoint, all-cause mortality.

Results: All-cause mortality during follow-up was 44% (90/206) and median follow-up to the primary endpoint was 314 days. Levels of OPG were divided into tertiles (first: 1.35-4.12, second: 4.12-6.04, third: 6.04-31.32 pg/ml). High OPG levels were associated with increased mortality, using the first tertile as reference, with a HR of 1.83 (CI 1.05-3.20) for the second tertile and HR of 1.72 (CI 0.95-3.09) for the third tertile. In survival analysis this was not statistically significant with an adjusted p-value of 0.08 and an adjusted p-value for trend of 0.07. In multivariate Cox-regression analysis only age and OPG in the second tertile were associated with increased mortality.

Conclusions: In contrast to previous studies, we were not able to demonstrate that high levels of OPG, in a cohort of HD patients with documented CVD, were associated with an increased mortality.

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-PO2605

Effect of Body Composition Moniter-Guided Volume Control on Inflammation and Adipokines in Hemodialysis Patients: A Prospective 16-Week Interventional Study Sejoong Kim,[1] Hayne C. Park,[2] Hajeong Lee,[2] Ho Jun Chin,[2] Dong Ki Kim,[2] Yon Su Kim,[2] Jin Suk Han,[2] Kwon Wook Joo.[2] *[1]Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea; [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

Background: Inappropriate volume control can be linked to an increased cardiovascular morbidity in hemodialysis (HD) patients, although there is no accurate method to moniter patients' water contents. Also, inflammation and adipokines are related to cardiovascular outcome in HD patients. We evaluated the effect of body composition moniter (BCM)-guided volume control on inflammation and adipokines in hemodialysis patients.

Methods: We enrolled 120 patients who received more than 3-month hemodialysis in major 3 dialysis centers. According to the amount of fluid overload, which was provided by BCM (Fresenius Medical Care Korea), we divided into 3 groups: overhydrated group (OH; fluid overload ≥ 1.1L), normohydrated group (NH; -1.1L ≤ fluid overload < 1.1L), and dehydrated group (DH; fluid overload < -1.1L) and optimized body weight towards an objective target for normohydration for 16 weeks.

Results: The proportion of OH group was 36.6% (44/120), and that of DH group was 15% (18/120). Intervention failure rate was 16% (10/62). In OH group, serum levels of IL6 and MCP1 were significantly decreased after 8-week intervention period (IL6: 1.17 ± 1.34 at week 0 vs. 0.06 ± 1.18 log [pg/mL] at week 8, $p<0.001$; MCP1: 5.66 ± 0.77 at week 0 vs. 5.33 ± 0.39 log [pg/mL] at week 8, $p=0.019$). After 8-week intervention period, serum leptin levels were decreased, while serum adiponectin levels were increased in OH group. Those changes were persistent until another 8-week observation. In DH group, serum adiponectin levels were significantly increased every 8 weeks.

Conclusions: This results showed that inflammatory markers and adipokines improved after the correction of patients' overhydrated status and adiponectin levels increased after the optimization of patients' dehydrated status in hemodialysis patients.

Funding: Pharmaceutical Company Support

SA-PO2606

Vascular Calcification Inhibitors Are Promising Markers of Subclinical Cardiovascular Disease in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in ESRD (PACE) Study Stephen M. Sozio,[1] Rulan S. Parekh,[1,2] Julia J. Scialla,[1] Tariq Shafi,[1] Bernard G. Jaar,[1] Miguel Santaularia-Tomas,[1] Lucy A. Meoni,[1] Wen Hong Linda Kao.[1] *[1]Johns Hopkins University; [2]University of Toronto.*

Background: Vascular calcification is common in ESRD and likely contributes to clinical cardiovascular disease (CVD) events. The effect of circulating regulators of this dynamic process on subclinical CVD is unknown.

Methods: We investigated the association of the calcification inhibitors osteopontin (OPN), osteoprotegerin (OPG), bone morphogenetic protein-7 (BMP-7), and fetuin-A with simultaneously measured coronary artery calcium score (CAC), pulse pressure (PP), carotid-femoral pulse wave velocity (PWV), aortic augmentation index (AIX), and ankle-brachial index (ABI) in an incident hemodialysis cohort of 82 PACE participants. Laboratory and clinical tests were performed on a non-dialysis day at the baseline visit. Linear regression was used to assess the association of each inhibitor and subclinical CVD process.

Results: Mean age was 53 with 59% male, 67% African-American, 54% diabetic, and 50% with prior ASCVD. Mean±SD level of each calcification inhibitor was: OPN 313±113 ng/ml, OPG 9.7±3.4 pmol/l, BMP-7 9.3±2.8 pg/ml, fetuin-A 0.49±0.13 g/L. OPN, OPG, and fetuin-A were associated with several of the CVD processes.

	Circulating Vascular Calcification Inhibitor*			
Subclinical CVD	**OPN**	**OPG**	**BMP-7**	**Fetuin-A**
Cardiac Disease				
CAC§	**0.77 (0.07, 1.48)†**	**0.99 (0.22, 1.76)†**	-0.46 (-1.28, 0.36)	**-1.00 (-1.71, -0.29)†**
Arterial Stiffness				
PP (mmHg)	1.56 (-2.65, 5.77)	**6.50 (2.47, 10.5)†**	-0.14 (-4.28, 4.01)	-0.67 (-4.77, 3.42)
PWV (m/s)	0.18 (-0.69, 1.05)	**1.40 (0.54, 2.27)†**	-0.38 (-1.24, 0.48)	0.07 (-0.76, 0.89)
AIX (%)	**5.86 (2.26, 9.45)†**	**4.46 (0.70, 8.21)†**	2.84 (-0.54, 6.22)	1.90 (-1.87, 5.67)
Peripheral Vascular Disease				
ABI	0.00 (-0.08, 0.07)	-0.06 (-0.32, 0.02)	0.03 (-0.07, 0.13)	0.02 (-0.05, 0.10)

*Coefficient (95% CI) from unadjusted linear regression per SD increase in each calcification marker §per log(CAC+1) †p<0.05

Conclusions: OPN and OPG are promising markers of subclinical CVD. If validated prospectively, individual vascular calcification marker profiles have the potential to identify patients at highest risk of potential CVD outcomes.

Funding: NIDDK Support, Private Foundation Support

SA-PO2607

Coronary Artery Calcifications and Cardiovascular Mortality in Hemodialysis Patients Roberto Palumbo,[5] Annalisa Noce,[1] Olga Durante,[1] Simone Manca di Villahermosa,[1] Michele Ferrannini,[5] Efstathia Athanasopoulou,[1] Sandro De Angelis,[3] Mariarita Dessì,[2] Pietro Sfregola,[4] Giorgio Splendiani,[3] Nicola Di Daniele.[1] *[1]Department of Internal Medicine, Tor Vergata University, Rome, Italy; [2]Department of Laboratory Medicine, Tor Vergata University, Rome, Italy; [3]Regional Agency for Transplantations and Related Pathologies, Rome, Italy; [4]Nephrology and Dialysis Service, Madonna delle Grazie, Velletri, Rome, Italy; [5]Nephrology and Dialysis Unit, S.Eugenio Hospital, Rome, Italy.*

Background: Coronary artery calcifications and long term overall cardiovascular mortality were investigated in maintenance hemodialysis (mHD) patients.

Methods: Between June and December 2002 two-hundred and five patients with no history of major acute cardiovascular event (MACE), aged 59.85±12.77 years, on mHD since 62.30±55.00 months were enrolled. All patients underwent a single cardiac Multi-layer spiral computed tomography (MSCT). Coronary calcium load was quantified according to the Agatston score (AS). According to AS patients were stratified into groups 1 (AS=0), 2 (AS 1 to 400), 3 (AS 401-1000) and 4 (AS>1000). All patients were followed up between January 2003 and January 2011. Primary endpoint of the study was mortality for MACE. Seven-year actuarial survival was calculated for patients of the four groups separately by Kaplan-Meyer equation. Patients who died for causes other than cardiovascular disease and transplanted patients were censored. The log rank test was employed to compare survival curves.

Results: One-hundred two patients (49.7%) died for cardiovascular disease during the follow up Seven-year actuarial survival was more than 90% for patients of groups 1 and 2, but failed to about 50% in patients of group 3 and to <10% in patients of group 4. Patients with AS>400 thus showed a significantly higher cardiovascular mortality compared to patients of with AS<400 (p<0.0001).

Conclusions: In mHD patients The presence of extended coronary artery calcifications detected with cardiac MSCT may be predictable of an elevated risk of cardiovascular mortality.

SA-PO2608

Correlation of Breast Arterial Calcification with Cardiovascular Disease in End Stage Renal Disease Na'Da Abouhassan, W. Charles O'Neill. *Renal Division, Emory University School of Medicine, Atlanta, GA.*

Background: Vascular calcification is common in end-stage renal disease (ESRD) and correlates with cardiovascular disease. However, since the imaging used to date cannot distinguish between medial and intimal (atherosclerotic) calcification, the clinical significance of medial calcification in ESRD is unknown. We have previously shown that breast arterial calcification (BAC) is exclusively medial and that BAC on mammography is a marker of generalized medial calcification

Methods: We obtained mammograms performed on women with ESRD at this institution. Age, race, cardiovascular disease, presence of diabetes mellitus (DM), and duration of renal replacement therapy were determined by review of the medical records. DM was defined as diabetic medication or diagnosis of DM. Coronary artery disease (CAD) was defined as a history of myocardial infarction, or coronary stenosis >50% on angiography. Peripheral artery disease (PAD) was defined as a history of bypass surgery or amputation, evidence of occlusion on imaging, or history of claudication. BAC was scored as present or absent by visual inspection of mammograms. Univariate analyses were performed using t-tests for continuous variables and the chi-squared test for categorical variables. Multivariate logistic regression analysis was performed with SPSS 17.0 software.

Results: We identified 91 women with ESRD and screening mammograms, all but 3 of whom were undergoing hemodialysis. BAC was detected on 57 mammograms (63 %). The prevalence of PAD in patients with BAC was 36.8 % as compared to 8.5% in patients without BAC (p = 0.003). The prevalence of CAD was 24.6 % in patients with BAC and 22.9% in patients without BAC. In a multivariate logistic model including age, diabetes, and duration of ESRD, only BAC (p = 0.006) and ESRD duration (p=0.031) were independent determinants of BAC.

Conclusions: BAC is strongly and independently correlated with peripheral arterial disease in ESRD. This indicates that medial arterial calcification is a risk factor for peripheral arterial disease and may have a pathophysiologic role.

Funding: Pharmaceutical Company Support

SA-PO2609

Use of Warfarin for Atrial Fibrillation (AF) in Patients on Hemodialysis (HD) Salina Juma,[1] Charmaine E. Lok,[2] Catherine M. Clase,[3] Peter G. Blake,[1] Benjamin Ka Thomson,[1] Louise M. Moist.[1] *[1]University of Western Ontario, London, ON; [2]University Health Network, Toronto, ON; [3]McMaster University, Hamilton, ON.*

Background: The risk/benefit ratio for warfarin use in AF among patients on HD is unclear. The objectives of this study are to: 1) measure Nephrologists' certainty about warfarin use and 2) determine the patient variables influencing warfarin use in AF among Canadian Nephrologists.

Methods: Nephrologists at Victoria Hospital, London reviewed their patients with AF on HD. Using a 7 point scale, they documented their certainty regarding the use or nonuse of warfarin pre and post a 10 min presentation on risk and benefits of warfarin based on current literature. A survey was then developed consisting of 6 cases of patients with AF with a gradient of thromboembolic and bleeding risk and sent to a random sample of Nephrologists selected from the Canadian Society of Nephrology (CSN).

Results: Warfarin was used in 52.5 % of patients with AF. Nephrologists expressed uncertainty regarding both the use and nonuse of warfarin and this did not change with an update of the available literature. The survey response rate was 62.2 % (56/90).

CSN Survey Responses

Case	CHADS2	HD	GI Bleed	Risk for Falls	Very likely to start/ continue warfarin (%)	Very unlikely to start/ continue warfarin (%)	Uncertain (%)
1	2	No	No	No	80.4	3.6	16.1
2	2	Yes	No	No	50	14.3	35.7
3	5	Yes	No	No	76.7	3.6	19.6
4	5	Yes	No	Yes	23.2	28.6	48.2
5	5	Yes	Yes	No	48.2	8.9	42.9
6	5	Yes	Yes	Yes	3.6	67.9	28.6

Cases 1-3: A 65 year old Caucasian male, with untreated sustained AF, HTN and NIDDM. Cases 4-6: A 79 year old Caucasian female, on warfarin therapy, with sustained AF, previous stroke, HTN, NIDDM, PVD. She is on chronic HD secondary to glomerulonephritis.

The presence of renal failure and HD, previous GI bleed, and risk for falls all increased uncertainty about warfarin use. 73.2% of nephrologists agreed to be at clinical equipoise and 83.9% would enroll their patients with AF on HD in a RCT on oral anticoagulation.

Conclusions: Nephrologists have significant uncertainty about the use and non use of warfarin for AF among patients on HD. A RCT on the use of oral anticoagulation in patients with AF on HD is needed.

SA-PO2610

Left Ventricular Diastolic Dysfunction in Dialysis Patients Assessed by Novel Speckle Tracking Strain Rate Analysis: Prevalence and Determinants Mihaly K. De Bie,[1] Nina Ajmone Marsan,[1] Arien Gaasbeek,[1] Victoria Delgado,[1] Bas A. Gabreels,[3] Jan H. Groeneveld,[2] Jeroen J. Bax,[1] Martin J. Schalij,[1] Ton J. Rabelink,[1] J. Wouter Jukema.[1] *[1]Leiden University Medical Center; [2]Medisch Centrum Haaglanden; [3]Rijnland Ziekenhuis.*

Background: Diastolic dysfunction is common among dialysis patients and is associated with increased morbidity and mortality. Novel echocardiographic speckle tracking strain analysis permits accurate assessment of left ventricular (LV) diastolic function, with higher sensitivity and specificity as compared to more conventional measurements. The aim of the study was to evaluate the prevalence of diastolic dysfunction in chronic dialysis patients using this novel technique, and to identify its determinants among clinical and echocardiographic variables.

Methods: Patients currently enrolled in the ICD2 study protocol were included for this analysis. Next to conventional echo measurements diastolic function was also assessed by global diastolic strain rate during isovolumic relaxation (SRIVR). The presence of diastolic dysfunction was assessed using a previously defined cut-of: E/SRIVR ≥ 236. Following the determinants of diastolic dysfunction were identified among the baseline clinical parameters.

Results: A total of 77 patients were included (age 67±8 years, 74% male). When defined as E/SRIVR ≥ 236, the prevalence of diastolic dysfunction was higher compared to more conventional measurements (48% vs. 39%). Multiple regression analysis demonstrated that left ventricular mass (OR 1.02, 95% CI 1.01 - 1.04, p=0.01) and pulse wave velocity (OR 1.31, 95% CI 1.05-1.79) were independent determinants of diastolic dysfunction.

Conclusions: Diastolic dysfunction is highly prevalent among dialysis patients with preserved ejection fraction and might be underestimated using conventional measurements. Among baseline clinical and echocardiographic parameters left ventricular mass and pulse wave velocity were the only determinants of diastolic dysfunction in these patients.

Funding: Pharmaceutical Company Support

SA-PO2611

Statin Exposure over Time in Chronic Dialysis: An Observational, National Study of Dually Eligible Patients James B. Wetmore, Theresa I. Shireman, Jonathan D. Mahnken, Qingjiang Hou, Purna Mukhopadhyay, Sally K. Rigler, Edward F. Ellerbeck. *University of Kansas School of Medicine, Kansas City, KS.*

Background: Although HMG CoA reductase inhibitors (statins) effectively lower cholesterol levels in patients on chronic dialysis, randomized trials have not consistently demonstrated a survival benefit. Statins are still widely prescribed in this population.

Methods: We examined factors associated with statin use over time in a large, national cohort of dually eligible, chronic dialysis patients from 2000-2005. Medication exposure was tracked through Medicaid prescription claims using the proportion of days covered (PDC) adjusted for inpatient and skilled nursing home stays. PDC was computed across the entire window of observation for each cohort member, reflecting medication exposure rather than adherence. Baseline characteristics and comorbidities were taken from linked USRDS Core Data.

Results: There were 18,757 dually-eligible, chronic dialysis patients taking statins during this period of observation (41.6% of the available cohort). The mean PDC for statin users was 0.57 (SD = 0.32). Statin PDCs increased with advancing age (10 year increments) but were lower among non-Caucasians ($p < 0.0001$). Persons with diabetes, CAD, or CVA at baseline also had higher PDCs ($p < 0.0001$). Cholesterol levels were not available for these study subjects, however, persons with higher BMIs had higher statin PDCs ($p < 0.0001$).

Conclusions: Even with their high potential for adverse cardiac outcomes, patients on chronic dialysis have inconsistent exposure to statins. In part, this may reflect the clinical equipoise during a period where clinical trials had not yet been completed. It may also reflect poor long-term adherence to statin therapy as has been demonstrated in other populations. Further examination of the effectiveness of statins in chronic dialysis is needed.

Funding: NIDDK Support

SA-PO2612

The Role of Statins and Cholesterol on Clinical Outcome of Continuous Ambulatory Peritoneal Dialysis Patients: A Retrospective Study Yong Kyu Lee, Tae Ik Chang, Sug Kyun Shin. *Nephrology Division, NHIC Ilsan hospital, Goyang, Geongido, Korea.*

Background: Patients who are on CAPD (Continuous Ambulatory Peritoneal Dialysis) show higher serum LDL cholesterol and Triglyceride compared to patients who are on hemodialysis. But higher cholesterol level does not seem to effect on raising mortality or cardiovascular morbidity and CAPD failure. On the contrary, lower serum cholesterol level in CAPD patients tends to raise mortality and morbidity due to poor nutritional status.

Methods: This study is a retrospective study designed to evaluate the effect of cholesterol level, statin on CAPD outcome and mortality. Patients who were on peritoneal dialysis for at least 6 months since March 1st, 2000 were included. A total of 467 patients were enrolled in this study. Patients' biological parameter, biochemical parameter and morbidity/mortality during CAPD maintenance period were collected.

Results: Patients whose initial cholesterol level were above 240 mg/dL shows significantly low CAPD failure rate compared to patients whose initial cholesterol level were below 200 mg/dL (OR= 0.469, p=0.049). Patients whose average LDL-cholesterol during CAPD period were over 100mg/dL showed significantly higher mortality compared to patients whose initial LDL-cholesterol level were below 100mg/dL (OR=1.848, p=0.024). Patients whose compliance to statin during CAPD period was over 80% showed significantly low mortality compared to patients who did not take statin during CAPD period (OD=0.556, p=0.020). Patients showed no significant difference in motality due to total cholesterol, HDL cholesterol levels and patients showed no significant difference in CAPD failure due to HDL/LDL cholesterol, statin usage.

Conclusions: In CAPD patients, serum total cholesterol level should be targeted higher than HD or CKD patients. On the contrary, similar to HD or CKD patients, Statin should be administered and LDL cholesterol should be lowered during CAPD period to lower mortality. To identify the difference in cholesterol mechanism of CAPD patients further, in depth study over adequate cholesterol level in CAPD patients needs to be proceeded.

SA-PO2613

***SIRT1 G*ene Polymorphisms Are Associated with Cholesterol Metabolism and Coronary Artery Calcification in Japanese Hemodialysis Patients** Toshimitsu Niwa,[1] Yasuhiko Shimoyama,[1] Hidehisa Shimizu.[1] *[1]Nagoya University Graduate School of Medicine; [2]Meiyo Clinic.*

Background: *Sirtuin 1 (SIRT1*), a longevity gene, protects cells against oxidative and genotoxic stress. This study aimed to investigate the association of *SIRT1* gene single nucleotide polymorphisms (SNPs), rs7895833, rs7069102 and rs2273773, with various laboratory data in 219 Japanese hemodialysis (HD) patients.

Methods: Genotyping of these polymorphisms was performed using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) assay.

Results: The A allele frequency of rs7895833 and G allele frequency of rs7069102 were significantly lower in HD patients (0.228 and 0.131, respectively) than those in 803 control subjects (0.289 and 0.181, respectively) (p=0.010 and p=0.012, respectively). However, the allele frequency of rs2273773 was not significantly different from that in the control subjects. Multivariate analysis adjusted for age and duration on HD demonstrated that the serum levels of total and low-density lipoprotein (LDL) cholesterol were significantly high in G allele carriers of rs7069102 compared with CC genotype in male HD patients. Coronary artery calcification score was significantly high in C allele carriers of rs2273773 in all and male HD patients.

Conclusions: This study first demonstrates that *SIRT1* polymorphisms, rs7069102 and rs2273773, are associated with cholesterol metabolism and coronary artery calcification, respectively, in Japanese HD patients, especially in males.

SA-PO2614

Divert to Ultra: Differences in Infused Volumes and Clearance in Two Treatments HDF On-Line (Conventional vs Ultra) Antonino Sidoti. *Italian Cooperative Study on High Volume On-Line HDF, Italy.*

Background: A consolidate evidence exists about the higher removal capabilities of HDF therapies with respect to conventional HD. It is also well known that postdilution HDF improves the removal rate of mid to high molecular weight uremic toxins leading to some clinical benefits, like less dialysis related amyloidosis, better control of patient's phosphatemia and likely better response to ESA. The aim of this study was to compare two different post-dilution On-Line HDF in regards to exchanged volumes (EV) and middle molecules dialysis efficiency: standard On-Line (sOL) and ULTRAcontrol (UC).

Methods: Thirty ESRD patients (pts, 6/24 F/M) were enrolled in this prospective sequential study from 8 centres. The pts underwent a 3-month sOL (fixed exchanged volume) followed by 3-month UC. AK200 ULTRA has been employed in all treatments. In sOL, the EV were set according to a filtration fraction greater than or equal to 25%. In UC the EV was driven by a biofeedback system controlling in a double loop the transmembrane pressure (TMP) and its set point. In both HDF, pts mantained the treatment time, the dialyzer, the blood flow rate and anticoagulant regimen unchanged. Primary response variables were: EV, ß2-microglobulin (ß2m) clearance (Kß2m) measured at 60 and 120 min. Secondary response variables were: ß2m in pre and post dialysis and phosphate clearance (Kp).

Results: A greater EV was infused in UC than in sOL (20.8±3.7 l vs 16.9±4.4 Liters; $p<0.001$). The average Kß2m values were higher in UC than in sOL (111.5±22 vs 123.3±24 ml/min, $p<0.002$), while the average Kp did not achieve a statistical significant difference (150.8±33.9 vs 156.2±38.9 ml/min, p=NS) despite they were higher in UC than sOL. The estimated ß2m concentrations at the beginning of dialysis were similar in both HDF (23.0±11.8 vs 22.1±15.0 mg/l, p=NS), while they significantly differed in the post dialysis (7.8±5.8 vs 5.8±4.2 mg/l, $p<0.019$).

Conclusions: This study shows that the biofeedback module, applied to the automatic control of TMP in On-Line HDF (ULTRAcontrol), allows higher EV and correspondingly higher Kß2m. This results were obtained by reducing both the technical complexity of HDF and staff workload.

SA-PO2615

Has the Introduction of Clinical Practice Guidelines Improved Dialysis Care? Helga K. Mogensen,[1] Runolfur Palsson,[1,2] Olafur S. Indridason.[2] *[1]University of Iceland, Reykjavik, Iceland; [2]Divison of Nephrology, Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland.*

Background: Several clinical practice guidelines for dialysis care have been issued over the past decade. In this study we examined if the introduction of the KDOQI and ERBP guidelines resulted in improvement of the quality of dialysis therapy at the University Hospital in Reykjavik.

Methods: This was a retrospective study of all patients receiving dialysis at the University Hospital's Dialysis Unit for >3 months from 2003 to 2008. Data were obtained from electronic medical records. The study period was divided in two 3-year intervals, 2003-2005 and 2006-2008. Chi-square test was used to compare the proportion of patients reaching therapeutic targets in each interval and to compare hemodialysis and peritoneal dialysis patients.

Results: A total of 177 patients underwent dialysis treatment during the study period. The target level for URR (>65%) was achieved in 80.4% of hemodialysis patients during the first interval and 72.6% during the second interval (p=0.80). In the first interval, 65% of patients reached the target for hemoglobin (110-130 g/L) and 67.5% during the second interval (p=0.68). The target for serum calcium (2.20-2.60 mmol/L) was achieved in 72.1% of patients during the first and 70.7% during the second interval. Only 53.0% of patients reached the target for serum phosphate (1.13-1.78 mmol/L) during the first interval and 55.7% during the second interval (p=0.67). The target for PTH (150-300 ng/L) was achieved in only 23.9% of patients during the first and 33.0% during the second interval (p=0.30). The target for serum bicarbonate (>21 mmol/L) was achieved in 94.9% of peritoneal dialysis patients and 65.6% of hemodialysis patients ($p<0.001$); otherwise no significant difference existed between the two dialysis modalities.

Conclusions: Introduction of clinical guidelines has not been associated with substantial improvement in the care of dialysis patients in Iceland, as measured by selected quality indicators. The use of well defined clinical pathways may be needed to facilitate the implementation of clinical practice guidelines and to improve the quality of dialysis care.

SA-PO2616

Outcomes of Enhanced Systematic Screening for Transient Ischemic Attack in Hemodialysis Albert J. Power, Dima Dahdaleh, Claire Edwards, Jan Sawyer, Harri Jenkins, David Taube, Neill Duncan. *Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Rapid recognition and management of transient ischemic attack [TIA] has been shown to reduce incident stroke in the general population but similar data are lacking in dialysis patients who form a high-risk group for cerebrovascular events. We hypothesised that systematic screening would reveal the true prevalence of TIA in hemodialysis [HD] and drive reductions in stroke incidence.

Methods: All patients on maintenance HD at one unit at our center were given verbal and written information about stroke symptoms [UK Dept of Health], staff attended an educational session and posters were distributed through the patient areas prior to study start. Prospective screening was performed once weekly using a dedicated questionnaire comprising all elements of the FAST test [UK Dept of Health], screening for sensory & visual symptoms and a record of any emergency attendances in an attempt to maximise yield.

Results: 304 patients were screened over 12 months from Nov 2009 [mean age 65.7±14.0yrs, 60% male, 53% Indo-Asian] with 2594 patient months follow-up. 44% were diabetic, 14% had prior cerebrovascular disease [stroke +/- TIA]. Mean HD length was 4.3±0.4hrs, single-pool Kt/V 2.0±0.3. Mean blood pressure was 140/78mmHg pre- & 135/75mmHg postdialysis. Mean hemoglobin was 12.2±0.9g/dL, mean weekly darbepoietin dose 0.56±0.37mcg/kg.

9504 questionnaires [mean 32±14/patient] were administered. 6 strokes occurred, 5 ischemic- an incidence rate of 23.1/1000 pt years. No patients screened positive for TIA despite a predicted rate of 4.2/1000 pt years [95% CI 1.4-9.7/1000 pt years]. One ischemic stroke was preceded by symptoms compatible with a TIA ascertained in retrospect alone.

Conclusions: In the first study of its kind to date systematic screening for TIA in HD patients cannot be relied on alone as a method of identifying HD patients at higher risk of stroke events. The confounding presence of symptoms attributable to uremia, neuropathy, dialysis hypotension and dysglycemia could reduce the sensitivity of established screening tests with significant implications for the detection and treatment of TIA in dialysis patients.

SA-PO2617

Improvement in Cardiac Strain Rate in Children on Chronic Hemodialysis after Carntine Supplementation Kristen Sgambat, Lowell Frank, Ahmad Ellini, Craig Sable, Asha Moudgil. *Nephrology, Childrens National, Washington, DC.*

Background: Carnitine is essential for transport of fatty acids into mitochondria and plays a key role in energy production in the myocardium. Carnitine deficiency may occur in patients on chronic hemodialysis (HD) due to removal by dialysis and inadequate intake and may contribute to cardiomyopathy. We prospectively assessed cardiac response to IV carnitine supplementation by standard echocardiographic (echo) and more sensitive parameters including left ventricular (LV) strain rate.

Methods: Carnitine levels (total,free,acyl,and acyl:free ratio) and cardiac function of 9 children on chronic HD were assessed before and after carnitine infusion (20mg/kg given 3 times a week for 6 months). Standard echo measures of LV size and systolic and diastolic function as well as circumferential and longitudinal strain rate analysis using speckle tracking were performed. Changes in echo parameters of a retrospective control group of 8 children on chronic HD not treated with carnitine were assessed for comparison.

Results: The study group had mean age of 12.7±1.9(range 9-16 years) and dialysis vintage 9.3±6.5 months,which did not differ from controls. After carnitine supplementation, total and free carnitine levels increased (48.9±1.67 vs. 260.3±13.1 µmol/l and 29.0 ± 1.19 vs.156.6 ± 8.44 µmol/l (p<0.001), whereas acyl:free ratio remained unchanged (0.73±0.04 vs. 0.69±0.05). There were no significant changes in standard echo measures of LV function including end diastolic dimension, mass index, ejection fraction, and shortening fraction after carnitine supplementation. However, there was a significant improvement in longitudinal strain rate (-1.48 ± 0.11 vs. -1.91 ± 0.12, p=0.01)after supplementation. There were no changes in blood pressure, interdialytic weight gain, or hemoglobin of study patients pre vs post treatment. No improvements in LV strain rate occurred in control subjects.

Conclusions: Carnitine supplementation improved total and free carnitine levels in children on chronic HD without impacting acyl:free ratio. LV performance improved after carnitine supplementation as assessed by strain rate analysis that was not obvious by standard echo measures.

Funding: Private Foundation Support

SA-PO2618

Aspirin Resistance: Prevalence, Affecting Factors and Effects on Cardiovascular Complication and Vascular Access Failure in Hemodialysis Patients Jong-Woo Yoon, Myung Jin Choi, Youngki Lee, Ja-Ryong Koo, Jung-Woo Noh. *Internal Medicine, Hallym University Hospital, Kidney Research Institute, Chuncheon, Gangwon, Korea.*

Background: Even though aspirin has been effectively used for prevention of cardiovascular(CV) complication, some patients experience CV events during aspirin use. Association of aspirin resistance(AR) and CV events have been reported in many CV disease and kidney transplant patients. The prevalence and effects of AR on CV complication and vascular access failure of hemodialysis(HD) patients is not known.

Methods: 119 HD patients from two hospital who took 100-200mg of aspirin more than 1 month were enrolled. Patients who used NSAIDs or anti-platelet agents and thrombocytopenia were excluded. To define AR, measurement of anti-platelet effects was assessed by VerifyNow® assay device. AR was defined as aspirin resistance unit (ARU)>550.

Results: Among 119 patients, female was 68(58.1%), mean age was 57, mean dialysis duration was 51.5 month and 68(57.5%) were diabetes. AR was observed in 19 of 119 patients(15.9%) and showed higher hsCRP(14.24±7.91 vs 5.04±0.90, *p=0.02*) and higher prevalence of CV complication(43.8% vs 30.6%, *p=0.02*), including cerebrovascular complication(25.8% vs 19.8%, *p=0.01*). But vascular access failure incidence was not different. It also showed positive correlation with serum 25(OH) Vitamin D, total level(*r=0.305, p=0.001*), negative correlation with calcium(*r=-0.271*, *p=0.003*), phosphate(*r=-0.177, p=0.05*) and Kt/V urea(*r=-0.196, p=0.036*). Multivariety analysis showed vitamin D level(OR 1.117, 95% CI 1.025-1.117, *p=0.01*), phosphate(OR 0.618, 95% CI 0.395-0.968, *p=0.04*), HDL(OR 1.065, 95% CI 1.010-1.124, *p=0.02*) as a independent factor. There were no relationship between AR with age, sex, dialysis duration, diabetes, BMI, smoking, aortic calcification.

Conclusions: AR was observed 15.9% in HD patients and was associated with higher incidence of CV disease but vascular access failure was not. Relative lower Kt/V urea and higher hsCRP suggest the possible influence of dialysis adequacy and inflammation. Correlation with some factors affecting vascular remodeling mechanisms such as Vit D, HDL, phosphate, and calcium need further investigations.

SA-PO2619

Randomized Controlled Trial on the Cardiovascular Efficacy of Active Vitamin D in Hemodialysis Patients (J-DAVID): Rationale and Study Design Tetsuo Shoji.[1,2] *[1]Department of Metabolism, Endocrinology and Molecular Midecine, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]J-DAVID Research Group.*

Background: Patients with stage 5 chronic kidney disease on maintenance hemodialysis are at an increased risk for cardiovascular disease. Observational cohort studies showed that use of active vitamin D sterols was associated with better survival and better cardiovascular outcome among dialysis patients. Since these patients have deficiency of active vitamin D which potentially plays protective roles in the cardiovascular system, we hypothesized that administration of active vitamin D would reduce the incidence of cardiovascular outcomes.

Methods: The Japan Dialysis Active Vitamin D Study (J-DAVID) is a prospective, randomized, open-labeled, blinded-endpoint (PROBE) trial. The intervention is oral alfacalcidol (starting dose of 0.5 micro g/day) versus no active vitamin D sterol (1:1 allocation). The main inclusion criteria were serum calcium ≤ 10.0 mg/dL, phosphate ≤ 6.0 mg/dL, intact PTH ≤ 180 pg/mL without use of active vitamin D sterol. The target number of subjects is 972, and the follow-up period is 4 years. The primary end-point is the composite of incident myocardial infarction, coronary revascularization, stroke, revascularization for PAD, amputation of ischemic legs, and congestive heart failure requiring hospitalization. The results will be analyzed on intention-to-treat principle.

Results: Between July 2008 and January 2011, a total of 976 subjects on maintenance hemodialysis were enrolled at approximately 100 dialysis centers in Japan, and they were randomly assigned to the two treatment arms.

Conclusions: The results of this study will provide important information on the cardiovascular protection of active vitamin D sterol in hemodialysis patients without increased intact parathyroid hormone level.

Funding: Private Foundation Support

SA-PO2620

The Effect of Intradialytic Vasopressin Infusion on Chronic Blood Pressure Control in Hypertensive Patients with ESRD: A Program To Develop a Decisive Randomized Controlled Trial Anjali Ganda,[1] John L.P. Thompson,[2] Joseph Schwartz,[3] Anthony M. Valeri,[1] Juan A. Oliver,[1] Alexandra Sanford,[2] Richard Buchsbaum,[2] John T. Bigger,[1] Bruce Levin,[2] Donald W. Landry.[1] *[1]Medicine, College of Physicians&Surgeons, Columbia University, New York, NY; [2]Biostatistics, Mailman School of Public Health, Columbia University, New York, NY; [3]Psychiatry & Behavioral Science, Stony Brook University, New York, NY.*

Background: Volume expansion is the central cause of interdialytic hypertension in patients with ESRD; ironically, hemodynamic instability during hemodialysis (HD) prevents adequate fluid removal and patients remain hypertensive between treatments. Non-pressor doses of exogenous arginine vasopressin (AVP) stabilize blood pressure (BP) and allow greater fluid removal during a single HD. We now hypothesize that chronic intradialytic AVP will lead to better chronic interdialytic BP control due to enhanced control of volume.

Methods: To demonstrate feasibility, we conducted a randomized, double-blinded, placebo-controlled 12-subject pilot study with 2-week follow-up, using the same randomization process, treatment groups, medication administration system, and outcome recording procedures that will be used in a 72-subject 12-week Phase II dose selection trial. The primary outcome was change in mean interdialytic 44-hour ambulatory systolic blood pressure (ABP). Dry weight was assessed via relative plasma volume monitoring.

Results: 12 hypertensive ESRD HD patients were randomized to 3 treatment groups. Mean age 73±8.1 years, 50% male, 60% Hispanic/Latino, 75% diabetic. Mean baseline (BL) interdialytic 44-hour systolic ABP 137.5 ±15.0 mmHg. Mean BL number of ABP observations 87.8±37.1. 10 patients completed all 6 study visits. There were no serious adverse events. Excluding 2 patients who lacked outcome data, mean change in systolic

ABP was -0.4±11.8 in the placebo group (N=4); -1.5±8.6 in the 0.15 mU/kg/min AVP group (N=3); and -3.7± 27.0 in the 0.30 mU/kg/min AVP group (N=3).

Conclusions: The pilot study shows that the study design and procedures are viable, and that the randomization, treatment administration, and data management systems function appropriately.

Funding: Other NIH Support - Columbia University CTSA funds

SA-PO2621

Magnesium: A Cardiovascular Risk Marker in Hemodialysis Patients Patrícia Matias,[1,2,3] Tiago Amaral,[1,2,3] Carina Ferreira,[1,2,3] Cristina Jorge,[1,2,3] Inês Aires,[1,2,3] Marco Mendes,[1,2,3] Célia Gil,[1,2,3] Aníbal Ferreira.[1,2,3] *[1]Dialysis Clinic, Hemodial, Vila Franca de Xira, Portugal; [2]Dialysis Clinic, Dialverca, Alverca, Portugal; [3]NIDAN, Lisboa, Portugal.*

Background: Hypomagnesemia seems to play a role in the pathogenesis of arterial hypertension, endothelial dysfunction and inflammation in the general population.

The aim of this cross-sectional study was to evaluate the relationship between pre dialysis magnesium (Mg) levels and inflammation, bone metabolism and cardiovascular risk markers, including pulse pressure (PP), left ventricular mass index (LVMI) and vascular calcifications (VC), in chronic hemodialysis (HD) patients.

Methods: We studied 206 HD patients with mean age (± SD) of 63.6±14.3 years, 45% female, 26% diabetics, with mean HD time of 42.3±38.6 months. All patients were under pre dilution hemodiafiltration with a dialysate Mg concentration of 1 mmol/L. Univariate and multivariate analysis were performed and a $p<0.05$ was considered significant.

Results: Mean serum Mg was 1.36±0.18 mmol/L and none of the patients presented hypomagnesemia (Mg < 0.6 mmol/L). Mg levels were negatively correlated with age (r= -0.44; p=0.006), diabetes mellitus (r= -0.42; p=0.007), iPTH (r= -0.33; p=0.02), PP (r= -0.36; p=0.01), LVMI (r= -0.37; p=0.01) and VC (r= -0.40; p=0.008). Mg was positively correlated with albumin (r= 0.57; p<0.001) and 25-hydroxyvitamin D3 (r= 0.45; p=0.004).

In multivariate analysis, lower Mg concentrations were predictors of an increased PP (> 65 mmHg) (p=0.002) and LVMI (> 140 g/m2) (p=0.03) and of a higher VC score (> 3) (p=0.01).

Conclusions: Pre dialysis Mg serum levels were associated with inflammation, bone disease and CV risk markers in HD patients.

SA-PO2622

Mechanical Circulatory Support in Dialysis Patients as a Bridge to Transplantation Jeffrey D. Wallach,[1] Gregory V. Warren,[1] Michael E. Anigbogu,[2] Krishna Nagendran,[2] T. Edward Hastings,[2] Nasir Z. Sulemanjee,[2] Andrew J. Boyle.[2] *[1]Division of Nephrology, Aurora St. Lukes Medical Center, Milwaukee, WI; [2]Division of Cardiology, Aurora St. Lukes Medical Center, Milwaukee, WI.*

Background: Advanced chronic kidney disease has generally been an exclusion criterion for cardiac transplant using standard listing criteria. Selected patients with end stage renal disease (ESRD) have undergone successful combined heart and kidney transplantation. However, the use of mechanical circulatory support as a bridge to transplantation has not previously been reported in patients with ESRD. We report the experience of a single transplant center where ESRD patients received continuous flow ventricular assist devices (VAD) as a bridge to transplant, and continued to receive dialysis in their usual outpatient setting.

Methods: 5 prevalent ESRD patients (3 in-center HD, 2 home PD, mean vintage 433 days) underwent VAD placement at our center between Nov 2009 and Oct 2010 as a bridge to transplantation. No ESRD patients were selected for destination therapy. Patients were observed from VAD placement until transplant or to 5/31/2011. Outcomes were length of stay (LOS) post-VAD placement, readmission days, and duration of circulatory support.

Results: Following VAD implantation initial LOS was 22 ±8 days. 2 patients were successfully weaned from dialysis post-VAD and did not require renal transplantation. Patients remained on VAD support for 202 ±72 days. No dialysis modality changes were made pre-transplant. There were 4 readmissions in 3 patients with diagnoses of peritonitis, ventricular tachycardia, driveline infection, and weakness. Following initial discharge post-VAD implantation, patients remained as outpatients for 98% of observed days. 4 patients received transplants and 1 patient remains listed.

Conclusions: 1)ESRD patients on HD or PD can successfully receive mechanical circulatory support with VAD as a bridge to transplantation. 2)Outpatient HD and PD can be safely performed in patients with VAD. 3)Clinical outcomes, LOS and readmissions in ESRD patients are similar to VAD patients without ESRD. 4)Following VAD implantation, an average of 98% of follow-up days were spent as outpatients.

SA-PO2623

Characteristics of the Dipper and Non-Dipper Type of Hemodialysis Patients -24-Hours ABPM Monitoring and Cardiovascular Complications over 10 Years Yuzuru Sato, Megumi Sato, Masanor Jotoku. *Internal Medecine, Sato Junkanki Hospital, Matsuyama, Ehime, Japan.*

Background: 24-hour blood pressure monitoring (ABPM) is useful to determine whether the patient is a dipper or non-dipper. But characteristics of dipper and non-dipper on hemodialysis patients, the relationship between the two types, and the cardiovascular complications on a long-term, are still unknown. In our study, ABPM was performed on 45 hemodialysis patients over 10 years.

Methods: 24-hour blood pressure monitoring (ABPM) was performed on 51 hemodialysis patients from 2000. Four patients were moved, two were transplanted and the other forty-five patients were followed for 10 years. ABPM started at the end of hemodialysis and BP was measured every 15 minutes at the daytime (defined from 6 AM to 9 PM) and every 1 hour at the nighttime (9 PM to 6 AM). Dipper status was defined on the changes between the average SBP from daytime and nighttime. We considered two groups, dippers (BP nocturnal fall superior to *10mmHg)* and non-dippers (BP nocturnal fall less than 10mmHg).

Results: The ABPM on 45 patients showed distinct groups, as 11 patients (24.4% of the whole patients) were dipper type (group A) and 34 patients (75.6%) were non-dipper type (group B). SBP of group A in the daytime was significantly higher than group B (163.7±16.8 vs. 150.2±19.4mmHg, $p<0.05$), but both group's SBP were similar in the nighttime (138.3±17.0 vs. 146.8±22.8mmHg). The aortic calcification scores (ACS) were the same according to computed tomography of the abdomen. Nine patients in group A and 13 in group B had cardiovascular complications for 10 years and the cumulative events-free rate of group B was significantly higher than group A's one (Kaplan-Meier methods p=0.016, Log-rank test). ACS of the patients in group B with cardiovascular complications were significantly higher than without them (63.9±24.0 vs. 34.6±25.4, $p<0.01$).

Conclusions: In this study, two types of hemodialysis patients were characterized over 10 years. Higher SBP in the daytime for dipper type patients and pre-existing severe aortic calcification for non-dipper type patients suggested several risk factors of cardiovascular complications in hemodialysis patients.

Methods: SA-PO2624

Early Interdialytic Fluid Retention Is Associated with Cardiovascular Outcomes in Incident Hemodialysis Patients Ji Yong Jung, Jae Hyun Chang, Sejoong Kim, Hyun Hee Lee, Wookyung Chung. *Department of Internal Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea.*

Background: Fluid retention is a major clinical problem in patients undergoing hemodialysis (HD) and is associated with cardiovascular outcomes. Patient with end stage renal disease have similarities to heart failure. Volume overload in heart failure is associated with worse outcome. Removal of fluid during the HD is the cornerstone of volume management in this population. Therefore, we hypothesized that greater inter-dialytic fluid retention (IDFR) is associated with poor cardiovascular event and survival in incident HD patients.

Methods: We analyzed the 174 patients who newly started and maintained the HD over 6-month in Gachon University Gil Hospital between January 1, 2003 and December 31, 2008. We did not take the first 3 month IDFR into account by reason of stabilized period in incident HD patients. According to the average IDFR of 4~6 month, we divided into 2 groups by the median value: Lower IDFR (< 2.17 kg), Higher IDFR (Fluid retention ≥ 2.17 kg). The associations of IDFR with cardiovascular outcomes were evaluated with the use of Cox proportional regression analysis.

Results: Higher IDFR showed higher prevalence of diabetes, better nutritional status (higher phosphorus and nPNA level). In univariate analysis, higher IDFR, hemoglobin (Hb), total cholesterol and LDL cholesterol level were associated with cardiovascular events. After multivariate adjustment, higher IDFR, low Hb, and high LDL cholesterol were associated with increased risk of cardiovascular events. The odds ratio (95% confidence interval) of cardiovascular events 2.213 (1.011 – 4.846, P = 0.047) in the higher IDFR, 0.557 (0.355-0.876, P = 0.011) in Hb level, and 1.106 (1.003 – 1.028, P = 0.014) in LDL cholesterol level, respectively. However, all-cause and cardiovascular mortalities were not significantly different between two groups.

Conclusions: In incident HD patients, early greater IDFR is associated with higher risk of cardiovascular events. Further research with large subjects needed to elucidate the pathophysiological mechanisms that link fluid retention to increased cardiovascular events.

SA-PO2625

Pre-Procedural Serum Albumin and C-reactive Protein Levels Predict Clinical Outcome after Endovascular Therapy in Hemodialysis Patients with Peripheral Artery Disease Fumiyo Kobayashi,[1] Hirotake Kasuga,[2] Keiko Kimura,[2] Chieko Matsubara,[2] Rei Okada,[2] Seiichi Matsuo.[3] *[1]Nephrology, Toyota Kyoritsu Clinic; [2]Nephrology, Nagoya Kyoritsu Hospital; [3]Nephrology, Nagoya University Hospital.*

Background: Although endovascular therapy (EVT) has become widely performed for peripheral artery disease (PAD), adverse events such as high restenosis rate or premature death after EVT remain major clinical problems in patients on hemodialysis (HD). On the other hand, malnutrition and chronic inflammation status are frequently observed, and linked to poor cardiovascular outcome in such population. We evaluated the possible prognostic values of serum albumin and C-reactive protein (CRP) levels on clinical outcomes after EVT in patients on HD.

Methods: A total of 450 HD patients successfully undergoing EVT for PAD were enrolled and were followed-up for up to 8 years. Serum albumin and CRP levels were measured prior to EVT. They were divided into tertiles according to serum albumin and CRP levels; the lowest tertile (T1), the middle tertile (T2) and the highest tertile (T3), respectively. We analyzed the incidence of major adverse cardiovascular events (MACE) as a composite endpoint including target lesion revascularization (TLR), amputation and all-cause death.

Results: During follow-up period (mean 36±31months), 206 MACE (46%) including 67 TLRs, 45 amputations and 94 deaths occurred. Event-free survival rate from MACE for 8 years was 23.1%, 25.9%, and 50.0% in T1, T2 and T3 of albumin ($p<0.0001$), and 41.2%,

36.3% and 18.9% in T1, T2 and T3 of CRP (p=0.0004), respectively. After adjustment, lower albumin (HR 2.00, 95%CI 1.29-3.10, p=0.0071 for T1 vs. T3) and elevated CRP (HR 1.93, 95%CI 1.29-2.90, p=0.0061 for T3 vs. T1) were independent predictors for MACE, respectively. In the combined setting of albumin and CRP, the risk of MACE was 5.22-fold (95%CI 2.34-11.64, p=0.0011) higher in the T1 of albumin with T3 of CRP compared to the T3 of albumin with T1 of CRP even after adjustment.

Conclusions: Lower albumin and elevated CRP levels could predict MACE after EVT in patients on HD. However, the combination of these variables is more markedly related to increased MACE than either variable alone.

SA-PO2626

Risk Factors of Progression of Aortic Arch Calcification in Patients with Maintenance Hemodialysis and Peritoneal Dialysis Hyun Gyung Kim, Young Ok Kim. *Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.*

Background: Vascular calcification is accelerated during the dialysis and known as an important risk factor for cardiovascular disease. Progression of AoAC can be simply estimated with an AoAC score (AoACS) on chest radiography. The objective of this study was to evaluate the risk factors of the progression of AoAC.

Methods: The enrolled subjects were 125 hemodialysis and 59 peritoneal dialysis patients, newly treated at the dialysis unit. In the patients who had undergone chest radiographies before initial dialysis therapy and every year, we estimated AoACS and then divided into two groups by the presence or absence of the AoAC progression. We also compared the baseline clinical and biochemical profiles in two groups.

Results: Eighty-five (46.2%) were men and the mean age was 58.6 ± 12.7 years. 76 patients (41.3%) had AoAC before initial dialysis with the mean AoACS of 13.0 ± 20.4%. The mean duration of follow-up of AoACS was 2.7 ± 1.0 years. The half of the patients (50%) had the progression and the others non-progression of AoAC. Old age more than 65 years (p=0.003), dialysis duration (p=0.004), diabetes (p=0.015) and the presence of AoAC at baseline (p=0.001) were related to the progression of AoAC. No significant association was detected between the AoAC progression and baseline clinical parameters including gender, obesity, hypertension and dialysis modality. In multivariate analysis, the duration of dialysis (p=0.004) and the presence of AoAC at baseline (p=0.001) were independent risk factors of the progression of AoAC in dialysis patients.

Conclusions: The duration of dialysis and the presence of AoAC before initial dialysis were significantly related to the progression of AoAC in dialysis patients. We suggest that we should focus on the through management from pre-dialysis stage to prevent the progression of AoAC and reduce cardiovascular morbidity in chronic dialysis patients.

SA-PO2627

Reduction of Nurse Intervention Using Blood Volume Tracking (BVT) System Maria Doria,[1] Simonetta Genovesi,[2] Antonio Santoro.[3] *[1]Policlinico S.Donato I.R.C.C.S., San Donato, Italy; [2]San Gerardo Hospital, Monza, Italy; [3]Sant'Orsola Malpighi Hospital, Bologna, Italy.*

Background: Intradialytic hypotension (IDH) still represents the most common acute complication of hemodialysis (HD) therapy accounting for up to 30% of HD sessions. BVT system is a tool capable of reducing the occurrence of IDH by avoiding sudden drop of BV below hazard values during HD by adjusting both UF rate and the dialysate sodium content. The present study was aimed to understand if BVT system is able to reduce the staff workload and job complexity associated to a reduction of IDH.

Methods: Ten HD patients (M/F: 5/5; age: 76.7±8.3 yrs) prone to IDH were selected for the study. The presence of cancer, mental illness, pregnant, residual diuresis greater than 500 ml/d, poor vascular access were considered an exclusion criterion. Each patient was treated with 39 conventional HD for 3 months and then was switched to 39 HD+BVT for further 3 months. The variables monitored were: number of session requiring nurse interventions (NI), effective treatment time (ETT), body weight loss (BWL), end-treatment BV, pre/post dialysis systolic arterial pressure and heart rate, number of sessions complicated by IDH and type of staff interventions.

Results: Throughout the HD and the HD+BVT period all episodes of IDH, the number of NI, the total volume of fluid infusions for therapeutic purposes, the infusions of 11.7% NaCl and all possible preterm disconnection of patients were reported on the dialysis records. There was no statistically significant differences between the two treatments with regard to the actual treatment time, the BWL achieved and the corresponding average UF rate. The number of HD sessions requiring intervention by the medical staff was approximately halved when the patient underwent HD+BVT (102 vs. 57, p<0.001). Using as parameter the ETT, the HD sessions were interrupted before the prescribed time in a percentage significantly greater than the HD+BVT (15 vs. 2, p<0.001).

Conclusions: This study confirms the usefulness of the BVT during HD to reduce the frequency of IDH episodes in hemodynamically unstable patients, reducing the staff workload with less nurse interventions and higher dialysis efficiency.

SA-PO2628

Renalase Was Not Related to Blood Pressure, but to Residual Renal Function in Haemodialysis and Peritoneal Dialysis Patients Edyta Zbroch, Jolanta Malyszko, Jacek S. Malyszko, Ewa Koc-Zorawska, Michal Mysliwiec. *Nephrology, Medical University, Bialystok, Poland.*

Background: Recently the new flavin-adenine dinucleotide containing hormone, secreted by the kidney and circulates in blood, named renalase was investigated. It degrades catecholamines and may play a role in the regulation of sympathetic tone and blood pressure.

Methods: The aim of the study was to assess the serum renalase concentration (RNLS) in a cohort of 104 treated with haemodialysis (HD group) and 26 treated with peritoneal dialysis (PD group) patients and its relationship to the blood pressure control, type of antihypertensive therapy and the presence of residual renal function (RRF).

Results: Abnormal blood pressure control was observed in 67% patients of the HD group and in 57,69% of the PD group. The main antihypertensive drugs were beta-blockers and calcium-channel blockers. RRF was present in 50% of HD and in 61,5% of PD patients. RNLS in the study cohort was 25,86µg/ml and it was significantly higher than in the control group 3,86µg/ml (p<0,001). There was the significantly higher RNLS in the HD group comparing to the PD group (27,53 vs. 19,24µg/ml). The results indicated a significant correlation between the duration of dialysis and RNLS (r=0.3175, p=0.002). It was higher in those patients who were maintained dialysis longer. The relationship between the presence of RRF and RNLS was found in whole study cohort (24,21 vs. 27,83µg/ml, p=0,006) and in HD patients (25,65 with vs. 30,11µg/ml, p<0,002). The significant inverse correlation between RNLS and RRF was also indicated: HD group r=(-0,327, p =0,001); PD group r=(-0.4286, p=0.02). No correlation between RNLS and blood pressure rate was found. RNLS was higher in male than female only in HD patients.

Conclusions: The elevated RNLS in dialysis patients may be rather related to kidney function (as time on dialysis passes, the residual renal function is lower) and due to the sympathetic nervous system hyperactivity found in this population and it may have an impact on the development of cardiovascular complications. Further studies are needed to prove or disprove the possible role of renalase in the pathogenesis of hypertension in patients with kidney diseases.

Funding: Government Support - Non-U.S.

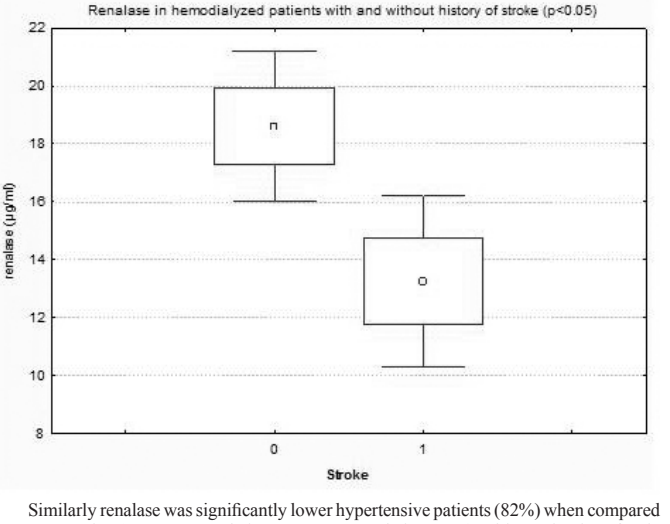
SA-PO2629

Renalase as a Possible Risk Factor of Cardiovascular Complications in HD Jolanta Malyszko,[1] Jacek S. Malyszko,[1] Ewa Koc-Zorawska,[1] Edyta Zbroch,[1] Michal Mysliwiec.[1] *[1]Nephrology, Medical University, Bialystok, Poland; [2]Dialysis Unit, NZOZ Centum, Mlawa, Poland.*

Background: Renalase, newly discovered hormone, is secreted by the kidney and circulates in blood. It was shown that renalase degrades catecholamines and may play the role in the regulation of sympathetic tone and blood pressure. Cardiac renalase deficiency may contribute to the increased susceptibility to myocardial injury due to ischemia and rhythm disturbances frequently found in CKD. The aim of the study was to assess the renalase relationship to cardiovascular status in hemodialyzed patients.

Methods: Renalase was assessed using commercially available assay from uscn. Life Sci, China. Echocardiography was performed in each patient.

Results: Serum renalase was significantly lower in patients with a history of stroke (21%) (13.25±3.97µg/ml vs. 18.61±6.91µg/ml, p<0.05), than in patients without it.



Similarly renalase was significantly lower hypertensive patients (82%) when compared to normotensive (16.47±5.86µg/ml vs. 22.34±8.91µg/ml, p<0.05), with proximal a-v fistula (29%) vs distal a-v fistula (16.55±6.39µg/ml vs. 24.61±5.14µg/ml, p<0.05), and higher in patients with glomerulonephritis (26%) vs other etiologies of ESRD (21.93±5.52µg/ml vs. 15.91±6.49µg/ml, p<0.05). Serum renalase correlated with creatinine, residual renal function, TSAT, and dysfunction of mitral valve. No correlation between serum renalase concentration, and blood pressure rate or haemodialysis vintage was found.

Conclusions: Our preliminary results suggest that renalase, due to the sympathetic nervous system hyperactivity could be associated with cardiovascular complications in hemodialzyed patients. However, further studies are needed to prove or disprove the possible role of renalase in the pathogenesis of these complications.

Funding: Government Support - Non-U.S.

SA-PO2630

Age May Explain the Association of an Early Dialysis Start with Poor Survival

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Background: Some studies postulated that early dialysis start may increase mortality. To examine this issue we measured survival associated with eGFR and age at dialysis initiation in our centre.

Methods: We studied the following variables at dialysis initiation: eGFR, age, gender, diabetes mellitus, serum albumin, hemoglobin, date of dialysis initiation, history of ischemic heart disease and stroke.

Results: Over the last 15 years 428 patients initiated dialysis therapy in our reference area. Mean age at dialysis start 63±13 years and 65% male. The three years survival rate was increasing within the different periods 63% 1995-99, 69% 2000-2004, 77% 2005-2009(p=0.003). Causes of death: heart disease(25%), infections(25%), neoplasms(11%), and stroke(9%). Mean eGFR dialysis start 8.16 mL/min. In the univariate analisis, increased eGFR, age, dialysis initiation 1995-99/2000-2004, diabetes and history of ischemic heart disease were associated with increased mortality in end-stage renal disease patients(ESRD) ($p<0.05$). Patients that started dialysis program with eGFR >8.16 were older than with eGFR<8.16 (66 vs 61y, $p<0.001$). In the multivariate Cox model(without age), eGFR, dialysis initiation period, serum albumin and history of ischemic heart disease were associated with mortality. The association between mortality and eGFR was lost when the model was adjusted by age.

Model without AGE included			Adjusted by age		
Variables included in the model	Relative risk	p	*Variables included in the model*	Relative risk	p
eGFR (ml/min/1.73)	1.05	0.004	eGFR (ml/min/1.73)	1.12	0.364
Period 1995-1999 (2005-2009=1)	2.16	<0.001	Period 1995-1999 (2005-2009=1)	2.39	<0.001
Period 2000-2004 (2005-2009=1)	1.63	0.029	Period 2000-2004 (2005-2009=1)	1.67	0.024
Albumin (g/l)	0.76	0.022	Albumin (g/l)	0.65	0.001
Ischemic heart disease	1.92	<0.001	Ischemic heart disease	1.58	0.005
			Age (years)	1.07	<0.001

Conclusions: History of ischemic heart disease, serum albumin and dialysis start before 2005 were risk factors for mortality in ESRD. Older age is usually associated with early dialysis initiation, so age adjustment is needed to perform studies aimed to calculate the effect of eGFR at dialysis initiation on survival.

SA-PO2631

Alteration of Autonomic Control during Hemodialysis in Peripheral Vascular Disease Patients

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Background: An association between the pulse pressure (PP) decrease induced by HD treatment and improved outcomes was recently described. Chronic HD patients with peripheral vascular disease (PVD) may be unable to decrease PP because of vascular stiffness and altered autonomic nervous system. Our aim is to compare PP and heart variability parameters in HD patients with and without PVD.

Methods: We studied 7 HD patients (pts) with PVD and 8 without PVD. Continuous blood pressure (BP) was recorded with a Finometer (FMS) in the first and last half-hour of a single HD session (HD_{begin} and HD_{end}). Beat-by-beat series SBP, DBP, PP were extracted from BP signals and subjected to a spectral analysis. Power in the very-low, low and high frequency bands (VLF, LF, HF) was computed, as well as total power, LF/HF ratio, LF% and HF%. VLF relates to local control, e.g. nitric oxide, LF to sympathetic activity, and HF to the mechanical coupling with respiratory activity. DBP is considered related to afterload.

Results: As expected, PVD pts were significantly older (70±9 vs 48±15 yrs). At HD_{end}, PP was higher and HR was lower in PVD pts compared to no-PVD pts. PP increased during HD in PVD pts (ΔPP: +16.2±14.5 mmHg), while this remained stable or decreased in no-PVD. Table 1 reports the significant results.

Table 1
Mean Values of Time and Frequency Domain Indices

	PVD pts		no PVD pts	
	Begin	End	Begin	End
PP (mmHg)	*81± 19*[a]	***97±28***[*]	*73±12*	***70±16***
DBP (mmHg)	51.0± 7.0	**60.9±9.3**[**]	*55.9± 12.1*[a]	***77.9± 11.4***
HR (bpm)	*58±7*	***58±11***[*]	*68±14*	***73±14***
VLF_{DBP} ($mmHg^2$)	***1060±821***[*]	*1249±2031*	***380±215***	*604±571*
$LF\%_{DBP}$	***32± 13***[*,a]	*55±8*	***58± 19***	*54± 15*
$LF\%_{SBP}$	***22±8***[*,a]	*47±16*	***44± 22***	*47± 16*
$LF\%_{RR}$	***19± 12***[**,a]	*42±18*	***65± 14***	*57± 23*

T-test: * P-value<0.05, ** P-value<0.01
Paired t-test: [a] P-value<0.05 (HD_{begin} vs HD_{end})

Conclusions: In this pilot study, chronic HD patients with PVD have an altered autonomic peripheral control system with a prevalence of local control (VLF_{DBP} higher in PVD pts). PVD induces a chronic elevation of afterload and is associated with both a PP increase during HD and a reduced sympathetic activity on the heart (lower $LF\%_{RR}$).

SA-PO2632

Adverse Effect of Chronic Fluid Overload on Left Ventricular Mass Index in Pediatric Patients Maintained with Chronic Peritoneal Dialysis

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Background: Pediatric (ped) patients (pts) with end-stage renal disease have increased cardiovascular mortality. Risk factors include chronic fluid overload (CFO), hypertension, no residual renal function, anemia and inflammation, which all contribute to abnormal cardiac remodelling, increased left ventricular mass index/left ventricular hypertrophy (LVMI/LVH) and subsequent diastolic dysfunction. Few ped studies have assessed the relationship between CFO and cardiac remodeling and function, and no ped study has assessed CFO prevalence in ped pts maintained on chronic peritoneal dialysis (CPD). This study aimed to determine the prevalence of CFO and its association with LVMI in ped CPD pts.

Methods: This study is a prospective monthly follow-up study of ped pts 6-25 years old with no underlying primary cardiac disease, who have been treated with home cycler CPD for >3 months. Fluid status was assessed by bioimpedence, weight (wt) and BP at each monthly visit. BP indices were calculated to normalize BP for age and height for statistical analysis. Annual ECHOcardiograms done. CFO was defined as >4% target dry wt for at least 3 months per 6 month period or median value >4% for the entire 6 month period.

Results: 11 pts have been enrolled to date with 5/11 followed at least 6 months. 3/11 (27%) had CFO; all 3 had increased LVMI. CFO was associated significantly with increased LVMI and BP indices, but not other ECHOcardiogram parameters.

	Age in years	Dialysis months	LVMI g/m^2.7	FS%	EF%	95%ile SBP index	95%ile DBP index
No CFO	13(4)	15(21)	42.6(16.02)	36.5(6.4)	58(11)	0.84(0.11)	0.78(0.14)
CFO	16(8)	37(40)	68.6(17.17)	33(13.2)	50(13)	1.11(0.25)	1.04(0.16)
p value	NS	NS	0.04	NS	NS	0.02	0.01

Values mean(SD)

Conclusions: CFO may occur frequently in ped CPD pts and when present, appears to be associated with high BP indices and abnormal cardiac remodeling. CFO per se is likely an important risk factor for cardiovascular disease in ped CPD pts.

SA-PO2633

Bedside Cardiovascular Testing as a Prognostic Tool in Dialysis Patients

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Background: Cardiovascular disease and sudden death are a barrier to better survival in dialysis patients. However, the underlying pathology is poorly understood, and seems to be different to the general population. We used bedside cardiovascular tests to identify possible pathological processes responsible for the high rate of mortality in dialysis patients.

Methods: A longitudinal assessment of outcome in a cohort of stage 5 CKD patients starting dialysis was undertaken. We analyzed baseline 12 lead ECG and trans-thoracic echocardiogram performed as part of transplant, pre-operative, or routine out-patient cardiology assessment. A sub-group had also undergone pulse wave analysis. Multivariate logistic regression was used to identify independent predictors of all-cause mortality, whilst accommodating clinical co-variates.

Results: There were 208 patients (mean age 67 years, BMI 26kg/m2, BP 141/77 mmHg, 42% male, 33% diabetes, 39% ischemic heart disease). The mean time from starting dialysis to investigations was 4.7 months. Mean follow up was 47 months. There were 93 deaths at a rate of 113 per 1000 patient years. The following ECG parameters were independent predictors of death when $\alpha=0.05$: QRS/T-wave axis discordance >60°(Odds ratio=1.6); resting tachycardia (OR=2.0); strain pattern repolarization in left ventricular hypertrophy (LVH) (OR=1.9); abnormal R wave progression (OR=2.3). Atrial fibrillation, QRS duration, and QTc were not. On echocardiogram, M-mode ejection fraction (EF) was predictive of

death (OR=1.6 per 10% fall in EF) but qualitative assessment of function was not. Mass-indexed LVH was predictive (OR=2.1); LVH was concentric in 74% of cases. Diastolic function, annular calcification, and PWA were not predictive.

Conclusions: LVH is important in the high mortality of dialysis patients. The higher risk of strain pattern shows the potential value of using ECG and echocardiography together in risk assessment tools. Strain indicates abnormal repolarization, as does axis discordance. This may suggest that aberrant repolarization is a precursor to fatal arrhythmia in dialysis patients.

SA-PO2634

Do Current Guidelines for Implantable Cardioverter Defibrillator Therapy Apply to Hemodialysis Patients? A Single-Centre Experience Michelle M. O'Shaughnessy, Sinead Kinsella, Emer Joyce, Donal N. Reddan, Matthew D. Griffin, David Lappin. *Departments of Nephrology and Cardiology, Galway University Hospitals, Galway, Ireland.*

Background: One in four dialysis patients will succumb to sudden cardiac death (SCD). Carefully selected non-dialysis patients at high risk for ventricular arrhythmia can derive mortality benefit from implantable cardioverter defibrillator (ICD) therapy. The benefit of this intervention in end-stage kidney disease is less clear and data from randomised controlled trials (RCTs) are lacking. We studied the utility of conventional ICD guidelines as applied to a hemodialysis (HD) population.

Methods: A single-centre prospective observational study. American College of Cardiology/American Heart Association/Heart Rhythm Society (ACC/AHA/HRS) ICD eligibility criteria were applied to prevalent HD patients. Patients were grouped according to ICD eligibility status: potential candidates for secondary preventative ICD (group 1); potential candidates for primary preventative ICD (group 2); non-candidates (group 3). Cause-specific mortality for each group was determined.

Results: Sixty-two patients (66.1% male, 62.1±15.6 years) were followed for 29 months. Outcomes were as follows:

Group 1 (n=2): 1 patient (EF 15%) received an ICD and died of heart failure 20 months later; 1 (EF 55%) declined an ICD and died of a fatal arrhythmia 7 months later.

Group 2 (n=4): No patient received an ICD. 3 had multiple contraindications to ICD therapy (reduced life expectancy, sub-optimal medical management, recurrent infection) and died from non-SCD causes; 1 is alive and continues to receive hemodialysis.

Group 3 (n=56): 3 patients experienced sudden cardiac arrests (2 of which were fatal).

Conclusions: Conventional guidelines which stratify eligibility for primary preventative ICD therapy according to systolic cardiac function should be interpreted with caution in dialysis populations. Dialysis patients have multiple non-traditional risk factors for SCD and may consequently benefit from ICD therapy despite having an EF >35%. Dialysis patients with an EF ≤35% have a high burden of competing risk. RCTs examining the utility of ICD therapy in this unique population are required.

SA-PO2635

CKD-MBD as the Risk Factors of Incident Cardiovascular Disease (CVD) and Fatality after CVD in a Nationwide Registry of Japanese Hemodialysis Patients Tetsuo Shoji,[1,6] Seiji Marubayashi,[2,6] Takashi Shigematsu,[3,6] Kunitoshi Iseki,[4,6] Yoshiharu Tsubakihara.[5,6] *[1]Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Ajina Tsuchiya Hospital, Hatsukaichi, Japan; [3]Wakayama Medical University, Wakayama, Japan; [4]University Hospital of The Ryukyus, Okinawa, Japan; [5]Osaka General Medical Center, Osaka, Japan; [6]Committee of Renal Data Registry, Japanese Society for Dialysis Therapy (JSDT-CRDR), Tokyo, Japan.*

Background: Although chronic kidney disease mineral and bone disorder (CKD-MBD) is a systemic disease affecting cardiovascular mortality, it is unknown whether it affects the risk of incident cardiovascular disease (CVD) or the risk of fatality after CVD. We examined the associations of some components of CKD-MBD and related medications with the risk of incident CVD and the risk of death after CVD in dialysis patients.

Methods: This is an observational cohort study using a standard analysis file provided by JSDT-CRDR (JRDR-09102) including 216612 dialysis patients at the end of 2004. We extracted subjects without previous history of CVD (myocaridal infarction, cerebral infarction, and/or cerebral bleeding) and with key variables including use of vitamin D receptor activator (VDRA) and outcomes at the end of 2005.

Results: A total of 49659 hemodialysis patients (59% men, 28% diabetes) were extracted. At baseline, the median age, serum corrected calcium, phosphate, and intact PTH levels were 63 years, 9.3 mg/dL, 5.4 mg/dL, and 136 pg/mL, respectively. VDRAs and phosphate binders were used in 57% and 85% of the cohort, respectively. Incident CVD events were recorded in 3134 patients, and 373 died after such events. In multivariate logistic regression models, a higher risk of incident CVD was associated with higher intact PTH, non-use of VDRA, and non-use of phosphate binders. A higher risk of death after CVD was associated with higher corrected calcium and non-use of phosphate binders.

Conclusions: Some components of CKD-MBD and related medications were independent predictors of incident CVD, death after CVD, or both in this large cohort of hemodialysis patients.

SA-PO2636

Associations between Serum Progesterone and Mortality in Hemodialysis Patients John Kyriazis,[1] Kostas Stylianou,[2] Ioannis P. Tzanakis,[3] George Lamprinoudis,[1] Eugene Daphnis.[2] *[1]Nephrology, General Hospital of Chios, Chios, Greece; [2]Nephrology, University Hospital of Heraklion, Heraklion, Crete, Greece; [3]Nephrology, General Hospital of Chania, Chania, Crete, Greece.*

Background: Recent epidemiologic data show that men and women with increased progesterone (PRG) levels are at a higher risk of cardiovascular disease (CVD) mortality (Gend Med 2009; 6: 433-443). In this study, we examined the possible associations of PRG levels with cardiovascular risk markers and subsequent mortality in hemodialysis (HD) patients.

Methods: One hundred and seventy-three HD patients (65±12 years, 111 men) after completion of baseline assessment, including sex hormones, were followed up for CVD and all-cause mortality.

Results: The median PRG concentrations in men 0.53 (0.25-1.20) ng/ml and women 0.43 (0.12-2.12) ng/ml did not differ from reported reference values for each sex. In the whole group, PRG levels were inversely related to HD vintage and positively related to prevalence of diabetes mellitus, diastolic blood pressure and serum phosphorus. Age and estradiol were inversely and positively associated with PRG in men and women, respectively. During a median follow up period of 49 months, 79 deaths occurred, 47 (59 %) of which were caused by CVD. Patients with PRG levels in the sex-specific highest tertile had increased CVD and all-cause mortality (crude hazard ratio: 2.94 [95% CI, 1.65 to 5.23] and 2.52 [95% CI, 1.62 to 3.93], respectively. Likewise, the risk for CVD and all-cause mortality increased by 51 % (1.51 [1.16-1.96]) and 45% (1.45 [1.18-1.78]), respectively, for each I SD increment in log PRG. The high serum PRG - mortality association remained essentially unchanged (in both analyses) after adjustment for age, sex, mass body index, serum albumin and c-reactive protein, clinical history of CVD at baseline, presence of diabetes and HD vintage. Addition of estradiol also failed to influence the results. Similar results were observed when looking at each sex separately.

Conclusions: Our results indicate that in HD patients, PRG levels positively correlate with all-cause and CVD mortality, as well as with cardiovascular risk markers. The clinical relevance for these findings needs further elucidation.

SA-PO2637

Involuntary Discharge from Dialysis Units (IVD): Who, Why, Where Abey K. Thomas,[1] Senthil P. Ramaiyah,[1] Brian T. Chu,[2] Carol Lyden,[3] George N. Coritsidis,[2] Chaim Charytan.[1] *[1]New York Hospital Medical Center of Queens, Flushing, NY; [2]Elmhurst Hospital Center, Elmhurst, NY; [3]IPRO ESRD Network of New York, Lake Success, NY.*

Background: Patients with behavioral, psychological or financial issues place an extra burden on the stretched resources of dialysis providers and may be at risk for IVD. IVD is a significant problem for patients, providers and payers and affects the quality of care. There is concern that the bundling policy may further increase IVD. There is a paucity of data about causes of IVD and their impact on ESRD care.

Methods: Data on IVD reported to ESRD Network 2(EN2) between July 2006 and March 2011 was analyzed for patient and facility characteristics and compared to the general EN2 population. Statistical analysis was performed by calculating IVD incidence rate in each group using average denominators. Comparison of proportions were carried out using the Fisher's exact test. p-values were calculated across all groups in each category.

Results:

	IVD	IVD Incidence %	Average EN2 population	p-value
Total	72	0.29	24518	
Age				
<50	30	0.61	4916	<0.0001
>50	42	0.21	19601	
Gender				
M	51	0.37	13832	0.017
F	21	0.20	10685	
Race				
African-American(AA)	57	0.45	12621	<0.0001
White	14	0.14	9890	
Other	1	0.05	2007	
Modality				
Hemodialysis	72	0.32	22602	0.015
Home Dialysis	0	0	1394	
Facility Size				
<50	1	0.06	1802	0.014
51-100	7	0.15	4720	
>101	63	0.33	18284	
Affiliation				
Large Dialysis Organization (LDO)	44	0.47	9328	<0.0001
Other	28	0.16	15478	
Profit status				
Profit	55	0.38	14482	0.002
Non Profit	17	0.16	10324	
Geography				
Downstate New York(NY)	40	0.25	15974	0.26
Upstate NY	29	0.33	8832	
Primary Insurance		IVD	%	
Medicaid		25	34.72	
Medicare		15	20.83	
Other		9	12.50	
None		3	4.17	
Unknown		20	27.78	
Reasons for IVDs				
Behavioral		55	76.39	
Nonpayment		12	16.67	
Noncompliance		2	2.78	
Unknown		3	4.17	

Conclusions: Our study in EN2 reveals an increased incidence of IVD among younger, male, AA patients treated at larger, for-profit and LDO facilities predominantly for behavioral and financial reasons. These observations may have important consequences for developing interventions to deal with IVD, particularly given the growth of minority ESRD population and LDO facilities. These observations need to be compared to the national experience and trends monitored in an ongoing manner.

SA-PO2638

MORRIS: Model for Optimising Renal Replacement Investment and Services; an Interactive Tool Predicting Future Patient Numbers and Associated Costs Mark Brady,[1] Daisy Wild,[2] Beverley Matthews,[3] Donal O'Donoghue.[2] *[1]Cumberland Infirmary, NHS, Carlisle, United Kingdom; [2]Department of Health, London, United Kingdom; [3]NHS Kidney Care, London, United Kingdom.*

Background: Predicting the future needs for a renal replacement therapy (RRT) programme, on a national or local level is essential to maximise efficiency and quality of care. This freely available interactive tool combines UK national census data with UK renal registry data to forecast annual RRT at both national and local levels until 2018, with the ability to use local data or alter proportions of patients on different modalities to examine the effect of investment in such switches.

Methods: MORRIS model uses 2008 UK Renal Registry data, Office of National Statistics (2001) census data incorporating age and ethnicity with expert advice from UK nephrologists. It is a dynamic simulation model for patients survival and movement between modalities over time. Interactive element allows projections based on local data, plans or needs with treatment costs based on tariff or reference costs.

Results: Figure 1 shows one possible output from the model, making national level projections for various scenarios, predicting the cost of RRT provision in the UK will exceed 1 billion pounds in 2018 and demonstrating the cost benefits of altering the proportion of patients on various modalities. Improving transplantation rates and home therapies uptake together could save 24-28 million pounds per annum on RRT costs alone in the medium and long-term (scenario 1 vs 3). Numbers of in-centre dialysis patients could be 9000 lower by 2018 in this projection.

Scenario	Take-on rate	Tx rates	Home Dx rates	Number of patients in 2018					Costs in year (£ millions)	
				CHD	HHD	PD	Tx	Total	2013	2018
1	High	Fixed	Fixed	24700	500	5700	30200	61100	868	1,040
2	High	Growth	Fixed	21860	466	4310	35329	61965	849	1,045
3	High	Growth	Growth	15981	2664	7991	35329	61965	821	1,012
4	Mod	Fixed	Fixed	22800	500	5300	29900	58500	849	983
5	Mod	Growth	Fixed	19300	400	4300	35300	59300	866	987
6	Mod	Growth	Growth	14400	2400	7200	35300	59300	840	959
7	Low	Fixed	Fixed	22073	514	4478	29756	56820	812	951
8	Low	Growth	Fixed	18408	374	3576	35317	57675	829	956
9	Low	Growth	Growth	13400	2200	6700	35300	57600	829	927

Figure 1. MORRIS model national UK level predictions for patient numbers on various modalities and associated costs for year end 2013 and 2018, with variations in take-on, transplant or home dialysis rates indicated (CHD centre HD = satellite or hospital, HHD home haemodialysis). The scenarios described 1-9 are based on: Fixed referring to 2008 levels, transplant growth = 50% increase in 5 years, home therapies growth = 10% HHD & 30% PD. High take-on rate represents 2.8% average growth reported by UK RR from 2001-2008. Moderate take-on rate represents 1.6% growth, including demographics + risk factors. Low take-on rate of 0.8% growth represents demographics alone (age and ethnicity).

Conclusions: The MORRIS model is a powerful interactive tool for predicting future RRT requirements in the UK, demonstrating the benefits of investment in transplantation and home dialysis for example, with the ability to use the model at both national and renal unit level.

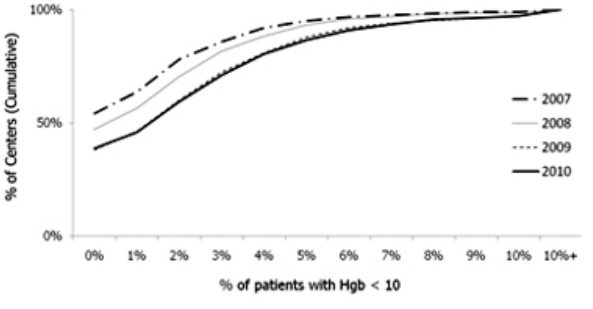
SA-PO2639

Impact of Baseline Year on PPS Payments under the Quality Improvement Program Andrew Barba, Randy Smith, Joe Weldon, LeAnne Zumwalt, Mahesh Krishnan. *DaVita Inc, Denver, CO.*

Background: In 2011, the CMS issued a final rule creating a Quality Incentive Program (QIP). Beginning in 2012, QIP penalizes dialysis facilities for underperformance by reducing payments up to 2%. QIP uses 3 weighted quality measures to generate a Total Performance Score (TPS): % of patients (pts) with hemoglobin (Hb) >12 g/dL, Hb <10 g/dL and urea reduction ratio (URR) ≥65%. The TPS compares a center's 2010 quality measures to the least stringent benchmark of either (i) the center's performance in 2007 (base year) or (ii) the 2008 national average. To assess the validity of the TPS, we determined the relative 2012 revenue impact of the QIP using the 2007 base year, and compared results to those using either 2008 or 2009 as the base year.

Methods: To model payments under QIP effective January 1, 2011, we estimated relative QIP impact per treatment for 1,682 centers using fiscal year (FY)10 claims from the DaVita database and current TPS calculations for Hb and URR. To reflect the CMS projected FY12 market basket update, we increased resulting estimates by 1.4%. We assessed QIP penalties for each facility according to their respective TPS. Facilities with insufficient clinical data were assigned an average TPS of 24 out of 30 possible points.

Results: The proportion of facilities that underperform using the <10 g/dL QIP measure is sizeable and differs by year (Figure). Between-year differences were due mainly to differences in Hb performance.



Conclusions: Lack of year-to-year consistency, particularly in anemia outcomes, throws into question the validity of the use of prospective payment quality measures that are calculated on temporally distant base years. Our results illustrate the need to modify QIP to use a baseline period as close as possible to the measurement period.

Funding: Clinical Revenue Support

SA-PO2640

The End-Stage Renal Disease (ESRD) Prospective Payment System (PPS) and Access to Care: Incremental Distance Traveled by Displaced Patients

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Background: The ESRD PPS will reduce payments to some dialysis facilities by more than the 2% as predicted by CMS and could lead to facility closures. Our objective is to estimate incremental distances patients may need to travel in the event of reduced access to dialysis care.

Methods: We estimated facility risk of closure/consolidation due to financial stress based on payment reduction under the PPS using the CMS flat file of PPS payment changes and a 10-point scale of ability to respond incorporating financial (e.g. payer mix), patient (e.g. case mix) and facility factors (e.g. ownership type). We categorized 5114 facilities in the 48 contiguous US as high, medium or low risk based on their combined scores. We estimated effects assuming a random sample of 10% of the high-risk group closes/consolidate from 2011-2013 (N=156). We assumed patients would migrate to the next-nearest facility and calculated incremental road miles and travel times.

Results: The incremental distance and time due to closure/consolidation of a small random sample of high-risk facilities could cumulate to 20,828,330 miles and 387,130 hours over a three-year period. By 2013, mean incremental travel distance would be 1084 miles per-patient-per-year (PPPY), nearly double for those in non-urban areas.

Incremental Distances Traveled if 10% of At-Risk Facilities Close/Consolidate 2011-2013

	2011	2012	2013	Total
Facility Closures % (N)	3% (48)	3% (48)	4% (60)	10% (156)
Displaced Patients				
Total	3453	6801	10,251	11,994
Rural/Suburban	1384	3081	4,376	5,135
Urban	2069	3720	5875	6859
Mean Incremental Miles Traveled Per Treatment (Round-trip)				
Total	10.7	12.0	11.8	11.7
Rural/Suburban	17.0	19.7	20.9	20.0
Urban	6.4	5.4	4.5	5.1
Mean Incremental Miles PPPY				
Total	754	1047	1084	961
Rural/Suburban	1219	1741	2008	1656
Urban	443	471	395	436

Conclusions: Prior research by DOPPS showed that reduced access as a result of increased driving distances may be associated with increased mortality and morbidity. Increased travel could have significant implications for clinicians and policy makers especially in rural/suburban areas.

Funding: Pharmaceutical Company Support

SA-PO2641

Urban Emergency Department (ED) Visits and Costs for Patients with End-Stage Renal Disease (ESRD), 2005-2009

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Background: Hospitalization of ESRD patients cost Medicare $35 billion in 2004-2008, not counting ED costs. Very little is known about the cost and utilization patterns of ED services by patients with ESRD.

Methods: We explored ED use and costs in a poor city, Camden, NJ, using a database of all ED contacts citywide, 2005-2009. We included only ED visits that did not result in hospitalization. We defined ESRD by ICD-9 code 585.6. We excluded patients less than 18 years old.

Results: Patients with ESRD accounted for 0.6% of the total patients in the study. The results are shown in Table 1 below.

Table 1

	Non ESRD	ESRD	P value
Unique patients	78219	488	
Total visits	289764	2876	
visits/patient	2(1-4)	4 (2-7)	<.001
charges/visit	$3539 ($1581-$7792)	$8980 ($3442- $17573)	<.001
receipts/visit	$372 ($0-$1130)	$1082 ($458-$2348)	<.001

*Mann-Whitney U test; Median [inter-quartile range]

We were also able to identify a subpopulation among the ESRD group as "super utilizers". We arbitrarily defined "super-utilizers" as patients with at least 10 ED visits in the 4 years. There were 71 such ESRD patients (14.5% of ESRD patients). They accounted for 1,475 (51%) of visits. Their median charge per visit was $36,899, 5.9 times that of the other ESRD patients. These super utilizers accounted for about 49.3% of the total charges and 46.5% of the total receipts. Acute dyspnea was the modal primary diagnosis for super-utilizers; the remaining patients did not have any one modal primary diagnosis in significant counts. Primary payers for "super-utilizers" were Medicare 47%, Medicaid 36%. For other ESRD patients, Medicare was the primary payer for 58%, Medicaid for 26%.

Conclusions: Thus, in this poor city, ESRD patients are disproportionate users of ED services, and their visits are costlier. A small minority account for the majority of visits and costs. Future interventions will target "super-utilizers" to diminish ED use by meeting their needs more cost effectively.

SA-PO2642

Healthcare Utilization and Costs in Patients with End-Stage Renal Disease Beginning Renal Replacement Therapy with Peritoneal Dialysis Versus Hemodialysis

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Background: To compare healthcare utilization and costs over 1 year between patients beginning renal replacement therapy with peritoneal dialysis (PD) versus hemodialysis (HD).

Methods: This retrospective study was conducted at a large US urban integrated health plan that maintains comprehensive records on the use of healthcare services by all of its members. Study subjects consisted of all persons aged 18-63 years who initiated PD or HD for end-stage renal disease (ESRD) between January 1, 2005 and December 31, 2008 ("study period"). Each patient's earliest noted date of receipt of dialysis was designated his/her "index date", and all patients were followed for 1 year thereafter. Each PD patient was matched to 2 HD patients using propensity scoring to control for differences in baseline characteristics, including age, gender, race, and pretreatment healthcare utilization. Healthcare utilization and costs were then compared over 1 year between matched PD and HD patients, using methods appropriate for matched samples.

Results: We identified a total of 162 patients who began dialysis during the study period (PD, n=26; HD, n=136). After matching, the final study sample included 26 PD patients and 52 HD patients. PD and HD patients were well-matched. Mean age of study subjects was 55 years, 58% were men, and 74% were African American. During the 1 year follow-up, HD patients were more likely to be hospitalized (67.3% vs 42.3% for PD; p=0.03). HD patients also averaged more physician office visits (17.2 vs 12.4, respectively; p=0.01) and emergency room visits (2.1 vs 1.1; p=0.04) over this period. Mean total healthcare costs over 12 months were significantly higher among HD than PD patients ($188,340 vs $127,981; p=0.01).

Conclusions: In patients initiating renal replacement therapy for ESRD, healthcare utilization and costs over 1 year may be lower in those receiving PD.

Funding: Pharmaceutical Company Support

SA-PO2643

Key Performance Indicators (KPIs) for Peritoneal Dialysis in Thailand: A Nationwide Survey

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Background: Implementation of the "Peritoneal Dialysis-first (PD first)" policy, mandating PD as a first modality of RRT for ESRD patients under universal health coverage since year 2008, leads to rapid growing of PD cases and centers in Thailand. To ensure quality of PD service paralleling the burst of PD cases, nationwide survey of key performance indicators (KPIs) was performed.

Methods: All PD centers in Thailand were invited to participate in the present study. PD nurse case managers in each center were asked to review medical records of all patients undergoing PD during October 1, 2009 to September 30, 2010 and submitted data to the main investigators

Results: About three-fourths of all PD-centers in Thailand (88 out of 121) participated in the present study. One hundred five nephrologists and 154 PD nurse specialists served 7,339 PD (279 APD and 7,060 CAPD) patients in these centers. Overall annual dropout rate was 24.8% and mortality rate was 17.1%. Nephrologist/patient ratio was 1:58, and certified PD nurse/patient (N/P) ratio was 1:85. Of these, 33.7% had N/P ratio > 1:60, while 66.5% and 41.9% had no dietitian and no nephrologist, respectively in their center. Overall exit-site infection rate was 1 episode/ 40.7 pt-mo (0.30 episodes/pt-yr) while overall peritonitis rate was 1 episode/ 25.8 pt-mo (0.47 episode/pt-yr). Of interest, larger (>100 cases), community-based, PD nurse-overloading, non-nephrologist coverage centers had higher peritonitis (0.56 *vs.* 0.39; 0.67 *vs.* 0.46; 0.53 *vs.* 0.37; 0.46 *vs.* 0.35 episodes/pt-yr, respectively). Culture negative was 32.1%. Almost patients (90.4%) received pre-dialysis education and the home visit rate was 37.82%.

Conclusions: Despite of the rapid growth of PD cases under the limited resource, the PD-related infection rates in Thailand are only small degree behind the goal of Asia-Pacific Key Performance Indicators (KPIs) Task Force.

Funding: Government Support - Non-U.S.

SA-PO2644

Association between Mode of Renal Replacement Therapy and Employment Status

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Background: It is poorly known how mode of renal replacement therapy affects probability of being employed. We analyzed the association of treatment modality with employment status among patients on dialysis and after transplantation in a cross-sectional study.

Methods: The employment status of all prevalent 15–64-year-old dialysis and kidney transplantation patients in Finland at end of 2007 (N=2637) was analyzed by combining the data from Finnish Registry for Kidney Diseases with the official individual-level

employment statistics of the Finnish government. Association between treatment modality and employment status was studied using multivariate logistic regression with adjustment for age, gender, cause of ESRD, duration of ESRD, and comorbidities.

Results: Altogether 19% of hemodialysis patients, 31% of peritoneal dialysis patients, and 40% of patients with a functioning transplant were employed. In adjusted analysis, patients on home hemodialysis (odds ratio 2.41), automated peritoneal dialysis (OR 3.48) or those with a kidney transplant (OR 3.14) were more likely employed than in-center hemodialysis patients. Patients with type 1 or type 2 diabetes or amylodosis as the cause of end-stage renal disease had the lowest probability of employment (OR 0.26–0.32 as compared to glomerulonephritis). Patients aged 25–54 years were more frequently employed than those younger than 25 or older than 54. Gender did not predict employment. Among transplant recipients, a longer time since transplantation associated with higher employment in addition to the aforementioned factors.

Conclusions: Home dialysis modalities predicted higher employment of ESRD patients, also after transplantation. Diabetes patients were less likely to be employed.

SA-PO2645

Costs of Care and Major Clinical Events among Chronic Dialysis Patients with and without Treatment for sHPT: A Descriptive Study of Claims Data

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Background: Secondary hyperparathyroidism (sHPT) is highly prevalent in chronic dialysis (CD) patients, yet little data describing costs of care for sHPT exists.

Methods: We examined direct medical costs in US CD patients covered by commercial or Medicare insurance with and without treatment for sHPT in a retrospective cohort study of 6-years-CD patient data from MarketScan. All patients were ≥ 18 years old with ≥ 2 CD claims ≥ 30 days apart. sHPT patients were identified as ≥ 3 claims within 2 weeks for therapeutic doses of vitamin D analog(s) and/or ≥ 1 claim for cinacalcet. Patients were followed up from first claim (sHPT patients) or from comparable dialysis vintage (non sHPT patients) to inpatient (IP) death, end of continuous insurance enrollment, parathyroidectomy (PTX) or end of study period. We summarized mean (SD) per patient (PP) annual total (TL), IP, outpatient dialysis (OPD), outpatient other (OPO), outpatient pharmacy (MED) cost categories, and the mean (SD) PP cost per hospitalization.

Results: A total of 26,371 non sHPT and 15,556 sHPT patients met study criteria. Based on 6 months of data prior to first claim, sHPT patients were younger (mean age 61.4 (SD 14.3) vs 66.2 (SD 14.1)) and had higher percentage of black patients (20.3 vs 16.8) than non sHPT patients. PP sHPT TL costs were 20.8% higher than non sHPT PP TL costs. Higher OPD and MED costs contributes to the higher TL costs for sHPT patients. For all CD patents, cost per fracture ranged from $16,990 for non-hip non-vertebral to $23,339 for hip fractures and cost per cardiovascular (CV) event ranged from $13,481 for heart failure to $25,484 for peripheral artery disease.

	Mean (SD) Annualized PP Costs					Mean (SD) PP Cost per Hospitalization		
N=41,927	IP	OPD	OPO	MED	TL	CV	Fracture	PTX
Non sHPT (n=26,371)	$25,117 (458)	$60,040 (531)	$20,455 (204)	$4,659 (46)	$110,271 (820)	$21,914 (40,092)	$18,991 (24,929)	N/A
sHPT (n=15,556)	$24,816 (523)	$83,142 (770)	$17,815 (228)	$7,431 (50)	$133,204 (1,053)	$23,906 (41,027)	$22,332 (27,837)	$18,010 (14,742)

Conclusions: The observed higher TL costs for sHPT patients may be an important consideration for reimbursement policy decisions.

Funding: Pharmaceutical Company Support

SA-PO2646

Cost-Effectiveness of Treating Chronic Anemia with Epoetin Alfa among Hemodialysis (HD) Patients in the United States (US)

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Background: Economic constraints have introduced uncertainty regarding the value of partial correction of anemia using Epoetin alfa. We assessed the value of targeting hemoglobin (Hb) of 10 or 11g/dL vs. 9g/dL among Medicare patients on HD.

Methods: A Markov model was developed with 1-year cycles over a 5-year period. Model inputs included clinical (e.g. morbidity, mortality) and economic (e.g. drugs, outpatient, inpatient) factors adjusted to 2010 $USD and discounted 3.5% annually. Model assumptions were determined by previously published data and included no difference in mortality based on TREAT ESRD patients, a differential in hospitalization and health utility based on past clinical trials and observational data, and a dose-response curve constructed from 7 randomized trials. Outcomes included incremental cost per: quality-adjusted life year (QALY) gained and hospitalizations avoided. Sensitivity analyses (SA) were conducted to test assumptions of differential mortality and hospitalization rates by Hb level to assess uncertainty of the assumptions and outcomes.

Results: Compared to Hb targets of 10 or 11g/dL vs. 9g/dL the model results were cost saving in Hb targets of 10 or 11g/dL compared to higher costs and lower effectiveness in the Hb target of 9 g/dL. Over a 5-year period hospitalizations avoided were 0.89 resulting in cost offsets of $23,593 for 11 vs. 9g/dL and 0.51 resulting in cost offsets of $15,340 for 10 vs. 9g/dL. In SA, the cost-effectiveness for 11 vs. 9g/dL ($27,294 per QALY gained) and 10 vs. 9g/dL ($22,764 per QALY gained) persisted with equal rates of hospitalization.

Conclusions: Treating HD patients with Epoetin alfa for partial correction of anemia to Hb targets of 11 or 10 vs. 9g/dL may be cost-effective when taking into account new research. Further SA's are needed to confirm the robustness of the results when varying assumptions pertaining to mortality, hospitalization, and health-related quality of life.

Funding: Pharmaceutical Company Support

SA-PO2647

Physician Reaction to Spontaneous Rises in Transferrin Saturation and Serum Ferritin Levels in End Stage Renal Disease Patients

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Background: Ferric citrate is an investigational phosphate binder (PB) to treat hyperphosphatemia in dialysis patients (pts). A Phase II randomized placebo-controlled trial demonstrated a significant increase in both transferrin saturation (TSAT) and ferritin (FN) levels in treated pts. We sought to determine how physicians titrate epoetin alfa (ESA) and intravenous iron (Fe) in response to the changes observed in this trial.

Methods: We analyzed data from DaVita pts experiencing rises in TSAT (>10%) and FN (15-25%) (6/1/08-12/31/10). Pts were: ≥18 years old; prescribed a PB; on dialysis ≥120 d; without significant change in Fe or ESA dose (-30% to 10%) from the prior mo; and without change in PB type. Per session ESA and Fe use was compared for 60 d before and after index date and stratified by baseline use.

Baseline Fe			Baseline ESA (U)			All
		1-<2000	2000-<4500	4500-<9000	≥9000	
0 to <16.0 mg	ESA	+772.1 (1,484.0)	+660.5 (2,456.6)	+51.8 (2,662.9)	-818.2 (4,811.6)	+360.9 (2,751.4)
	Fe	-0.92 (5.75)	-0.98 (4.66)	-0.07 (6.68)	-0.23 (8.81)	-0.67 (6.13)
16 to <32.0 mg	ESA	+542.0 (1,485.4)	-51.0 (1,884.7)	-1,081.8 (2,675.0)	-1,328.6 (4,079.2)	-387.7 (2,635.9)
	Fe	-4.23 (8.53)	-3.76 (9.50)	-3.38 (8.78)	-4.54 (11.01)	-3.92 (9.37)
≥ 32.0 mg	ESA	+226.7 (1,197.6)	-274.0 (1,659.6)	-1,332.8 (3,174.6)	-3,080.7 (5,583.8)	-1,715.7 (4,288.3)
	Fe	-15.19 (16.05)	-13.16 (17.93)	-11.32 (17.36)	-17.33 (22.05)	-14.66 (19.65)
All	ESA	+616.1 (1,463.9)	+181.6 (2,121.5)	-812.8 (2,883.7)	-2,146.7 (5,124.8)	-500.2 (3,316.5)
	Fe	-3.87 (9.57)	-4.60 (11.43)	-4.75 (12.42)	-10.30 (18.85)	-5.79 (13.62)

Results: 2,037 concurrent rises in FN and TSAT were found. Over the 2 mo after index date, decreases in ESA (-500 U) and Fe (-5.79 mg) use were observed. ESA reductions were greater for pts at higher baseline ESA and Fe doses (-3080 U), indicating an interaction effect. No such effect was seen for Fe.

Conclusions: Physicians may respond to rises in TSAT and FN by reducing ESA and Fe doses, particularly in pts with high baseline use. If approved, ferric citrate may reduce medication usage and have incremental economic value within the 2014 Medicare bundle.

Funding: Pharmaceutical Company Support

SA-PO2648

Erythropoiesis-Stimulating Agents (ESAs) and Mortality in Hemodialysis Patients. Relationship among ESAs Dose, Hemoglobin, and C-reactive Protein Concentrations. A 36 Months Observational Study

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Background: ESAs responsiveness and inflammationis are associated, leading to increase ESAs dosage (ESAdose) in order to reach hemoglobin (Hb) targets. While the relationship between inflammation, evaluated by C-reactive protein (CRP), and mortality is known, the relationship between ESAdose and mortality is not. Aim of the study: prospective evaluation, over a period of 36 months, of the role of CRP, ESAdose, and Hb on mortality in chronic hemodialysis (HD) pts.

Methods: 100/116 prevalent HD pts (mean age 68±13 years, 58 men, dialysis vintage 12-408 months) on ESA therapy, were enrolled and followed-up for 36 months. Demographic, co-morbidity, laboratory data, and causes of death were recorded. ESAdose (U/Kg/W), Hb (g/dL), serum ferritin (F) (ng/mL), %TSAT, CRP (mg/L) were measured at the start, and at the end of the study, or at death (on average 15±9 days before the event). Data are expressed as mean±SD, Linear and multiple regression analysis were employed. Survival was evaluated according to Kaplan-Meier analysis and Cox regression analysis. $p < 0.05$.was considered significant.

Results: CRP was 8.3±6.1 mg/L, Hb 11.2±0.6 g/dL, %TSAT 32±16%, F, 578±404 ng/mL, ESAdose 131±36 U/Kg/sett. 39 pts died during follow-up. The causes were: 51% cardiovascular, 30% infective, 19% neoplastic. Mortality was directly correlated either with CRP ($p<0.0001$), or with ESAdose ($p< 0.0001$). Kaplan-Meier survival analysis by dividing pts according to CRP quartiles (< 3.3; 3.3-6.5; 6.6-12.3; >12.3 mg/L) and ESAdose quartiles (< 104; 104-130; 131-157; > 157 U/Kg/W) showed that the higher values of CRP and ESAdose were associated with the higher mortality risk ($p <0.001$ for both variables). Cox regression analysis showed that CRP and ESAdose were independent significant factors associated with increased risk of desth(CRP: $p<0.0001$; ESA dose: $p< 0.001$).

Conclusions: High CRP levels and high ESAdoses, but not Hb concentrations, are the parameters that independently are associated with an increased mortality risk in HD pts.

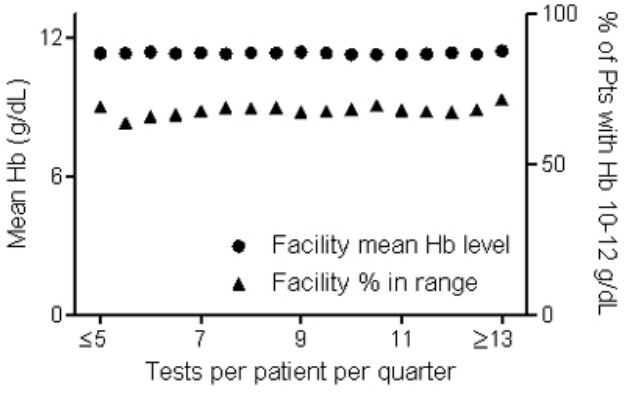
SA-PO2649

Association of Frequency of Hb Testing on Anemia Outcomes Irina Goykhman, Carey Colson, Steven M. Wilson, David B. Van Wyck. *DaVita Inc, Denver, CO.*

Background: A paucity of information about the optimal hemoglobin (Hb) testing frequency required to achieve target anemia outcomes likely contributes to wide practice variation in the frequency of orders for lab tests within the health care community. However, the medical resource consumption that arises from frequent lab testing has gained increasing consideration with the recently implemented US end-stage renal disease (ESRD) prospective payment system. We assessed facility-level Hb testing patterns and their impact on anemia outcomes.

Methods: We reviewed 2010 data from a large US dialysis provider's database to categorize dialysis facility Hb testing patterns as well as the percentage of patients with Hb levels in range (between 10 and 12 mg/dL). We categorized facilities by the mean number of reported Hb lab tests per patient dialyzing in that facility per quarter. We used patient-weighted Generalized Linear Models (GLM) to predict the percent of patients in range and mean Hb levels from number of Hb tests.

Results: The frequency of Hb testing based on physician ordering preference varied from 2.5 to 19.5 tests/quarter. Most (96%) facilities tested Hb 6-12 times per quarter, accounting for 97% of patients. Despite this, mean Hb levels did not vary significantly with greater Hb testing frequency (p=0.28; Figure).



Conclusions: When examined over a range from weekly to monthly, Hb test frequency shows no discernable relationship to Hb outcomes. This finding bears directly on the design and evaluation of anemia management protocols.

Funding: Clinical Revenue Support

SA-PO2650

Association of Erythropoietin Dosing and Mortality in the Maintenance Pediatric Dialysis Population Rachel M. Lestz,[1] Barbara A. Fivush,[2] Meredith A. Atkinson.[2] *[1]Pediatric Nephrology, Children's Hospital of Los Angeles, Los Angeles, CA; [2]Pediatric Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: Treatment with exogenous erythropoietin (EPO) has transformed the management of anemia in patients undergoing maintenance dialysis therapy. In recent years, the effort to normalize hemoglobin levels with increasing does of EPO has revealed an association of higher EPO doses with adverse events and increased mortality in the adult CKD and dialysis populations. However, this relationship has not yet been documented in pediatric patients.

Methods: Retrospective study of prevalent pediatric (age≤ 18 years) dialysis patients receiving maintenance EPO, using the Centers for Medicare & Medicaid ESRD Clinical Performance Measures Project 2005 linked with the USRDS mortality records for the following year. Four EPO categories were created based upon the distribution of EPO dosing (unit/kg/week): 0-25th %: 0-<92 u/kg/wk), 25-50th:92-<192 u/kg/wk(reference group), 50-75th%:192-<356 u/kg/wk , >75th %: ≥ 356 u/kg/wk. Cox-proportional hazards regression was performed to determine if EPO dose category was associated with an increased risk death over 12 months. The adjusted model controlled for race, age, gender, diagnosis, hemoglobin ≥11, albumin ≥3.5 (BCG)/3.2 (BCP), dialysis modality and access type, Kt/V and time on dialysis.

Results: Of the 1014 children included in the analysis, 35 died during the 12 month observation period. In the adjusted analysis, pts in the highest EPO category had a 4.2 times higher risk of death compared to those receiving 92-<192 units/kg/wk (25th-50th%) EPO dosing.

Adjusted Risk of Mortality

EPO Category	Hazard Ratio	95% CI	p-value
0-25th%: 25th-50th%	1.26	0.25-6.36	0.78
50-75th : 25th-50th%	3.07	0.85-11.12	0.09
>75th : 25th-50th%	4.21	1.12-15.10	0.03

Conclusions: Although mortality in general is a relatively rare outcome among children compared to adults on dialysis, we have shown that pediatric dialysis pts. who received the highest EPO doses demonstrated an increased risk of death. Further study, including prospective analyses, are required to determine whether the higher EPO doses have an independent causal affect in dialyzed children.

SA-PO2651

Evaluation of the Predictive Value of Commonly Used Anemia Management Indicators in Incident Hemodialysis Patients Jochen G. Raimann,[1,2] Len A. Usvyat,[1,2] Stephan Thijssen,[1,2] Peter Kotanko,[1,2] Nathan W. Levin.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center.*

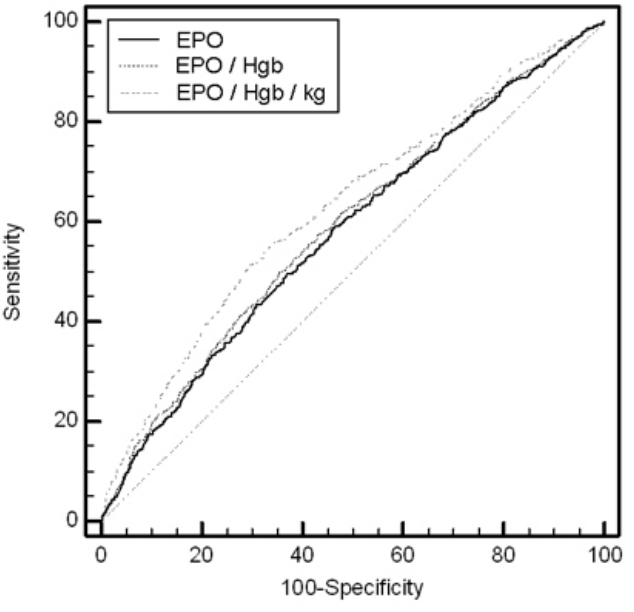
Background: Adequate anemia management improves survival and hospitalization rates (Locatelli 2004) in hemodialysis (HD) patients (pts). Erythropoietin (EPO) resistance, reflected by the ratio of EPO dose per hemoglobin (Hgb) concentration, was associated with inflammation (Stenvinkel 2002) and survival (Kilpatrick 2008). The best predictor of survival in terms of anemia management is controversial. This analysis aims to compare the predictive performance of different indicators.

Methods: Pts starting HD between 1/1/2001 and 7/30/2008 in RRI clinics were included. Average EPO dose per treatment was computed over the first 3 months of HD and survival monitored over one year. Predictive power of 1) EPO dose per treatment, 2) EPO dose per treatment per g/dL Hgb and 3) EPO dose per treatment per g/dL Hgb per kg of body weight (BW) was assessed as the area under the curves (AUC) of the respective receiver operating characteristics (ROC) curves. Youden index was employed to compute optimal discriminatory thresholds; positive and negative likelihood rations (LR+; LR-) were calculated.

Results: 6580 incident HD patients (56% males, 44% black, 47% white, 50% diabetic, age 61.5±15.4 years) were studied. Mortality rate was 92.6 per 1000 pts per year. AUC differed between the respective ROC curves (P<0.0001; **Figure 1**): (1) EPO dose per treatment: 0.58 (95% CI 0.57 to 0.59); (2) EPO dose normalized to Hgb: 0.59 (95% CI 0.58 to 0.60); (3) EPO dose normalized to Hgb and BW: 0.63 (95% CI 0.62 to 0.64).

Average EPO per g/dL per kg of BW was 11.4 (95% CI 11.2 to 11.6); a threshold of 12.9 allowed to predict mortality (LR+ 1.7; LR- 0.7).

Conclusions: These data suggests that the normalization of EPO doses to Hgb and BW has the most predictive power. This result supports the use of ERI analyses of survival and anemia management.



SA-PO2652

Outpatient (OP) Red Blood Cell (RBC) Transfusion Payments among Patients on Chronic Dialysis Matthew Gitlin, Shaowei Wan, Xue Song, Jeffrey L. Carson, David M. Spiegel, Brian Custer, Akhtar Ashfaq, Helen V. Varker, Katherine A. Cappell.

Background: Estimate OP RBC transfusion payments among chronic dialysis patients.

Methods: This retrospective economic analysis is based on a conceptual model of transfusion associated resource use to estimate OP RBC transfusion payments in dialysis patients using MarketScan data (1/1/02-10/30/10). All patients had ≥ 2 chronic dialysis claims ≥ 30 days apart and ≤ 1 year (1st claim defined as index date), ≥ 6 months pre-index data to measure comorbidities, and ≥1 OP RBC transfusion and ≥30 days post-transfusion follow-up. Total payments per RBC transfusion episode included pre/post screening/monitoring (+/- 3 days), blood acquisition/administration (within 2 days) and associated complications (acute within 3 days, e.g., circulatory overload, acute lung injury and hyperkalemia; 45 days for chronic, e.g., hemolytic reaction).

OP RBC Transfusion Payments:

	Mean (SD)	Median (25%, 75%)
Per Episode per Patient ($) (N=7049)		
Screening/Monitoring	191 (616)	34 (0, 179)
Blood Acquisition/Administration	615 (1237)	289 (11, 801)
Complications	175 (4790)	0 (0, 0)
Total	951 (5038)	427 (54, 1074)
Per Unit ($) per Episode (N=340)		
Screening/Monitoring	244 (425)	117 (0,341)
Blood Acquisition/Administration	433 (495)	374 (0, 608)
Complications	156 (1918)	0 (0, 0)
Total	827(2127)	551 (163, 999)

Results: A total of 3283 patients were included in this preliminary analysis, with a mean age of 60.9 (SD 15.0), 56.4% men, and 40.9% with Medicare supplemental insurance. Mean Charlson comorbidity index was 4.3 (SD 2.5). During a mean length of follow up of 552 days (SD 503), a patient on average had 3.1 (SD 4.3) OP RBC transfusion episodes. In the subgroup who had specific procedure claims for RBC unit level data of blood acquisition/administration, the mean (median) number of units per transfusion episode was 1.1 (1).

Conclusions: Preliminary results show payments for OP RBC transfusions are driven by blood acquisition/administration, followed by screening/monitoring. Although infrequent, transfusion complications increase costs substantially when they occur.

Funding: Pharmaceutical Company Support

SA-PO2653

A Comparison of Projected ESRD Incidence and Prevalence with Recent Data David T. Gilbertson,[1] Craig Solid,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: The USRDS published projections of the ESRD population to 2015 in 2005, and updated these projections to 2020 in the 2009 Annual Data Report (ADR). A primary message of those projections was that while incident rates of ESRD were slowing in many age and race groups, counts continued to increase. Here we compared actual incidence and prevalence numbers with those projected in the 2009 ADR that used information available through 2007.

Methods: A non-stationary Markov model was used, incorporating census projections and expected changes in both demographics and diabetes prevalence, to project ESRD incidence and prevalence through 2020. USRDS data through 2007 were used to obtain transition probabilities as well as past incident and prevalent counts.

Results: Incident counts were virtually constant from 2006-2008, but increased 3.3% in 2009, somewhat larger than projected. Prevalent counts have been consistently increasing approximately 4% per year, and are also ahead of projected numbers. Using data through 2007 and assuming relatively constant incidence rates based on recent trends, the model consistently projected increasing incident/prevalent counts through 2020.

ESRD Projected and Actual Incidence/Prevalence

	Incidence		Prevalence	
	Projection	Actual	Projection	Actual
2006		111,110		508,461
2007		111,193		528,666
2008	113,198	112,664	545,128	549,265
2009	115,295	116,395	563,173	571,414
2010	117,425		581,352	
2015	129,555		676,343	
2020	142,858		774,386	

Conclusions: Modeling assumptions were generally conservative, assuming flattening or even decreasing incident rates in some age/race groups. However, the post WWII population bulge is beginning to move into age groups with increasing chronic disease and increasing numbers of patients requiring RRT are inevitable. If incident rates increase in some groups and ESRD death rates continue to decline, the total number of patients requiring RRT will exceed these projections. This increasing population, along with changing incentives under the Medicare payment bundle implemented in Jan 2011, may increase the use of home therapies in the dialysis population.

Funding: NIDDK Support

SA-PO2654

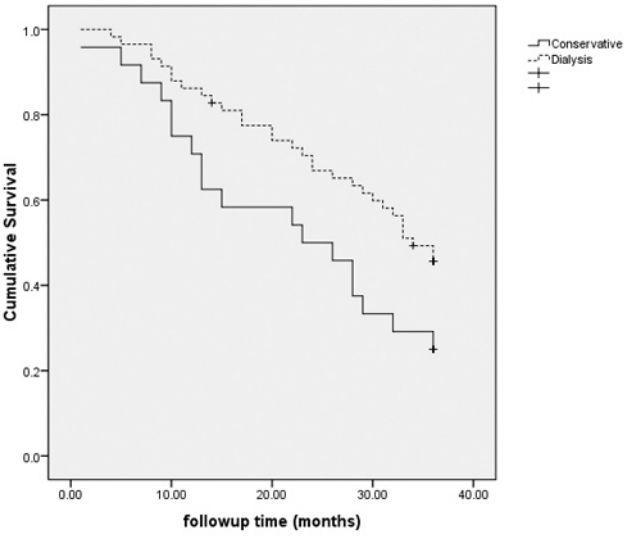
Does Dialysis Improve Survival in Elderly Patients? Darren Green, Philip A. Kalra. *Vascular Research Group, Manchester Academic Health Sciences Centre, University of Manchester, Salford Royal Hospital, United Kingdom.*

Background: It is unclear whether dialysis provides significant survival benefit to elderly CKD patients. Dialysis planning begins when patients reaches stage 5 CKD. It would be of benefit to be able to give prognostic information from this point.

Methods: We undertook a prospective observational study of outcome in patients who reached CKD stage 5 aged >75 years. Patients were sorted by whether they had chosen dialysis or conservative care, and were followed for 3 years. Patients were excluded if they suffered from malignancy, end-stage heart failure, or dementia.

Results: 82 patients were included (24 conservative care, 44 hemodialysis, 14 peritoneal dialysis). Dialysis patients were younger (79.4 vs. 83.4 years, p=0.000) and less likely to have coronary artery disease (21.6% vs. 45.5%, p=0.016). There was no difference in blood pressure, diabetes, heart failure, COPD, Karnofsky performance score, weight, smoking or alcohol intake. Patients who chose conservative care tended to be those who were widowed (40.0% vs. 10.4%, p=0.40) or lived alone (45.5% vs. 11.9%, p=0.36). 3 year survival was 45.6% for dialysis patients versus 25.0% for conservative care.

Figure 1. Survival in stage 5 CKD: dialysis versus conservative care.



This survival difference was independent of age. Patients who chose hemodialysis spent more time in hospital and underwent more operations that peritoneal dialysis or conservative care patients.

Table 1. Days spent in hospital per year by dialysis modality choice.

	Conservative	Hemodialysis	Peritoneal
Inpatient	15	27	19
Outpatient	8	64	20
Operations	0.0	1.4	0.7

Outpatient days include hemodialysis sessions. Operations included all dialysis access. Values are annual mean over the 3 years.

Conclusions: This study shows that there may be a survival benefit to choosing dialysis over conservative care in elderly patients with stage 5 CKD. Choosing dialysis appeared to be as strongly dependent on social factors as medical ones.

SA-PO2655

Exploring Preventable Hospitalizations of Dialysis Patients John R.C. Wheeler,[1] Richard Hirth,[1] Kathryn Meyer,[2] Joseph M. Messana.[1] *[1]University of Michigan, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Background: Hospitalization rates are high (1.9 per pt yr) among ESRD pts. We investigated whether some types of hospitalization could be prevented by high quality dialysis care.

Methods: To identify causes of hospitalizations that may be candidate quality measures, we identified the most frequent diagnoses (dx) among ESRD pts and the frequency of dx deemed potentially preventable by AHRQ, i.e., Prevention Quality Indicators (PQIs). Medicare inpatient claims data for admissions in 2008 among ESRD pts were used. Admissions and time at risk were counted during time when the pt had Medicare as their primary payer (n=316,066 pts, with 238,266 yrs at risk and 447,972 admits).

Results: The most common discharge dx among ESRD pts were: (1) comp of device, implant or graft; (2) congestive heart failure (CHF); and (3) hypertension (HTN). The most common PQI dx were: (1) CHF; (2) DM long term complications; and (3) lower extremity amputation. AHRQ potentially preventable admissions comprised over 18% of all hospitalizations for ESRD pts, compared to 15% for all Medicare beneficiaries. Further, some non-PQI dx common among ESRD pts, such as comp of device, graft, or implant, may reflect preventable hospitalizations among ESRD pts. Finally, there is a high degree of variation across facilities in hospitalization rates for PQI dx (CHF inter-quartile range is 4% to 17%).

PQI Hospitalizations Among ESRD Medicare Primary ESRD Pts in 2008

Potentially Preventable Admission Indicator	N	% of Admits	Admits per 1,000 Enrollees
CHF	28,158	6.67	89.1
DM long term comp	17,650	4.18	55.8
Bacterial pneumonia	15,225	3.60	48.2
Lower extremity amputation	7,081	1.68	22.4
UTI	4,174	0.99	13.2
COPD	3,213	0.76	10.2
Dehydration	2,293	0.54	7.3
DM short term comp	2,071	0.49	6.6
Adult asthma	1,236	0.29	3.9
Angina	571	0.14	1.8
HTN	431	0.10	1.4
DM uncontrolled	355	0.08	1.1
Perforated appendix	153	0.04	0.5

Conclusions: PQI Hospitalizations account for more than 18% of admissions among ESRD pts. Further research may identify those potentially preventable hospitalizations that may reflect specific practice patterns in the dialysis setting, not currently identified using the AHRQ-PQI preventable hospitalization methodology.

Funding: Other U.S. Government Support

SA-PO2656

Hospital 30 Day Readmission Rates in Spanish Versus English Speaking End Stage Renal Disease Patients in a Medical Practice That Has Implemented An Ambulatory Care Language Concordance Program Mateo Levine Ledezma, Kwun-Yee T. Poon, Scott A. Rasgon. *Division of Nephrology and Hypertension, Kaiser Permanente, Los Angeles, CA.*

Background: Nationally there is a disparity in health outcomes in Hispanic compared with non-Hispanic patients with end-stage-renal-disease (ESRD)

Objective: To determine whether there is a difference in 30 day hospitalization readmission rates in Spanish vs English speaking ESRD patients in a medical group that has implemented a language concordance program.

Methods: We conducted a retrospective analysis of automated medical record database for admissions in Kaiser Permanente, Southern California hospitals in 2010 (n 366,225). Kaiser Permanente provides health care for more than 3.4 million patients, implemented an ambulatory care language concordance program in 2008. Non-English speaking patients where assigned primary care physicians fluent in preferred language (concordance 91.3%. The study cohort was adults (mean age 64.3) with a non obstetric admission (n 88,502); diagnosis ESRD (n 3,166), and Spanish (n 5,012). The demographics: gender (54% female), BMI (mean 29), diabetes (29%), and ethnicity (white 52%, black 15%, Hispanic 22%, and Asian 5%.

Results: Among study cohort patients who had an inpatient hospitalization in the measurement year (n 88,502), 9.8% had a second hospitalization within thirty days (n 8,617). Patients appeared older (mean 67), likely white (55%) or African American (17%), and tended to be English (vs Spanish) speaking (95.1%, $p<0.0014$). Patients with a diagnosis of ESRD (n 3,166), who had a 30 day readmission (n 679), tended to be white (35%) vs Hispanic (27%), male (56%), and older (67). The multivariate analysis of the independent variable in association with the outcome using logistic regression demonstrated Spanish versus English (OR 0.791, $p<0.0001$), gender female versus male (OR 0.914, $p<0.0001$), history of diabetes (OR 1.44, $p<0.0001$) and ESRD (OR 2.292, $p<0.0001$).

Conclusions: In a medical practice that has an ambulatory care language concordance program, Spanish speaking patients are 21% less likely to have a 30 day hospital readmission compared to English speaking patients, after adjusting for gender and history of ESRD.

SA-PO2657

Rehospitalizations in Prevalent 2009 Hemodialysis Patients Tricia L. Roberts,[1] David T. Gilbertson,[1] Craig Solid,[1] Allan J. Collins.[1, 2] *[1]USRDS Coordinating Center, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: Rehospitalizations contribute to the Medicare cost burden and indicate need for improved quality of care. Recent literature reported 30-day rehospitalization rates of nearly 20% among general Medicare patients; however, current rates among the dialysis population remain unknown.

Methods: We calculated rehospitalization rates in adult Medicare prevalent hemodialysis patients in 2009. Live hospital discharges were included from January 1 to December 1. We excluded rehabilitation claims, transfers, and discharges with a same-day admission to long-term care and critical access hospitals. Events were first rehospitalization and the combined endpoint of rehospitalization or death (rehos/death). Rates showed the percent of live discharges with an event within 30 days after discharge. Rates were computed by cause-specific groups for the discharge and rehospitalization. Rates from 1998 to 2009 were adjusted for age, gender, race, and primary diagnosis with direct adjustment.

Results: Results included 365,348 discharges from January 1 to December 1 in 2009. Overall, 36 and 39% of all-cause discharges were followed by an all-cause 30-day rehospitalization and rehos/death, respectively. The highest all-cause rehospitalization and rehos/death rates were among the youngest patients (age 20-44, 43 and 44%) and African Americans (38 and 40%). All-cause rehospitalization rates were highest after discharge from a cardiovascular hospitalization (37%), compared to infection (34%) and vascular access infection (31%). Among patients age 20-44, rehospitalization occurred after nearly half (47%) of discharges from a cardiovascular hospitalization. Cause-specific rehospitalizations were highest after a discharge with the same cause. Adjusted rehospitalization rates were stable from 1998 to 2009 and ranged from 35 to 36%.

Conclusions: Rehospitalization rates among hemodialysis patients were strikingly high: greater than one third of discharges were followed by at least one 30-day rehospitalization. Rates have not improved in the last decade, and identification of high risk groups, such as young patients and African Americans, could focus efforts to reduce rehospitalizations.

Funding: NIDDK Support

SA-PO2658

Long Term Renal Outcomes Following Autologous Stem Cell Transplant for Multiple Myeloma Siobhan Glavey,[1] Nelson Leung.[1] *[1]Nephrology, Mayo Clinic Rochester, Rochester, MN; [2]Hematology, Mayo Clinic Rochester, Rochester, MN.*

Background: Autologous Stem Cell Transplant (SCT) following high dose chemotherapy (HDC) has been established as optimal therapy for patients with multiple myeloma (MM) for over a decade. At our institution 19% of patients have a serum creatinine over 2g/dL at time of presentation with MM. The current literature pertaining to the outcome in terms of renal recovery following SCT is conflicting, particularly with regard to gaining independence from dialysis.

Methods: We conducted a retrospective analysis of the medical records of all patients undergoing SCT for MM between 2000 and 2010 at our institution. Inclusion criteria: Patients who had an elevation in serum creatinine (SCr) over 3mg/dLor were dialysis dependent at the time of SCT were selected. Exclusion criteria: Patients who had known chronic kidney disease preceeding the onset of MM were excluded.

Results: Fifteen patients (15/30) were found to have a serum creatinine over 3mg/dL in the pre-SCT period but were not dialysis dependent.Median SCr prior to SCT in this group was 3.5mg/dL. One month following SCT this increased to 6.1mg/dL.Four patients (26.6%) had progression to ESKD in the long term requiring dialysis. None of these patients regained independence from dialysis by time of death or last clinical review. Fifteen patients (15/30) were dialysis dependent at the time of SCT.Of these only one was able to attain independence from dialysis post SCT. This patient had improving renal function prior to SCT with an iothalamate clearance of 19ml/min and went on to discontinue dialysis 17 days after SCT.

Conclusions: Previous studies have indicated that SCT may have a favourable impact on renal outcome in MM.

We have not found this to be the case and on retrospective analysis of our experience in SCT for treatment of MM over ten years we found no benefit in terms of freedom from RRT or progression of renal pathology and impairment. Furthermore we found for patients who were close to commencing dialysis prior to SCT there was a 26.6% progression to ESKD requiring RRT in the long term. We propose caution prior to SCT in advising patients that there is likely to be an improvement in renal function.

SA-PO2659

Factors Affecting the Decision To Start Renal Replacement Therapy: Results of a Survey among European Nephrologists Moniek Van de Luijtgaarden, Marlies Noordzij, Wim Van Biesen, Christoph Wanner, Charles Tomson, Kitty J. Jager. *ERA-EDTA Registry Investigators, AMC, Amsterdam, The Netherlands.*

Background: The level of residual renal function is likely to determine the timing of start of renal replacement therapy (RRT).The patient's clinical status is suggested to play an *at least as* important role in this decision, but the importance of specific factors is unknown.We evaluated current opinions of European nephrologists on the decision making process on when to start RRT and whether opinions differed by nephrologist or facility characteristics

Methods: We distributed a web-based survey among nephrologists in 11 European countries with questions on the target level of renal function related to the start of RRT,factors bringing forward/postponing RRT,and nephrologist/facility characteristics.We used chi-square tests and multivariate linear regression to study associations and determinants of estimated glomerular filtration rate (eGFR)

Results: We received 433 completed surveys. Overall, renal function was not considered as the most important factor in the decision to start RRT, but for uncomplicated patients,54% of respondents did regard eGFR as the most important factor.The majority (88%) believed that a start at eGFR>10.5 mL/min/1.73m^2 was only beneficial if symptoms are present.Factors bringing forward the start of RRT were hyperkalemia (100%), pericarditis (98%) and fluid overload (97%). Patient preference (69%) and vascular dementia (66%) postponed the start.The median eGFR on which respondents aimed to start RRT in uncomplicated patients was 10.0 ml/min/1.73m^2 (IQR 8.0-10.0).We found higher target levels adjusted for confounders for respondents from countries with high vs low RRT incidence (eGFR 10.3 [9.8-10.8] vs 9.1) and from for-profit vs non-profit centers (eGFR 10.0 [9.4-10.7] vs 9.4;$p<0.05$)

Conclusions: Signs and symptoms rather than eGFR are important in the decision on when to start RRT. Nephrologists from countries with high RRT incidence and from for-profit centres aim to start at slightly higher eGFR levels. Although we gained insight in the decision making process, prospective studies are needed to further study current practice and its association with patient outcomes

SA-PO2660

The Rate of Wearing a Mask among HD Outpatients, Seroconversion Rates for Novel Influenza HA and Seasonal Influenza Vaccines, and Infection Status in the Pandemic between 2009 and 2010 Toru Hyodo,[1] Naoyuki Sato,[2] Daisuke Ishii,[1] Kazunari Yoshida,[1] Shiro Baba.[1] *[1]Urology, Kitasato University, Sagamihara, Kanagawa, Japan; [2]Nursing, Atsugi Clinic, Atsugi, Kanagawa, Japan.*

Background: To determine the rate of wearing a mask, seroconversion rates for novel and seasonal influenza vaccines, and rate of influenza infection in the pandemic of novel influenza (pandemic A[H1N1] 2009).

Methods: The rates of wearing a mask and the seroconversion status for A/Brisbane/59/2007(H1N1), A/Uruguay/716/2007(H3N2), and B/Brisbane/60/2008, which were used for inoculation in October 2009, and A/California/7/2009(H1N1) used for inoculation in December 2009 was compared before inoculation, and one month and six months after inoculation in 103 dialysis patients (average age: 64.0±13.4, mean dialysis history: 6.9±6.0 years, 75 men, 28 women, 42 diabetic patients, and 61 non-diabetic patients). Titers of at least 1:40 were within the safe range. The rate of influenza infection among HD patients was evaluated based on whether titers six months after inoculation were at least four times those one month after inoculation.

Results: Among the subjects, only 3.1±1.3% did not wear a mask between October 2009 and the end of April 2010. The proportion of subjects with titers of at least 1:40 for A/California was 8% before inoculation, 35% one month after inoculation, and 18% six months after inoculation, for A/Brisbane was 36% before inoculation, 45% one month after inoculation, and 38% six months after inoculation, for A/Uruguay was 24% before inoculation, 43% one month after inoculation, and 26% six months after inoculation, and for B/Brisbane was 25% before inoculation, 35% one month after inoculation, and 36% six months after inoculation. The rate of influenza infection was 1% (1 in 103 subjects, positive for type B).

Conclusions: It was suggested that the seroconversion rate for A/California used for inoculation between 2009 and 2010 was slightly lower than that for A/Uruguay or A/Brisbane. Given the seroconversion rates for vaccines among HD patients, wearing a mask appeared to be very effective because the number of infected subjects was extremely small.

SA-PO2661

Low Prevalence of Occult Hepatitis B Virus Infection in Chronic Hemodialysis and Kidney Transplant Patients Seema Baid-Agrawal,[1] Ralf Schindler,[1] Petra Reinke,[1] Ulrich Frei,[1] Thomas Berg.[2] *[1]Dept of Nephrology and Medical Intensive Care, Campus Virchow Clinic, Charite Medical University, Berlin, Germany; [2]Division of Hepatology, University Clinic Leipzig, Leipzig, Germany.*

Background: Occult hepatitis B virus (HBV) infection is defined as presence of HBV DNA in serum, liver or peripheral blood mononuclear cells (PBMC) in patients whose sera test negative for HBsAg. It may be a potential source of nosocomial transmission in chronic hemodialysis (CHD) patients and kidney transplant recipients (KTxR). This is the first study to date investigating its prevalence in large cohorts of these patients.

Methods: In this cross-sectional study, 391 patients undergoing CHD (Group 1), 417 NTxR (Group 2), and 20 HBsAg-positive non-HD non-KTx patients (positive controls, Group 3) and 40 HBsAg-negative healthy subjects (negative controls, Group 4) were enrolled.HBV DNA was determined in both serum and PBMC using Cobas TaqMan™ (Roche Diagnostics).

Results: Group 1 (CHD): 387/391 patients were HBsAg-negative (Group 1a), 4 were HBsAg-positive (Group 1b). The overall prevalence of HBsAg-positive infection was 1% (4/391). Occult HBV infection was found only in 1/387 (0.2%) HBsAg-negative patients and that also in serum and not in PBMC (HBV DNA-positive in serum). Group 2 (KTxR): 400/417 KTxR were HBsAg-negative (Group 2a), remaining 17 (4.1%) were HBsAg-positive (Group 2b). Occult HBV infection was found in 2/400 (0.5%) HBsAg-negative KTx recipients only in serum. Of note, HBV DNA was not detected in PBMC in any of CHD and KTx patients.

	HBV DNA positive in		
	serum only	both serum and PBMC	PBMC only
Group 1a (N=387)	1 (0.2%)	0	0
Group 1b (N=4)	1* (25%)	0	0
Group 2a (N=40)	2 (0.5%)	0	0
Group 2b (N=17)	4* (23.5%)	0	0
Group 3 (N=20)	5* (25%)	11(55%)	0
Group 4 (N=40)	0	0	0

* Most of the HBsAg+ patients were receiving anti-HBV therapy

Conclusions: We found a low prevalence (<1%) of occult HBV infection in CHD and KTx patients suggesting that laborious and expensive testing of HBV DNA in PBMC is not required for screening and diagnosis in these populations in our region. However, our results may not be generalizable to other populations with a higher prevalence of HBV where the role of occult HBV infection needs to be clarified.

Funding: Private Foundation Support

SA-PO2662

Antibodies to Hepatitis B Virus Surface Antigen and Interleukin 12b Gene Polymorphism in Hemodialysis Patients Alicja E. Grzegorzewska,[1] Piotr M. Wobszal,[1,2] Pawel P. Jagodzinski.[2] *[1]Department of Nephrology, Transplantology and Internal Diseases, University of Medical Sciences, Poznan, Poland; [2]Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznan, Poland.*

Background: In hemodialysis (HD) patients, interleukin (*IL*) *18* -1297CC rs360719 genotype is associated with development of antibodies to surface antigen (anti-HBs) of hepatitis B virus (HBV). IL18 shares biological properties with IL12 in promoting Th1 system. A predominant Th1 response may inversely affect the Th2 humoral response. Our aim was to check the association between *IL12B* 3' untranslated (UTR) region polymorphism and anti-HBs development in HD patients with respect to *IL18* promoter polymorphism.

Methods: Polymerase chain reaction restriction fragment length polymorphism was used to detect *IL12B* rs3212227 3'UTR A/C and IL18 -1297C/T rs360719 polymorphisms in 364 HD patients and 242 controls. HD patients were categorized by *IL12B* genotypes (AA n = 226 AC n = 126, CC n = 12). Anti-HBs development in response to HBV transmission or anti-HBV vaccination was compared in these groups.

Results: There were no differences in genotype frequencies of *IL12B* or *IL18* and a gene-gene interaction in all HD patients and controls, and in *IL18* genotype frequencies in HD patients categorized by *IL12B* genotypes. Positive antibodies to HBV core antigen (anti-HBc) without developed anti-HBs were the most frequent in *IL12B* CC carriers (62.5%), and this frequency was higher than that in AC (12.9%, p = 0.009) or AA/AC (24.7%, p = 0.036) carriers. Positive anti-HBc without anti-HBs could be predicted by *IL12B* CC genotype (OR 3.00, 95%CI 1.86-4.84, p = 0.039) or *IL12B* AC genotype (OR 3.60, 95%CI 1.11-11.7, p = 0.033). A gene-gene interaction between *IL12B* and *IL18* was of borderline significance in anti-HBc positive patients categorized by anti-HBs development (p = 0.09) or "isolated" pattern (p = 0.08). Anti-HBs development in vaccinated patients was not *IL12B* genotype dependent.

Conclusions: *IL-12B* CC genotype, occurring in 3.3% of HD patients, seems to be related to impaired development of anti-HBs in response to HBV infection, but not in response to anti-HBV vaccination.

Funding: Supported by Poznań University of Medical Sciences, grant No 502-01-01124182-07474.

SA-PO2663

Use of Hepatitis C Nucleic Acid Testing Improves Surveillance in a United Kingdom Prevalent Haemodialysis Population Virginia L. Prout, Richard W. Corbett, Alison D. Cox, Mark Atkins, Neill Duncan. *West London Renal and Transplant Centre and Department of Virology, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: The prevalence of Hepatitis C virus (HCV) infection is significantly higher in haemodialysis patients, with a wide disparity between countries. Though previous work supports the use of nucleic acid testing by polymerase chain reaction (PCR) for surveillance of infection in prevalent haemodialysis patients in areas with high levels of carriage, the evidence for its use in units with a lower prevalence is less clear.

Methods: Prospective data was collected over a twelve month period on all patients receiving maintenance haemodialysis across our satellite dialysis centres. In addition to monthly HCV antibody (3rd generation enzyme immunoassay- EIA) testing, four-monthly pooled samples from each unit were submitted for nucleic acid testing (HCV-RNA PCR). Each pool contained five patients, who were then re-tested individually in the presence of a positive pool result. Individuals known to carry HCV were not excluded.

Results: 1352 patients were tested on more than one occasion, in total 51 individuals (3.8%) were found to be either HCV-positive by PCR or EIA testing.

Prevalence of HCV antibodies and HCV-RNA in a prevalent haemodialysis population

	HCV-RNA (+) by PCR	HCV-RNA (-) by PCR
Anti-HCV (+) by EIA	42	7
Anti-HCV (-) by EIA	2	1307

All 7 patients who proved to be EIA+ / PCR- had at least two non-pooled negative HCV PCR results, separated by six months. All 44 patients found to be PCR+ have had non-pooled confirmatory testing and viral genotyping. The pooled screening program successfully identified the 42 EIA+ / PCR+ patients known to be viraemic prior to commencement of the study. The two EIA- /PCR+ patients had not been previously identified prior nor had had abnormal ALT levels and appeared to have longstanding infection with moderate viral loads.

Conclusions: Occult HCV infection is a problem even in low prevalence haemodialysis populations within the United Kingdom. Pooled HCV-RNA PCR surveillance allows efficient, robust and accurate identification of viraemic patients within a dialysis program.

SA-PO2664

Cessation of Renal Replacement Therapies with Improved Kidney Function in the ESRD Medicare Program Edwin D. Huff, Marianne Neumann, The FFBI Data Committee. *Fistula First Breakthrough Initiative, IPRO ESRD Network of NY, Lake Success, NY.*

Background: ESRD has meant "Loss of kidney function, with need for kidney replacement treatments", however, as evidence will show, some patients may not actually be afflicted with ESRD in the first place, and may instead be individuals suffering from types of acute kidney injury.

Methods: A total of 205,399 incident ESRD hemodialysis patients from 2008 & 2009 were tracked over two years in the CMS SIMs registry. Start dates were initialized with incident dialysis. Recovery, if reported, was marked from dialysis facility updates transmitted through ESRD Networks and entered into the SIMs central repository on a daily basis.

Results: The rate of renal recovery over two years was 6.67%, with median time to recovery 74 days (3.3% recovery rate). Approximately 75% recover within 120 days (4.9%). The rates of renal recovery in those years were 6.62% in 2008 and 6.69% in 2009. Significantly fewer patients were noted to have permanent accesses placed at incident dialysis, with over 94% utilizing catheters. The origin of their kidney diseases are similar, though more have Acute Tubular Necrosis. Patients who recover kidney function are half as likely (27% vs. 56%) to have had pre-dialysis nephrology consultations. Of those patients who were able to discontinue renal replacement therapies, 14% restart treatment within 2 years.

Conclusions: 13,673 patients over two years were reported to have regained adequate kidney function within 74 days on average. While many aspects of clinical presentation appear similar, there are noted differences suspected in disease onset and early treatment that

are consistent with many of these patients having acute kidney diseases. Fewer nephrology referrals and higher catheter rates are consistent with these cases having a shorter time horizon in their onset, not like those with chronic CKD patients.

Funding: Other U.S. Government Support

SA-PO2665

Workload Demand and Caseload Disparities of Dialysis Social Workers in the United States Joseph R. Merighi,[1] Teri Browne.[2] [1]*School of Social Work, Boston University, Boston, MA;* [2]*College of Social Work, University of South Carolina, Columbia, SC.*

Background: Every dialysis unit in the United States must have a Master's level social worker as part of its patient care team. This study examines regional variations in perceived workload demands and patient caseloads of these dialysis social workers. Studies indicate that high social worker caseloads are associated with decreased ability to provide psychosocial interventions that can help improve patient outcomes (Merighi & Ehlebracht, 2005).

Methods: A 130-item online survey was conducted to assess renal social workers' caseloads, job-related resources, and workload demands. 1,055 full-time social workers completed the surveys from all five National Kidney Foundation (NKF) regions: 1 (Northeast), 2 (Southeast), 3 (Midwest), 4 (South), and 5 (West and Southwest). Respondents were recruited between 3/31/10 and 6/21/10 using the Council of Nephrology Social Worker listserv.

Results: One-way ANOVA was used to examine mean workload demands and caseloads across all five NKF regions. Findings yielded significant main effects for caseload, $F(4, 1035) = 5.46$, $p = .001$, and workload demands, $F(4, 939) = 3.06$, $p = .016$. Post hoc tests revealed significantly higher mean workload demands in Region 1 (21.3) compared to Region 3 (20.1) and Region 5 (19.9), and Region 4 had a significantly higher mean caseload (125) than Region 3 (112). Geographic Information Systems (GIS) technology was used to map workload demands and caseloads using respondents' unique zip code boundary. The GIS maps display a distinctive pattern at the state level of high workload demands in Region 1 (esp. NH, MA, and CT) and high caseloads in Region 4 (esp. AR and MS).

Conclusions: This study represents an important national effort to assess dialysis social workers' workload demands and caseloads and document regional disparities. High caseloads may prevent social workers from helping patients ameliorate psychosocial barriers to optimal kidney disease outcomes. These findings also have implications for all dialysis units because high social work caseloads may lead to facility citations for failure to comply with the Medicare's Conditions for Coverage.

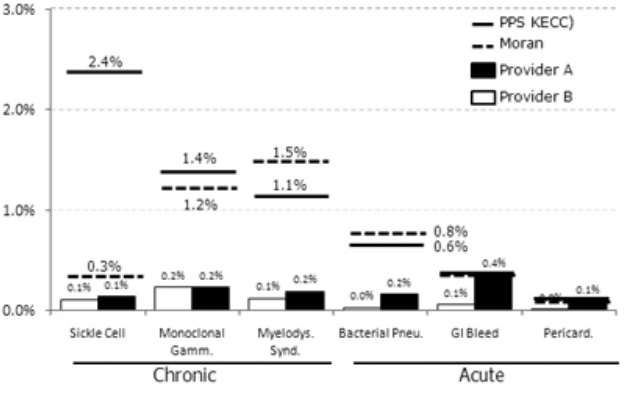
SA-PO2666

Case Mix Adjusters Continue To Be Difficult To Detect Tracy Jack Mayne, Jeremy Jonckheere, LeAnne Zumwalt, Mahesh Krishnan. *DaVita Inc, Denver, CO.*

Background: Beginning in 2011, the ESRD prospective payment system (PPS) increased the number of case mix adjusters (CMA) from 3 to 11, including 6 for co-morbid conditions. The additional CMAs were based on an analysis by the University of Michigan Kidney Epidemiology and Cost Center (KECC). Our objective was to replicate the prevalence reported by KECC in other currently available data sources.

Methods: The Moran Group (Moran) analyzed the 2008 5% Medicare Standard Analytic Files (SAFs) for the 6 CMA comorbidities in US dialysis patients. We searched for the ICD-9 codes for each adjustor as identified in the final rule, including non-nephrologist physician and hospital claims. In acute conditions, we added 4th quarter 2007 ESRD, physician, and hospital claims. In chronic conditions, we added all of 2007 ESRD, physician, and hospital claims. We then searched all available paper and electronic records at each of the two large dialysis organizations (Provider A and B) for documentable diagnoses of these 6 conditions.

Results: The prevalence of the 6 co-morbid case mix adjusters detected in the medical records of the two dialysis organizations was significantly lower than the prevalence reported by KECC (Figure). The Moran analysis of the Medicare 5% SAF found significantly less sickle cell disease, somewhat less monoclonal gammopathy, slightly more myelodysplastic syndrome and bacterial pneumonia, and equivalent GI bleed with hemorrhage and pericarditis.



Conclusions: The results demonstrate the difficulty for dialysis providers to document CMA prevalence at the level used to estimate the 2011 bundled rate. Were CMS to provide dialysis organizations with the prevalence of these conditions from the SAFs, this would correct the inequity in all areas except sickle cell disease.

Funding: Clinical Revenue Support

SA-PO2667

Actual Cost of Dialysis Drugs: A Step towards the Final Bundle Payment System in 2014 Megha Shah,[2] Michelle Malabanan,[2] Wajid M. Choudhry,[1] Vijay K. Jain.[1] [1]*Nephrology, Unity Health/University of Rochester, Rochester, NY;* [2]*Medicine, Unity Health System, Rochester, NY.*

Background: CMS have implemented the new ESRD bundled payment system on January 1, 2011. Medicare provides a single bundled payment of $229.63 per dialysis inclusive of the following:

1. Composite rate including labor to deliver dialysis and routine supplies
2. Part B ESRD injectable drugs and their oral Part D equivalent
3. ESRD lab. tests ordered by a nephrologist

The CMS however have delayed including Part D oral-only ESRD medications to the bundle base rate until 2014. There is insufficient data at a local and national level about the cost of these oral drugs.

Methods: We conducted a cross-sectional chart review of all patients in 2 hemodialysis units, one suburban (N=133) and one inner city (N=95) and the associated peritoneal dialysis program (N=35) in Rochester, NY. Our main objectives are to determine the actual cost of: 1) Part D ESRD prescribed oral-only drugs 2) injectable drugs and their oral equivalent. Data was collected over a 31- day period at these 2 hospital-owned dialysis units staffed by a single nephrology group. We determined average calcium and phosphorus levels, quarterly PTH levels and the last hemoglobin of the month to evaluate the effectiveness of current prescribing practices.

Results: The average patient age (66.6 years) is similar in all groups. Inner city unit had substantially higher black patients compared to the suburban unit (70.5% vs 23.3%). The calcium, phosphorus, PTH and hemoglobin control were similar in the three groups and were within the acceptable range (8.4-8.5 mg/dl, 4.8-5.1 mg/dl, 380-384 pg/ml, 11-11.3g/dl, respectively).

Cost per patient per dialysis	Suburban Unit	Inner City Unit	PD
Oral-Only Drugs ($)	47.4	44.2	39.7
Injectables + Vit D* ($)	50.5	50.5	18.8

*Included in the current bundle

Conclusions: Our data suggests that the average cost of ESRD oral drugs ranges between $39-$47 today. Data like ours at a local level will hopefully enable Medicare to make appropriate adjustments to the final bundle payment rule in 2014. This will help avoid any unintentional consequences on quality of care, such as alteration of prescribing practice based on economic reasons.

Funding: Private Foundation Support

SA-PO2668

Poor Representation in the Medical Evidence Report (CMS-2728) of the Nephrology Care Received by Patients Approaching End-Stage Renal Disease: A Validation Study Jane Paik, Manisha Desai, Glenn M. Chertow, Wolfgang C. Winkelmayer. *Medicine, Stanford University, Stanford, CA.*

Background: In 2005, the Centers for Medicare and Medicaid Services added a new item to the Medical Evidence Report querying providers on the timing of the patient's first nephrologist consultation prior to initiation of renal replacement therapy (RRT). This item is also being used for monitoring disease-specific goals in the Healthy People 2020 initiative. The accuracy of the reported information, however, is unknown.

Methods: We defined a cohort of 41,455 patients initiating RRT between 7/2005 and 12/2006, who were ≥67 years old; among those, 28,778 had 2 years of uninterrupted Medicare (primary payer) coverage prior to RRT. We report accuracy of medical reporting using claims data as the gold standard. Using linear and Cox regression, we assessed the associations between the magnitude of discrepant reporting and patient characteristics as well as patient survival.

Results: Agreement between the two sources of ascertainment regarding timing of visit was present in only 32.9%, where the Kappa statistic was 0.13 when timing was defined as a 4-category variable and 0.30 when it was dichotomized as having had a visit over 6 months ago vs within 6 months. With an overall accuracy of 65%, accuracy was associated with race (p-value=0.001) and underlying cause of disease (p-value<0.001). In particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, cystic kidney was higher at 72% compared to hypertension and diabetes at 65%. Compared with patients receiving accurate reporting of their pre-dialysis nephrology care, inaccurate reporting was associated with a 22% increase in 1-year mortality.

Conclusions: We found substantial disagreement between information from the Medical Evidence Report form and Medicare physician claims on the timing of earliest pre-dialysis nephrologist care. In addition, inaccurate reporting contained prognostic information about these patients. This work casts doubt on the utility of the Medical Evidence Report for research and public health surveillance.

SA-PO2669

Adaptated Exercice Training Program for Dialysis Patients Philippe Chauveau,[1] Tomas Labat,[1] Stanislas Trolonge,[1] Christian Combe,[2] Catherine Deforges-Lasseur.[1] *[1]Aurad-Aquitaine, Bordeaux, France; [2]Hopital Pellegrin, CHU de Bordeaux, Bordeaux, France.*

Background: A sedentary lifestyle is frequent in hemodialysis (HD) patient and is associated with an increased relative risk for death. Evidence exists that increased physical activity (PA) is associated with better quality of life, better nutritional and inflammatory status and rehabilitation.

If an increased of PA should be one of the goals of care management several barriers prevent implementation of PA in dialysis units, related to patients but also to dialysis staff, notably lack of time, of knowledge or competence.

Methods: Since, march 2010, all 150 pts in AURAD Aquitaine have been informed about the opportunity to participate in an individualized program of PA (IPA) carried out by an Adapted Physical Activity Coach (APAC). After an Initial screening (consultation with the APAC, PA questionnaire (GPAQ), 6-minute walk test, balance test, handgrip strength, measure of quality of life (KDQOL36), assessment of body composition using the Body Composition Monitor® (BCM), biological nutritional parameters) and a 8-days period of observation with podometer and walkbook, an home-based individualized exercise program is determined for each patient and group-activity sessions were proposed. Supervision and support were provided according to patient's request.

Same complete screening is performed every 6 months.

Results: 32 patients (22%) enter the program (45±15 years, BMI 24±5 kg/m2) and only 2 stop at one year. At start, 6-min walk test was 541±110 m (320-885), mean steps per day using 8-days podometers was 5674 ± 2123steps/d: only 2 of them had a recommended PA according to age. 6-months evaluation is available for the 15 first patients (7 men , aged 41±15 years (20-65 years), and demonstrate a 37% increase of PA.

Conclusions: An individualized home-based PA program could be easily and safely initiated in dialysis unit using a trained qualified PA-health care professional. A combination of individual support and activity group maintain the adherence to the program. Preliminary results demonstrate an increase in physical activity and well-being.

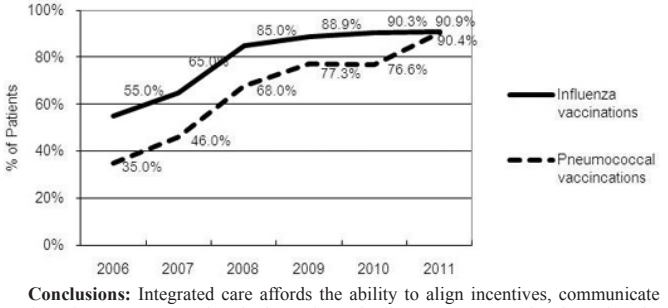
SA-PO2670

Effect of an Integrated Care Delivery System on Improving Immunization Outcomes Susan Rizkalla, Steve Mitchener, Barrett J. Johnson, David B. Van Wyck, Mahesh Krishnan, Allen R. Nissenson. *DaVita Inc, Denver, CO.*

Background: Pneumococcal vaccination and seasonal influenza vaccination each lower the risk of hospitalization and death in dialysis patients (pts). Those pts who receive both vaccines show the lowest risk. Using an integrated care intervention model similar to that proposed for accountable care organizations under healthcare reform, we undertook a systematic effort over the past 3 years to improve vaccination rates among in-center hemodialysis (HD) pts.

Methods: At the onset, clinical leadership recommended a goal of 90% of pts to receive pneumococcal vaccination (1x in past 5 years or 2x in lifetime), and 90% to receive seasonal influenza (annually). Operational leadership assigned a high priority to the effort, coordinated communications and management, provided implementation resources, applied process engineering approaches to identifying barriers and best practices, and assured adequate vaccine supply. IT systems were developed to report facility performance and identify unvaccinated pts in real time. Facility interdisciplinary care teams used teaching tools and daily exception reports during the flu vaccination season.

Results: Vaccination rates in the population of ~120,000 pts improved progressively during the period of intervention. By March 31, 2011, the goal of 90% seasonal and pneumococcal vaccination rate was achieved (Figure). The percentage of pts who declined influenza vaccination for allergy was very low (0.6%) in 2010.



Conclusions: Integrated care affords the ability to align incentives, communicate consistently, report comprehensively, and standardize care. Our results show that the integrated care model can be dramatically successful in improving vaccination rates within a large population of HD pts, and that care processes within large dialysis providers are well-suited to serve as potential accountable care organizations.

Funding: Clinical Revenue Support

SA-PO2671

Regular Screening for Staphylococcus Aureus Colonisation in a Satellite Haemodialysis Population: Lessons Learnt from a One-Year Single Centre Study David Makanjuola,[1] Glynn Richardson,[1] Jonathan Dick,[1] Angela Wright,[1] Maggi Steele,[1] Ginny Quan,[1] John Clark.[2] *[1]Renal Unit, St. Helier Hospital, Surrey, United Kingdom; [2]Microbiology Department, St. Helier Hospital, Surrey, United Kingdom.*

Background: Methicillin resistant Staphylococcus aureus (MRSA) is an important pathogen in the haemodialysis (HD) population and clinically significant infection is strongly associated with skin colonisation. In a bid to reduce MRSA bacteraemias, the UK department of health advocates screening for MRSA in all HD patients; however, the cost implications and utility of this strategy are unknown.

Methods: 76 patients on HD at a satellite unit were screened at 0,1,2,3,6 and 9 months for staphylococcus aureus (SA) carriage. Universal hygiene precautions were observed. Due to limited availability, SA positive patients could not always be isolated during HD sessions. SA eradication was attempted using 4% Chlorhexidine solution on a maximum of two separate occasions for any single patient.

Results: 66 patients completed the study. 4% were MRSA positive at baseline and 9% were positive on at least one occasion during the study. Transitory, rather than persistent carriage was observed. 5 different strains of MRSA were detected. 2 patients were colonised with a similar strain, but they dialysed on different days, making direct spread unlikely. In the MRSA positive patients who responded to decolonisation therapy, upon becoming re-colonised with MRSA, it was found to be with the same strain as they originally had.

34% were methicillin sensitive SA positive on at least one occasion during the study. Of these, 4 were consistently positive through the study in spite of eradication therapy.

Conclusions: The limited spread of MRSA in our cohort suggests that even where there is a dearth of isolation rooms, barrier nursing and universal hygiene precautions are sufficient to prevent spread of MRSA.

The differing variety of MRSA strains suggests that patients are acquiring MRSA from diverse sources in the community or other healthcare settings, rather than from the dialysis unit. MSSA decolonisation was of limited benefit.

SA-PO2672

Comparison of Life Participation Outcomes between Renal Replacement Therapy Modalities: A Systematic Review Tanjala S. Purnell,[1] Priscilla Auguste,[1] Deidra C. Crews,[1] Julio Lamprea,[1] Temitope Olufade,[1] Raquel Greer,[1] Patti Ephraim,[1] Johanna Sheu,[1] Neil R. Powe,[2] Hamid Rabb,[1] L. Ebony Boulware.[1] *[1]Johns Hopkins University; [2]University of California San Francisco.*

Background: The effect of renal replacement therapy (RRT) modality on patients' limitations in life participation (physical function, travel, recreation, freedom, work) has been poorly explored.

Methods: We performed a systematic review to explore the association of RRT (hemodialysis-HD, peritoneal dialysis-PD, kidney transplant-TX) with patients' life participation. We searched PubMed (English language, after 1980) and hand-searched bibliographies to identify studies that compared relevant outcomes by RRT. Independent reviewers used standard criteria to evaluate the quality of studies' research design and methods (internal and external validity), and we calculated the Cohen's d effect size estimate to assess the magnitude and direction of differences. We considered non-statistically significant results to indicate that neither treatment was favored for that comparison.

Results: Of 270 potential studies, 42 studies met our inclusion criteria, ranging from low to moderate quality (Table 1). These studies included 81 relevant comparisons by RRT (28 HD-PD; 28 HD-TX; 20 PD-TX; 5 Dialysis-TX). Studies were conducted in diverse racial/ethnic groups and included 2 cohort, 39 cross-sectional, and 1 pre-post study designs. Patients who received a TX consistently reported more favorable outcomes than patients on HD or PD.

Table 1. Summary of Published Evidence Comparing Life Participation Outcomes by RRT Modality

RRT Modalities Compared	HD vs. PD			HD vs. TX			PD vs. TX			Dialysis* vs. TX		
Life Participation Outcome Domains	# of outcomes that			# of outcomes that			# of outcomes that			# of outcomes that		
Study Design (Total Published Studies)	Favors HD	Favors Neither	Favors PD	Favors HD	Favors Neither	Favors TX	Favors PD	Favors Neither	Favors TX	Favors Dialysis	Favors Neither	Favors TX
Physical Function												
Randomized Trial (0)												
Longitudinal Cohort (2)		2										
Cross-Sectional (15)	1	5			4	9		2	6			3
Pre-Post Transplant (1)						1						
Travel												
Randomized Trial (0)												
Longitudinal Cohort (1)		1										
Cross-Sectional (0)												
Recreation												
Randomized Trial (0)												
Longitudinal Cohort (1)		1										
Cross-Sectional (7)		5			2	2			3			1
Freedom												
Randomized Trial (0)												
Longitudinal Cohort (1)		1										
Cross-Sectional (5)		4				4			4			1
Work												
Randomized Trial (0)												
Longitudinal Cohort (1)		1										
Cross-Sectional (8)	2	4	1			6		1	4			
Total Published Study Comparisons Favoring Each RRT Modality	3	24	1	0	6	22	0	3	17	0	0	5

Note: *Dialysis includes both HD and PD patients.

Conclusions: Evidence suggests patients who receive a TX report fewer limitations in life participation outcomes than dialysis patients, and there are no significant differences between HD and PD patients. However, the overall quality of evidence is low. Rigorously performed studies are needed to better inform patients about the effect of RRT on these important patient reported outcomes.

Funding: NIDDK Support

SA-PO2673

Effects of Antioxidant Therapy in CKD: Systematic Review & Meta-Analysis Min Jun,[1] Vinod Venkataraman,[2] Mona Razavian,[1] Sophia Zoungas,[1,4] Angela C. Webster,[3] Bruce A. Cooper,[2] Toshiharu Ninomiya,[1] Vlado Perkovic.[1] *[1]George Institute for Global Health, Uni of Sydney, Australia; [2]Royal North Shore Hospital, Australia; [3]School of Public Health, Uni of Sydney, Australia; [4]School of Public Health & Preventive Med, Monash Uni, Australia.*

Background: Trials of antioxidants show inconsistent effects on cardiovascular (CV) risk in chronic kidney disease (CKD) so we performed a systematic review and meta-analysis to synthesize the available evidence.

Methods: We systematically searched MEDLINE, EMBASE, and Cochrane Library for prospective RCTs (1950-March 2011) assessing antioxidant therapy compared to placebo for CV outcomes. Summary RRs and 95% CIs were calculated using a random effects model. Study quality was judged based on specific variables. Outcomes analysed were CV death, CV events, coronary events, cerebrovascular events, peripheral vascular disease (PVD), end-stage kidney disease (ESKD), and adverse events.

Results: We identified 4 trials with 1500 participants (327 CV events, 209 coronary events, 298 deaths). Studies were of mixed quality with some studies lacking information regarding randomization process, allocation concealment, and intention-to-treat analyses. Compared to placebo, antioxidant therapy had no overall effect on risk of CV death (RR 0.95; 95% CI: 0.70-1.27, p=0.712), cerebrovascular events (0.91;0.63-1.32, p=0.632), PVD (0.55;0.27-1.21, p=0.099), or ESKD (0.57;0.31-1.06, p=0.075). Data for adverse outcomes were sparse and reported no difference between the antioxidant and placebo arms. There was significant heterogeneity in the magnitude of the effect across studies for CV events (I^2=76.4%, p=0.01) with no effect in the early CKD population (1.04;0.83-1.32) but a beneficial effect in dialysis patients (0.57;0.41-0.80, p=0.001). Similar heterogeneity was identified for coronary events (I^2=64.1%, p=0.062).

Conclusions: Overall, there is no evidence that antioxidant therapy reduces risk of CV death, all-cause death, or major CV events but it is possible there is benefit for patients undergoing dialysis. Sufficiently sized trials in patients with advanced CKD are needed to better understand the effects of antioxidant therapy.

SA-PO2674

Impact of a Low-Glucose Peritoneal Dialysis Regimen on Inflammatory and Fibrotic Mediators in Effluent Dialysate Susan Yung, Chris Ng, Andy Yim, Maggie Ma, Sing-Leung Lui, Daniel Tak Mao Chan. *Department of Medicine, University of Hong Kong, Hong Kong SAR, Hong Kong.*

Background: Constant exposure of the peritoneum to bio-incompatible peritoneal dialysis fluids (PDF) results in deleterious changes to the peritoneal structure and function, which leads to technique failure and unfavorable clinical outcomes. While low-glucose PDF may have improved biocompatibility compared with conventional glucose based dialysates, their impact on biomarkers of inflammation, fibrosis and peritoneal membrane integrity remains to be investigated.

Methods: New PD patients who had entered the PD programme within 1 month were randomized to receive either a low-glucose regimen (1 exchange of Nutrineal®, 2-3 exchanges of Physioneal® and 1 overnight exchange of Extraneal®) for 12 months and then Dianeal® for 6 months (PEN Group, n=43), or 3 to 4 exchanges of Dianeal® for 18 months (Control Group, n=37). Overnight PDF was collected from all patients for the assessment of CA125, and inflammatory and fibrotic markers.

Results: Patients in the PEN Group showed a progressive increase in dialysate CA125 level, which was significantly higher than that in Controls after 12 months treatment [mean (range): 56.50 (16.00-166.00) IU/ml vs 20.50 (1.00-84.00) IU/ml, PEN vs Control, P<0.01)], and which reverted after switching back to Dianeal®. Similarly, dialysate levels of decorin, MMP-9, adiponectin, ICAM and VCAM were 1.35-, 2.52-, 1.79-, 1.93- and 1.59-folds respectively higher in the PEN Group compared to Controls after 12 months, but the two groups showed similar levels after the PEN Group changed back to Dianeal®. In contrast, dialysate MIF level was significantly lower in the PEN Group during the first 12 months [0.46 (0.06-1.22) pg/ml vs 0.77 (0.00-1.95) pg/ml, P<0.05], but was comparable between the two groups at 18 months.

Conclusions: Our data demonstrate that the low-glucose dialysis regimen is associated with reduced intra-peritoneal inflammatory and fibrotic response, and thus potentially result in better preservation of the structural and functional property of the peritoneum during long-term peritoneal dialysis.

Funding: Government Support - Non-U.S.

SA-PO2675

Relationship between Truncal Fat Mass and Serum High-Sensitivity C-Reactive Protein in Hemodialysis Patients — Metabolic Syndrome and Inflammation Eiji Ishimura,[1] Senji Okuno,[2] Naoki Tsuboniwa,[2] Shinya Nakatani,[1] Mitsuru Ichii,[1] Tomoyuki Yamakawa,[2] Shigeichi Shoji,[2] Yoshiki Nishizawa,[1] Masaaki Inaba.[1] *[1]Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Shirasagi Hospital, Osaka, Japan.*

Background: The relationship between fat mass distribution and chronic inflammation in dialysis patients is unknown, although chronic inflammation is related to central obesity in general population.

Methods: The fat mass and lean mass (total, truncal, and non-truncal) of 452 maintenance hemodialysis patients (age: 64±11 years; hemodialysis vintage: 89±77 months; 37% diabetics) without significant infection were measured by dual X-ray absorptiometry, and their association with high sensitivity C-reactive protein (hsCRP) was examined.

Results: Total fat mass and truncal fat mass of the high hsCRP group (n=346) were significantly higher than those of the normal hsCRP group (n=106) (p<0.05, respectively); while there were no significant differences in non-truncal fat mass or in lean mass between the two groups. In all patients, there were significant positive correlations between serum hsCRP and total fat mass (r = 0.186, p < 0.0001), and between serum hsCRP and truncal fat mass (r = 0.202, p < 0.0001), although there was none between serum hsCRP and lean mass. In a multiple regression analysis, truncal fat mass was significantly and independently associated with serum hsCRP levels after adjustment for several confounders, whereas non-truncal fat mass was not.

Factors affecting serum hsCRP levels (multiple regression analysis)

	β	p-value
Age (per 1 year)	0.102	0.034
Gender (male 0, female 1)	0.152	0.001
Hemodialysis duration (months)	0.018	0.693
Diabetes (absent 0, present 1)	- 0.048	0.312
Albumin (per 1 g/dL)	- 0.278	< 0.001
Truncal fat mass (per 1 kg)	0.227	0.003
Non-truncal fat mass (per 1 kg)	- 0.007	0.926
R^2	0.137	< 0.001

Conclusions: Fat mass, particularly truncal fat mass, but not lean body mass, was significantly associated with serum hsCRP levels. The results indicate that truncal fat mass exhibits a distinct effect on chronic inflammation in hemodialysis patients, suggesting the effect of metabolic syndrome on chronic inflammation in dialysis patients.

SA-PO2676

Elevated Serum Concentrations of Maillard Amino Acid Amides Can Be Reduced by Hemodialysis with HCO1100 Dialyzer Membranes Matthias Girndt,[1] Roman Fiedler,[1] Christian Henning,[2] Christof Ulrich,[1] Felix Neugebauer,[1] Eric Seibert,[1] Marcus A. Glomb.[2] *[1]Department of Internal Medicine II, Martin-Luther-University Halle, Halle, Germany; [2]Institute of Chemistry - Food Chemistry, Martin-Luther-University Halle, Halle, Germany.*

Background: Protein glycation by Maillard reactions leads to advanced glycation end products (AGE). In plasma of hemodialysis (HD) patients AGEs are found at high concentrations. We studied plasma concentrations of a novel group of free amino acids with amid structure in HD patients and put emphasis on the ability of different dialysis techniques to remove them.

Methods: Amid-AGEs were quantified by mass spectrometry (LC/MS/MS) in plasma of HD patients obtained prior to a dialysis session. The patients studied were included in a randomized double blind cross over study testing efficacy and safety of a high cut-off dialyzer membrane (Gambro HCO1100™) compared to a high-flux membrane (Gambro Polyflux H™) in 17 patients (age 68.1±10.9 years). Treatment was performed over 2 weeks (6 consecutive dialysis sessions). Healthy individuals of two age ranges (young=27.2±6.2 years, elderly=65.1±4.3 years) were tested as controls.

Results: In healthy controls the plasma concentrations of the amino acid adducts *N*-formyllysin, *N*-acetyllysin, *N*-lactoyllysin, and *N*-glycerinyllysin increased with age. In HD patients the adducts showed higher plasma concentrations than in both healthy groups (formyllysin in pmol/ml, young=125±43; elderly=172±112; dialysis=309±113; p<0.0001 by Kruskal-Wallis test). Predialysis concentrations of formyllysin und acetyllysin could significantly be reduced by 2 weeks of high cut-off dialysis (formyllysin, polyamid 309±113; HCO 245±75; p=0.014 by Wilcoxon test).

Conclusions: Free amino acids with amid modifications are an important novel group of Maillard AGEs that are renally excreted in the healthy. HD dialysis patients have elevated plasma levels reminding of earlier findings of AGE proteins in these patients. High cut-off dialysis is the first intervention that proves to reduce these substances significantly from patient's plasma. This clears the way to interventional trials to better understand the relevance of free amino acid adducts in CKD.

Funding: Government Support - Non-U.S.

SA-PO2677

Enhanced Suppressive Function of Tregs from ESKD Patients Using a New a High Cutoff HD Technique Pascal Meier. *Division of Nephrology, RSV - Hôpital du Valais, Sion, Switzerland.*

Background: Considering the effect of oxLDL accumulation on Tregs viability and function in chronic HD patients, a new generation of dialyzers, with very large pores (HCO 1100 Gambro), could be useful to clear oxLDL and thus improve Tregs survival and function. The aims of this study were to make *in vivo* assessments of several hemodialyzers (i.e. Polyflux 21 L – Polyflux 210 H [regular membranes: RM] *vs.* HCO [high cutoff] 1100) in plasma oxLDL clearing, and to analyze the Tregs suppressive function in ESKD patients.

Methods: Thirty ESKD patients on chronic HD (3 x 4 hours weekly) were studied in three equal groups during three months. Plasma and dialysate oxLDL concentrations were measured using a mAb-4E6–based ELISA. To determine the frequencies and phenotypes of Tregs, multicolor flow cytometry was performed. Apoptosis was indirectly assessed by Fas staining and flow cytometry and then confirmed by DNA fragmentation determination.

Results: Patients on RM exhibited elevated plasma level of oxLDL compared with HCO 1100 at three months (P = 0.002, ANOVA). In parallel, Tregs from patients on RM represented 1.34 to 1.73% of $CD4^+$ T cells whereas the frequency of Tregs in patients on HCO 1100 was 3.42±0.19%; P < 0.01. After PHA stimulation, increment in Tregs response was much more substantial in patients on HCO 1100 than in patients on RM. In

all experimental conditions, the apoptotic DNA fragmentation significantly increased in FAS-sorted Tregs from patients on RM compared with those on HCO 1100 (P = 0.005), in accordance with the decline in cell viability. Finally, Tregs from patients on RM did not efficiently suppress the proliferation of the co-cultured $CD4^+$/CD25- T cells (1:1; 21153±2045 cpm). In striking contrast, Tregs from patients on HCO 1100 were able to strongly inhibit the proliferation of $CD4^+$/CD25- responder T cells (1:1; 4172±382 cpm; P < 0.001). Unlike RM, the suppressive capacity of Tregs after one HCO 1100 HD session was significantly increased compared with that of Tregs before session.

Conclusions: In ESKD patients on chronic HD using HCO 1100, Tregs number, viability and suppression capacity were significantly improved compared with RM without significant side effects.

Funding: Clinical Revenue Support

SA-PO2678

Monocyte Transcriptome Analysis in Hemodialysis Patients: Identification of a Role for a $CD16^+$ and $CX3CR1^+$ Subpopulation Eva Schepers, Annemieke Dhondt, Raymond C. Vanholder, Griet L.R.L. Glorieux. *Internal Medicine/Renal Division, University Hospital Gent, Gent, Belgium.*

Background: The risk for cardiovascular morbidity and mortality is increased in chronic kidney disease patients, whereby micro-inflammation plays an essential role. Numerous culprits have not yet been identified. In this study transcriptome analysis of monocytes was used to identify in an unbiased manner discriminative factors in hemodialysis (HD) patients.

Methods: Forty gender- and age-matched, non-diabetic, non-smoking subjects with a CRP<2mg/L were recruited, 9 healthy controls, 10 patients with GFR > 60 mL/min/1.73m^2 and a prevalent cardiovascular event (CVE), 11 patients on HD without prevalent cardiovascular event (HD) and 10 HD patients with prevalent cardiovascular event (HD/CVE). Monocytes were isolated, by a positive selection using MACS and submitted to transcriptome analysis using an in house-array containing ca. 700 genes associated with macrophage activation (Van den Berghe R et al: Retrovirol.7:53,2010)

Results: A significant differential expression was observed for FCGR3A (CD16;fold-change HD/CVE vs CVE; 2.73;p=0.005), CYTH1 (cytohesin 1;2.05;p=0.006), BMP2K (bmp2 inducible kinase;1.67;p=0.01) and CX3CR1 (chemokine receptor;1.53;p=0.049). For CD16 and for CX3CR1, this finding could be confirmed by qRT-PCR (HD and HD/CVE vs control and vs. CVE,p<0.05 and p<0.01,resp). Both CD16 and CX3CR1 relative expression correlated with CRP (r^2=0.1179;p<0.05 and r^2=0.3387;p<0.0001,resp). Flow cytometric analysis revealed a significant increase in the percentage CD16 positive monocytes both in the HD and HD/CVE group vs control and vs. CVE (p<0.01). No discrimination, based on CD16 expression, could be made between HD patients with or without cardiovascular disease.

Conclusions: The present study indicates the importance of pro-inflammatory $CD16^+$ monocyte subpopulation in HD patients who also have elevated CRP. The co-expressed chemokine receptor, CX3CR1, is involved in monocyte recruitment and survival in atherosclerotic plaques. For future, discriminative factors should be explored in well defined and purified monocyte subpopulations which play an important role in the inflammatory status of HD patients.

Funding: Government Support - Non-U.S.

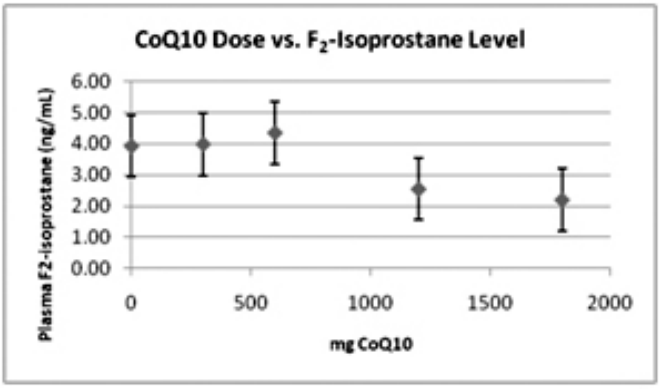
SA-PO2679

Safety, Tolerability, and Efficacy of the Dietary Supplement Coenzyme Q10 in Hemodialyisis Patients Catherine K. Yeung,[1] Adam Claessens,[1] Lori Linke,[2] Crystalyn Austin Clark,[2] Talat Alp Ikizler,[3] Danny D. Shen,[1] Jonathan Himmelfarb.[2] *[1]Department of Pharmacy, University of Washington, Seattle, WA; [2]Kidney Research Institute, University of Washington, Seattle, WA; [3]Division of Nephrology, Vanderbilt University, Nashville, TN.*

Background: Administration of coenzyme Q10 (CoQ10) has been shown to improve mitochondrial coupling of respiration to oxidative phosphorylation, improve oxidative stress, and may be associated with clinical cardiovascular benefits in diverse patient populations. To date, there are no studies evaluating the pharmacokinetics, safety tolerability and efficacy of CoQ10 administration in the hemodialysis population.

Methods: We performed a multiple ascending dose study in 20 hemodialysis patients. CoQ10 supplement (Vitaline) was successively administered at doses of 300 mg, 600 mg, 1200 mg, and 1800 mg for 14 days each. Safety and tolerability were assessed. Plasma CoQ10 concentrations were measured by LC/MS. Plasma F2-isoprostane concentration was measured by GC/MS to assess systemic oxidative stress.

Results: 15 of 20 subjects (75%) completed the entire sequence. CoQ10 supplementation was generally well tolerated and no severe adverse events were attributed to CoQ10. All laboratory values were within normal limits. CoQ10 was not significantly removed from circulation by hemodialysis. Mean Plasma F2-isoprostane concentration decreased following the 1200 and 1800 mg CoQ10 administration.



Conclusions: CoQ10 up to 1800 mg daily was shown to be safe and well tolerated in most subjects. The observed decrease in plasma levels of F2-isoprostanes suggests that CoQ10 is a promising antioxidant in dialysis patients. A double-blinded, randomized, placebo-controlled pilot study to assess the efficacy of CoQ10 in hemodialysis patients is warranted.

Funding: Other NIH Support - NCCAM 5R21 AT004265-02

SA-PO2680

IL-17: A Novel Player in Peritoneal Dialysis Treatment Marta Ruiz-Ortega,[1] Raquel Rodrigues-Diez,[1] Luiz S. Aroeira,[2] José A. Jimenez,[2] Raúl R. Rodrigues Díez,[1] Sandra Rayego-Mateos,[1] Guadalupe González- Mateo,[2] Manuel Lopez-Cabrera,[2] Jesus Egido,[3] Alberto Ortiz,[3] Rafael Selgas.[2] *[1]Universidad Autonoma; [2]IDIPAZ; [3]ISCFJD, Madrid, Spain.*

Background: The classical view of the immune response regulation has been enlarged by the recent discovery of IL-17-producing T helper (Th) cells, named Th17 cells. Growing evidence suggests that IL-17, besides participating in immune-mediated diseases, is also involved in chronic inflammatory diseases. In peritoneal dialysis (PD) patients, a chronic inflammatory response and an imbalanced Th1/Th2 response have been described, but there is no data about the Th17 response.

Methods: Human peritoneal biopsies of PD patients were analyzed by immunohistochemistry. To study the regulation of Th17 responses in the peritoneal membrane an experimental PD model in mice, that resembles human situation, was done.

Results: In human peritoneal biopsies of PD patients, IL-17 expression associated to T lymphocyte infiltration was found. In the mice model, exposure of the peritoneum to PD fluid (PDF) for 7 days resulted in an inflammatory response, characterized by infiltrating T lymphocytes, including CD4+/IL-17 expressing cells in the submesothelial zone. Elevated gene and protein levels of IL-17 were found in the peritoneum of PDF-treated mice compared to untreated mice. In contrast, tissue levels of INF-γ and IL-4 (Th1 and Th2 hallmark cytokines, respectively) were not changed between groups. Th17 cell differentiation in mice is regulated by a combination of cytokines (IL-6 and TGF-β) and by several transcription factors (RORγt and STAT3). PDF-treated mice presented upregulation of TGF-β and IL-6, and activation of RORγt and STAT3 in peritoneum compared to controls. These data show an activation of the Th17 response in the peritoneum exposure to PDF. After 30 days of daily PDF instillation increased peritoneal thickness, angiogenesis and functional alterations were observed. At this time point, IL-17 levels remained elevated, both in the peritoneum and in the peritoneal lavage, correlated with peritoneal thickness.

Conclusions: These data suggest that local Th17 response could participate in dialysis-fluid induced damage to the peritoneal membrane.

Funding: Government Support - Non-U.S.

SA-PO2681

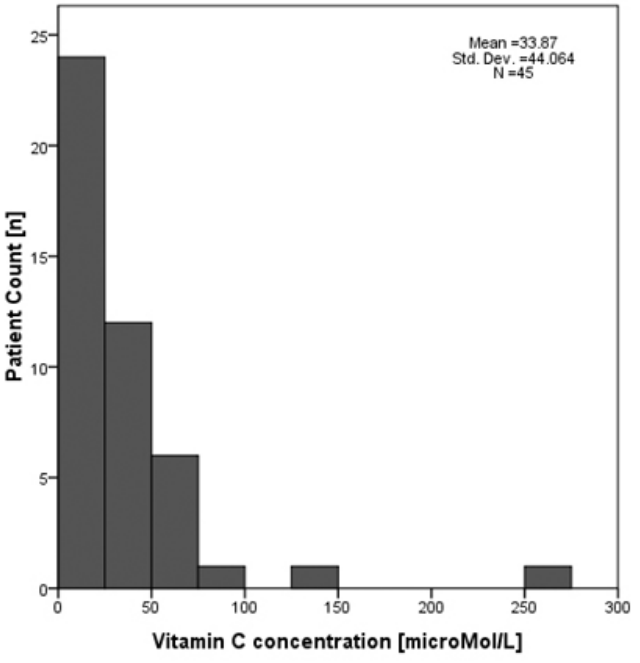
Ancillary Study to the Frequent Hemodialysis Network (FHN) Trial: Cross-Sectional Assessment of Vitamin C Concentrations in Chronic Hemodialysis Patients at Baseline Jochen G. Raimann,[1,2] Li Liu,[1,2] Samer Rateb Abbas,[1,2] Len A. Usvyat,[1] Stephan Thijssen,[1,2] Garry J. Handelman,[3] Peter Kotanko,[1,2] Nathan W. Levin,[1,2] And FHN Trial.[4] *[1]Renal Research Institute; [2]Beth Israel Medical Center; [3]University of Massachusetts; [4]NIDDK.*

Background: Vitamin C status in hemodialysis (HD) patients may be low because potassium-rich foods are restricted and potentially also because vitamin C is removed during dialysis. In the non HD population vitamin C levels are between 20-80 μmol/L and fatigue, as a first symptom of acute deficiency occurs below 20 μmol/L. Vitamin C deficiency has been associated with elevated oxidative stress, anemia and secondary hyperparathyroidism.

Methods: This analysis examined cross-sectional vitamin C levels in HD patients at baseline. Samples were taken prior to HD, processed with metaphosphoric acid as a preservative and immediately frozen at -70°C. Measurements were performed using HPLC. The Shapiro Wilk Test was used to test for normality.

Results: 45 chronic HD patients (age 49±12 years, 27 males, 31 blacks, body mass index 28.9±7.0 kg/m^2) were studied. The mean vitamin C concentration was 33.9±44.1 μmol/L, data was not normally distributed (**Figure 1**) and 22 patients (48.9 %) were below the level of 20 μmol/L where symptoms can be expected.

Conclusions: Vitamin C deficiency is frequent in HD patients. Almost 50% of the enrolled patients showed vitamin C levels below the symptom level. Associations with clinical parameters of anemia management, mineral and bone metabolism and inflammation require additional analyses.



SA-PO2682

Incidence and Mortality Risk Factors Associated with Non-Occlusive Mesenteric Ischaemia in Patients Undergoing Haemodialysis Borja Quiroga, Eduardo Verde, Soraya Abad, Almudena Vega, Marian Goicoechea, Javier Reque, Juan Manuel Lopez Gomez, Jose Luno. *Nephrology, Hospital Gregorio Marañón.*

Background: Non-occlusive mesenteric ischaemia (NOMI) is an emergent pathology in haemodialysis (HD) patients. Although it is associated with a poor outcome, it has not been studied enough.

Methods: A retrospective study was conducted between 2003 and 2011 in HD patients. During follow-up time, all cases of NOMI were registered, and demographic, clinical, biochemical and HD parameters were collected. Then, analysis was performed between this group and a control one (n=100). Risk factors associated with the NOMI development and prognosis were studied and survival curves were established between both groups.

Results: There were fifty-seven episodes of NOMI in 44 patients (mean age 72.9±8.9 years; 56% male). Thirty percent were diabetic. The incidence of NOMI was 2.29 episodes/100-patients/year. Cardiovascular risk factors were present in 72% of the patients. The patients with NOMI had leukocytosis (16945±4672/μL), mild acidosis (bicarbonate 22.6±4.3 mEq/L), even though the episodes were posthaemodialysis, and serum lactate (2.6±1.8 mg/dL), LDH (441±649 UI/L) and CPR (21.4±15.5 mg/dL) increase. The caecum was the most frequently (42%) affected segment, followed by diffuse bowel involvement. Nineteen patients (33%) were surgically treated. Twenty six patients (59%) did not survive the acute episode of NOMI.

Caecum damage was the only protective factor related with mortality in the univariate and multivariate analysis (RR 0.16; p=0.009). The incidence of NOMI was related to age (RR 1,11; p<0,001), diabetes mellitus (RR 2,61; p=0,037) and duration of dialysis (RR 1,01; p=0,006), when compared with the control group. Those patients who survived the acute episode (41%) were compared with the control group (47%), showing a higher mortality at 5-year follow-up (Log Rank 26.48; p<0.001).

Conclusions: NOMI is associated with age, diabetes mellitus and long time undergoing haemodialysis. Caecum damage is the most frequent location, but is related with better prognosis. Mortality is very high both in the acute episode and long-term.

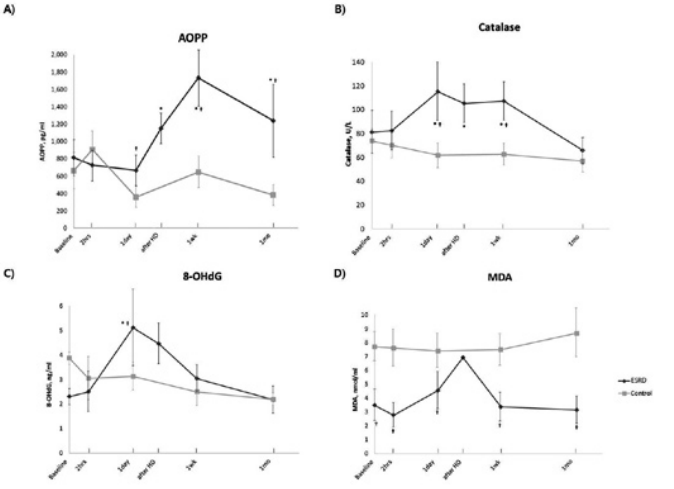
SA-PO2683

Iodinated Contrast Media Can Induce Long-Lasting Oxidative Stress in Hemodialysis Patients Soo Young Yoon, Seun Deuk Hwang, Yoon Ji Kim, Sang Heun Lee, Deok Kyu Cho, Yun Hyeong Cho, Sung Jin Moon, Sang Choel Lee. *Internal Medicine, Kwandong University, Goyang, Republic of Korea.*

Background: Due to their comorbidities, dialysis patients have many chances to undergo diagnostic and therapeutic procedures using iodinated contrast media. Iodinated contrast has been regarded as a safe agent in these patients who have little risk of contrast-induced nephropathy. Oxidative stress plays a major role in toxicity of contrast media. We hypothesized that contrast induces more severe systemic oxidative stress in hemodialysis (HD) patients than in non-dialysis population. We assessed time-sequenced blood oxidative stress level after contrast media exposure in anuric HD patients compared to those in the controls.

Methods: We included 21 anuric HD patients (ESRD group) and 23 controls (Control group) scheduled for coronary angiography (CAG) and assessed 4 oxidative stress markers (AOPP, advanced oxidation protein products; catalase; 8-OHdG, 8-hydroxydeoxyguanosine and MDA, malondialdehyde) before and after CAG, and subsequently up to 28 days.

Results: In the Control group, only AOPP increased immediately after CAG and returned to baseline within one day. However, in the ESRD group, AOPP, catalase and 8-OHdG significantly increased from one day after CAG, and remained elevated longer than in the Control group.



Changes in oxidative stress markers after coronary angiogram
* p<0.05 vs. baseline; † p<0.05 vs. Control

Conclusions: Our study showed that iodinated contrast media induces severe and prolonged oxidative stress in HD patients. Further studies are needed to determine the effectiveness of the preventive measures using antioxidants such as N-acetylcysteine.

SA-PO2684

Elevated Serum Osteoprotegerin Levels Are Associated with Inflammation, Malnutrition, and New-Onset Cardiovascular Events in Peritoneal Dialysis Patients Shin-Wook Kang, Mi Jung Lee, Dong Ho Shin, Seung Jun Kim, Dong Eun Yoo, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ, Seoul, Korea.*

Background: Osteoprotegerin(OPG) has been known to be associated with cardiovascular events(CVE) and malnutrition in non-dialysis chronic kidney disease patients. Malnutrition-inflammation-atherosclerosis(MIA) syndrome is commonly found in dialysis patients, but the association between OPG levels and MIA syndrome has been largely unexplored in these patients. This cross-sectional study was undertaken to investigate the association between OPG levels and malnutrition, inflammation, and CVE in prevalent peritoneal dialysis(PD) patients.

Methods: A total of 176 prevalent PD patients were included. At baseline, OPG, hs-CRP, and albumin concentrations were measured. In addition, percent lean body mass(%LBM) was determined by creatinine kinetics. Subjective global assessment(SGA) was also performed. Based on the median levels of OPG, patients were divided into 2 groups, and CVE and patient survival were compared by Kaplan-Meier analysis. Moreover, Cox regression analysis was conducted to determine risk factors for CVE.

Results: Age, hs-CRP, and Charlson comorbidity indiex were significantly higher, while serum albumin levels, %LBM, and SGA score were significantly lower in patients with high OPG levels(H group) compared to patients with low OPG levels(L group). The rates of newly developed CVE were significantly higher in the H group compared to the L group(40.9 vs. 17%, P<0.01). Overall mortality rates were also higher in H group compared to L group, without statistical significance. Univariate Cox regression analysis revealed OPG as a risk factor for new-onset CVE(per an increase by 1 in log OPG; HR, 2.34; 95% CI, 1.35-4.04; P=0.002), which remained significant after adjustment for age, sex, diabetes, and PD duration(HR, 2.00; 95% CI, 1.13-3.89; P=0.02).

Conclusions: Serum OPG levels were significantly correlated with markers of systemic inflammation and malnutrition and was revealed as a significant predictor of new-onset CVE in PD patients, suggesting OPG might be a prognostic indicator of MIA syndrome in PD patients.

SA-PO2685

Circulating S100A12 (EN-RAGE) Levels Predict Cardiac Dysfunction by Tissue Doppler Echocardiography in Peritoneal Dialysis Patients Tae Yamamoto,[1] Shirley Yumi Hayashi,[1,2] Abdul Rashid Tony Qureshi,[1] Marcelo M. Nascimento,[1] Ayumu Nakashima,[1] Lars-åke Brodin,[2] Björn Anderstam,[1] Britta Lind,[2] Miguel C. Riella,[3] Astrid Seeberger,[1] Bengt Lindholm.[1] *[1]Renal Medicine and Baxter Novum, Karolinska Institute, Stockholm, Sweden; [2]Med Eng, School of Technol and Health, Royal Institute of Technology, Stockholm, Sweden; [3]Pro-Renal Foundation, Curitiba, Brazil.*

Background: Cardiovascular disease is common in patients (pts) on peritoneal dialysis (PD), and Tissue Doppler imaging (TDI) is a useful tool for assessing cardiac function. In PD pts, dialysis solutions may lead to formation of advanced glycation end-products (AGEs), which may result in cardiovascular toxicity. Therefore, we examined the possible link between circulating AGEs and cardiac function in PD pts.

Methods: Plasma concentrations of soluble form of receptor for AGEs (sRAGE), its ligand S100A12, and other biomarkers were measured in 51 pts undergoing PD. The myocardial systolic (PSV, peak systolic velocity) and diastolic (E', early diastolic velocities) velocities were assessed by TDI. M-mode echocardiography was also performed. Pts were divided into two groups by E' value.

Results: Among 51 pts, 95% (n=48) had diastolic dysfunction (E' <8.0 cm/s). The medium plasma levels of S100A12 and sRAGE were 64 ng/ml and 2594 pg/ml. S100A12 levels correlated with PSV and E', both of which were negatively associated with troponin-1 and left ventricular mass index. High E' group had lower S100A12 and higher sRAGE levels compared to low E' group, while other inflammatory and oxidative stress markers such as CRP, IL-6, TNF and 8OHdG did not differ. Stepwise multiple regression analysis identified S100A12 (β=-0.38, p=0.002) and age (β=-0.52, p<0.001) as independent determinants of E' in a model (r^2=0.40).

Predictors of early diastolic velocities (E', cm/s)

	Unadjusted (β, P) (r2=0.09)	Model 1 (β, P) (r2=0.38)	Model 2 (β, P) (r2=0.39)	Model 3 (β, P) (r2=0.40)
logS100A12	-0.33 (0.017)	-0.38 (0.001)	-0.41 (0.001)	-0.38 (0.002)
Age (years)		-0.55 (<0.001)	-0.53 (<0.001)	-0.52 (<0.001)
Albumin (g/dl)			0.16 (0.167)	0.15 (0.200)
Troponin-I				-0.12 (0.29)

Conclusions: Circulating S100A12 is an independent predictor for cardiac dysfunction in PD pts, suggesting an important role of AGEs.

Funding: Pharmaceutical Company Support

SA-PO2686

Monocyte Chemoattractant Protein-1 Production Is Diminished by Mycophenolic Acid Mirjam Schuchardt,[1] Jasmin Pruefer,[1] Matthias Höhne,[2] Markus van der Giet,[1] Markus Tolle.[1] *[1]Med. Klinik mit SP Nephrologie, Charite - Campus Benajmin Franklin, Berlin, Germany; [2]Novartis Pharma AG, Nürnberg, Germany.*

Background: For the initiation and maintenance of atherogenesis or in transplant vasculopathy, the production of monocyte chemoattractant protein-1 (MCP-1) is crucial. There is evidence that the immunosuppressive regime influences vascular alterations. In the last years there is increasing evidence that mycophenolic acid (MPA), routinely used after kidney transplantation, is more than just an immunosuppressant. In this study we wanted to investigate the influence of MPA on the thrombin-induced MCP-1 production in vascular smooth muscle cells (VSMCs).

Methods: MCP-1 formation was measured via real-time PCR and Luminex™ technology. Superoxide production was detected in dihydroethidium (DHE)-labeled cells via fluorescence microscopy and quantified in a multimode-reader. H2O2 production was measured in 5,6-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA)-labeled cells via flow cytometry. Rac1 activation was detected via ELISA. NADPH consumption rate was measured photometrically.

Results: Thrombin induced MCP-1 expression and secretion in VSMCs. Costimulation with MPA dose-dependently and significantly decreased the thrombin-induced MCP-1 production. Next, we investigated the production of superoxide in DHE-labeled cells treated with thrombin ± MPA. MPA significantly diminished the superoxide production as well as the H2O2 production in H2DCFDA-labeled VSMCs. The NADPH oxidase is a dominant source of reactive oxygen species in the vasculature. Therefore, we measured the NADPH consumption. MPA significantly reduced the thrombin-induced NADPH consumption rate in VSMCs. To gain further evidence pointing to NADPH oxidase as target, we measured Rac1 activation. MPA significantly diminished the thrombin-induced Rac1 activation significant.

Conclusions: MPA attenuates the production of the pro-inflammatory chemokine MCP-1 in VSMCs, directing an anti-inflammatory effect of MPA in a redox-sensitive manner. In summary, MPA is not only an immunosuppressant substance, but might also play a relevant role in anti-inflammatory response.

SA-PO2687

Combined High Serum Ferritin and Low Iron Saturation Predicts Infection in Hemodialysis Patients André Fragoso, Ana Pinho, Anabela Malho, Ana Paula Silva, Elsa Morgado, Pedro Neves. *Nephrology, Hospital de Faro EPE, Faro, Portugal.*

Background: Serum ferritin is a classical marker of iron status and chronic inflammation. More recently, the combination of high serum ferritin and low iron saturation has been associated with inflammation. The aim of our paper was to study the relationship between these inflammatory markers and infection events.

Methods: We included all patients of a dialysis unit on treatment for more than 3 months by the 31st of January 2009. Patients were prospectively followed for 12 months and divided in two groups (G1: patients with infection events; G2: without infection events).

Results: We followed 169 patients (63,3% males) with a mean age of 61,87±16,32 years in hemodialysis for 73,26±112,14 months. G-1 patients showed lower serum iron (63,56±22,21 vs 75,70±29,01 mcg/dl; p<0,05), serum ferritin (527,42±246,65 vs 673,56±349,79 ng/ml; p<0,05) and iron saturation ratio (25,53±8,31 vs 34,06±23,13%; p<0,05). No differences were found between the two groups regarding CRP, albumin, BMI, KT/V and IV iron dose. In a logistic regression model, after adjustment for Diabetes, BMI, serum albumin and CRP, patients with combined high serum ferritin (>600ng/ml) and low iron saturation ratio (<25%) presented a 5-fold risk for an infection event (p=0,023).

Conclusions: In our population, known markers of chronic inflammation independently predicted infection events. To our knowledge this relationship has only been demonstrated in peritoneal dialysis patients. Further studies are needed to better understand the mechanisms that link inflammation to classical infection in the hemodialysis setting.

SA-PO2688

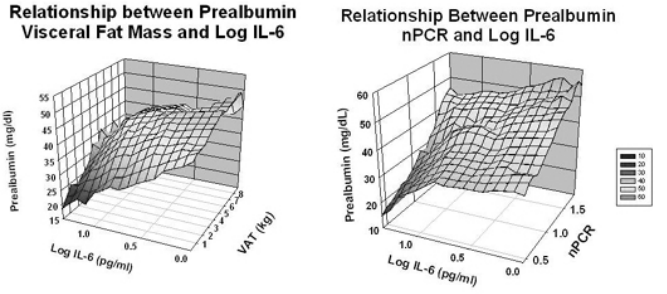
Prealbumin Is Associated with Visceral Fat Mass in Hemodialysis Patients George A. Kaysen,[1,4] Alessio Molfino,[1,2] Steven B. Heymsfield,[3] Fansan Zhu,[4] Peter Kotanko,[4] Nathan W. Levin.[4] *[1]Medicine, UC Davis, Davis, CA; [2]University of Rome, Italy; [3]PBRC, Baton Rouge, LA; [4]RRI, NY, NY.*

Background: Both Albumin (ALB) and Prealbumin (PreAlb) are associated with malnutrition and inflammation. Each have a residual effect on mortality outcomes when included in regression models that include the other. PreAlb, but not ALB is inversely associated with infectious and directly associated with vascular access hospitalizations. PreAlb has been reported to be increased in the obese mouse model as a consequence of stabilization of PreAlb by Retinol Binding Protein 4 (RTB4), secreted by adipocytes. We questioned if PreAlb was associated with adiposity in dialysis patients, independent of the effects of inflammation or nutrition.

Methods: We evaluated body composition in 48 prevalent hemodialysis (HD) patients by MRI, measuring total skeletal muscle mass (SM), visceral and subcutaneous adipose tissue (VAT and SAT), and serum ALB, PreAlb, RTB4, interleukin -6 (IL-6) concentrations. We used normalized protein catabolic rate (nPCR) to report nutrition and separately analyzed the determinants of ALB and then of PreAlb by multiple stepwise regression.

Results: 32 subjects were women, 16 were diabetic, median age 54.5, range 40-69, 10th 90th percentile). Median BMI 27.3, range 22.5-37.2 10th 90th percentile), Median TAT 24.3 kg range 11.5-40.1 kg 10th-90th percentile), Median VAT 3.25 kg range 0.5 5.8 kg 10th-90th percentile). PreAlb was positively associated with VAT (P = 0.002), and nPCR (P=0.0001) and RTB4 P = 0.05, and negatively associated with IL-6 (P<0.003) and white race (P< 0.001). r^2 for the model was 0.66.

Prealbumin is Independently Associated with Inflammation, Visceral Adiposity and nPCR



By contrast ALB was positively associated only with nPCR and negatively with Log IL=6. r^2 for the model was 0.18.

Conclusions: PreAlb, like ALB, is associated with markers of nutrition (nPCR) and inflammation (IL-6), but unlike ALB, PreAlb is strongly associated with adiposity.

SA-PO2689

Increase in Lymphocyte β-catenin, IL-10 and Cortisol Serum Levels May Play a Role in the Protection of the Central Nervous System Against the Inflammation in Hemodialysis Patients with Cognitive Impairment Sabrina Degaspari,[1] Geison Stein Meirelles Ramos,[2] João Paulo L.B. Martins,[2] Elzo R. Junior,[2] Carmen B. Tzanno-Martins,[2] Carolina D. Munhoz,[1] Cristoforo Scavone,[1] Elisa M. Kawamoto.[1,3] *[1]Pharmacology-ICB, USP, Brazil; [2]Cine; [3]Laboratory of Neurosciences, NIA, NIH; [4]Nephrology, URGS, Porto Alegre, Rio Grande do Sul, Brazil.*

Background: A high prevalence of cognitive impairment (CI) in hemodialysis patients (HD-P) is well established. Inflammatory process has been shown to be associated with chronic kidney disease and may play a role in the development of CI. WNT signaling has been described in pathological conditions, such as neuroinflammation, and recent data have suggested that WNT signaling could participate in anti-inflammatory response. The purpose of this study is to evaluate the role of cytokines and the WNT signaling in HD-P with or without CI.

Methods: Thirty six HD-P were submitted to cognition test through Modified Mini Exam of Mental State and Kidney Disease Quality of Life. Blood was collected before and after hemodialysis (HD) procedure. Serum was used to analyze IL-6, IL-10, TNF and cortisol levels by ELISA test. Lymphocytes were isolated to verify the WNT proteins by Western Blotting analysis.

Results: Forty percent of patients showed CI. We observed that IL-6 (control=7.1±0.4 pg/ml) and TNF (control=14.4±4.8 pg/ml) levels were increased in HD-P (IL-6=227.8±92.1 pg/ml*; TNF=53.7±9.4 pg/ml*) without CI before HD, whereas IL-10 (control=7.4±2.4 pg/ml) and cortisol (control=0.16±0.03 mg/dl) levels were increased in HD-P-CI before (IL-10=22.6±8.5 pg/ml*; cortisol=0.32±0.03 mg/dl*) and after (IL-10=32.4±9.1 pg/ml*; cortisol= 0.28±0.04 mg/dl*) HD (*p<0,05). We also observed an increase of β-catenin in lymphocytes of HD-P-CI compared with control and HD-P groups.

Conclusions: HD-P without CI show an increase in proinflammatory cytokines levels and the increase of IL-10 and cortisol in HD-P-CI could be an organic response against an inflammatory state. The increase of β-catenin in HD-P-CI demonstrates the activation of the canonical WNT signaling that may play a role in the protection of the central nervous system against the neuroinflammation.

Funding: Government Support - Non-U.S.

SA-PO2690

Variations in the Prevalence of HIT Antibodies during the Period 2004-2011. Relevance to Heparin Contaminants Vinod K. Bansal,[1] Debra Hoppensteadt,[2] Jawed Fareed.[2] *[1]Nephrology, Loyola University Medical Center; [2]Pathology, Loyola University Medical Center; [3]Thoracic and Cardiovascular Surgery, Loyola University Medical Center.*

Background: During the period of November 2007 - April 2008 several batches of unfractionated heparin were recalled due to the unexpected adverse reactions. These heparins were characterized to contain such contaminants as oversulfated chondroitin sulfate and high molecular weight dermatan sulfate. Several centers also reported a high prevalence of heparin induced thrombocytopenia (HIT) during this time. In order to compare the prevalence of HIT antibodies in ESRD patients, plasma samples from different time periods were analyzed.

Methods: In conjunction with an ongoing program to investigate the prevalence of HIT antibody (HIT Ab) in ESRD, blood samples were collected from patients (n=53-89) on maintenance dialysis and retrospectively analyzed for the presence of these antibodies during the period February-April 2004-2011.Specified cut off levels were used to determine the positive and negative results. An ELISA method (GTI. Brookfield, WI) was used to quantitate the HIT antibodies.

Results: The prevalence of the HIT Ab during the period 2004-2011 was < 15% with the exception of 2007 where it was 35% and in 2008 it was 30%. A decreased prevalence was noted during the period 2009-2011 (13-15%). The antibody titers as quantified by optical density measurement were relatively higher during the year 2007 and 2008. Moreover, the subtyping of these antibodies in 2007 and 2008 showed a higher proportion of IgG subtype. Similar trends were noted in the prevalence of HIT antibodies when other methods to detect HIT antibodies were used.

Conclusions: The oversulfated glycosaminoglycans such as oversulfated chondroitin sulfate and other non-heparin glycosaminoglycan derivatives are most likely to contribute to this increased prevalence and seroconversion of HIT antibodies. These observations suggest that heparin contaminants have contributed to the higher prevalence of HIT antibodies during the period 2007-2008. The improved quality measures resulting in more purified heparins after this recall have resulted in a decreased prevalence of these antibodies.

Funding: Private Foundation Support

SA-PO2691

Characteristics of T Cell Mediated Immunity in Patients with End-Stage Renal Disease Byung Ha Chung, In O Sun, Hoon Suk Park, Sun Ryoung Choi, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea.*

Background: Patients with end-stage renal disease (ESRD) exhibit immune dysregulation, but the precise immunological profile has not been investigated fully. We evaluated T cell-associated immune characteristics in ESRD patients.

Methods: Thirty-eight ESRD patients (22 on hemodialysis (HD) and 16 in predialysis) and 24 healthy volunteers were included. We compared the laboratory findings and immune profiles associated with T cells in these patients.

Results: Among the effector T cell subset, the percentages of Th2 cells and Th17 cells were significantly higher in the ESRD group than in the healthy controls ($P<0.05$). The frequencies of Th1 cells did not differ significantly between these groups. The percentages of Th1, Th2 and Th17 cells did not differ significantly ($P>0.05$) between the two subgroups within the ESRD group. The $CCR4^{-}CCR6^{+}$ /$CD4^{+}$T cell percentage was also significantly higher in the ESRD group. The naïve T cell ($T_{naïve}$) percentage was significantly lower in the ESRD group, and the difference between patients and controls was greater in the predialysis patients than in the HD patients ($P<0.05$, for each comparison). By contrast, the percentages of central memory T cells (T_{CM}) and effector memory T (T_{EM}) cells were significantly higher in the ESRD group. Interleukin-17 production by T_{EM} cells was significantly higher in the ESRD group. The severity of uremia was related negatively to the $T_{naïve}$ cell percentage but positively to the T_{CM} and T_{EM} cell percentages. The percentages of T_{EM} and $CD45RA^{+}$ T effector memory subsets of $CD8^{+}$ T cells were significantly higher in the ESRD group ($P<0.05$).

Conclusions: The result of this study showed significantly altered T cell-associated immunity and that it could not be corrected with hemodialysis.

SA-PO2692

Insulin Resistance in Non-Diabetic, Non-Obese Patients on Peritoneal Dialysis Dong Eun Yoo, Seung Jun Kim, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang, Dae-Suk Han. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Adipose tissue produces adipokines, which are closely associated with insulin resistance(IR). However, in patients with end-stage renal disease(ESRD), even though they are not obese, IR is commonly observed and is known to be attributed to uremia-associated factors, such as chronic inflammation and oxidative stress. This study was conducted to elucidate which factor, between adipokines or chronic inflammation, is more important in the pathogenesis of IR in non-diabetic, non-obese ESRD patients on peritoneal dialysis(PD).

Methods: This cross-sectional study included 55 non-diabetic, non-obese PD patients. Homeostatic model assessment-insulin resistance(HOMA-IR) was calculated, and leptin, adiponectin, high-sensitivity C-reactive protein(hsCRP), and IL-6 levels were measured. The patients were divided into 2 groups according to the median value of HOMA-IR, and clinical findings and laboratory data were compared between groups. Pearson's correlation analysis was performed to determine the relationship of clinical and laboratory data with HOMA-IR, and multiple linear regression analysis was used to identify independent factors associated with HOMA-IR.

Results: The mean age was 47.3 years, 50.9% were male, and the mean PD duration was 83.1 months. Male gender was significantly more prevalent, and hsCRP and IL-6 concentrations were significantly higher in patients with high HOMA-IR($p<0.05$). In addition, correlation analysis revealed that HOMA-IR was positively related to log hsCRP($r=0.499$, $p<0.001$), log IL-6($r=0.367$, $p=0.006$), and age($r=0.370$, $p=0.005$), whereas it was negatively correlated with Kt/Vurea($r=-0.296$, $p=0.031$). However, there was no correlation between HOMA-IR and adipokines, such as leptin and adiponectin. In multiple linear regression analysis, log hsCRP was the only significant independent factor associated with HOMA-IR(β±SE, 1.402±0.619; $p<0.05$).

Conclusions: This study shows that chronic inflammation rather than adipokines plays a major role in the pathogenesis of IR in non-diabetic, non-obese ESRD patients on PD, suggesting attenuating chronic inflammation may improve IR in these patients.

SA-PO2693

Kidney Failure Is Associated with an Imbalance between Pro- and Anti-Oxidant Agents Bertrand Gondouin,[1,2] Laetitia Dou,[2] Claire Cerini,[2] Stephane Burtey,[1,2] Philippe Brunet,[1,2] Regis Guieu,[3] Bertrand Dussol.[1] *[1]Service de Nephrologie et Transplantation Renale, Hôpital Conception, Marseille, France; [2]INSERM U 608, Faculté de Pharmacie, Marseille, France; [3]Service de Biochimie et Biologie Moleculaire, Hôpital la Timone, Marseille, France.*

Background: Patients with kidney failure display an accelerated atherosclerosis related to non-classical cardiovascular risk factors, including oxydative stress. Numerous studies have shown elevated levels of pro-oxidant agents whereas few studies have evaluated levels of antioxidant agents. This study evaluated the main parameters of oxidative stress in hemodialysis patients (HD).

Methods: Fifty non diabetic clinically stable HD, and thirty healthy subjects (HS) were included. HD were 30 male and 20 female, 66±15 years old with a dialysis vintage of 9±6 years. HS were 8 male and 22 females, 62±9 years old. We performed dosages of (i) anti-oxidant agents: super oxide dismutase (SOD), vitamins C, A, E, zinc (Zn), copper (Cu) and selenium (Se), (ii) pro-oxidant agents: malondialdehyde (MDA), ischemia modified albumin (IMA), and (iii) enzymes: xanthine oxidase (XO), red blood cells glutathione reductase (RBC-GR), and plasmatic glutathione reductase (P-GR). Results are expressed in mean +/- SD.

Results: Plasma levels of vitamin C (5.6±3.9 mg/L vs 16.7±7.1, $p<0.0001$), SOD (350±100 vs 416±65 U/g of Hb, $p<0.01$), Zn (69±14 vs 95±15 µg/dl, $p< 0.0001$), and Se (47±15 vs 73±17µg/L, $p <0.0001$) were lower in HD. Vitamin A levels were higher in HD (1.35±0.57 vs 0.73±0.17 mg/L, $p<0.0001$). Vitamin E and Cu were not statistically different.

Activities of RBC-GR (19.4±7.4 vs 10.2±3.3 U/g of Hb, p <0.0001), and XO (6.2±1.7 vs 5.0±1.3 µM/min, p<0.01) were higher in HD but not P-GR activity. MDA (256±72 vs 140±30 nmol/L, p<0.0001), and IMA (104±13 vs 90±9UI/L, p<0.0001) were higher in HD.

Conclusions: These results evidenced an imbalance in favour of oxidative stress in HD patients. The increased activity of xanthine oxidase led to the formation of numerous free radicals and its inhibition by allopurinol could be a therapeutic option to reduce oxidative stress in HD patients. A 2-years prospective follow-up is in progress to link those biological abnormalities to clinical events.

Funding: Government Support - Non-U.S.

SA-PO2694

Plasma Gelsolin Correlates with All Cause Hospitalization in Hemodialysis Patients Laura Rosales,[1] Yanna Dou,[1,2] Len A. Usvyat,[1] Georges Ouellet,[3] Stephan Thijssen,[1,2] Viktoriya Kuntsevich,[1,2] Mary Carter,[1] Nathan W. Levin,[1] Peter Kotanko.[1,2] *[1]Renal Research Institute, NY; [2]Beth Israel Medical Center, NY; [3]Maisonneuve-Rosemont Hospital, Montreal, Canada.*

Background: Plasma Gelsolin (pGNS) is a circulating protein that has been implicated in acute and chronic inflammation and tissue injury. Low pGNS may reflect the occurrence of clinical adverse events since its protective action at removing circulating actin declines. This prospective study explored the relationship between pGNS and hospitalizations (hosp) from all cause in hemodialysis (HD) patients (pat) over a 12 month period.

Methods: pGSN levels were determined with the 2C4 pGSN ELISA kit (Critical Biologics, Cambridge, MA) in maintenance HD pat from 2 HD centers; demographics (age, HD vintage, race, gender, BMI), co-morbidities: diabetes (DM), cardiovascular disease (CVD), HIV, and hepatitis C were recorded. Hosp and cause of admission were documented for 1 year. Cox proportional and Anderson Gill hazard models adjusted for age, gender, height, BMI, DM, vintage, HIV and hepatitis C status were used to compute hazard ratios (HR) for mortality, first and multiple hosp, and cause of admission such as CVD, infection and vascular access events.

Results: 104 stable HD pat followed for 1 year (mean±SD; age 61±15 years; HD vintage range 0.3-17.5 years; 52% female; 49% Blacks, 53% DM). pGSN was 195 mg/L ± 52.6 mg/L. pGSN quartiles were: Q1: <153.7 mg/L; Q2: 154.8-190.3 mg/L, Q3: 190.4 to 225.3 mg/L; Q4: > 225.4 mg/L.

In multivariate Cox analysis, Q1 of pGNS was associated with a high risk for all cause first hosp (HR 2.2, 95%CI 1.14 to 4.32, p<0.05).

Hazard ratio of pGNS for first hospitalization

Quartiles	HR	95% CI	P value
1	2.21	1.14 to 4.32	<0.05
2	1.19	0.59 to 2.39	0.62
3	1.00	0.49 to 2.05	0.99
4	1	reference	

In Anderson Gill analysis for multiple hosp the HR was not significant. Low pGNS did not show a significant relationship with mortality or cause of hosp.

Conclusions: Low pGNS levels in stable chronic HD pat were associated with time to first hosp from all causes; no relationship was found with recurrent hosp, cause of hosp, and mortality. In chronic HD pat pGNS may have a predicting value in the short term.

SA-PO2695

Influence of HCO1100™ – Dialysis Membrane on Monocyte Subpopulations and Soluble Inflammatory Parameters in Hemodialysis Patients with Elevated CRP Levels Roman Fiedler, Christof Ulrich, Felix Neugebauer, Eric Seibert, Matthias Girndt. *Department of Internal Medicine II, Martin-Luther-University Halle, Halle, Germany.*

Background: Chronically elevated CRP levels are a negative predictor for survival in hemodialysis (HD) patients because they indicate inflammation, atherosclerosis, and malnutrition. Inflammation-inducing cytokines are only insufficiently dialyzed due to their middle molecular weight (15-45 kD). As a result, cellular activation with enhanced levels of proinflammatory monocytes (CD14+CD16+, Mo3) and elevated ACE-expression (CD143) on this cells go along with high cardiovascular mortality in HD.

Methods: In a double-blind randomized crossover study we tested the impact of the High-cut-off (HCO)- Membrane 1100™ (Membrane B, Gambro, Hechingen, Germany) on removal of different cytokines (IL-1, IL-6, IL-10, TNFα) as well as on changes of the inflammatory monocyte subpopulation compared to the conventional high-flux membrane Polyflux 11S (Membrane A, Gambro, Hechingen, Germany). 17 patients (11 male, 6 female), aged 68.1 ± 10.9 years and on HD since 3.7 ± 2.8 years, were investigated over a study period of 2 weeks (6 HD consecutive sessions) on each membrane type. After normalisation data were analyzed by a one-way analysis of variance (ANOVA) for repeated measurements.

Results: Treatment with membrane B resulted in a non-significant decrease of Mo3 from 18.7 ± 7.4 to 17.7 ± 7.1 % (membrane A: 16.2 ± 7.7 to 17.9 ± 9.0 %, p=0.31) and in an increase of CD143-expression (MFI) from 11.2 ± 3.8 to 12.1 ± 5.5 (membrane A: 12.5 ± 5.1 to 12.0 ± 5.4 %, p=0.23). All other examined parameters were not different between both groups.

Conclusions: HCO 1100™ short-time dialysis treatment seems to be without effect on inflammatory parameters, at least regarding different important cytokines and pro-inflammatory monocyte subpopulations. Since trends indicate that the treatment intensity and duration might have been too low, additional studies with larger sample sizes and longer treatment duration are justified.

Funding: Government Support - Non-U.S.

SA-PO2696

Vitamin D Deficiency Is a Predictor of Inflammatory State in Hemodialysis Patients Syed Atif Mohiuddin, Omer H. Ali, Ravindra Rajakariar, Mark Blunden, Magdi Yaqoob. *Royal London Hospital.*

Background: High inflammatory burden is associated with morbidity and mortality in dialysis patients. Recent studies have indicated that vitamin D deficiency in dialysis patients may be associated with increased inflammatory markers which may be potentially reversed by vitamin D supplementation. In this cross sectional study we analysed the impact of vitamin D deficiency on inflammatory markers in a large multi ethnic cohort of haemodialysis patient in East London.

Methods: All patients admitted to the hospital or acute infections were excluded from the analysis. Vitamin D deficiency was defined as total 25 hydroxy vitamin D level less than 30 nmol/L, level between 30 and 79 defined insufficiency and level ≥80 was regarded as normal.

Results: 704 patients (32% Caucasian, 30% black,28% south Asian) were analysed. Results are shown in the table.

table1

	Normal	Insufficiency	Deficiency	Significance(p Value)
No (%)	115(16)	372(53)	217(31)	
Age±SD	58 ±16	60±14	56±15	0.27
Sex (male%)	58	64	53	0.04
Weight Kg±SD	70±18	73±18	70±19	0.9
Dialysis VintageYears±SD	3.2±2.7	3.1±2.6	3.8±2.6	<0.001
Dialysis catheter(%)	34	41	42	0.63
Mean Total Vitamin D nmol/l ±SD	118±31	47±13	21±5	<0.001
Mean PTH ±SD	43±42	47±42	53±60	0.09
Mean Calcium mmol/l±SD	2.34±0.18	2.29±0.19	2.29±0.2	0.01
Mean Phosphate mmol/l±SD	1.49±0.46	1.62±0.5	1.64±0.55	0.01
CRP>5 (%)	48	61	63	0.02
Albumin<35 g/l(%)	3	7	7	0.26
Mean KT/V±SD	1.55±0.26	1.5±0.32	1.55±0.35	0.9

Although there was no difference in albumin levels in different groups due to low prevalence of albumin deficiency, a strong association was found between low vitamin D levels and low albumin in regression analysis(p value <0.001). Vitamin D levels less than 80 nmol/l were also associated with high CRP levels (OR 1.75, p value 0.005). This association persisted after adjustment for comorbidity. A trend towards high PTH, low calcium and high phosphate was also seen with low vitamin D levels.

Conclusions: Vitamin D deficiency in haemodialysis population is associated with raised inflammatory markers. A multi-centre intervention study to look at the effect of vitamin D replacement on the inflammatory markers, morbidity and mortality is warranted.

SA-PO2697

Oral L-Carnitine Administration Could Lower Plasma Levels of Total Homocysteine in Patients on Hemodialysis Masataka Tsunoda,[1] Ryota Ikee,[2] Naomi Sasaki,[3] Megumi Sato,[4] Nobuo Hashimoto.[3] *[1]H. N. Medic Sapporo-Higashi, Sapporo, Japan; [2]H. N. Medic Kitahiroshima, Kitahiroshima, Japan; [3]H. N. Medic, Sapporo, Japan; [4]Jinzonaika Megumi Clinic, Sapporo, Japan.*

Background: Hyperhomocysteinemia has been consistently demonstrated in patients with renal failure. Recent studies suggested that increased levels of total homocysteine (tHcy) may result in both atherosclerosis and osteoporosis in HD- and non-HD patients through increased oxidant stress. On the other hand, there are some reports suggesting that L-carnitine may have antioxidant and anti-inflammatory properties. In this retrospective cohort study, we investigated effect of L-carnitine administration on clinical parameters including tHcy in HD patients.

Methods: We enrolled 10 males and 7 females treated with HD for at least 1 year (age 65 ± 11 years, HD duration 166 ± 137 months) to this study. L-carnitine was orally administered at 300 mg before each dialysis session. Plasma levels of tHcy, white blood cell (WBC) count, hemoglobin (Hb), albumin (Alb), high sensitivity C-reactive protein (hsCRP), and lipid profile (total cholesterol [TC], triglyceride [TG], and high-density lipoprotein cholesterol [HDL-C]) were evaluated every 3 months for 6 months.

Results: The mean value of tHcy at baseline was 61.8 ± 40.8 nmol/mL. After L-carnitine administration, plasma levels of tHcy significantly decreased. The mean reduction of tHcy at month 6 was 21.3 ± 20.0%, and the maximum reduction was 70.9%. Other parameters did not change during this follow-up period (Table 1).

Table 1. Effects of L-carnitine administration in HD patients.

	Baseline	Month 3	Month 6
tHcy (nmol/mL)	61.8 ± 40.8	56.8 ± 34.5*	47.7 ± 24.7*
WBC count (/µL)	5538 ± 1696	6581 ± 4420	5639 ± 1859
Hb (g/dL)	11.4 ± 0.92	11.3 ± 1.05	11.2 ± 0.58
Alb (g/dL)	3.86 ± 0.38	3.79 ± 0.37	3.76 ± 0.31
hsCRP (mg/dL)	0.09 ± 0.16	0.38 ± 0.94	0.18 ± 0.34
TC (mg/dL)	138 ± 31	140 ± 30	138 ± 34
TG (mg/dL)	108 ± 49	112 ± 40	115 ± 41
HDL-C (mg/dL)	46 ± 12	47 ± 13	46 ± 13

*p<0.05 compared with baseline.

Conclusions: We found a significant reduction of plasma tHcy levels after L-carnitine administration in HD patients. L-carnitine may prevent atherosclerosis and bone fracture by reducing plasma tHcy.

SA-PO2698

Electronegative LDL and Its Autoantibodies Correlated with Inflammatory Markers in Hemodialysed Patients Julie Lobo,[1] Milena Barcza Stockler-Pinto,[1] Najla Elias Farage,[2] Tanize Do Espirito Santo Faulin,[4] Dulcinéia Saes Parra Abdalla,[4] João Paulo Torres,[1] Denis Fouque,[5] Denise Mafra.[3] *[1]Federal Univ. of Rio de Janeiro, Brazil; [2]RenalCor Clinic, Brazil; [3]Federal Univ. Fluminense, Brazil; [4]Université Claude Bernard, Lyon, France; [5]University of São Paulo, Brazil.*

Background: A modified circulating electronegative LDL subfraction [LDL (-)], which has chemotactic, cytotoxic and immunogenic properties, is increased in hemodialysis (HD) patients and has been implicated in inflammation and atherosclerosis. However, antibody against LDL(-) may play a protect role, although the mechanisms involved have not yet been elucidated. Our aim was to investigate the association between LDL(-) and anti LDL(-) autoantibodies with inflammatory markers in HD patients.

Methods: Forth seven HD patients (29M/18F; 54.3 ± 12.6 yr, body mass index (BMI) 24.4 ± 4.1 Kg/m^2, average dialysis time 57.5 ± 50.1 months) from a private Clinic in Rio de Janeiro, Brazil, were studied and compared to 20 healthy subjects (9M/11F, 51.6 ± 15.6 yr, BMI 25.5 ± 3.9 Kg/m^2). LDL(-) and Anti LDL(-) IgG autoantibodies were determined by ELISA. TNF-α, IL-6, VCAM-1 and ICAM-1 were measured by a multiplex assay kit manufacture by R&D Systems®. The SPSS (version 11.0) was used as statistical program.

Results: HD patients present higher levels of inflammation and LDL(-) and lower levels of anti LDL(-) IgG autoantibodies when compared with health subjects (Table 1). There was a positive correlation between LDL(-) and IL-6 (r=0.25, p=0.004) and ICAM-1 (r=0.36; p=0.003) and a negative correlation between anti-LDL(-) autoantibodies and TNF-α (r=-0.37; p=0.003) and VCAM-1 (r=-0.50; p= 0.0001).

Table 1: Biochemical characteristics of the HD patients and healthy individuals

Parameters	HD Patients	Healthy individuals
LDL(-) (U/L)	0.32 ± 0.30*	0.09 ± 0.10
Anti LDL(-) (mg/L)	0.02 ± 0.01*	0.05 ±0.03
TNF-α (pg/ml)	5.5 ± 2.1*	2.4 ± 1.1
IL-6 (pg/ml)	4.1 ± 1.6*	2.6 ± 0.2
VCAM-1 (ng/mL)	48.5 ± 8.5*	23.8 ± 5.5
ICAM-1 (ng/mL)	20.5 ± 15.9*	7.2 ± 1.2

* p<0.04.

Conclusions: Our data seem to suggest that the association between LDL(-) and inflammation and the lower levels of anti-LDL(-) autoantibodies are important risk factors related to atherosclerosis in CKD.

Funding: Government Support - Non-U.S.

SA-PO2699

Novel Anti-Inflammatory Natural Products: Potential Agents for Reducing Inflammatory Signaling during Hemodialysis (HD) Thomas A. Vander Jagt, Lucy A. Hunsaker, David L. Vander Jagt, Vallabh O. Shah. *Uni of New Mexico.*

Background: The process of hemodialysis produces oxidative stress and secretion of pro-inflammatory agents, resulting in chronic low-grade inflammation in patients undergoing hemodialysis. Chronic inflammation can lead to downstream risks of CVD which are linked to increased patient mortality. Bacterial contamination of dialysis media and/or equipment or physical progression of blood leukocytes past artificial surfaces can initiate proinflammatory reactions. Several factors may contribute to the inflammatory response during HD, such as activation of the pro-inflammatory transcription factor NF-κB in leukocytes. Recently, we used lipopolysaccharide (LPS) stimulation of blood leukocytes to screen a natural product library (TimTec480) in order to identify novel anti-inflammatory agents ideally for use in dialysis media (Shah et al. 2010; Shah et al. 2011). These initial studies identified cardiac glycosides (strophanthidin, oubain, proscillaridin A, digoxin, digitoxin and lanatoside C) as inhibitors of the activation of NF-kB that also prevent expression of TNFα and other pro-inflammatory cytokines in whole blood in response to LPS.

Methods: In the current study a natural product library from Green Pharma (GPNCL240) was initially screened for inhibition of LPS-induced TNFα secretion in whole blood at 4 μg/ml. Agents that significantly prevented TNFα secretion were then screened using a luciferase reporter assay to determine whether these natural products also inhibited the activation of NF-κB.

Results: Two natural products (taspine and 10- hydroxy-camptothecin) were identified from this library. Both taspine and 10-hydroxy-camptothecin, similar to the cardiac glycosides, demonstrate low micro-molar IC50 values for inhibition of TNFα secretion in whole blood in response to LPS and for inhibition of NF-κB activation in reporter assays.

Conclusions: The mechanism appears mainly to involve inhibition of the activation of pro-inflammatory NF-kB. It appears reasonable to propose that a safe natural product can be identified that can be added to dialysis media to protect blood leukocytes and reduce chronic inflammatory signaling during hemodialysis.

Funding: Other NIH Support - NCRR -NEW Mexico - INBRE

SA-PO2700

Vascular Calcification: Is There a Role for Ferritin? André Fragoso, Ana Pinho, Anabela Malho, Ana Paula Silva, Elsa Morgado, Pedro Neves. *Nephrology Department, Hospital de Faro, Epe, Faro, Portugal.*

Background: Vascular calcification predicts cardiovascular events and death in ESRD patients. The aim of our study was to identify predictors of vascular calcification in our hemodialysis population.

Methods: We included all patients of a dialysis unit on treatment for more than 3 months by the 31st of January 2009. Patients were prospectively followed for 12 months. Vascular calcification was assessed and the population divided in two groups according to the Adragão score (G-1: score < 2; G-2: score ≥ 2.

Results: We followed 169 patients (63,3% males) with a mean age of 61,87±16,32 years in hemodialysis for 73,26 ±112,14 months. G-2 patients were older (65,60 ±13,86 vs 56,89±18,23 years, p = 0,001) and showed higher levels of ferritin (723,50±340,90 vs 569,31± 313,94 ng/ml, p= 0,003) despite lower IV iron doses (39,80±36,78 vs 53,64±40,69 mg/week, p= 0,023). The number of received RBC units was also higher (5,37± 10,23 vs 2,63 ± 3,43 units, p= 0,033) in the G-2. Additionally, these patients showed lower levels of serum Pi (4,14 ±1,06 vs 4,61 ±1,44 mg/dl, p= 0,019) and albumin (4,16±0,29 vs 4,33±0,33 g/dl; p= 0,001). Furthermore, the number of hospital admissions (0,93±1,20 vs 0,50±0,82 adm/patient, p=0,011) and mean in-hospital stay (5,19±8,9 vs 1,49±3,30 days, p = 0,001) were higher in the G-2 group. In a logistic regression model each ferritin quartile represented a 1.4-fold risk for a vascular calcification score ≥ 2 (p = 0,014; 95% CI: 1,079-1,957), which in turn represented a 1.3-fold risk for cardiovascular events (p=0,012, 95% CI: 1,062-1,640).

Conclusions: Vascular calcification is a known risk factor for cardiovascular events in the renal population. The encountered relationship between ferritin and vascular calcification puts inflammation and oxidative stress as central players in the genesis of cardiovascular disease.

SA-PO2701

Low Free Triiodothyronine and Hospitalization in Chronic Hemodialysis Patients Yanna Dou,[1,2] Laura Rosales,[1] Len A. Usvyat,[1] Georges Ouellet,[1,2,3] Stephan Thijssen,[1,2] Mary Carter,[1] Nathan W. Levin,[1] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York; [3]Maisonneuve-Rosemont Hospital, Canada.*

Background: Low levels of free triiodothyronine (fT3) are associated with inflammation, cardiovascular disease and mortality in hemodialysis (HD) patients. This study investigates the relationship between fT3 and multiple hospitalizations (hosp), first hosp and the cause of first hosp (cardiovascular events, infection) in HD patients.

Methods: In this prospective study chronic HD patients were recruited from two HD centers. fT3 was measured pre-HD at baseline by direct chemiluminescense (ADVIA Centaur, Deer field, IL). Mortality, time to first hosp, the cause of first hosp and multiple hosp were analyzed by Cox proportional and Andersen-Gill hazard models adjusted for age, gender, height, BMI, diabetes, dialysis vintage, HIV and hepatitis C status.

Results: 104 chronic HD patients were studied (54 females; 51 blacks; 55 diabetes; mean age 61±15 years). Mean level of fT3 was 2.4±0.4 pg/ml (normal range 2.27 to 4.22 pg/ml). During one-year follow up, 9 patients died and 191 hosp events occurred. In multivariate hazards model analysis, only Q1 of fT3 was associated with an increased risk of both time to first hosp and multiple hosp.

Hazard Ratio (HR) of fT3 for hospitalization

Quartiles of fT3 [pg/mL]	HR for first hosp	95% CI	P value	HR for multiple hosp	P value
Q1: <2.2	3.03	1.47 - 6.27	<0.01	2.4	0.02
Q2: 2.2-2.4	1.55	0.72 - 3.34	0.26	1.4	0.32
Q3: 2.4-2.6	2.20	0.93 - 5.17	0.07	2.56	0.02
Q4: >2.6	reference				

Q1 of fT3 did not show a significant relationship with mortality or cause of hospitalization.

Conclusions: Low fT3 level is an independent predictor of all-cause hospitalization in chronic HD patients.

SA-PO2702

The Immunosuppressivum 6-mercaptopurine Increases Calcification of Vascular Smooth Muscle Cells Jasmin Pruefer,[1] Mirjam Schuchardt,[1] Markus Tolle,[1] Matthias Höhne,[2] Markus van der Giet.[1] *[1]Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany; [2]Novartis Pharma AG, Nürnberg, Germany.*

Background: The cardiovascular mortality is dramatically increased in patients with chronic kidney disease and kidney transplanted patients. Vascular alterations like arteriosclerosis contribute to the increased cardiovascular risk. The mineralization process in the vascular wall is characterized by an transformation of vascular smooth muscle cells in the media to osteochondrocyte-like cells. There is evidence that different immunosuppressive therapy affect the development of arteriosclerosis. In this study we investigated the influence of 6-mercaptopurine on the mineralization process of rat vascular smooth muscle cells (VSMCs).

Methods: In vitro calcification in rat VSMCs were induced with calcification medium (CM). Calcium deposition was monitored by Alizarin staining and quantified by O-cresolphthalein complexone method. ALP gene expression was measured by real-time PCR.

Results: Cultivation of VSMCs in CM in vitro induced mineralization of VSMCs visualized by Alizarin Red staining quantified by measuring the extracellular calcium content. This process could be time-dependently and significantly increased in the presence of 6-MP [100 μmol/L]. To verify this effect, we used aortic rings and stimulated it for 14 days in CM±6-MP ex vivo. 6-MP could significantly increase the calcium content of the aortic rings compared to CM alone. For the precipitation of calcium phosphate, the activation of alkaline phosphate (ALP) is necessary. CM led to a significant increase in ALP enzyme activity, which is enhanced by pretreatment with 6-MP. Besides ALP activity, ALP expression was investigated. The 6-MP-induced ALP expression is significantly and dose-dependently increased in VSMCs after 48 h.

Conclusions: 6-MP treatment increases the mineralization of VSMCs in vitro and ex vivo. The data let suggest that 6-MP treatment may contribute to the high cardiovascular risk by enhancing vascular mineralization and arterial stiffening.

SA-PO2703

Eosinophilia after an Initiation of Dialysis Is Associated with Cardiovascular Morbidity and Mortality Jung-Hwa Ryu, Kyungjin Kim, Mina Yu, Dong-Ryeol Ryu, Seung-Jung Kim, Kyu Bok Choi, Duk-Hee Kang. *Division of Nephrology, Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul, Republic of Korea.*

Background: Dialysis-associated eosinophilia(DAE) has been reported frequently with incidence of 19~57%, however the clinical significance of DAE is not determined, especially in terms of the association of DAE with morbidity or mortality. In this study, we investigated the incidence of DAE, factors determining the development of DAE and an association of DAE with cardiovascular disease(CVD).

Methods: One hundred sixty seven patients on dialysis(HD or PD) more than 3M at Ewha Medical Center with available data on eosinophil count before and after 3 and 12M from an initiation of dialysis were enrolled(M:F=72:85, age 60.3 years, dialysis duration 50.8M). Eosinophilia is defined as a presence of eosinophil>5% of peripheral leukocytes or eosinophil count>350/mm^3. Definition of DAE was new development of eosinophilia after an initiation of dialysis at 3M(DAE3), 12M(DAE12), and both initial 3 and 12M(DAE3+12).

Results: DAE was observed in 50 patients(29.9%) with a comparable incidence in HD(30.2%) and PD(28.8%). There was no association of eosinophil count with age, gender, underlying renal disease, history of allergy or asthma or any specific medication. Subject with DAE had higher prevalence of CVD compared to DAE(-) subject. In subgroup analysis, both all-cause and cardiovascular mortality was higher in DAE12 compared to DAE(-) subject(all-cause: 58.8% vs. 24.3%, p=0.006, cardiovascular: 35.3% vs. 7.1%, p=0.006). Interestingly, all-cause and cardiovascular mortality of subject with DAE12 was significantly higher than subject with DAE3 or DAE3+12. In multiple regression analysis adjusted by age, gender, diabetes, and hypertension, DAE12 was an independent risk factor for both overall and cardiovascular mortality(overall: OR4.577, 95%CI; 1.284-16.311, cardiovascular: OR5.255, 95%CI 1.405-19.657). Kaplan-Meier survival analysis also revealed a lower survival rate in subject with DAE12(p=0.025) compared to other groups.

Conclusions: These results suggest that the development of late-onset eosinophilia may be associated with cardiovascular morbidity and mortality.

SA-PO2704

Genotype/Phenotype Correlation in FHHNC Caused by Mutations in Either CLDN16 or CLDN19: Long-Term Follow-Up in 125 Patients Lea Haisch,[1] Karl P. Schlingmann,[1] Bodo B. Beck,[2] Pierre Cochat,[3] Martin Konrad.[1] *[1]University Children's Hospital, Muenster, Germany; [2]University Children's Hospital, Cologne, Germany; [3]Edouard-Herriot Hospital, Lyon, France.*

Background: Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a tubular disorder caused by mutations in genes coding for the tight junction proteins Claudin-16 and Claudin-19. Both proteins play a pivotal role for the paracellular reabsorption of cations in the thick ascending limb. Interestingly, FHHNC leads to chronic renal failure and may be associated with extrarenal symptoms. This study compared the clinical course of FHHNC caused by mutations in CLDN16 or CLDN19.

Methods: We investigated 125 patients with FHHNC (CLDN16, n=100; CLDN19, n=25). We identified 20 different mutations in CLDN16 and 7 in CLDN19 including 6 novel mutations. We developed a standardized questionnaire to assess the long-term clinical course. Heterologous expression studies were carried out to investigate the cellular localization of the novel mutations.

Results: Immunohistostainings showed that most of the new mutants were retained in subcellular compartments. The decline in renal function showed no significant difference between the two cohorts, with a tendency toward a slower progression in CLDN19 patients (0.35 ml GFR loss/year vs 3,8 ml in CLDN16). Polyuria/polydipsia, an additional symptom in FHHNC, was reported by 85% of all patients, but salt craving (CLDN19: 57%; CLDN16: 8%; $p < 0.01$) and bedtime drinking (CLDN19: 75%; CLDN16: 45%; $p < 0,05$) significantly differed between the groups. 63% of all patients reported urinary tract infections without a difference between the two cohorts or gender. Most patients with CLDN19 defects seeked medical assistance for eye involvement which was the first symptom of FHHNC in this group. Moreover, 11/13 patients with CLDN19 defects showed retinal abnormalities compared to 0/19 of CLDN16 patients with documented fundoscopy.

Conclusions: Clinical course of FHHNC depends on the genotype. FHHNC due to defects in CLDN19 differs from CLDN16 not only by the ocular involvement but also by a more pronounced renal salt loss and a slower progression of chronic renal failure.

Funding: Government Support - Non-U.S.

SA-PO2705

Significance of Henle's Loop as a Responsible Nephron Segment of Impaired Mg Reabsorption in Renal Interstitial Damages Taisuke Shimizu, Hajime Hasegawa, Kaori Takayanagi, Yosuke Tayama, Juko Asakura, Takatsugu Iwashita, Koichi Kanozawa, Yuta Kogure, Tota Kiba, Shimpei Okazaki, Minoru Hatano, Tetsuya Mitarai. *Dept of Nephrol & Hypertens, Saitama Med Center, Saitama Med Univ, Kawagoe, saitama, Japan.*

Background: Hypermagnesiuria-induced hypomagnesemia is known to be occasionally developed in renal interstitial disorders (RID), and has been demonstrated to be correlated with severity of interstitial damages, suggesting that evaluation of hypermagnesiuria might be a clinical parameter of RID which frequently lacks significant urine abnormalities. In kidney, it is known that Mg is reabsorbed in mTAL by 70% of filtered Mg and in DCT by 10%. The aim of this study is to elucidate the responsible tubular segment for impaired Mg reabsorption in RID by use of unilateral ureter obstruction (UUO) model.

Methods: Kidneys were sampled 1 week after ligation of left ureter of male SD rats. Interstitial damages and changes in expression of Mg transporting molecules were assessed by histological analysis, immunohistochemistry and RT-PCR in control (C) and obstructed (O) groups.

Results: In results, studies of ratio of interstitial area (C: 0.19, O: 0.32), number of ED-1 positive cells (C: 32.5, O: 235.8/field), expression of MCP-1 (C: 105.0, O: 302.6%), and expression of TGF-beta (C: 101.1, O: 338.9%) revealed development of significant RID in O group in addition to an increase in FEMg (C: 2.1, O: 12.7%). The expression of Mg transporting molecules in mTAL showed significant decrease (C:100.3, O:59.3% in paracellin-1, C:103.1, O: 8.38% in NKCC2), whereas those in DCT, including TRP6, 7 and NCC, were not changed. Those results of gene expression were agreed with immunohistochemistry.

Conclusions: In conclusion, obtained results may suggest that the responsible tubular segment of impaired Mg reabsorption in RID would be mTAL, not DCT. Decreased expression of paracellin-1 in tight junction of mTAL, resulting in the decreased trans-tubular Mg permeability, might be implicated in the impaired Mg reabsorption associated with diminished driving force of Mg transport by suppression of lumen-positive charge due to decreased NKCC2 expression.

SA-PO2706

Genome-Wide Association Study Identifies Multiple Novel Common Variants Associated with Serum Calcium Concentration Conall M. O'Seaghdha,[1] Karen Kapur,[2] Qiong Yang,[3] Ian Deary,[4] Caroline Hayward,[6] Anna Kottgen,[7] Lorna Lopez,[4] David Siscovick,[9] Toshiko Tanaka,[10] Alexander Teumer,[11] Veronique I. Vitart,[6] Caroline S. Fox,[1] Murielle Bochud.[2] *[1]NHLBI's Framingham Heart Study; [2]University of Lausanne, Switzerland; [3]Boston University; [4]University of Edinburgh, United Kingdom; [5]University of Rotterdam, Netherlands; [6]MRCHGU, Edinburgh, United Kingdom; [7]Freiburg University, Germany; [8]Inst. Pop. Genetics, Italy; [9]Cardiovascular Health Research Unit, Seattle; [10]National Institute on Aging, Baltimore; [11]University of Greifswald, Germany.*

Background: Serum calcium levels are highly heritable. Genome-wide association studies (GWAS) have recently demonstrated association of SNPs within the calcium-sensing receptor gene (CASR) with serum calcium levels. However, the role of common genetic variation in calcium homeostasis is largely undetermined.

Methods: We performed GWAS in 17 population-based studies (n=47718) to identify common genetic variation associated with serum calcium level. The analysis was performed in 39400 individuals of European ancestry and 8318 of Asian ancestry for ~2.5 million genotyped and imputed single-nucleotide polymorphisms, adjusted for age, sex and study center.

Results: We confirmed the previously identified association of rs1801725 in the CASR gene; serum calcium levels were 0.07 mg/dL higher per copy of the minor allele. We also identified 7 new loci (p-value range 4.80 x10-08 to 2.53 x 10-12), at 2q37.1, 2p23.3, 6q13, 7q11.21, 10p14, 11p15.4 and 20q13.2. These SNPs identify several potential loci of interest, including genes related to the metabolism of vitamins D (CYP24A1) and K (VKORC1-L1). Results were similar for albumin-corrected calcium and were reproducible between European and Asian populations.

Conclusions: We have identified several common novel loci that are associated with calcium concentration in European and Asian adult populations. While preliminary and in need of independent replication, these findings highlight known pathways and suggest several novel pathways in association with calcium homeostasis.

Funding: Other NIH Support - NHLBI

SA-PO2707

Post-Hepatectomy Hypophosphatemia in Rats Kengo Nomura, Sawako Tatsumi, Yuji Shiozaki, Seiichi Yamaguchi, Tatsuya Kamatani, Hiroko Segawa, Shinsuke Kido, Ken-Ichi Miyamoto. *Molecular Nutrition, University of Tokushima, Tokushima, Japan.*

Background: Significant hypophosphatemia is common after major hepatic resection. Recently, the increase of renal fractional excretion of phosphate (FE Pi) has been reported to implicate in post-hepatectomy hypophosphatemia. In order to clarify the mechanism of hypophosphatemia caused by hepatectomy, we investigated rats received a partial hepatectomy (PH; about 70%).

Methods: We used male Wistar rats (9 wk old) for experiments. Thyroparathyroidectomy (TPTX) surgery was performed on male Wistar rats (6 wk old), which were purchased from SLC (Shizuoka, Japan). The blood and urine biochemical marker were measured before and on postoperative hours 6, 12, 24 in PH and sham rats. The expression of NaPi-IIa and NaPi-IIc in renal proximal tubules were analyzed by western blotting and immunohistochemistry.

Results: Within 24 hours after PH, serum phosphate levels were significantly decreased in PH rats. Urine Pi/creatinine ratio and FE Pi levels markedly increased. These results are consistent with hepatectomy patients. The Na+ -dependent phosphate uptake in renal brush border membrane vesicle declined about 40-50% at 6 hours after PH. Indeed, the amounts of NaPi-IIa and NaPi-IIc protein were significantly decreased in the PH rats.

The levels of serum fibroblast growth factor 23 (FGF23) were decreased about 50%, but the intact parathyroid hormone (PTH) concentration significantly increased in 6 hours after PH. To verify whether elevation of plasma PTH after PH causes hypophosphatemia, we analyzed the effects of PH on thyroparathyroidectomy (TPTX) rats. The present study showed that PH-TPTX rats still exhibited hypophosphatemia with down-regulation of NaPi-IIa and NaPi-IIc transporters.

Conclusions: These results imply that other factors rather than FGF23 and PTH, may contribute hypophosphatemia in PH rats.

Funding: Government Support - Non-U.S.

SA-PO2708

Estrogen Causes Phosphaturia through Specific Downregulation of NaPi-IIa and Via the Activation of Both Estrogen Receptors ERα and ERβ Faraaz Siddiqui,[1] Moshe Levi,[2] Hassane Amlal.[1] *[1]Internal Medicine, University of Cincinnati, OH; [2]Internal Medicine, University of Colorado Health Sciences Center, Denver, CO.*

Background: We have previously demonstrated that estrogen (E2) treatment of ovariectomized (OVX) rats caused a significant urinary Pi wasting and hypophosphatemia. This effect resulted from downregulation of apical sodium-dependent phosphate cotransporter (NaPi-IIa). However, the effects of E2 on other Pi transporters and the respective roles of estrogen receptors ERα and ERβ in this effect remain unknown.

Methods: To address these issues, we first examined whether ERα and ERβ are expressed in the kidney proximal tubule using RT-PCR and immunoblotting. Next, and because of the anorexic effect of E2 or the activation of ERα vs. ERβ, we performed two experiments. First, 3 groups of OVX rats were treated with either E2, 4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl) *tris*phenol (PPT), a selective ERα agonist, or vehicle in pair feeding protocol. In a second experiment, OVX rats were injected with 2,3-*bis*(4-Hydroxyphenyl)-propionitrile (DPN), a highly potent ERβ agonist, or its vehicle, and the expression of NaPi-IIa and other Pi transporters was examined by Northern hybridization and immunoblotting.

Results: Molecular studies demonstrated both ERα and ERβ are expressed in the kidney PT cells, and while NaPi-IIa mRNA and protein were significantly reduced (-62% and -58% vs. vehicle, P<0.03), as expected, the expression levels of NaPi-IIc (P>0.05), Pit1 (P>0.05) and Pit2 (P>0.05) were unchanged in E2, as compared to pair-fed vehicle rats. Further, PPT caused a significant decrease in NaPi-IIa mRNA (-31%, P<0.003) but did not significantly alter its protein abundance, as compared to pair- fed vehicle. Interestingly, DPN treatment caused a significant decrease in the protein abundance of NaPi-IIa (-61%, P<0.007), however, its mRNA expression levels was not significantly decreased, as compared to vehicle (P>0.05).

Conclusions: These studies demonstrate that the phosphaturic effect of E2 is mediated solely and specifically through downregulation of NaPi-IIa and that this effect involves a complex mechanism involving the activation of both receptors ERα and ERβ.

Funding: NIDDK Support

SA-PO2709

Renalase Regulates Renal Phosphate Excretion Daria Sizova, Gary V. Desir. *Medicine Nephrology, Yale School of Med & VACHS, New Haven, CT.*

Background: Renalase, a FAD/NADH dependent amine oxidase that metabolizes catecholamines, including dopamine (DA), plays an important role in the regulation of blood pressure and cardiac function. It is expressed in kidney, heart, liver, and skeletal muscle, and the highest levels are detected in proximal tubules. The protein is secreted plasma and urine. In the renalase KO mouse, plasma and urinary DA are markedly elevated. Furthermore, changes in dietary phosphate (Pi) modulate expression and activity of renalase in WT kidney, along with MAO-A and MAO-B, suggesting that these enzymes participate in renal Pi metabolism by regulating renal and urinary DA levels. The KO mouse was studied to determine the contribution of renalase to renal Pi handling.

Methods: KO and WT mice were maintained either on a low Pi diet (0.02% Pi) for 4 days or on a high Pi diet (1.25%). Serum Pi were determined at baseline and prior to sacrifice. Kidney were harvested and gene expression was measured by quantitative PCR.

Results: Figure 1a shows that serum phosphate is significantly lower in KO mice maintained on a low Pi diet. On a high Pi diet, serum phosphate is also significantly lower in KO mice. These data suggest that KO mice excrete Pi at a higher rate than WT, and are unable to conserve Pi on a low Pi diet in spite of significant fall in serum Pi. Gene expression analysis using qPCR revealed that low Pi intake was associated with increased COMT (27%, n=5, p<0.01, fig 1b) and MAO-B (45%, n=5, p<0.05) in KO compared to WT.

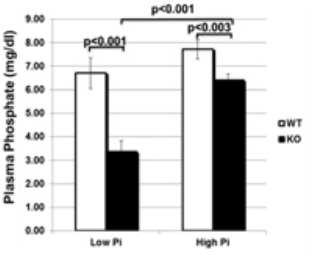


Figure 1a: Diet induced hypophosphatemia in renalase KO mice (n=5) maitained on low Pi for 4 days

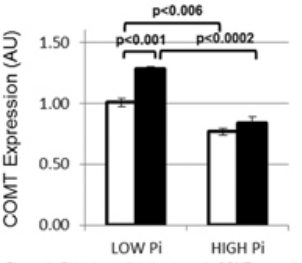


Figure 1b: Pi intake mediated changes in COMT expression in WT (n=5, clear box) and KO (n=5, filled box) mice.

Conclusions: In the absence of renalase, a low Pi diet fails to decrease urinary DA even though both COMT and MAO-B are upregulated, suggesting that renalase is the key factor that regulates urinary DA levels. Elevated urinary DA in the renalase KO drives urinary Pi excretion and leads to low serum Pi. These data support the notion that renalase plays a critical role in renal phosphate metabolism by regulating urinary DA.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2710

Adverse Effects of Simulated Hyper- and Hypo-Phosphatemia on Endothelail Cell Function and Viability Ai Peng,[1,4] Tianfu Wu,[2] Cai-Hong Zeng,[3] Dinesh Rakheja,[4] Zhi-Hong Liu,[3] Jiankun Zhu,[2] Nosratola D. Vaziri,[5] Chandra Mohan,[2] Xin J. Zhou.[4] *[1]Medicine, Shanghai Tenth Hospital of Tongji University, Shanghai, China; [2]Medicine, UT Southwestern Medical Center, Dallas, TX; [3]Research Institute of Nephrology, Nanjing University School of Medicine, Nanjing, China; [4]Pathology, UT Southwestern Medical Center, Dallas, TX; [5]Medicine, UC Irvine, Irvine, CA.*

Background: Dysregulaiton of phosphate homeostasis as occurs in chronic kidney disease is associated with cardiovascular complications. It has been suggested that both hyper- and hypo-phosphatemia can cause cardiovascular disease. The molecular mechanisms by which high or low serum phosphate levels adversely affect cardiovascular function are poorly understood. The purpose of this study was to explore the mechanisms of endothelial dysfunction in the presence of non-physiologic phosphate levels.

Methods: We studied the effects of simulated hyper- and hypophosphatemia in human umbilical vein endothelial cells in vitro. The effects of inorganic phosphate on cell proliferation and apoptosis were measured by flow cytometry. Nitric oxide (NO) production and endothelial NO synthase were determined. The effects of inorganic phosphate on cell signaling protein expressions were determined using reverse-phase protein microarry.

Results: We found both simulated hyperphosphatemia and hypophosphatemia decrease eNOS expression and NO production. This was associated with reduced intracellular calcium, increased protein kinase C β2 (PKCβ2), reduced cell viability, and increased apoptosis. While simulated hyperphosphatemia was associated with decreased Akt/p-Akt, Bcl-xl/Bax ratios, NFkB and p-Erk abundance, simulated hypophosphatemia was associated with increased Akt/p-Akt and Bcl-xl/Bax ratios and p-Mek, p38, and p-p38 abundance. This is the first demonstration of endothelial dysfunction with hypophosphatemia.

Conclusions: Our data provides the basis for further studies to elucidate the relationship between altered phosphate homeostasis and cardiovascular disease. As a corollary, our data suggests that the level of phosphate in the culture media, if not in the physiologic range, may inadvertently affect experimental results.

Funding: Other NIH Support - NIAMS, Private Foundation Support, Government Support - Non-U.S.

SA-PO2711

Association of 24-Hour Urine Dopamine with Serum and Urine Phosphorus in Persons with Coronary Heart Disease: The Heart and Soul Study Nisha Bansal,[1] Chi-Yuan Hsu,[1] Mary Whooley,[1] Joachim H. Ix.[2] *[1]UCSF; [2]UCSD.*

Background: Urine dopamine (DA) is produced in the proximal tubule and has been found to increase in response to dietary phosphorus (Pi) intake, and to contribute to greater urinary Pi excretion in animal models. Whether urine DA is associated with Pi homeostasis in humans is uncertain.

Methods: Urine DA and Pi were measured in 24 hour urine collections among 963 outpatients with stable coronary heart disease (CHD). Fasting morning serum was used to measure Pi and kidney function. We examined cross-sectional associations between urine DA and serum Pi, 24-hour urine Pi, (as an indicator of dietary Pi intake), and fractional excretion of phosphorus (FePi) using linear regression. Models were adjusted for age, sex, race, eGFR by cystatin C, albuminuria, hypertension, heart failure, tobacco use, body mass index and diuretic use.

Results: Mean age was 67±11 years, 83% were men, and mean eGFR was 71±23 ml/min/1.73m^2. Mean urine DA was 192.8±85.1 µg/day, mean serum Pi was 3.7 ± 0.6 mg/dL, mean 24-hour urine Pi was 46.8 ± 25.1 mg/day, and mean FePi was 7.3 ± 8.6%. Higher urine DA was associated with younger age, male sex, black race, higher eGFR, and lower albuminuria (all P< 0.05). In adjusted models, each standard deviation higher DA was associated with higher serum Pi, higher urine Pi, and lower FePi (Table 1).

Conclusions: These data suggest that higher dietary Pi intake is associated with higher urine dopamine in persons with CHD, consistent with animal models. However, these changes were not associated with higher fractional excretion of phosphorus, suggesting that urine DA increases in parallel with phosphorus intake, but may not be responsible for greater urinary Pi excretion.

Table 1. Association of Urine DA (per SD) with Serum Pi, 24-Hr Urine Pi and FePi

	Unadjusted	Adjusted*
Serum Pi (mg/dL)	0.02 (95% CI: -0.02, 0.06), p=0.3	0.04 (95% CI: 0.003, 0.09), p=0.04
24-Hour Urine Pi (mg/day)	4.8 (95% CI: 3.2, 6.4), p<0.001	3.6 (95% CI: 1.7, 5.6), p<0.001
FePi (%)	-2.3 (95% CI: -2.9, -1.8), p<0.001	-0.8 (95% CI: -1.5, -0.2), p=0.007

*Adjusted for age, race, sex, eGFR, albuminuria, hypertension, heart failure, tobacco use, body mass index, and diuretic use

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SA-PO2712

Dietary Phosphate Modifies Life Span in Drosophila Clemens Bergwitz,[1] Joanne Huang,[1] Mark J. Wee,[1] Harald Jueppner.[3] *[1]Endocrine Unit, Massachusetts General Hospital, Boston; [2]Dept. of Genetics, Harvard Medical School, Boston; [3]Dept. of Pediatrics, Massachusetts General Hospital, Boston.*

Background: Drosophila, owing to the large number of genetic tools and its short life span, has been used as model organism to study the effects of the insulin/IGF-1 signaling, reactive oxygen species, and autophagy pathways on longevity.

Methods: To test the hypothesis that dietary phosphate modifies life span in Drosophila just like in higher organism and humans, we supplemented Standard medium (yeast/corn/malt/sucrose/agar)(SM), with 30 mM sodium-phosphate (pH 6.0)(Pi30), 30 mM sodium-sulfate (pH 6.0)(Si30), or 1% sevelamer (Sev1%).

Results: Median lifespan of male yellow white flies (wild-type) at 25 degrees C was 45±2.5, 36±3.9, 44±2.9, and 51±3.4 days in SM, Pi30, Si30, and Sev1%, respectively (n>100 flies per condition). Findings were similar for females and for different fly strains, including W1118, Canton S, and Oregon R. Hemolymph phosphate concentration was 21±0.3, 23±1.7, and 23±1.6 mg/dl in SM, Pi30, and Sev1%, respectively. Excretion of phosphate was 2.5±0.4, 9.0±2.5, and 0.6±0.05, and ng/fly/60 min., and whole fly phosphate content was 285±7, 273±28, and 285±5 ng/fly, respectively. We next prepared a defined medium supplemented with 1.5 mM potassium-phosphate (pH 6.0), 27 mM Pi and 53 mM Pi, in which total osmolarity was kept constant using potassium sulfate. Survival of yellow white males was 22±0.3, 20±0.3, and 17±0.9 days in these media, respectively. All media supported larval development and eclosion of adult flies after 11 days, except for Sev1%, which caused developmental delay.

Conclusions: Our findings suggest that the effects of dietary phosphate on life-span are conserved between fly and man. Life-span in flies is extended by 40% in Sev1% when compared to Pi30 medium, and by 10% when compared to SM. By adjusting phosphate excretion under different dietary conditions, homeostatic mechanisms appear to be present in fly intended to maintain stable hemolymph and whole fly phosphate concentrations. Drosophila may therefore be a suitable model organism to understand the molecular basis of dietary phosphate toxicity in vivo.

Funding: NIDDK Support

SA-PO2713

OSR1 in the Regulation of Proximal Tubuluar Phosphate Transport Michael Föller,[1] Ganesh Pathare,[1] Kerim Mutig,[2] Jakob Völkl,[1] Sebastian C. Bachmann,[2] Florian C. Lang.[1] *[1]Institute of Physiology, University of Tuebingen, Germany; [2]Insitute of Anatomy, Charité, Berlin, Germany.*

Background: Gain of function mutations of WNK1 and WNK4 kinases lead to Gordon's syndrome resulting in hypertension and hyperkalemia. WNK kinases phosphorylate and thereby activate the serine/threonine oxidative stress kinase 1 (OSR1) and the closely-related STE20/SPS1-related proline/alanine-rich kinase (SPAK). Both, OSR1 and SPAK have been shown to participate in the regulation of renal salt excretion and blood pressure by stimulating the $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) and the Na^+/Cl^- cotransporter (NCC). The present study explored whether OSR1 is expressed in proximal renal tubules and influences proximal tubular phosphate transport.

Methods: Immunohistochemical analysis showed OSR1 expression in murine proximal tubular epithelial cells. To elicit the in vivo significance of OSR1-sensitive phosphate transport, knock-in mice carrying WNK kinase-resistant OSR1 ($OSR185A/^+$) were compared to wild type mice ($OSR^+/^+$).

Results: Despite a significantly lower serum phosphate concentration the urinary phosphate excretion was significantly larger in $OSR185A/^+$ mice than in $OSR^+/^+$ mice. The serum calcium concentration was not significantly different between the genotypes whereas the urinary calcium excretion tended to be lower in $OSR185A/^+$ mice compared to $OSR^+/^+$ mice. Under a low phosphate diet, the serum phosphate concentration was again significantly lower and the urinary phosphate excretion significantly higher in $OSR185A/^+$ mice compared to $OSR^+/^+$ mice. Neither plasma PTH nor serum calcitriol levels were significantly different between the genotypes.

Conclusions: In conclusion, OSR1 is expressed in proximal tubular epithelial cells and participates in the regulation of Napi-IIa-dependent renal phosphate reabsorption.

SA-PO2714

Regulation of the Type IIa Sodium Phosphate Cotransporter by AMP Activated Protein Kinase Eleanor D. Lederer,[1,2] Francesca Pribble,[2] Syed J. Khundmiri.[2] *[1]Medical Service, Robley Rex VA Medical Center, Louisville, KY; [2]Medicine, University of Louisville School of Medicine, KY.*

Background: The physiologic functions of AMP activated protein kinase (AMPK), a kinase implicated in cell polarity and energy metabolism, in proximal tubule are not known. Based on the critical role of phosphate in cell metabolism and the documented role of AMPK in regulation of sodium pump expression, we hypothesized that AMPK regulated apical membrane trafficking of the type IIa sodium phosphate cotransporter (Npt2a).

Methods: OK (opossum kidney) cells, a model for renal proximal tubule, were grown in monolayers. Total and activated (phospho) AMPK were detected by Western blot in whole cell lysates and membrane preparations. Npt2a expression was detected by Western blot. Phosphate transport was measured by uptake of radiolabeled phosphate in transport medium.

Results: Treatment of cells with 1 mM AICAR, an AMPK activator, resulted in a progressive increase in pAMPK expression detectable at 2h and rising by 75% at 24 hours, analyzed as pAMPK to total AMPK. Exposure of cells to medium containing no phosphate had no effect on AMPK activity in membrane or cytosol fractions, measured by pAMPK to total AMPK. Treatment with phosphate-free medium or with AICAR for two hours had no effect on AMPK activation in brush border membrane (BBM). AICAR alone had no significant effect on Npt2a expression or phosphate uptake. Phosphate-free medium increased Npt2a expression by 44% at 2 hours. However, treatment of cells with phosphate-free medium in the presence of AICAR blocked the ability of phosphate-free medium to increase BBM expression of Npt2a. Despite the increase in Npt2a expression in the presence of phosphate-free medium, phosphate uptake did not change.

Conclusions: We conclude that 1) OK cells express AMPK, 2) ambient phosphate does not regulate AMPK activation, and 3) activated AMPK has no effect on basal Npt2a expression but inhibits Npt2a apical membrane trafficking stimulated by low ambient phosphate. The dissociation of Npt2a expression and function in phosphate-free medium suggests differential regulation of these properties by as yet unknown mechanisms.

Funding: Veterans Administration Support

SA-PO2715

Secondary Hyperparathyroidism and Hyperphosphaturia in Mice Lacking Adenylyl Cyclase 6 Timo M. Rieg,[1,3] Tong Tang,[3] H. Kirk Hammond,[1,3] Volker Vallon.[1,2,3] *[1]Dept. of Medicine, University of California San Diego, CA; [2]Dept. of Pharmacology, University of California San Diego, CA; [3]VA San Diego Healthcare System, San Diego, CA.*

Background: Adenylyl cyclase isoform 6 (AC6) is expressed in all renal tubular segments and catalyzes the synthesis of cAMP. Parathyroid hormone (PTH) is coupled to a Gs protein-coupled receptor and upon activation Na+/phosphate cotransporters are retrieved from the membrane of the proximal tubules resulting in increased phosphate excretion. Fibroblast growth factor 23 (FGF23) was identified as novel phosphaturic hormone. To define the role of AC6 in Pi and Ca2+ homeostasis, we studied AC6 wild-type (WT, n=10) and knockout (AC6-/-, n=10-13) mice.

Methods: Twentyfour hour metabolic cage studies were performed with free access to food (2% Ca2+, 1% Pi) and water. Blood was taken from the retrobulbar plexus. Plasma and urine were analyzed for Pi and Ca2+. A separate plasma sample was analyzed for PTH, FGF23, and 1,25-Dihydroxy Vitamin D. To test for primary hyperparathyroidism we applied the calcimimetic (NS568, 30 mg/kg by oral gavage) to activate Ca2+ sensing receptors and study suppression of endogenous PTH after 30 min.

Results: Metabolic cage studies identified greater urinary Pi excretion in AC6-/- (32±5 vs. 4±1 µmol/day, P<0.001) which was associated with, and likely secondary to, higher plasma levels of PTH (255±60 vs. 39±6 pg/ml, P<0.005) and FGF23 (466±36 vs. 275±13 pg/ml, P<0.001), which may have suppressed 1,25-Dihydroxy Vitamin D levels in AC6-/- (141±17 vs. 215±16 pmol/l, P<0.01). Urinary Ca2+ excretion (2.5±0.2 vs. 2.6±0.2 µmol/day), plasma Pi (WT: 1.5±0.1 vs. 1.5±0.1 mmol/l in AC6-/-) and Ca2+ (WT: 2.7±0.1 vs. 2.7±0.1 mmol/l in AC6-/-) were not different. NS568 suppressed endogenous PTH to similar levels in both genotypes (WT: 14±1 and AC6-/-: 14±2 pg/ml).

Conclusions: Our results imply AC6 in the regulation of Pi homeostasis and exclude primary hyperparathyroidism as the cause for phosphaturia. PTH- and/or FGF23 induced inhibition of renal Pi reabsorption do not require AC6. Further studies are needed to determine the cause of secondary hyperparathyroidism including primary alterations in Ca2+ homeostasis or intestinal phosphate uptake.

Funding: Private Foundation Support

SA-PO2716

Calcium Sensitive Dicarboxylate Transport in Mouse Proximal Tubule Cells Kathleen S. Hering-Smith,[1,2,3] Glenn T. Nagami,[4] L. Lee Hamm.[1,2,3] *[1]Medicine, Tulane University, New Orleans, LA; [2]Physiology, Tulane University, New Orleans, LA; [3]THRCE, Tulane University, New Orleans, LA; [4]Nephrology Section, VA Greater Los Angeles Health Care System, Los Angeles, CA.*

Background: Urinary citrate is the most important endogenous inhibitor of calcium nephrolithiasis and is regulated by proximal tubule reabsorption. Urinary citrate increases with alkalosis and with increased urinary calcium. We recently reported a novel calcium sensitive dicarboxylate citrate transport process (AJP 2010) in the OK (opossum proximal tubule) cell line. This transport differed from that found with NaDC1, the known apical citrate transporter.

Methods: To determine whether this calcium sensitive process is also found in other species, ^{14}C-citrate uptake was measured in the proximal tubule specific cell lines S1 and S2 derived from large T-antigen transgenic mice. Cell were grown on permeable supports with uptake measured from the apical aspect only.

Results: In both S1 and S2 cells, lowering extracellular calcium stimulated citrate uptake (0.70 ± 0.16 to 1.35 ± 0.33 pmole/well in S1, and 0.075 ± 0.01 to 0.10 ± 0.02 pmole/well in S2, $p< 0.05$ for both). Succinate uptake (which occurs via NaDC1 also) was not affected by calcium. Uptake was not sensitive to 2,3-dimethylsuccinate, an inhibitor of basolateral type dicarboxylate transporters. NaDC1 was present in both cell types by Westerns in equivalent amounts despite the nearly 10 fold difference in citrate uptake.

Conclusions: Mouse proximal tubule cells contain an apical calcium sensitive citrate transporter demonstrating that this novel citrate transport process is present in a variety of mammalian species. Despite the presence of NaDC1, this process is unlikely to be NaDC1 since we have previously demonstrated that NaDC1 is not calcium sensitive. The proximal tubule likely contains several citrate transporters reflecting regulation of reabsorption by several pathways.

Funding: Other NIH Support - COBRE

SA-PO2717

L-WNK1 Controls ARH-Dependent ROMK Endocytosis by Phosphorylation

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Background: In states of dietary potassium deficiency, ROMK renal potassium channel is marked for endocytosis by a clathrin adaptor protein, ARH, and regulated by a kinase mutated in a familial disease of hyperkalemia and hypertension, WNK1 (Fang et al, Journal of Clinical Investigation '09). In the present study, we explore the mechanism by which ARH is regulated by L-WNK1. In the absence of L-WNK1, ARH is polyubiquitinated and highly liable, decaying in very short time frame by the proteasome. Co-expression of L-WNK1 rendered ARH insensitive to degradation, and increased ARH phosphorylation. In vitro studies with purified proteins revealed ARH is substrate of L-WNK1 phosphorylation, but a signaling intermediate was found to be required for maximal activity. The primary phosphorylation site was mapped a serine at the N-terminal region, which is important for protein stability. In conclusion, L-WNK1 phosphorylates ARH and blocks proteasomal degradation of ARH. This mechanism provides a likely explanation for how ARH protein abundance is augmented in states of dietary potassium deprivation to stimulate ROMK endocytosis and reduce urinary potassium loss.

Funding: NIDDK Support, Private Foundation Support

SA-PO2718

High Potassium Intake Upregulates Transepithelial Potential Difference and Potassium Secretion in Mouse Cortical Collecting Duct

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Background: K^+ secretion via constitutively active ROMK and flow-stimulated maxi-K channels in aldosterone-sensitive distal nephron including late DCT, CNT and CCD are important for K^+ homeostasis. Patch-clamp and immunological studies showed that a high K^+ diet increased the density of ROMK and ENaC in rat CCD. In vitro microperfusion study of rabbit CCD showed that flow-stimulated K^+ excretion presumably via maxi-K is upregulated by high K^+ intake. In the study in rabbit CCD, baseline K^+ excretion presumably via ROMK was not increased by a high K^+ diet, raising the possibility of species differences of K^+ secretion in CCDs. Genetic mouse models are invaluable for studying K^+ handling in renal tubules, yet little information is available for dietary regulation of K^+ secretion in the mouse CCD.

Methods: C57BL/6 mice were fed either a control K (CK) (1% K^+) or high K (HK) diet (5.25% K^+) for 3 weeks. CCDs dissected from CK and HK mouse kidneys were microperfused in vitro under no transepithelial osmotic gradients. Tubular fluid samples were collected at a low physiological flow rate (1-2 $nl_{[sup]*[/sup]}min^{-1}$,$mm^{-1}$). The lengths of perfused segments ranged from 0.4 to 0.6 mm (mean 0.5 mm) in both CK and HK mice. K^+ and Na^+ concentrations in perfusate and collected fluids were analyzed using ion selective electrodes (ISE).

Results: Lumen-negative transepithelial PD were increased in HK versus CK CCDs (-20.1 ± 3.3 vs. -0.4 ± 0.1 mV, $p < 0.01$). High K^+ diet greatly increased secretory K^+ fluxes (J_k: 28.1 ± 8.8 $pmol_{[sup]*[/sup]}min^{-1}$,$mm^{-1}$ in HK mice versus 1.0 ± 0.5 pmol,min^{-1},mm^{-1} in CK). The increase in J_k was completely abolished by amiloride (100 μM).

Conclusions: A high K^+ diet increases transepithelial PD and enhances K^+ excretion in the mouse CCD. The mechanism for the increase in PD and whether an increased abundance of ROMK contributes to the increased K^+ secretion remain to be determined. Future studies will include investigation of regulation of K^+ secretion in CCDs by WNK kinases using genetic mouse models. Finally, ISE is a reliable method for measuring K^+ and Na^+ in small volume of fluid collection in microperfusion.

Funding: NIDDK Support

SA-PO2719

Inhibition of Src-family Protein Tyrosine Kinase (SFK) and Serine/threonine Phosphatase Stimulates ROMK Channels in the Rat Cortical Collecting Duct (CCD)

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

Background: Previous studies have demonstrated that low Na intake or aldosterone infusion did not increase ROMK channel activity in the CCD despite of a high aldosterone level. The aim of the present study is to test the hypothesis that a high SFK activity may be responsible for suppressing the effect of aldosterone on ROMK channels.

Methods: We used the Western blot to examine the effect of low Na intake and a high K intake (HK) on the expression of WNK4, c-Src and phosphorylated-c-Src^{Y416} (P-c-Src^{Y416}) in the rat kidney. Also, we used the patch-clamp technique to examine the effect of inhibiting SFK and serine/threonine phosphatase on ROMK channels in the CCD of rats on a low Na diet or a HK diet.

Results: A HK intake significantly decreased c-Src expression and P-c-Src^{Y416} level but had no effect on WNK4 expression in the kidney. In contrast, low Na intake had no effect on c-Src expression and P-c-Src^{Y416} level in comparison to the control animals, suggesting that SFK activity is higher in the kidney of rats on a low Na diet than those on a HK diet. Patch-clamp experiments demonstrated that inhibition of SFK activated ROMK channels in 7 patches of total 10 experiments and increased channel activity, defined by NP_o from 0.9±0.2 to 3.0±0.5. In contrast, inhibition of SFK had no effect on ROMK channels in the CCD of rats on a HK diet. Also, patch-clamp experiments showed that inhibition of phosphatases with calyculin A (5 nM) increased ROMK channel activity (NP_o= 1.9±0.3) in the CCD of rats fed low Na diet but not in rats on a HK diet. This suggests the role of serine/threonine phosphatases in suppressing ROMK channel activity in rats on a low Na diet.

Conclusions: We conclude that SFK and serine/threonine phosphatases play a role in suppressing ROMK channels in the CCD of rats on a low Na diet.

Funding: NIDDK Support

SA-PO2720

Protein Phosphatase 1 (PP1) Binds to With-no-Lysine Kinase 4 (WNK4) and Regulates the Effect of WNK4 on ROMK Channels

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Background: WNK4 inhibited ROMK channels and the inhibitory effect of WNK4 was abolished by SGK1 but restored by c-Src. The aim of this study is to explore the role of serine/threonine protein phosphatases in modulating the interaction among SGK1, c-Src and WNK4.

Results: Immunoprecipitation experiments demonstrated that serine/threonine phosphatase, PP1, binds to WNK4 at amino acid (aa) residues 695-699 ($PP1^{\#1}$) and at aa1211-1215 ($PP1^{\#2}$) in HEK cells transfected with flag-tagged WNK4 constructs. Coexpression of c-Src decreased the association of PP1 to WNK4 at $PP1^{\#1}$ but increased the association at $PP1^{\#2}$. Expression of WNK4 mutants, $WNK4^{-PP1\#1}$, $WNK4^{-PP1\#2}$ or $WNK4^{-PP1\#1+2}$ in which the $PP1^{\#1}$, $PP1^{\#2}$ or both $PP1^{\#1}$ and $PP1^{\#2}$ binding sites were deleted or mutated, inhibited ROMK channels as potent as WNK4. However, inhibition of phosphatases with calyculin A enhanced WNK4's inhibition of ROMK channels in the cells transfected with $WNK4^{-PP1\#1}$ but diminished the WNK4-induced inhibition in the cells transfected with $WNK4^{-PP1\#2}$. In contrast, neither the basal activity of ROMK channels nor WNK4's inhibition of the K channel was affected by calyculin A treatment in HEK cells transfected with wt WNK4. Moreover, c-Src restored the inhibitory effect of WNK4 but not $WNK4^{-PP1\#1}$ on ROMK channels in the presence of SGK1. Expression of c-Src inhibited SGK1-induced phosphorylation of WNK4 but not $WNK4^{-PP1\#1}$ at Ser^{1196}. In contrast, coexpression of c-Src restored the inhibitory effect of $WNK4^{-PP1\#2}$ on ROMK in the presence of SGK1 and inhibited SGK1-induced WNK4 phosphorylation at Ser^{1196} in cells transfected with $WNK4^{-PP1\#2}$.

Conclusions: We conclude that WNK4 is associated with PP1 at two sites and that PP1 binding to aa 695-9 is directly responsible for removal of SGK1-mediated phosphorylation of WNK4 while PP1 at aa1211-5 is responsible for removal the phosphorylation of WNK4 whose phosphorylation enhanced WNK4's inhibition of ROMK channels.

Funding: NIDDK Support

SA-PO2721

A-kinase Anchoring Protein (AKAP) Regulates BK Channel-Mediated K^+secretion (JK) in the Cortical Collecting Duct (CCD)

Wen Liu, Carlos Schreck, Lisa M. Satlin. *Department of Pediatrics, Mount Sinai School of Medicine, NY, NY.*

Background: The apical BK channel in CCD principal cells (PC) is tonically inhibited by PKA under low flow conditions (Liu et al, AJP Renal 297:F904, 2009), an observation that led us to speculate that BK channel-mediated flow-induced net K secretion (FIKS) requires release of channel inhibition by this kinase. Emerging evidence suggests that interactions between integral membrane proteins (e.g., ENaC) and protein kinases are stabilized at the plasma membrane by anchoring proteins, including AKAP.

Methods: To examine whether AKAP contributes to the tonic inhibition of the BK channel under basal conditions, we measured JK and net Na absorption (JNa; each in pmol/min.mm) in NZW rabbit CCDs microperfused in vitro at slow (~1) and fast (~5 nl/min.mm) flow rates in the presence of St-Ht31 (25 μM), a cell permeant AKAP inhibitor peptide that disrupts PKA anchoring on AKAP, added to bath and lumen. Control studies were performed using a control peptide (CP; 25 mM) of similar sequence which does not inhibit the interaction between PKA and AKAP.

Results: We found that JK was higher in CCDs treated with Ht31 (-19.0±1.9; n=8) than CP (-7.3±2.9; n=3; p<0.01) when perfused at slow flow rates; JNa in these same Ht31 and CP treated CCDs did not differ (21.7±3.1 vs. 13.3±1.9; p=0.13). A 5-fold increase in luminal flow rate did not further increase JK in 3 Ht31-treated tubules (-17.6 ±0.2 to -21.5±0.2; p=NS).

Conclusions: Our observation that the BK channel blocker iberiotoxin (50 nM) inhibited the Ht31-induced stimulation of JK in 5 CCDs perfused at slow flow rates (-19.8±2.9 to -10.1±1.7; p<0.05), without significant effect on JNa (19.5±3.7 vs. 16.6±4.6; p=NS), suggests that AKAP contributes to suppression of BK channel activity under low flow conditions, perhaps by anchoring PKA to the ion channel at the apical membrane.

Funding: NIDDK Support

SA-PO2722

The Kir4.1 Knock-Out Mouse Shows Decreased Na^+/Cl^- Cotransporter Expression Daniel A. Gray, Michael W. Cypress, Talia Holtzman. *Nephrology Unit, University of Rochester, NY.*

Background: Mutations in the Kir4.1 potassium channel underlie the SeSAME/EAST syndrome, an autosomal recessive condition characterized by seizures, sensorineural deafness, ataxia, developmental abnormalities and a hypokalemic hypomagnesemic metabolic alkalosis. To better understand the pathophysiology of this syndrome, we have been investigating the renal phenotype of a generalized Kir4.1 knock out mouse.

Methods: Serum and spot urine electrolytes were measured in 3-6 day old pups and paraformaldehyde-fixed kidney sections were probed with antibodies to distal nephron transporters.

Results: Our previous work showed that the Kir4.1 knock out mouse displays a reduced serum [K^+] associated with an elevated transtubular K^+ gradient and decreased urine osmolality. We now show that serum [Mg^{+2}] is also significantly reduced from 2.9 ± 0.3 mEq/L in WT to 2.3 ± 0.1 mEq/L in the knock out (n=6-7, p < 0.05) and is associated with an inappropriately elevated urine [Mg^{+2}]. Serum [Ca^{+2}] was normal. Light microscopy did not demonstrate any structural abnormalities of the distal nephron in the knock out. However, decreased Na^+/Cl^- cotransporter (NCCT) expression was observed by immunofluorescence in kidney sections probed with an NCCT antibody. No increase in aquaporin-2 immunofluorescence was seen to account for the decreased osmolality observed in the knock out.

Conclusions: Our results suggest that the renal phenotype of the SeSAME/EAST syndrome is largely recapitulated in the Kir4.1 knock out mouse. In addition, the decreased NCCT expression observed suggests a means by which loss of basolateral Kir4.1 function results in failure to reabsorb Na^+ and Cl^- apically.

Funding: NIDDK Support

SA-PO2723

A Case of EAST/Sesame Syndrome with Tubulopathy as First Manifestation Rosa Vargas-Poussou,[1] Djamal Djeddi,[2] Xavier Jeunemaitre.[1] *[1]Genetics, AP-HP Hôpital Européen Georges Pompidou, Paris, France; [2]Pediatrics, CHU d'Amiens, Amiens, France.*

Background: EAST (epilepsy, ataxia, sensorineural deafness,tubulopathy) or SeSAME (Seizures, sensorineural deafness, ataxia, mental retardation, electrolyte imbalance)is a rare autosomal recessive disease caused by mutations at the KCNJ10 gene coding for potassium channel kir 4.1. First manifestations of index cases of the 7 families described to date are neurological, in particular generalized seizures in the first months of life.

Methods: We describe clinical and genetic characterization of a French patient with this disease, in whom the first manifestations were linked to tubulopathy.

Results: Patient was the first child of a consanguineous union, born at term of an uncomplicated pregnancy. At 6 months of age, she was hospitalized for failure to thrive and a profound hypokalemia (2.2 mmol/L) was detected. Enteral nutrition and oral potassium were instituted. During a new hospitalization 1 month later for persistent hypokalemia, clinical and biological data were: weight 4.6 kg, size 60 cm, blood pressure: 99/49 mmHg, axial hypotonia and psychomotor retardation. Blood analysis: pH 7.52, electrolytes (mmol/L): Na 140, K 3.1, HCO3 26, Cl 98, Ca 2.65 and Mg 0.97. Urine electrolytes (mmol/L): Na 59, K 82, and Cl 102. Calcium/creatinine 0.03 mmol/mmol. Plasma renin and aldosterone: 480 and 284 pg/mL. Renal ultrasound was normal. The diagnosis of Bartter/Gitelman syndrome was suggested, haplotype analysis showed homozygosity at CLCNKB locus but sequencing and MLPA were negative. Neurological evaluation at 9 months showed a rotatory nystagmus and a mild ataxia; cerebral tomography and visual evoked potential were normal; hearing evaluation showed bilateral absence of otoacoustic emission. At age 1 year she had generalized seizures with recurrence 2 weeks later. During last hospitalization auditory evoked potential showed mixed hearing loss.KCNJ10 sequencing showed a known homozygous missense mutation (p.Arg175Gln), for which in vitro expression showed an impaired channel function (Reichold M et al. PNAS 2010).

Conclusions: EAST/SeSAME syndrome can be first presented as a salt wasting tubulopathy followed by severe neurological manifestations.

Funding: Government Support - Non-U.S.

SA-PO2724

PAR2 Promotes Potassium Sparring and Controls Plasma Potassium Concentration Luciana Morla, Gaëlle Brideau, Lydie Cheval, Gilles Crambert, Suresh Krishna Ramakrishnan, Alain Doucet. *UMRS 872, team 3, UPMC Univ Paris 06 and INSERM and CNRS, Paris, France.*

Background: We previously showed that activation of PAR2 by trypsin or an agonist peptide increases sodium reabsorption in isolated rat cortical collecting duct (CCD) without promoting potassium secretion. We therefore evaluated the role of PAR2 in maintaining potassium balance *in vivo* and in controlling the activity of the potassium-secreting channel ROMK in CCD.

Methods: For this purpose, we compared plasma potassium level and potassium handling in $PAR2^{-/-}$ and wild type (WT) mice under basal state and potassium depletion *in vivo* and in *in vitro* microperfused CCDs.

Results: Under basal state, $PAR2^{-/-}$ mice displayed normal potassium excretion and blood concentration. Within two days of potassium depletion, $PAR2^{-/-}$ mice showed a blunted ability to maximally decrease their urinary excretion of potassium, as compared to WT mice. This inappropriate ability to conserve potassium was associated with decreased plasma potassium concentration in $PAR2^{-/-}$ mice (in mM ± SE; WT, 3.9 ± 0.2; $PAR2^{-/-}$, 3.2 ± 0.1; p<0.025). To evaluate the effect of PAR2 on ROMK activity, we compared the effect of trypsin on AVP-stimulated potassium secretion in isolated CCDs. In absence of AVP, rat CCDs neither reabsorbed sodium nor secreted potassium. AVP treatment induced sodium reabsorption and potassium secretion. Pre-treatment with trypsin did not alter AVP-stimulated sodium reabsorption but abolished potassium secretion, suggesting an inhibitory effect on ROMK. This inhibition was further supported by the finding that trypsin induced the phosphorylation of ERK in CCD, a pathway known to induce the endocytosis of ROMK.

Conclusions: In conclusion, we showed that activation of PAR2 inhibits potassium secretion in CCD and thereby participates in potassium sparing during potassium restriction.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

SA-PO2725

Progesterone Is Required for Efficient Renal Adaptation to Chronic Dietary K+ Restriction in Male by Stimulating H,K-ATPase Type 2 Expression Gilles Crambert, Amel Salhi, Aurelie Edwards. *UMRS872 Team 3, INSERM/UPMC/CNRS, Paris, France.*

Background: Modern dietary habits are characterized by high-Na and low-K intakes, each of which has been correlated with a higher risk for hypertension. In this study, we examined whether long-term variations in the intake of Na+ and K induce lasting changes in the plasma concentration of circulating steroids.

Methods: We developed a mathematical model of adrenal steroidogenesis in mice that permits to predict concentration of all steroids based on the level of expression of the steroidogenic genes. These predictions were then confirmed by experimental measurements using RIA. The investigations of the renal effects of progesterone in the context of a dietary K restriction has been done in mice and using a cell line (mCCD).

Results: Our mathematical model predicted that male mice increase their plasma progesterone levels in response to K+ depletion. This prediction was confirmed by experimental measurements showing a 3-time increase of progesterone after 8 days of K restriction. Our results indicated a relationship between the ability to produce progesterone and the efficiency of the renal retention of K. Moreover, adrenalectomized mice exhinited a 2-time higher loss of potassium when placed under low-K diets and have an increase mortality rate after 10 days of this treatment.

The mechanism by which progesterone stimulates renal K retention involved an increased expression and activity of HKA2. This effect was proved by using HKA2 null mice that turned to be "resistant" to the anti-kaliuretic effect of progesterone whereas wild-type mice reduced their K excretion by 15-20% after progesterone injection.

Finally, we determine that the progesterone-dependent ability to retain K+ efficiently is RU486-sensitive , indicating the involvement of the nuclear progesterone receptor in this process.

Conclusions: Our results suggest the existence of a hereto unknown regulatory process involving progesterone, its nuclear receptor, the HKA2 and renal K+ retention.This effect of progesterone, in male as in female, is mineralcorticoid-independent (aldosterone is low in the K-depleted state) and may represent a "hidden" regulatory pathway in hypokalemic states.

Funding: Government Support - Non-U.S.

SA-PO2726

Claudin-16 Knock Down Mice Modulate Pendrin and vH-ATPase Abundance in Distal Nephron To Prevent Volume Depletion Nina Himmerkus,[1] Jan E. Behrends,[1] Magdalena A. Gutowska,[1] Paul S. Steels,[2] Markus Bleich.[1] *[1]Institute of Physiology, Christian-Albrechts-University, Kiel, Germany; [2]Biomed, University Hasselt, Diepenbeek, Belgium.*

Background: Mutations in Claudin-16 are associated with the hereditary disease Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC). In Claudin-16 knock down mice (KD) we have previously shown that young KD mice compensate for NaCl loss in the TAL by enhanced downstream reabsorption in the cortical collecting duct (AJP,2008; 295(6):F1641-7).

Methods: We now investigated a cohort of aged (1.5-2 years) mice to assess long term compensation. We examined the expression pattern and membrane representation of chloride bicarbonate exchangers (Pendrin and AE1) and the vH^+-ATPase by confocal immunofluorescence (IF). IF data are given as % intensity of IF in the membrane area compared to the cytosolic signal.

Results: KD showed the key symptoms of FHHNC and were 15% lighter, 10% shorter and showed a slightly increased hematocrit. Urine osmolality was 1.5-fold higher and the ratio of urinary Na^+ to K^+ concentrations was 30% decreased, indicating an increased sodium and water reabsorption in the distal nephron. The urine of KD as well as WT was acidic (pH 5.8 +/- 0.2 vs. pH 5.8 +/- 0.1). In kidney slices of KD 64% of the Pendrin IF was localized in the luminal membrane of cortical intercalated cells compared to 46% in WT. Freshly isolated cortical collecting ducts showed a similar pattern and, in addition, basolateral membrane expression of vH^+-ATPase was increased in KD with 41% compared to 21%, respectively. In the medulla 58% of vH^+-ATPase IF intensity was localized in the luminal membrane of KD intercalated cells compared to 42% in WT. We did not find any difference in the distribution of the IF signal for AE1.

Conclusions: We conclude that aged KD suffer from long term volume depletion. Functional upregulation of Pendrin serves compensatory NaCl reabsorption downstream of the TAL. The increased cortical bicarbonate secretion is counterbalanced by an activation of H^+ secretion in the medulla.

Funding: Government Support - Non-U.S.

SA-PO2727

The Leaky Epithelial Cell Culture Model, OK Cells, Express Claudin-4, Whose Gene Dosage Dictates Transepithelial Resistance R. Todd Alexander, Jelena Borovac, Reid S. Barker, Emmanuelle Cordat. *University of Alberta, Edmonton, AB, Canada.*

Background: The study of paracellular flux across leaky epithelial, such as the proximal tubule, has been limited by the lack of a properly characterized cell culture model. We therefore set out to determine the molecular and electrophysiological properties of the leaky epithelial cell culture model, opossum kidney (OK) cells.

Methods: The electrophysiological properties of OK cells were assessed on confluent monolayers grown on semi-permeable filters, mounted in Ussing chambers, by measuring transepithelial resistance (TER) and dilution potentials. To determine the molecules mediating the electrophysiological properties we identified, cloned and then measured the relative expression, by quantitative PCR, of the claudins (a family of tight junction proteins, which dictate paracellular permeability characteristics). To validate the model system we over-expressed claudin-4 (identified as being endogenously present) and then repeated the expression and electrophysiological studies.

Results: OK cells demonstrate a low transepithelial resistance (TER = 10.2 ± 1.4 Ωcm^2), slight cation selectivity (pNa/pCl = 1.07 ± 0.002), a Eisenman sequence for cations of $K^+ > Cs^+ > Rb^+ > Na^+ > Li^+$ and for anions of $Cl^- > I^- > Br^-$. This mirrors the published electrophysiological properties of the proximal tubule *in vivo*. At the molecular level OK cells express claudin 4 > 1 > 6 > 20 > 9 > 12 > 11 > 15. We were surprised that claudin-4, a barrier forming claudin, was significantly expressed in this model system of a leaky epithelia. We therefore generated stable cell lines over-expressing claudin-4. Immunofluorescence microscopy confirmed that claudin-4 appropriately localized to the tight junction when over-expressed. All the clones stably expressing claudin-4 demonstrated significantly increased TER, without an alteration in pNa/pCl. They also displayed significantly increased claudin-1, -6 and -9 expression.

Conclusions: Together these results establish OK cells as a leaky epithelial model system to study claudin function. They also implicate claudin-4 in forming a paracellular barrier, whether directly or via affects on other claudin expression remains to be elucidated.

Funding: Government Support - Non-U.S.

SA-PO2728

Differential Effects of Extracellular ATP on Chloride Transport Via Calcium Activated Chloride Channels in Kidney Cortical Collecting Duct CELLS Madhumitha Rajagopal,[1,2] Alan C. Pao,[1,2] Jonathan Widdicombe.[3] *[1]Nephrology/ Medicine, Stanford University, Stanford, CA; [2]Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; [3]Physiology and Membrane Biology, University of California, Davis, Davis, CA.*

Background: The cortical collecting duct (CCD) of the kidney plays a critical role in fine-tuning sodium chloride (NaCl) balance and is subject to extensive regulation by hormones such as aldosterone. Recent studies have demonstrated that extracellular nucleotides are also important in regulating the epithelial Na^+ channel (ENaC) in the CCD, thereby contributing to NaCl balance and blood pressure regulation under high dietary salt conditions. Extracellular nucleotides also regulate Cl^- transport through Ca^{2+} activated chloride channel (CACC) in CCD cells, although the relationship between CACC and ENaC activity is not clearly defined.

Methods: We studied a CCD ($mpkCCD_{cl4}$) cell line in Ussing chambers to determine the effects of ATP on CACC-mediated Cl^- transport under varying levels of ENaC activity.

Results: To simulate conditions associated with salt overload, we clamped transepithelial voltage (V_{te}) to 0 mV and then treated $mpkCCD_{cl4}$ cells with amiloride to inhibit ENaC. The addition of ATP caused a robust and transient increase in Cl^- secretion, which could be inhibited by flufenamic acid (FFA, a CACC inhibitor) or BAPTA-AM (a Ca^{2+} chelator). The V_{te} of aldosterone-stimulated $mpkCCD_{cl4}$ cells was ~ -50 mV. Therefore, to mimic conditions of the DOCA-salt model of hypertension, where both aldosterone and ATP are elevated, we treated $mpkCCD_{cl4}$ cells with aldosterone, clamped the V_{te} at -50 mV, and then treated cells with ATP. We found that ATP induced a robust and transient increase in transcellular Cl^- absorption, which could be inhibited by FFA or BAPTA-AM.

Conclusions: These findings illustrate that the direction of CACC-mediated Cl^- transport in CCD cells, which is dependent on V_{te} and ENaC activity, and suggest that extracellular ATP, in combination with aldosterone, differentially regulates CACC activity under conditions that reflect varying states of NaCl balance.

Funding: Other NIH Support - K08, Private Foundation Support

SA-PO2729

Chemical Library Screening for Drugs To Correct Intracellular Mislocalization of R8L Mutant Barttin Naohiro Nomura, Shotaro Naito, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental Universtiy, Bunkyo-ku, Tokyo, Japan.*

Background: Barttin, a gene product of *BSND*, is one of four genes responsible for Bartter syndrome. Previously, we showed that one of the disease-causing mutant barttin, R8L, was mis-localized to cytoplasm and could not reach to plasma membranes in MDCK cells and R8L knockin mouse. In addition, we reported that curcumin and 17-allylamino-17-demethoxygeldanamycin (17-AAG) could rescue the mis-localization of R8L barttin and partially corrected the phenotypes in the R8L barttin knockin mice. These results suggested that the aberrant intracellular localization of R8L barttin is a major cause of this disease, and to find more potent drugs to correct the mislocalization could be a promising strategy to treat this disease. For this purpose, we screened a chemical library consisting of about 20,000 chemical compounds in our university.

Methods: The screening was performed using MDCK cells stably expressing GFP-tagged R8L barttin. Cellular localization of the GFP signal after treatment with the compounds was assessed by using ArrayScan (Thermo Scientific), an automated fluorescence microscopic imaging system designed for high throughput screening.

Results: As a result of this screening, we identified four candidates. We confirmed the effects of these compounds by cell surface specific biotinylation assay. Furthermore, we injected one of these compounds to R8L knockin mice intraperitonealy, and could observe the improvement of plasma membrane signal of R8L barttin in the kidney.

Conclusions: We expect that these chemical compounds can be seeds for drugs to treat Bartter syndrome type IV caused by R8L barttin mutation, and that they can also be effective for treating diseases caused by mislocalization of mutant proteins, such as CFTR-ΔF508.

Funding: Government Support - Non-U.S.

SA-PO2730

Chloride Channels CLIC1 and CLIC4 Function in Proximal Tubule Endocytosis John C. Edwards, Yao-Wen Cheng. *UNC Kidney Center and Department of Medicine, University of North Carolina, Chapel Hill, NC.*

Background: CLIC proteins can function as chloride channels but their normal physiologic role remains uncertain. CLIC4 has been implicated in intracellular membrane traffic in tubulating endothelial cells. Whether CLICs are important in other intracellular membrane pathways is unknown. CLIC1 and CLIC4 are both highly expressed in the apical region of kidney proximal tubule cells and a role in proximal tubule endocytosis has been proposed. We examined mice carrying targeted disruption of either CLIC1 or CLIC4 for alterations in levels of proteinuria which could be a reflection of proximal tubule endocytosis.

Methods: Urine and blood was collected from groups of *Clic1*$^{(-/-)}$ or Clic4$^{(-/-)}$ mice and matched WT controls. Plasma albumin, plasma and urine creatinine, and urine total protein were determined by commercially available clinical testing. Urine albumin concentration was determined by western blotting. Plasma and urine β-2 microglobulin (β2M) were determined by ELISA assay. Values are reported as mean ± SEM. P values determined by T-test.

Results: Creatinine, albumin, and β2M values in plasma were not different among the groups.

	Clic1 WT(n=5)	Clic1(-/-) (n=5)	P value	Clic4 WT (n=5)	Clic4 (-/-) (n=5)	P value
Urine pro/ creat (mg/ mg)	0.265±0.048	0.279±0.011	(NS)	0.172±0.028	1.332±0.615	0.096
Urine alb/creat (normalized arbitrary units)	1.00±0.029	1.42±0.086	0.0016	1.00±0.047	1.39±0.14	0.025
Frac Exc β2M (%)	1.44±0.4	6.71±2.1	0.04	(nd)	(nd)	

nd: not determined

Mice lacking either CLIC1 or CLIC4 showed changes in urinary protein. *Clic1*$^{(-/-)}$ mice had no significant change in total urine protein but have significantly increased albuminuria and β2microglobunuria. *Clic4*$^{(-/-)}$ mice had a tendency to increased urine pro/creat ratio that did not reach significance have significantly increased albuminuria.

Conclusions: Both CLIC1 and CLIC4 appear to contribute to clearance of protein from the glomerular filtrate. In particular the increased fractional excretion of β2M in the *Clic1*$^{(-/-)}$ mice indicate decreased endocytic uptake from the proximal tubule lumen. The data are consistent with the hypothesis that CLICs 1 and 4 function as chloride channels in vesicles along the endocytic pathway.

Funding: Other NIH Support - NIHLB

SA-PO2731

A Novel Conditional Knockout Mouse Model of the Proximal Tubule Endocytic Receptors Megalin and Cubilin Kathrin Weyer,[1] Tina Storm,[1] Jingdong Shan,[2] Seppo J. Vainio,[2] Erik I. Christensen,[1] Pierre J. Verroust,[1] Rikke Nielsen.[1] *[1]Department of Anatomy, Aarhus University, Aarhus, Denmark; [2]Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland.*

Background: The megalin and cubilin receptors are highly expressed in the kidney proximal tubules. Previous studies using animal models have demonstrated their synergistic and important role for normal proximal tubule endocytic recovery of filtered proteins in vivo. Studies using mouse models are however restricted by the fact that cubilin-deficient mice die in utero and most megalin-deficient mice die perinatally.

Methods: Using the *Cre/loxP* gene knockout system, we have developed a viable megalin and/or cubilin knockout mouse model, where the Cre gene is driven by the Wnt4 promoter which is expressed in the embryonic kidney. Kidney tissue was analysed using immunohistology and Western blotting. Urine samples were analysed for excreted proteins by SDS-PAGE, ELISA and Western blotting.

Results: The $Meg^{lox/lox}$;Wnt4-Cre, $Cub^{lox/lox}$;Wnt4-Cre, and combined $Meg^{lox/lox}$,$Cub^{lox/lox}$; Wnt4-Cre transgenic mice were all viable, fertile, and transmitted the transgenes in a Mendelian fashion. Kidney tissue analysis by immunohistology and Western blotting demonstrated a highly reproducible and efficient (≥88%) knockdown of megalin and cubilin in proximal tubule cells. Consistent with the loss of megalin and cubilin expression in the kidneys, the mice were found to excrete increased amounts of low molecular weight proteins in the urine. Because this model allows the production of a large number of mice we were further able to statistically analyse groups of mice for urinary albumin excretion.

Conclusions: We anticipate that these mice will be a valuable tool to study the role of megalin/cubilin-defects in the pathogenesis of proximal tubulopathies.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2732

Altered Prostanoid Receptor Signaling, but Not Decreased PGE2 Production, Explains the Significant Resistance of $P2Y_2$ Null Mice to Li-Induced Polyuria Ioana L. Pop,[1] Yue Zhang,[1] Noel G. Carlson,[2] Bellamkonda K. Kishore.[1] *[1]Medicine, Nephrology, VAMC & Univ of Utah; [2]Neurovirology & GRECC, VAMC & Univ of Utah, Salt Lake City, UT.*

Background: Previously we showed that genetic deletion of $P2Y_2$ receptor results in significant resistance to Li-induced polyuria, without altering blood or renal medullary Li levels. Here we document that this resistance is not due to decreased production of PGE2, which is widely regarded as a causative factor in Li-induced polyuria, but instead due to altered prostanoid receptor (EP-R) signaling.

Methods: Groups of wild type (WT) and $P2Y_2$-R KO mice were fed normal (ND) or Li-added (LD) diets (40 mmol/kg food) for 14 days with free access to water and euthanized.

Results: Li-induced polydipsia, polyuria, and decreases in urine osmolalities and AQP2 protein abundance in renal medulla were significantly less in KO mice vs. WT mice ($P < 0.05$). Interestingly, genetic deletion of $P2Y_2$-R did not suppress Li-induced increased urinary excretion of PGE2 ($P < 0.05$). Since PGE2 induced effects are mediated through EP-Rs, using semi-quantitative immonoblotting, we determined the protein abundances of EP1, EP2, EP3 and EP4 receptors in the inner medulla (EP2-R not detectable). EP3-R protein abundance in ND fed KO mice was markedly low as compared to the ND fed WT mice (29%; $P < 0.001$), while levels of EP1-R and EP4-R were not altered across the groups (EP2-R not detectable). Li-feeding caused a modest decrease in EP3-R protein in WT (67%; $P > 0.05$), and KO mice (82%). Furthermore, *ex vivo* stimulation of medullary collecting ducts (mCD) with PGE2 generated significantly more cAMP in LD-fed KO mice vs. LD-fed WT mice (130%; $P < 0.03$), while no such differences were seen when the mCD from these groups of mice were stimulated by dDAVP.

Conclusions: Taken together these data suggest that genetic deletion of $P2Y_2$-R offers significant resistance for the development of Li-induced polyuria by alteration of EP-R signaling, but not by decreasing the production of PGE2. Incidentally, this study reveals that the decreased protein abundance of EP3-R in P2Y2-R KO mice may also contribute to the increased urinary concentrating ability reported in these mice.

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SA-PO2733

Acute Lithium Administration Increases Water Excretion through Activation of MAP Kinases Francesco Trepiccione,[1] Trairak Pisitkun,[2] Jason D. Hoffert,[2] Robert A. Fenton,[1] Soren Nielsen,[1] Mark A. Knepper,[2] Birgitte M. Christensen.[1] *[1]Department of Biomedicine, Water and Salt Research Center, Aarhus University, Aarhus, Denmark; [2]Epithelial Systems Biology Laboratory, NHLBI, NIH, Bethesda, MD.*

Background: Acute lithium (Li^+) treatment impairs the water permeability of the kidney collecting duct. Changes in phosphorylation status are a fast way of regulating protein function. Here we used a phosphoproteomic approach to detect the early molecular targets of Li^+ in inner medullary collecting duct (IMCD).

Methods: Rats were treated with a gavage of either LiCl or NaCl (2.4 mmol/Kg of BW). After 4 or 9 hours rats were euthanized and IMCD suspensions were prepared. Phosphopeptides from IMCD isolated after 9 hours of Li^+-treatment were enriched by immobilized-metal affinity chromatography (IMAC) and then analysed on a LTQ-orbitrap LC-MS/MS system. Label-free relative quantification of MS1 peak integration was carried out by QUOIL software.

Results: Nine hours after LiCl gavage, rats showed a significantly increased urine volume and decreased urine osmolality ($p<0.01$ n=8). A total of 1222 unique phosphopeptides were identified in 578 proteins. DAVID analysis of the obtained phosphopeptide database highlighted a MAPK cluster of proteins. In addition, phosphopeptides in the Li^+-treated group with ≥ 1.5 fold increased abundance shared a common "proline-directed" kinase motif as determined by Motif-X analysis, further evidence that MAP kinases may be regulated by acute Li^+. The phosphopeptides in this group included AQP2 phosphorylated at S261, a potential MAPK target.Western blot analysis of IMCD isolated 4 hours after LiCl gavage showed increased phosphorylation of p38 and ERK1/2. No changes were observed in pS261-AQP2 and pJNK1/2 at 4 hours. After 9 hours of LiCl gavage pS261-AQP2 and pS256-261-AQP2 were significantly increased, while no changes in pS256-AQP2 or phosphorylated MAPKs were observed.

Conclusions: We conclude that MAPK pathways are an early target of Li^+ action in IMCD and the Li^+-related polyuric effect may involve the associated increase in pS261-AQP2.

SA-PO2734

Thiazide Attenuates Lithium-Induced Nephrogenic Diabetes Insipidus Independently of the Sodium-Chloride Co-Transporter Anne P. Sinke,[1] Marleen L.A. Kortenoeven,[2] Peter M.T. Deen.[1] *[1]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Anatomy, University of Aarhus, Aarhus, Denmark.*

Background: Thiazide diuretics are commonly used to reduce urine output in patients with lithium-induced nephrogenic diabetes insipidus (Li-NDI). As thiazides block NaCl-cotransporter (NCC) mediated sodium uptake, it is generally thought that the observed AQP2 expression levels with treatment are due to hypovolemia-induced increases in vasopressin release. As Li-NDI is already anticipated to go with increased vasopressin levels, we investigated the role of NCC in this paradoxical anti-diuretic effect.

Methods: Polarized mouse cortical collecting duct (mpkCCD) cells show deamino-8 D-arginine vasopressin (dDAVP)-induced expression of endogenous AQP2, which is reduced upon treatment with clinically-relevant (1 mM) levels of ltihium for 24-48 hours. Interestingly, this lithium-induced reduction in AQP2 levels was reversed by thiazide treatment for 48 hours. Moreover, while lithium inactivated Gsk-3β, mainly by increasing its S9-phosphorylation, thiazide treatment reduced intracellular levels of lithium and led to increased total levels of Gsk-3β, while Gsk-3β phosphorylation was not changed, indicating that thiazide treatment partly impairs lithium influx resulting in more active Gsk-3β. Interestingly, however, NCC expression was not observed at protein or mRNA level, suggesting that the thiazide effect was NCC independent.

Results: To test the physiological relevance of these data in vivo, we examined the effects of thiazide on Li-NDI in NCC knockout mice and found that thiazide still reduced urine volume, and increased urine osmolality and AQP2 abundance in NCC knockout mice as compared to lithium-treated NCC knockout mice.

Conclusions: Our in vitro and in vivo data reveal that at least part of the antidiuretic effect of thiazide in Li-NDI is NCC independent.

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SA-PO2735

Pharmacological Blockade of $P2Y_{12}$ Receptor Increases Urinary Concentration in Rats Yue Zhang,[1] Ioana L. Pop,[1] Noel G. Carlson,[2] Bellamkonda K. Kishore.[1] *[1]Medicine, Nephrology, VAMC & Univ of Utah; [2]Neurovirology & GRECC, VAMC & Univ of Utah, Salt Lake City, UT.*

Background: $P2Y_{12}$ receptor, a G protein-coupled ADP receptor, is expressed predominantly in blood platelets, and the brain (microglia and astrocytes). Apart from Rap1b and Akt-mediated effects that contribute to platelet aggregation, signaling through $P2Y_{12}$-R also inhibits adenylyl cyclase. Hence, we investigated the expression and activity of $P2Y_{12}$-R in rat kidney.

Methods: Real-time RT-PCR and Western blotting (antibody and blocking peptide from AnaSpec) were used to detected the expression of $P2Y_{12}$-R. Groups of rats were administered clopidogrel bisulfate (Plavix®; 20 mg/kg bw/day) in drinking water for 2 weeks and euthanized. Water intake and urine output were monitored and kidney tissue analyzed.

Results: $P2Y_{12}$-R mRNA and protein are expressed in all regions of normal rat kidney, albeit at a much lower level (10-12-fold) than in the brain. Administration of Plavix® resulted in a significant increase in urine osmolality ($P < 0.02$), associated with significant decreases in urine output ($P < 0.05$) and water consumption ($P < 0.04$), and a modest increase in solute-free water absorption (24%; $P = 0.06$) as compared to the control group of rats. These changes in whole body water metabolism were matched with significant increases in the protein abundance of AQP2 water channel in the inner medulla (1.9-fold; $P < 0.002$), and cortex (2.7-fold; $P < 0.02$), but not in the outer medulla. Plavix® did not alter the protein abundances of AQP1 water channel or $P2Y_{12}$-R in the kidney. Interestingly, Plavix® administration significantly increased the urinary excretion of AVP (2-fold; $P < 0.01$) and decreased urinary PGE2 (~40%; $P < 0.02$). Finally, Plavix® administration (80 mg/kg/day for 2 weeks) resulted in significant increase in urinary concentration in $P2Y_2$ receptor null mice also, thus ruling out the possible contribution of $P2Y_2$ receptor blockade in mediating the observed effects.

Conclusions: Further studies are needed to delineate the renal and extra-renal actions of $P2Y_{12}$-R in urinary concentration and its potential as a target to treat water-losing conditions, such as nephrogenic diabetes insipidus.

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SA-PO2736

Molecular Mechanisms of Proton Permeation across Membranes: Differential Effects of Cholesterol Mark L. Zeidel,[1] Aaron Carrithers,[2] John Mathai.[1] *[1]Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [2]Transylvania University, Lexington, KY.*

Background: Despite years of study the mechanisms by which H+ permeate lipid membranes remains unclear (1, 2). H+ flux differs from that of other ions in that H+ conductance is not dependent on the actual [H+] in the solution.

Methods: Combining careful proton permeability measurements with structural analyses of lipid bilayers using X-ray diffraction, we have developed models of water, solute and H+ permeation across membranes, comprising various headgroups, chain lengths and extent of unsaturation(3). We compared H+ permeability with physical parameters of the lipids, such as area/lipid, hydrocarbon thickness, bending modulus and compressibility modulus.

Results: Like water and solutes, in membranes composed of a single phospholipid, H+ permeability varied linearly with area/lipid, and was unrelated to other physical parameters. On this basis, in single component lipid systems, the rate limiting step for H+ permeation is penetration of the proton from the aqueous medium into the lipid bilayer. When cholesterol is a component of the bilayer, water permeability decreases (15.8 ±0.58 x 10-3 cm/s and 6.8 ± 0.57 x 10-3 cm/s in absence and presence of cholesterol respectively) , but H+ permeability increases as the proportion of cholesterol increases and as area/lipid decreases (0.056 ± 0.006 cm/s in absence and in presence of cholesterol 0.113 ± 0.005 cm/s).

Conclusions: We conclude that mechanisms of H+ permeability differ markedly from those of water and solutes. We have developed and are testing a new model for H+ permeation, which defines how cholesterol enhances H+ flux, while impeding water and solute fluxes.

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SA-PO2737

Gas Permeability of the Urothelium Mark L. Zeidel,[1] Florian Zocher,[2] Peter Pohl,[2] Tung-Tien Sun,[3] John Mathai.[1] *[1]Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [2]Biophysics, Johannes Kepler University Linz, Linz, Austria; [3]Cell Biology, New York University School of Medicine, New York, NY.*

Background: Mammalian urinary bladder, which maintains enormous chemical gradients between blood and urine exhibits extremely low permeability (P) to water and solutes like urea and other metabolites in the urine. The bladder permeability barrier resides in the umbrella cell (UC) apical membrane (AM) with its specialized lipid composition and dense array of uroplakin complexes. Umbrella cell's of uroplakin II/III knockout mice showed a marked diminution of plaque surface area and significantly enhanced water and urea P which suggested that uroplakins represent a significant component of the AM barrier to water and solute flux across the bladder. Under many conditions PCO2 of urine reaches 80 – 100 mm Hg, 3 – 4 fold higher than the blood PCO2. This high urinary PCO2 suggests that bladder P to CO2 must be very low. We have shown that gases such as CO2 and H2S are freely permeable across the lipid membrane and are limited only by the unstirred layer adjacent to the membrane. Since the umbrella cell apical membrane forms a barrier to water and solute flux, we hypothesized that this specialized membrane may also act as a barrier to CO2 flux.

Results: We measured the CO2 flux across normal urothelium and urothelium in which apical membrane barrier function was disrupted. We found that disrupting apical membrane barrier function gave water and urea P's that were 7 to 8 fold higher than in wild type mice with intact urothelium. However these interventions had no impact on CO2 P's across the bladder (P, 0.0115 cm/s). To test if the observed permeability was due to an unstirred layer effect or due to kinetics of CO2 hydration, we measured the CO2 permeability in MDCK cells in presence (P, 0.035 cm/s) and absence of carbonic anhydrase inhibitor (P, 0.366 cm/s).

Conclusions: Our studies show that the lowered permeability of CO2 across the bladder is mainly due to a lack of carbonic anhydrase in the urothelium and the large unstirred layer created by the 4-5 cell layers of the urothelium.

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SA-PO2738

Functional Implications of Inner Medullary Vascular and Loop-of-Henle Architecture Thomas Pannabecker,[1] William H. Dantzler,[1] Anita T. Layton.[2] *[1]Physiology, University of Arizona, Tucson, AZ; [2]Mathematics, Duke University, Durham, NC.*

Background: A region-based mathematical model of the medullary concentrating mechanism was extended to represent new anatomic findings on vascular architecture in rat inner medulla (IM). We also hypothesize that the terminal aquaporin-1 null segment of the descending thin limb may express a urea-Na+/Cl- co-transporter.

Methods: Radial organization of outer medullary (OM) and IM nephrons and vessels is represented by 4 or 3 interconnected concentric regions, respectively. Water and solute conservation equations incorporate medullary blood flow. Equations embody mass conservation of solute and water, and represent transport of solutes and water by single-barrier equations that represent double-barrier transepithelial or transendothelial transport. Transmural solute diffusion is characterized by solute permeabilities; active transport is represented by saturable Michaelis-Menten kinetics. Water flux is osmotically-driven, or, for ascending vasa recta, is pressure-driven advection.

Results: Model results suggest that, despite compartmentalization, IM interstitial fluid composition is substantially more homogeneous compared to OM. Despite a reduction in IM collecting duct (CD) water permeability, more water is reabsorbed along the IM CD in diuresis, resulting in larger IM blood flow, owing to a much larger transepithelial osmolality gradient. In antidiuresis, as urea diffuses from urea-rich papillary interstitium into descending thin limb lumen, NaCl is secreted via hypothetical urea-NaCl co-transport against its concentration gradient. NaCl is reabsorbed near the loop bend, raising interstitial fluid osmolality and promoting water reabsorption from CDs.

Conclusions: Marked radial organization of the upper IM, centered on the CD clusters, results in sequestration of urea in and near CD clusters. Indirect effects of vasopressin on CDs have much smaller impact on medullary blood flow than direct vasoconstrictive actions. The model predicts that the urea-Na+ or urea-Cl- co-transporter improves the concentrating ability by facilitating cycling of NaCl within the IM, and yields a loop-bend fluid composition consistent with experimental data.

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SA-PO2739

Vasopressin-Induced Phosphorylation at Ser261 in AQP2-P262L May Underlie Nephrogenic Diabetes Insipidus Christiane Trimpert,[1] Enno Klussmann,[2] Peter M.T. Deen.[1] *[1]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Max Delbrueck Center for Molecular Medicine Berlin, Berlin, Germany.*

Background: Vasopressin regulates water homeostasis through insertion of aquaporin-2 (AQP2) water channels in the apical plasma membrane of collecting duct principal cells. AQP2 mutations cause nephrogenic diabetes insipidus (NDI), a disease in which the antidiuretic response is lacking, resulting in polyuria. While most AQP2 mutants in recessive NDI are misfolded, retained in the endoplasmatic reticulum and unable to interact with wild-type (wt)-AQP2, cell culture studies suggest that AQP2-P262L in recessive NDI folds properly and interacts when co-expressed with wt-AQP2, but is impaired in its vasopressin-induced translocation from vesicles to the apical membrane when co-expressed with other recessive NDI mutants. It was recently shown that besides S256, vasopressin-induced translocation of AQP2 also coincides with AQP2 phosphorylation at S264 and S269 and dephosphorylation at S261. Since P262 lies adjacent to S261, we tested vasopressin-induced phosphorylation of AQP-P262L.

Results: In MDKC and mpkccd cells, wt-AQP2 only shows an unglycosylated 29kDa band, while AQP2-P262L is expressed as a double 29/30kDa band. Using AQP2 antibodies insensitive to phosphorylation, dDAVP increased the presence of the 30kDa band. This band felt back to 29kDa upon treatment with phosphatases, indicating that phosphorylation of AQP2-P262L underlies the appearance of the 30kDa band. Using phospo-specific antibodies, it appeared that, similar to wt-AQP2, dDAVP increased pS256 and pT269 in human AQP2-P262L. However, in contrast to wt-AQP2, pS261 was hardly detectable with control AQP2-P262L and was extensively increased with dDAVP. Moreover, compared to the 29kDa band, 30kDa AQP2-P262L was strongly phosphorylated at S256 and S261.

Conclusions: Our data reveal that vasopressin induces phosphorylation at S261 in AQP2-P262L and that this difference from wt-AQP2 may underlie its inability to sort to the plasma membrane and to cause NDI in the patients.

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SA-PO2740

Collecting Duct-Specific Knockout of Adenylyl Cyclase Type VI Causes Urinary Concentration Defect in Mice Karl P. Roos,[1] Kevin A. Strait,[1] Mitsi A. Blount,[2] Donald E. Kohan.[1] *[1]Department of Medicine, Division of Nephrology, Univeristy of Utah School of Medicine, Salt Lake City, UT; [2]Department of Medicine, Renal Division, Emory University School of Medicine, Atlanta, GA.*

Background: Adenylyl cyclase VI (AC6) has been implicated in arginine vasopressin (AVP)-stimulated renal water reabsorption. To evaluate the role of AC6, we generated mice with collecting duct (CD)-specific KO of AC6 using the Cre/lox system.

Methods: Renal papillary AC6 mRNA was determined. Inner medulla aquaporin (AQP2), urea transporter A1 (UT-A1), and urea transporter A3 (UT-A3) protein expression was analyzed. Inner medullary CD (IMCD) were acutely isolated to evaluate AVP-stimulated cAMP accumulation. KO and floxed controls were studied on normal, high and low water intake, as well as a DDAVP clamp (25 ng/hr). KO and control mice were fed a standardized gel diet containing all daily food and water. All data is expressed as % reduction of KO from control and are all significant ($p<0.05$).

Results: There was a 43% reduction in AC6 mRNA expression in renal papilla (sample contains non-CD cell types). There was a reduction in inner medulla AQP2, UT-A1, and UT-A3, (44%, 21% and 44% respectively), and 66% reduction of AQP2 expression in outer medulla. In acutely isolated IMCD, AVP-stimulated cAMP accumulation was reduced by 44%. Baseline (normal Na diet and free access to water) urine osmolality (Uosm) was reduced 26%. After 18 hr water deprivation, Uosm was reduced by 18%. There was no change in Uosm with chronic water loading. With DDAVP infusion, there was no difference in Uosm under any of the conditions. There was no difference in urine

volume or fluid intake under any of the conditions. After being fed standardized gel diet, plasma AVP levels were unchanged. There was no difference in urine urea excretion on the baseline water intake diet.

Conclusions: In summary, CD-specific KO of AC6 leads to a urinary concentration defect in mice. This is associated with decreased AVP-stimulated cAMP accumulation and reduced expression of AQP2, UT-A1, and UT-A3. These data indicate that AC6 in the CD mediates, at least in part, AVP regulation of water and urea transport pathways.

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SA-PO2741

Familial Azotemia Is Caused by a Duplication of Urea Transporter-B Gene

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Background: Facilitated urea transport in the kidney is regulated by urea transporters UT-A and UT-B. UT-A is expressed in different nephron segments, UT-B in the descending vasa recta and on erythrocytes. Autosomal dominant familial azotemia has been reported in two kindreds. It is characterized by high plasma urea due to an isolated impairment of urea excretion. Here, we report a family with autosomal-dominant azotemia segregating with a duplication of the UT-B gene.

Methods: Case report: The index patient, her brother and her son have isolated azotemia (plasma urea 46 mmol/l) due to strongly diminished fractional excretion of urea of 1.4% (normal 30%). Creatinine clearance and other tubular functions are normal.

Results: CGH array analysis revealed a duplication on chromosome 18q12.3-21.1, which includes the gene coding for UT-B. A kidney biopsy revealed isolated UT-B staining in the vasa recta in the affected child and a control biopsy indicating orthotopic expression. UT-B content by Western-blot in biopsy material was twice that of a control. This coincided with increased UT-B expression on erythrocytes (142% of controls) and a threefold acceleration of urea-induced hemolysis.

Conclusions: Impaired fractional excretion of urea segregates with a duplication in the UT-B gene. This leads to increased expression of UT-B in kidney and on erythrocytes. Functional studies demonstrate increased urea transport in erythrocytes. This is suggestive of an increased urea transport in the descending vasa recta, while ectopic UT-B expression was ruled out. We hypothesize that increased delivery of urea to the inner medulla exceeds UT-A mediated excretion in Henle's loop causing backflow to the systemic circulation via the ascending vasa recta.

SA-PO2742

Demeclocyclin Attenuates Hyponatremia by Reducing Adenylate Cyclase and Aquaporin-2 Expression

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Background: Demeclocycline is a bacteriostatic antibiotic of the tetracycline group, which has been shown to cause water diuresis and nephrogenic diabetes insipidus and is used to treat hyponatremia. Although demeclocycline is thought to act in the distal part of the renal nephron, the exact mechanism is not known.

Methods: Mouse cortical collecting duct (mpkCCD) cells were treated with deamino-8 D-arginine vasopressin (dDAVP) to induce endogenous AQP2 expression, and treated with demeclocycline for the last 24 hours. Demeclocycline reduced AQP2 abundance in mpkCCD cells in a time and dose dependent manner. This effect was abolished by inhibiting protein synthesis, implying that a decrease in AQP2 production explains the AQP2 down-regulation. Using a AQP2-promoter-luciferase construct, it was shown that demeclocycline decreases AQP2 transcription. Further analysis revealed that demeclocycline decreased adenylate cyclase III and V/VI expression and inhibited vasopressin induced cAMP production.

Results: To test the physiological relevance of these data, rats receiving liquid diet in combination with dDAVP and with or without demeclocyclin were examined. In these hyponatremic rats, demeclocyclin increased urine volume (44.8 ± 9.3 vs 91.3 ± 15.9 ml/kg/24 hours; $p<0.05$), decreased urine osmolality (1374 ± 117 vs 820 ± 148; $p<0.05$), and reduced hyponatremia (117.3 ± 4.5 vs 98.7 ± 0.8 mmol/l; $p<0.05$). Immunoblotting revealed reduced AQP2 and adenylate cyclases in the inner medulla only.

Conclusions: Our in vitro and in vivo data reveal that demeclocycline causes diuresis and reduces hyponatremia by affecting adenylate cyclases III or V/VI expression resulting in reduced cAMP and AQP2 expression in the inner medullary collecting duct.

Funding: Government Support - Non-U.S.

SA-PO2743

The AVP-Stimulated NaCl Absorption in Mouse Medullary Thick Ascending Limb Is Abolished by the V2 Receptor Antagonist Satavaptan

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Background: Arginin-vasopressin (AVP) activates water and electrolyte absorption in the collecting duct as well as electrolyte absorption in the thick ascending limb (TAL). In all renal tubules, which respond to AVP the hormone causes increases of cAMP and intracellular Ca^{2+}. The V2 receptor is critical for most renal tubular actions of AVP. However, other AVP receptors (V1) are known to be expressed in TAL of certain mammalian species.

Objective: Investigate the effect of the highly specific V2 receptor antagonist satavaptan on AVP-stimulated NaCl transport in mouse thick ascending limb.

Methods: We used isolated, perfused mouse mTAL to electrically measure Na^+ absorption. By electrodes we determined the transepithelial voltage (V_{te}) and the transepithelial resistance (R_{te}) and via these the transepithelial Na^+ absorption (equivalent short circuit current, I_{sc}).

Results: Non-stimulated mTALs showed a lumen-positive transepithelial voltage (V_{te}) of +6.26±1.02 mV and a transepithelial resistance (R_{te}) of 3.05±0.45 Ω cm^2 resulting in an equivalent short circuit current (I_{sc}) of 2196±356 μA/cm^2 (n=5). Within 5-10 minutes basolateral AVP (10 nM) triggered a pronounced and ongoing activation of transport as seen by an increase of V_{te} to +11±0.71 mV and I_{sc} to 3460±475 μA/cm^2. R_{te} remained unaltered. In paired experiments satavaptan (1 μM) completely abolished the AVP-induced increase in V_{te} and I_{sc} (n=5). Satavaptan did not influence baseline mTAL transport.

Conclusions: These are the first functional data in isolated perfused mTAL to quantify the effect of specific V2 antagonists on NaCl transport. They indicate that V2 receptor blockage is sufficient to suppress the entire AVP-activated NaCl absorption in mouse mTAL. Other vasopressin receptors appear not to be involved. Thus, the critical role of the V2 receptor for AVP-mediated transport activation is confirmed.

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SA-PO2744

Cold-Induced AQP2 Basolateral Membrane Accumulation in Polarized MDCK Cells

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Background: AQP2 accumulates in the apical plasma membrane of collecting duct principal cells upon stimulation by vasopressin (VP). However, basolateral AQP2 has been reported in vivo, and forskolin (FK) or VP-induced AQP2 plasma membrane distribution is different among cultured cell lines. Here, we report experimental conditions that affect the polarized expression of AQP2 in MDCK cells.

Methods: We initially performed domain-specific biotinylation assays and immunofluorescence (IF) on filter-grown MDCK cells before and after stimulation with FK.

Results: By surface biotinylation, AQP2 was clearly detected in the basolateral membrane under basal conditions, and this expression was not increased by FK. However, by IF, AQP2 was not detected in the basolateral membrane, but instead accumulated only in the apical membrane after FK. This discrepancy, together with a previous finding that basolateral AQP2 appeared after cold incubation (4°C for 4 h) in rat kidney slices, led us to hypothesize that cold exposure (4°C), necessary for biotinylation, might induce basolateral AQP2 accumulation. Indeed, by IF, AQP2 was clearly accumulated in MDCK cell basolateral membranes after 15 min at 4oC, even in the absence of VP or FK. After rewarming to 37°C, AQP2 was internalized into vesicles, suggesting that MDCK cells were not irreversibly damaged, but had been temporarily modified by the low temperature. This cold-induced AQP2 basolateral membrane accumulation was not seen in transfected LLC-PK1 cells, suggesting that the effect is cell-specific. Furthermore, the effect was not reproduced at 37°C after microtubule disruption by colchicine, ruling out one possible explanation for our data (MT are depolymerized at 4°C).

Conclusions: These results suggest that a basolateral targeting pathway for AQP2 is present in MDCK cells and can be revealed by cold treatment. This system may be useful in the further understanding of this targeting process, which also occurs in some renal tubule segments. In addition, our results raise a note of caution for interpretation of basolateral signals in domain-specific biotinylation assays.

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SA-PO2745

Membrane Fusion Protein SNAP23 Mediates Constitutive NKCC2 Trafficking in Thick Ascending Limbs

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Background: Vasopressin stimulates NaCl reabsorption and NKCC2 in the thick ascending limb (TAL) by enhancing cAMP. Trafficking of NKCC2 to the apical surface is required for ion transport and this is stimulated by cAMP. In all cells, vesicle fusion is mediated by proteins of the SNARE family. Vesicle Associated Membrane Proteins (VAMPs) recognize two other SNAREs in the target membrane: a SNAP (Synaptosome Associated Protein) and a syntaxin. We previously found that VAMP3 mediates constitutive delivery of NKCC2 to the surface while VAMP2 mediates cAMP-stimulated NKCC2 trafficking. The target membrane SNAREs involved in NKCC2 trafficking are unknown. Four SNAP isoforms (23,25,29 and 47) have been described. SNAP23 is present in the kidney and binds VAMP2 and VAMP3 in other cells. We hypothesized that SNAP23 colocalizes with NKCC2 and mediates constitutive and cAMP-stimulated NKCC2 trafficking in TALs.

Methods: TALs were transduced in vivo with adenoviruses coding for a dominant-negative SNAP23 (SNAP23ΔC8) injected into the outer medulla. TAL suspensions were obtained from the left kidney (SNAP23ΔC8-transduced) and the control right kidney (GFP-transduced), and incubated with vehicle or forskolin+IBMX to increase intracellular cAMP. Surface NKCC2 was measured by surface biotinylation of TAL suspensions obtained from transduced kidneys. Immunolabeling of SNAP23 and NKCC2 was performed in isolated, perfused TALs.

Results: SNAP23 colocalized with NKCC2 in the apical membrane and subapical space. Expression of dominant negative SNAP23ΔC8 decreased constitutive surface NKCC2 by 36±6% ($p<0.01$). However, cAMP increased surface NKCC2 to similar extent

in control and SNAP23ΔC8-transduced TALs (GFP: 91±23%, SNAP23ΔC8: 140±20% increase from baseline, n=5). SNAP23ΔC8 did not affect total NKCC2 levels (5±13% of control). A Western blot screening in TALs revealed expression of SNAP23, 29 and 47 but not SNAP25.

Conclusions: We conclude that SNAP23 is involved in constitutive (biosynthetic) but not in cAMP-stimulated NKCC2 trafficking in the TAL, most likely by pairing with VAMP3. Other SNAP isoforms are expressed and may be responsible for cAMP-stimulated NKCC2 trafficking.

Funding: Other NIH Support - NHLBI, Private Foundation Support

SA-PO2746

Decreased Abundance of Urinary Exosomal Aquaporin-2 after Renal Ischemia-Reperfusion in Rats and Humans Hiroko Sonoda,[1] Naoko Yokota-Ikeda,[2] Shigehiro Uezono,[2] Sayaka Oshikawa,[1] Masahiro Ikeda.[1] *[1]Veterinary Pharmacology, University of Miyazaki, Japan; [2]Nephrology, Miyazaki Prefectural Miyazaki Hospital, Miyazaki, Japan.*

Background: Urinary exosomes are small vesicles secreted by all tubular epithelial cells and are known to contain membrane proteins. Several studies have shown that urinary exosomal proteins have potential to be used as biomarkers for renal diseases. So far, we have reported that urinary exosomal aquaporin-2 (AQP2) is continuously decreased in cisplatin-induced acute kidney injury (AKI) in rats. However, it has not been examined whether urinary exosomal AQP2 levels are affected by renal ischemia-reperfusion (I/R) that is also known to cause AKI. In this study, we examined the abundance of urinary exosomal AQP2 following renal I/R in a rat model and in kidney transplantation patients.

Methods: Rats were subjected to unilateral renal I/R (I/R-rats) or sham operation (sham-rats). Urinary exosomes were isolated by differential ultracentrifugation.

Results: In I/R-rats, plasma creatinine concentration was slightly but significantly higher than in sham-rats. As judged by immunoblot analysis, urinary exosomal AQP2 level was significantly lower in the urine collected at 0-6 h after renal I/R, in comparison with the sham-rats. However, this reduction was not further observed in the urine collected at later times. Renal AQP2 protein level was significantly decreased at 6 h after I/R in the cortex but not in the outer and the inner medulla. In 9 human recipient patients, all exosomal AQP2 levels in urine collected within 2 days after kidney transplantation through the urinary catheter for 30 min were less than 4% of the mean value for 3 healthy volunteers. On the other hand, such reduction in urinary exosomal AQP2 was only observed in 4 out of 49 patients with various renal diseases (IgA nephritis, minimal change diseases, and etc.) other than AKI and end-stage renal disease.

Conclusions: These results indicate that urinary exosomal AQP2 abundance decreases at an early time after renal I/R along with decrease in renal cortex AQP2 content, suggesting urinary exosomal AQP2 as a biomarker for the early detection of renal I/R-induced AKI.

SA-PO2747

PPARγ Controls Urine Concentrating Ability Independently of Vasopressin Tianxin Yang,[1,2] Alexandra Panasiuk,[1] Maicy Downton,[1] Li Zhou,[1,2] Zhanjun Jia.[1,2] *[1]Internal Medicine, University of Utah; [2]VA Medical Center.*

Background: The synthetic PPARγ agonists, thiazolidinediones (TZDs), are highly effective in managing type 2 diabetes but are limited by a major side effect of fluid retension. Despite intensive investigation, the role and mechanism of PPARγ regulation of fluid metabolism is still incompletely understood. The present study was undertaken to investigate the integrative role of PPARγ in regulation of fluid homeostasis.

Methods: The inducible whole-body PPARγ knockout mice were created by crossing floxed PPARγ mice with β-actin-Cre/ESR mice, followed by tamoxifen treatment. The phenotype in these mice was analyzed.

Results: The KO mice had grossly normal phenotype except a modestly increased food intake (WT: 5.26±0.439g vs. KO: 4.46±0.152g, $p<0.05$). Strikingly, the KO mice developed severe urine concentrating defect as evidenced by marked increases in water intake (WT: 5.3±0.72 mL vs. KO: 7.2±1.28 mL, $p<0.05$) and urine volume (WT: 1.45±0.216 mL vs. KO: 2.52±0.497 mL, $p<0.01$) accompanied with reduced urine osmolality (WT: 2796.7±439.72 mOsm/kg H_20 vs KO: 1488.0±282.79 mOsm/kg H_20, $p<0.01$). The urine concentrating defect persisted after pair-feeding or in combination with pair-drinking. Surprisingly, plasma vasopressin concentration assessed by ELISA was not different between the genotypes. After 24-h water deprivation, urine osmolality increased 27% in WT mice but failed to rise in the KO mice. However, the responsiveness to acute (0.06 ng/kg i.p.) and chronic (1μg/kg/day) deamino-8-D-arginine vasopressin (dd-AVP) treatment remained intact in these mice.

Conclusions: Together, we identified PPARγ as an essential regulator of urine concentrating ability that appears to be independent of vasopressin.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2748

Quantitative Phosphoproteomics of Bilateral Ureteral Obstruction Induced Nephrogenic Diabetes Insipidus Sookkasem Khositseth,[1] Panapat Uawithya,[2] Poorichaya Somparn.[3] *[1]Department of Pediatrics, Faculty of Medicine, Thammasat University, Pathumthani, Thailand; [2]Department of Physiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand; [3]Biomedical Science, Graduate School, Chulalongkorn University, Thailand.*

Background: Nephrogenic diabetes insipidus (NDI) is a common complication in patients with obstructive uropathy.

Methods: We employed phosphoproteomics of rat inner medullary collecting duct (IMCD) to investigate the mechanism of bilateral ureteral obstruction (BUO)-induced NDI. IMCD isolated from 7 12-hr BUO rats and 7 sham-operated rats were compared (n=3). The samples were enriched for phosphopeptides using Ga^{3+}immobilized metal affinity chromatography, and analyzed by liquid chromatography-tandem mass spectrometry using an LTQ-OrbitrapVelos machine. Phosphopeptide identification was performed using SEQUEST algorithm and label-free quantification was executed using QUOIL software (NHLBI Proteomics Core Facility).

Results: We identified 30 increased phosphorylation sites and 84 decreased phosphorylation sites in the BUO. The increased phosphorylation sites present in proteins involved in cytoskeletal reorganization (Lima1, Plec, Pxn, Twf1, Sept2), vesicle trafficking (Ebag9), transcriptional regulation (Ebag9, Eif4b), and nucleotide binding (Hnrnpk, Pds5b, Rbm39, Top2a). The decreased phosphorylation sites present in channel proteins (Aqp2 and Slc14a2), cytoskeletal proteins (Add1, Cnn3, Ctnna1, Ctnna2, Cttn, Myh9), nucleotide binding proteins (Hdgf, Hnrnpc, Hnrpd, Nucks1, Psip1), small G protein (Fgd2), and steroid binding protein (Pgrmc2). Down-regulation of aquaporin-2(Aqp2) phosphorylation at S256, S261, and S264 (by 33.5%), and urea transporter (Slc14a2) phosphorylation at S62, and S63 (by 17.4%) were observed. Immunoblotting showed a significant reduction in total Aqp2 (82%, $P<0.001$), pS256-Aqp2 (95%, $P<0.01$), and total UT-A1 (68.2%, $P<0.001$) in BUO IMCD compared to the sham control.

Conclusions: Two key proteins in urine concentrating mechanism, Aqp2 and Slc14a2, were found decreased in both total and phosphorylated forms in BUO-induced NDI model in rat. Other signaling molecules that might play important role in this disease process were also identified.

Funding: Government Support - Non-U.S.

SA-PO2749

Impaired Renal Water Reabsorbtion Mediated by Vascular Disrupting Agent Anja Bille Bohn,[1,2] Rikke Norregaard,[3] Yan Wang,[3] Lotte Bonde Bertelsen,[2] Michael R. Horsman,[1] Hans Stødkilde-Jørgensen,[2] Jorgen Frokiaer.[3] *[1]Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark; [2]MR-Research Centre, Aarhus University Hospital, Denmark; [3]Institute of Clinical Medicine, Aarhus University, Denmark.*

Background: Combretastatin A-4 phosphate (CA4P) is a vascular disrupting agent shown to mediate its effect primarily on tumor blood vessels. We have previously shown that CA4P results in a significant increase in hematocrit and in mean arterial blood pressure in non-tumour bearing mice. Based on this we now want to examine whether this is associated with molecular changes in renal water handling.

Methods: Non-tumour bearing male Wistar rats were injected intraperitoneally with CA4P (30 mg/kg) or saline (controls). Blood samples and urine was collected and analyzed. Tissue water content was assesed using DCE-MRI. Renal tissue was examined for AQP2, the active phosphorylated AQP2 (pAQP2) and vasopressin V2 receptor (V2R) expression in inner medulla (IM). Furthermore, medullary osmolality and cAMP was determined.

Results: Total hemoglobin increased 1h after CA4P treatment (11.1±0.3 vs. 9.2±0.3 mmol/l, $p<0.01$). Tissue water content in muscle, brain, or renal tissue following CA4P administration did not change significantly. Urinary water and salt excretion in the CA4P treated rats increased significantly during the first hour after treatment (200.1±20.6 vs. 90.0±12.1 μl/min/kg, $p<0.01$ and 16.9±3.8 vs. 3.9±0.9 μmol/min/kg, $p<0.01$, respectively). Urine and medullary osmolality decreased 2h after CA4P. AQP2 mRNA level in IM did not change in response to CA4P treatment. However, immunohistochemistry showed an apical localization of pAQP2 in control rats compared to a more diffuse pAQP2 distribution within the IM principal cells in the CA4P treated rats. V2R expression and medullary cAMP concentration did not change 1h after CA4P. Three hours after CA4P treatment the AQP2 mRNA level and the pAQP2 protein level was significantly increased compared to controls.

Conclusions: The data are compatible with the notion that CA4P affects the urine concentrating ability through a local renal effect on colleting duct water permeability due to changed AQP2 trafficking.

SA-PO2750

Urine Concentration Defect in the Absence of Urea Transpoters-A1/A3 May Involve an Inability to Phosphorylate Aquaporin2 at Serine 256 Titilayo O. Ilori, Jeff M. Sands, Janet D. Klein. *Medicine, Emory University School of Medicine, Atlanta, GA.*

Background: Polyuria is a significant clinical manifestation of diabetes mellitus yet diabetics rarely go into hypovolemic shock. Upregulation of aquaporin 2 (AQP2) and urea transporters increase water and solute reabsorption. In the absence of the urea transporters UT-A1 and UT-A3, AQP2 cannot stimulate increased urine osmolality even with vasopressin. In this study we looked for changed in phosphorylated AQP2 in response to diabetes in wild type (WT) and UT-A1/A3 knockout (KO) mice.

Methods: WT and A1/A3 KO mice were injected 2 times with 100mg/kg streptozotocin. After 24 h hyperglycemia was verified. Metabolic cages were used to collect 24 h urines for osmolalities. Mice were sacrificed at 7 days or when humane end points were reached. Lysates of inner medullas were analyzed by western blot probing for total AQP2 and pser256- and pser261-AQP2. Thereafter, WT and A1/A3 KO were treated with vasopressin for 7 days via minipump. Aquaporin 2 levels were then measured by western blot.

Results: There was a 40% survival of UT-A1/A3 KO 7d post-STZ injection compared to 70% in WT. AQP2 levels are decreased 30% in UTA1/A3 KO mice compared to WT mice and did not increase in the diabetic A1/A3 KO mice, compared to a 133% increase in WT diabetic mice. Vasopressin treatment (7-days) up-regulated AQP2 abundance in the UT-A1/A3 KO mice, but did not correct the KO's polyuria. Pser256 AQP2 was lower in the

UT-A1/A3 KO vs WT mice and did not increase in either WT or KO mice with diabetes. Pser261-AQP2 tended to be decreased with diabetes in both KO and WT mice consistent with increased vasopressin levels in diabetic animals.

Conclusions: We conclude that despite increased AQP2 in response to vasopressin, in the absence of UT-A1 and UT-A3 the diabetic animal is unable to optimally activate AQP2 consistent with decreased pser256-AQP2 in both normal and diabetic KO vs WT mice. An inability to activate AQP2 despite increased protein may explain the lack of vasopressin-mediated urine concentration in the absence of UTA1/A3.

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SA-PO2751

Urinary Exosomal Aquaporin-2 Excretion Is Altered by the Treatment with Furosemide or Acetazolimide, in Association with Vasopressin Yoshiki Higashijima, Hiroaki Kondo, Kanako Shigemura, Saki Takahashi, Hiroko Sonoda, Masahiro Ikeda. *Veterinary Pharmacology, University of Miyazaki, Japan.*

Background: Aquaporin-2 (AQP2) is the only AQP regulated by vasopressin. In response to vasopressin, AQP2 traffics to the apical plasma membrane to increase water permeability in collecting ducts. Exosomes are membranous vesicles delivered to the urine from all renal epithelial cell types and this urinary exosomes are known to contain membrane proteins including AQP2. So far, we reported that urinary exosomal AQP2 level was continuously decreased in rats treated with cisplatin, suggesting urinary exosomal AQP2 as a biomarker for the detection of cisplatin-induced acute kidney injury (2010 ASN). However, at present the mechanisms underlying the excretion of urinary exosomal AQP2 are largely unknown. In order to elucidate the mechanism, this study examined the effects of diuretics on the excretion of urinary exosomal AQP2.

Methods: A 2-h urine was collected from rats treated with saline (control), furosemide (20 mg/kg), acetazolamide (50 mg/kg), or OPC-31260 (10 mg/kg, a V2 receptor antagonist). Urinary exosomes were isolated from the collected urine by differential ultracentrifugation.

Results: Western blot analysis revealed that urinary exosomal AQP2 abundance was dramatically increased in the furosemide and the acetazolamide groups, in comparison with the control group, while OPC-31260 had no significant effect on the urinary exosomal AQP2 excretion. In the furosemide and acetazolamide groups, renal AQP2 protein level was significantly decreased in the cortex but not in the outer and the inner medulla, and this decrease was accompanied with the enhancement of the apical expression of AQP2. Co-administration of OPC-31260 with furosemide or acetazolamide completely inhibited the increased excretion of urinary exosomal AQP2 in response to furosemide or acetazolamide.

Conclusions: Our results indicate that basal excretion of urinary exosomal AQP2 is independent of vasopressin, but its increased excretion by a decrease in body fluid volume through treatments with diuretics is initiated by vasopressin.

SA-PO2752

Decreased Aquaporin 2 and Urea Transporters in a Rat Model of Nephrotic Syndrome Raed Bou Matar,[2] Jeff M. Sands,[1] Janet D. Klein.[1] [1]*Renal Division, Emory University, Atlanta, GA;* [2]*Department of Pediatrics, Emory University, Atlanta, GA.*

Background: Urea transporters and aquaporins play an important role in the regulation of water homeostasis. Nephrotic syndrome is associated with significant primary renal salt and water retention. This study investigates the role of urea transporters and aquaporins in nephrotic syndrome associated water retention. We tested the effect of doxorubicin-induced nephrotic syndrome on the abundance of urea transporters UT-A1, UT-A3, aquaporin-2 (AQP2), and Na-K-Cl Cotransporter 2 (NKCC2) in the rat kidney tissue.

Methods: Three cohorts of male Sprague Dawley rats (total n = 18 treatment, 13 control) were injected intravenously with doxorubicin and urinary protein excretion was monitored. Upon confirmation of high grade proteinuria using spot urine protein to creatinine ratios (Pr/Cr), we determined urine osmolality, serum cholesterol, and serum creatinine by specific chemical assays. A Pr/Cr of 80 was used as the endpoint indicator. Kidney tissue was dissected and analyzed for the abundances of UT-A1, UT-A3, AQP2, and NKCC2 using western blot.

Results: Within 2 weeks following doxorubicin injection, all treated rats developed features of nephrotic syndrome, with an average 9-fold increase in urine Pr/Cr ratio (from 15 ± 2 to 130 ± 10; $p < 0.001$) and significant hypercholesterolemia (from 103 ± 2 mg/dl to 276 ± 19 mg/dl). Urine osmolalities were reduced by 38 ± 5% in the doxorubicin treated group (983 ± 84, treatment vs. 1570 ± 134, control). UT-A1 and UT-A3 protein abundances in IM tip tissue were significantly decreased (44 ± 8% and 31 ± 5% respectively; $p < 0.001$) in nephrotic rats. AQP2 protein abundance was also significantly reduced (65 ± 7%, $p < 0.001$) in the nephrotic group as compared to control rats. A 72 ± 5% reduction in the abundance of the NKCC2 was noticed in outer medullary tissue of nephrotic rats as compared to controls.

Conclusions: AQP2, UT-A1, UT-A3, and NKCC2 are decreased in doxorubicin-induced nephrotic syndrome and may represent a response to avid reabsorption of sodium at the level of the cortical collecting duct, coupled with a general disruption of the normal corticomedullary osmotic gradient.

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SA-PO2753

Enhanced Renal Expression of *SGLT2* Gene in Response to Diabetes Niloofar Moeini Tabatabai, Rajendra Kishore Kothinti. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Background: SGLT2 is the major glucose transporter in the kidney and is expressed on the apical side of the proximal tubule cells. It has been suggested that SGLT2 expression may be enhanced in type 2 diabetic patients. The goal of this study was to examine temporal changes in renal expression of *SGLT2* gene in response to diabetes.

Methods: Zucker Diabetic Fatty (ZDF) (*fa/fa*) rats, a model of type 2 diabetes, and non-diabetic *fa/+* controls were obtained. Five male 5 weeks (wk) old controls and 5, 8, 12, 15, and 19 wk old *fa/fa* were used. Overnight urine was collected from fasting rats. Rat was anesthetized, blood was collected from the heart, and left kidney was removed. Serum and urine concentrations of glucose and serum insulin levels were measured. To examine *SGLT2* gene expression, total RNA from kidney tissues were prepared, reverse transcribed, and cDNA samples were used in real time polymerase chain reaction with SYBR Green reagents. Amplification of small subunit ribosomal protein 15 (S15) cDNA was used as endogenous control and fold difference between *fa/fa* groups and controls were determined.

Results: Fasting serum glucose in 5 wk old control and *fa/fa* were 109 and 195 mg/dL, respectively. In *fa/fa*, serum glucose increased to 221 mg/dL by 8 wk of age, it reached 301 mg/dL in 12 wk old rats and remained as high in 15 and 19 wk old *fa/fa*. Glucose excretion was only ~0.018 g/day in 5 wk old control and *fa/fa*; however, 8 wk old *fa/fa* were glucosuric and excreted 9 g of glucose/day. Glucose excretion almost doubled by 12 wk, reached 21 g/day by 15 wk, and then declined. In 5 wk old *fa/fa*, serum insulin was 1.5 ng/ml, reached maximum of 11 ng/ml at 8 wk of age, and declined to ~2 ng/ml in older rats. Relative expression of *SGLT2* mRNA in the kidneys of 5 wk old *fa/fa* was 1.6 (arbitrary unit, A.U.) and it increased 1.6 folds to maximum of 2.5 A.U. by 8 wk of age. *SGLT2* expression in 12 and 15 wk old *fa/fa* were 1.53 and 1.76 A.U., respectively, and decreased to 0.82 A.U. by 19 wk of age.

Conclusions: Results of this study show that renal expression of *SGLT2* gene was enhanced in the early stages of the development of type 2 diabetes in ZDF *fa/fa* model. Increased expression of SGLT2 may augment symptom of hyperglycemia.

SA-PO2754

Hypertonicity Increased Glutathione Content in MDCK Cells and Had Protective Effect Against H_2O_2 Induced Cell Damage Masaru Horio. *Functional Diagnostic Science, Osaka University Graduate School of Medicine, Suita, Japan.*

Background: Oxidative stress in renal tubular cell is a possible factor inducing the cell damage. Hypertonicity is one of the main causes of the oxidative stress. Glutathione (GSH) is an important antioxidant, but the content after hypertonic exposure has not been well studied. GSH content after hypertonic exposure and effect of preincubation with hypertonic medium on oxidative stress were evaluated.

Methods: ROS generation was detected using carboxy-H_2DCFDA**.** GSH content, activity of glutamate-cysteine ligase (GCL), the rate-limiting enzyme of GSH synthesis, and contents of amino acids, which included primary sources of GSH, were measured. Cell damage was assessed by LDH activity in culture medium.

Results: Hypertonicity increased reactive oxygen species (ROS) in an osmolality dependent manner. Hypertonicity of 400 and 450 mOsm significantly increased GSH content up to 1.5 fold the value of isotonic cells after 8h. Higher osmolality (500 and 600mOsm) did not show further increase of GSH, but decrease the GSH content. Significant increase of GCL activity was not observed after 8h of hypertonicity of 350, 400 and 450 mOsm. Contents of glutamate, glycine and cysteine which were precursors of GSH, were significantly increased 7, 3 and 2 fold the value of isotonic cells after 8h of hypertonicity, respectively. This might be a mechanism of inducing GSH content by hypertonicity. H_2O_2 showed cell damage in MDCK cells in dose dependent manner assessed by LDH activity in culture medium. 24h-preincubation of 400mOsm significantly protected the H_2O_2-induced cell damage.

Conclusions: Preincubation of mild hypertonicity has a protective effect on H_2O_2 induced cell damage in MDCK cells. Increase of GSH content by hypertonicity may contribute to the protective effect.

Funding: Government Support - Non-U.S.

SA-PO2755

Removal of Indoxyl Sulfate by Hybrid Kidney with Human Organic Anion Transporter-3 Over-Expressing Cells Hisashi Yamamoto,[1] Shuichi Tsuruoka,[1 2 3] Takashi Ioka,[1] Yoshikatsu Kanai,[4] Hitoshi Endou,[5] Akio Fujimura,[2] Eiji Kusano.[1] [1]*Nephrology, Jichi Medical University, Tochigi, Japan;* [2]*Clinical Pharmacology, Jichi Medical University, Tochigi, Japan;* [3]*Nephrology, University of Tsukuba, Ibaraki, Japan;* [4]*Pharmacology, Osaka University, Osaka, Japan;* [5]*Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan.*

Background: Indoxyl sulfate (IS), one of the protein-bound uremic toxins and a substrate of organic anion transporter (OAT)-3, is cleared poorly by conventional hemodialysis. One of the reasons for the high incidence of cardiovascular disease in dialysis patients is accumulation in the body of uremic toxins. We have previously

reported a hybrid-type artificial kidney by introducing drug transporter gene and succeeded experimental treatment of drug intoxication. We evaluated the efficacy of a hybrid kidney with introducing OAT-3 gene for IS removal in vitro and in vivo.

Methods: The proximal tubule cell line over-expressing human OAT-3 (S2-hOAT3) and control gene (S2-DNA) were cultured onto the hollow fiber module. Transport of IS from the capillary to pericapillary side were evaluated in vitro. The hybrid kidney system was further connected to 7/8-nephrectomized uremic dogs and blood was circulated for 4-hour through the system. Arterial and venous blood concentrations of IS were monitored during the treatment.

Results: In vitro clearance of IS in hollow fiber module with S2-hOAT3 (45 ± 3.6 ml/min) was significantly higher than in S2-DNA (7.1 ± 1.6 ml/min) and no cells (12.6 ± 1.4 ml/min), respectively. When we applied this module to 7/8-nephrectomized uremic dogs, the arterial IS levels decreased from 0.53 ± 0.06 to 0.16 ± 0.02 mg/dl by 4-hour treatment of S2-hOAT3. In vivo clearance of IS was significantly higher in S2-hOAT3 (46.4 ± 3.0 ml/min) than in S2-DNA (0.8 ± 0.5 ml/min) and no cells (6.17 ± 1.8 ml/min), respectively.

Conclusions: These results demonstrated that our hybrid kidney with S2-hOAT3 can selectively remove IS in uremic dog. This might be useful for the treatment of chronic renal failure patients to avoid cardiovascular complications.

SA-PO2756

Inhibitory Effect of Overexpression of Aquaporin-11 on Intracellular Ca^{2+} Release in Transfected Mammalian Cells Ryuji Nishimura, Kanako Muta, Saki Takahashi, Hiroko Sonoda, Masahiro Ikeda. *Veterinary Pharmacology, University of Miyazaki, Japan.*

Background: The aquaporins (AQPs) are a family of membrane protein that are selectively permeable to water and glycerol. So far, thirteen AQPs (AQP0 - AQP12) have been identified in mammals, and among them the recently identified molecule AQP11 is known to be localized in the endoplasmic reticulum (ER), an intracellular Ca^{2+} store. Furthermore, AQP11-null mice die in the neonatal period due to renal failure with progressive renal cyst formation. However, the molecular function of AQP11 is largely unknown. In this study, we examined whether AQP11 affected intracellular Ca^{2+} response in transfected mammalian cells.

Methods: Intracellular Ca^{2+} concentration was monitored with Fluo4 in CHO-K1 cells transfected with each expression plasmid. Cultured cells were observed 24 h post-transfection using a confocal microscope.

Results: The elevation of intracellular Ca^{2+} concentration in response to ATP was completely inhibited in both the presence and absence of extracellular Ca^{2+} in cells expressing N-terminal DsRed monomer-tagged AQP11 (DsRM-AQP11). Similar inhibition was also observed in cells expressing myc-AQP11 or AQP11-DsRM. On the other hand, no inhibition of ATP-induced Ca^{2+} increase was observed in cells expressing DsRM-AQP1. Under extracellular Ca^{2+}-free condition, ionomycin is known to mobilize Ca^{2+} from intracellular Ca^{2+} stores, depending on Ca^{2+} content of the stores. When cells expressing DsRM-AQP11 was treated with ionomycin in the absence of extracellular Ca^{2+}, ionomycin-induced Ca^{2+} response was also significantly inhibited. In order to identify the amino acid region involved in inhibiting the Ca^{2+} response, we examined the effect of the overexpression of deletion mutant (DsRM-AQP11-E230X, -L196X, -V161X, -G111X, -H74X, -E50X) on ATP-induced Ca^{2+} increase. As a result, reduced inhibition of ATP-induced Ca^{2+} -increase was observed only in cells expressing DsRM-AQP11-E50X.

Conclusions: These data indicate that AQP11 inhibits ATP-induced Ca^{2+} release via the depletion of intracellular Ca^{2+} stores, suggesting the importance of AQP11 in the regulation of intarcellular Ca^{2+} concentration.

SA-PO2757

Aquaporin-11 Expression in the Brain: Choroid Plexus and Capillaries with Blood-Brain Barrier Function Shin Koike,[1] Yasuko Tanaka,[1] Yoshiyuki Morishita,[2] Kenichi Ishibashi.[1] *[1]Meiji Pharmaceutical University, Japan; [2]Jichi Midical School, Japan.*

Results: We found that AQP11 was expressed in the cytosol of choroid plexus epithelia and at endothelia of brain capillaries by immunohistochemistry selectively as absent in other capillaries outside the brain. AQP1 was also expressed at choroid plexuses and capillaries while AQP4 was expressed at astrocytes around capillaries. AQP11 was initially expressed at pia mater and little at capillaries in newborns (P1), and the former expression shifted to the latter with growth, which was quantified by a capillary deletion method: the ratios of capillary to parenchymal fractions increased by 2.5 folds from P1 to P28. AQP1and 11 were highest at P1 and decreased at P7 to P28 by one-eighth in AQP1 and by one-third in AQP11in the brain, while AQP4 increased 6 folds from P1 to P7. The brain of AQP11-null mice revealed numerous intracellular vacuoles in the choroid plexus but the other area of the brain appeared normal. The water contents of the brain were similar (78.61% vs. 78.96%). The two-dimentional protein electrophoresis analysis also revealed no obvious changes of protein expression patterns. Blood-brain-barrier (BBB) function examined by biotin injected into the heart appeared to be normal with no leakage in AQP11-null mice even with a lipopolysaccharide pre-administration. Interestingly, the expression of AQP4 was decreased by half in the brain of AQP11-null mice by real-time PCR with normal AQP1 expression. When osmotically challenged by addition of sodium (serum Na199 mEq/l) or water (Na123), the decreased AQP4 mRNA in AQP11-null mice was restored to normal levels. Hypotonia (Na123) enhanced the expansion of perivascular Virchow-Robin spaces in AQP11-null mice. In wild mice, mannitol administration (Na156) decreased AQP1,4,11 mRNAs in the brain. Both hyponatremia (Na121) and hypernatremia (Na154) significantly decreased AQP11 mRNA with stable AQP4 mRNA.

Conclusions: As AQP4 plays an important role in the regulation of brain edema, these findings suggest that both AQP4 and AQP11 may function in concert at BBB in response to osmotic changes. AQP11 can be a target for drugs to modulate brain edema as well as BBB function.

Funding: Government Support - Non-U.S.

SA-PO2758

Inhibitors of the Uric Acid Transporter URAT1 Require URAT1 Phenylalanine 365 for High Affinity Interaction Philip K. Tan, David L. Hyndman, Jeffrey N. Miner. *Ardea Biosciences, San Diego, CA.*

Background: URAT1 is the target of many uricosuric agents that block uric acid reabsorption. Ardea Biosciences has recently identified a class of next generation inhibitors that specifically inhibit URAT1 at nanomolar potency. Here we report on a residue in URAT1 that is required for this high affinity interaction.

Methods: Human URAT1 has a 74% protein sequence identity with the ortholog rat uric acid transporter rRST, yet the compounds inhibit rRST with a 1000-fold lower affinity. We focused on residues that differ between these transporters and systematically converted residues within URAT1 into the corresponding RST residues, and vice versa, and measured the activity and sensitivity of these mutants to URAT1 inhibitors. We also developed a binding assay to URAT1 membranes using a radiolabeled next generation Ardea compound.

Results: Conversion of a single residue in URAT1 at position 365, from phenylalanine to the RST residue tyrosine, results in a mutant URAT1 uric acid transporter that is 100-fold less sensitive to inhibition. Conversely, conversion of RST residue tyrosine-365 to the URAT1 phenylalanine produces an RST mutant that is 10-fold more sensitive to inhibition. For the binding assay, binding of the radiolabeled compound is specific and saturable to URAT1 and occurs at a nanomolar affinity, showing that the compound directly binds with high affinity to URAT1. Xenopus Oocyte microinjection experiments were also conducted demonstrating that the binding site is available from both the inside of the cell and the outside.

Conclusions: URAT1 phenylalanine-365 is both necessary and sufficient for the high affinity inhibition by inhibitors, and this inhibition is perturbed by the addition of a tyrosine hydroxyl group. URAT1 phenylalanine-365 is either involved in a direct hydrophobic interaction with these molecules that is blocked by the tyrosine hydroxyl group, or it is important to maintain a particular structure within URAT1 that is required for high affinity binding. We suggest that URAT1 phenylalanine-365 is within a substrate binding pocket that also interacts with Ardea's compound inhibitors.

Funding: Pharmaceutical Company Support

SA-PO2759

Protective Effects of N-acetylcysteine (NAC) in Renal and Lung Function in Old Rats Maria Heloisa M. Shimizu, Rildo A. Volpini, Ana C. de Bragança, Lucia Andrade, Antonio C. Seguro. *Nephrology, School of Medicine - University of Sao Paulo, Sao Paulo, Brazil.*

Background: Oxidative stress increases with age and is associated with alterations in kidney and lung functions. Previous studies demonstrated that NAC, an antioxidant drug, protects animals from kidney and lung injury.

Methods: The aim of this study was to evaluate the effects of NAC on sodium and water transporters in kidney and lung of old rats. Normal 8-month-old male Wistar rats were treated (n=6) or not (n=6) with NAC (600 mg/L in drinking water) and followed for 16 months. At the end of follow-up we measured thiobarbituric acid reactive substances (TBARS, a lipid peroxidation marker), urine volume (UV) and inulin clearance (In Cl). In addition we performed immunoblotting for renal proteins (NKCC2, αENaC and AQP2) and for lung proteins (αENaC , NKCC1 and AQP5).

Results: Mean NAC ingestion was 24±2 mg/day. As we can see in table 1, NAC decreased serum TBARS and ameliorate inulin clerance in old rats.

	In Cl (ml/min/100 g BW)	U V(µl/min)	FE H2O (%)	TBARS (nmol/ml)
Old Rat	0.308±0.012	23.1±8.0	0.63±0.09	10.6±0.7
Old Rat +NAC	0.459±0.041 *	7.6±0.4 **	0.29±0.02**	8.4±0.3 *

* $p<0.01$; ** $p<0.05$ vs. Old Rat

NKCC2 and AQP2 renal expression was significantly 69% and 42% higher in old+NAC rats than in old rats. There was no change in αENaC renal expression. In old+NAC rats, lung expression of αENaC and AQP5 was 32% and 30% higher than in old rats, whereas the NKCC1 was lower in 46% than the old rats.

Conclusions: NAC treatment in old rats ameliorated renal function and upregulated NKCC2 and AQP2 renal expression. These effects may increase urine concentration and dilution capacity in old rats and consequently decrease the incidence of hypo or hyperosmolar states in this population. In addition, we found that NAC profoundly influences the sodium and water transport in alveolar epithelial cells and these effects may decrease the incidence of lung edema in old population.

Funding: Government Support - Non-U.S.

SA-PO2760

Sodium Myoinositol Cotransporter-1 Knockout Mice Are Not at Increased Risk for Osmotic Renal Injury Ajay Kher, S. Ananth Karumanchi, Samir M. Parikh. *Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: Previous studies have shown that (1) loss of the tonicity-responsive transcription factor NFAT5/TonEBP results in renal atrophy, (2) NFAT5 is a critical positive regulator of the tonicity-responsive sodium-myoinositol co-transporter-1 (SMIT-1), and (3) injection of a small-molecular SMIT inhibitor into rodents induces acute kidney injury. We therefore hypothesized that SMIT-1 knockout mice (KO) would have increased susceptibility to the osmotic stress of dehydration.

Methods: 10-12 week KO, heterozygotes and wild-type male mice were evaluated at baseline and after 48hours of dehydration for serum electrolytes, BUN and creatinine by iSTAT analyzer and urine osmolarity. Mice also underwent radiocontrast injury by intraperitoneal injection of LNAME and Indomethacin (each 10mg/kg) followed 1 hour later by an intravenous injection of iohexol 10ml/kg.

Results: Baseline renal function was indistinguishable between KOs, heterozygotes, and wild-type male littermates. After 48 hours of dehydration, mice of all three genotypes had similar percent weight loss (WT 14.2 +/- 0.8, Het 15.9 +/- 1.8, KO 15.9 +/-1.3), serum sodium (WT 152+/-1.6, Het 153+/-4.6, KO 152+/-4.0 meq/L) and BUN (WT 46+/-5.6, Het 48+/-5.0, KO 54+/-11.9 mg/dl). SMIT1 KO mice were also able to increase their urine osmolarity to a similar extent (WT n=4, 3975 mosm/L, Het n=6, 3925 mosm/L, KO n=2, 4170 mosm/L). After mouse radiocontrast renal injury, male wildtype (n=13), heterozygote (n=9) and knockout (n=8) mice developed similar BUN elevation (WT 73+/-30, Het 73+/-29, KO 74+/-32).

Conclusions: We conclude that loss of SMIT-1 does not lead to renal atrophy and that SMIT-1 knockout mice do not appear to be at increased risk of osmotic renal injury. Our results suggest the importance of other NFAT5 transcriptional targets either singly or, more likely, in combination.

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SA-PO2761

The Effect of Hyperosmolality on the Proliferation and Cell Cycle Regulation of Gastrointestinal and Bladder Epithelial Cell Lines Bradley P. Dixon, Brian J. Siroky, John J. Bissler. *Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Children with complex urogenital abnormalities require surgical reconstruction of their urinary tract, and the gastrointestinal tissues often utilized in such reconstructions are at increased risk for cancer. We have previously shown that the hyperosmolal bladder microenvironment may attenuate the DNA damage response in gastrointestinal, but not bladder, cells. Hyperosmolal conditions in the bladder may also induce alterations in cellular proliferation and cell cycle regulation in susceptible tissues, which combined with a faulty DNA damage response could, in part, explain the tissue-specific susceptibility to cancer in the augmented bladder. We sought to characterize the cellular proliferation and cell cycle regulation of gastrointestinal and bladder epithelial cells adapted to a hyperosmolal microenvironment.

Methods: Conditionally immortalized colon (YAMC) and bladder (ULTI) epithelial cells were gradually adapted to either isoosmolal or hyperosmolal conditions. Cellular proliferation was measured by detachment and counting on a hemocytometer, as well as crystal violet DNA binding assay. Cell cycle analysis was performed by propidium iodide staining and flow cytometry.

Results: The YAMC colon cells demonstrated continued cellular proliferation from 450mOsm/kg to 600mOsm/kg, whereas in ULTI bladder cells, cellular proliferation plateaued after 450mOsm/kg. Additionally, YAMC cells adapted to hyperosmolal sodium chloride had marked abnormalities in cell cycle distribution by 450mOsm/kg and especially 600mOsm/kg, whereas the cell cycle distribution of ULTI cells was preserved to 600mOsm/kg.

Conclusions: Gastrointestinal cells under hyperosmolal conditions were less likely to reduce cellular proliferation despite, and perhaps due to, dysregulation of the cell cycle, whereas these processes remained intact in bladder cells. These findings, coupled with an attenuated DNA damage response under such conditions, could ultimately explain the susceptibility to carcinogenesis experienced by gastrointestinal tissues in the augmented bladder.

Funding: NIDDK Support

SA-PO2762

High Throughput Quantification of Apical Versus Basolateral Trafficking Javier E. Mazzaferri,[1] Stephane Lefrancois,[1,2] Santiago Costantino.[1,3] *[1]Centre de Recherche, Hôpital Maisonneuve-Rosemont, Montréal, Canada; [2]Département de Médicine, U. de Montréal; [3]Département d'Ophtalmologie et Institut de Génie Biomédicale, U. de Montréal.*

Background: Aquaporins (AQP) 2, 3 and 4 are water permeable membrane channels expressed in principal cells of the collecting duct of the kidney. AQP2 localizes to the apical membrane (in response to vasopressin secretion caused by dehydration) allowing water reabsorption from urine. Mutations in AQP2 have been implicated in Nephrogenic Diabetes Insipidus. AQP3 and AQP4 are localized to the basolateral membrane constituting potential water exit pathways from the cell. The complex protein trafficking machinery that maintains these segregated distributions of biomolecules in the apical and basolateral membranes is still not completely understood. Studies that have attempted to characterize the molecular mechanisms implicated in AQP trafficking have often relied on observations made with single or time-lapse fluorescence microscopy images. However, the lack of adequate image analysis techniques makes many of these observations potentially biased and not statistically significant.

Methods: We propose to address this by accurately quantifying the dynamics of AQPs trafficking vesicles using video-rate optically-sectioned in-vivo images. The commercially available optical sectioning techniques do not meet these requirements; therefore we proposed to use an approach that takes advantage of typical features of vesicles. In fact, cargo vesicles are near diffraction limited, belonging to the high-frequency range of the images' spatial spectrum, and are naturally axially localized. High-frequency filtering provides optically-sectioned images of cargo vesicles at frame rates near 10 times faster than confocal microscopy.

Results: We present in-vivo images of MDCK cells transfected with various GFP-tagged AQPs, obtained with the proposed approach.

Conclusions: We have obtained high-resolution, axially-resolved video-rate images of GFP-AQP trafficking vesicles. This will enable us to perform quantitative studies to further elucidate the molecular mechanisms required to sort AQPs to either the apical or basolateral membranes of polarized renal epithelial cells.

Funding: Government Support - Non-U.S.

SA-PO2763

Trends in Hyponatremia Management & Outcomes in Hospital: Interim Results from a Prospective, Observational, Multi-Center, Global Registry Arthur Greenberg,[1] Joseph G. Verbalis,[2] Alpesh Amin,[3] Joseph Dasta,[4] Sandra Chase.[5] *[1]Division of Nephrology, Duke University Medical Center, Durham, NC; [2]Georgetown University Medical Center, Washington, DC; [3]University of California, Irvine, CA; [4]University of Texas, Austin, TX; [5]Otsuka America Pharmaceutical, Inc, Princeton, NJ.*

Background: Hyponatremia (HN) is the leading electrolyte abnormality of hospitalized patients (pts), and an independent predictor of increased mortality. This registry is designed to observe currently utilized management modalities for HN, characterize their relative efficacy, and assess their impact on hospital resource utilization.

Methods: After informed consent or waiver, medical records of pts meeting the registry entry criteria, principally age ≥18 years and euvolemic or hypervolemic HN (serum sodium ([Na]) ≤130 mmol/L) were abstracted. Accrual to date is approximately 40% of the projected US enrollment; data are summarized by sample size (n) and percentage (%) for categorical data, and mean and standard deviation for continuous data.

Results: A total of 483 of the 634 pts enrolled at 96 US sites between study initiation in Sept 2010 and May 2011 had sufficient data for analysis. The mean entry and discharge [Na] values were 125±4.8 mmol/L and 131.3±7.6 mmol/L, respectively. The average length of stay was 9.8±8.7 days.

Demographics, Etiologies, Chronicity and Outcomes	
Baseline Characteristics (n=483)	**%**
Male	49%
White	75%
Age ≥ 65 years	49%
Etiology of HN (n=483)	
SIADH	25%
Heart Failure	28%
Cirrhosis	12%
Nephrotic Syndrome	4%
More than One Hypervolemia Dx	31%
Onset/Duration of HN (n=483)	
Chronic HN	42%
HN as Admitting Dx	19%
HN Developed during Admission	29%
HN on a Prior Admission	29%
Pts treated and discharged with [Na] <130 mmol/L (n=118)	
Any Drug	43%
Fluid Restriction	57%

A total of 116 pts received drug therapy, and 122 fluid restriction alone. A drug was added in 36% of pts initially receiving fluid restriction alone, while only 12% of pts initially receiving drug therapy were treated with a second drug.

Conclusions: These interim data suggest that HN is often chronic or recurrent, fluid restriction alone is frequently not adequate, and pts are often discharged without resolution of HN particularly when the HN is not drug treated. Additional data will be forthcoming as enrollment and analysis continue.

Funding: Pharmaceutical Company Support

SA-PO2764

Renoprotective Mechanisms for AII Blockade in CKD Involve AMPK and Autophagy Joseph Satriano, Aihua Deng, Roland C. Blantz. *UC San Diego, VASDHS, San Diego, CA.*

Background: In the subtotal nephrectomy (STN) model of chronic kidney disease (CKD) we previously observed similar physiologic, metabolic and histologic renal protection with administration of ACE inhibitor + AII receptor blockers (AII-b), or via induction of hypoxia inducing factor-1 (HIF-1) with cobalt chloride (Co) or dimethyloxalyglycine

(DMOG) (Deng, et al, AJP:Renal 2010). Here we further investigate the molecular mechanisms involved in this protection.

Methods: Physiologic and protein expression (Western blotting) changes were evaluated in the rat STN model at 1-week post 5/6 nephrectomy.

Results: Our studies reveal a reduction of renoprotective AMPK activity in STN (>3 fold*). AMPK inhibition of mTOR promotes autophagy, a mechanism required for defense against proteotoxicity and metabolic stress. In accord with a decrease in AMPK in STN we observe an increase in mTOR activity, reflected by the increase of p70S6K expression (>40 fold*), and a reduction of autophagy, via the LC3-II/LC3-I ratio (>7 fold*) and the accumulation of the autophagy linker protein p62 (1.5 fold*). Importantly, the administration of either AII-b or DMOG effectively normalizes AMPK activity, p70S6K, autophagy and p62 levels in STN animals (*). Interestingly, Co, although a HIF-1 inducer like DMOG, did not affect AMPK activity, autophagy or p62 levels, but did provide beneficial kidney effects. Co does, however, induce HO-1 levels (45 fold*), unlike AII-b or DMOG that do not increase HO-1.

Fibrosis and apoptosis are observed in CKD. We see a marked increase in profibrotic PAI-1 expression in STN (>8 fold*). All three agents prevent this response (*). The expression of the execution caspase-3 of apoptosis also increases with STN (1.6 fold*), an event normalized with Co or DMOG administration (*). AII-b tends to decrease caspase-3 from STN alone levels, but is not statistically significant.

(* = $p<0.05$)

Conclusions: Normalization of AMPK/autophagy in STN animals by AII-b and DMOG correlates with beneficial physiologic changes in GFR, RBF and renal metabolic efficiency (QO2/TNa). Thus, preventing the decrease in the renoprotective AMPK pathway network could afford kidney protection and normalization of function in CKD.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2765

Angiotensin II Generates Oxidative Stress in the Kidney Proximal Tubule Via EGFR-ERK Pathway Vinayak Ramanath, Ravi Nistala, Adam Whaley-Connell, James R. Sowers. *Internal Medicine/Nephrology, University of Missouri-Columbia, Columbia, MO.*

Background: Oxidative stress is a major contributor to both short term and long term kidney function decline that ultimately leads to chronic kidney disease and its attendent problems. Proximal tubule oxidative stress is increasingly recognized as a major player in the early stages of kidney damage. Activation of NADPH oxidase enyzme and mitochondria contribute towards generation of oxidative stress in the proximal tubule. Several moieties have been implicated in the causation of oxidative stress. The role of the renin-angiotensin-aldosterone system has been well documented in the generation of kidney oxidative stress. However, the mechanisms for linking Angiotensin II (Ang II) to the generation of the superoxide free radical are not well known. **Hypothesis**: Ang II generated oxidative stress via transactivation of the EGFR-ERK pathway.

Methods: A opossum proximal tubule cell line (OKP) was used as a model system to study the signaling mechanisms activated by Angiotensin II. 24-hour starved OKP cells were treated over a time-course of 5min to 24hrs. The 30min and 24hr timepoints were chosen to look at the acute and chronic effects of Ang II. Superoxide generation was measured by lucigenin assays while NADPH oxidase activity was measured via Cayman radical assay. Protein measurements were via Western blots and immunoprecipitation. siRNA assays were performed according to the manufacturer (SABiosciences).

Results: Ang II stimulated superoxide production in a biphasic manner with 2 peaks at 30min and 24hrs. This was primarily via activation of NADPH oxidase enzyme. Ang II transctivated the EGF receptor and led to further activation of ERK. Blockade of EGFR with AG1478 and siRNA reversed Ang II-mediated activation of oxidative stress. Furthermore, blockade of Ang II-mediated oxidative stress improves albumin endocytosis via improvement in megalin function.

Conclusions: Ang II mediates oxidative stress in the kidney proximal tubule primarily via transactivation of the EGFR. Generation of oxidative stress and alteration in megalin function via Ang II may explain Ang II-mediated proteinuria at the proximal tubule level.

Funding: Private Foundation Support

SA-PO2766

Deletion of the Ace2 Gene Exacerbates Kidney Injury in Mice after Unilateral Ureteral Obstruction Fei Fang,[1] James W. Scholey,[1] George Chu Liu,[1] Xiaohua Zhou,[1] Gavin Oudit,[3] Rohan John.[2] *[1]Institute of Medical Science, University of Toronto, ON, Canada; [2]Department of Pathology, University of Toronto, ON, Canada; [3]Division of Cardiology, University of Alberta, Edmonton, AB, Canada.*

Background: Activation of the renin-angiotensin system (RAS) is a well-established risk factor for chronic kidney disease, and blockade of the RAS has proven beneficial in this regard. ACE2 is a homologue of ACE that is highly expressed in the kidney, and can degrade angiotensin II (AngII) to produce the vasodilating peptide angiotensin-(1-7). Deletion of the Ace2 gene in male mice has been associated with an age-dependent development of glomerulosclerosis, which was prevented with AT1R blocker irbesartan. ACE2 has also shown to be a protective factor in diabetic glomerular injury. However the role of ACE2 in modulating tubulointerstitial kidney injury is not as well defined. Unilateral ureteral obstruction (UUO) is an accelerated model of chronic kidney disease associated with the rapid development of inflammation and fibrosis in the tubulointerstitium of the kidney, in part due to activation of the RAS. Accordingly we sought to determine whether deletion of the Ace2 gene would exacerbate kidney injury in mice subjected to UUO.

Methods: Two groups of male mice were studied: 8 week old wild-type C57BL/6J mice and mice with a deletion in the gene for Ace2. Mice from each group were subjected to sham operation or UUO of the left kidney, and gene expression measured by real time PCR in the renal cortex after 7 days.

Results: As expected, UUO was associated with an increased expression of pro-inflammatory genes (IL6, TNFα, and MCP-1), pro-fibrotic genes (collagen 1α1, TGFβ1, PAI-1, and αSMA) seven days after surgery. Deletion of the Ace2 gene did not affect expression in sham-operated mice, but exacerbated the increase in expression levels for both the inflammatory and fibrotic genes following UUO.

Conclusions: Deletion of the ACE2 gene promotes inflammation and fibrosis in the kidneys of mice after UUO, suggesting that ACE2 is also protective in the kidney tubulointerstitium.

Funding: Government Support - Non-U.S.

SA-PO2767

Effect of Combination Therapy with Low Dose Darbepoetin and High Dose ARB on Established Glomerulosclerosis in 5/6 Nephrectomized Mice Keizo Matsushita, Haichun Yang, Agnes B. Fogo. *Department of Pathology, Vanderbilt University Medical Center, Nashville, TN.*

Background: Very early intervention with low dose erythropoietin, independently of effects on anemia, may attenuate progressive kidney failure. We now assessed effects of combination therapy with low dose darbepoetin and high dose ARB on established glomerulosclerosis.

Methods: 129/Sv male mice underwent 5/6 nephrectomy (5/6Nx). Body weight (BW), systolic blood pressure (SBP), urinary albumin creatinine ratio (ACR) and hematocrit (Hct) were measured. At wk8 mice underwent renal biopsy and were randomized to groups with equal starting moderate glomerulosclerosis (GS), and treated with darbepoetin alpha (0.1 μg/kg sc once a week, DAR; n=9), losartan (200 mg/L DW; ARB; n=10), DAR+ARB (n=8), or vehicle (VEH; n=9) for 4 wks until sacrifice.

Results: At wk8 after 5/6Nx, BW, SBP, ACR, Hct and GS were similar among groups by study design. Survival at wk12 was higher in all treatment groups compared to VEH (DAR+ARB 100%, ARB 90%, DAR 80%, vs VEH 67%). SBP at wk12 was reduced in ARB and DAR+ARB (94±9 and 114±8 mmHg), but increased in DAR (191±10 mmHg) compared to VEH (172±11 mmHg). Hct was significantly decreased in VEH and DAR (34.5±3.3 and 30.1±2.5%), but ARB (37.2±0.8%) and combination (40.6±1.5%) prevented anemia. ARB and DAR+ARB reduced proteinuria (ACR 28.6±9.8 and 29.9±13.8 mg/g), compared to VEH and DAR (1014±440 and 812±263 mg/g). GS showed progression in untreated VEH (0-4 scale, mean 1.3±0.3 at wk12, 91±55% increase from biopsy). DAR alone had no effect on progression (90±38% increase GS). In contrast, ARB showed significantly less sclerosis (30±25% increase, with regression of sclerosis in 3 mice at wk12 vs wk8), and DAR enhanced this effect (0±23% increase, regression in 5 mice). Podocyte number (WT-1/glomerulus) in VEH was 4.7±0.6 with no benefit of DAR alone (5.7±0.4). In contrast, ARB and DAR+ARB increased podocyte number (6.5±0.5, 6.6±0.6; $p<0.05$ vs VEH).

Conclusions: Low dose darbepoetin per se has no effect on progression, but in combination with high dose ARB halts progressive renal anemia and enhances regression of glomerulosclerosis.

Funding: Pharmaceutical Company Support

SA-PO2768

Combined Losartan (L) and Hydrochlorothiazide (H) Therapy Arrests Renal Injury and Reverses Cell Events in the Remnant Kidney Model (Nx) Simone R. Costa, Carla P. Valente, Cristiene Okabe, Flavia G. Machado, Camilla Fanelli, Claudia R. Sena, Grasiela P. Barlette, Vivian L. Viana, Denise M. Malheiros, Roberto Zatz, Clarice K. Fujihara. *Univ Sao Paulo, Brazil.*

Background: We showed previously that L+H treatment arrests renal injury and reverses tubulointerstitial proliferation in Nx even when started at very late phases. Here we investigated further the mechanisms of this protection, focusing on 1) the role of hypertension (HTN). 2) the nephron segments involved in the antiproliferative action. 3) the possible role of myofibroblasts.

Methods: Adult male Munich-Wistar rats underwent Nx and received no treatment for 4 mo. At this time, tail-cuff pressure (TCP, mmHg), serum creatinine (Scr, mg/dL), glomerulosclerosis (%GS), % cortical interstitium (%INT), % interstitial smooth muscle actin (%α-SMA_{int}), tubular PCNA+ cells ($PCNA_{tub}$, cells/mm^2) and distal tubule PCNA+ cells ($PCNA_{dist}$, cells/mm^2) were assessed in 15 pretreatment controls (Nx_{pre}). The remaining rats were divided in: $\mathbf{Nx_V}$ (untreated); $\mathbf{Nx_{LH}}$ (L, 50 mg/kg/d and H, 6 mg/kg/d); and $\mathbf{Nx_{AHHd}}$ (Amlodipine, 5 mg/kg/d, H, 6 mg/kg/d, Hydralazine, 12 mg/kg/d). Age-matched sham-operated rats (**S**) were also studied. Results (Mean±SE) after 3 mo of treatment (^{a,b,c,d}; $p<0.05$ vs. S, Nx_{pre}, Nx and Nx_{AHHd}, respectively)

Results:

	TCP	S_{Cr}	%GS	%INT	%αSMA_{int}	$PCNA_{tub}$	$PCNA_{dist}$
S	141±1	0.6±0.1	1±1	0.7±0.1	0.1±0.1	9±1	0.2±0.1
Nx_{pre}	207±3^{a}	1.4±0.1^{a}	26±3^{a}	4.2±0.4^{a}	6.4±0.9^{a}	68±6^{a}	3.9±0.9^{a}
Nx_V	212±4^{a}	2.4±0.1^{ab}	58±4^{ab}	6.9±0.4^{ab}	6.7±0.8^{a}	70±9^{a}	2.6±0.3^{a}
Nx_{AHHd}	172±9^{abc}	2.1±0.1^{abc}	47±7^{abc}	5.5±0.6^{ac}	7.8±1.2^{a}	55±6^{a}	2.1±0.4^{ab}
Nx_{LH}	164±3^{abc}	1.6±0.1^{acd}	25±3^{acd}	4.0±0.4^{ac}	4.5±0.7^{ad}	26±2^{abcd}	0.2±0.1^{bcd}

Nx_{pre} exhibited severe HTN, GS and INT expansion, marked tubular (partly distal) cell proliferation, and increased αSMA expression. Renal injury worsened in Nx_V. LH treatment arrested renal injury at pretreatment levels, and reversed both distal and total tubular cell proliferation. Despite similar TCP reduction, AHHd only slightly ameliorated renal injury and tubular hyperplasia.

Conclusions: Cell events predominate over HTN in the pathogenesis of advanced renal injury in Nx. Myofibroblast infiltration, likely originated in part from proximal tubular cells, may play a key role in this process.

SA-PO2769

Inhibitory Effect of Spironolactone (SPL) on Renal Injury and Intrarenal Angiotensinogen (AGT) Expression in Salt-Loaded, Nitric Oxide-Deficient Rat Takaichi Suehiro, Kazuhiko Tsuruya, Toshiaki Nakano, Masatomo Taniguchi. *Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: Intrarenal renin-angiotensin system (RAS) may be involved in the pathogenesis of kidney injury, and AGT is thought to have an important role for the intrarenal RAS activation. The effect of RAS blockade for intrarenal RAS is evaluated in some animal models, but little is known about mineralocorticoid receptor (MR) blockade. The aim of this study is to investigate the effect of MR blocker for kidney injury and AGT in nitric oxide-deficient rats with salt loading, and to evaluate the relationship between kidney damage and AGT.

Methods: Male Wistar rats were divided into the following three groups; the control rats given vehicle (Cont), L-NAME rats given 50 mg/kg/day L-NAME in drinking water and 4% salt in chow (L-NAME), and SPL-treated L-NAME rats given 100 mg/kg/day SPL in chow in addition to L-NAME rats (SPL).

Results: Following treatment for 6 weeks, L-NAME rats developed hypertension, proteinuria and kidney injury. Without reduction of blood pressure (BP), SPL prevented proteinuria (14, 112, and 27 mg/day in Cont, L-NAME, and SPL, respectively; $p<0.05$), glomerulosclerosis, interstitial fibrosis (IF), osteopontin (OPN) expression determined by real time PCR and immunohistochemistry, and urinary excretion of 8-OHdG. Plasma angiotensin II and aldosterone (Ald) were comparable among the three groups, but urinary AGT excretion (208, 6697, and 1317 ng/day in Cont, L-NAME, and SPL, respectively; $p<0.05$) and AGT expression in the proximal tubules were increased in L-NAME rats, while these changes were inhibited by SPL. Urinary AGT was positively correlated with proteinuria, IF and OPN expression ($r = 0.87$, 0.69, and 0.73, respectively; $p<0.05$).

Conclusions: In conclusion, the present study demonstrated that SPL ameliorated kidney injury through reduction of AGT expression in the kidney independent of plasma Ald and BP, and intrarenal AGT was related to renal injury. These results suggest that intrarenal AGT may be activated through MR pathway and potentially involved in the kidney injury during nitric oxide deficiency and salt-loaded status.

SA-PO2770

Ghrelin Suppresses Angiotensin II-Induced Premature Renal Senescence by Reducing Oxidative Stress Keiko Fujimura, Shu Wakino, Koichi Hayashi. *Department of Medicine, Keio University, Tokyo, Japan.*

Background: Premature senescence is one of the main pathways towards the deterioration of renal function. Angiotensin II (AngII) induces renal premature senescence by multiple mechanisms including by increasing oxidative stress. Recent study revealed that GH secretagogue ghrelin exerts anti-senescence effects by reducing mitochondria-derived reactive oxygen spieces (ROS) levels. In this study, we examined whether ghrelin inhibits AngII-induced renal senescence and damages.

Methods: Renal senescence was induced by infusion of AngII in C57BL/6 mice with osmotic mini-pump. Ghrelin was administered by the daily intraperitoneal injection. 8 weeks after the treatment, kidneys were removed and utilized in the various experiments. In *in vitro* experiment, cultured human proximal cell line, HK-2 cells were incubated with 1 mM of AngII for 72 hrs and ghrelin were administered 30 mins prior to AngII stimulation.

Results: AngII infusion induces senescence and oxidative stress as accessed by senescence-associated (SA) b-Gal and 4-hydroxy-2-nonenal (4-HNE) staining, respectively. The expressions of markers for senescence, p21 , p53 as well as senescence-associated cytokines, TGF-b , PAI-1 in the kidney were increased by AngII. AngII infusion also induced renal damages as assayed by the urinary excretion of renal tubular marker, N-acetyl-glucosaminide (NAG) and neutrophil gelatinase-associated lipocalin (NGAL) as well as by urinary protein excretion. Finally, AngII infusion provoked interstitial fibrosis as evaluated by Masson-trichrome staining. These changes were attenuated by the treatment with Ghrelin. In HK-2 cells, the receptors for both Ghrelin and AngII were expressed. SA b-Gal assay revealed tubular cell senescence by AngII. AngII also increased the expression of p21 and p53 as well as TGF-b and PAI-1. These changes were attenuated by pretreatment with Ghrelin. In addition, AngII reduced the mitochondria number, which was restored by Ghrelin as measured by staining cells with mitochondria-specific fluorescent dye.

Conclusions: Our data indicated that ghrelin suppressed AngII-induced renal premature senescence and AngII-induced renal damages presumably through modulating the mitochondrial ROS production.

SA-PO2771

Endogenous Angiotensin-(1-7) Protects Against Tubulointerstitial Fibrosis in Mice with Unilateral Ureteral Obstruction Danielle L. Zimmerman, Alex Gutsol, Anthony Carter, Rhian Touyz, Kevin D. Burns. *Kidney Research Centre, Division of Nephrology, Dept. of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada.*

Background: Angiotensin-(1-7) [Ang-(1-7)] is a heptapeptide product of the renin angiotensin system that is generated by angiotensin-converting enzyme 2 (ACE2)-dependent degradation of Ang II. In proximal tubular cells, Ang-(1-7) inhibits Ang II-stimulated mitogen-activated protein kinase signaling, suggesting that it may counteract the profibrotic and proinflammatory effects of Ang II. We determined if endogenous Ang-(1-7) protects against tubulointerstitial injury in a mouse model of renal fibrosis.

Methods: Male C57BlK6/J mice underwent unilateral ureteral obstruction (UUO) and were treated with or without the Ang-(1-7) antagonist A779 (31 ug/kg/hr by osmotic mini-pump), starting at day 0 until sacrifice at day 10. Blood pressures were monitored by tail cuff plethysmography and tissues were harvested for histologic and immunoblot analyses.

Results: Blood pressure was not affected by UUO or treatment with A779. UUO caused a significant increase in tubulointerstitial injury scores compared to unobstructed kidneys, and this was exacerbated in mice treated with A779 (UUO: 1.38 ± 0.1 vs UUO+A779: 2.07 ± 0.2, $p<0.01$, $n=7$).). By immunoblots, expression of fibronectin, pro-collagen I, and transforming growth factor-ß (TGF-β) was increased in obstructed kidneys, compared to unobstructed kidneys, with a further significant increase in A779-treated mice (fibronectin: UUO: 1.17±0.09, vs UUO+A779: 2.80±0.30, $p < 0.001$; pro-collagen I: UUO: 0.84±0.06 vs UUO+A779: 1.16±0.14, $p < 0.05$; TGF-β: UUO: 0.9±0.2 vs UUO+A779: 1.8±0.2, $p <0.01$, $n=7$). Expression of α-smooth muscle actin was also increased in obstructed kidneys with A779 treatment ($p<0.05$, $n=7$). Protein expression of ACE2 and ACE was significantly decreased in obstructed kidneys ($p<0.001$, $n=7$), but A779 had no effect on expression.

Conclusions: These data indicate that the Ang-(1-7) antagonist A779 worsens renal fibrosis in mice with UUO. The results suggest that endogenous Ang-(1-7) protects against tubulointerstitial injury *in vivo*, perhaps via inhibition of profibrotic pathways stimulated by Ang II.

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SA-PO2772

Dependence of Renal Fibrosis on a Cell Cycle Regulator Judit Megyesi, Adel Tarcsafalvi, Robert L. Safirstein, Peter M. Price. *Internal Medicine, UAMS, Little Rock, AR.*

Background: A major cause of progressive renal failure occurs during repair from kidney injury that results in the formation of fibrotic lesions rather than tissue replacement. The molecular causes of this maladaptive growth are not yet understood, but recent studies have suggested potential pathways.

Methods: We used several mouse models of renal fibrosis, either mimicking effects of acute kidney injury or effects of loss of renal mass.

Results: A mouse model of subtotal nephrectomy induced parameters of chronic kidney failure, i.e. systemic hypertension, glomerular sclerosis, interstitial fibrosis, and reduced glomerular filtration. Fibrosis was evident 6 weeks after nephrectomy and functional renal failure occurred 14-16 weeks after surgery. A cell cycle regulatory protein, $p21^{WAF1/Cip1}$, was induced in the remnant kidney, accompanied by hypertrophy in the tubular epithelial cells. In homogygous p21 knock-out mice, the filtration rate of the remaining nephrons did not decline, and sclerosis did not develop. We have since developed transgenic mice in which p21 expression could be induced in kidney proximal tubules. We hypothesized that p21 over-expression will induce more severe fibrosis and tested this in models of AKI in which renal fibrosis developed several weeks after injury. Comparing wild-type with transgenic mice 14 days after unilateral ureteral obstruction, more α-smooth muscle actin was seen around the kidney tubules of transgenic mice. Similarly, more macrophage infiltration was seen in the p21 these mice. We also used an *in vitro* model of fibrosis using 0.5 mg/ml aristolochic acid treatment of mouse proximal tubule cells (TKPTS) in which 6.3 mg collagen was secreted by the cells per ml of medium after 48 hours. p21 regulates the cell cycle by inhibiting progression into S and M phases, and the effect of p21 induction correlated with collagen production as evidenced by TKPTS cells being arrested in G2 phase preceding collagen induction.

Conclusions: We conclude that p21 induction is a causative mechanism for development of fibrosis and chronic renal failure, either from its activity to prevent cell cycle progression or from its activity to inhibit phosphorylation of pro-fibrotic substrates of cell cycle kinases.

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SA-PO2773

Attenuated Glomerular Arginine Transport Provokes Renal Injury in the Pregnant Uremic Rat Doron Schwartz, Idit F. Schwartz. *Nephrology, Tel Aviv Sorasky Medical Center, Tel Aviv, Israel.*

Background: Pregnancy worsens renal function in females with renal failure (CRF) through an unknown mechanism. Reduced nitric oxide (NO) generation induces renal injury. Arginine transport by cationic amino acid transporter-1 (CAT-1), which governs endothelial NO generation, is reduced in both renal failure and pregnancy. We hypothesize that attenuated maternal glomerular arginine transport promotes renal damage in CRF pregnant rats.

Methods: Rats underwent a two stage 5/6 nephrectomy (interval of one week). All experiments were performed 6 weeks following the second operation.

Results: In uremic rats, pregnancy induced a significant decrease in glomerular arginine transport and c-GMP generation (a measure of NO production) compared to CRF or pregnancy alone and these effects were prevented by L-arginine. While CAT-1 abundance was unchanged in all experimental groups, PKCα and phosphorylated PKCα (CAT-1 inhibitor), were significantly augmented in CRF, pregnant, and pregnant CRF animals, phenomena which were prevented by co-administrating L-arginine. α-tocopherol (PKC inhibitor) significantly increased arginine transport in both pregnant and CRF pregnant rats, effects which were attenuated by ex vivo incubation of glomeruli with PMA (a PKC stimulant). Renal histology revealed no differences between all experimental groups. Creatinine clearance failed to augment and renal cortical expression of HIF-1α significantly increased in CRF pregnant rat, both phenomena were prevented by arginine.

Conclusions: These studies suggest that in CRF rats, pregnancy produces a profound decrease in glomerular arginine transport, through post translational regulation of CAT-1 by PKCα, resulting in attenuated NO generation. These events provoke renal damage manifested by upregulation of renal HIF-1α and loss of the ability to increase GFR during gestation and may be responsible for the accelerated deterioration in renal function during pregnancy in uremic females.

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SA-PO2774

Periostin Is a Novel Marker of the Severity of Hypertensive Nephropathy Dominique Guerrot, Sandrine Placier, Mouna Mael-Ainin, Jean-Claude Dussaule, Christos Chatziantoniou. *INSERM UMR 702, Tenon Hospital, Paris, France.*

Background: The aim of our study was to identify novel determinants of progression and regression of chronic kidney disease.

Methods: Rats were treated with the NO synthase inhibitor L-NAME. When proteinuria exceeded 1 g/mmol creatinine (5-6 wk), a group of animals was used to estimate renal hemodynamics and morphology parameters just before the beginning of therapy (LN 6w group). The remaining animals were divided into two groups for an additional experimental period of 4 wk: in the first group L-NAME was given alone (LN 10w); in the other group L-NAME was co-administered with Losartan. At the end of Losartan treatment, some animals showed normal creatininemia (REGR), whereas other animals escaped therapy (creatininemia ≥70 µmol/l, ESC group).

Results: Differential transcriptomic analysis of renal cortex between REGR and LN 10w groups revealed that periostin, a gene non expressed in normal kidneys (but strongly involved in heart remodelling and fibrosis), was highly upregulated in the LN 10w group. Quantitative evaluation of periostin mRNA and protein expressions in the kidney indicated that periostin was continuously increasing during hypertensive nephropathy, it was significantly decreased in the REG group, whereas it remained increased in the ESC group in spite of 4 wk losartan treatment. Immunohistochemistry revealed that hypertensive nephropathy was characterized by a strong increase in periostin staining in the media and the adventitia of renal vessels. Interestingly periostin also exhibited a focal de novo interstitial expression in close vicinity to the most severe lesions.

To further evaluate the relevance of periostin as a marker of progression of renal disease, we performed regression analyses with classical functional and histological parameters of hypertensive nephropathy. We found a very strong positive association (r≥0.70) between periostin expression and creatininemia, proteinuria, renal blood flow decrease, and histological lesion scores.

Conclusions: Periostin expression in the kidney is associated to the development of hypertensive renal disease. We propose periostin as a novel marker determining progression/regression of chronic kidney disease.

Funding: Government Support - Non-U.S.

SA-PO2775

Epiphyseal Growth Plate Growth Hormone Receptor Signaling Is Impaired in a Rat Model of Chronic Kidney Disease Related Growth Retardation Yael Segev,[1] Ariel Troib,[1] Debbie Wiezel,[1] Ralph Rabkin,[2] Daniel Landau.[3] *[1]Shraga Segal Dept of Microbiology and Immunology, Ben Gurion University, Beer Sheva, Israel; [2]Medicine, Veterans Administration, Stanford University, Palo Alto, CA; [3]Pediatrics, Ben Gurion University, Beer Sheva, Israel.*

Background: Linear growth retardation with reduced muscle mass is a major problem in children and adults with chronic kidney disease (CKD) and is ascribed to insensitivity to GH. This resistance has largely been attributed to low IGF-I bioavailability and to a decrease in liver GH receptor (GHR) expression and signaling. No data is available on bone GHR expression and action in CKD. We investigated the role of local GH-IGF system in developing bones of young growth retarded CKD rats.

Methods: Twenty-day old rats underwent a 2 step 5/6 nephrectomy (CKD) or sham operation. Control rats (C) were pair-fed with CKD. Animals were sacrificed 2 weeks after second surgery.

Results: There was no difference in the amount of food consumed, yet there was a significant difference in weight gain and longitudinal growth between C and CKD rats. Serum creatinine (225±11% of C) and albuminuria were significantly higher in CKD rats. No change was observed in serum GH, yet serum IGF1 was decreased significantly in CKD. Epiphyseal growth plate (EGP) morphometry showed no change in the proliferative zone but a wider hypertrophic zone in CKD rats, with a disturbance in cell order (but not in cell count) in that zone. EGP type 10 collagen mRNA increased in CKD (375±56% of C, $p< 0.05$). EGP GH receptor (GHR) mRNA was unchanged, but JAK2 mRNA and phosphorylated (p-) STAT5 were decreased, while SOCS2 mRNA was increased in CKD. IGF1 mRNA levels were unchanged.

Conclusions: Young rats with moderate CKD develop significant growth retardation despite the enlargement of EGP hypertrophic zone, hinting for a chondrocyte maturation arrest. The decrease in JAK2 mRNA and p-STAT5 and the increase in SOCS2 mRNA in the EGP of CKD rats in spite of unchanged GHR mRNA suggest a bone GHR signaling impairment in CKD as an additional mechanism of CKD mediated growth retardation.

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SA-PO2776

AZGP1 Inhibits the Development of Renal Fibrosis Inga Soerensen, Nathan D. Susnik, Hermann G. Haller, Roland Schmitt. *Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: With age the kidney becomes more susceptible to acute and chronic injury and looses its regenerative potential. Additionally, old kidneys show more interstitial fibrosis and tubular atrophy. Zinc-α2-glycoprotein 1 (AZGP1) is a secreted glycoprotein whose function has not yet been fully elucidated. In vivo knockdown of AZGP1 in old mice was associated with increased epithelial proliferation and amplification of interstitial fibrosis. Therefore, our hypothesis was that AZGP1 has an antifibrotic effect.

Methods: UUO as a common fibrosis model was induced in AZGP1-/- and AZGP1 +/- mice. At different time-points kidneys were analyzed using histology, immunohistochemistry, qPCR and immunoblot. For in vitro studies we used different renal epithelial cell lines and rat renal fibroblasts. Epithelial cells were transiently transfected with an AZGP1 expression construct. The resulting conditioned medium was transferred to cultured rat renal fibroblasts. The expression of myofibroblast markers was analysed using immunoblot and qPCR.

Results: Renal fibrosis developed in both groups of mice but AZGP1 deficiency was associated with a significant attenuation of renal tubulointerstitial damage. We found that α-Smooth muscle actin, as a marker for fibrosis, was significantly higher in UUO kidneys of AZGP1 -/- mice. Additionally we found a significant reduction of the epithelial marker E-Cadherin in AZGP1 -/- UUO kidneys at one and two weeks, whereas Vimentin was highly upregulated on protein and mRNA level. The number of infiltrating inflammatory (CD45+) cells were elevated in AZGP1 -/- kidneys. In vitro we found that the conditioned medium obtained from AZGP1 overexpressing epithelial cells contains factors that inhibit TGFβ-dependent activation of fibroblasts .

Conclusions: In summary our data indicate that epithelial expression of AZGP1 attenuates the development of fibrosis in the UUO model. In vitro studies suggest that the secretome of AZGP1 overexpressing renal epithelial cells exhibits an inhibitory effect on the activation of renal fibroblasts.

Funding: Government Support - Non-U.S.

SA-PO2777

The Role of Autophagy in Unilateral Ureteral Obstruction Rat Model Yong Kyun Kim,[1] Wan-Young Kim,[2] Sun-Ah Nam,[2] Ho Cheol Song,[1] Euy Jin Choi,[1] Jin Kim.[2] *[1]Internal Medicine, Catholic University of Korea, Seoul, Korea; [2]Department of Anatomy and MRC for Cell Death Disease Research Center, Catholic University of Korea, Seoul.*

Background: Autophagy is a cellular process of degradation of damaged cytoplasmic components and regulates cell death or proliferation. Unilatreral ureteral obstruction (UUO) is a model of progressive renal fibrosis in the obstructed kidney. And UUO is followed by compensatory cellular proliferation in the contralateral kidney. We investigate the role of autophagy in the obstructed kidney and contralateral kidney after UUO.

Methods: To obtain the evidence and the patterns of autophagy during UUO, the rats were sacrificed 3, 7 and 14 days after UUO. To examine the efficacy of the autophagy inhibitors, 3-MA , the rats were treated with 3-MA. The rats were divided into the four groups: (1) the rats that underwent sham operation and were treated with vehicle, (2) the rats that underwent sham operation and were treated with 3-MA (3) the rats that underwent UUO and were treated with vehicle and (4) the rats that underwent UUO and were treated with 3-MA. The rat were sacrificed 7 days after UUO.

Results: After UUO, autophagy was induced in the obstructed kidney in a time-dependent manner. Autophagy is induced in the obstructed kidney early after UUO and before tubular cell apoptosis and tubulointerstitial fibrosis and then it decreased toward the basal levels at day 14 after UUO. Inhibition of autophagy by 3-MA enhance tubular cell apoptosis and tubulointerstitial fibrosis in the obstructed kidney. In the contralateral kidney, autophagy was also induced and prolonged during UUO. Inhibition of autophagy by 3-MA increased the protein expression of proliferating cell nuclear antigen significantly in the contralateral kidney. The Akt-mammalian target of rapamycin (m-TOR) signaling pathway was involved in the induction of autophagy after UUO in both kidneys.

Conclusions: Taken together, our present results support that autophagy induced by UUO has a renoprotective role in the obstructed kidney and regulatory role of compensatory cellular proliferation in the contralateral kidney through Akt-mTOR signaling pathway.

SA-PO2778

Epithelial-to-Mesenchymal Transition of Tubular Epithelial Cells Associates with Deciliation and Loss of Claudin-2 Expression Punita Dhawan, Amar B. Singh. *Vanderbilt University.*

Background: After sustained injury, tubular epithelial cells undergo epithelial-to-mesenchymal transition (EMT), which plays critical role in the evolution of renal fibrosis. Loss of functional tight junction (TJ) and polarity characterizes EMT. Tight Junction helps maintain polarity, which also appears necessary for ciliogenesis. Importantly, proteins that regulate polarity and/or ciliogenesis are localized at the TJ. However, role of TJ integral protein, claudins, in renal injury and associated EMT is poorly understood.

Methods: MDCK-II cells were subjected to chronic hypoxia (1% Oxygen). To induce EMT MDCK-II cells were also subjected to chronic EGF receptor (EGFR) activation. To silence claudin-2 expression anti-claudin-2 shRNA was used.

Results: Exposure of MDCK-II cells to chronic hypoxia led to the EMT. Cells lost cilia and epithelial morphology. E-cadherin expression decreased while Vimentin expression increased. Membrane distribution of $Na\text{-}K^+$-ATPase was lost while apical permeability

increased (P<0.001) and thus suggested loss of polarity. However, the most profound decrease was observed in claudin-2 expression, a TJ protein, which decreased to undetectable levels and this decrease preceded the decrease in E-cadherin expression. We observed a similar decrease in claudin-2 expression to undetectable levels when EMT was induced in MDCK-II cells by chronic EGF receptor (EGFR) activation, either using exogenous EGF (100 ng/ml) or stable expression of soluble HB-EGF, an EGFR ligand. Re-expression of claudin-2 in MDCK-II cells expressing soluble HB-EGF rescued the epithelial phenotype including cilia growth. In contrast, stable silencing of claudin-2 expression in MDCK-II cells resulted in sharp decreases in cilia length (P<0.001) and delayed tight junction formation following Ca^{++}-switch. Furthermore, stable expression of claudin-2 in MDCK-I cells, a clonal variant of MDCK-II cells deficient in claudin-2 expression and cilia formation, resulted in cilia formation in these cells.

Conclusions: Our findings support an important role for claudin-2 in the regulation of renal tubular injury and associated EMT through its role in the regulation of epithelial cell morphogenesis including polarity and cilia formation.

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SA-PO2779

Hypoxia-Induced Down-Regulation of MicroRNA-34a Promotes Epithelial-Mesenchymal Transition by Targeting Notch Signal in Tubular Epithelial Cells Rui Du, Shiren Sun. *Deparment of Nephrology, Xijing Hospital, FMMU, Xi'an, China.*

Background: Hypoxia-induced renal tubular cell Epithelial–mesenchymal transition (EMT) is an important reason for renal fibrosis. MicroRNAs (miRNA) are small non-coding RNA molecules which bind to their mRNA targets thereby leading to repression of translation. The role of miRNA in the hypoxia-induced EMT is largely unknown.

Methods: miRNA profiling was performed to identify differentially expressed miRNAs in HK2 cells under normal and low oxygen and the results were then verified by quantitative real time RT-PCR (qRT-PCR). Specific miRNA inhibitors and mimics transfection was performed to assess the function of miRNA in hypoxia-induced renal tubular cell EMT. Luciferase report gene assay and western blot was performed to validate the target genes of miR-34a. siRNA against Notch1 was designed to investigate the role of miR-34a-Notch pathway in hypoxia induced renal tubular cells EMT.

Results: miRNA-34a was identified as a down-regulated miRNA in hypoxic renal tubular epithelial cells. Inhibition of miR-34a expression in HK2 cells, which highly express endogenous miR-34a, promoted a mesenchymal phenotype accompanied by reduced expression of the epithelial marker E-cadherin and increased expression of the mesenchymal markers α-SMA and vimentin. Whereas, miR-34a mimic effectively reversed hypoxia-induced EMT. MiRNA-34a transfection in HK2 cells under hypoxia abolished hypoxia-induced expression of Notch1 and Jagged1 as well as Notch downstream signal, such as snail and slug. Western blot and luciferase report gene assay showed direct evidence for miR-34a targeting Notch1 and Jagged1. Notch1IC transfection could activate Notch signal and promote EMT phenotype, while siRNA against Notch1 effectively reversed hypoxia and miR-34a inhibitor-induced tubular epithelial cells EMT.

Conclusions: Our study provides evidence that hypoxia-induced decrease of miR-34a expression could promote renal tubular epithelial cells EMT by directly targeting Nothch1 and Jagged1, and subsequently activation of Notch downstream signal.

SA-PO2780

MicroRNA-324-3p Is a New Mediator of Kidney Fibrosis in Progressive Nephropathy and a Potential Target of ACE Inhibition-Induced Renoprotection Daniela Macconi,[1] Susanna Tomasoni,[1] Paola Romagnani,[3] Piera Trionfini,[1] Fabio Sangalli,[1] Benedetta Mazzinghi,[3] Paola Rizzo,[1] Giuseppe Remuzzi,[1,2] Ariela Benigni.[1] *[1]Mario Negri Institute, Bergamo, Italy; [2]Ospedali Riuniti, Bergamo, Italy; [3]University of Florence, Florence, Italy.*

Background: Munich Wistar Fromter (MWF) rats develop spontaneous proteinuria, glomerulosclerosis and tubulointerstitial fibrosis with age. So far efforts in finding candidate genes causing progressive renal damage have been inconclusive. Recent studies have suggested that post-transcriptional regulation of genes by microRNA (miRNA), may have a pathogenetic role in chronic kidney disease.

Methods: Untreated or lisinopril-treated MWF rats (40 to 60 weeks of age) were studied together with Wistar rats as controls. miRNA expression was assessed by microarray and real time RT-PCR in microdissected glomeruli and by in situ hybridization in the whole kidney. miRNA targets were predicted by in silico approach.

Results: Analysis of miRNA expression profiling showed miR-324-3p as the most up-regulated miRNA in glomeruli of MWF rats compared to Wistar. Expression of miR-324-3p was confined to podocytes and parietal cells of the Bowman's capsule in the glomerulus, but was also present at tubular level. Among predicted targets of miR-324-3p, we focused on prolyl endopeptidase (Prep) mRNA encoding a serine peptidase involved in the metabolism of angiotensins and in the synthesis of the anti-fibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP). In MWF rats up-regulation of miR-324-3p was associated with marked reduction of Prep in both glomeruli and the tubular epithelium, loss of Ac-SDKP expression and enhanced collagen deposition. ACE-inhibition down-regulated miR-324-3p in Bowman's capsule and in cortical tubules with a parallel increase in renal Prep expression. Lisinopril-treated MWF rats also showed increased plasma levels of Ac-SDKP and reduced renal fibrosis.

Conclusions: Dysregulation of post-transcriptional events linked to miR-324-3p/Prep pathway contributes to kidney fibrosis in progressive nephropathy and suggests miR-324-3p as a potential therapeutic target of ACE inhibition.

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SA-PO2781

MiR-30e Antagonizes TGFb1-Induced EMT by Targeting UCP2 in Kidney Tubular Epithelial Cells Lei Jiang, Chunsun Dai, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate target gene expression at the posttranscriptional level. The role and mechanisms of miRNAs in kidney tubular cell EMT and renal scar formation after injury remain unclear. Mitochondrial uncoupling protein 2 (UCP2) are members of mitochondrial anion carrier proteins (MACP). UCP2 separates ATP synthesis with energy dissipated as heat, also referred to as the mitochondrial proton leak. The role and mechanism of UCP2 in Epithelial-Mesenchymal Transition of tubular epithelial cells is also unknown.

Results: In this study, we found that miR-30e expression was declined and UCP2 protein expression was increased in unilateral ureter obstruction (UUO) kidneys. TGF-β1 could downregulate miR-30e and upregulate UCP2 expression in cultured NRK-52E cells. MiR-30e mimics largely abolished TGF-β1-induced UCP2 upregulation and tubular cell EMT, while miR-30e inhibits mimicked TGF-β1's effect. A mammalian expression vector in which the luciferase reporter cDNA linked to a 3'UTR segment of UCP2 was created. The miR-30e mimics significantly reduced TGF-β1-induced luciferase activity, which indicates that UCP2 is the direct target of miR-30e. We then explore the role of UCP2 in TGFb1-induced EMT and kidney fibrosis. UCP2 siRNA transfection could remarkably downregulate UCP2 expression and block TGF-β1-induced EMT in NRK-52E cells. In vivo, UCP2 knockout mice were more resistant to UUO-induced kidney fibrosis compared to its control littermates.

Conclusions: Taken together, it is concluded that miR-30e antagonizes TGFb1-induced EMT and fibrosis by targeting UCP2 in tubular epithelial cells.

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SA-PO2782

The Relation between Renal Parameters and Urinary microRNAs in Patients with Renal Diseases Tsuneo Konta, Kazuko Suzuki, Ami Ikeda, Yusuke Mashima, Emiko Nishidate, Kazunobu Ichikawa, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

Background: Renal microRNAs modulate the development and the progression of renal diseases by regulating various gene expressions in kidney. In this study we examined whether urinary microRNAs reflect renal changes in patients with renal diseases.

Methods: We extracted and quantified microRNAs in morning spot urine in 28 patients with renal diseases (16 IgA nephropathy, 6 crescentic glomerulonephritis and 6 diabetic nephropathy) and 5 healthy controls, and examined the relation between clinical and pathological parameters and urinary microRNAs including miR-133b, miR-192, miR-200 family and miR-30c. The localization of microRNAs expression was examined using in situ hybridization in renal biopsy specimens and urinary sediments.

Results: The urinary excretion of total microRNAs was significantly higher in the patients with renal diseases than controls (median 389 vs. 182 μg/gCr, P=0.014). Urinary protein excretion was related positively with urinary total microRNAs and miR-133b, and negatively with miR-192 and 200a, respectively. Glomerular proliferative changes were negatively related with urinary miR-192, miR-200 family and miR-30c. Glomerular sclerotic changes were positively related with urinary total microRNAs. There was no significant relation between GFR and interstitial fibrosis, and urinary microRNAs. In situ hybridization revealed that the expressions of microRNAs were detected mainly in glomerular cells, infiltrating cells and renal tubular epithelial cells in biopsy specimens and urinary sediments.

Conclusions: This study showed that urinary microRNAs were related with renal changes and might be utilized for the diagnosis and the evaluation of renal diseases.

Funding: Government Support - Non-U.S.

SA-PO2783

SOCS-3 Protein Expression Correlates with Kidney Function In Vivo and Differentially Regulates Proliferation and Differentiation Via STAT1 and -3 in Human Proximal Tubule Cell Hannes Neuwirt, Michael Rudnicki. *Dept. of Internal Medicine IV, Innsbruck Medical University, Innsbruck, Tyrol, Austria.*

Background: Proliferation and epithelial-mesenchymal-epithelial (EME) cycling are the underlying mechanisms of kidney repair and fibrosis, respectively. Suppressor of cytokine signaling (SOCS-)3 is a negative regulator of STAT1 and -3 signaling pathway for which both pro- and antifibrotic as well as pro- and antiproliferative effects have been shown depending on the cellular context.

Methods: We have analysed the expression of SOCS-3 in two kidney cell lines (HK-2, LLC-PK1) and in human renal biopsies, and we studied its effects on STAT1/3 activation and upon cytokine stimulation. For this purpose we established cellular models with SOCS-3 overexpression and SOCS-3 knock-down.

Results: In renal biopsies from patients with glomerular diseases SOCS-3 expression was inversely correlated with creatinine at time of biopsy and follow up. In vitro modulation of SOCS-3 levels was sufficient to alter basal activity of STAT1 and -3. SOCS-3 knock-down in LLC-PK1 cells resulted in pSTAT1/3 upregulation. This was associated with epithelial differentiation and inhibition of proliferation. On the other hand SOCS-3 overexpression in human HK-2 cells inhibited pSTAT1 but not pSTAT3, which was associated with induction of epithelial differentiation and inhibition of proliferation.

Conclusions: Our data suggest that SOCS-3 regulates STAT1/3 phosphorylation in renal tubule cells and that pSTAT3 stimulates epithelial differentiation and proliferation while pSTAT1 might have a dominant role in inhibition of these cellular functions in HK-2 cells. Downregulation of SOCS-3 and following activation of STAT1 signalling may inhibit proliferation and epithelial differentiation leading to progressive decline of renal function.

SA-PO2784

Tubular Deficiency of von Hippel-Lindau Attenuates Renal Disease Progression in the Anti-GBM Glomerulonephritis Franziska Theilig,[1] Anne Enke,[1] Brigitte Scolari,[1] Sebastian C. Bachmann,[2] Robert Koesters.[3] *[1]Medicine, Anatomy, Fribourg, Switzerland; [2]Charite, Anatomy, Berlin, Germany; [3]INSERM/University Pierre et Marie Curie, Paris, France.*

Background: In many kidney diseases, the original insult primarily involves the glomerulus and may then pass onto the tubulointerstitium. Several hypothesis link the glomerular disease to tubular injury, one of the foremost is the chronic tubular hypoxia. The effects of hypoxia and consecutive stabilization of hypoxia-inducible factors (HIF) however, are discussed controversially. Hypoxia was shown to induce interstitial fibrosis but also to have beneficial effects on renal disease progression when HIF was activated pharmacologically.

Methods: To analyze the impact of HIF´s on the tubulointerstitial disease development in a primary glomerular affection transgenic von Hippel Lindau (VHL)-knockout mice were generated and induced before the onset of an anti-glomerular basement membrane glomerulonephritis (GN).

Results: Tubular VHL-knockout and therefore local HIF-a stabilization increased renal production of VEGF, TGF-b_1 and PDGF-B resulting in augmented formation of capillaries, interstitial matrix and conversion of fibroblasts into myofibroblasts. Within the glomerular disease VHL-knockout reduced the glomerular damage and attenuated tubulointerstitial injury albeit similar characteristics. Likewise, proteinuria, plasma urea concentration and tubulointerstitial matrix were decreased in VHL-knockout with GN.

Conclusions: These findings demonstrate that tubular HIF-a stabilisation in a glomerular disease is beneficial for the disease outcome. In comparison to VHL-knockout alone GN is a much stronger activator of fibrosis so that other stimuli than hypoxia may be considered for renal disease progression.

Funding: Government Support - Non-U.S.

SA-PO2785

Aspirin Inhibits the Development of Vascular Calcification through Induction of Heat Shock Protein 72 in Human Aortic Smooth Muscle Cells Tzong-Shi Lu,[1] Kenneth Lim,[1,2] Guerman Molostvov,[2] Daniel Zehnder,[2] Li-Li Hsiao.[1] *[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom.*

Background: Vascular calcification (VC) is a significant contributor to cardiovascular mortality in patients with chronic kidney disease (CKD) and coronary artery disease (CAD). Acetylsalicylic acid (Aspirin, ASA) is a widely used medication in artery intima disease. However, the role of ASA in the development of VC is unknown. There is emerging evidence that ASA could function as an inducer of the cardio-protective factor, heat shock protein 72 (HSP72). It is possible that ASA may also exert cardio-protective effects through HSP72.

Methods: Human vascular smooth muscle cells were treated with control or calcification medium containing 5mM calcium chloride and 5mM β-glycerolphosphate with/without ASA (4mM) treatment for 21 days.

Results: We have shown for the first time that ASA can inhibit the development of VC through the induction of HSP72 in our long-term VC model, *in vitro*. Furthermore, heat shock treatment (HST), an established inducer of HSP72 significantly inhibited the development of VC. Anti-calcific effects of both ASA and HST were abolished by HSP72 SiRNA. We also found marked reduction of HSP72 expression in arteries from patients with CKD and CAD, compared to healthy controls, *in vivo*. This confirms the role of HSP72 as an endogenous calcification inhibitor. We next showed that ASA, through a HSP72-dependent pathway, suppressed major promoters (Cbfa1, Osteocalcin, and Alkaline Phosphatase) of VC and also stabilized the contractile smooth muscle cell phenotype through maintaining myocardin and serum response factor in our long-term VC model. Organ culture of arteries from CKD and CAD patients, confirmed that despite initially reduced expression, retained their ability to induce HSP72 by HST.

Conclusions: In conclusion, our study shows that ASA is a powerful inhibitor of VC and its anti-calcific effects function through a HSP72-dependent pathway. We suggest treatment strategies involving ASA or other inducers of HSP72 as a new approach to inhibit localized or diffuse VC.

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SA-PO2786

AMPK Activity as a Biomarker of CKD Status and Its Association with Metformin's Effects on CKD Aihua Deng,[1,2] Joseph Satriano,[1,2] Roland C. Blantz.[1,2] *[1]Medicine, University of California at San Diego, San Diego, CA; [2]Nephrology/Hypertension, VA San Diego Healthcare System, San Diego, CA.*

Background: Our previous studies have shown that both Angiotensin II blockade (AIIB) and hypoxia inducible factor induction with DMOG can significantly correct renal metabolic deficiency and improve renal function in rat renal ablation&infarction (A/I) model of chronic kidney disease(CKD)[Deng & Blantz, AJP:Renal,2010]. In this study , we investigated the regulation of AMPK activity in the CKD model as well as the therapeutic effects of metformin, an activator of AMPK.

Methods: The rat A/I model of CKD was produced by removing the right kidney and ligating two branches of the left renal artery. AMPK activity (p-AMPK-α) was tested by Western blot. GFR, RBF, renal oxygen consumption (QO2) and sodium transport(TNa) were measured. An increase in QO2/TNa indicates renal metabolic deficiency.

Results: Untreated A/I rats showed reductions of GFR, RBF and renal metabolic efficiency, accompanied with a major decrease in AMPK activity, i.e. a reduction of p-AMPK-α(P<0.01). AIIB and DMOG both improved renal fuction, corrected renal metabolic deficiency and normalized AMPK activity (P<0.01). Metformin also normalized AMPK activity (P<0.01), while significantly correcting renal metabolic deficiency and improving kidney function.

The effects of 3 different treatments on renal function, renal metabolic efficiency in A/I rats

	GFR ml/min	RBF ml/min	QO2 ml/min	QO2/TNa ml/mmole
Normal n=6	1.05 ±0.11	6.72±0.47	0.15±0.01	1.08±0.11
A/I n=7	0.54±0.05*	3.39±0.44*	0.12±0.01	1.70±0.10*
A/I + AIIB n=8	0.84±0.04†	6.64±0.23†	0.11±0.01	0.98±0.07†
A/I + DMOG n=7	1.48±0.19†	7.2±0.55†	0.20±0.03†	1.03±0.12†
A/I + Metformin n=8	1.10±0.06†	6.26±0.24†	0.17±0.01	1.13±0.10†

* P<0.05 vs normal; †P<0.05 vs A/I

Conclusions: Untreated A/I kidney demonstrated reduced AMPK activity , associated with reductions in renal function and renal metabolic efficiency. The three agents (AIIB, DMOG and Metformin) produced similar beneficial effects associated with normalization of AMPK activity, suggesting that renal AMPK activity is an indicator of CKD status, regardless of their initial molecular pathways. Metformin may be an additional effective agent for CKD treatment.

Funding: NIDDK Support

SA-PO2787

NLRP3 Functions in Renal Tubular Epithelial-Mesenchymal Transition Independent of Cytokine Production Wenjie Wang,[1] Aylin Sar,[2] Akosua Vilaysane,[1] Sharon Ann Clark,[1] Kiril Trpkov,[2] Daniel A. Muruve.[1] *[1]Medicine, University of Calgary, Calgary, AB, Canada; [2]Pathology, University of Calgary, Calgary, AB, Canada.*

Background: Interstitial inflammation and fibrosis are strongly associated with the outcome of chronic kidney disease. We recently demonstrated that the NOD-like receptor containing a pyrin domain 3 (NLRP3) not only mediates renal inflammation but also potentially plays a direct role in fibrogenesis. We investigated whether NLRP3 plays a role in tubular epithelial-mesenchymal transition (EMT).

Methods: Human proximal and murine tubular epithelial cell were cultured in the presence or absence of transforming growth factor-β (TGF-β, 10ng/ml). Tubular EMT was determined by the expression of α-smooth muscle actin and matrix metalloproteinase. NLRP3 and MMP gene transcription and expression were assessed by semi-quantitative PCR or western blotting. NLRP3, apoptosis-associated speck-like protein containing a CARD domain (ASC), Caspase-1 and MyD88 knockout tubular epithelial cell was studied to determine the contribution of inflammasome formation and proinflammatory cytokine on MMP expression. In vivo progressive fibrosis model of unilateral ureteic obstruction was carried out in NLRP3$^{-/-}$ or littermate mice. After 14 days, renal MMP activity and histological change were analyzed.

Results: TGF-β induced tubular EMT and concomitant NLRP3 expression in a time-dependent fashion as evident by de novo expression of α-smooth muscle actin and upregulation of both MMP-2 and 9. The induction of MMP-9 was decreased in both NLRP3 and ASC knockout tubular epithelial cell, suggesting an essential role for NLRP3 in tubular EMT. The effect of NLRP3 and ASC on MMP-9 expression was inflammasome-independent as neither MyD88 or Caspase-1 knockout, nor adding IL-18 to NLRP3$^{-/-}$ tubular epithelial cell affected MMP-9 expression. Finally, NLRP3 knockout mice demonstrated decreased MMP-9 expression and developed less fibrosis compared to wild type controls after unilateral ureteric obstruction.

Conclusions: These data suggest a role for epithelial NLRP3 in promoting renal fibrosis independent of the inflammasome formation and also independent of subsequent pro-inflammatory cytokine production.

SA-PO2788

CKD Changes the Composition of Exhaled Gasses and Gaseous Products of the Gut Microbiome Simone Meinardi,[2] Kyu-Bok Jin,[1] Donald R. Blake,[2] Nosratola D. Vaziri.[2] *[1]Medicine, Inje Univ Haeundae Paik Hospital; [2]Medicine/Chemistry, UC Irvine, Irvine, CA; [3].*

Background: Few gases are produced by cellular metabolism and many by intestinal flora. While much is known about the effect of CKD on composition of body fluids little is known on its impact on the gases in exhaled breath or those produced by the gut microbiome. We showed that uremia significantly changes the structure-function of the gut microbiome. We hypothesized that uremia-induced changes in cellular metabolism and intestinal microbiome may modify gaseous metabolites found in the exhaled breath and generated by the intestinal flora.

Methods: SD rats were randomized to the CKD (5/6 nephrectomy) or control (sham operation) groups and observed for 6 wks. Exhaled breath was collected in evacuated stainless steel canisters at wks 0, 1, 2, 4, and 6. The animal was enclosed in a 10 L glass chamber flushed with clean air for 1 hr, the chamber was then sealed with animal inside for 45 min and 2 L of air were collected. At wk 6 feces were collected, dissolved in milliQ water, incubated at 37º C in glass reactor for 24 hr and the headspace air collected. The gases collected from exhaled breath and cultured feces were analyzed by a gaschromatographic system equipped with 6 different column/detector combinations.

Results: More than 50 gases were detected in the animals' exhaled breath and cultured feces ranging from pptv to ppmv of which 7 gases in exhaled breath and 8 collected from the cultured feces were significantly different between the 2 groups at week 6. Longitudinal studies revealed an early rise in isoprene and a late fall in concentration of linear aldehydes in the exhaled breath in the CKD rats. Numerous sulfur and oxygen containing gases were identified in both exhaled breath and cultured feces. Compared to controls cultured feces from the CKD animals released larger amounts of dimethyldisulfide and dimethyltrisulfide, and three thioesters which were absent in the controls.

Conclusions: CKD significantly changes the composition of exhaled breath and gaseous products of intestinal flora. Further studies are needed to determine the utility of these gases as biomarkers of CKD and its complications.

SA-PO2789

CKD Results in Depletion of H_2S, Accumulation of Homocysteine, and Downregulation of the Related Enzymes in the Kidney Mohammad A. Aminzadeh, Nosratola D. Vaziri. *Medicine/Nephrology, University of California, Irvine, CA.*

Background: Oxidative stress and inflammation are constant features and major mediators of progression of chronic kidney disease (CKD) and its cardiovascular complications. Hydrogen sulfide (H2S) is an endogenous signaling gas which has potent anti-hypertensive, anti-oxidant, and anti-inflammatory properties. In contrast, homocysteine has pro-oxidant and pro-inflammatory effects. H2S is produced by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulphurtransferase (MST) while homocysteine is removed by CBS and methylenetetrahydrofolate reductase (MTHFR). Plasma H2S is reduced in humans with hypertension, atherosclerosis, and end-stage renal disease and tissue H2S is diminished in experimental animals with atherosclerosis, hypertension, and acute kidney injury. The effect of CKD on intra-renal metabolism of H2S and homocysteine is unknown and was studied here.

Methods: SD rats were subjected to 5/6 nephrectomy or sham operation and observed for 6-12 weeks. Kidney tissue H2S-producting capacity, homocysteine content, and CBS, CSE, MST and MTHFR expressions were determined.

Results: The CKD group exhibited significant reduction in plasma H2S concentration (33.30±2.40 vs44.65±1.56, P<0.001) and oxidative stress in the remnant kidney. This was associated with marked elevation of homocysteine (0.19±0.02 vs 0.05±0.02 P<0.001), significant reductions in CBS (46%, P<0.006), CSE (76%, P<0.004), MST (60%, P<0.021 and MTHFR (86%, P<0.001) expressions and H2S-production capacity (28%, P<0.001) in the remnant kidney.

Conclusions: Progression of renal disease and oxidative stress in CKD are associated with depletion of H2S and accumulation of homocysteine in the remnant kidney which are due to down-regulation of H2S-producing and homocysteine-metabolizing enzymes. Given the potent anti-oxidant, anti-inflammatory and cytoprotective properties of H2S and pro-oxidant and pro-inflammatory effects of homocysteine, the observed abnormalities can contribute to progression of CKD.

SA-PO2790

Dabigatran, a Direct Thrombin Inhibitor, Demonstrates Antifibrotic Effects on Kidney Fibroblasts James W. Tanner, Danielle M. Fowler, Jorge L. Villalona, Glenn A. Reinhart, Joanne Van Ryn, Scott MacDonnell. *Cardiometabolic Diseases Research, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT.*

Background: Involvement of the coagulation cascade, via thrombin mediated activation of the PAR-1 receptor, has been shown to play a role in the activation and development of lung fibrosis, but is undocumented in the context of renal fibrosis. Objectives include, investigating the profibrotic activity of thrombin in renal fibroblasts by assessing myofibroblast formation and extracellular matrix (ECM) deposition and determine whether the direct thrombin inhibitor, dabigatran, interferes with these signaling events.

Methods: Rat kidney fibroblasts (NRK49F) were treated with thrombin at 0.5U/ml-2.5U/ml for 24, 48, and 72 hours. Functional parameters examined included kinetics (cell proliferation), differentiation (αSMA expression), and ECM deposition (collagen I and fibronectin expression). RT-PCR and immunohistochemistry was used to characterize expression of profibrotic genes and PAR-1 in both isolated fibrobalsts and rat kidney tissue.

Results: In fibroblasts, thrombin induced both αSMA and fibronectin expression, stimulated collagen synthesis (hydroxyproline and immunostaining) in a time and dose dependent manner (P<0.05), and had no significant proliferative effects. Thrombin induced collagen deposition was comparable to TGF-b (5ng/ml) stimulation at 72 hours despite a significantly lower expression of fibrotic genes. Dabigatran, at concentrations of 10 and 1000ng/ml inhibited thrombin induced myofibroblast activation and ECM accumulation in a dose dependent manner (P<0.05). The inhibitory effects of dabigatran were not observed when fibroblasts were stimulated with TGF-b (5ng/ml) while ALK5 inhibition failed to prevent thrombin induced collagen production. In a model of rat obstructive nephropathy (UUO), PAR-1 mRNA expression was increased 4-fold, in kidney tissue.

Conclusions: Dabigatran inhibited thrombin induced activation and ECM deposition in kidney fibroblasts. These effects were independent of TGF-b signaling. Thus direct thrombin inhibition with dabigatran may warrant study as an antifibrotic drug for the treatment of renal fibrosis.

Funding: Pharmaceutical Company Support

SA-PO2791

Effects of the Receptor Tyrosine Kinases Inhibitor BIBF1000 on Renal Fibrosis in Rodents Steven M. Weldon,[1] Haichun Yang,[2] Yiqin Zuo,[2] Cheng-Kon Shih,[1] Susan Goldrick,[1] Hong Wang,[1] Xiaoyu Jiang,[1] Glenn A. Reinhart,[1] Agnes B. Fogo.[2] *[1]Cardiometabolic Disease, Boehringer Ingelheim Pharm. Inc., Ridgefield, CT; [2]Division of Renal Pathology, Vanderbilt University Medical Center, Nashville, TN.*

Background: Tubular interstitial fibrosis (TIF) is a major factor contributing to the progression of chronic kidney disease (CKD) and subsequent renal failure. Presently only ACE inhibitors and ARBs are approved CKD therapy. We used unilateral ureteral obstruction (UUO) in rodents to study mechanisms and potential modulation of TIF. Receptor tyrosine kinase inhibitors (RTKi) such as Gleevec (imatinib) exhibit anti-fibrotic activity in animal models including UUO. BIBF1000 (BIBF) is a novel RTKi that attenuates PDGF,VEGF and FGF signaling and fibrosis in the rat bleomycin lung fibrosis model but has not been evaluated in renal fibrosis. Therefore, we evaluated BIBF in the UUO model of renal fibrosis.

Methods: SD rats and C57BLKS mice were treated 1 day prior to ureter ligation with vehicle (VEH), enalapril (ENA, 30 mg/kg), Gleevec (GLE, 100 mg/kg) or BIBF (25 or 50 mg/kg) by gavage (5 days). TIF was assessed by sirius red morphometry (SRM) and a panel of fibrotic and inflammatory biomarkers (BMs) were evaluated by immunostaining (IHC) or qPCR (mRNA).

Results: In mice, BIBF attenuated TIF by 18.8 and 19.1% (*p<0.05) for the 25 and 50 mg/kg doses, respectively. By comparison, GLE exhibited 17%* inhibition while ENA showed only 7% inhibition. In rats, BIBF inhibited TIF in a dose-depend manner, 16% and 25%*, respectively. GLE reduced TIF by 21%* and ENA by 13%. BIBF, GLE and ENA showed differential effects on fibrotic and inflammatory BMs in mice and rats. Finally, plasma levels of GLE were nearly identical in both mice and rats; ENA exhibited nearly 3-fold higher levels in rats while BIBF showed ~ 10-fold higher exposure in mice.

Conclusions: In conclusion, these are the first data to show a novel RTKi such as BIBF1000 attenuates renal tubulointerstitial fibrosis assessed quantitatively using SR morphometry. As such, RTK's and downstream signaling pathways may offer a novel alternative for targeting fibrotic renal disease associated with CKD and subsequent renal failure.

Funding: Pharmaceutical Company Support

SA-PO2792

Peritoneal Dialysate-Induced Down-Regulation of E-cadherin Is Mediated by Oxidative Stress Via an Activation of Mitochondrial Respiration, Independent of NAPDH Oxidase Duk-Hee Kang, Eun Sun Ryu, Mina Yu, Yang-Hee Jang. *Division of Nephrology, Ewha Womans University School of Medicine, Seoul, Korea.*

Background: Chronic exposure of the peritoneum to unphysiologic dialysate leads to peritoneal membrane dysfunction and fibrosis. Recent studies indicate phenotypic transition of peritoneal mesothelial cells as an early mechanism of peritoneal fibrosis. Especially, E-cadherin, which was formerly viewed exclusively as a structural protein mediating cell-cell adhesion, is now regarded as a key molecule to regulate the epithelial phenotype. Glucose-degradation product in conventional dialysate has been blamed for peritoneal dialysis-induced changes in peritoneum, and a use of low-GDP solution leads to rapid remesothelialization and less EMT.

Methods: In order to investigate the mechanism of peritoneal fibrosis after a prolonged use of high GDP dialysate, we examined an alteration in E-cadherin mRNA and protein expression in primarily isolated peritoneal mesothelial cells exposed to conventional high GDP or low GDP solution. We also investigated the effect of anti-oxidants on E-cadherin expression of MCs.

Results: Conventional dialysate (Perisis^{R}, Boryung Pharm, Korea) induced a down-regulation of E-cadherin with an enhanced generation of ROS assessed by DCF-DA staining. Interestingly, the exposure of MCs to low-GDP dialysate (PeriPlus^{R}, Boryung Pharm, Korea) resulted in a less decrease in E-cadherin expression both in mRNA and protein levels and less increase in ROS production compared to conventional dialysate. *N*-acetylcysteine or mitoQ, a novel mitochondria-specific antioxidant inhibited conventional dialysate-induced

down-regulation of E-cadherin, however apocynin, an inhibitor of NAPDH oxidase, showed no significant effect on E-cadherin expression. Treatment of MCs with H_2O_2 or glucose oxidase per se induced phenotypic transition with a decrease in E-cadherin expression.

Conclusions: Taken together, oxidative stress induced by GDP seems to be one of the mechanisms of peritoneal damage in PD patients, and novel mitochondrion-targeted antioxidant can be a therapeutic option for preserving mesothelial phenotype.

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SA-PO2793

Renal Mass Reduction (RMR) Impairs Recovery from Acute Kidney Injury (AKI) and Promotes the Mechanisms of Chronic Kidney Disease (CKD) Progression Aaron Polichnowski,[1] Rongpei Lan,[2] Hui Geng,[2] Karen A. Griffin,[1] Anil K. Bidani,[1] Manjeri A. Venkatachalam.[2] *[1]Loyola U Med Ctr; [2]U TX.*

Background: Clinical studies suggest that AKI in CKD settings increases the risk for progression to end stage. Limited experiments testing this concept have been conflicting.

Methods: Sprague-Dawley rats were prepared for blood pressure radiotelemetry (BP) and normotensive RMR by uninephrectomy (UNx), UNx + excision of 2 poles from other kidney (3⁄4 Nx), or no Nx. Two wks later, AKI was induced by 40' ischemia/reperfusion (I/R) and rats followed for 4 wks post I/R.

Results: SCr and BP in Pre I/R 3⁄4 Nx rats were modestly increased. Without I/R, this effect of RMR was stable over 4 wks (not shown). Judged by SCr at 48h post I/R, AKI severity was similar in all groups. But, recovery was impaired in 3⁄4 Nx relative to other groups (SCr 7d).

Pre I/R				Post I/R (1st wk)		Post I/R (4th wk)	
Group	SCr mg/dl	BP mmHg	Uprot mg/24h	SCr 48h	SCr 7d	BP	Uprot mg/24h
No Nx (n=10)	0.44±0.03*	114±1.9	4.6±0.4	4.6±0.4	0.8±0.07	119±1.8	6.1±0.7
UNx (n=12)	0.60±0.04*	109±2.0	4.5±0.3	4.3±0.4	0.9±0.05	120±3.8	23.1±12.4
3/4 Nx (n=12)	0.85±0.04*	130±3.4*	8.6±1.2	4.5±0.3	1.8±0.2*	152±8.2*	57.6±14.5*

mean±SE; * $p < 0.05$ vs all other groups. BP and Uprot data 1st wk post I/R not shown

SCr remained significantly higher than pre I/R values over 4 wks in 3⁄4 Nx rats; this was mirrored by more extensive tubule atrophy and fibrosis 4 wks post I/R compared to other groups. BP was similar before I/R and 1 wk post I/R in 3/4 Nx rats, but showed large increases by 4 wks. This was accompanied by markedly increased Uprot. Overall, the severity of tubulo-interstitial fibrosis correlated better with BP and Uprot than with SCr. Data in UNx rats showed similar trends but exhibited much greater variability.

Conclusions: Repair is less complete and fibrosis more extensive after ischemic AKI in the setting of severe RMR. By reducing functional renal mass further, such tubulo-interstitial fibrosis after AKI accelerates the development of hypertension and augments proteinuria, setting the stage for CKD progression. Our data suggest that RMR models are useful to investigate the mechanisms responsible for incomplete repair, regeneration and fibrosis in CKD progression after AKI.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2794

The Role of Erk Activation in the Subtotal Nephrectomy Model of Kidney Fibrosis Faith Hannah Nutter, John Haylor, Arif Khwaja. *Academic Unit of Nephrology, University of Sheffield, Shefffield, United Kingdom.*

Background: The Ras-Mek-Erk signalling pathyway plays a key role in many cellular pathways underpinning kidney fibrosis including cell proliferation, apoptosis, epithelial to mesenchymal transition and accumulation of extracellular cellular matrix. Phosph-Erk (p-Erk) is upregulated in fibrotic kidney disease and in vitro data suggests Erk activation amplifies TGF-β mediated pro-fibrotic effects. We hypothesised that Erk is a therapeutic target in fibrotic kidney disease and the MEK inhibitor CI-1040 would attenuate renal fibrosis in the subtotal nephrectomy (SNx) model.

Methods: Male Wistar rats were subjected to either 5/6 SNx or sham operation under isofluorane anaesthesia. SNx rats received intraperitoneal injections of either CI-1040 (100mg/kg/day) or vehicle alone from day 1. Animals were sacrificed 130 days later. Renal function was assessed by serum creatinine, creatinine clearance and urinary protein excretion. Fibrosis and myofibroblast number were assessed by Masson's Trichrome and α-SMA staining. Immunohistochemistry and western blotting for p-Erk was performed on lymphocyte and kidney homogenates. Cell proliferation was assessed by BrdU uptake.

Results: A 5-fold upregulation of p-Erk levels ($p<0.0001$) was observed in renal tissue and lymphocytes throughout the duration of the model with CI-1040 almost completely inhibiting p-Erk activity in the kidney (4.78 ± 0.8 vs. 0.14 ± 0.1 OD/mm2, $p<0.0001$) and in lymphocytes. Despite inhibition of p-Erk, CI-1040 had no effect on either the development of renal fibrosis (17.3 ± 3.6 vs. $24.6 \pm 4.6\%$) or the increase in α-SMA positive cells (17.1 ± 3.3 vs. $20.8 \pm 4.5\%$). Furthermore CI-1040 had no impact on creatinine clearance, proteinuria or on cell proliferation.

Conclusions: Erk activity is significantly upregulated following SNx yet complete inhibition of Erk activation by CI-1040 had no impact on kidney function, fibrosis or myofibroblast number. This suggests that kidney fibrosis in SNx is not dependent on Erk activation. It is possible that upregulation of other mitogen activated protein kinases (MAPKs) pathway may compensate for the loss of Erk activation and future work will examine activity of JNK and p38 MAPKs.

SA-PO2795

Renal Growth Hormone Receptor Signaling Is Impaired in a Rat Model of Chronic Kidney Disease Daniel Landau,[1] Debbie Wiezel,[2] Ariel Troib,[2] Ralph Rabkin,[3] Yael Segev.[2] *[1]Pediatrics, Ben Gurion University, Beer Sheva, Israel; [2]Shraga Segal Dept. of Microbiology and Immunology, Ben Gurion University, Beer Sheva, Israel; [3]Medicine, Veterans Administration Hospital and Stanford University, Palo Alto, CA.*

Background: Linear growth retardation with reduced muscle mass is a major problem in children and adults with chronic kidney disease (CKD) and is ascribed to insensitivity to GH. Treatment with exogenous GH has been accepted as standard therapy in children with CKD and short stature. However, concerns have been raised in the past on the potential fibrogenic effects of GH on the kidney since transgenic mice over-expressing bGH develop glomerulosclerosis. We have previously described the renal GH-IGF –I signaling in a whole animal model of diabetic kidney disease. No data is available on this renal pathway in CKD.

Methods: To investigate the renal GHR signaling pathway of growth retarded young CKD rats, 5/6 nephrectomized (CKD) and pair-fed control (C) 20 day old rats were sacrificed after 2 weeks of CKD. Bovine GH (IV 100μg/kg) was given 15 min prior to sacrifice.

Results: There was no difference in the amount of food consumed, yet there was a significant difference in weight gain and longitudinal growth between C and CKD. Serum creatinine increased to 225±11% of C and albumin excretion was significantly higher in CKD. Glomerular volume was higher in CKD vs. C (252±12.5 % of C, $p< 0.05$). No change was observed in serum GH, yet serum IGF-I was decreased significantly in CKD vs. C (60.4±5 % of C, $p < 0.05$). Kidney and liver IGF-I mRNA levels were unchanged.

A significant decrease was seen in kidney GHR mRNA and protein, as well as phosphorylated (p-)JAK2 and p-STAT5 in CKD vs. C. An increase in SOCS3 mRNA was seen in CKD vs. C.

Conclusions: Young rats with moderate CKD develop significant growth retardation. The decrease in GHR expression as well as the subsequent proteins of its signaling pathway in the kidney in association with the increase in the GHR inhibitor SOCS3 shows a GHR signaling impairment in the kidneys of CKD rats. This may explain the relative insensitivity of kidney tissue to the potential adverse effects of exogenous GH in CKD patients.

Funding: Government Support - Non-U.S.

SA-PO2796

Renal Failure, Endothelial Dysfunction, Hypotension and Anemia in Mice with Homozygous Deletion of the Gene Encoding the Canonical Transient Receptor Potential 1 (TRPC1) Channel: Role of Hypoplasia Kai Lau,[1] Pedro Lozano,[1] Qilong Wang,[1] Bonnie Eby,[1] Becky Pennington,[1] Richard Matthew Atkins,[1] Meghan Pantalia,[1] Joel Abramowitz,[2] Lutz Birnbaumer,[2] Leonidas Tsiokas.[3] *[1]Medicine, University of Oklahoma, Oklahoma City, OK; [2]Intramural Research, NIEHS, Research Triangle Park, NC; [3]Cell Biology, University of Oklahoma, Oklahoma City, OK.*

Background: TRPC is a superfamily of cation channels implicated in many cell functions. A key role for TRPC1 was found in cardiac hypertrophic signaling, VSM proliferation & mesangial cell contraction. Its normal physiologic functions are unknown.

Methods: Male TRPC1 +/+ & -/- mice were studied by cardiac & renal ultrasound, clearance (C) of creatinine (cre) by HPLC & inulin, tailcuffs/intra-arterial blood pressure (BP) readings & chamber studies of aorta relaxation.

Results: At 2 mon, null mice had smaller heart (0.45 vs. 0.52% of body weight [BW]) & kidney (1.4 vs. 1.5%). At 7 mon, they had reduced left ventricle end-diastolic & end-systolic diameters & volumes. Stroke index (SI) & cardiac output (CO) (14 vs. 21 ml/min) were reduced. Systolic (in torr) (113 vs. 121), diastolic (77 vs. 86) & mean arterial (MA) BP (89 vs. 98) were lower. Arterial resistance [MABP/CO] was high. Arterial stiffness was elevated 2 fold & compliance down 50%. Heart was 20% smaller at 22 mon. Aortic relaxation were normal at 2 mon but reduced at 22 mon. Hematocrit was reduced by ~20% from 10-22 mon. At 7 mon, kidney volume (KV) by echo & KW:BW were reduced. From 11-20 mon, KV stayed lower by 18% & KW:BW ratio down by 28%. Renal echogenicity was similar at 7 & 11 mon but elevated by 37 % at 20 mon, consistent with scarring. Normal at 12 mon, serum cre (0.1 vs. 0.06 mg%) was elevated at 17 mon. Ccre (ml/min) was down by ~50% (0.7 vs. 1.1/mouse; 1.7 vs. 3.4/100g BW). Renal failure was confirmed by ~46% reduced Cinulin (μl/min) (214 vs. 384/mouse; 603 vs. 1,155/100g BW; 362 vs. 681/g KW).

Conclusions: 1. TRPC1 deficiency retards renal & heart growth, produces hypoplastic nephropathy, reduces CO & BP, increases arterial resistance & stiffness & causes endothelial dysfunctions & anemia. 2. Our data indicate its key roles in normal development.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support, Clinical Revenue Support

SA-PO2797

DNA Methyltransferase 1, 3a, 3b and 3L Are Transiently Upregulated during the Development of Renal Fibrosis Benjamin Arthur Vervaet, Anne Soontjens, Anja Verhulst, Patrick C. D'Haese, Annelies De Beuf. *University of Antwerp.*

Background: The cell biological process underlying all chronic renal disorders is fibrosis. Crucial in this process is the activation of fibroblasts, resulting in their proliferation and excessive production of extracellular matrix. Recently, it has been shown that DNA methylation, performed by DNA methyltransferases (Dnmt's), plays an important role

in the perpetuation of this fibroblast activation. Little is known, however, on (i) which Dnmt's are involved and (ii) what their temporal expression is in the kidney during the development of fibrosis. Hereto, the present study aimed at a time course evaluation of the expression of the most prominent Dnmt's (Dnmt1, 3a and 3b) and the similar, but non-functional, Dnmt3L in two different mice models of fibrosis: aristolochic acid nephropathy and ischemia reperfusion (I/R) injury.

Methods: Mice (20-25g) received a single intraperitoneal injection of aristolochic acid I (AAI, 3.5 mg/kg) or PBS or underwent unilateral left ischemia during 30 minutes. Mice were sacrificed after 1, 3, 6 and 12 weeks. The expression level of Dnmt1, 3a, 3b and 3L was assessed by quantitative real-time PCR on total renal tissue mRNA.

Results: All Dnmt's were significantly upregulated during the development of renal fibrosis in both animal models from week 1 on, reaching maximum expression levels 3 weeks after AAI injection and 6 weeks after reperfusion. At week 12, when fibrosis was most prominent, the expression of the Dnmt's decreased towards control levels in both models.

Conclusions: Dnmt 1, 3a, 3b and 3L are all upregulated shortly, but not permanently, after AAI injection or I/R injury, supporting a transient role for these enzymes in the development of renal fibrosis following both an acute toxic and ischemic insult. Interestingly, these findings contrast the recently reported observation that only Dnmt1 (but not Dnmt3a and Dnmt3b) was upregulated in a folic acid induced mouse model of renal fibrosis (Bechtel et al. Nat. Med. 2010;16(5):544). Our data, therefore, indicate that the involvement of Dnmt's might depend on the stage of renal histopathology and/or the type of insult.

Funding: Private Foundation Support

SA-PO2798

Indoxyl Sulfate Induces Cbp/p300-Interacting Transactivator, with Glu/Asp-Rich Carboxy-Terminal Domain, 2, and Impairs Hypoxia Response in Proximal Tubular Cells Tetsuhiro Tanaka,[1] Toshiro Fujita,[2] Masaomi Nangaku.[2] *[1]Division for Health Service Promotion, University of Tokyo, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Japan.*

Background: Hypoxia in the tubulointerstitium is common in progressive renal diseases. Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor composed of α- and β- subunits and controls the expression of genes involved in glycolysis, angiogenesis and erythropoiesis in hypoxia. Results from recent studies suggest that HIF, while obviously expressed, may be insufficiently activated in experimental chronic kidney disease (CKD) models.

Methods: In this study, we investigated the effect of indoxyl sulfate (IS), a uremic toxin which accumulates in CKD patients and is responsible for renal pathology, on cellular response to hypoxia in cultured proximal tubular cells. Hypoxia response by HIF-1 was measured by luciferase reporter assay. Quantitative changes in HIF-1α, Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 (CITED2) and HIF-target genes were analyzed by real-time PCR and Western blotting. HIF-1 binding to the enhancer region was evaluated by chromatin immunoprecipitation (ChIP) assays. Protein interaction between HIF-1α and its coactivators was estimated by mammalian two-hybrid assays.

Results: Transient transfection with the hypoxia-responsive luciferase reporter (HREluc) revealed that IS impaired hypoxia response in HK-2 cells, which was associated with a decrease in the expression of HIF-1 target genes. Immunoblotting using whole cell lysates demonstrated no significant changes in HIF-1α expression, and ChIP assays revealed no effect of IS on HIF-1 binding to the promoter region. However, IS induced the expression of CITED2 via post-transcriptional mechanisms and impaired activity of the HIF-1α C-terminal transactivator domain (CTAD) by blocking the recruitment of its coactivator p300.

Conclusions: Results of the present study uncover a novel mechanism through which IS dampens hypoxia response mediated by HIF-1, and may provide an insight into clinical problems such as insufficient erythropoietin (EPO) production in uremic patients.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2799

Role of HSP47 in Epithelial-Mesenchymal Transition of Renal Tubular Epithelial Cell and Extracellular Matrix Accumulation Huixin Bi,[1] Ruihong Liu,[1] Lin Sun,[1,2] You-Ming Peng,[1] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]Departments of Pathology and Medicine, Northwestern University, Chicago, IL.*

Background: Epithelial-mesenchymal transition (EMT) and extracellular matrix (ECM) accumulation play an important role in renal tubulointerstitial fibrosis. Previous studies have showed the close relationship between the increased expression of HSP47 and the progression of tubulointerstitial fibrosis. However whether HSP47 plays a role in the process of EMT and ECM accumulation is unclear.

Methods: HSP47 antisense oligonucleotides(ODNs) was introduced in the kidney with an ultrasound microbubbles system by injecting it into a renal artery at the day of inducing UUO, sense ODNs served as a control. Rats were sacrificed at day 14 after UUO or sham operation. The expressions of HSP47, vimentin, ZO-1 and ColI were detected by immunohistochemical, Western blot and Real-time PCR, respectively. In vitro studies, HK-2 cells were exposed to 10ng/ml TGF-β1. Furthermore, HK-2 cells were transfected with HSP47 siRNA and siRNA negative control before exposing to TGF-β1. The expressions of HSP47, vimentin, ZO-1, ColI, Smad3 and p-Smad3 were examined by Western blot, Real-time PCR, and/or immunofluorescence, espectively.

Results: Compared to that in control, after treatment of HSP47 antisense ODNs resulting in an efficient and specific inhibition of HSP47 expression in the kidney of UUO model, the vimentin and ColI expression was significantly decreased and ZO-1 expression was significantly increased in the obstructed kidneys. Compared to the TGF-β1 group, inhibition of HSP47 expression in HK-2 cells up-regulated the expression of ZO-1 and down regulated the expressions of vimentin, ColI. Compared to the TGF-β1 group, inhibition of HSP47 expression down regulated the expression of p-Smad3/Smad3 in HK-2 cells.

Conclusions: HSP47 could promote the EMT of renal tubular epithelial cells in vivo and vitro via modulation the activation of Smad3, and suppression of HSP47 would be a possible therapeutic target against tubulointersitial fibrosis.

Funding: Government Support - Non-U.S.

SA-PO2800

TGF-b1 Mediates Macrophage-Induced Tubular Epithelial-to-Mesenchymal Transition in Fibrotic Nephropathy Yang Zhou, Chunsun Dai, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Macrophage infiltration is a common feature of chronic kidney disease, which suggests a pathologic function for macrophage in renal fibrosis. Tubular epithelial cell sparks renal fibrogenesis through epithelial-to-mesenchymal transition (EMT). Whether macrophage infiltration causes EMT and the exact underlying mechanisms remain to be determined.

Results: In this study, the effect of macrophage infiltration on kidney tubular EMT was investigated in unilateral ureteral obstruction (UUO) mice and cultured tubular cells. Macrophage infiltration was remarkably increased in the obstructive kidney. RT-PCR and ELISA analysis showed robust increased TGF-b1 mRNA expression and protein secretion in primary cultured macrophages isolated from UUO mice. Culture media from these activated macrophages could induce Smad signaling activation and EMT in tubular cells (NRK-52E), as demonstrated by loss of E-cadherin, de novo expression of a-SMA and upregulation of fibronectin, suggesting a critical role for activated macrophage in promoting EMT. Moreover, macrophage-induced Smad3 phosphorylation and tubular EMT were largely abolished by TGF-b1 neutralization antibody.

Conclusions: Hence, these results indicate that TGF-b1 plays an essential role in macrophage-induced tubular EMT in fibrotic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2801

Transforming Growth Factor-β Receptor Inhibition Preserves Renal Tubular Mass and Reduces Formation of Atubular Glomeruli Following Ureteral Obstruction Robert L. Chevalier,[1] Michael S. Forbes,[1] Barbara A. Thornhill,[1] Dae-Kee Kim.[2] *[1]Department of Pediatrics, University of Virginia, Charlottesville, VA; [2]College of Pharmacy, Ewha Womans University, Seoul, Korea.*

Background: Chronic kidney disease is characterized by parenchymal loss and interstitial fibrosis. Unilateral ureteral obstruction (UUO) is the most widely used model of chronic kidney disease, and we have recently reported that murine UUO results in rapid formation of atubular glomeruli (ATG) (March 2011, doi:10.1152/ajprenal.00022.2011). Transforming growth factor-β1 (TGF-β1) is a central regulator of renal cell death and collagen deposition following UUO.

Methods: Mice were subjected to UUO and placement of subcutaneous pellets for the continuous release of vehicle or IN-1130 (ALK5 TGF-β Type I receptor inhibitor), 30 mg/kg/day. Kidneys were harvested after 14 days, and proximal tubules, tubular atrophy and ATG were identified by *Lotus tetragonolobus* lectin. Collagen was identified by Sirius red. Relative distribution of renal parenchymal thickness, glomerular area, proximal tubular area, and interstitial collagen deposition were determined by quantitative histomorphometry.

Results: Weight and parenchymal thickness of the obstructed kidney were 42% and 51% greater in IN1130-treated than control mice, respectively ($p<0.05$). Glomerular area was 27% greater in IN1130 than in control mice ($p<0.05$). The fraction of intact glomeruli was 42±5% in IN1130-treated mice, but only 23±3% in controls ($p<0.05$); the volume fraction of proximal tubules was 30±5% with IN1130 vs. 18±2% in controls ($p<0.05$). Renal interstitial collagen accumulation was 2.9±0.3% with IN1130, but increased to 5.2±0.3% in controls ($p<0.05$).

Conclusions: We conclude that in addition to its role in interstitial fibrosis, TGF-β signaling mediates proximal renal tubular injury leading to the formation of ATG following UUO. ATG are formed in many renal disorders, including IgA and membranous nephropathy, cystinosis, diabetes, renovascular hypertension and renal allograft rejection (JASN 19:197, 2008). Inhibition of TGF-β type I receptor kinase may therefore prove clinically effective in the preservation of functional nephron number in chronic kidney disease.

Funding: NIDDK Support

SA-PO2802

Gremlin Is a Mediator of High Glucose and TGF-β-Induced Fibrosis in Renal Cells Sergio A. Mezzano,[1] Raquel Rodrigues-Diez,[2] Carolina Lavoz,[2] Sandra Rayego-Mateos,[2] Raúl R. Rodrigues Díez,[2] Alberto Ortiz,[2] Jesus Egido,[2] Marta Ruiz-Ortega.[2] *[1]Nephrology, Universidad Austral, Valdivia, Chile; [2]Universidad Autonoma Madrid, Spain.*

Background: Accumulation of extracellular matrix (ECM) is a hallmark of renal fibrosis. Fibroblasts are the main matrix-producing cells. Epithelial mesenchymal transition (EMT) is a source of activated fibroblasts. Gremlin is a developmental gene upregulated in diabetic nephropathy and in renal cells in response to high glucose and TGF-β1, but its role in renal fibrosis is unclear. We studied the role of gremlin in fibrogenic events in cultured renal cells.

Methods: Murine fibroblasts or human tubuloepithelial cells (HK2) were stimulated with recombinant gremlin, TGF-β or high glucose conditions. Changes in ECM or EMT proteins were evaluated by western blot and confocal microscopy. Endogenous gremlin was targeted with small interfering RNA (siRNA). The involvement of TGF-β in gremlin actions was assessed with a TGF-β neutralizing antibody.

Results: In murine fibroblasts stimulation with recombinant gremlin upregulated profibrotic genes, such as TGF-β1 and CTGF, and increased the production ECM proteins, including type I collagen. In human tubular epithelial cells gremlin also increased ECM-related proteins and induced EMT changes: phenotypic modulation to myofibroblast-like morphology, loss of epithelial markers (E-cadherin and cytokeratin) and induction of mesenchymal markers (vimentin and fsp-1). In both fibroblasts and tubular epithelial cells TGF-β1 induced gremlin production. We have tested whether gremlin is a downstream mediator of the profibrotic effects of TGF-β1 and high glucose. The blockade of endogenous gremlin with small interfering RNA inhibited TGF-β1 and High glucose-induced ECM upregulation and EMT.

Conclusions: Gremlin could participate in renal fibrosis, acting as a downstream mediator of high glucose and TGF-β1. Our data suggest that gremlin is a novel mediator of renal fibrosis and could be a target for anti-fibrotic therapies.

SA-PO2803

Marinobufagenin Induces CD40 Expression in Experimental Uremic Cardiomyopathy Steven T. Haller,[1] George Budny,[1] Jiang Liu,[1] Olga Fedorova,[2] Alexei Bagrov,[2] Deepak K. Malhotra,[1] Christopher J. Cooper,[1] Joseph I. Shapiro.[1] *[1]Medicine, University of Toledo Collage of Medicine, OH; [2]National Institute on Aging, Baltimore, MD.*

Background: We have shown that experimental uremic cardiomyopathy causes cardiac fibrosis, the generation of reactive oxygen species (ROS), and is associated with increased levels of the cardiotonic steroid marinobufagenin (MBG), a ligand of the Na/K-ATPase. CD40, a type I transmembrane receptor and member of the TNF receptor superfamily, is expressed by a variety of cells including endothelial cells and renal proximal tubular epithelial cells. CD40 receptor expression has been shown to play a vital role in inflammation. Recent evidence in renal transplant models suggests that activation of CD40, by its ligand (CD40L), results in activation of profibrotic signaling cascades. CD40 expression in the setting of experimental uremic cardiomyopathy has not been defined.

Methods: Male Sprague Dawley rats weighing between 250-300 gms were used for these studies. Western blot analysis was performed on kidney cortex tissue derived from partially nephrectomized (PNx) rats, and rats administered MBG at a dose of 10μg/kg/day by osmotic minipump infusion. In vitro experiments were performed using a pig renal proximal tubular cell line (LLC-PK1 cells).

Results: The PNx and MBG treated animals exhibited a substantial increase in CD40 expression compared to controls along with concurrent increases in collagen type-I expression. LLC-PK1 cells treated with MBG, ouabain, and glucose oxidase (a hydrogen peroxide inducer) showed a similar response. The increase in CD40 expression was mitigated by treatment with a monoclonal anti-MBG antibody (3E9).

Conclusions: MBG and ROS stimulate CD40 expression, which may contribute to the development of renal fibrosis in the setting of experimental uremic cardiomyopathy.

SA-PO2804

Carbamylated Proteins Accumulate in Tissues in Mice with Chronic Renal Failure Christine Pietrement,[1, 2] Laetitia Gorisse,[2] Philippe Rieu,[2, 3] Stephane Jaisson,[2, 4] Philippe Gillery.[2, 4] *[1]Department of Pediatrics, University Hospital, Reims, France; [2]UMR CNRS/URCA n°6237, Faculty of Medicine, Reims, France; [3]Department of Nephrology, University Hospital, Reims, France; [4]Laboratory of Pediatric Biology and Research, University Hospital, Reims, France.*

Background: Carbamylation is a late post-translational modification of proteins resulting from the nonenzymatic binding of isocyanic acid, a urea by-product, to specific free functional groups. This reaction alters protein structural and functional properties. Many studies have suggested the involvement of carbamylated proteins in chronic renal failure (CRF) and atherosclerosis but their *in vivo* accumulation or degradation is still incompletely known. We tested the hypothesis of the *in vivo* carbamylated proteins accumulation in a CRF animal model.

Methods: C57Bl/6J mice were subjected to a 75% reduction of total renal mass or to sham operation, and were sacrificed 10 weeks after surgery. Carbamylated proteins were evaluated in plasma and tissues by homocitrulline (Hcit) measurement with LC-MS/MS method after acid hydrolysis.

Results: In CRF mice group, mean serum urea concentration was 16.92 mmol/L and hematocrit 38% *vs* 8.24 mmol/L and 41% in control group. Hcit concentration was increased in CRF mice plasma *vs* control (0.97 and 2.07 mmol/mmol of amino acids respectively). Plasma urea and Hcit were positively correlated (r^2=0.8). Hcit content was increased in CRF mice *vs* controls in heart (x2.2), lung (x2.2), aorta (x2.1), brain (x2.0), spleen (x1.8), muscle (x1.7), liver (x1.6), skin (x1.5), kidney (x1.5), bone (x1.5). Blood urea concentration was positively correlated with Hcit content in muscle, lung, brain, spleen, heart, kidney, liver ($0.66 < r^2 < 0.91$). Hcit was increased in purified collagen from skin and tail tendons in CRF mice *vs* controls (x2.1 and x1.7, respectively) with a positive correlation to blood urea concentration (r^2=0.42 and 0.43).

Conclusions: These results indicate that carbamylated proteins in CRF increase in plasma and accumulate in tissues. The accumulation of these functionally and structurally altered proteins may contribute to the extra-renal consequences and progression of CRF.

SA-PO2805

Electroacupuncture and Moxibustion Hinder the Progression of Renal Disease by Modulating Systemic and Renal Renin-Angiotensin System (RAS) Josne Carla Paterno, Dulce Elena Casarini, Fernanda Fernandes Barrinha, Zaira Palomino Jara, Nestor Schor, Anaflávia De Oliveira Freire, Vicente de Paulo Castro Teixeira. *Medicine, UNIFESP, Sao Paulo, Brazil.*

Background: Systemic and renal RAS are pivotal for the development and maintenance of renal disease. Traditional Chinese Medicine (TCM) is increasingly recognized as an effective therapy in several fields of medicine. Among its therapeutic strategies are eletroacupuncture (EA) and moxibustion (MO). We investigate the effects of EA and MO on RAS in an experimental model of hypertensive and progressive renal disease (PRD).

Methods: Male wistar rats were submitted to 5/6 nephrectomy (5/6 nx) and studied along eight weeks. There were three groups: 5/6 nx (NX), 5/6 nx and EA-MO session in sham-points(NX-AS); and 5/6 nx and EA-MO session in three real acupuncture points (NX-AM). We evaluated 24h-proteinuria, tail-cuff blood pressure (TBP), mean arterial blood pressure (MAP), plasma and renal Ang I, Ang II and Ang^{1-7} and plasma renin activity.

Results:

Biochemical and Blood Pressure Studies

Parameters	NX	NX-AS	NX-AM
24h-proteinuria (mg/24h) initial	12.3 ± 3.1	11.9 ± 4.4	13.8 ± 3.7
24h-proteinuria (mg/24h) final	187.2 ± 25.1	179.5 ± 46.8	68.4 ± 15.1*
TBP (mmHg)	188.7 ± 15.2	176.7 ± 15.2	127.4 ± 9.4*
MAP (mmHg)	190.3 ± 12.8	182.7 ± 14.8	125.3 ± 19.8*
Plasma Ang I (ng/mL)	50.4 ± 13.5 Ψ	24.9 ± 6.6	88.1 ± 21.6 #
Plasma Ang II (ng/mL)	58.7 ± 19.9 Ψ	22.5 ± 6.2	32.3 ± 9.1 #
Plasma Ang 1-7 (ng/mL)	4.2 ± 2	5.9 ± 1.3	15.8 ± 7.2*
Renal Ang I (ng/g)	26.6 ± 9.2	20.1 ± 5.9	21.4 ± 4.7
Renal Ang II (ng/g)	8.8 ± 4.1 Ψ	19.1 ± 5.4	14.2 ± 4.7
Renal Ang 1-7 (ng/g) (ng/g)	12.4 ± 3.2	7.9 ± 2.1	43.3 ± 9.4*
Plasma Renin Activity (pmol/h)	22.9 ± 7.2	40.1 ± 0.9	29.8 ± 4.6*

(p=<0,05) vs NX and NX-AS; # (p=<0,05) vs NX-AS; Ψ(p=<0,05) vs NX-AS and NX-AM

Conclusions: The acupuncuture-treated group presented significant improvement in all measured functional parameters. EA and MO modulated RAS leading to higher production of systemic and renal Ang^{1-7}. Since A^{1-7} is protective against endothelial dysfunction and has vasodilatory and antifibrotic effects, our findings suggest that it could contribute to the improvement of the PDR in this model.

Funding: Other NIH Support - CNPq- Brazil

SA-PO2806

Antioxidant Inflammation Modulators (AIMs) Increase Glucose Metabolism in Muscle Cells Rhesa D. Stidham,[1] Thomas Palaia,[2] Ron Bumeister,[1] Deborah A. Ferguson,[1] Christian Wigley,[1] Louis Ragolia.[2] *[1]Reata Pharmaceuticals, Inc., Irving, TX; [2]Winthrop University Hospital, Mineola, NY.*

Background: Oxidative stress induced by chronic hyperglycemia is involved in the development of CKD and insulin resistance. The Antioxidant Inflammation Modulator (AIM) drug class potently induces Nrf2, a regulator of many antioxidant and cytoprotective genes.

Bardoxolone methyl is an AIM that recently completed a 52-week randomized, placebo-controlled trial for treatment of chronic kidney disease (CKD) in type 2 diabetics. In this trial, 54% of bardoxolone methyl-treated patients experienced muscle spasms, which were most frequent during the first two months of treatment and generally mild to moderate in severity. Muscle spasms were not associated with markers of muscle injury and improved while on drug. Similar muscle spasms have been reported with insulin treatment in diabetics, suggesting an association with muscle glucose metabolism.

Methods: To test whether a similar mechanism occurs in response to AIMs, we investigated their effect on glucose metabolism in cultured skeletal muscle cells. Uptake of 2-deoxyglucose (2-DG) and GLUT4 translocation to the plasma membrane were monitored in differentiated L6 muscle cells treated with RTA 405, a bardoxolone methyl analog. In differentiated C2C12 muscle cells, levels of the glycolytic products pyruvate and lactate were measured in response to both bardoxolone methyl and RTA 405 using biochemical assays. The extracellular acidification rate (ECAR) was also measured in response to RTA 405.

Results: RTA 405 increased 2-DG uptake in a dose-dependent manner. This effect correlated with translocation of GLUT4 to the plasma membrane. Pyruvate and lactate concentrations also increased in a dose-dependent manner following treatment with both bardoxolone methyl and RTA 405. RTA 405 also increased the extracellular acidification rate, further suggesting elevated glycolytic flux.

Conclusions: Taken together, these observations are consistent with treatment-induced increases in muscle glucose uptake and anaerobic glycolysis, which could result in enhanced acidification and correlate with muscle spasms, similar to that observed transiently following bouts of extreme exercise.

Funding: Pharmaceutical Company Support

SA-PO2807

Progenitor Cell Secretory Products Exert Additive Renoprotective Effects When Combined with ACE Inhibitors in Experimental CKD Darren A. Yuen, Yanling Zhang, Kim Connelly, Andrew Advani, Richard E. Gilbert. *St. Michael's Hospital, Toronto, ON, Canada.*

Background: Bone marrow-derived early outgrowth cells (EOCs) and their secreted factors markedly attenuate the functional and structural manifestations of experimental CKD. However, with blockade of the renin-angiotensin system (RAS) as established therapy in progressive kidney disease, any new therapy would need to show incremental efficacy. Accordingly, we tested whether administration of EOC-derived factors provides additive renoprotective effects when used in combination with RAS blockade for the treatment of late-stage CKD.

Methods: Cell free conditioned medium (CFCM) was generated by incubating F344 rat EOCs with serum-free EBM-2 medium to collect their secreted factor(s). Subtotally nephrectomized (SNX) F344 rats were randomized at a late stage of disease (8 wks post-SNX) to receive enalapril 0.5 mg/L in drinking water or vehicle. 4 wks later, enalapril-treated rats were randomized to receive thrice weekly iv injections of CFCM or EBM-2 for 2 wks on top of continued enalapril administration. Three groups were thus studied: (1) No Therapy, (2) Enalapril and EBM-2, and (3) Enalapril and CFCM. GFR, urinary protein, and systolic BP were assessed serially.

Results: Compared to vehicle, 4 wks of enalapril treatment lead to a slight reduction in urinary protein at 12 wks post-SNX (vehicle vs enalapril: 117 ± 30 vs 89 ± 20 mg/d). Following treatment with EBM-2, the Enalapril and EBM-2 group experienced a further rise in proteinuria and decline in GFR to levels similar to those of the 'No Therapy Group'. In contrast, the 'Enalapril and CFCM Group' demonstrated a sustained reduction in proteinuria, and a higher GFR. No differences in systolic BP were noted between treatment groups (Table 1).

	No Therapy	Enalapril and EBM-2	Enalapril and EOC CM
GFR (µL/min/g body wt)	2.4 ± 0.3	2.5 ± 0.3	3.7 ± 0.5 *
Urinary protein (mg/d)	139 ± 25	101 ± 31	59 ± 14
Systolic BP (mm Hg)	158 ± 9	152 ± 9	163 ± 7

* p < 0.05 vs No Therapy

Conclusions: These data demonstrate that EOC-derived factors exert additive renoprotective effects on top of ACE inhibitor therapy in experimental CKD, providing the rationale for clinical trials of EOC-based therapies for CKD.

Funding: Government Support - Non-U.S.

SA-PO2808

Abstract Withdrawn

SA-PO2809

Repeated Treatment with Progenitor Cell Secretory Products Maintains Long-Term Renoprotection in Experimental CKD Darren A. Yuen,[1] Yanling Zhang,[1] Kim Connelly,[1] Andrew Advani,[1] Richard E. Gilbert.[1] *[1]St. Michael's Hospital, Toronto, ON, Canada; [2]Toronto, ON, Canada; [3]Toronto, ON, Canada.*

Background: Bone marrow-derived early outgrowth cells (EOCs) are thought to mediate organ protection via paracrine effects. Given the dysfunction of autologous cells from patients with CKD and the uncertainties surrounding allogeneic cells, we considered whether repeated administration of a cell-free but EOC-derived product might provide a viable alternative strategy for kidney protection.

Methods: The secretory output of EOCs was harvested by incubation with serum-free EBM-2 medium to generate cell free conditioned medium (CFCM). Subtotally nephrectomized (SNX) F344 rats were randomized 4 wks post-SNX to receive 3x weekly iv injections of CFCM or EBM-2 over 2 wks. Rats were retreated if they showed signs of recurrence, as defined by a 4-fold rise in proteinuria above initial post-treatment values. Three groups were studied, according to whether they were administered CFCM: once (Initial Therapy Group), twice (Repeated Therapy Group) or not at all (No Therapy Group). GFR, urinary protein, and systolic BP were assessed serially.

Results: Proteinuria rose progressively in the 'No Therapy Group' (1740 ± 468 mg/d) but was lower in rats initially treated with CFCM (881 ± 138 mg/d) at 10 wks post-SNX. Following CFCM retreatment at this time point, the 'Repeated Therapy Group' showed sustained improvements in renal function, whereas the 'Initial Therapy Group' experienced a fall in GFR and rise in proteinuria to levels similar to those of the 'No Therapy Group' at 14 wks post-SNX (Table 1). Systolic BP did not differ between groups.

	No Therapy	Initial Therapy	Repeated Therapy
GFR (µL/min/g body wt)	1.41 ± 0.25	1.70 ± 0.15	2.14 ± 0.20 *
Urine protein:creatinine ratio (mg/mmol)	2674 ± 602	2536 ± 471	1912 ± 311
Systolic BP (mm Hg)	178 ± 20	183 ± 10	173 ± 10

* p < 0.05 vs No Therapy

Conclusions: EOC-derived factors, whilst reno-protective, have a limited duration of action. Repeated administration of these factors was, however, able to extend the duration of efficacy. These findings suggest that while CFCM may be a viable alternative to cell infusion, the need for their repeated administration seems likely.

Funding: Government Support - Non-U.S.

SA-PO2810

A Protective Role for Type 1 Angiotensin Receptors on Macrophages in Renal Fibrosis Jiandong Zhang, Johannes Stegbauer, Young-Soo Song, Thomas M. Coffman, Steven D. Crowley. *Duke University.*

Background: In current studies, we examined the actions of type 1 angiotensin (AT_1) receptors specifically on macrophages during progressive renal fibrosis in the unilateral ureteral obstruction (UUO) model.

Methods: To this end, we intercrossed mice carrying a floxed gene for the AT_{1A} receptor (*Agtr1a*$^{flox/flox}$) with mice harboring *Cre recombinase* under the control of the LysM promoter to remove AT_{1A} receptor-mediated responses from macrophages alone (*Cre*$^{+}$ *Agtr1a*$^{flox/flox}$ = MKO). Compared to *Cre*$^{-}$ *Agtr1a*$^{flox/flox}$ littermates (WT), the MKOs had a ~75% reduction in AT_{1A} receptor mRNA expression in macrophages ($p<0.0001$) but had preserved AT_{1A} expression in B and T cells, kidney, and heart (p=NS).

Results: 7 days following UUO, staining of kidneys from MKO mice showed 80% more collagen-producing myofibroblasts (9.4±0.6% vs 5.2±0.4% a-SMA+; $p<0.001$) and 64% more interstitial fibrosis than UUO WT kidneys (4.1±0.4% vs 2.5±0.2% picrosirius red; p=0.003). Moreover, at day 7 UUO, renal mRNA expressions of Col I and TGF-β1 were enhanced by 129% (p=0.02) and 40% ($p<0.04$), respectively, in MKOs compared to WTs. Macrophage infiltration was slightly greater in obstructed MKO kidneys than WTs (10.7±1.3% vs. 7.8±0.8%;$p<0.07$). Early in the course of UUO (day 3), MKO kidneys compared with WTs had increased expression of pro-inflammatory M1 macrophage markers (IL-1β +92%,$p<0.02$;TNF-α +42%;p=0.01) but similar levels of anti-inflammatory M2 macrophage markers (YM-1, IL-1R2). Moreover, peritoneal macrophages cultured from MKO mice had enhanced IL-1β and TNF-α mRNA levels (+ >100%;$p\leq0.01$ for each) but similar M2 marker levels to WT controls, suggesting that AT_1 receptors on macrophages suppress their M1 differentiation during UUO and thereby protect the kidney from injury. Consistent with this possibility, in renal tubular cells (RTCs) co-cultured with MKO macrophage supernatants, TGF-β1 and PAI-1 mRNA levels were enhanced by 106% and 156%, respectively, compared to RTCs co-cultured with WTs ($p\leq0.006$ for each).

Conclusions: We conclude that AT_1 receptors on macrophages ameliorate fibrotic CKD by limiting their polarization toward the pro-inflammatory M1 phenotype.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2811

Human Embryonic Mesenchymal Stem Cell Derived Conditioned Medium Rescues Kidney Function in Rats with Established Chronic Kidney Disease Arianne van Koppen,[1] Jaap A. Joles,[1] Bas W.M. Van Balkom,[1] Dominique De Kleijn,[2] Marianne Christina Verhaar.[1] *[1]Nephrology&Hypertension, University Medical Center, Utrecht; [2]Experimental Cardiology, University Medical Center, Netherlands.*

Background: Chronic kidney disease (CKD) is a major health care problem. New interventions to slow CKD progression are needed. Mesenchymal stem cell (MSC) derived conditioned medium (CM) plays a protective role in acute renal failure. In a model of acute myocardial infarction, both human MSC-derived CM and exosomes effectively decreased infarct size, suggesting exosome-mediated repair. We studied functional and structural effects of human MSC-derived CM and exosomes in a rat model of established CKD.

Methods: CKD was induced by 5/6 nephrectomy (SNX) in Lewis rats and progression was accelerated with L-NNA and 6% NaCl diet. Six wk after SNX, CKD rats received 250µl conditioned medium (CM) (N=13), 250µl non-CM (NCM) (N=13), 7µg exosomes in 250µl PBS (N=8) or 250µl PBS (vehicle) (N=8) IV twice daily for 4 consecutive days. Systolic blood pressure (SBP) and 24h-proteinuria were assessed fortnightly. Six wk after treatment, glomerular filtration rate (GFR; inulin clearance) and effective renal plasma flow (RPF; PAH clearance) were determined. Renal injury and inflammation were scored. CM, NCM, exosomes and PBS were also tested *in vitro* (angiogenesis and scratch-wound closure).

Results: At 5 wk after treatment, SBP was lower in CM- compared to both NCM- and PBS-treated rats: 145±17 vs. 163±21 and 172±15 mm Hg ($P<0.05$). Proteinuria tended to decrease after CM treatment. After 6 wk, CM was functionally effective vs. both NCM and PBS: GFR: 0.43±0.08 vs. 0.34±0.09 and 0.32±0.07 ml/min/100gr ($P<0.05$) and RPF: 1.48±0.22 vs. 1.25±0.62 and 0.99±0.34 ml/min/100gr ($P<0.05$). CM treatment decreased glomerulosclerosis and tubular damage ($P<0.05$). Exosomes influenced neither kidney function nor morphological damage. Both CM and exosomes stimulated angiogenesis and wound closure vs. respective controls *in vitro*.

Conclusions: Human MSC-derived CM decreases progression of CKD and hypertension, and ameliorates morphological injury in a rat model of established CKD. In this CKD model, these beneficial effects appear not to be due to exosomes.

Funding: Private Foundation Support

SA-PO2812

Role of the Transcription Factor ETS-1 in the Regulation of Pro-Inflammatory and Pro-Fibrotic Mediators in Response to Ang II Wenguang Feng,[1] Phillip H. Chumley,[1] Ping Hua,[1] Gabriel Rezonzew,[1] Edgar A. Jaimes.[1,2] *[1]Department of Medicine, University of Alabama at Birmingham, AL; [2]VA Medical Center, Birmingham, AL.*

Background: The transcription factor ETS-1 is an important mediator of growth-related responses and inflammation in different models of injury. We previously demonstrated that ETS-1 mediates macrophage infiltration, cell proliferation, mesangial expansion and oxidative stress in response to Angiotensin II (Ang II) in vivo (AJP '08, ASN '10). Herein, we tested the hypothesis that ETS-1 mediates these effects by regulating the expression of pro-inflammatory and pro-fibrotic mediators upregulated by Ang II.

Methods: C57BL/6 mice (n=6/group) were infused with vehicle (Veh), Ang II (1.4mg/kg/day SQ), Ang II and an ETS-1 dominant negative peptide (DN, 10 mg/kg/day SQ) or Ang II and an ETS-1 mutant peptide (MU 10 mg/kg/day SQ) for 4 weeks. Kidneys were saved for immunofluorescence (IF), real time PCR and western blot (WB).

Results: Ang II increased the mRNA expression of the pro-inflammatory cytokines IL-4, IL-5, IL-6 and CCL3, the growth factors TGF-β and CTGF and the NADPH oxidase NOX4 (table). DN reduced the mRNA expression of these mediators ($p<0.05$ vs Ang II), except IL-6 while MU had no effect. Ang II resulted in a 15-fold increase in TGF-β protein expression (WB), which was reduced by 49% by DN ($p<0.01$) but not by MU. Ang II also increased CTGF expression (IF): Veh: 0.7±0.25, Ang II: 560±299 ARU ($p<0.05$), which was reduced by DN: 4.7±2.8 ARU ($p<0.01$) but not by MU: 149±98.7 ARU.

Table

	TGFβ	CTGF	NOX4	IL4	IL5	CCL3	IL6
Veh	1.0±0.06	1.0±0.14	1.0±0.27	1.0±0.22	1.0±0.05	1.0±0.12	1.0±0.30
Ang II	1.5±0.23*	2.6 ±0.38*	2.4 ±0.29*	2.1±0.19*	2.0±0.16*	1.5±0.22*	2.1±0.37*
Ang II+DN	0.8±0.18#	1.7±0.26#	1.6±0.31#	0.9±0.13#	0.8±0.14#	0.6±0.15#	3.0±0.90
Ang II+MU	1.3±0.02	1.9±0.15	1.9±0.24	1.9±0.26	2.1±0.16	0.8±0.30	4.2±1.52

Data are shown as mean±SE, mRNA expression was normalized by 18s RNA. * $P<0.05$ vs control; # $P<0.05$ vs Ang II

Conclusions: Our studies demonstrate that the transcription factor ETS-1 mediates the expression of several pro-inflammatory and pro-fibrotic mediators induced by Ang II making ETS-1 a potential novel target in the treatment and prevention of end-organ injury in hypertension.

Funding: Veterans Administration Support

SA-PO2813

Conditioned Medium Generated from Early Outgrowth Bone Marrow Cells Reveals Renoprotective Effects in Type 2 Diabetes Yanling Zhang, Darren A. Yuen, Andrew Advani, Kim Connelly, Richard E. Gilbert. *St. Michael's Hospital, Toronto, Canada.*

Background: Our previous study shows that early outgrowth cells (EOCs) cultured from the bone marrow preserve kidney structure and function in chronic kidney disease including diabetic nephropathy. The benefits of the cells appear not to be a consequence of engraftment into the kidney, but rather systemic antioxidant and antifibrotic effects. Accordingly, we postulated that EOCs might mediate their beneficial effects by secreting soluble renoprotective factor(s). To test the hypothesis in the *in vivo* setting we conducted proof of principle studies using a cell free preparation in which the EOCs had been grown.

Methods: EOCs were grown from donor db/m mice bone marrow and conditioned medium (CM) was generated by incubating EOCs with serum-free EBM-2 culture medium. db/db mice were randomized to receive thrice weekly tail vein injections of either 10 x concentrated EOC CM or EBM-2 for 2 weeks. Outcome parameters including mesangial expansion, glomerular hypertrophy and oxidative stress were assessed 4 weeks after the first injection. The *in vitro* antioxidant activity of EOC CM was assessed by DCFDA.

Results: EOC CM demonstrated robust antioxidant activity *in vitro*, reducing the accumulation of reactive oxygen species (ROS) within NRK cells cultured in 25 mM glucose ($p< 0.05$). EBM-2 treated db/db mice developed mesangial matrix expansion and collagen type IV accumulation and increased ROS ($p<0.05$). EOC CM treatment attenuated mesangial and peritubular matrix expansion and collagen type IV accumulation, as well as ROS accumulation.

Conclusions: Our data demonstrate that EOC conditioned medium can mimic the effects of cell infusion, possibly acting via antifibrotic and antioxidant mechanisms. These findings may be translatable to offer a new cell free therapeutic strategy for diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2814

Chronic Nicotine Exacerbates Sub Pressor Ang II (SP-AngII) Induced Hypertension (HTN) and Renal Injury Kiran B. Chandrashekar,[1] Arnaldo F. Lopez-Ruiz,[1] Andrea P. Soljancic,[1] Ruisheng Liu,[2] Istvan Arany,[3] Luis A. Juncos.[1,2] *[1]Division of Nephrology, University of Mississippi Medical Center, Jackson, MS; [2]Physiology, University of Mississippi Medical Center, Jackson, MS; [3]Pediatrics, University of Mississippi Medical Center, Jackson, MS.*

Background: Smoking, in tandem with HTN, are strong risk factors for the development and progression of renal disease. Nicotine (Nic) is one of the main contributing factors to the deleterious effects of smoking. To evaluate the interaction between Nic and HTN in the progression of chronic kidney disease, we hypothesized that long term Nic administration exacerbates the functional and parenchymal damage induced by SP-AngII-induced HTN, a clinically relevant model of essential HTN.

Methods: To test this, male SD rats were pretreated for 12 days with Nic [12.5μg/ml; dose needed to reach a steady state cotinine (a Nic metabolite) level similar to chronic human smokers) or vehicle (saccharine-Sac2%) in drinking water. We then randomized these animals into four groups: 1) Control-Sac, 2) Nic + Sac, 3) SP-AngII (200ng/kg/min) + Sac, 4) SP-AngII + Nic + Sac. Each of these groups received either vehicle (saline) or SP-AngII by SQ osmotic minipumps, for an additional 22 days. Systolic blood pressure (SBP) was measured by tail cuff throughout the duration of the study. At the end of the protocol, laser doppler probes were used to measure outer medullary renal blood flow and the resistance (OM-RVR) was calculated. Renal function was evaluated by plasma creatinine. The kidney tissue was then harvested to evaluate injury (KIM-1), inflammation (MCP-1, TGF-β1) and apoptosis (Cytochrome-C).

Results:

	SBP mmHg	OM-RVR TPU/mmHg	Creatinine mg/dl	KIM-1 pg/μg	Cyt-C ng/μg	MCP-1 pg/mg	TGF-β1 ng/μg
Sac	110±3	4.5±0.2	0.4±0.05	58±5	8±1	523±47	13±2
Nic	111±5	4.9±0.2	0.6±0.13	253±9*	12±3	996±103*	24±2.5*
SP-AngII	164±2*†	6.6±0.3*†	1.2±0.14*†	537±76*†	26±2*†	1518±147*†	23.9±2.8*
SP-AngII+Nic	175±3*‡†	9.5±0.5*‡†	1.8±0.1*‡†	874±68*‡†	71±7*‡†	2193±207*‡†	34.5±2.1*‡†

$p< 0.05$; * vs. Sac; ‡ vs. SP-AngII; † vs. Nic

Conclusions: Nicotine initiates a pro-inflammatory and pro-fibrotic state, which consequently exacerbates SP-AngII-induced HTN, renal dysfunction and damage.

Funding: Other NIH Support - NIH DK073401

SA-PO2815

Stromal Cell-Derived Factor-1 Preserves Renal Function in Chronic Kidney Disease Li-Hao Chen,[1] Darren A. Yuen,[1] Yanling Zhang,[1] Manish M. Sood,[2] Ian W. Gibson,[2] Michael Kuliszewski,[1] Howard Leong-Poi,[1] Kim Connelly,[1] Richard E. Gilbert,[1] Andrew Advani.[1] *[1]Keenan Research Centre, St. Michael's Hospital, Toronto, ON, Canada; [2]Health Sciences Centre, University of Manitoba, Winnipeg, ON, Canada.*

Background: While the homeostatic chemokine stromal cell-derived factor-1 (SDF-1) plays an essential role in renal vascular development, its function in the adult kidney and in chronic kidney disease (CKD) is unclear. In the present study, we hypothesized that the pro-angiogenic properties of SDF-1 would serve to promote microvascular health in both the normal and chronically ischemic kidney.

Methods: SDF-1 expression was determined in human biopsies, rat kidneys and cultured cells. Antagonism and augmentation of SDF-1/CXCR4 signaling were achieved with either AMD3100 or gene transfection.

Results: While SDF-1 was expressed by podocytes, fibroblasts, collecting ducts and also corticomedullary proximal tubular cells, its cognate receptor CXCR4 was primarily restricted to endothelial cells of glomerular and peritubular capillaries. Unlike in biopsies from patients with diabetic nephropathy, SDF-1 expression was not upregulated in kidneys from individuals with CKD due to secondary focal segmental glomerulosclerosis (FSGS). Moreover, SDF-1 expression was decreased in renal fibroblasts exposed to the pro-fibrotic growth factor transforming growth factor-β and in the kidney cortices of subtotally nephrectomized (SNx) rats, a rodent model of CKD reminiscent of FSGS. The CXCR4 antagonist AMD3100, which attenuated SDF-1 induced endothelial tube formation in vitro, increased proteinuria in normal rats and accelerated renal injury in SNx animals associated with a decline in capillary growth and an increase in matrix deposition. Conversely, increasing SDF-1 activity, achieved by ultrasound-guided gene transfection using plasmid-bearing microbubbles, attenuated proteinuria progression in SNx rats.

Conclusions: Collectively, these observations indicate that SDF-1 plays a homeostatic role in the adult kidney and in the setting of progressive, proteinuric kidney disease. Therapeutic strategies that augment SDF-1 signaling represent a novel approach to slow the decline of kidney function in patients with CKD.

SA-PO2816

Blood or Urine? A Systematic Comparison of Information Content and Analytical Practicality of Different Sample Types for Targeted Metabolomics Ulrika Lundin, Klaus M. Weinberger. *Biocrates Life Sciences AG, Innsbruck, Austria.*

Background: Right now there is a controversy going on since the majority of metabolomics studies are based on either only urine or only blood fluid (serum or plasma) samples. In nephrology, urine might seem like the obvious sample type of choice, because of the metabolic activity of the kidneys and the urine being excreted would show big changes in the composition of its metabolites. We intended to do a systematic review to see which sample type is really the most informative for targeted metabolomics, particularly in nephrology.

Methods: The comparisons between sample types were done in three different studies; on db/db mice, a rodent model of hyperphagia-associated type II diabetes with little kidney damage, puromycin-induced nephrotoxicity in Sprague-Dawley rats and a clinical study on the progression of chronic kidney disease in diabetic and non-diabetic patients. Targeted metabolomics was used to quantify metabolites from plasma and urine including the classes amino acids, biogenic amines, polyamines, acylcarnitines, phosphatidylcholines, sphingomyelins, eicosanoids, bile acids and energy metabolism intermediates in the presence of isotopically labeled internal standards and determined by flow injection analysis (FIA)-

and high performance liquid chromatography (HPLC)-tandem mass spectrometry with multiple reaction monitoring (MRM) using an AB Sciex 4000 QTRAP® with electrospray ionization. Additionally, free fatty acids were quantified by GC-MS.

Results: We performed a large a number of comparisons to assess sensitivity and specificity on the datasets of urine, blood and both combined. Because of the study design of the cohorts, we could look at the data both in a nephrology and non-nephrology aspect to be unbiased and remove any organ-specific information. With discriminant analysis and receiver operating characteristics analysis we assessed the information in the different datasets and summarized the outcomes.

Conclusions: We found that that blood is much more informative than urine, and almost as good as the combined dataset, the reason probably being that there is a strict homeostatic regulation in the blood, whereas urine does not possess this property.

Funding: Government Support - Non-U.S.

SA-PO2817

Reduced Klotho Expression Level in Kidney Aggravates Renal Interstitial Fibrosis Hidekazu Sugiura,[1] Takumi Yoshida,[2] Junko Kohei,[1] Shunji Shiohira,[1] Michihiro Mitobe,[1] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Fourth Department of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan; [2]Yoshida Medical Clinic, Tokyo, Tokyo, Japan.*

Background: Klotho is associated with the suppression of several aging phenotypes. The renal expression of the *Klotho* gene is markedly suppressed in chronic renal disease patients. Renal fibrosis is a major risk factor of chronic kidney disease (CKD). We explored the relation between renal interstitial fibrosis and Klotho in mice and cultured renal cell lines. We previously reported The plasma urea nitrogen and phosphate concentrations were significantly elevated, the renal a-SMA level was significantly upregulated, and renal interstitial fibrosis was severer in Klotho-deficient mice (*kl/kl* mice). In addition, TGF-β1 reduced Klotho expression in a time-dependent manner in epithelial cells.

Methods: Expression of fibrotic factors was by Real time PCR, Western blotting, ELISA and immunohistochemical staining. To determine whether reduction in Klotho expression predisposes the kidney to renal fibrosis, we introduced renal fibrosis to *kl/+* mice by unilateral ureteral obstruction (UUO) and compared them with wild-type mice.

Results: The expression levels of a-SMA (1.6-fold, $p<0.05$), fibronectin (1.6-fold, $p<0.05$) and TGF-β1(1.4-fold, $p<0.05$) were higher and the renal interstitial fibrosis was severer in the *kl/+* mice than those in the wild-type mice. In cultured renal fibroblast cells (NRK49F cells), expression levels of a-SMA and PAI1 (0.5-fold, $p<0.05$) were significantly suppressed by addition of recombinant Klotho protein to the medium. The expressions of a-SMA and PAI-1 were significantly increased in NRK49F cells cultured in the presence of TGF-β1 for 24 hours, although these elevations in the a-SMA and PAI-1 levels (0.6-fold, $p<0.05$) were significantly blunted in NRK49F cells cultured in the presence of TGF-β1 with recombinant Klotho protein. These effects were also suppressed by a TGF-β1 receptor inhibitor (ALK5 inhibitor).

Conclusions: Klotho is downregulated by the progression of renal fibrosis and chronic kidney disease via TGF-β1. Reduced Klotho expression levels in the kidney aggravate renal interstitial fibrosis via TGF-β1.

Funding: Government Support - Non-U.S.

SA-PO2818

Xeno-Klotho Administration Retards the Progression of Adriamycin Nephropathy Tsuneo Takenaka, Tsutomu Inoue, Hirokazu Okada, Yoichi Ohno, Takashi Miyazaki, Hiromichi Suzuki. *Saitama Medical University.*

Background: Recent data reveal that elevating free klotho in proximal tubular lumen reduces phosphate reuptake, and suggest that the gain-of-function in klotho gene slows the progression of chronic kidney diseases, at least partly through the inhibition of TGF signaling and fibrosis.

Methods: In the present study, effects of exogenous administration of recombinant human klotho protein on renal injury was assessed in two groups of rats (n=6 for each group); male Wister rats treated with intravenous adriamycin (5 mg/Kg) to induce focal segmental glomerulosclerosis through tail vein as a control (C), those treated with recombinant human klotho (10 μg/Kg/day) by osmotic pump (K). Saline was used as a vehicle. Four weeks later, rats were killed, and harvested kidney for analysis.

Results: Chronic administration of klotho increased serum free klotho (870±65 vs. 186±16 pg/ml, $p<0.01$). Albuminuria was lower in K (5.3±1.2 mg/day) than C group (15.2±3.1 mg/day, $p<0.01$). Creatinine clearance tended to be lower in C (1.6±0.2 ml/min) than K group (2.0±0.3 ml/min, $p=0.07$). Serum calcium was similar (9.9±0.2 vs. 10.0±0.2 mg/dl), but phosphate was reduced in K group (6.4±0.2 vs. 7.6±0.3 mg/dl, $p<0.05$). Phosphate excretion was also increased in K group (9.0±0.7 mg/day), compared to C group (5.8±0.5 mg/day, $p<0.05$). Although serum levels of fibroblast growth factor (FGF) 23 and parathyroid hormone were similar between two groups, 1,25-dihydroxy-vitamin D was lower in K than C group (164±9 vs. 201±10 pg/ml, $p<0.05$). However, western blotting demonstrated that exogenous klotho infusion failed to alter both renal klotho and 1-alpha-hydroxylase expression.

Conclusions: The present data demonstrate that adding-on circulating free klotho suppresses the production of 1,25-dihydroxy-vitamin D independently of parathyroid hormone, FGF23 and renal 1-alpha-hydroxylase expression. Our findings indicate that an elevated circulating klotho declines serum phosphate associated with increased phosphate excretion without affecting renal klotho expression. The current results have provided the evidence that free klotho reduces albumin excretion, showing renoprotective actions in adriamycin nephropathy.

Funding: Clinical Revenue Support

SA-PO2819

Ronacaleret Retards Renal Injury with Preserving Klotho in 5/6-Nephrectomized Rats Tsuneo Takenaka, Tsutomu Inoue, Hirokazu Okada, Takashi Miyazaki, Hiromichi Suzuki. *Saitama Medical University, Iruma, Saitama, Japan.*

Background: We have previously reported that vitamin D increases renal expression of klotho in rats with normal kidney function, and that calcimimetics reduces it.

Methods: In the present study, effects of ronacaleret, a calcilytic agent, on renal injury was assessed in four groups of rats (n=6-10 for each group); 5/6-nephrectomized (uninephrectomy with ligation of 2 renal artery branches in contralateral kidney) Wister rats as a control (C), those treated with ronacaleret (150 mg/kg/day) (R), rats treated with calcitriol (30 ng/kg/day) (V), and rats treated with both ronacalcet and calcitriol (R+V). Three months later, rats were killed with over-anesthesia, and harvested remnant kidney for analysis.

Results: Albuminuria was lower in R (4.2±0.6 mg/day), V (3.8±0.5 mg/day) and R+V (4.2±0.6 mg/day) than C group (13.2±1.4 mg/day, $p<0.05$). Creatinine clearance (Ccr) was elevated in R (2.2±0.2 ml/min) and V (1.9±0.2 ml/min) than C group (1.5±0.2 ml/min, $p<0.05$). Serum calcium was increased in R+V (10.1±0.1 mg/dl) than C group (9.4±0.1mg/dl, $p<0.05$). Fractional phosphate excretion was increased in R (13±2 %), V (13±2 %) and R+V (16±2 %) compared to C group (7±1 %, $p<0.05$), and serum phosphate was reduced in R group (6.1±0.3 mg/dl vs 7.8±0.3 mg/dl (C)). FGF23 was lower in R (371±18 pg/ml) and higher in V (675±58 pg/ml) and R+V (671±54 pg/ml) than C group (500±33 pg/ml, $p<0.05$). However, parathyroid hormone did not significantly differ among 4 groups. RT-PCR and western blot analyses revealed that compared to C ($p<0.05$), renal klotho expression was elevated in R (1.8-2.1 fold) and V (1.7-2.0 fold) groups.

Conclusions: The present data indicate that ronacaleret preserved klotho expression and renal function with reduction in serum phosphate and albuminuria in 5/6-nephroctomized rats. Our findings demonstrate that vitamin D prevented declines in klotho expression and renal function, suppressing albuminuria. The current results provide the evidence that hypercalcemia decreases klotho expression, participating in the progression of renal dysfunction.

Funding: Clinical Revenue Support

SA-PO2820

Renal Nrf2 Expression Is Decreased in Klotho Deficient Mouse Tetsuro Kusaba, Eiko Matsuoka, Yasukiyo Mori. *Department of Nephrology, Kyoto Prefectural University of Medicine, Kyoto, Japan.*

Background: Klotho is anti-aging humoral factor and abundantly expressed in the kidney and has an antioxidative effect. Nuclear factor erythroid-2-related factor (Nrf2) is a cytoplasmically localized transcription factor and also has an antioxidative effect. Nrf2 translocates to the nucleus in response to oxidative stress and regulates the expression of genes encoding antioxidant enzymes. Although both Klotho and Nrf2 have an antioxidative effect on kidney, the relationship between these molecules has not been well understood previously.

Methods: Tissue extract of kidney was obtained from Klotho deficient and wild-type mice and nuclear Nrf2 expression was assessed by Western blot analysis. The tubular epithelial cells were primarily cultured from these mice and assessed the Nrf2 expression. 300mM of H_2O_2 was added to these cells for 3hours and nuclear Nrf2 translocation was assessed by the immunofluorescence analysis. For investigating the association between Nrf2 and Klotho protein further, immunoprecipitation between these molecules were performed.

Results: Nrf2 expression in tissue lysates of kidney is significantly decreased in klotho deficient mouse compared with wild type mouse ($p < 0.05$). Nrf2 expression in primarily cultured epithelial cells is also significantly decreased in klotho deficient mouse. Nuclear Nrf2 translocation after H_2O_2 stimulation was remarkably decreased in primarily cultured epithelial cells from klotho deficient mouse compared with wild type mouse. In western blot analysis, the H_2O_2 stimulation did not affect the Nrf2 expression in klotho deficient mouse, whereas increased in those from wild type mouse. However, immunoprecipitation analysis revealed that klotho protein did not bind to Nrf2.

Conclusions: Our data suggested that anti-oxidative effect of klotho was partially and indirectly through Nrf2 expression and its nuclear translocation.

SA-PO2821

Serum Klotho Levels Are Associated with Kidney Function but Not with Calcium Phosphorus Metabolism in CKD Eric Seibert, Daniel Radler, Christof Ulrich, Sylvia Hanika, Roman Fiedler, Matthias Girndt. *Department of Internal Medicine II, Martin-Luther-University Halle, Halle, Germany.*

Background: Klotho protein which is expressed on renal distal tubular cells is an essential co-receptor for FGF-23 and a key regulator of phosphate excretion. Its disturbed expression in mice leads to a syndrome resembling premature human aging. Its secreted form modulates expression of ion-channels and the sensitivity of growth factors which may be key factors for its anti-aging properties. In chronic kidney disease (CKD), impaired renal klotho expression has been demonstrated by Western blot and immunohistochemic analyses. Therefore, soluble klotho is expected to be reduced as well and is likely to contribute to accelerated aging.

Methods: We performed a cross-sectional study of soluble klotho serum levels using a recently established ELISA in 156 subjects with a kidney function ranging from normal to end-stage renal disease.

Results: Klotho levels were significantly lower in HD patients than in healthy adults and those with moderate CKD (368.3±99.0 pg/ml vs. 468.1±205.8, p<0.01 and 368.3±99.0 pg/ml vs. 498.7±221.9, p<0.01). There was a significant correlation with eGFR (p=0.0005, r=0.31). In end stage renal disease there was a weak but significant correlation with bone alkaline phosphatase and 25-OH-Vitamin D levels. Correlations to age, calcium or phosphorus levels could not be established. Klotho levels were significantly higher in females (463.0±202.6 pg/ml vs. 387.6±132.0 pg/ml, p=0.02), and acute kidney injury (567.6±294.4 pg/ml, vs. 403.5±152.5 pg/ml, p<0,01).±

Conclusions: Serum klotho levels are associated with kidney function. In CKD, impaired klotho levels may contribute to accelerated aging and may be a measure of viable renal tubular cells mass.

Funding: Government Support - Non-U.S.

SA-PO2822

Induction of Phosphaturia Accelerates Chronic Kidney Diseases in Mice

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Background: Lowering serum phosphate(Pi) levels by Pi binders improves clinical outcome of chronic kidney disease (CKD). Because serum Pi levels can be lowered also by promoting phosphaturia, we tested if induction of phosphaturia would delay CKD progression in mice. Klotho is expressed in the kidney where it functions as a co-receptor for fibroblast growth factor-23 (FGF23) and plays a critical role in Pi metabolism. The extracellular domain of Klotho is subject to ectodomain shedding and secreted into blood and urine. Secreted Klotho has phosphaturic activity independent of FGF23. Klotho injection ameliorates acute kidney injury (AKI), and transgenic overexpression of Klotho alleviates soft tissue calcification in CKD. This study is to test whether Klotho injection alleviates CKD in mice.

Methods: Eight weeks old mice (129S1/SvImJ males) had received uninephrectomy plus ischemic reperfusion injury in contralateral kidney followed by feeding with 2% Pi diet. Mice were treated with secreted Klotho (0.02mg/kg, i.p, every other day) or vehicle for 12 weeks.

Results: Mice given recombinant secreted Klotho exhibited more prominent renal damage than vehicle-treated mice in functional (higher serum creatinine (Cr) and lower Cr clearance), histological (more severe nephrocalcinosis, renal interstitial fibrosis), and molecular parameters (higher levels of TGF-β1, phosphorylated Smad2/3, α-smooth muscle actin, collagen-1 and p21). Previous animal studies have furnished a model that kidney damage induced by dietary Pi overload correlates better with Pi excretion per nephron than blood Pi. It is conceivable that Klotho injection further increases urinary Pi excretion that is already high due to dietary Pi overload; with increased CaPi crystal formation in the tubules and nephrocalcinosis.

Conclusions: Thus, we propose that a delicate balance between the beneficial effects of phosphaturia v.s. overloading kidney with phosphate should be considered in Pi-lowering therapies.

Funding: NIDDK Support

SA-PO2823

Myocardial Expression of Fibroblast Growth Factor Receptor-1 and Renin-Angiotensin System Genes in Hypertrophic Hearts of Uremic Rats

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Background: Cardiac hypertrophy (CH), common in CKD, associates with increased mortality. CH causes are multifactorial including disturbances of the renin-angiotensin system (RAS) and fibroblast-growth factor (FGF)-23. However, expression of RAS components in myocardium (M) of uremic animals has not been studied; furthermore, the coreceptor Klotho, essential for FGF-23 action, is not expressed in M. Since the vitamin D analog paricalcitol (Pc) improves uremic CH, suppresses C-RAS in non-uremic animals and interacts with FGF23 in other tissues, we studied Pc effects on the M expression of these genes in uremic rats.

Methods: CH and left ventricular (LV) gene expression of RAS components and the FGF receptor 1 (r1) were evaluated in 5/6 nephrectomized rats treated 8 weeks with Pc 0.3 μg/kg 3x/week IP or enalapril (E) 5 mg/kg/day enterally vs. untreated uremic (U) and sham-operated animals (S).

Results: PC and E improved hypertension (U 204±18 mm Hg; Pc 156±8; E 144±9.5; p<0.0001) and renal insufficiency (creatinine, U 1.67±0.7 mg/dl; Pc 0.76±0.11; E 0.76±0.06; p<0.002). Cardiac weight↑in U was similarly↓by Pc and E (p<0.02). U↑mRNA expression of LV angiotensinogen by 30-fold (p<0.01 vs.S), attenuated by Pc and E (p<0.006); AT1R↑2.4-fold in U, was suppressed markedly by E and unmodified in Pc. ACE mRNA was similar in all groups. Renin mRNA expression and brain natriuretic peptide (BNP) protein in LV↑in U and suppressed similarly by Pc and E (p<0.05). FGFr1 mRNA was↑6-fold in U, and while Pc↓only mildly this gene, E↓values similar to S .

Conclusions: 1) CH is associated with increased LV renin and angiotensinogen gene expression and M upregulation of FGFr1 mRNA. 2) M renin mRNA and BNP were similarly suppressed by Pc and E. Evaluation of direct effects of PC and E, independent of those related to improvement of uremia and hypertension in this model, requires further studies.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

SA-PO2824

Serum Levels of Soluble Secreted αKlotho Are Modulated by Renal Function, Age, FGF23, and May Play a Key Role in CKD-MBD in Chronic Kidney Disease Patients

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Background: αKlotho was first identified as an aging gene and was later shown to be a regulator of phosphate metabolism. Fibroblast growth factor 23 (FGF23) is the important regulator of phosphate. FGF23 was reported to markedly increase in patients of CKD. αKlotho protein is a co-receptor specific for FGF23. But serum level of soluble αKlotho in CKD patients is still undermined, especially in relationship to FGF23, creatinine, and phosphate levels. This study was designed to investigate whether serum αKlotho are modulated by renal function, age, FGF23, and study a potential role in CKD-MBD in CKD. This study is the first report on the utility of serum αKlotho in human CKD.

Methods: We made a continuously survey of CKD patient characteristics and outcomes in Kochi prefecture (Western Japan). Patients of CKD (N=101) were enrolled. Serum sample were collected and measured FGF23 and soluble αKlotho by using an ELISA kits. In addition, serum creatinine, hemoglobin, albumin, Ca and Pi were measured. This study was approved by Kochi University review board. All patients provided written informed consent.

Results: FGF23 correlated positively to age(P<0.01:r=0.296) and creatinine (P<0.0001:r=0.861)and negatively to hemoglobin(P<0.01:r=-0.340) and albumin(P<0.0001:r=0.861). FGF23 was clearly associated with CKD stage. αKlotho negatively correlated to age(P<0.001:r=-0.368)and positively correlated to hemoglobin (P<0.05:r=0.246). αKlotho had a tendency to associate negatively with serum creatinine. Interestingly, αKlotho levels significantly decreased early phase of CKD (stage 2) as compared with stage1. In addition, αKlotho dramatically decrease according to the progression of CKD, especially stage 4 and 5. However, αKlotho did not show statistically significant correlation with Pi, Ca and FGF23.

Conclusions: Our data indicate that serum αKlotho could be a new predictive marker in progression of CKD, especially in early stage, and αKlotho and FGF23 may play a key role in pathogenesis of CKD-MBD.

SA-PO2825

Atheromatous Disease and Resistance to the Phosphaturic Action of FGF23 Contribute to the Severiry of Vascular Calcification in Non-Dialyzed Kidney Disease Patients

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Background: Vascular calcification (VC) is an important contributor to the high mortality rates in chronic kidney disease (CKD), and hyperphosphatemia is a well recognized cause of enhanced VC in these patients. However, a recent study in hemodialysis patients has shown that not only hyperphosphatemia, but also atheromatous disease contributes to carotid calcification

Methods: This cross-sectional study analyzes the impact of abnormalities in phosphate (P) metabolism and atheromatous disease on abdominal aortic calcification (AAC) measured by the Kauppila index (KI) in 186 patients CKD stages 3, 4, and non–dialyzed stage 5.

Results: In multivariate analyses, both the presence of AAC and its severity (Moderate: KI≤5; Severe: KI>5) associated positively with age, a higher stage of CKD, and more significantly, with atheromatosis disease (higher carotid intima-media thickness and presence of carotid plaques), and negatively with female sex. Intriguingly, no association was found between either AAC or KI values with well recognized risk factors from abnormal mineral metabolism (serum levels of Ca, P, PTH, soluble a-Klotho, 25(OH)D and 1,25(OH)2D). Furthermore, serum FGF23 associated positively only with the severity but not with the presence of AAC, while the fractional excretion of P (FEP) associated negatively with both. The correlation between FEP and serum FGF23 showed that the highest FGF23 levels associated with KI > 5 only in a small group of patients in which FEP was far below the expected FEP for a given serum FGF23 in both non calcified and moderately calcified patients.

Conclusions: Atheromatous disease and an impaired phosphaturic response to high serum FGF23 contribute to the severity of VC at CKD stages 3, 4 and non-dialyzed stage 5.

SA-PO2826

Impaired Angiogenesis and Rarefied Peritbular Capillaries Participated in Renal Tubularintersitial Fibrosis in Experimental Chronic Renal Ischemic Rats Jiaming Zhu, Yi Fang, Xiaoqiang Ding. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: Chronic tissue hypoxia is regarded as a contributing factor in the progression of renal failure, impaired angiogenesis and loss of peritubular capillaries in the late phase of renal diseases gives evidence of renal hypoxia.

Methods: Unilateral clamping of the left renal artery to induce chronic ischemia of the kidney tissue was utilized in this study. The experimental rats and respective sham-operated controls were sacrificed at week 1 and 12, and the renal tissues were harvested for histological study.

Results: The results showed that the blood pressure and serum creatinine of the experimental rats was elevated at week 6 and keep a high level in late stages. The left renal tissue with artery stenosis showed proximal tubuler epithelium vacuole and granulation degeneration with inflammatory cells infiltration in tubulerinterstitium in week 1 group. Hypoxyprobe™-1 labelling showed significant staining in out medulla and cortex, while the hypoxic probe was only weak stained in outer medulla in the control rats. Interestingly, VEGF and both of its receptors Flk and Flt were markedly upregulated in one week ischemic renal cortex compared with the control($P<0.01$. But in advanced stage (week 12), the chronic ischemic renal tissue exhibited atrophic tubular epithelium, widened tubularinterstitium with obvious collagen I expresssion. The Hypoxyprobe™-1 labelling showed limited staining only in medulla while negative staining in cortex. Coincidence with Hypoxyprobe™-1, VEGF and its receptors staining in cortex weakened significantly compared with that of early stage(week 1)($P<0.01$). Further investigation revealed that the endothelial cell marker CD31 was markedly reduced and showed a significant loss of peritubular capillaries in cortex area, and also decreased endothelial progenitor cell marker CD34 expression with lack of vascular repair compared with that of One week group.

Conclusions: We concluded persistent ischemia would finally destroy the ability of angiogenesis in renal tissues, which accelerated ischemia extent of the kidney, led to interstitial fibrosis and attributed to renal failure in advanced stages.

SA-PO2827

Paradoxical Pro-Growth Gene Expression Patterns in Rat Kidney Endothelial Cells Characterized by Limited Growth Potential David P. Basile,[1] Pingyu Zeng,[2] Jessica Friedrich.[1] *[1]Cellular & Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN; [2]Pediatrics, Indiana University School of Medicine, Indianapolis, IN.*

Background: The renal vasculature is characterized by limited regenerative potential following both acute and chronic injuries, and peritubular capillary loss is a consistent feature in the setting of interstitial fibrosis leading to worsened hypoxia. The current study sought to investigate the growth properties of rat kidney endothelial cells and determine factors that may lead to their impaired regenerative capacity.

Methods: Rat kidney endothelial cells (KEC) were isolated by CD31 immuno-isolation techniques and maintained in long-term cultures.

Results: When compared with endothelial cells isolated from the pulmonary microvasculature (i.e., PMVEC), KECs did not respond to VEGF, had significantly slower growth rates and limited clonogenic capacity. The expression of 84 VEGF signaling pathway genes was compared in KEC and PMVEC by quantitative PCR arrays. Despite their low growth rates, KECs showed significant and dramatic enhancement in 18 VEGF pathway genes ($\log_2>2$ vs PMVEC), including Flt-1 (~50 fold), FLK-1/KDR (~60 fold) and NOS-3 (~200 fold). In contrast, only 5 genes were expressed at sigificantly lower levels in KEC vs. PMVEC, and these included VEGF-A and C. We further screened the expression of 384 rat microRNAs and compared KECs and PMVECs. The vast majority of miRNAs were either undetectable or showed no difference between KECs and PMVECs; However, 4 miRNAs were significantly greater in KECs including the pro-angiogenic miR126, which was expressed at ~ 10,000 fold greater levels in KEC vs. PMVEC. Moreover, the pro-apoptotic miR24 was highly expressed in PMVECs and was 3-fold lower KECs.

Conclusions: Taken together, rat KECs display a prominent enhancement of genes typically associated with endothelial proliferation and angiogenesis, despite their low growth rates, suggesting that unidentified factors may impose strong negative growth regulation in these cells.

Funding: NIDDK Support

SA-PO2828

Reduced γ-Carboxylase Activity in Uremia- A Possible Mechanism of Uremic Vascular Calcification Nadine Kaesler,[1] Thomas Schettgen,[2] Elke Magdeleyns,[3] Vincent Brandenburg,[4] Cees Vermeer,[3] Jurgen Floege,[1] Thilo Krueger.[1] *[1]Nephrology and Clinical Immunology, University Clinic of the RWTH Aachen, Aachen, Germany; [2]Institute and Outpatient Clinic of Occupational Medicine, University Clinic of the RWTH Aachen, Aachen, Germany; [3]VitaK BV, University Maastricht, Maastricht, Netherlands; [4]Cardiology, Pneumology, Angiology and Internal Medicine Intensive Care, University Clinic of the RWTH Aachen, Aachen, Germany.*

Background: Vascular Calcification (VC) is present in chronic kidney diseases. This can be inhibited by matrix gla protein (MGP), which achieves full activity by carboxylation by the vitamin K dependent γ-carboxylase. Its inhibition by warfarin leads to augmented vascular calcification. The vitamin K regeneration cycle is formed by DT-diaphorase, VKOR and γ-carboxylase. Vitamin K deficiency is present in dialysis patients and so we investigated whether uremia reduces enzyme activities.

Methods: 10 Wistar rats in each group were fed a) standard diet b) 100mg/kg vitamin K2 c) 0,75% adenine or d) 0,75% adenine + 100mg/kg vitamin K2. Finally, serum parameters, extent of VC, uncarboxylated (uc)MGP and in kidney an liver activities of diaphorase, VKOR and γ-carboxylase were measured.

Results: After 4 weeks of treatment, creatinine, urea (7-fold) and phosphate were higher in adenine groups (c+d) than in controls (a+b). Calcium was unchanged. Aortic calcium content was higher in c+d than in controls. Systolic blood pressure was unaltered in all groups. DT-diaphorase activity was significantly higher (70, 72%) in groups c+d. VKOR activity was unchanged; γ-Carboxylase was more active in groups c+d, significantly. This also led to significant higher levels of ucMGP (12.2 vs 8.6, 7.4 μM).

Conclusions: Experimental uremia inhibits the key enzyme of the vitamin K dependent carboxylation, γ-Carboxylase. This is accompanied by higher levels of ucMGP and calcium deposition in the aorta. Even though there is reduced enzyme activity, vascular calcification and ucMGP can be reduced by dietary vitamin K2 (group d). Our data identifies a new mechanism of uremic vascular calcification and supports the rationale of our vitamin K2 interventional study VitaVasK.

SA-PO2829

Low-Dose Erythropoietin Increases Superoxide Production in Normal Rat Aorta and Endothelial Cells Chieko Ihoriya, Minoru Satoh, Hiroyuki Kadoya, Yuko Nishi, Kengo Kidokoro, Hajime Nagasu, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: High-dose erythropoietin (EPO) affords vascular and tissue protection with non-hematopoietic effects, but it is still controversial whether the therapeutic dosage of EPO is beneficial for cardiovascular protection. High-dose EPO has been reported to increase nitric oxide (NO) production via activation of the phosphatidylinositol-3-kinase/Akt pathway. However, the clinical dosage of EPO sometimes leads to an increase in blood pressure in humans. This study was designed to investigate whether the therapeutic dosage of EPO accentuates oxidative stress and modulate endothelial function.

Methods: In in vivo experiments, normal male Sprague-Dawley (SD) rats were treated with either EPO (20 IU/kg/week, subcutaneously) 3 times per week or darbepoietin (D-EPO; 0.1 μg/kg/week, subcutaneously) 1 time per week for 4 weeks. The endothelial-dependent vasodilatory response, NADPH oxidase activity, and gene expression of ICAM-1 and TNF-αwere assessed. To explore whether EPO is involved in superoxide production, in in vitro experiments, we stimulated human umbilical vein endothelial cells with EPO and assessed NADPH oxidase activity and NO production.

Results: The used doses of EPO and D-EPO had no effect on hemoglobin level. In the normal SD rats, the acetylcholine-dependent vasodilatory response decreased significantly in both the EPO and D-EPO treatment groups. NADPH oxidase activity as well as aortic gene expression of ICAM-1 and TNF-αincreased significantly, and to the same extent, in both groups.We confirmed EPO-mediated superoxide production in vitro. EPO increased NO levels as previously reported. However, because of parallel superoxide production, NO was consumed during the production of peroxynitrite associated with activation of NADPH oxidase.

Conclusions: Administration of EPO and D-EPO increased oxidative stress and impaired endothelial function in normal rats and in human endothelial cells.

Funding: Other NIH Support - The Kidney Foundation

SA-PO2830

Hyperphosphataemia Impairs Relaxation in Resistance Vessels; an Effect Which Is Partially Reversed in the Presence of a Phosphodiesterase Inhibitor Kathryn K. Stevens, Elisabeth C. Beattie, William A. Sands, Christian Delles, Alan G. Jardine. *Renal Research Group, ICAMS, University of Glasgow, United Kingdom.*

Background: The mechanism of action of phosphate as a risk factor for cardiovascular disease is unclear. This study looks at the effect of altered phosphate concentration on the function of rat resistance vessels

Methods: Resistance vessels were dissected from the mesentery of 12 week old male WKYs and incubated overnight in physiological saline solution (PSS) with normal (1.18mM) or high phosphate concentration (2.5mM). Vessels were mounted on a wire myograph. Vasoconstriction response to phenylepherine (PE) and vasorelaxation response to carbachol were measured. L-NAME was added and contractile response to PE measured again. In a separate experiment, following contraction with PE, vasorelaxation response to sodium nitroprusside (SNP) was measured. Experiments were repeated in the presence of zaprinast (phosphodiesterase 5 (PDE5) inhibitor). Concentration-response curves were constructed for each vessel for PE +/- L-NAME, carbachol and SNP.

Results: Vessels in high phosphate relax less well in response to both carbachol and SNP than those in normal phosphate PSS ($p<0.001$ and $p=0.029$). The contractile response to PE, in the absence of LNAME, between the 2 groups is similar. In normal phosphate PSS, with the addition of LNAME, a significant difference is seen in the concentration-response curve to PE ($p=0.05$). This difference is not seen in the vessels in high phosphate PSS. In the presence of zaprinest, there is improved relaxation in the vessels in high phosphate ($p=0.006$).

Conclusions: Elevated phosphate decreases endothelium dependent and independent vasorelaxation. This may be a marker of oxidative stress and endothelial dysfunction. The presence of a PDE5 inhibitor, which increases cyclic GMP, improves the relaxation response. Elevated phosphate may result in a combination of reduced production of basal nitric oxide within endothelial cells and cyclic GMP production or guanylate cyclase expression in vascular smooth muscle cells. These experiments mimic a uraemic state and may offer an explanation for elevated serum phosphate as a cardiovascular risk factor.

SA-PO2831

CD36-Na/K ATPase Signal Complex Mediates a Pro-Inflammatory Signaling Loop in Kidney David J. Kennedy,[1] Wenxin Huang,[1] Jiang Liu,[2] Zi-Jian Xie,[2] Joseph I. Shapiro,[2] Roy L. Silverstein.[1] *[1]Cell Biology, Cleveland Clinic, Cleveland, OH; [2]Medicine, University of Toledo, OH.*

Background: Pro-atherogenic, hyperlipidemic (HL) states are accompanied by increases in circulating ligands for scavenger receptor CD36 (e.g. oxLDL) and the signaling Na/K ATPase (e.g. ouabain-like cardiotonic steroids). These factors increase inflammation, oxidative stress, and progression of chronic kidney disease. We tested the hypothesis that ligands generated in HL accelerate renal inflammation through activation of a CD36-Na/K ATPase signaling complex, including potentiation of an inflammatory paracrine loop between proximal tubule (PT) cells and their associated macrophages (MΦ).

Methods: CD36$^-$ and CD36+(WT) mice on an apoE$^-$ background were fed a high-fat diet (HFD) for 32wks.

Results: Compared to WT, CD36$^-$ kidneys had less glomerular and tubulointerstitial Mφ accumulation, glomerular foam cell formation and mesangiolysis. CD36$^-$ Mφ also demonstrated decreased production of proinflammatory cytokines and ROS in response to oxLDL, ouabain, or plasma from HFD mice. Both oxLDL and ouabain increased attachment of WT but not CD36$^-$ Mφ to tissue culture plastic. In a modified Boyden chamber migration assay, WT Mφ showed increased migration to cell-free conditioned media from HK2 PT cells treated with either oxLDL or ouabain. OxLDL, ouabain, and HFD plasma stimulated ROS production in LLC-PK1 PT cells and this was inhibited by either N-acetyl-cysteine or apocynin. OxLDL induced ROS were also proportionally attenuated by RNAi-induced knock-down of the Na/K ATPase α-1 subunit in A411 cells (40% Na/Kα-1 knockdown) and PY17 (90% Na/Kα-1 knockdown). Cells transfected with control vector showed no effect. OxLDL and ouabain increased CD36 and Na/Kα-1 co-localization in both LLC-PK1 and Mφ as demonstrated by co-IP and a cell surface fluorescence proximity ligation assay. Finally, oxLDL and ouabain increased the content of both CD36 and Na/Kα-1 in HK2 PT early and late endosomes.

Conclusions: These data suggest that a CD36-Na/K ATPase signaling complex in both PT and Mφ facilitates the development of chronic inflammation that underlies the renal dysfunction common to HL states.

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SA-PO2832

Selective Estrogen Receptor Modulator Inhibits Fatty Acid-Induced Inflammasome Activation and Attenuates Proteinuria-Induced Tubular Injury in Mice Yuko Nishi, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Proteinuria is an independent risk factor for progressive renal diseases by initiating or aggravating tubulointerstitial injury. Albumin-bound free fatty acid (FFA) overloaded in proximal tubule evokes inflammatory responses. However, the mechanisms underlying the induction of inflammation have not yet been fully elucidated. Recent study showed that inflammasomes-dependent inflammatory responses were triggered by FFA and mitochondria-derived reactive oxygen species (ROS) were required for this response in adipose tissue. We hypothesized that albumin-bound FFA would trigger inflmmasomes activation through mitochondrial ROS production and raloxifene, selective estrogen receptor modulator, could ameliorate tubular injury by reducing inflammasomes-activation associated with mitochondrial oxidative stress.

Methods: Female ICR-driven glomerulonephritis (ICGN) mice, an inbred strain with hereditary nephrotic syndrome, underwent ovariectomy and treatment with raloxifene. Human renal proximal epithelial cells were cultured with human fatty acid-bearing human albumin (FA-HSA) or human fatty acid free human albumin (Free-HSA) for 24 h with or without raloxifene and antiestrogen, ICI 182,780.

Results: ICGN mice showed tubular activation of inflammasomes and elevated imflammasomes-dependent cytokines. Raloxifene attenuated these changes and ameliorated tubular damages. Moreover, raloxifene reduced mitochondrial ROS production, prevented morphological changes of mitochondria, and improved mitochondrial respiratory function. FA-HSA but not Free-HSA caused loss of mitochondrial membrane potential, increased oxidative stress, and inflammasomes activation. Pretreatment with raloxifene improved the FA-HSA-induced changes via amelioration of mitochondrial function. These beneficial effects of raloxifene were blocked by co-incubation with ICI 182,780.

Conclusions: Albumin bound FFA activates inflammasomes in tubular cells through induction of mitochondrial ROS production. Thus, Inflammasomes could be regarded as a novel and promising therapeutic target for proteinuria-induced renal injuries.

SA-PO2833

Disintegration of Colonic Mucosal Tight Junction in Uremia: A Likely Cause of CKD-Associated Inflammation Nosratola D. Vaziri, Ardeshir Rahimi, Jun Yuan, Hyder Said, Veedamali Subramanian. *Medicine/Nephrology, University of California, Irvine, CA.*

Background: Inflammation is a constant features and a key mediator of progression of CKD and its cardiovascular complications. Inflammation in CKD has been attributed to uremic toxins, co-morbid conditions, infections, dialysis procedure etc. However, little attention is paid to the potential role of the gut and its microbial flora in the CKD-induced inflammation. CKD patients frequently exhibit endotoxemia without detectable infection. Intestinal epithelium and its tight junction form a barrier which prevents entry of microbes and their byproducts in the internal milieu. We tested the hypothesis that uremia may impair structure and function of intestinal tight junction, facilitating leakage of toxic/pro-inflammatory byproducts of the microbial flora in the circulation.

Methods: SD rats were randomized to undergo 5/6 nephrectomy (CKD) or sham-operation (control) and observed for 8 weeks. In a separate experiment SD rats were rendered uremic by addition of 0.7% adenine to their food for 2 weeks and observed for 2 weeks. Rats consuming regular diet served as controls. The animals were euthanized and colon was harvested and processed for expression of the key constituents of the tight junction using RT-PCR, Western blot analysis and immunohistology.

Results: The animals with CKD exhibited azotemia, reduced creatinine clearance and marked reductions ($P<.001$) in protein expressions of claudin-1 (70-90%), occludin-1(50%-70%), ZO- 1 (80%-90%) and phosphorylated myosin light chain in ascending and descending colon. The reduction of the given proteins was confirmed by immunohistological examinations. In contrast, mRNA abundance of claudin-1, occludin-1 and ZO-1 were elevated or unchanged pointing to the possible post-transcriptional/post-translational modification as a cause of the observed tight junction protein depletion.

Conclusions: The study revealed for the first time that uremia results in disintegration of the colonic tight junction, a phenomenon which can contribute to the systemic inflammation and common occurrence of endotoxemia in advanced CKD.

SA-PO2834

Spironolactone (Sp) Prevents Chronic Kidney Disease (CKD) Induced by Acute Kidney Injury (AKI) Jonatan Barrera-Chimal, Rosalba Pérez-Villalva, Roxana Rodriguez, Juan Reyna, Norma Bobadilla. *Molecular Physiology Unit, Instituto de Investigaciones Biomédicas, UNAM and Instituto Nacional de Ciencias Médicas y Nutrición SZ, Mexico, Mexico.*

Background: AKI has been recognized as a risk factor to develop CKD, however the mechanisms involved have not been elucidated yet. We previously demonstrated that Sp prevents renal injury induced by ischemia/reperfusion (I/R). This study was designed to: 1) develop an experimental model that leads to CKD induced by AKI, 2) study the mechanisms by which AKI may lead to CKD and 3) determine if preventing AKI with Sp protects against CKD.

Methods: Forty Wistar rats were divided in: 1) Sham-operated, 2) Sp-treated group (20mg/Kg), 3) rats underwent to bilateral ischemia (45') and 4) rats receiving Sp before I/R (Sp+I/R). All groups were followed through 270 days. Proteinuria (UProtV) and urinary Kim-1 (UKim) were evaluated every 30 days. At the end, creatinine clearance (CrC) and renal blood flow were measured. Right kidney was used for molecular studies and the left for histopathological analysis.

Results: Rats underwent to I/R exhibited increased mortality rate by 57% and developed CKD characterized by a progressive increase in UProtV and UKim, together with a fall in CrC and RBF. Glomerular hypertrophy and focal sclerosis, extensive tubular dilation, tubular proliferation and tubule-interstitial fibrosis were also observed. These alterations were associated with an up-regulation of αSMA and TGFβ and its downstream effectors: p-Smad 3, collagen-I, and fibronectin. Also, an up-regulation of TGFα and inflammatory cytokines were observed. In Sp+I/R group mortality, Uprot, UKim, renal dysfunction and structural injure were prevented. Renal architecture preservation was associated with prevention of αSMA up-regulation, reduction of pro-inflammatory cytokines, profibrotic factors and its target genes.

Conclusions: Here we show a new model of CKD induced by AKI. In this model, the mechanisms responsible of CKD progression were mediated by up-regulation of αSMA, TGFα and TGFβ, together with a greater activation of TGFβ pathway and inflammatory response. Intriguingly, we show for the first time that Sp is a novel treatment to prevent CKD induced by AKI.

Funding: Government Support - Non-U.S.

SA-PO2835

Clinical Implication of Tubulointerstitial Inflammatary Cell Infiltration in IgA Nephropathy Haifeng Ni, Linli Lv, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: IgA nephropathy is the most common glomerular disease which could progressively progressed to end stage renal failure. While inflammatary cell infiltration in tubulointerstitium is commonly involved in IgA nephropathy (IgAN) , the importance of such involvement is not well defined. In the present study, we evaluated the infiltration of inflammatory cells in renal tubulointerstitium with IgAN and its clinical implication.

Methods: We have evaluated the infiltrating immune cells in renal biopsies from 33 patients with IgAN using immunohistochemical techniques with antibodies against T cells (CD3,CD4, CD8,), B cells (CD20), macrophages (CD68) and follicular dendritic

cells (CD21). Progression in renal disease was defined as an elevation of serum creatinine above the normal limit and over 20% from baseline.

Results: It was shown that positive rates in progressive disease were 100% for CD3,100% for CD4,89% for CD8, 33.33% for CD20, 11.11% for CD21. Those of CD3, CD4, CD8 and CD68 were significantly higher than in Stable disease. Clinical pathological analysis showed that a positive correlation between the number of interstitial CD3+T cell, CD68+ macrophages and the level of serum creatinine (p=0.09,p=0.005) and proteinuria (p=0.011,p=0.007) at the time of biopsy was found. Moreover, In the renal tubulointerstitium, the number of CD3+, CD4+, CD68+cells correlated with interstitial fibrosis (p=0.004,p=0.01,p=0.008) and the number of CD8+cells correlated with segmental glomerulosclerosis (p=0.03). In the multivariate analysis, the number of tubulointerstitial CD3+ and CD68+ cells were independently associated with progressive disease in IgAN.

Conclusions: Our study demonstrated that interstitial CD3+ and CD68+macrophages play an important role in causing the progression of IgAN. It might hold a great promise in predicting the prognosis of IgAN patients by evaluating inflammatary cells infiltration in renal tubulointerstitium.

Funding: Government Support - Non-U.S.

SA-PO2836

Bardoxolone Methyl Transcriptionally Regulates Transaminase Levels and Increases Glutathione Levels Gregory A. Miller, Ron Bumeister, Jeffrey Laidlaw, Pritam Kambuj, Brandon Probst, Deborah A. Ferguson, W. Christian Wigley. *Reata Pharmaceuticals, Inc., Irving, TX.*

Background: Bardoxolone methyl is the lead molecule from the Antioxidant Inflammation Modulator (AIM) class that potently induces Nrf2, a transcriptional regulator of many antioxidant and cytoprotective genes. In a 52-week randomized, placebo-controlled clinical trial in patients with chronic kidney disease and type 2 diabetes, 98% of patients treated with bardoxolone methyl had transaminase elevations above baseline levels, while 71% had transaminase elevations ≥2X the upper limit of normal. The transaminase elevations were transient, peaking within 2 to 4 weeks of treatment initiation or dose escalation, and generally resolved without drug discontinuation. Transaminase elevations did not recur once resolved and were not associated with liver toxicity.

Methods: Studies were undertaken to investigate the molecular mechanism underlying these observations.

Results: Treatment with bardoxolone methyl and other AIMs increased transaminase mRNA and protein levels in hepatocytes, myocytes, renal cells, and macrophages in a dose- and time-dependent manner. Treatment of HuH-7 human hepatoma cells and AML-12 mouse hepatocytes with bardoxolone methyl resulted in time- and dose-dependent increases in glutathione (GSH) levels. Transaminase enzymatic reactions produce GSH, an essential antioxidant molecule. Consistent with this, microarray analysis of bardoxolone methyl-treated HepG2 hepatoma cells revealed induction of several genes involved in GSH production, as well as the production of GSH precursors. We observed that siRNA knockdown of ALT1 in AML-12 mouse hepatocytes resulted in reduced glutamate levels. Furthermore, siRNA knockdown of ALT2 in HuH-7 human hepatoma cells resulted in up to a 50% reduction in total GSH levels.

Conclusions: These data suggest that transcriptional elevation of transaminases may reflect increased demand for glutamate as a result of bardoxolone methyl-mediated increases in glutathione production.

Funding: Pharmaceutical Company Support

SA-PO2837

CD11c Positive Cells Recruitment Is Critical for the HFD-Induced Kidney Injury Wenjian Wang,[1] Wei Shi,[1] Xinling Liang,[1] Farhad R. Danesh.[2] *[1]Division of Nephrology, Gaungdong Genral Hospital, Gaungzhou, Guangdong, China; [2]Division of Nephrology, Baylor College of Medicine, Houston, TX.*

Background: CD11c is a type I transmembrane protein found at high levels on most human dendritic cells, but also on monocytes macrophages, neutrophils, and some B cells that induces cellular activation. Recently, a specific subset of CD11c positive macrophages was shown to be recruited to obese adipose and muscle tissue. This subset expresses CD11c and produces high levels of proinflammatory cytokines that are linked to the adipose tissue injury. CD11c+ cell ablation leads to a marked decrease in inflammatory markers, both locally and systemically, as reflected by gene expression and protein levels.

Methods: With a method by which mice were subjected to unilateral nephrectomy and followed by either high-fat diet (HFD) or normal diet (ND) for 16 weeks, we successfully established a HFD-induced kidney injury model evidenced by significant deposition of lipid, fibronectin, collagen I, and collagen III in the kidneys.

Results: Those HFD-induced kidnry injury mice displayed markedly increased plasma levels of MCP-1, IL-6, and TNF-α and reactive oxygen species production in the kidneys compared with ND mice. Interestingly, a significantly increased recruitment of specific subset CD 11c positive cells were observed in the kidneys of HDF mice compared to the volume of ND mice. Importantly, when the HDF mice were pretreated with 500 rad of total body irradiation, a significantly decreased numbers of CD11c positive inflammatory cells as well as a markedly ameliorated kidney injury locally, and a marked decrease in proinflammatory cytokines systemically were observed compared to the no pretreated mice.

Conclusions: Our findings provide new insights into the role of CD11c positive cells in lipid-induced kidney injury, and offer a potential novel target in preventing the progression of chronic kidney disease elicited by HDF-induce hyperlipidemia.

Funding: Government Support - Non-U.S.

SA-PO2838

Differentiating Profibrotic and Anti-Inflammatory Effects of TGF-β1 by Targeting β-Catenin Guoping Zheng,[1] Xinrui Tian,[2] Jianlin Zhang,[2] Thian Kui Tan,[1] So Ra Lee,[1] Tim Tzu-Ting Hsu,[1] Ya Wang,[1] Qi Cao,[1] Dong Zheng,[1] Yiping Wang,[1] Changqi Wang,[1] Vincent W.S. Lee,[1] David C. Harris.[1] *[1]Centre for Transplantation and Renal Research, University of Sydney, Sydney, NSW, Australia; [2]Shanxi Medical University, Taiyuan, Shanxi, China.*

Background: TGF-β1 is known to be both anti-inflammatory and profibrotic. Epithelial-mesenchymal transition (EMT) is an important mechanism for TGF-β1-mediated fibrosis, in which β-catenin plays a role. However, the dependence on β-catenin of TGF-β1-induced EMT has never been fully explained. Whether β-catenin plays a role in the anti-inflammatory effects of TGF-β1 is unknown.

Methods: A protein knockdown chimera (F-Trcp-Ecad) was used for specific degradation of cytosolic β-catenin in C1.1 renal tubular epithelial cells and J774 macrophages. TGF-β1-induced EMT in C1.1 cells, inhibition of J774 activation (by LPS/IFN-γ), β-catenin/p-Smad/LEF-1 interactions and β-catenin Topflash activity were analysed.

Results: TGF-β1-induced EMT, E-cadherin promoter repression, snail transcription and MMP-9 activity were reduced in C1.1 cells expressing F-TrCP-Ecad. F-TrCP-Ecad selectively degraded cytosolic β-catenin, and blocked TGF-β1-induced Smad3/β-catenin complex formation with no involvement of β-catenin/LEF-1 complex and Top/Fop flash activity. In contrast, the steady state level of β-catenin in J774 macrophages was low and was not changed when exposed to IFN-γ or LPS with or without TGF-β1. TGF-β1 inhibition of LPS-induced TNF-α and IFN-γ-stimulated iNOS mRNA expression was not affected in J774 cells expressing wild type β-catenin or F-TrCP-Ecad. Neither β-catenin/Smad3 or β-catenin/LEF-1 complex formation or β-catenin signaling was involved in the TGF-β1 inhibition of macrophage.

Conclusions: TGF-β1 induces EMT through a β-catenin/p-Smad3 dependent mechanism in C1.1 cells, but inhibits macrophage activation independently of β-catenin in J774 cells. Degradation of cytosolic β-catenin inhibits TGF-β1-induced EMT, but not anti-inflammatory effects of TGF-β1. β-catenin dependency of profibrotic but not anti-inflammatory actions of TGF-β1 expose β-catenin as a key therapeutic target.

Funding: Government Support - Non-U.S.

SA-PO2839

Do Cytokines at Moderately Elevated Concentrations in CKD Really Induce Leukocyte Activity? Nathalie Neirynck, Griet L.R.L. Glorieux, Eva Schepers, Raymond C. Vanholder. *Internal Medicine, Nephrology, University Hospital Ghent, Ghent, Belgium.*

Background: Oxidative stress induced by uremic retention products is one of the mechanisms involved in the pro-inflammatory status and the cardiovascular morbidity and mortality of CKD. IL6, TNFα, IL1β and IL18 are moderately elevated in CKD. In observational studies, IL6 and TNFα were associated with increased cardiovascular mortality in CKD. Although extensively studied at high concentrations as in sepsis, low concentrations as observed in uremia have to the best of our knowledge rarely been evaluated. The present study investigated whether IL6, TNFα, IL1β and IL18, as occurring in CKD, induced oxidative burst in leukocytes.

Methods: Whole blood of healthy volunteers was incubated in vitro with different concentrations of cytokines, ranging from 5 to 102.8pg/ml for IL6, from 20 to 1400pg/ml for TNFα, from 20 to 400pg/ml for IL1β and from 75 to 1200pg/ml for IL18. Oxidative burst in leukocytes in basal conditions and after stimulation with fMLP, E.coli and PMA, was measured by flow cytometry.

Results: At baseline, TNFα and IL6 increase the percentage of ROS-producing monocytes and granulocytes from the lowest concentration on (P<0.05), while no effects were seen with IL1β and IL18. After stimulation with fMLP, TNFα increased the percentage of ROS-producing monocytes and granulocytes in uremic concentrations, while IL6 suppressed oxidative burst in granulocytes at various uremic concentrations (P<0.05). After stimulation with E.coli, IL18 increased the percentage of ROS-producing cells (P<0.05, all leukocyte cell types). After E.coli stimulation, the mean ROS-production per cell in monocytes increased with IL6 (P<0.05 at 21.9pg/ml and 95.4pg/ml), while it was suppressed by TNFα from 70pg/ml on (P<0.05). After stimulation with PMA there were no significant effects.

Conclusions: Our data indicate that although cytokines appear to influence inflammatory status, there is certainly no consistent evolution in uremic concentrations. First of all, in basal conditions only some cytokines (TNFα and IL6) are pro-inflammatory while others (IL1β and IL18) are not. In activated leukocytes, some cytokines appear to have stimulatory and inhibitory effect as well.

Funding: Government Support - Non-U.S.

SA-PO2840

Protective Effect of Electrolyzed Water with High Dissolved Hydrogen (H_2) on the Development of Cardiorenal Syndrome by Aging in Dahl Salt Sensitive Rat Wan-Jun Zhu,[1,2] Masaaki Nakayama,[1,3] Shigeru Kabayama,[1,2] Sadayoshi Ito.[1] *[1]Center for Advanced and Integrated Renal Science, Tohoku University, Sendai, Japan; [2]Department of Blood Purification, Tohoku University Hospital, Sendai, Japan; [3]Fukushima Medical University, Fukushima, Japan.*

Background: Electrolyzed water (EW) exhibits high dissolved H_2 (DH). We recently reported that EW protects kidney and heart tissue from injury induced by ischemia reperfusion. Oxidative stress and inflammation play a crucial role for chronic kidney injury by aging. The present study aims to test the effect of EW drinking on the development of cardio-renal tissue injury by aging.

Methods: Dahl salt sensitive male rats (n=90) were divided into three groups: filter water (FW; DH 0.0mmol/L) (TI9000, Nihon Trim, Osaka), de-gass EW (DW; DH 0.35 mmol/L), and EW (DH 0.0 mmol/L) for *ad lib* drinking (n=30 each). They were fed with 0.5% salt diet during the study. Blood pressure (BP) were measured by tail cuff method every 4weeks. Echocardiography, and tissue samplings of kidney and heart, were performed at 16th, 24th, and 48th week.

Results: There were no differences during the study in body weight, water and food consumption among the groups, but the BP was the lowest in EW ($p<0.05$). Regarding the test parameters, no differences were found at 16th, 24th week, but the following parameter levels or changes (vs. 16th week) were significantly less in EW as compared to counterparts at 48th week ($p<0.05$); (Heart) left ventricle posterior wall thickness: FW 14.4%, DW 12.7%, EW 6.3%; cardiomyocyte size (μm): FW 43.2%, DW 14.0%, EW 0%; heart tissue fibrosis: FW 300.4%, DW 155.2%, EW 25.1%; ED1 staining (number/slice): FW 28.1±1.46, DW 20.5±1.29, EW 12.1±2.41; malondialdehyde (MDA) staining (%/field):FW 63.7±2.64, DW 70.0±3.94, EW 44.6±2.92, (Kidney): ED1 cells in cortex (number/slice): FW 113.5±13.11, DW 88.2±11.85, EW: 75.3±3.04; MDA staining in cortex (%/field): FW 60.5±1.18, DW 66.2±1.48, EW 57.2±1.20.

Conclusions: *Ad lib* drinking of high H_2 water could suppress the development of cardiorenal tissue injury of Dahl Salt sensitive rat by aging, at least partly, through the mechanism of attenuating inflammation and oxidative stress.

Funding: Pharmaceutical Company Support

SA-PO2841

Uremic Toxins Inhibit Glucuronidation in Human Proximal Tubule Epithelial Cells Henricus A.M. Mutsaers,[1,2] Dorien Reijnders,[1] Martijn J. Wilmer,[1] Hanneke Wittgen,[1] Lambertus Vd Heuvel,[3] Joost G. Hoenderop,[2] Rosalinde Masereeuw.[1] *[1]Pharmacology & Toxicology, Radboud University Nijmegen Medical Centre, Netherlands; [2]Physiology, Radboud University Nijmegen Medical Centre, Netherlands; [3]Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Metabolic enzymes play a key role in the clearance and bioavailability of drugs. During chronic kidney disease (CKD), metabolism is affected leading to altered drug disposition. Furthermore, there is a progressive accumulation of uremic retention solutes due to impaired renal clearance. Here we investigated the impact of uremic toxins on the functionality of an important class of phase II enzymes, *viz.* UDP-glucuronosyltransferases (UGTs).

Methods: Gene expression of phase I and II enzymes in conditionally immortalized renal proximal tubule epithelial cells (ciPTEC) was studied via a qPCR array and UGT protein expression was investigated via Western blot. In addition, the glucuronidation activity of a subset of UGTs in ciPTEC, either untreated or exposed to uremic toxins for 48h, was studied by high performance liquid chromatography using 7-hydroxycoumarin (7-OCH) as a substrate.

Results: Our results showed that ciPTEC express a wide variety of metabolic enzymes, similar to primary proximal tubule epithelial cells, including cytochrome P450 enzymes and UGTs. Especially UGT1A1, 1A9, 2B7 and 2B28 are highly expressed in ciPTEC (Ct: 25, 17, 24 and 25, respectively; Ct GAPDH: 23) and UGTs were demonstrated to be functionally active (7-OCH-glucuronide Km: 12 ± 2 μM, Vmax: 242 ± 10 pmol/min.mg). Furthermore, exposure of ciPTEC to non-toxic concentrations of indoxyl sulfate, oxalate, putrescine, p-toluenesulfonic acid or a mix of these toxins significantly decreased the glucuronidation of 7-OHC with 20%, 14%, 18%, 16% and 41%, respectively. Moreover, UGT1A and 2B protein expressions remained unaltered following exposure to uremic toxins, suggesting that the observed inhibition occurs via a direct enzyme interaction.

Conclusions: In conclusion, uremic toxins inhibit UGT function in ciPTEC, thereby affecting the metabolic capacity of the kidney. This may have a clinically significant impact on pharmacokinetics in CKD patients.

Funding: Private Foundation Support

SA-PO2842

Elevated Soluble Flt1 Mediates an Anti-Angiogenic State in Patients with ANCA-Associated Vasculitis Caroline Vercel,[1] Ruth J. Pepper,[2] H. Terence Cook,[3] Alan D. Salama,[2] Fadi Fakhouri.[1] *[1]Nephrology and Immunology, CHU de Nantes, Nantes, France; [2]Centre for Nephrology, University College London, London, United Kingdom; [3]Centre for Complement & Inflammation Research, Division of Immunology and Inflammation, Department of Medicine, Imperial College London, United Kingdom.*

Background: Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) are a group of necrotizing small vessel vasculitis. Little is known regarding endothelium survival and repair in these diseases.

This study aims to demonstrate that elevated levels of sFlt1 (soluble fms like tyrosine kinase1), an inhibitor of VEGF, induce an anti-angiogenic state that could impair vascular regeneration.

Methods: Circulating sFlt-1 levels were determined by ELISA, during active disease and remission, in plasma from patients with Proteinase 3 (PR3)-AAV(n=40), Myeloperoxydase(MPO)-AAV (n=23), and healthy controls (n=18).

To assess the anti-angiogenic activity of these plasma; we used the chick chorioallantoic membrane(CAM) model of angiogenesis. To determine the source of sFlt-1 in AAV patients, HUVEC and monocytes were incubated with: PR3 and MPO-AAV patients' plasma drawn during acute or remission phase and monoclonal anti-PR3 and anti-MPO antibodies. For each condition, sFlt-1 level was measured in cells supernatants.

Results: sFlt1 serum levels increased during active AAV in patients with PR3-ANCA(mean 7304 [321-47355] pg/ml; $p<0,001$ vs controls(mean 120 [82-168] pg/ml))and MPO-ANCA(mean 2242 [31-16851] pg/ml; $p<0,001$ vs controls). sFlt1 levels decreased during remission but remained higher than controls. Plasma from patients with acute AAV displayed an anti-angiogenic effect in the CAM model, an effect prevented by incubating serum with an excess of VEGF.

Monoclonal anti-PR3 antibodies and plasma from patients with acute PR3-AAV induced a significant and sustained sFlt1 release from monocytes. Anti-MPO antibodies and plasma from acute MPO-AAV had no effect.

Conclusions: Anti-PR3 antibodies mediate an increase in sFlt1 during acute ANCA-associated vasculitis that sustains an anti-angiogenic state.sFlt1 may be an optimal tool for the assessment of AAV prognosis and blocking sFlt1 may enhance renal recovery in AAV patients.

SA-PO2843

Cyclosporine A Treatment for idiopathic Membranous Nephropathy: A Systematic Review and Meta-Analysis Ge Xiao, Liya Yang, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: Treatment of idiopathic membranous nephropathy (IMN) is still a challenge for nephrologists. Cyclosporine A (CSA) has recently been used as an commonly immunosuppressant in the treatment of IMN. However, its clinical effect has not been clear. In this study, a systematic review and meta-analysis were performed to evaluate the efficacy and adverse effect of CSA in treatment of IMN.

Methods: Publications in the English literature were searched with the keywords or text words: 'cyclosporine', 'glomerulonephritis', 'membranous', 'membranous nephropathy' , 'membranous glomerulopathy', 'membranous glomerulonephropathy', 'idiopathic membranous nephropathy', 'idiopathic membranous glomerulonephritis' for clinical trials in electronic databases. Primary outcome was relative risks (RRs) of complete and total renal remission at the end of study period. Secondary outcome included RRs of deterioration of renal function, relapse, development of end-stage renal disease (ESRD) and hypertension.

Results: Five randomized controlled trials (RCTs) and two clinical controlled trials (CCTs) involving 353 patients were included. CSA offers better efficacy in inducing complete renal remission rates than other regimens (RR 1.68, 95%CI 1.18 - 2.18, p=0.002). But CSA did not increase total renal remission rates compared with control group (RR 1.18, 95%CI 0.85 - 1.64, p=0.31). After removing the Kosmadakis trial whose follow-up was less than 1 year, the total renal remission rates was also higher in patients receiving CSA than those receiving other regimens(RR 1.46, 95%CI 1.20 - 1.77, p=0.0001). CSA tended to deteriorate renal function (RR 1.44, 95%CI 0.98 - 2.14, p=0.07) compared with control group. However, there's no significant difference between two groups in the risks of relapse (RR 1.26, 95%CI 0.35 - 4.49, p=0.72), hypertension(RR 1.50, 95%CI 0.80 - 2.81, p= 0.20)and ESRD (RR 0.63, 95%CI 0.25 - 1.56, p= 0.32).

Conclusions: This study suggested that CSA was effective in inducing complete remission in IMN than other regimens. However, CSA appears to cause a decline of renal function which recovers after dosage reduction or discontinuation.

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SA-PO2844

New Retinoic Acid Receptor Agonists for Treatment of Kidney Disease Yifei Zhong,[2] Ruijie Liu,[1] Peter Y. Chuang,[1] John C. He.[1] *[1]Medicine, Mount Sinai School of Medicine, New York, NY; [2]Nephrology, Shanghai University of Traditional Chinese Medicine, Shanghai, China.*

Background: There is a lack of the treatment options for kidney glomerular disease including HIV-associated nephropathy (HIVAN). Podocyte injury is a major cause of glomerular disease. Thus, the strategy to develop effective drugs to protect podocytes

from injury is critical for treating patients with glomerular disease. Retinoic acid reduces proteinuria and glomerulosclerosis in multiple animal models of kidney disease. However, the clinical studies are limited because all-trans retinoic acid (ATRA) has significant side effects. Animal studies suggest that ATRA attenuates proteinuria likely through protection of podocytes from injury. The protective effects of ATRA are though binding to the retinoic acid receptor-alpha (RARa). We hypothesize that the RARa specific agonists might be potential drugs for treating patients with kidney disease with significantly less side effects than ATRA.

Methods: To test our hypothesis, we first designed a new lead compound (BD4), which can bind to RARa receptor specifically and has much lower toxicity based on the structure prediction and in vitro cell toxicity assay. We examined the effects of BD4 in podocyte differentiation in vitro. We tested the effects of BD4 in vivo by treating the animal model for HIVAN (Tg26) with either BD4 or vehicle from age of 4 weeks to 10 weeks. Then, we assessed proteinuria and kidney histology in these mice.

Results: BD 4 is a unique compound as this is the first retinoids containing boronic acid. We found that BD4 induces expression of podocyte differentiation markers including synaptopodin, nephrin, and WT-1 in podocytes similar to the effects of ATRA. We confirmed that BD4 reduces proteinuria and improves kidney injury in HIV-1 transgenic mice, a model for HIV-associated nephropathy. BD4 treated mice did not develop any obvious toxic or side effects.

Conclusions: Our data suggest that BD4 is a potential new RARa agonist with less toxicity for treatment of patients with kidney disease including HIVAN.

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SA-PO2845

KLF15 Reduces the Threshold for Podocyte Injury Sandeep K. Mallipattu, Ruijie Liu, Peter Y. Chuang, Yan Dai, John C. He. *Nephrology, Mount Sinai School of Medicine, New York, NY.*

Background: Podocyte injury resulting from a loss of podocyte differentiation has been implicated in many glomerular diseases. It has been previously shown that Retinoic Acid (RA) induces podocyte differentiation via stimulation of cAMP/PKA/CREB pathway. Previous computation analysis revealed that Krupper-Like Factor 15 (KLF15), a kidney enriched CREB targeted nuclear transcription factor, is highly regulated in RA mediated podocyte differentiation and binds to the promoter region of many podocyte specific genes. RA was shown to increase KLF15 expression in cultured wild-type and HIV infected podocytes. KLF15 expression was suppressed in in vitro and in vivo models of HIV-associated nephropathy. KLF15 over-expression stimulated the expression of podocyte differentiation markers in wild-type and HIV-infected murine podocytes. KLF15 binds to the promoter region of slit diaphragm proteins in RA treated murine podocytes. Although, KLF15-/- mice have minimal podocyte injury at baseline, we hypothesize that KLF15 may reduce the threshold for podocyte injury.

Methods: We used two known murine models of podocyte injury to test our hypothesis. Initially, wild-type and KLF15-/- mice were administered low dose Lipopolysaccharide (LPS) (10ug/g) and urine was collected and mice were sacrificed at 48 hours. Similarly, wild-type and KLF15-/- mice were administered Adriamycin (20mg/kg) and urine was collected and mice were sacrificed at four weeks.

Results: Compared to the control group, we observed that LPS treated KLF15-/- mice had a 4-5X increase in albuminuria with significant increase in podocyte effacement. Similarly, the Adriamycin treated KLF15-/- mice had a 15-20X increase in albuminuria with significant podocyte effacement and glomerular basement membrane thinning.

Conclusions: Although a lack of KLF15 expression results in minimal podocyte injury, additional injury (LPS/Adriamycin) results in significant podocyte effacement and albuminuria. This indicates that KLF15 plays a vital role in reducing the threshold for podocyte injury.

Funding: NIDDK Support

SA-PO2846

Podocyturia in Patients Treated with Anti-VEGF Therapy for Cancer Correlates with the Level of Proteinuria Juan C. Calle,[1] Iasmina Craici,[1] Steven Wagner,[1] Aminah Jatoi,[2] Eddie L. Greene,[1] Joseph P. Grande,[3] Vesna D. Garovic.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Oncology, Mayo Clinic, Rochester, MN; [3]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Background: Proteinuria is a recognized side effect of anti-VEGF (Vascular Endothelial Growth Factor) medications. Similarly, podocyturia (excretion of viable podocytes in the urine) has been observed in various proteinuric diseases including patients treated with anti-VEGF therapy. In this study, we report the largest series to date analyzing podocyturia in patients undergoing anti-VEGF therapy with and without proteinuria.

Methods: The aim of the study was twofold: 1. to demonstrate the presence of podocyturia in patients developing proteinuria while undergoing anti-VEGF therapy and 2. quantitate the observed higher level of viable podocyte excretion in the urine of patients with greater than 500 mg of proteinuria/24 hours calculated by the protein to creatinine ratio.

For identification of viable podocytes, overnight cultures of urine sediment followed by staining with podocin antibodies and a secondary FITC–labeled antibody were performed.

Results:

	Proteinuria ≤500 mg/24 hours	Proteinuria ≥500 mg/24 hours
# of patients	28	12
Age range (mean)	34-79 (59) years	51-73 (63) years
Female n (%)	19 (68)	7 (58)
Type of cancer		
Lung cancer and pulmonary carcinoids	3	1
Ovarian cancer	3	3
Gastrointestinal system cancer and carcinoids	12	4
Hemangioendothelioma	2	0
Central nervous system	2	2
Breast cancer	2	0
Melanoma	1	0
Renal cell carcinoma	3	2
Type of anti-VEGF treatment		
Bevacizumab	23	11
Bevacizumab + Sunitinib	1	0
Bevacizumab + Sorafenib	3	0
Sunitinib	0	1
Sorafenib	1	0
Podocyturia (cells/mg of creatinine)		
Range	**0-1.64**	**0-14.55**
Mean*	**0.17**	**2.67**

***p value = 0.0068. 95% CI for proteinuria ≤500 mg/24 (0.06–0.17); ≥500 mg/24 hour (0.82–3.56)**

Conclusions: Our data demonstrate two major findings: 1. the presence of podocyturia accompanies proteinuria in patients undergoing anti-VEGF therapy regardless of the type of cancer and 2. levels of podocyturia are higher in patients with proteinuria ≥500 mg/24 hours when compared to patients excreting ≤500 mg/24 hours.

SA-PO2847

Serum Phosphate in the Nephrotic Syndrome Varies with Degree of Proteinuria but Is Independantly Associated with High Pulse Wave Velocity Donal John Sexton, Sinead Kinsella, Joseph A. Eustace. *Department of Renal Medicine, Cork University Hospital, Cork, Ireland.*

Background: The prevalence and determinants of abnormal vascular stiffness in the Nephrotic Syndrome is not well defined.

Methods: We conducted a cross-sectional study of 42 prevalent non-diabetic adult nephrotics, measuring carotid-femoral Pulse Wave Velocity (PWV) (MicroMedical Pulse Trace v2.12) at a median (intra quartile range (IQR)) of 23 (10 - 47) months of follow-up. PWV was dichotomized using age specific cutoffs (Eur Heart J, 2010). Relationship between variables from time of renal referral, at time of PWV testing and time averaged (Time Av) over the above interval were quantified by non-parametric (Spearman's) correlation and linear regression using SPSS v18.

Results: Mean age was 44(18) yrs, 74% were male, 38% had FSGS, 19% MN, 12% MCD; 40.5% had an early full remission, 28.6% never remitted and 31% relapsed. 48% were on current immunosupression.

At presentation median (IQR) CKD-EPI eGFR and spot urine protein creatinine ratio (urPCR) was 80 (56-90) ml/min and 4.4 g/g (2.9-7.1); mean (sd) serum phosphate (P), total cholesterol (TC) and albumin were 1.34 (0.23) mmol/L, 8.2 (3.3) mmol/L and 25 (9.8) g/dl; referral urPCR correlated with P, TC and albumin, all $p<0.01$. P was associated with urPCR on univariate analysis ($\beta=0.43$, $p=0.007$) and when adjusting for eGFR ($\beta=0.42$, $p<0.01$) but not with eGFR.

Median (IQR) PWV was 7.1(4-11) m/sec. PWV did not correlate with the serum TC, any lipoprotein subfraction, mean arterial pressure, eGFR, albumin or urPCR at referrral, follow-up or Time Av values. PWV only correlated with follow-up P ($r=0.49$, $P=0.001$) and Time Av P ($r=0.51$, $P=0.001$).

An elevated, age-specific, PWV was present in 52.4% (22/42) overall and in 65% (13/20) of those <40 year old. Time Av P was independantly associated with PWV ($\beta=1.58$, $p<0.001$) simultaneously adjusting for age, gender and eGFR and separately when adjusting for Time av {eGFR, TC and urPCR} ($\beta=1.72$, $p=0.002$)

Conclusions: PWV is elevated in nephrotics. Serum phosphate levels vary with the degree of proteinuria independantly of GFR and associate with high PWV independantly of blood pressure, dyslipidaemia, GFR or proteinuria.

SA-PO2848

Measurement of D-dimer Levels in the Nephrotic Syndrome Donal John Sexton, Marek J. Mazur, Joseph A. Eustace. *Department of Renal Medicine, Cork University Hospital, Cork, Ireland.*

Background: The Nephrotic Syndrome is associated with a significant increased risk of thromboembolism. D dimer is commonly used as a screening test for thrombosis in general practice, but prevalent D dimer levels -and its associations- in the nephrotic syndrome in the absence of overt thrombosis is poorly characterised.

Methods: We conducted a cross sectional study of 97 patients with the nephrotic syndrome. Ddimer were measured using either BIOPOOL MiniQuant or Siemens INNOVANCE assays. No patient had clinical evidence of thrombosis or was on anticoagulation. Univariate and multivariate linear regression models were used to examine associations with Ln-transformed D dimer (Ln- DDimer) levels, expressed as percentage of the assay specific reference range, using SPSS v18 and a type one error rate of 0.05.

The descriptive statistics are expressed as median (25-75th centile) unless otherwise stated.

Results: Study characteristics were (n=97) MDRD eGFR: 49 ml/min (30-90), protein:creatinine ratio (PCR) 2.0 g/g (0.8-4.4), , urate 434 umol/L (332-545), mean (sd) age 55yrs (20), mean (sd) serum albumin 34g/dl (8.4). 5% had a raised CRP. On separate univariate linear regression models the β (95% CI) for the association with Ln-DDimer was: age (decades) 0.54 (0.02-0.03), eGFR -0.34 (-0.02, -0.005), Ln PCR 0.59, (0.31, 0.55) and serum albumin -0.49 (-0.07, -0.03), all P<0.01. Ln-DDimer was not associated with wbc count, urate, gender, diagnosis of membranous nephropathy, or diabetes mellitus. On multivariate modeling using backward stepwise regression the final model β (95% CI) was age (decades) 0.36 (0.01, 0.03) p<0.001, Ln-PCR 0.33 (0.309, 0.39) p=0.002 and Serum Albumin -0.23 (-0.05, -0.004) p=0.02. Ln-D dimer was not independently associated with eGFR (p=0.3). When analysis was performed stratified by DDimer assay type, lnPCR correlation with DDimer in both separate analyses (P=0.0001).

Conclusions: The standard reference range of Ddimer for evaluation of thrombosis should not be used for patients with the nephrotic syndrome. Elevated Ddimer levels in the NS is associated with PCR rather than eGFR.

SA-PO2849

1,25-Dihydroxyvitamin D(3) Ameliorates Podocytes Injury Via Inhibiting CD80 Expression Jianchao Ma, Wei Shi, Wenjian Wang, Shuangxin Liu, Lixia Xu, Zhilian Li, Zhiming Ye, Yuan Han Chen, Xinling Liang. *Nephrological Department, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Background: Accumulating studies have demonstrated that 1,25-Dihydroxyvitamin D(3) reduces podocytes loss and slows the decline of kidney function in chronic kidney disease. Recent evidences showed that CD80 expressed on injured podocytes and may play a critical role in proteinuria. In this study, we hypothesized that vitamin D protects podocytes via modulating CD80 expression.

Methods: SD rats were subjected to 5/6 nephrectomy, and then were randomly allocated into two groups: 5/6 nephrectomy and 5/6 nephrectomy + 1,25-Dihydroxyvitamin D(3) (0.3ug/kg.day) groups. Albuminuria was monitored at 2 weeks, 4weeks, 8weeks and 12weeks after treatment. *In vivo*, immortalized mouse podocytes were cultured and randomized into groups: control, PAN and PAN +1,25-Dihydroxyvitamin D(3) (10^{-9}ug/L) treated groups . CD80 ,VDR and synaptopodin were tested in both protein and mRNA level. The podocyte apoptosis was checked by immunofluorescence.

Results: A significant decrease of proteinuria and podocytes apoptosis were observed in 5/6 nephrectomy +1,25-Dihydroxyvitamin D(3) group compared to 5/6 nephrectomy rats. In vivo, 1,25-Dihydroxyvitamin D(3) also exhibited a decrease of apoptosis cell nuber in PAN-induced podocytes. Importantly, the expression of CD80 was markedly inhibited by 1,25-Dihydroxyvitamin D(3) in both protein level and mRNA level. Consistent with the results observed in vivo, the same effect of 1,25-Dihydroxyvitamin D(3) on the podocytes injury induced by PAN was also confirmed in vitro.

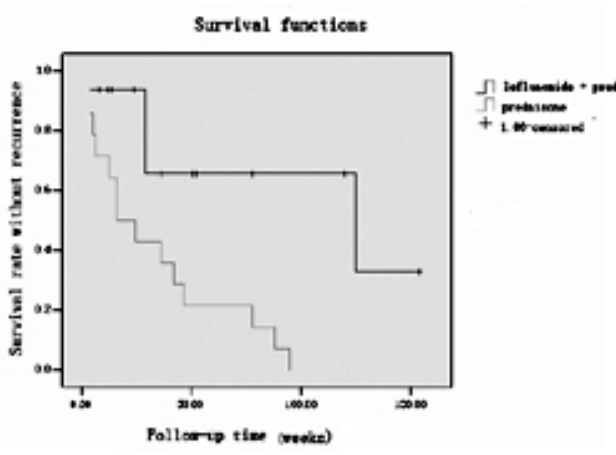
Conclusions: Our findings provided new insights of the renopretection role of 1,25-Dihydroxyvitamin D(3) in podocytes injury, and offer a potential target in prevent the progression of CKD by inhibiting CD80 expression.

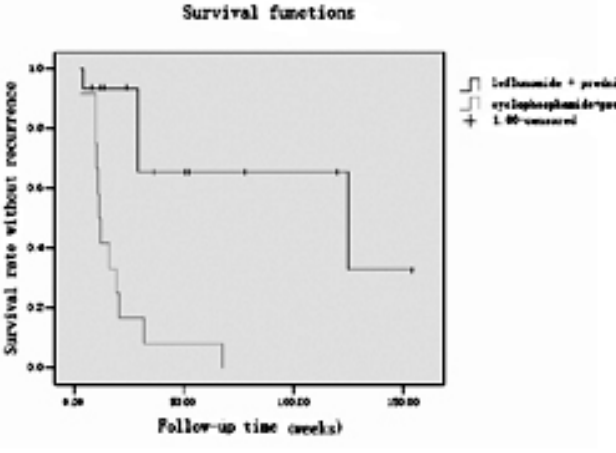
SA-PO2850

Efficacy and Safety of Leflunomide in the Treatment of Steroid Dependent or Resistant Minimal Change Disease: A Single-Centre Experience Jun-Hua Zhou, Yi-Miao Zhang, Gang Liu, Jun Li, Rong Xu, Jing Huang. *Renal Division, Peking University First Hospital, Beijing, China.*

Methods: Fourteen patients with steroid-dependent MCD (minimal change disease) and two patients with steroid-resistant MCD, who had been treated with leflunomide, were retrospectively analyzed.The initial dose of leflunomide was 10-20mg/d combined with prednisone of initial dose of 0.25-1.0mg/kg/d and gradually tapering after 8 weeks. Clinical data of baseline, 2nd, 4th, 8th, 12th, 24th, 48th week was analyzed comparing with previous prednisone monotherapy and prednisone combined with cyclophosphamide therapy.

Results: Total effective rate of the steroid-dependent patients was 100% (14/14), and complete remission rate was 92.8% (13/14).Besides,two steroid-resistant patients achieved complete remission by leflunomide combined with prednisone.During the treatment,eight patients had adverse effects which could be well tolerated.Comparing with prednisone monotherapy,the dose of prednisone to maintain remission was reduced dramatically (from median 22.5mg/d to median 7.5mg/d,P=0.041),relapse rate during follow-up time decreased from 100% to 31.3% (*P*=0.002), the median time before relapse increased from 20.3 weeks to 32.5 weeks.





Comparing with prednisone combined with cyclophosphamide,the dose of prednisone to maintain remission could be reduced significantly (from median 22.5mg/d to median 5.0mg/d, *P*=0.003), relapse rate during follow-up time decreased from 100% to 31.3% (*P*=0.001),the median time before relapse increased from 11.7 weeks to 32.5 weeks.

Conclusions: Leflunomide might be effective in steroid-resistant and steroid-dependent minimal change disease.It may reduce the amount of prednisone to maintain remission and reduce relapse rate comparing with prednisone monothrerapy and prednisone combined with cyclophosphamide therapy.This strategy might be usually well tolerated by the patients.

SA-PO2851

Synthetic ACTH Is Less Effective Than Cyclophosphamide in Patients with Idiopathic Membranous Nephropathy Julia M. Hofstra,[1] Hans S. Brink,[2] Jos J. Van de Kerkhof,[3] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [2]Internal Medicine, Medisch Spectrum Twente, Enschede, Netherlands; [3]Internal Medicine, Bernhoven Hospital, Veghel, Netherlands.*

Background: Therapy with alkylating agents in idiopathic membranous nephropathy (iMN)is effective, but associated with serious side effects. Synthetic ACTH may be advantageous with reported remission rates up to 85% and no significant side effects.

Methods: We conducted a prospective cohort study in patients with iMN and high risk for renal failure (NCT00694863). Patients with iMN, a nephrotic syndrome, eGFR> 60 ml/min and high risk for progression (elevated urinary β2m levels) were treated with i.m. injections of synthetic ACTH during 9 months (m.). Maximal dose was 1 mg tetracosactide-hexacetate (Synacthen Depot®) twice a week. For comparison, we selected historical controls, treated with cyclophosphamide (CP 1.5 mg/kg/day for 12 m.) and steroids, matched for serum creatinine, proteinuria, age, sex and previous immunosuppressive treatment.

Results: We compared 16 patients (M/F 13/3, age 52 ±15yr, sCr 105 ±20 μmol/l, sAlb 23 ±7g/L, Prot./Cr.Ratio 8.3 ±3.5 g/10mmol) treated with ACTH and 16 patients treated with CP (M/F13/3, age 48 ±13 yr, sCr 102 ±23 μmol/l, sAlb 22 ±5 g/L, PCR 9.6 ±3.5 g/10mmol). At the end of treatment, 7 (44%) patients treated with ACTH developed a partial remission of proteinuria (PCR < 2.0g/10mmol) versus 15 (94%) patients treated with CP (p= 0.02). At the end of follow-up (20±6 m.in the ACTH group and 75±30 m. in the CP group), relapses had occurred in 3 of 7 patients (43%) in the ACTH group and 4 of 15 patients (27%) in the CP group (log rank p<0.01). Although all patients had side effects on ACTH, these were minor and necessitated dose reduction in only one patient compared with 5 patients treated with CP.

Conclusions: Treatment with synthetic ACTH is less effective than CP in inducing an initial remission of proteinuria in high risk patients with iMN. Sustained remission was induced in only a minority of patients. These data suggest that synthetical ACTH has limited value in the treatment of high risk patients with iMN.

Funding: Private Foundation Support

SA-PO2852

Efficacy of Rituximab in Steroid Resistant & Dependent Nephrotic Syndrome Arvind Bagga,[1] Aditi Sinha,[1] Asha Moudgil,[2] Ashima Gulati,[1] Stanley C. Jordan.[3] *[1]All India Institute of Medical Sciences, New Delhi, India; [2]Children's National Medical Center, Washington; [3]Cedars Sinai Medical Center, Los Angeles.*

Background: To evaluate the efficacy of rituximab (RTX) in inducing & maintaining remission in difficult steroid resistant (SRNS) & steroid dependent nephrotic syndrome (SDNS)

Methods: Data on 74 patients receiving RTX (375 mg/m^2 IV weekly) for SRNS (4 doses) or SDNS (2 doses) & followed ≥6-months is presented. Therapy with prednisone & calcineurin inhibitors (CNI) was tapered. For SRNS, remission was defined as complete (CR), partial (PR) or non response (NR).

Results:

SRNS. Following onset at a mean age of 4 yr, 38 patients received RTX at 10 yr. Failure of IV steroids & failure (26) or toxicity (12) to CNI was present. Histology showed MCD (n=18) or FSGS (20). Four weeks post-RTX, 17 (45%) patients showed CR or PR, allowing CNI taper (100%) & discontinuation (61%). Remission rates were similar for initial (35%) or late (50%) steroid resistance & CNI failure (38%) or toxicity (44%). More patients with MCD (60%) than FSGS (24%) attained remission (P=0.04). At 2-yr, 47% had favorable outcome (CR/PR 7, steroid sensitive relapses 11); 24% had impaired GFR.

SDNS. Following onset at a mean age of 3-yr, 36 patients received RTX at 12-yr. Patients had failed therapy with levamisole, cyclophosphamide, MMF or CNI. Following therapy, there was decrease in relapses (difference 3.5 episodes/yr; P<0.001). The mean duration of remission was 13 (5-38) months; 3, 18, 7 cases relapsed at <6, 7-12 & ≥12 months respectively. Therapy with CNI or MMF was discontinued; steroids were withdrawn in 79%. At 19 months follow-up, 28% had sustained remission, 16% infrequent relapses & 56% frequent relapses. Relapse-free survival was 92%, 32% & 16% at 6, 12 & 18 months respectively. Repeat doses of RTX (n=11) had similar benefit.

CD19 depletion was found in all cases.

Conclusions: RTX effectively induced remission in patients with refractory SRNS & maintained 6-months remission in over 90% cases with SDNS. Since a high proportion of patients with SDNS relapsed after 6-months, studies should examine interventions that prevent relapses following RTX.

Funding: Clinical Revenue Support

SA-PO2853

Urinary Exosomes May Represent a New Diagnostic Tool for Early Diagnosis of Renal Damage Anja Susanne Muhlfeld,[1] Silvia Spanu,[2] Bernd Denecke,[3] Jurgen Floege.[1] *[1]Department of Nephrology and Clinical Immunology, University Clinic of the RWTH Aachen University, Aachen, Germany; [2]Department of Nephrology, University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3]IZKF, RWTH Aachen University, Aachen, Germany.*

Background: Exosomes are membrane vesicles, that are secreted from the surface of all cells types after fusion of the multivesicular body with the cell membrane. They contain proteins and mRNA of the cells of origin. Because of the surrounding cell membrane intraexosomal proteins and mRNA are well protected from digesting enzymes. In the present study we have evaluated the expression profile of intra-exosomal mRNAs as a non-invasive tool for the diagnosis of renal disease.

Methods: Toxic podocyte damage was induced by puromycin aminonucleoside in male Sprague Dawley rats. Urinary exosomes were isolated by differential centrifugation at time points 0 (before disease induction), at the onset of proteinuria (day 5) and during maximal disease activity (day 10). Exosomal mRNA was isolated, amplified, and the mRNA species were globally assessed by gene array analysis.

Results: Rats treated with puromycin aminonucleoside developed acute kidney damage, indicated by an increase in serum creatinine and BUN and the development of proteinuria. Messenger-RNA isolated from urinary exosomes revealed 887 differentially expressed genes with an at least 1.5-fold change and 77 genes with an at least 3-fold change in their level of expression on days 5 and 10 as compared to baseline expression on day 0. These genes were mainly involved in the maintenance and reorganization of the cytoskeleton, in the response to oxidative stress and in the control of the apoptotic pathway. Out of these genes we identified a number of genes that were regulated only in the initial phase of the disease (day 5), i.e. at the onset of proteinuria, and therefore bear the potential to act as early markers of glomerular damage.

Conclusions: The gene expression pattern in urinary exosomes of rats with puromycin aminoglycosid nephrosis may serve to identify early markers of renal disease that may lend themselves to both diagnostic and pathogenetic studies.

Funding: Government Support - Non-U.S.

SA-PO2854

Superior Results with Tacrolimus Than Ciclosporine in Children with Steroid Resistant Nephrotic Syndrome Mara Medeiros, Yolanda Fuentes, Saul Valverde, Ana M. Hernández. *Hospital Infantil de México Federico Gomez, Mexico, DF, Mexico.*

Background: The aim of the study was to evaluate if Prednisone (PDN) and Tacrolimus (Tac) therapy administered during a 12 month period achieves a greater rate and prolonged remission of proteinuria in pediatric patients with sterioid resistant nephrotic syndrome SRNS, compared to those treated with the standard prednisone and Cyclosporine(CyA) regimen."

Methods: A comparative, randomized trial was conducted in children with SRNS; the protocol was approved by the IRB, parent and children consent/assent was obtained in all cases. They received prednisone (PDN) 60 mg/m2/day for 1 month and 30 mg/m2/day every 48 hours, for 5 months, group I received CyA 5 mg/kg/day, target levels 100 to 200 ng/ml. Group II received Tac 0.10 mg/kg/day,adjusted to target levels 5-10 ng/ml). Monthly visits were scheduled for clinical and laboratory evaluations (blood and urine).

Results: Twenty patients were included, ten in each group. Clinical an laboratory data is depicted in Table 1. All Tac treated patients had complete remission at 6 months of treatment, whereas only four patients in the CyA group.

Table 1. Clinical and Laboratory Data

	CyA (n=10)	Tac (n=10)	p value
Age (median years, range)	5.9 (2.2, 17)	7.2 (2.7, 15.8)	0.76
Baseline serum albumin (mg/dL)	2.9 (0.7,3.3)	1.6 (0.4,3.2)	0.51
6 months serum albumin (mg/dL)	3.6 (2.8,3.9)	3.7 (3.4,4.1)	0.62
New onset hypertension (n,%)	8 (80%)	1 (10%)	0.005
Partial or complete remission (n.%)	7 (70%)	10 (100%)	0.21
Complete remission (n,%)	4 (40%)	10 (100%)	0.01

CyA: Cyclosporin, Tac: Tacrolimus, NS: Nephrotic syndrome.P value obtained either by Fisher Exact test or Mann Whitney test.

No difference was found in serum creatinine and eGFR.

Conclusions: The rate of complete remission is higher with tacrolimus than with cyclosporine at six months of treatment in SRNS children.

Patients treated with cyclosporine had significantly higher rate of new onset hypertension.

Tacrolimus seems to be better theraputic option in SRNS than Cyclosporine.

Funding: Government Support - Non-U.S.

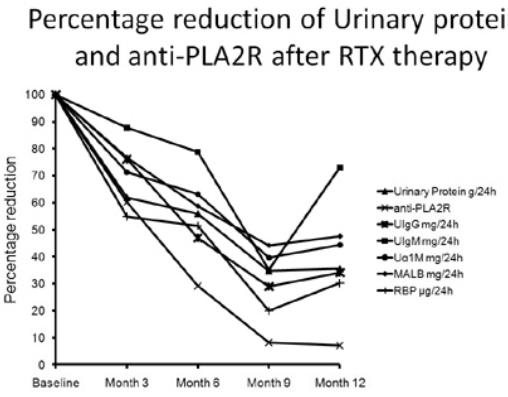
SA-PO2855

Urinary Biomarkers as Predictors of Response to Rituximab in Patients with Membranous Nephropathy Maria V. Irazabal,[1] Alfonso Eirin,[1] John C. Lieske,[1] Laurence H. Beck,[2] John J. Dillon,[1] Patrick H. Nachman,[3] Sanjeev Sethi,[1] Stephen B. Erickson,[1] Daniel C. Cattran,[4] Fernando C. Fervenza.[1] *[1]Division of Nephrology, Mayo Clinic, Rochester, MN; [2]Department of Medicine, Boston University School of Medicine, Boston, MA; [3]Division of Nephrology, University of North Carolina, Chapel Hill, NC; [4]Division of Nephrology, Toronto General Hospital, Toronto, ON, Canada.*

Background: Rituximab (RTX) reduces proteinuria (P) in membranous nephropathy (MN). However, given the cost and potential side-effects, it would be useful to limit treatment to patients likely to respond to RTX. Recent data suggest that urinary markers (e.g. IgG) predict prognosis in MN, but whether they can predict P response to RTX is unknown.

Methods: Urinary (U) excretion of retinol binding protein (RBP), alpha-1 microglobulin (α1M), albumin (alb), IgG, and IgM at baseline were correlated with P at 12 and 24 mo, in 20 MN patients treated with RTX (375mg/m^{2}x4) with retreatment at 6 mo. Anti-phospholipase A2 receptor antibody (anti-PLA2R) was also tested.

Results: At 24 mo, complete remission (CR=UP<0.3g/24h) occurred in 4 patients, partial remission (PR=UP<3.5g but >0.3g/24h) in 12, limited response (LR=>50% UP reduction but UP>3.5g) in 1, non-response (NR=<50% UP reduction) in 2, and 1 relapsed. Baseline UIgG (mg/24h), fractional excretion (FE) of IgG, Uα1M (mg/24h) and URBP (μg/24h), significantly correlated with change in P at 12 mo (p=0.04, 0.05, 0.04, and 0.03), but not at 24mo (p=0.55, 0.42, 0.29 and 0.20 respectively). Correlation between baseline UIgM, FEIgM, Ualb, FE alb and response at 12 or 24 mo was not significant. In the 16 patients in which the anti-PLA2R antibody were positive, the decline correlated with the reduction in these protein markers.



Conclusions: Quantification of low, medium and high molecular weight UP may predict short but not long-term response to RTX.

Funding: Private Foundation Support

SA-PO2856

Cd2ap Regulates Actin Cytoskeleton in Podocytes by Regulating RhoA Activity Hani Suleiman,[1] Andrey S. Shaw.[1] *[1]Department of Pathology and Immunology, Washington University, Saint Louis, MO; [2]Institut für Anatomie und Zellbiologie, Universitätsmedizin Greifswald, Greifswald, Germany.*

Background: CD2-associated protein (CD2AP) is a scaffold protein which plays a critical role in the maintenance of the kidney filtration barrier. The integrity of the kidney filtration barrier is regulated through the fine organization of the actin cytoskeleton. Since CD2AP has been implicated in actin regulation, we were interested to determine whether CD2AP deficient podocytes exhibit any changes in actin-myosin machinery; and whether these changes in actin-myosin machinery could explain the kidney phenotype found in the Cd2ap (-/-) mice.

Methods: Imaging, FRET sensor based live imaging, Immunohistochemistry, Western blot, Immunoprecipitation.

Results: We find here that podocytes lacking Cd2ap (-/-) have less prominent actin stress fibers, and disorganized localization of Non-muscle myosin IIa. Consistent with the defect in Non-muscle myosin IIa, when we measured the actin-myosin cytoskeleton force across the cells, we found that podocytes lacking Cd2ap exhibit less cell tension. Biochemically, Cd2ap (-/-) cells had reduced myosin light chain phosphorylation (pMLC) on Ser19, a key regulator of non-muscle myosin II activity. We measured the RhoA activity RhoA using a FRET sensor and found that RhoA activity is being reduced, which could explain the pMLC defect. Potentially explaining the defect, the RhoGAP, p190, was found to be hyperphosphorylated in CD2ap deficient cells. Furthermore, we have evidence suggesting that Cd2ap and p190RhoGAP interact together, which could be responsible for proper activation/inhibition of p190RhoGAP.

Conclusions: We conclude that Cd2ap regulates the actin-myosin machinery by regulating RhoA activity.

SA-PO2857

The Occurrence of Cancer in Patients with Membranous Nephropathy Sophia Lionaki,[1,2] Sean Barbour,[3] Yichun Hu,[1] Susan L. Hogan,[1] Caroline E. Jennette,[1] Ronald J. Falk,[1] Daniel C. Cattran,[3] Patrick H. Nachman,[1,4] Heather N. Reich.[3,4] *[1]UNC Kidney Center, University of North Carolina; [2]Nephrology, Laiko Hospital, Greece; [3]University of Toronto, Canada; [4]Contributed equally, .*

Background: To describe the frequency and character of cancers (CA) in patients with biopsy proven membranous nephropathy (MN), in relation with clinical parameters, and long term outcomes.

Methods: We studied 898 patients with MN from the Glomerular Disease Collaborative Network (N=412) and the Toronto Glomerulonephritis Registry (N=486). Clinical and laboratory values at biopsy were included in the analysis. Smoking and immunosuppressive therapies for MN were studied as predisposing factors for cancer.

Results: Among 898 patients with MN, a total of 85 malignancies were recorded in 64 patients (7.1%) [56 patients (6.2%) excluding non-melanoma skin CA]. Of these, 39 (45.9%) occurred within 5 years of biopsy diagnosis. The most common cancers were of the skin (20), prostate (10), breast (9), lung (9), GI tract (6) and hematologic (6). Excluding non-melanoma skin CA, patients with CA were older.

Characteristic Total, N=898	Patients with CA, N=56	Patients without CA, N=842	P value
Age (years)	60.8±11.9	48.4±15.8	<0.0001
Caucasian, n(%)	42(80.8)	556(70.6)	0.25
Male, n(%)	33(58.9)	522(62)	0.67
24hr prot (g/day)	7.9±5.8	7.7±5.5	0.92
Serum albumin (g/dl)	25.49±7.51	25.96±7.58	0.81
Duration of proteinuria prior to Bx (months)	4.9±8.7	3.4±34.8	0.14
eGFR adjusted for age (ml/min/1.73m2)	77.2±31.3	72.8±31	0.31
Smoking, ever, n(%)	27(65.85)	330(60.44)	0.62
Patients treated with Immunosuppression, n (%)	30(53.7)	486(57.7)	0.58
Patients reaching ESRD, n(%)	8(14.3)	96(11.4)	0.52
Time to ESRD (months)	62.0±70.4	56.35±61.2	0.9

There were no statistical differences in serum albumin, eGFR adjusted for age, proteinuria or duration of proteinuria prior to biopsy between those with and without CA. Cancers occurred in 3.6% of patients <60 y.o. and 12.9% in those ≥ 60 y.o.

Conclusions: There is an increased frequency of cancers among patients with MN older than 60 years**.** We found no distinguishing clinical features at presentation between patients with and without cancer. Presence of cancer did not affect renal survival.

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

SA-PO2858

Performance of Anti-Phospholipase A2-Receptor Antibody Testing for Membranous Nephropathy in Routine Clinical Practice Ulf Schoenermarck,[1] Peter Eichhorn,[2] Thomas Sitter,[3] Thorsten Wendler,[1] Volker Vielhauer,[3] Stephan R. Lederer,[4] Kai Fechner,[5] Michael Fischereder.[1] *[1]Medical Clinic I, Nephrology Div., University Hospital Munich-Grosshadern, Munich, Germany; [2]Institute of Clin. Chemistry, University Hospital Munich, Munich, Germany; [3]Medical Policlinic, Dept. of Nephrology, University Hospital Munich-Innenstadt, Munich, Germany; [4]KfH Nierenzentrum Laim, Munich, Germany; [5]Institute of Experimental Immunology, Euroimmun AG, Luebeck, Germany.*

Background: Autoantibodies against the M-type phospholipase A2 receptor (anti-PLA2R) have been recently identified in patients with primary membranous nephropathy (MN). Aim of the study was to evaluate the measurement of anti-PLA2R-antibodies with a commercially available serologic test in routine clinical practice.

Methods: All patients with the diagnosis of bioptically proven MN seen between 10/2010 and 06/2011 were tested for the presence of anti-PLA2R-antibodies. As control group patients with secondary forms of MN and other proteinuric renal diseases were analysed. For detection of circulating anti-PLA2R-antibodies an indirect immunoflourescence assay was used (EUROIMMUN AG, Lübeck, Germany). A specific cytoplasmic fluorescence of transfected HEK 293-cells at a dilution of 1:10 or higher was considered to be positive.

Results: The anti-PLA2R-antibody test was negative in all patients (n=10) with secondary forms of MN (hepatitis B, n=1, tumor-assoc., n=1, assoc. with connective tissue disease and SLE, n=7, MPO-ANCA-assoc. MN, n=1) and patients with other proteinuric diseases (n=14). 11 of 16 patients with primary MN were positive for anti-PLA2R-antibodies. The antibody titer ranged from 1:10 to 1:3200 and was present up to 8 years after first diagnosis of MN. There was no correlation between the antibody level and proteinuria or renal function. Further 3 patients with primary MN tested at time or after kidney transplantation were also negative for anti-PLA2R-antibodies, although one patient presented with nephrotic proteinuria due to bioptically proven recurrent MN.

Conclusions: Anti-PLA2R-antibodies have a high sensitivity and 100% specificity for primary MN. The serologic test appears to be a useful tool in routine clinical testing.

SA-PO2859

The Role of Gene Polymorphisms of the Renin-Angiotensin-Aldosterone and Inflammation Pathways in the Progression of Glomerular Diseases: A Validation Study Isabelle Chapdelaine, Remi Goupil, Jean-Philippe Rioux, Stephanie Raymond-Carrier, Francois Madore, Stephan Troyanov. *Nephrology, Hôpital du Sacré-Coeur, University of Montreal, Montreal, QC, Canada.*

Background: Clinical risk factors of progression in glomerular diseases such as proteinuria and blood pressure predict outcome imperfectly. Previous studies have proposed various polymorphisms of genes implicated in the pathophysiology of glomerulopathies as clinical tools in risk assessment. We sought to validate their impact on the rate of renal function decline in a prospective cohort study receiving usual anti-hypertensive and anti-proteinuric therapies.

Methods: Using Medline, we identified 9 candidates polymorphisms of known genes, mostly of the renin-angiotensin-aldosterone and inflammation pathways: MCP-1 A2518G, TGF-ß1 T869C and C-509T, ACE I/D, AGT M235T, AT1R A1166C, TSC-22 A-396G, eNOS 4b/a C344T.

Results: We prospectively recruited 93 predominantly male (73%) and Caucasian (85%) patients with an age of 64 ± 13 years and eGFR of 34 ± 21 mL/min/1.73m2 at baseline. Patients were matched to 50 healthy controls of similar ethnic background. Sixty-one percent of patients had diabetic nephropathy, almost all received renin-angiotensin-aldosterone blockade (91%) and none immunosuppressive therapy. The average MAP during follow-up was 94 ± 7 mm Hg with a urinary protein to creatinine ratio of 0.15 (interquartile range from 0.05-0.30) g/mmol. The rate of renal function decline was -2.9 ± 4.5 mL/min/1.73m2/yr over a median 34 months period. Proteinuria and blood pressure strongly predicted progression.

However, none of the proposed polymorphism predicted renal function decline nor showed simple or partial correlations with proteinuria or MAP. A summation variable of the number of simultaneous polymorphisms also showed no predictive trend. Finally, the distribution of each allele was almost identical in the patients versus the control group.

Conclusions: This study does not validate an important role for these 9 candidate polymorphisms in the progression of glomerular diseases receiving standard therapy.

Funding: Government Support - Non-U.S.

SA-PO2860

The Serum Cholestanol, a Marker of Cholesterol Absorption, Is Elevated in Patients with Nephrotic Syndrome Masao Kikuchi, Shouichi Fujimoto, Yuji Sato, Kazuo Kitamura. *University of Miyazaki Hospital, Faculty of Medicine, Miyazaki, Japan.*

Background: In generally, nephrotic syndrome-associated hypercholesterolemia is considered due to increased production of lipoprotein by the mechanism of compensation for hypoproteinemia. But, there have been reports that cholesterol synthesis was not increased in nephrotic animal or human. There is still no convincing evidence for this hypothesis, especially in human.

Methods: In the present work, we investigated the serum cholestanol, campesterol and sitosterol, as markers of cholesterol absorption, and lathosterol as a marker of cholesterol synthesis in nephrotic patients not treated with a cholesterol lowering drug. Furthermore, we did same examinations in patients inducing complete remission. We made a comparison those results in patients in the nephrotic state (NS, n=19) and those in patients in the complete remission state (CR, n=8).

Results: The serum lathosterol, a marker of cholesterol synthesis, was elevated in NS, compared to CR (NS vs. CR, 3.85±0.42 vs. 2.39±0.32 μg/ml, p=0.04). As for markers of cholesterol absorption, the serum campestanol and sitosterol were not significantly different in both state, but the serum cholestanol, which is less affected by diet, were significantly elevated in NS, compared to CR (NS vs. CR, 5.52±0.50 vs. 3.35±0.34 μg/ml, p=0.01). The serum lathosterol and cholestanol were not correlated with the severity of nephrotic syndrome (ie: serum total protein and albumin, urinary protein concentration, serum total and low-density lipoprotein cholesterol, etc.).

Conclusions: In nephrotic syndrome, not only synthesis but also absorption of cholesterol should contribute to hypercholesterolemia.

SA-PO2861

Development of Chronic Kidney Disease Was Not Predicted by Urinary Testing or Renal Biopsy at Liver Transplantation for Hepatitis C Virus-Induced Cirrhosis David Patrick Newton, Brendan M. McGuire, Bruce A. Julian. *Medicine, University of Alabama at Birmingham, AL.*

Background: We have shown that urinalysis and quantitative proteinuria are often normal despite biopsy-proven immune-complex glomerulonephritis at the time of liver transplantation for cirrhosis due to chronic infection with hepatitis C virus (HCV) (Ann Intern Med 2006;144:735). It is uncertain whether the renal outcome for such transplant recipients can be predicted based on urinalysis or renal histology at the time of engraftment.

Methods: We reviewed the long-term clinical follow-up of 30 patients who had undergone renal biopsy at time of liver transplantation for HCV cirrhosis in 2004-2005.

Results: Four patients died within 2 years of transplantation, including 1 on dialysis at engraftment. The second patient on dialysis at transplantation now has eGFR 65 mL/min/1.73 m2. Seven patients have developed stage 4 chronic kidney disease at last follow-up. The Table compares this subgroup to the 19 other surviving patients. Microscopic hematuria was defined as >4 rbc/hpf; proteinuria was defined as dipstick 1+ or urinary protein/creatinine ratio at least 0.30.

eGFR at last follow-up	> 30 mL/min/1.73 m2	< 30 mL/min/1.73 m2
n	19	7
AT LIVER TRANSPLANTATION		
Age, yr, mean	50.3	55.7
Gender, male, n (%)	17 (89)	2 (29)
Race, Causcasian, n %	16 (84)	6 (86)
eGFR, mL/min/1.73 m2 (±SD)	62 (25)	61 (31)
HCV viral load, copies/mL, mean	537,931	598,904
Proteinuria, n (%)	4 (21)	1 (14)
Hematuria	10 (53)	2 (29)
Renal histology, n (%)		
Membranoproliferative glomerulonephritis	9 (47)	2 (29)
IgA nephropathy	3 (16)	3 (43)
Mesangioproliferative glomerulonephritis	5 (26)	1 (14)
Minor glomerular changes	2 (11)	1 (14)
AT LAST FOLLOW-UP		
Interval post transplant, mo (±SD)	68 (20)	69 (17)
Proteinuria, n (%)	10 (53)	6 (86)
Change in eGFR, % (±SD)	27 (69)	-53 (20)

Conclusions: Development of chronic kidney kidney disease was common within a relatively short interval after liver transplantation for HCV cirrhosis. However, this complication was not predicted by severity of urinary abnormalities, eGFR, or renal histological features at the time of liver transplantation.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2862

Antiproteinuric Action of Cinacalcet in Children with Steroid-Sensitive Nephrotic Syndrome Betti Schaefer,[1] Jun Oh,[2] Franz S. Schaefer,[1] Burkhard Toenshoff,[1] Claus P. Schmitt.[1] *[1]Center for Pediatric and Adolescent Medicine, Heidelberg; [2]University Children's Hospital, Hamburg.*

Background: CaSR is expressed in human podocytes. In vitro, exposure of podocytes to the calcimimetic R-568 stabilizes the actin cytoskeleton and reduces PAN induced apoptosis. In vivo it attenuates proteinuria, podocyte and GFR loss and glomerulosclerosis. Clinical data have not yet been obtained.

Methods: 4 children (3-9 years) with idiopathic nephrotic syndrome (NS), who showed up with initial manifestation (n=2) and second relapse (n=2), respectively, and who objected to steroid therapy, were treated with the calcimimetic Cinacalcet (Cin). Initial dose was 15 mg/m²BSA/d, gradually increased by 5 mg/m²BSA according to the antiproteinuric effect. Calcium was supplemented at an initial dose of 2x500 mg/m²BSA/d.

Results: The first patient received a single dose of Cin (5 mg/m²). Protein- and albuminuria were reduced (570 to 398 and 378 to 255 g/mol crea) within 24 hours (normal range <20 and <3). In the remaining 3 patients proteinuria was 1618, 821 and 763 g/mol crea and declined by 92, 71 and 73% to a nadir of 130, 235 and 209 within 8, 5 and 3 days. Albuminuria was 1347, 648 and 746 g/mol crea and declined by 96, 73, and 76% to a nadir of 58, 172 and 178. Serum albumin was 26.7, 26.9 and 29.1 g/l and increased to a maximum of 27.4, 28.6 and 31.8. Oedema disappeared in all 3 patients within 6-13 days. Ca^{++}- and phosphate excretion did not change; serum Ca^{++} remained in the normal range. The maximal doses of Cin were 26.1, 21 and 28.3 mg/m²BSA, the treatment was well tolerated. All 3 children experienced a relapse after 10, 23 and 19 days; which was associated with an upper airway infection in 2 patients. Cin was discontinued and prednisolone therapy initiated, which induced remission in all 3 patients within 7-9 days.

Conclusions: Cinacalcet markedly reduces proteinuria in children with idiopathic NS, albeit without inducing a complete and stable remission.

SA-PO2863

Correlation between Podocyte Loss and Albuminuria in a PAN-Induced Model of Glomerulonephropathy Stefan Wawersik, Stephen O'Brien, William Weber, John N. Vassiliadis, Steven R. Ledbetter, Susan Schiavi, Cynthia M. Arbeeny. *Endocrine and Renal Sciences, Genzyme, Framingham, MA.*

Background: Developing therapeutics for glomerular disease requires testing in pre-clinical models, often relying on albuminuria as an indicator of efficacy. Podocyte loss has recently been recognized as a critical step in glomerulopathy progression, and measurement of podocyte number has consequently emerged as an additional disease endpoint. However, the correlation between podocyte loss and albuminuria has not been carefully explored.

Methods: To better understand the relationship between these two endpoints, we carried out a time-course study in a puromycin aminonucleoside (PAN) rat model of glomerular injury. Uninephrectomized rats were dosed with PAN or with PBS as a control. Urine, serum, and kidneys were collected at weeks 1-5, 7, and 12.

Results: Average ACR in PAN-treated rats peaked at 4 weeks, after which ACRs declined but remained elevated relative to PBS controls. Podocyte loss is evident in PAN-treated rats over the course of the study, measured as number of WT-1 positive nuclei per glomerular area. A 42% reduction in podocyte density was observed at week 12 relative to controls or to PAN-treated rats at week 1. The largest drop in podocyte density was observed between weeks 2 and 3, slightly preceding the peak in average ACR. When all samples are analyzed together, ACR and podocyte number show a modest but statistically significant correlation (R^2=0.158, P<0.002). However, no correlation between ACR and podocyte number is observed when early PAN-treated samples (from weeks 1-5, peak proteinuria) are considered separately, and early PAN data are statistically distinct from late PAN- (weeks 7-12) and PBS-treated samples (P<0.01). Accordingly, the ACR/podocyte loss correlation improves when early PAN-treated samples are removed (R^2=0.472, P<0.001).

Conclusions: Taken together, these data suggest that the high levels of albuminuria seen in the early weeks of toxin-induced renal injury models may not reflect long term disease progression, and that care must therefore be taken in using such models to predict the efficacy of candidate therapeutics for renal disease.

Funding: Pharmaceutical Company Support

SA-PO2864

Treatment of Resistant Glomerular Diseases with ACTH Gel: A Prospective Trial Andrew S. Bomback, Pietro A. Canetta, Jai Radhakrishnan, Gerald B. Appel. *Columbia University Medical Center, New York.*

Background: Adrenocorticotropic hormone (ACTH) has shown promising results as second- and third-line therapy for idiopathic glomerular diseases resistant to conventional therapies, but the data reported to date has solely been from retrospective, observational studies.

Methods: In this prospective, open-label, pilot study (NCT01129284), 15 patients with resistant glomerular diseases were treated with ACTH gel (80 units SC twice weekly) for 6 months. Resistant membranous nephropathy (MN) was defined as failure to achieve sustained remission with at least 2 immunosuppressive regimens, resistant MCD/FSGS was defined as failure to achieve sustained remission with corticosteroids and at least 1 other immunosuppressive regimen, and resistant IgA nephropathy was defined as ≥1 g/day proteinuria despite effective renin angiotensin system blockade. Complete remission was defined as stable or improved renal function with proteinuria falling <500 mg/day. Partial remission was defined as stable or improved renal function with ≥50% reduction in proteinuria and final proteinuria 500-3500 mg/day.

Results: The study included 5 patients with resistant MN, 5 patients with resistant MCD(n=2)/FSGS(n=3), and 5 patients with resistant IgA nephropathy. Two resistant MN patients were in partial remission at the end of 6 months of therapy, although 3 achieved immunologic remission of disease (PLA2R antibody disappeared by 4 months of therapy). One patient with resistant FSGS achieved complete remission at 6 months; one patient with resistant MCD achieved a partial remission at 6 months but relapsed within 4 weeks of stopping ACTH. Three of 5 patients with resistant IgA nephropathy demonstrated ≥50% reductions in proteinuria while on ACTH, with proteinuria consistently <1 g/day by 6 months. Three of 15 patients reported significant steroid-like adverse effects with ACTH, including weight gain and hyperglycemia, prompting early termination of therapy without any signs of clinical response.

Conclusions: ACTH gel is a promising treatment for resistant glomerular diseases. This therapeutic option should be studied further in randomized, controlled trials against currently available therapies for resistant disease.

Funding: Pharmaceutical Company Support

SA-PO2865

Anti-Viral Therapy in Hepatitis B-Associated Membranous Nephropathy

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Background: Lamivudine is effective for treatment of hepatitis B-associated membranous nephropathy (HBV-MN), but prolonged use leads to the emergence of drug-resistant variants. Also, new drugs such as entecavir, adefovir or clevudine have been introduced to treat the chronic hepatitis B, but there are few data on its efficacy in HBV-MN. We describe our experiences about treatment of HBV-MN with various anti-viral drugs.

Methods: From 1996 to 2010, biopsy-proven MN was diagnosed in 89 patients, and 10 patients had HBsAg. We investigated the clinical courses and therapeutic responses, and prognoses of patients with HBV-MN.

Results: The incidence of HBV-MN was 10.1%. The mean age of the patients was 33 years (range, 19-55), and 8 patient (80%) were men. All patients had HBsAg, hepatitis B e antigen (HBeAg) and HBV DNA. Of the patients, 6 received anti-viral drugs, and 4 were treated by supportive care. One out of four patients who received supportive care had a spontaneous remission. Out of six patients who received anti-viral drugs, four were treated by lamivudine, and the other two by entecavir. Two of the four patients treated by lamivudine achieved complete remission with seroconversion to anti-HBeAg, whereas the other two patients experienced lamivudine-resistant strains with mutations at the at the tyrosine-methionine-aspartate-aspartate (YMDD) motif of DNA polymerase, which were detected at 22 and 23 months after lamivudine treatment, respectively. Therefore adefovir was added in one patient, and lamivudine was switched to clevudine in the other patient. Afterward two patients have been kept remission state of proteinuria. Two patients who received entecavir as an initial therapy went into complete remission, and resistance to entecavir didn't occur during follow-up period. No side-effects were seen in the patients who received anti-viral drugs.

Conclusions: New nucleoside analogues such as entecavir, adefovir or clevudine could be effective at treatment of HBV-MN including lamivudine resistant strains.

SA-PO2866

Factors Influencing Treatment Choice in Idiopathic Membranous Nephropathy (IMN)

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Background: The treatment of patients with IMN may include immunomodulation in patients resistant to conservative therapy alone, or perceived at high risk of progression. This study assesses the factors influencing the decision to treat with immunomodulators and the choice of treatment between cyclophosphamide (CP) and calcineurin inhibitors (CNI).

Methods: An inception cohort of 720 adults with biopsy-proven IMN (from 1976-2005) was derived from the Glomerular Disease Collaborative Network (N=328) and the Toronto Glomerulonephritis Registry (N=392). Subjects were allocated to groups based on initial treatment: no immunotherapy, CP, CNI, glucocorticoids (GC) only, or other immunosuppressant. The second treatment was considered in patients who initially received GC alone. Factors influencing decision to treat were analyzed using logistic regression, and those influencing choice of treatment by multinomial logit.

Results: Changes in choice of first therapy were observed over time. GC alone was the predominant approach until about 1994, at which time CP became the predominant choice. CNI were used in a minority of patients. Serum albumin, proteinuria, and site were significantly different between those treated (66.3%) and those not treated (33.7%).

Factors affecting decision to treat IMN

	Adjusted Odds Ratio	p-value
Site	0.45	0.001
Female	0.73	0.18
Age at biopsy (per increasing year)	0.99	0.11
Race		
-White	1	
-Other	0.86	0.57
Serum albumin <2.5 mg/dL	1.88	0.007
24 hour proteinuria (per g/day)	1.12	<0.001
eGFR (per 10 mL/min/1.73m2)	0.97	0.45
MAP (per mmHg increase)	1.00	0.72

None of the factors analyzed with regards to choice of treatment was significant.

Conclusions: Serum albumin, proteinuria, and site were the only independent determinants of the decision to treat with immunomodulators. No factors were associated with the specific choice of therapy between CP and CNI; however, the sample size of those treated was most likely too small to detect differences.

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

SA-PO2867

Urinary Interleukin-6 (IL-6)and Epidermal Growth Factor (EGF) as Independent Risk Factors for the Progression of Primary Chronic Glomerulonephritis (CGN)

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Background: The aim of the study was to estimate whether initial urinary IL-6 and EGF excretions in patients with newly diagnosed CGN are associated with clinical course and the outcome of the disease during 4-year follow-up.

Methods: 150 Caucasian patients (114 men and 36 women) with biopsy proven primary CGN were included into the study.One day before kidney biopsy urinary excretion of IL-6 (UIL6)and EGF (UEGF)were measured using the ELISA methods (R&D System.106 patients were treated with steroids alone and in 78 patients immunosuppressive therapy was included.

Results: UIL-6 excretion was significant higher (p<0.00001)than in healthy subjects. Significant negative correlation between initial U IL-6 and eGFR was found in patients with CGN (Sr = -0.40). UIL-6 was significantly higher but UEGF excretion was significantly lower in patients with eGFR <60 ml/min/1,73m2 when compared to patients with eGFR > 60 ml/min/1,73m2. After 4-year of follow-up, patients were divided into two groups: progressors (PG) - loss of eGFR > 5ml/min/1,73m2 /year and nonprogressors (NPG) with stable kidney function (loss of eGFR < 5ml/min/1,73m2/year). UIL-6 was significantly higher in PG when compared with the NPG (p<0,0005) and control subjects. On the contrary, UEGF excretion was significantly lower in PG in comparison to NPG patients (p<0,02).

Conclusions: Logistic regression analysis of the UIL-6 and UEGF excretions and traditional risk factors for the progression of the disease (eGFR< 60ml/min/1,73m2, male gender, age > 60 years, glomerulosclerosis > 30% and interstitial fibrosis 2+) showed that the most important independent risk factors for the deterioration of renal function are initial high (>11,8 pg/mgCr) UIL-6 excretion, initial low (<15.468 pg/mgCr) UEGF excretion and male gender. The initial value of IL-6/EGF> 0.46 increased the risk of progression of the disease 7,8 times in patients with newly diagnosed primary CGN.

Funding: Clinical Revenue Support

SA-PO2868

Clinical Outcome of Renal Primary Systemic Amyloidosis after Stem Cell Transplantation

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Background: Regarding the treatment strategy of primary amyloidosis, autologous stem cell transplantation after high dose melphalan shows similar patient survival than melphalan plus dexamethasone, but little is known on the renal outcome when complete remission is achieved.

Methods: Nine patients (sex-ratio F/M=3,7; age 60±9) with biopsy-proven AL amyloidosis and renal manifestations (defined by proteinuria ≥ 0,5 g/d or nephrotic syndrome, and/or a glomerular filtration rate (GFR) ≤ 30 ml/min) were treated in our center. 8 patients received high-dose melphalan and autologous stem cell transplantation.

Results: Renal manifestations included proteinuria (n=8) (median 10 g/d [range 1,5-47]), nephrotic syndrome (n=6) and GFR ≤ 30 ml/min (n=2). Multiple myeloma was associated in 6 patients (75%), at stage II (n=5) and III (n=1) according to the International Staging System. The mean follow-up was 62 ± 19 months; 2 patients died without any hematological or renal response. Among the 6 surviving patients, 4 achieved stringent complete remission (sCR), 1 reached partial remission (PR), and 1 relapsed after 4 years (digestive tract relapse without renal involvement). The patient showing PR, reached terminal renal failure after 33 months and initiated hemodialysis. Renal response defined as a proteinuria ≤ 0,5 g/d was achieved by the 5 other patients, in a mean time of 21 months. All renal responders experienced transitory renal impairment. By month 12, the lowest kidney function was noted with a mean GFR of 35 ml/min (n=5), as compared to 76 ml/min (n=5) on diagnosis. We observed a late improvement with a mean GFR of 54 ml/min (n=4) after 6 years.

Conclusions: In primary systemic amyloidosis treated with high-dose melphalan followed by autologous stem cell transplantation, achieving complete hematologic response is associated with an excellent renal response. Our unique patient with partial remission doesn't allow us to draw conclusions on the renal prognosis in this event. Interestingly we observed worsening renal function during the 1st year, followed by a late improvement.

SA-PO2869

Membranous Nephropathy Associated with Monoclonal Immunoglobulin-M
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Background: Membranous nephropathy (MN) is the most common cause of primitive nephrotic syndrome in adults. MN is characterized by IgG and C3 deposits on urinary side of glomerular basal membrane. Recently autoantibodies against PLA2R have been described associated with idiopathic MN in 70% of the cases. It remains 30% of MN without aetiology. The aim of our work is to study the association of monoclonal gammapathy with MN.

Methods: Retrospective study of all the MN diagnosed in an universitary hospital in France. The time lapse of the study was from January 2000 to July 2010. A monoclonal gammapathy, lupus biomarkers, hepatitis B and C, and HIV serology were systematicly searched for all the patients.

Results: We identified 94 patients with MN, 11 MN were associated with lupus. In the remaining 83 patients, 58 males and 25 females of mean age 53.5 years were studied. In 7 patients we found a monoclonal gammapathy. All the 7 were IgM. In two cases, Waldenström Disease (WD) was diagnosed, in the remain 5 cases, it was concluded to a IgM MGUS. The two patients with WD were treated with success and the MN is in full remission. One patient received a Ponticelli protocol with success on MN associated with a decrease in IgM level. One patient received only rituximab without effect on MN and IgM level. The last 3 patients received supportive care and experienced partial remission. On kidney biopsy we identified IgM deposits associated with IgG and C3 deposits in 5 cases.

Conclusions: Our data suggested that monoclonal IgM could be associated with MN. Full remission of nephrotic syndrome was obtained only when level of monoclonal IgM decreased and we identified IgM deposits in the kidney, thus suggesting a relationship between the hematological disease and nephropathy. The prevalence of MGUS IgM in our cohort of MN is 6% compared to 0,5% in general population in favor of a none fortutious association between monoclonal IgM and MN.

In conclusion, monoclonal IgM could play a role in pathogenesis of MN.

SA-PO2870

Renal Involvement in Patients with Primary Myelofibrosis: Fact or Fiction?
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Background: Some small reports suggest that primary myelofibrosis (PMF) can be associated with renal dysfunction. However, the prevalence of renal disease including glomerulopathy in PMF remains poorly defined.

Methods: Retrospective cohort analysis of all patients with PMF listed in the Mayo Clinic Myelofibrosis Database. Patients without renal function data or those who underwent stem cell transplant were excluded.

Results: Among a total of 565 patients with PMF (mean age 65±12 years, 366 men), the mean serum creatinine (Scr) at referral was 1.16±0.36 mg/dL, eGFR was <60 mL/min/1.73 in 210 patients (37%). Follow-up data were available for 383 patients, and over a mean follow-up period of 34±36 months, the average Scr increased from 1.13±0.35 to 1.17±0.6 mg/dL ($p<0.05$). Only 22 (5.7%) had a Scr ≥2 mg/dl and 5 (1.3%) had ESRD requiring hemodialysis. A total of 241 patients had complete urine analysis at the end of the follow up, and the mean proteinuria was 866±213 mg/day. Proteinuria ≥1 gr/day and ≥3 gr/day was seen in 29 (12%) and 13 (5.3%) patients, respectively. Survival data were obtained by chart review for the follow-up patients. Mortality rates were not different for patients without and with an eGFR <60 mL/min/1.73m2 from the time of the initial visit (mean follow-up time 63±50 months). However, mortality rates from the time of their last follow-up visit (mean follow-up time 29±42 months) were significantly higher for patients who sustained a decrease in eGFR ≥30 mL/min/1.73m2 from the initial to the last follow-up visit (63 vs. 38%, $p<0.01$).

Five kidney biopsies were reviewed. Myeloproliferative-neoplasm (MPN)-related glomerulopathy was the most common finding in four. One biopsy showed ATN. In two patients, the biopsies also showed extramedullary hematopoiesis.

Conclusions: This is the largest study on renal function in patients with PMF and indicates that a decline in eGFR may predict mortality. Contrary to plasma cell dyscrasias, glomerulopathy is rare in PMF and the most common histologic findings are MPN-related glomerulopathy and extramedullary hematopoiesis.

Funding: Private Foundation Support

SA-PO2871

Rituximab *vs.* Calcineurin Inhibitors in Steroid Dependent Nephrotic Syndrome
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Background: There are no studies comparing the efficacy of calcineurin inhibitors (CNI, cyclosporine CsA, tacrolimus Tac) & rituximab (RTX) in maintaining remission in steroid dependent nephrotic syndrome (SDNS). We report a retrospective comparison of their efficacy in SDNS.

Methods: Patients, 1-18 yr-old, with SDNS, failing therapy with ≥2 alternative agents (levamisole, cyclophosphamide, MMF) were eligible. Those receiving subsequent therapy with RTX (375 mg/m^2 IV for 2 weekly doses) or CNI (CsA 4-6 mg/kg/d; Tac 0.1-0.2 mg/kg/d) & followed ≥12 months were included. Relapses were treated with prednisone (2 mg/kg/d until remission, then tapered).

Results: 27 patients received CNI (CsA 14, Tac 13) and 10 RTX. Baseline features were similar (Table).

	Calcineurin inhibitors, N=27	Rituximab, N=10
Baseline		
Age at onset, yr	2.5 (1-7)	4.3 (1-6)
Age at therapy, yr	10.1 (4-17)	12 (8-15)
Relapses/yr	3 (1-7)	3 (1-5)
Cumulative prednisone, mg/kg/6-mo	70 (7-138)	69 (16-127)
Body mass index (BMI) SDS	1.8 (-0.6-4.3)	2.0 (0.5-3.6)
Effect of therapy		
Sustained remission at 1-yr	15 (56%)	6 (60%)
Time to first relapse, months	11 (1-35)	7 (2-32)
Relapses		
At 1-yr	1 (0-5)	0 (0-3)
At last follow up	2 (0-10)	2 (0-3)
Off prednisolone at 1-yr[$]	8 (30%)	8 (80%)
Prednisone dose, mg/kg/6-mo		
0-6 months[$]	50 (24-83)	19 (11-69)
6-12 months*	22 (2-116)	8 (0-69)
BMI SDS	1.6 (-0.3-4.2)	1.4 (0.2-2.7)

*P<0.05; [$]P<0.01

The median follow up was 26 (range 12-50) months in CNI & 14 (12-36) months in RTX groups. Patients treated with RTX had a shorter duration of remission (P=0.3). Relapse rates & proportions of patients with sustained remission & frequent relapses were similar. Patients in the RTX group received significantly lower doses of steroids in the following year. Adverse effects were infusion reactions (4); nephrotoxicity (5) & cosmetic effects (11).

Conclusions: Therapy with RTX was as effective as CNI in reducing relapses in patients with difficult SDNS. Therapy with the former resulted in significant steroid sparing, but the duration of sustained remission was shorter. Prospective studies are required to compare the efficacy of these agents and examine interventions that sustain the effect of RTX therapy.

Funding: Clinical Revenue Support

SA-PO2872

Novel Treatment of Steroid Resistant Nephrotic Syndrome in Children with Oral Galactose
Kristen Sgambat, Asha Moudgil. *Nephrology, Childrens National, Washington, DC.*

Background: Children with steroid resistant nephrotic syndrome(SRNS)typically have FSGS, but may rarely have minimal change histology. These patients are resistant to immunosuppressive(IS) drugs with poor long-term outcome. Immune mediated FSGS has been associated with the presence of a circulating permeability factor(FSPF), thought to increase glomerular permeability to albumin leading to proteinuria and focal sclerosis. Galactose binds to FSPF in vitro, but the effect in vivo is unknown. We prospectively investigated the effect of oral galactose on change in FSPF and clinical response in children with SRNS.

Methods: 52 children with primary SRNS or biopsy proven FSGS were screened. Children meeting inclusion criteria{nephrotic proteinuria(UPC above 2) despite IS therapy and ACE/ARB more than 12 weeks,stable Cr and estimated GFR above 60 ml/min/1.73m2} were tested for FSPF. Those with positive FSPF were treated with oral galactose(0.2g/kg/dose BID)for 16 weeks. Pre and post treatment FSPF levels, UPC, serum albumin(sAlb),and eGFR were assessed.

Results: FSPF was tested in 6 children(8.8±3.8 years old; 3 male,3 female). Of these,5 had FSGS (1 with post transplant recurrence) and 1 had minimal change. Patients with FSGS had positive FSPF, whereas patient with MCNS was negative for FSPF. Three children with baseline eGFR 93.9±26.7 completed 16 weeks of therapy without any side effects. These children were resistant to previous treatments with tacrolimus (3), ACE (2), rituximab(2), cyclosporine(1), and MMF(1). Time from onset of NS to FSPF was 31±8.7 months. Post-therapy, FSPF decreased from 0.74±0.16 to 0.35 + 0.21 and became negative in 2 of 3 children. UPC did not improve after therapy (12.6±9.4 pre and 27.4±31.9 post-therapy). Pre and post treatment eGFR (93.9±26.7 versus 89.4±50.7) and sAlb (2.4±1.3 versus 2.2±0.6) remained unchanged. One child with post-transplant recurrent FSGS entered chronic dialysis at the end of therapy.

Conclusions: Oral galactose was well tolerated in 3 children with SRNS and was able to decrease FSPF to negative range in 2 children. However, no clinical improvement was observed. It remains to be determined whether galactose will be clinically beneficial if used early in the course of SRNS.

Funding: Private Foundation Support

SA-PO2873

Columbia FSGS Classification Predicts Renal Outcome
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Background: Columbia classification (CC)has been proposed as a predictor of worse renal outcome in FSGS. We have analyzed clinico-laboratorial features and renal survival of Brazilian patients according to CC.

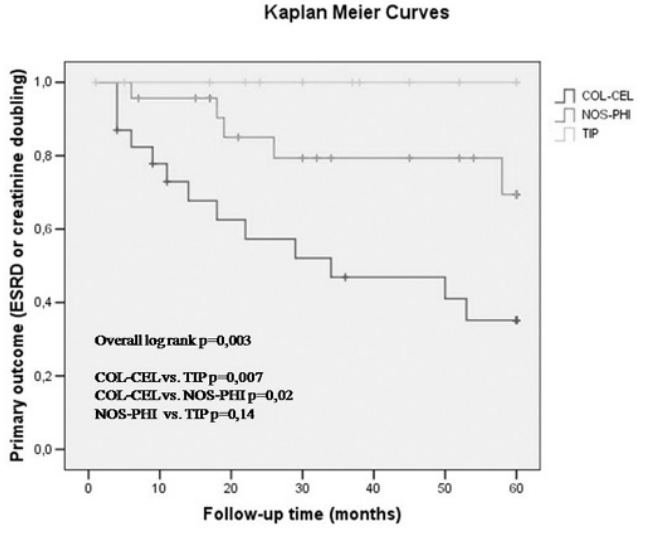
Methods: Inclusion criteria were primary FSGS from 99-09, no dialysis at presentation, and follow-up time >4m. Of 80 patients, 57 had available material for CC reclassification. Data was retrospectively collected. Primary outcome (PO) was defined as ESRD or creatinine doubling.

Results: Of 57 cases, 23 (40,3%) had collapsing or cellular (COL-CEL), 23 (40,3%) had peri-hilar or not otherwise specified (NOS-PHI) and 11 (19,4%) had tip lesion variants (TIP). Baseline variables were similar between groups. The PO was significantly related to age, renal fibrosis, non-response to treatment and to CC. Cox regression showed that CC was significantly associated with PO, even after adjustments for age and fibrosis.

Logistic regression models on the risk of ESRD or creatinine doubling.

	HR	Lower 95%CI	Upper 95%CI	p
Univariate				
Age	0,95	0,91	1,00	0,05
Renal fibrosis	2,54	1,08	5,93	0,03
Non-response at 4th month	5,10	1,66	15,66	0,004
CC (TIP vs. NOS-PHI vs. COL-CEL)	3,48	1,48	8,18	0,004
CC (COL-CEL vs. OTHER)	3,81	1,44	10,04	0,01
CC (non-TIP vs. TIP)	28,68	0,19	4260,27	0,19
Multivariate				
CC (TIP vs. NOS-PHI vs. COL-CEL)	3,20	1,36	7,54	0,01
Age	0,96	0,91	1,01	0,08
CC (TIP vs. NOS-PHI vs. COL-CEL)	3,00	1,25	7,24	0,01
Renal fibrosis	0,01	0,69	4,59	0,24
CC (TIP vs. NOS-PHI vs. COL-CEL)	2,64	1,07	6,52	0,04
Age	0,96	0,92	1,01	0,09
Renal fibrosis	1,79	0,67	4,78	0,25

Kaplan-Meier curves showed that the COL-CEL group had a worse outcome.



Conclusions: CC was an independent predictor of renal outcome in our study.

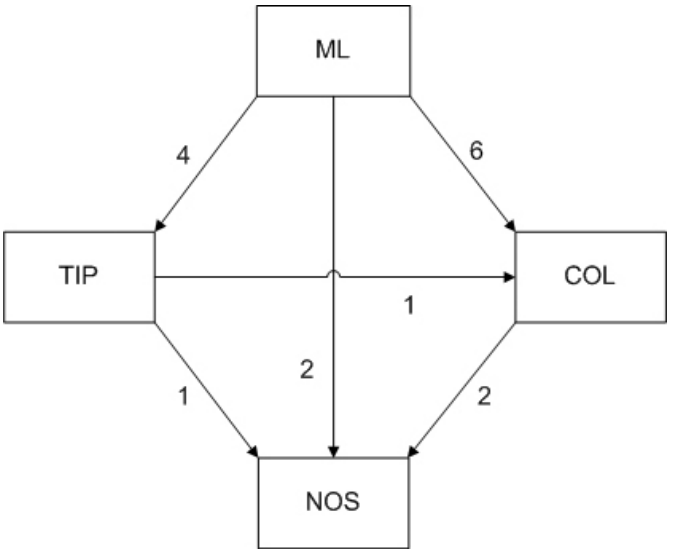
SA-PO2874

Evolution and Concordance of Focal Segmental Glomerulosclerosis Histologic Lesions in Native Kidney and Renal Allograft Biopsies Rutger J. Maas,[1] Eric Steenbergen,[2] Bart Smeets,[2] Elisabeth Cornelissen,[3] Jeroen Deegens,[1] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [2]Pathology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [3]Pediatric Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

Background: The Columbia classification of FSGS defines five mutually exclusive histologic variants. It is unclear if these variants reflect pathogenetic differences. We questioned if the classification of FSGS remains unchanged in an individual patient. Therefore, we compared repeated biopsies, performed in the native kidney as well as in the allograft during recurrence after transplantation.

Methods: We identified 30 patients with a biopsy proven diagnosis of FSGS in the native kidney and FSGS recurrence in a renal graft. Twelve patients were excluded because slides from only one kidney biopsy were available. Biopsy specimens from native (n=22) and transplant kidneys (n=45) of 18 patients were reviewed according to the Columbia classification. Samples without FSGS lesions were classified as minimal lesions (ML).

Results: Median age at FSGS diagnosis of these patients was 15 (range 4-57) years. We evaluated 25 repeated biopsies (8 native kidney, 17 transplant kidney). A change in variant was observed in 16 (64%, figure1). Fourteen patients with known native kidney histology developed a recurrent FSGS in 18 transplants. In the majority of patients the first allograft biopsy showed a ML pattern. We did not find any correlation between the variant in the native kidney and the first or second allograft.



Conclusions: In the majority of patients, there was no concordance in FSGS variants between repeated biopsies or between native and transplant kidney biopsies. These data suggest that the morphological variants must reflect different stages in the evolution of lesions

SA-PO2875

Arterial Stiffness in Childhood Nephrotic Syndrome Christine Sethna, Michael Gottesman, Rachel Frank, Lulette Infante, Suzanne M. Vento, Howard Trachtman. *Division of Pediatric Nephrology, Cohen Children's Medical Center of New York, New Hyde Park, NY.*

Background: Nephrotic syndrome (NS) may place pediatric patients at increased risk for cardiovascular disease (CVD). Arterial stiffness is an independent risk factor for CVD and is associated with proteinuria and hyperlipidemia; however, the relationship between arterial stiffness and childhood NS has not been explored. The objective was to investigate arterial stiffness measured by pulse wave velocity (PWV) and to test the hypothesis that children with NS have higher PWV than healthy controls.

Methods: Carotid-radial PWV was measured using applanation tonometry (AtCor Medical) in 35 subjects with NS and 25 healthy controls. Variables associated with increased pulse wave velocity in subjects with NS were evaluated by Pearson correlation and multiple regression analysis.

Results: NS subjects included 9 F/26 M, age 13.1 ± 3.9 yr, range 6-18 yr. Control subjects consisted of 5 F/20 M, age 12.6 ± 4.0 yr, range 6-18 yr. Age and gender did not differ between groups. NS diagnoses included: 17 MCNS, 11 FSGS, 2 MPGN and 3 Membranous. Median (range) time since diagnosis of NS was 2.3 (0.1-16) yr. Fourteen subjects (40%) had urine protein/creatinine >0.2; the rest were in remission. NS subjects had greater BMI ($p < 0.01$), systolic BP ($p < 0.01$) and diastolic BP ($p < 0.01$) compared to controls. PWV did not significantly differ between NS and controls (7.86 ± 1.47 vs. 7.54 ± 1.62 ms, $p = 0.44$) with no alteration after adjustment for age, gender, race and BMI. PWV in NS subjects positively correlated with age ($r = 0.47$, $p < 0.01$), height ($r = 0.44$, $p < 0.01$), weight ($r = 0.49$, $p < 0.01$), BMI ($r = 0.37$, $p = 0.03$), systolic BP ($r = 0.56$, $p < 0.001$), diastolic BP ($r = 0.5$, $p < 0.01$), and diagnosis of FSGS vs other NS ($r = 0.37$, $p = 0.03$). Cholesterol, proteinuria, time since diagnosis and medications were not associated with PWV.

Conclusions: Contrary to expectation, pediatric patients with NS do not have increased arterial stiffness related to its key features (proteinuria, hypercholesterolemia, treatment). However, the diagnosis of FSGS is associated with higher PWV and may place these patients at increased risk for CVD.

Funding: Other U.S. Government Support

SA-PO2876

Glucocorticoids Alone or with Cyclosporine Is Effective in Korean Patients with Idiopathic Membranous Nephropathy Shin-Wook Kang, Dong Ho Shin, Mi Jung Lee, Seung Jun Kim, Dong Eun Yoo, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Idiopathic membranous nephropathy(IMN) is a frequent cause of nephrotic syndrome(NS) in adults. An accumulating evidence has suggested that combined regimen of glucocorticoids and alkylating agents is beneficial to treat IMN in Western patients. However, glucocorticoids alone or with cyclosporine(CsA) have been reported to be effective for treating IMN in some populations. This study was undertaken to investigate the effects of glucocorticoids alone or with CsA treatment on proteinuria and renal function in Korean IMN.

Methods: A total of 179 Korean NS patients with IMN were included. The primary endpoint was regarded as complete remission of proteinuria(CR), and the secondary endpoints as a decline in eGFR>50% or initiation of dialysis. Cox regression analysis was

conducted to identify the independent power of glucocorticoids alone or with CsA treatment in reaching the primary endpoint, and Kaplan-Meier curves were constructed to compare the difference in attaining the primary and secondary endpoints between groups.

Results: Among the patients, 122 patients(68.2%) were treated with glucocorticoids alone or with CsA, and 57(31.8%) did not receive immunosuppressant treatment. Serum albumin concentrations were significantly lower, while serum cholesterol levels were significantly higher in the treatment group(P<0.05). During the mean follow-up duration of 58 months, CR occurred more frequently in the treatment group compared to the non-treatment group(72.1 vs. 40.4%, P<0.01). In multivariate analysis adjusted for age, gender, blood pressure, initial proteinuria, and serum creatinine levels, the probability of reaching the primary endpoint was significantly higher in the treatment group(OR, 2.75; 95% CI, 1.68-4.49; P<0.01). Kaplan-Meier analysis also revealed that CR rates and event-free rates for the secondary endpoints were significantly higher in the treatment group(P<0.05).

Conclusions: This study shows that glucocorticoids alone or with CsA were effective in inducing CR and preserving renal function in Korean IMN patients.

SA-PO2877

A Steroid-Sparing Therapeutic Option for Minimal Change Disease in Adults: An Initial Report of Tacrolimus Combined with Low-Dose Corticosteroid Therapeutic Trial Yong Chul Kim,[1,2,3] Ho Seok Koo,[2,4] Hajeong Lee,[1,2] Suhnggwon Kim,[1,2] Taewoo Lee,[2,3,5] Ho Jun Chin.[1,2] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea; [2]Kidney Research Institute, Seoul National University, Seoul, Republic of Korea; [3]Biomedical Sciences, Seoul National University Graduate School, Seoul, Republic of Korea; [4]Internal Medicine, Inje University College of Medicine, Republic of Korea; [5]UNC Kidney Center, Unitversity of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Minimal change disease (MCD) in adults not only takes longer to remit compared to children, but the response to high-dose corticosteroid treatment also tends to be less predictable with frequent relapse and drug-dependency. Avoiding prolonged exposure to steroids is challenging in practice. We conducted a single-arm, prospective, interventional study to examine the efficacy and safety of tacrolimus plus low-dose corticosteroid regimen for adult MCD with steroid dependent (SD), frequent-relapsing (FR), and/or intolerance to steroids.

Methods: Eighteen patients (mean age ± SD, 39.1 ± 19.3) with renal biopsy-proven MCD (mean serum albumin, 2.25 g/dL; mean urine protein-creatinine ratio (UPCR), 10.46) were enrolled. Tacrolimus was given orally at a dose of 0.03 to 0.05 mg/kg/day (target trough level, 5-10 ng/dL) for 16 weeks. Oral prednisolone was also simultaneously prescribed at a dose of 0.3 to 0.5 mg/kg/day with slow tapering.

Results: Proteinuria was significantly and consecutively reduced starting from the first week (median [range] of UPCR; 10.45 [3.1-18.4] at baseline, 3.07 [0.03-13.82] at 1 week, 0.14 [0.02-8.28] at 2 weeks; *P*<0.01). Complete remissions (UPCR < 0.3) were attained in 16 out of 17 patients after 16 weeks of the treatment and mean time to complete remission was 3.5 weeks (range, 1 to 16 weeks). One patient dropped out of the study. No significant adverse events were observed.

Conclusions: The therapeutic effect of tacrolimus plus low-dose corticosteroid was shown early with an excellent response rate. This regimen can be considered as a therapeutic option for patients with MCD showing intolerance and/ or resistance to standard corticosteroid therapy.

Funding: Private Foundation Support

SA-PO2878

Focal and Segmental Glomerulosclerosis: An Emerging Glomerulophaty in Jalisco, Mexico. Clinical and Histological Presentation Karina Renoirte, Xochitl Guerrero, Maria Concepcion Oseguera-Vizcaino, Guillermo G. Garcia. *Nephrology, Hospital Civil de Guadalajara, Guadalajara, Jalisco, Mexico.*

Background: Focal segmental glomerulosclerosis (FSGS) is found in 7-15% of patients undergoing evaluation for proteinuria, and is the 3rd cause of nephrotic syndrome wordlwide.There is scare data on this pathology in Mexico. We estimated its frequency, clinical and histological presentation, response to prednisone in patients from Jalisco, Mexico.

Methods: We reviewed 364 native kidney biopsies performed at Hospital Civil, a tertiary-care facility between Jan 2000 and March 2011. Age, gender, BP, degree of proteinuria, hematuria, eGFR, clinical and histological presentation, response to PDN and renal survival were recorded. Renal survive was defined as the doubling of Scr or the start of RRT. Complete remission (CR) was defined as urine proteine < 0.2 g/d; partial remission (PR) as < 3.4g/d but >0.2g/d or < 50% of initial value; steroid resistant (SR) those cases that didn't respond.

Results: FSGS was found in 164(45%) of the cases, followed by Lupus (16%) and membranous (11%) nephropaties. 111 record were available for review. Mean follow up was 27 months (range 1-90). Average glomeruli per biopsy for non-nephrotic patients was 12, range 5-47, and for nephrotic patients 9, range 3-26, p=0.004. No significant difference was observed between both groups for glomerular lesion (tip lesion, podocitopathy, nodular or collapsing type) nor chronicity index.

Clinical characteristics and and renal survival

N	No Nephrotic, N=55	Nephrotic, N= 56	p
Age(y)	25.2±9.3	271±3.7	0.04
Male	29(53%)	31(55%)	0.39
HTN	14(25%)	24(43%)	0.02
eGFR ml/min/1.73m2	74.49±32.1	74.12±41.0	0.95
Proteinuria(%)	1.44±0.75	6.38±3.88	0.0000001
Hematuria(%)	6(11%)	20(36%)	0.0003
Prednisone Rx (%)	46(84%)	56(100%)	0.03
CR(%)	21(41%)	15(28%)	0.08
PR(%)	13(25%)	19(35%)	0.13
SR(%)	14(27%)	17(31%)	0.3
Doubling Scr(%)	4(7%)	9(16%)	0.08

Conclusions: FSGS emergerged as the most most common lesion responsible of proteinuria in native kidney biopsies. Histological lesion, response to prednisone and renal survival did'nt differ among non-nephrotic and nephrotic patients, and is similar as reported in other series. HTN and hematuria were more common in nephrotic patients.

SA-PO2879

Urinary Excretion of Th1, Th2 and Th17 Cytokines in Idiopathic Focal Segmental Glomerulosclerosis. Preliminary Results from a Single Center Maria Stangou, Michael Spartalis, Aikaterini A. Papagianni, Dimitrios Moisiadis, Helen Rizopoulou, George Efstratiades, Demitrios Memmos. *Department of Nephrology, Aristotle University of Thessaloniki, Hippokration General Hospital, Thessaloniki, Greece.*

Background: Idiopathic Focal Segmental Glomerusclerosis (FSGS) usually leads to end stage renal disease. Cytokines excreted in the urine may represent immunological mechanisms and predict response to treatment.

Methods: First morning urine samples, collected at day of renal biopsy from 20 FSGS patients [M/F 15/5 age 43.4yrs (16-75)], and 10 healthy controls, were used to detect IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, INF-γ, G-CSF, GM-CSF, MCP-1, MIP-1B, TNF-β, by a multiplex cytokine assay. Histological findings evaluated were: percentage of global sclerosis, mesangial hypercellularity, interstitial infiltration and tubular atrophy. Renal function and degree of proteinuria were estimated at the day of renal biopsy and at the end of follow up.

Results: FSGS patients, comparing to controls had increased urinary levels of IL-1B (0.02±0.04 vs. 0.008±0.004fg/dl, p=0.05), IL-5 (0.02±0.02 vs. 0.01±0.004fg/dl, p=0.05) IL-12 (0.08±0.07 vs. 0.04±0.01fg/dl, p=0.05) GM-CSF (4.9±9 vs. 0.3±0.7ag/dl, p=0.03), and MCP-1 (1.2±0.9 vs. 0.2±0.2fg/dl, p<0.0001). During follow up, (68.4±40.5months), renal function deteriorated (Screat increased from 1.9±0.9 to 5±4.1mg/dl, p=0.009) and proteinuria reduced Ualb from 5±3.2 to 1.3±1.6g/24hr, p=0.01.

Cytokines which showed significant correlations with histology were mainly produced by Th2 cells. Global sclerosis correlated with: IL-10 (r=0.6, p=0.009), IL-13 (r=0.5, p=0.03) and TNF-beta (r=0.5, p=0.03). Degree of mesangial hypercelullarity had negative correlation with IL-4(r=-0.6, p=0.01), IL-5 (r=-0.6, p=0.03), IL-10 (r=-0.7, p=0.002) and GM-CSF(r=-0.5, 0.04). Patients who did not respond to treatment and progressed to ESRD had increased IL-6 (0.13±0.06 vs. 0.07±0.04fg/dl, p=0.05), MCP-1(1.7±0.9 vs. 0.9±0.8fg/dl, p=0.02) and reduced IL-17 0.03±0.03 vs. 0.05±0.1fg/dl).

Conclusions: Th2 cytokines seem to participate to the progression of histological lesions in FSGS and their urinary levels may predict disease outcome.

Funding: Private Foundation Support

SA-PO2880

A Retrospective Study of Adult-Onset Minimal Change Nephrotic Syndrome Rutger J. Maas, Jeroen Deegens, Jack F. Wetzels. *Nephrology, Radboud University Nijmegen Medical Center (on behalf of the study group), Nijmegen, Netherlands.*

Background: Few studies have addressed the clinical course of adult-onset minimal change nephrotic syndrome (MCNS)

Methods: We retrospectively studied the clinical characteristics, treatment response and outcome of 89 patients with adult-onset MCNS treated since 1985 in eight Dutch hospitals. Median follow-up was 5.0 years (range 0.3-25.7 years)

Results: Baseline characteristics are shown in the table. Acute renal failure with need for dialysis occurred in five patients at presentation. Twelve patients (13%) achieved a complete remission without immunosuppressive therapy after a median of 126 days (range 15-748 days). Seventy-two patients (81%) received initial high-dose corticosteroids (CS), and five cyclophosphamide. In general, tapering of CS was started 2 weeks after attaining partial remission. Total treatment duration averaged 277 days. With initial CS treatment, 61 patients (85%) achieved a complete remission after a median of 42 days (range 7-393 days), 6 (8%) achieved a partial remission after 46 days (range 12-111 days), 4 (4%) did not achieve a remission and 1 died during acute renal failure. All four patients who did not reach a remission on initial CS, ultimately reached a complete remission after additional immunosuppression. Overall, 34% of CS treated patients had persistent remission, relapses

occurred in 66%, median number of treated relapses was 2 (range 1-14).

Conclusions: Acute renal failure is a serious complication of adult-onset MCNS. Long-term outcome is good. Spontaneous remission rate is high. CS treatment results in complete remission in the majority of patients.

Characteristics at onset (mean ± SD)

Clinical characteristics (n=89)	
Age (yr)	48.1 ± 17.5
Gender (male/female)	38/51
Serum creatinine (micromol/l)	101 ± 68
eGFR by modified MDRD (ml/min per 1.73 m2)	79 ± 31
Serum albumin (g/l) (n=88)	19.2 ± 6.6
Urine protein (g/d) (n=84)	10.4 ± 5.3

SA-PO2881

Comparative Response of inmunosuppresive Treatment on Proteinuria in Adults with Primary Focal and Segmental Glomeruloesclerosis Diana Elvia Ceja Villanueva. *Nephrology and Hemodialysis, IMSS, D.F., Mexico.*

Background: The objective was to determine the difference on proteinuria in patients with GSFS treated with cyclophosphamide, mycophenolate mofetil or cyclosporine at 12 and 24 weeks of tretament, reduction according to treatment response and improvement of serum albumin.

Methods: Retrospective clinical study conducted in the Nephrology departament of the Specialty Hospital Raza, IMSS. We analyzed 58 cases of patients registered with FSGS in a period of five years. The cases were divided into three different groups depending on the treatment received. Ciclophosphamide, mycophenolate mofetil or cyclosporine. Albumin and albuminuria were compared to 12 and 24 weeks of treatment, and response to treatment as partial, full or no response.

characteristics of the cases studied

feature	cyclophospamide	mycophenolate mofetil	cyclosporine	value P
Male	12	8	8	
female	12	10	8	
interstitial fibrosis, %	23	32	24	
Tubular atrophy, %	21	19	18	
serum albumin, mg/dl	3.8	3.2	3.6	0.031
serum creatinine, mg/dl	1.47	1.25	1.19	
baseline albuminuria g/L	6.31	7.13	5.43	
albuminuria 12 weeks, g/L	3.59	5.45	4.52	0.013
albuminuria 24 weeks, g/L	3.67	4.93	3.34	0.090
complete response 12 weeks, %	3/24 (12.5)	1/18 (5.5)	1/16 (6.2)	
complete response 24 weeks, %	5/24 (20.8)	0/18 (0.0)	1/16 (6.2)	

The data are presented in number,% and standard deviation,P value .05 with nonparametric Kruskal-Wallis

Results: Significant difference in the different groups of treatment on albuminuria at 12 weeks of treatment (p=031) y improvement of serum albumin (p=013). In the analysis between groups showed significant difference with a value of P= .005 in reducing albuminuria at 12 weeks and improvement of serum albumin, P=.009 in CFA group compared with MMF. Were not significant when compared with the CSA group.

Conclusions: CFA reduces albuminuria no statistical difference in the CsA group, improved both serum albumin, MMF group does not improve the amount of serum albumin compared with CFA and CsA. Mycophenolato mofetil group reported minimal partial response without achieving complete remission.

Funding: Clinical Revenue Support

SA-PO2882

Interstitial Nephritis Is the Commonest Renal Pathology in HIV Infected Patients in West London Jeremy B. Levy,[1] Heather Isenman,[1] H. Terence Cook,[1] Rachael Jones.[2] *[1]Imperial College Renal & Transplant Centre, Imperial College NHS Trust, London, United Kingdom; [2]Dept of HIV, Chelsea & Westminster NHS Foundation Trust, United Kingdom.*

Background: The survival of patients with HIV has been transformed by highly active anti-retroviral therapy (HAART): patients survive longer, develop non-HIV related chronic disease, and are exposed to potentially toxic therapies.

Methods: We retrospectively reviewed the data on all patients with HIV in West London who had a renal biopsy over the last 12 years.

Results: 39 patients had a renal biopsy between 1998 & 2010 (10 female, 29 male; mean age 44). Mean creatinine at presentation was 3.4 mg/dL (sd 3.6). Mean time from diagnosis of HIV to renal biopsy was 8.6 years but 9 patients presented with renal disease as the indicator illness of HIV (diagnoses HIV associated nephropathy (HIVAN; 2), tubulo-interstitial nephritis (TIN; 4), immune complex nephritis (2), advanced scarring (1)). The most common diagnosis overall was acute TIN: 8 patients (21%) as primary diagnosis and 5 (13%) as a major but probably secondary feature. 92% of patients with TIN were taking either AZT or abacavir and mean creatinine was 3.4 mg/dL. 3/13 required dialysis but the remainder recovered renal function with steroids and change of ART. Acute tubular damage was the 2nd commonest finding (6 patients) 7-25 years after HIV diagnosis, all with controlled HIV on ART. Mean creatinine 6.4 mg/dL, 1 required acute dialysis and all recovered renal function. All were taking protease inhibitors and 3/6 tenofovir. HIVAN was uncommon and only seen in 5 patients (13%): all black, mean creatinine 4.5 mg/dL with heavy proteinuria, all uncontrolled HIV with viral loads > 50. None recovered renal function. Other renal diagnoses included IgA, immune complex GN, FSGS, membranous, advanced scarring, focal necrotising GN.

Conclusions: Renal biopsy findings in HIV are very variable with important prognostic features: in West London acute TIN and tubular damage are the commonest findings mostly related to drug toxicity and with a good outcome. There may be an association of AZT & abacavir with TIN, and PIs with ATN. HIVAN is rare in W London and has a poor outcome. Other glomerular diseases are uncommon.

SA-PO2883

Time-Average Proteinuria >2g/day as a Valid Predictor of Worse Renal Outcome in Idiopathic Membranous Nephropathy Masahiro Eriguchi, Toshiaki Nakano, Masatomo Taniguchi, Kazuhiko Tsuruya. *Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: The benefit of achieving a partial remission of proteinuria has not been well described with idiopathic membranous nephropathy (IMN). To assess the partial remission associated with good renal prognosis and establish its prognostic value, we examined the effects of sustained exposure to proteinuria in patients with IMN.

Methods: We studied consecutive 61 patients with biopsy-proven IMN. Baseline data were collected at the time of renal biopsy, and follow-up data (blood pressure, proteinuria, serum creatinine (S-Cr) and total cholesterol) were collected every 6 months and used as time-averaged models. Linear regression analysis was used to determine independent predictors of the rate of glomerular filtration rate (GFR) decline. Cox proportional hazards model was used to evaluate the influence of the time-average proteinuria (TA-P) on the occurrence of renal end-points (EP; exceeding 1.5-fold of the baseline S-Cr level). The subjects were divided into 4 categories according to TA-P (<1 g/d; 1-2 g/d; 2-3.5 g/d; >3.5 g/d), and the rate of GFR decline and renal EP were analyzed among the 4 categories.

Results: With a mean observation period of 7.1 years, 47 patients (77%) achieved <1 g/d of proteinuria. At the last observation, GFR declined at -0.32 ± 0.52 mL/min/1.73m^2/mo overall, 13 patients (21%) had reached renal EP and 5 patients (8%) had reached renal death. Multivariate linear regression analysis and Cox proportional hazards model revealed that TA-P was the most important predictor of the rate of GFR decline and renal EP. When corrected for other parameters with analysis of covariance, the rate of GFR decline differed significantly among the 4 categories of TA-P, and the difference in rate of decline occurred between the 1-2 and 2-3.5 g/d of TA-P. Having <1 g/d of TA-P was similar to 1-2 g/d, but >2 g/d of TA-P was associated with worse renal outcome.

Conclusions: Sustained proteinuria >2 g/d was the strongest predictor of the progression of renal disease in IMN, and the treatment goal of proteinuria <2 g/d is considered to be valid as the partial remission.

SA-PO2884

The Treatment of Hepatitis B Virus-Associated Nephritis with Variation or Refratory to Lamivudine Yongze Zhuang, Xiaorong Zhong. *Dept of Nephrology, Fuzhou General Hospital of Nanjing Command of PLA, Fuzhou, Fujian, China.*

Background: Hepatitis B virus-Associated nephropathy(HBV-GN) is a common secondary glomerulonephritis in China. Lamivudine is often used to treat patients with HBV-GN, but the effect of lamivudine is not found in a few patients.The treantment of Hepatitis B virus-Associated nephropathy with variation or refractory to lamivudine is very difficult.

Methods: 9 cases with Hepatitis B virus-Associated nephropathy had variation or refractory to lamivudine and high level of serum HBV-DNA(≥1.0x10^{5}copy/ml).The quantitation of proteinuria was more than 1.5g/d after treatment of lamivudine.Those patients were treated with entecavir(0.5mg/d).The other drugs ,which were used during the treatment of lamivudine, were continuously used(ACEI or ARB) or stopped(Tripterygium wilfordii).The therapeutic effect of entecavir and the level of serum HBV-DNA were observed during the follow-up time.

Results: Nephrotic syndorme(NS) was found in 7 cases and proteinuria combined with microscopic heamaturia in 2 cases. Among 9 cases, membranous nephropathy was in 3 cases, mesangial proliferative nephritis in 3 cases, membranoproliferative nephritis, IgA nephropathy and focal segmental mesangial proliferative nephritis in 1 case respectively.The patients were treated with lamivudine for 14.1±10.3 months.The corticosteroids was used in 6 caess.After the treatment of lamivudine, partial remission(PR) appeared in 5 cases and no responsive(NR) in 4 cases(NS in the patients).But, after the patients were treated with entecavir for 12 months, complete remisson(CR) was found in 6 cases, PR in 2 cases and NR in 1 case.Among 7 cases whose serum HBV-DNA were detected, the level of serum HBV-DNA decreased apparently and was normal in 5 cases.After follow-up for between 19 months and 27 months, CR was found in 7 cases and NR in 2 cases.The side effects of entecavir were not found during the follow-up.

Conclusions: Entecavir combined with the other renal protection drugs is a safe and effective scheme for the treatmenr of Hepatitis B virus-Associated nephropathy with variation or refractory to lamivudine and may be used continuously for no less than 18 months.

Funding: Pharmaceutical Company Support

SA-PO2885

Treatment with Mizoribine Followed by Low-Dose Prednisone Is Highly Effective in Patients with Idiopathic Membranous Nephropathy and Nephrotic Syndrome Yoshihiro Matsumoto, Yasushi Shimada, Youichi Nojima. *Nephrology and Dialysis, Shizuoka City Hospital, Shizuoka, Japan.*

Background: Idiopathic membranous nephropathy (IMN) patients with persistent high-grade proteinuria are at the highest risk of developing end-stage renal failure. We have previously reported the effects of treatment with mizoribine followed by low-dose prednisone in 4 IMN and nephrotic syndrome patients. The purpose of this study was to elucidate these results by using a larger study group consisting of patients who have been recently diagnosed with IMN and nephrotic syndrome.

Methods: We selected 12 consecutive patients who visited our hospital from 2005 to 2010 and were diagnosed with IMN and nephrotic syndrome. After 2 months of observation without any other treatment, mizoribine was started at a dose of 150 mg/day. After 2 months of mizoribine monotherapy, 20 mg/day prednisone was combined with 150 mg/day mizoribine. After that, the dosage of prednisone and/or mizoribine was tapered gradually according to the urinary protein-to-creatinine ratio (P/C), which is closely correlated to the daily protein excretion. After initiating combination therapy, the urinary P/C and the levels of serum albumin were measured on a monthly basis for 12 months.

Results: Before treatment, the P/C and serum albumin levels of the patients ranged from 3.7 to 15.9 g/g and from 1.6 to 3.4 mg/dl, respectively. Although no patient showed a decrease in the P/C during mizoribine monotherapy, all patients showed a decrease in the P/C with time during combination therapy. At 1, 3, and 9 months after combination therapy, a P/C of <1.0 was observed in 17%, 25%, 75% of the patients, respectively. By month 12, the P/C decreased to <0.4 g/g in 10 of 12 patients, and the remaining 2 patients showed a P/C of 1.1 and 1.4 g/g; in addition, the serum albumin levels increased to >3.5 g/dl in 9 of 12 patients.

Conclusions: The addition of prednisone after an initial treatment with mizoribine alone can have beneficial effects in all IMN patients with nephrotic syndrome. The risks associated with immunotherapy can be decreased by initially using mizoribine alone, which acts as a base for establishing therapy, followed by low-dose prednisone.

SA-PO2886

Fabry Disease in Shanghai --A Survey from Chinese Single Center Zhaohui Wang, Xiaoxia Pan, Hong Ren, Wen Zhang, Nan Chen. *Nephrology, Ruijin Hospital, Shanghai, China.*

Background: Fabry disease is an X-linked lysosomal storage disease with morbility of 1/40,000 in male newborns. The deficiency of lysosomal hydrolase a-galactosidase A (a-Gal A) leads to accumulation of globotriaosylceramide in multiple organs including kidney, skin, cornea and heart which results in variable clinical manifestations.

Methods: Since 2002, 39 families of Fabry disease have been recruited in Nephrology Department of Ruijin hospital in Shanghai, China. 207 related family members have been screened and 85 living patients have been diagnosed finally from 18 provinces in China. 39 hemizygotes and 46 heterozygotes were identified in all pedigrees. Among 39 probands, 31 were male and 8 were female with average age of 29.7 ys and 22 cases were biopsy-proven. Clinical manifestations varied from cerebovascular disease(5%), angiokeratoma(26%), renal insufficiency(28%), hypo-/anhidrosis(38%), acroparesthesia(38%), left ventricular hypertrophy(49%) , ophthalmological lesion(51%) and proteinuria(79%). 32 probands including 7 females and 25 males had renal lesions. 31/32 probands had proteinuria including 2 nephrotic syndrome. 8/32 (25%) patients present microscopic hematuria. 11/32 (34.4%) suffered from renal insufficiency including 5 ESRD.

Results: Almost all the main diagnostic technologies of Fabry disease have been set up in our department such as renal pathology, enzyme activity measurement with both peripheral leukocytes and dried blood spots sampled in filter paper and gene analysis. we screened 1544 hemodialysis (HD) patients and 118 peritoneal dialysis (PD) patients using enzymatic measurement and identified 1 male from HD and PD patients, respectively.

Conclusions: By gene sequencing 18 mutations which included 12 novel ones comprised of 13 missense mutations, 2 nonsense mutations, 1 splicing mutation and a single nucleotide deletion, a gross deletion.

SA-PO2887

Albumin Excretion Fraction for Monitoring Proteinuria When Protein Plasma Level Is Manipulated Gianluigi Ardissino, Francesca Tel, Alice Monzani, Sara Testa, Fabio Paglialonga, Alberto Edefonti. *Pediatric Nephrology Unit, Fondazione Ca' Granda Osp Maggiore Policlinico, Milano, Jamaica.*

Background: Urinary protein/creatinine ratio (uPr/uCr) or protein excretion rate are the most common indicators for monitoring nephrotic syndrome and the efficacy of treatments. However, these indicators are very much influenced by changes in plasma protein level induced by plasma or albumin infusion as well as by plasmaexchange (PEX). In these conditions, it becomes difficult to detect improvement, as a response to treatment, since the increase in plasma protein per se causes an increase in proteinuria.

As an example, in a 12 yo boy with clinically stable FSGS without specific treatment but weekly albumin infusion, uPr/uCr was 15, 95 and 34 at baseline, 1 hr and 24 hrs after albumin infusion, respectively.

In the present paper we explore the working hypothesis that albumin excretion fraction (FeAlb) is more accurate for measuring proteinuria whenever plasma protein level is manipulated.

Methods: A 19 yo girl with FSGS unresponsive to standard treatment (steroids, tacrolimus) was addressed to high volume PEX with albumin solution 4.5 gr/dL (thrice weekly).

Results: Following the first few sessions of PEX, uPr/uCr ratio increased (from 11 to 15) as a consequence of the increase in plasma protein level induced by PEX itself. Following are the basic laboratory before, during and after the PEX treatment. After the 3rd PEX the efficacy of the treatment was clearly detected by FeAlb but not by uPr/uCr (line 4 in table).

Day	sAlbumin	uPr/uCr	FeAlb
0 (Baseline)	1.7	11.3	8.9
6 (PEX 2)	2.5	14.9	10.8
7 (PEX 3)	2.7	15.0	8.2
11 (PEX 4)	2.4	11.3	4.8
14 (PEX 5)	2.9	7.7	2.7
20 (PEX 6)	3.1	8.9	2.4
26	2.9	4.4	1.3

Conclusions: In conclusion in selected conditions (whenever plasma protein level is manipulated), FeAlb seems a better indicator of protein loss than protein excretion rate or uPr/uCr. More studies are needed to test its efficacy

SA-PO2888

Low Glomerular Density with Glomerulomegaly Characterizes Renal Biopsies of Obesity-Related Glomerulopathy Nobuo Tsuboi, Go Kanzaki, Yasunori Utsunomiya, Tetsuya Kawamura, Tatsuo Hosoya. *Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.*

Background: Obesity-related glomerulopathy (ORG) is a secondary form of glomerular disease that may occur in individuals with obesity. It is histologically characterized by a marked glomerulomegaly closely related to glomerular hyperfiltration. However, there is likely to be factors other than obesity that contribute to the development of this renal complication since only a minority of obese individuals actually manifests renal injury. This study examined the glomerular density (GD; non-sclerotic glomerular number/renal cortical area of biopsy) in ORG to determine if the difference in the glomerular number is associated with the pathogenesis.

Methods: Obesity and overweight were defined as BMI≥30 and 25≤BMI<30kg/m^2, respectively. ORG was morphologically defined as obesity-associated glomerulomegaly with or without FSGS lesions. Patients with any evidence of other renal diseases or eGFR<60 ml/min/1.73m^2 at biopsy were excluded. The GD and the glomerular volume were measured using a computed imaging analyzer.

Results: The distribution of the GD in the biopsies of ORG was extremely low (1.7±0.6/mm^2, n=20). This was quite different than the widely-distributed GD in biopsies of kidney transplantation donors (3.1±1.0/mm^2 , n=20). However, an analysis of autopsy cases without renal diseases showed that the distribution of the GD in overweight (2.9±0.7/mm^2, n=15) or obese (3.1±1.1 x$10^6\mu m^3$, n=8) subjects was similar to that in the non-obese subjects (3.1±0.6 x$10^6\mu m^3$, n=25). The biopsies of patients with ORG showed marked glomerulomegaly (6.3±1.8 x$10^6\mu m^3$) compared with those of kidney transplantation donors (2.4±0.6 x$10^6\mu m^3$). However, only a modest increase in glomerular size was found in the overweight (3.8±1.5x$10^6\mu m^3$) or obese (3.7±1.5x$10^6\mu m^3$) autopsy kidneys without renal diseases. The comparison of the GD in the normotensive ORG patients and hypertensive ORG patients did not show significant difference.

Conclusions: Kidneys with a low GD may be a potential characteristic of individuals who are susceptible to obesity-induced glomerular enlargement and subsequent renal injury.

SA-PO2889

Influence of a Functional Polymorphism of Aldosterone Synthase Gene on Membranous Nephropathy Christos Bantis, Peter J. Heering, Nicoletta-Maria Kouri, Maria Stangou, Christina Schwandt, Nicola Kuhr, Lars C. Rump, Katrin Ivens. *Department of Nephrology, Heinrich-Heine University, Düsseldorf, Germany.*

Background: The C-344T polymorphism of the aldosterone synthase gene was associated with serum aldosterone levels and the development of arterial hypertension. In the present study we evaluated its influence on primary membranous nephropathy.

Methods: Fifty-nine patients with biopsy-proven primary membranous nephropathy were followed up for 8.0 ± 11.8 years. Patients were classified according to their slope of reciprocal serum creatinine (≥ or < -0.1 dl*mg-1 *year-1) into groups A (slow progressors, n=42) and B (fast progressors, n=17). One hundred healthy volunteers were analysed as controls. Aldosterone synthase gene C-344T polymorphism was determined by PCR amplification in all patients and controls. Serum aldosterone levels were determined by ELISA in 57 patients with chronic glomerulonephritis.

Results: Aldosterone synthase gene C-344T polymorphism influenced the serum aldosterone levels (CC/CT: 106.8 ± 70.4, TT: 243.2 ± 323 pg/ml, p<0.05). The genotype frequencies were similar in patients and control subjects (ns). Age, renal function, proteinuria and blood pressure did not differ significantly at the time of renal biopsy between patients with different genotypes (ns). The genotype distribution was similar patients in group A (CC/CT: 71.4%, TT: 28.6%) and to group B (CC/CT: 76.5%, TT: 23.5%, ns). Furthermore, there was no significant difference regarding the actual rate of progression in patients with different genotypes (CC/CT genotypes: -0.099 ± 0.151, TT: -0.050 ± 0.066 dl*mg-1 *year-1; ns). Aldosterone synthase C-344T polymorphism had also no impact on the kidney survival rate in the Kaplan Meier analysis (ns).

Conclusions: Our results indicate that the functional aldosterone synthase gene C-344T polymorphism does not influence the clinical course of membranous nephropathy.

SA-PO2890

Abstract Withdrawn

SA-PO2891

Effect of the Initial Type of Hemodialysis Access Planned in the Elderly on Mortality Outcomes Ranil N. Desilva, Bhanu K. Patibandla, Yael Vin, Akshita Narra, Alexander S. Goldfarb-Rumyantzev. *Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background: Patients with fistulas that are functional at the time of hemodialyis initiation are known to have superior survival compared to those with grafts and catheters. Because some of the AV fistulas and AV grafts fail to be functional at the time of dialysis start, we measured outcomes associated with the type of access first placed in preparation for hemodialysis (as opposed to the access used at hemodialysis initiation) in the elderly population.

Methods: We included incident hemodialysis patients who started dialysis from 2005 to 2008, ≥67 years of age, from the United States Renal Data System. Medicare Claims from 2003-2008 were used to identify placement of the first vascular access. Primary variable of interest was the type of vascular access first placed (i.e., fistula, graft, or central catheter), with primary outcome of all-cause mortality (time to death) measured from the first out-patient hemodialysis. Data analyzed using Cox regression model adjusted for age, sex, race, co-morbidity index, BMI, cause of ESRD, and diabetic status.

Results: 115,425 subjects were identified with first access placed being AV fistulas (n=21,436), AV grafts (n=3,472), and catheters (n= 90,517). Significant mortality benefit was found in those with fistula or graft placed as a first vascular access compared to those with catheters [HR 0.506, p<0.001 and HR 0.562, p<0.001 respectively]. Cohort was then stratified by age into three groups: 67-79, 80-89, and ≥90. Each of these three groups demonstrated statistically significant benefit from initial placement of an AV fistula or AV graft compared to catheter: for AVF [HR 0.506 (p<0.001), HR 0.527 (p<0.001), and HR 0.509 (p<0.001), respectively for each age group] and for AVG [HR 0.562 (p<0.001), HR 0.513 (p<0.001), and HR 0.522 (p=0.0013), respectively for each age group].

Conclusions: First access placed in an elderly prospective hemodialysis patient being an AV fistula or AV graft is associated with better mortality outcomes compared to catheters.

SA-PO2892

Effect of the Initial Type of Hemodialysis Access Used in the Elderly on Mortality Outcomes Ranil N. Desilva, Gurprataap Singh Sandhu, Jalaj Garg, Alexander S. Goldfarb-Rumyantzev. *Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background: Though the "fistula first" initiative is intended to be applied across all population subgroups, we hypothesized that certain subpopulations (i.e., elderly and those with greater co-morbidity) might not benefit from it.

Methods: Study cohort included incident hemodialysis patients from 2005 to 2007 ≥70 years old, derived from the United States Renal Data System. Primary variable of interest was the type of vascular access used at first outpatient hemodialysis (i.e., fistula, graft, or central catheter), with primary outcome of all-cause mortality (time to death measured from the first out-patient hemdialysis).

Results: Of the 82,202 elderly patients, 82.2% were dialyzed initially by catheter, 13.5% by AV fistula, and 4.2% by AV graft. Cohort was stratified by age (70-79, 80-89, and ≥90). Each of these age groups demonstrated a survival benefit with use of an AV fistula compared to catheter [HR 0.56 (p<0.001), HR 0.55 (p<0.001), and HR 0.69 (p=0.007) respectively]. The cohort was further sub-stratified based on specific co-morbidities: those ≥90 lost significant benefit of a fistula compared to a catheter if history of malignancy (HR 0.45, p=0.182), peripheral vascular disease (HR 0.70, p=0.350) or diabetes (HR 0.67, p=0.146) was also present. Comparing graft to a catheter, both groups 70-79 and 80-89 had significant benefit compared to catheter (HR 0.73, p<0.001 and HR 0.74, p<0.001 respectively). However, significance was lost in those ≥90 (HR 0.83 p=0.354). Graft use, in 80-89 years old with a history of malignancy or peripheral vascular disease also did not reach significant benefit compared to a catheter (HR 0.88, p=0.423 and HR 0.85 p=0.221 respectively).

Conclusions: While fistulas and grafts are generally advantageous to survival, there are subgroups of hemodialysis patients (≥90 years old with peripheral vascular disease, malignancy, or diabetes), where their use may not be of clear benefit compared to catheters.

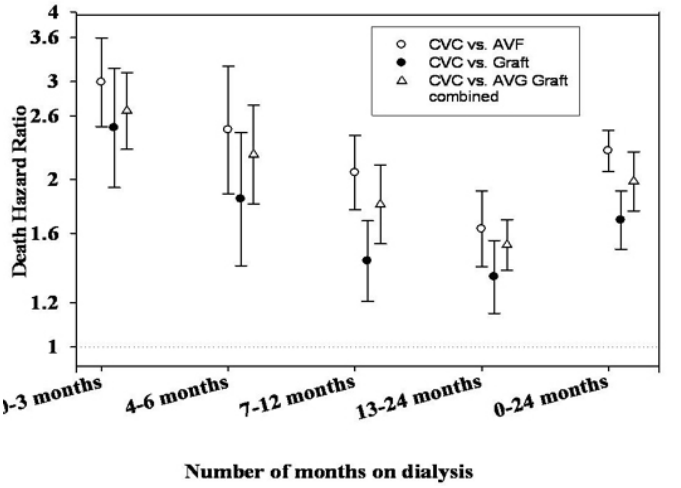
SA-PO2893

Vascular Access Type and Early Death in the First Two Years of Hemodialysis Treatment Lilia R. Lukowsky,[1,2] Leeka I. Kheifets,[2] Onyebuchi A. Arah,[2] Allen R. Nissenson,[3] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Torrance, CA; [2]UCLA School of Public Health, Los Angeles, CA; [3]DaVita Inc., Denver, CO.*

Background: Mortality is exceptionally high during the first year of hemodialysis treatment. There are limited data about the role of dialysis access type in early mortality of hemodialysis patients. We hypothesized that central venous catheter (CVC) is associated with stronger death risk in the first several months of dialysis therapy initiation.

Methods: We identified 18,707 incident MHD patients, who had started MHD treatment from the first week of therapy in a DaVita clinic (prior to Gambro acquisition) between 7/1/2001 and 6/30/2006. We calculated the risk of death at the time periods of 0-3, 4-6, 7-12, and 13-24 months after starting of dialysis therapy comparing patients with CVC to those with different types of vascular access.

Results: The incident MHD patients had a mean age of 63±15 years and included 45% women, 24% African Americans and 14% Hispanics. Use of CVC was associated with increased mortality during all time periods, but the death hazard ratio was even higher during the first 3 months of the therapy (2.7 [95% CL: 2.3-3.1]) compared to all other types of vascular access; it was 3.0 (2.5-3.6) compared to arteriovenous fistula (AVF) and 2.5 (1.9-3.2) compared to graft.



Conclusions: Among incident MHD patients, mortality hazard was almost 3 fold higher for patients with CVC compared to patients with AVF. Examining interventions to reduce use of CVC at the start of dialysis therapy and its impact on survival are indicated.

Funding: NIDDK Support

SA-PO2894

The Effect on Cardiac Function of AVF Flow in Hemodialysis Patients Misaki Moriishi,[1] Hideki Kawanishi.[2] *[1]Internal Medicine, Tsuchiya general hospital, Hiroshima, Japan; [2]Surgery, Tsuchiya general hospital, Hiroshima, Japan.*

Background: Cardiac failure is majority of deaths in hemodialysis patients. Some studies demonstrated that increase of the blood flow by AVF flow might cause left ventricle overload, resulting left ventricular failure. This study was to evaluate if AVF flow contributes to an increase in left ventricular mass (LVM).

Methods: 43 hemodialysis patients with ejection fraction (EF) more than 50% who had first AVF access created, entered the study. All were performed echo cardiography prior to AVF access creation and again at 12, 24 months post-hemodialysis initiation. Blood flow of brachial artery (BA Flow) as AVF blood flow was measured by echography prior hemodialysis initiation and again at 12, 24 months post-hemodialysis induced. Date were analysed usig Student's t-test, correlation coefficients and regression.

Results: The mean BA Flow was 420.1±175.6 mL/min prior hemodialysis initiation, and increased to 486.5±233.2 mL/min at 24 months post-hemodialysis initiation (p<0.01). The mean LVM was not changed at 24 months post-hemodialysis initiation(prior : 251.6±69.2 g , post-24 months 232.±84.3 g). BA Flow at 24 months did not correlate with percent change in LVM. Percent change in LVM correlated with percent change in percent change in EF.

Conclusions: Our study shows that blood flow of AVF had not a effect on left ventricular mass in hemodialysis patients with normal cardiac function.

SA-PO2895

Effect of the "Fistula First" Paradigm on the Population Attributable Fraction (PAF) for Mortality from Vascular Access (VA) in the Australian and New Zealand (ANZ) Hemodialysis (HD) Population Mark R. Marshall,[1] Stephen P. McDonald,[2] Peter G. Kerr,[3] Kevan R. Polkinghorne.[3] *[1]Department of Renal Medicine, Counties Manukau District Health Board, Auckland, New Zealand; [2]Australia and New Zealand Dialysis and Transplant Registry (ANZDATA), Adelaide, Australia; [3]Department of Nephrology and School of Medicine, Monash Medical Centre and Univeristy, Clayton, Australia.*

Background: Arteriovenous fistulas (AVFs) are associated with lower mortality than arteriovenous grafts (AVGs) and central venous catheters (CVCs). The "Fistula First" paradigm emphasizes the need to increase use of AVFs. We determined the PAF for mortality attributable to AVGs and CVCs pre and post "Fistula First" in prevalent stable HD patients in ANZ.

Methods: Using the ANZDATA Registry, we included all adults commencing renal replacement therapy (RRT) between 1999-2007 and on HD at 1 year post-RRT inception. We defined the primary exposure as VA recorded in the next ANZDATA survey. We

categorized patients into eras (1999-2002, 2003-7). The primary outcome was death. We used Cox regression with shared frailty by hospital, to determine PAF attributable to AVGs and CVCs in each era adjusting for baseline characteristics.

Results: We analyzed 1936 deaths in 5079 pts (18502 pt-years) from 70 hospitals. Compared to 1999-2002, there was a lower prevalence of AVGs but a higher prevalence of CVCs in 2003-2007. Adjusting for age, sex, ethnicity, late referral, eGFR at RRT inception, BMI, flux, HD time & dose, primary renal disease, diabetes and co-morbidity, the HR for death (relative to AVFs) was 1.64 (1.41-1.90) for AVGs and 1.78 (1.10-2.87) for CVCs. 354 of the deaths (PAF 18.3%) were attributable to VA other than AVFs:

	Pre "Fistula First" (1999-2002)		Post "Fistula First" (2003-2007)	
	n (%)	PAF (95% CI)	n (%)	PAF (95% CI)
All pts	2423	-	2656	-
AVF	1,737 (71.6)	-	1909 (71.9)	-
AVG	268 (11.1)	6.3% (4.1% - 8.4%)	203 (7.64)	4.6% (3% - 6.2%)
CVC	418 (17.25)	11.9% (4% - 19.2%)	544 (20.48)	13.9% (4.6% - 22.3%)

Conclusions: The lower PAF due to less AVGs post "Fistula First" has been offset by the higher PAF due to more CVCs. We suggest a complementary paradigm of "Line Last", and re-emphasize establishing AVFs before HD inception to avoid ongoing CVC use.

Funding: Government Support - Non-U.S.

SA-PO2896

Haemodialysis (HD) Via a Functioning Native Arteriovenous Fistula (AVF) Is Associated with a Lower Erythropoietin (EPO) Requirement in Camparison to HD Via a Central Venous Catheter (CVC) Muhammad Umair Sharif,[1] David Lappin,[2] Donal N. Reddan.[2] *[1]Nephrology, Mayo General Hospital, Castlebar, Ireland; [2]Nephrology, University College Hospital, Galway, Ireland.*

Background: EPO use is a significant cost in patients requiring long-term haemodialysis (HD). Higher doses of EPO are known to be an independent predictor of mortality & morbidity in this patient population. Due to difficulties in obtaining and maintaining a functioning native AVF many patients in our HD centre dialyse using a CVC. In this study, we examined the relative amount of EPO use in HD patients dialysing either via an AVF or CVC.

Methods: EPO requirement for 53 maintenance HD patients was calculated & then matched with their dialysis access, whether native AVF or CVC. The following conversion factors were applied when converting other forms of EPO to similar dose equivalents of epoetin alpha or beta.

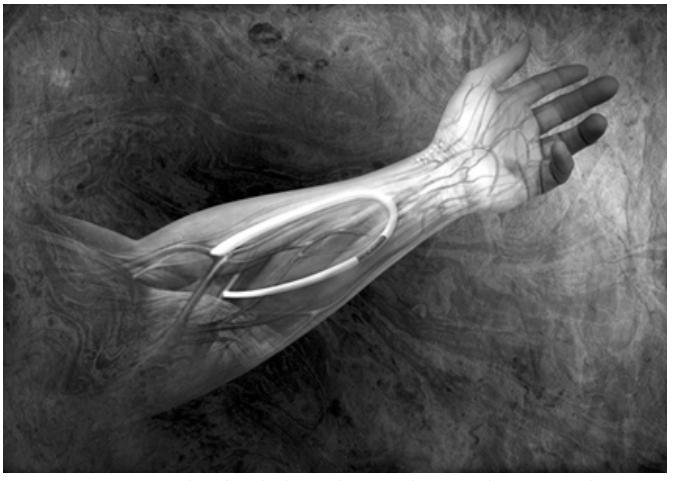
Epoetin = Epoetin alpha = Epoetin beta

1 mg of Darbepoetin (Aranesp) = 200 units of Epoetin

1 microgram Methoxy polyethylene glycol-epoetin beta (MIRCERA) = 200 units of Epoetin

Results: In our study patients with a native AVF required an average of 7919 Units of Epoetin/week as compared to patients dialysing via a CVC who required an average of 10125 units of Epoetin /week.

	AVF	CVC
Age	55+_27	62+_28
No. of Patients	32	21
Hemoglobin	11.1+_2.1	11.6+_2.7
Average EPO Requirement/ Week/Patient	7919 Units	10125 Units



Conclusions: HD via a functioning native AVF, in comparison to HD via a CVC, results in significant cost savings of approximately 72 euros per patient per month or 850 euros per patient per year.

SA-PO2897

Non-Programmed Vascular Access Is Associated with Greater Mortality in Patients Who Return to Hemodialysis with a Failing Renal Graft Gustavo Laham, Carlos H. Diaz, Gervasio Soler Pujol, Mario Davalos, Ana M. Cusumano, Antonio R. Vilches. *Nephrology Section, Department of Medicine, CEMIC, Buenos Aires, Argentina.*

Background: There is an increasing number of Pts entering dialysis because of a failing graft. Although the use of a catheter for vascular access (VA) in Pts entering a hemodialysis program (HD) is associated with increased mortality it is not known whether this association also holds in the case of failed renal transplants (Tx). The purpose of our study was to assess the relationship between the type of VA and mortality in Tx patients re-entering our HD program.

Methods: Between 1/1995 and 05/2010, 131 incident Pts started HD after a failed Tx. The cohort was divided into 2 groups according to the type of VA: 1) Planned VA (PVA), A-V fistula or a graft; and 2) Unplanned VA (UPVA), (catheter). Pts were censored at the time of re-transplantation or loss of follow up (FU). Co-morbid conditions were weighted using Khan's index based on age, presence of diabetes and organ-specific conditions. Cox regression analysis was used to establish mortality predictors and Kaplan-Meir's method for survival comparisons.

Results: Mean age was 44 years; 67.9% were males and median follow up was 52 months, IQR 14-105. Median Tx survival was 106 months, IQR 62-162, and the serum creatinine at the start of HD was 6.2 mg/dl +/- 2.4. 48/131 Pts (36.6%) died during FU.There were significant differences in age ($p<0.04$), Khan index ($p<0.008$), time on HD after re-started on the program ($p<0.0001$), and survival when the PVA (n=82) and UPVA (n=49) groups were compared. Mortality was 26.8% and 54.2 5%, respectively, Log Rank test $p<0.0001$. Multivariate Cox regression analysis showed that catheter use was independently associated with a greater mortality after adjusting for the other variables also associated with mortality in this cohort, such as Khan Index and donor type; Odds ratio 6.5; 95%; Confidence Interval 2.9-14.

Conclusions: In this retrospective study patients entering our institutional hemodialysis program after a failed renal Tx using a catheter, as opposed to a previously fashioned permanent access, showed a greater all cause mortality.

SA-PO2898

Hemodialysis Vascular Access Patency: Results from a Single Centre Initiative Janet Lynn Graham, Peter Magner, Swapnil Hiremath. *Division of Nephrology, Ottawa Hospital, Ottawa, ON, Canada.*

Background: Guidelines recommend the arteriovenous (AV) fistula as the vascular access of choice. The data on patency rates is from studies done over two decades ago; since then the hemodialysis population has changed with increasing proportion of older patients with diabetes and vascular comorbidities. At our academic centre, we have an active vascular access monitoring program, comprising of regular access flow surveillance, weekly rounds with interventional radiology +/- vascular surgeons and early intervention. The aim of our study was to examine the patency rates of AV access with this system of active surveillance.

Methods: This was a retrospective cohort study examining all patients who had creation of a vascular access at our centre over the period 2003 to 2010. Data was abstracted from our administrative database. Access survival was calculated as time from access placement to abandonment, including intervening manipulations (surgical or endovascular). Intervention-free access survival was measured as the time from access placement to any intervention designed to maintain or re-establish access patency. Kaplan-Meier survival analysis and the life table method were used to calculate access patency rates.

Results: 1064 patients, with 62% men with a mean age of 63 +/- 18 years had a first vascular accesses created over the period of study, with 960 AV fistula and 104 grafts. 319 accesses failed over the period of study with thrombosis being the commonest cause (68.5%). The median survival for fistula at 6.21 years was much longer than grafts at 1.05 years. 175 patients had a 2nd attempt at a vascular access (81% fistula, 17% graft), 35 patients had a 3rd access (83% fistula, 17% graft) and 5 patients had a 4th vascular access (all fistula). 482 patients (45%) required at least one intervention to assist in maintaining patency, an angioplasty being the commonest intervention (418/482, 87%). The intervention-free survival of the vascular accesses was also higher for fistula (4.4 years) compared to grafts (0.58 years).

Conclusions: A system of active vascular access surveillance results in a high access survival rate despite the increasing vascular comorbidity burden in the hemodialysis population.

Funding: Clinical Revenue Support

SA-PO2899

Who Is Eligible for a Fistula? A Survey of Vascular Surgeons Andra Nica,[1] Charmaine E. Lok,[2] Timmy C. Lee,[3] Jeremy R. Harris,[1] Michele H. Mokrzycki,[4] Ivan D. Maya,[5] Miguel A. Vazquez,[6] Louise M. Moist.[1] *[1]London Health Sciences Center -Victoria Hospital, London, ON, Canada; [2]University of Toronto, ON, Canada; [3]University of Cincinnati, OH; [4]Albert Einstein College of Medicine, NY; [5]University of Alabama, AL; [6]UT Southwestern Medical Center, TX.*

Background: The arteriovenous fistula (AVF) is the recommended vascular access (VA). Currently there are no criteria to determine which patients are eligible for an AVF.

Methods: We conducted an international survey of VA surgeons to assess the patient, vessel and process considered to decide the type of VA created, as well as contraindications and barriers to VA.

Results: The 131 responses had marked variability in the pre-operative assessment of patients. We found variability in the minimum vein diameter that surgeons consider for AVF creation. In general, surgeons accept a smaller cephalic vein, but require a larger basilic vein for a basilic vein transposition. The use of venous duplex ultrasound mapping is done in 70-90% of patients regardless of age, gender or co-morbidities. There was significant variability in its influence on the final VA decision.

Patient co-morbidities were the biggest deterrent for fistula creation, while a history of one failed VA increased the use of grafts rather than AVFs.

Absolute contraindications to a permanent VA were patient life expectancy less than 1 year, left ventricular ejection fraction (LVEF) <15%, and dementia. No absolute contraindications were indicated by 40% of surgeons. Figure 1 shows perceived barriers to the creation and use of an AVF.

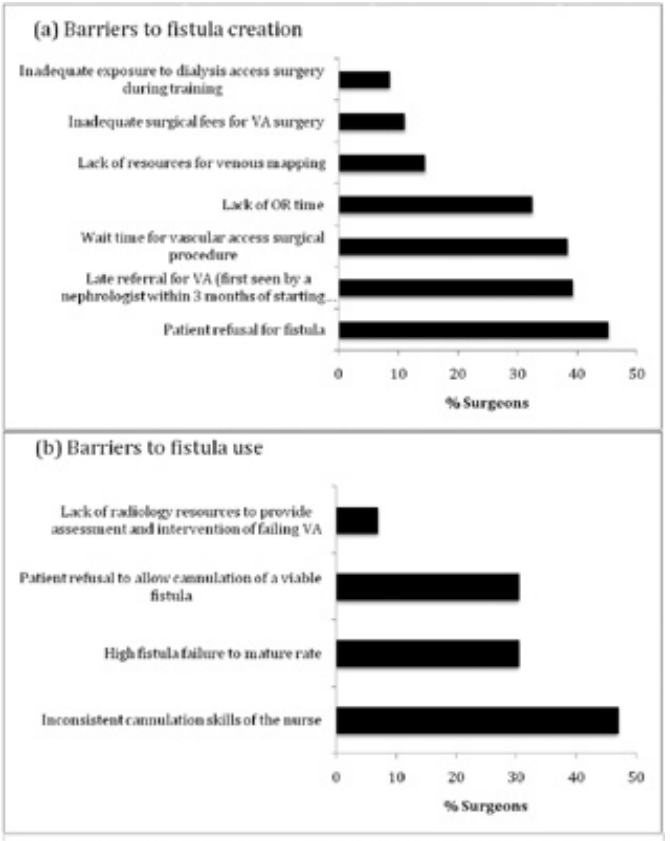


Figure 1. (a) Perceived barriers to establishing a mature AVF. (b). Perceived barriers to use of a created AVF

Conclusions: This study demonstrates great variability in the pre-operative assessment of patients, and in the criteria used to determine which type of VA a patient receives. Establishing guidelines for VA access eligibility is an important future step in optimizing patient care.

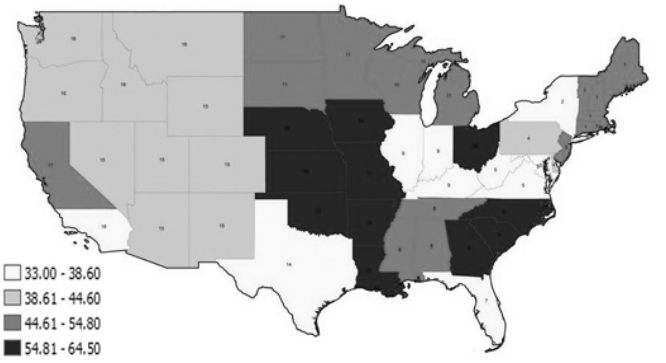
SA-PO2900

Surgical Specialist Practice Pattern Variation in AVF Placement across ESRD Networks for Late Stage CKD and ESRD Medicare Patients Edwin D. Huff, Sumit Mohan, William M. McClellan, The FFBI Data Committee. *Fistula First Breakthrough Initiative.*

Background: Given variation in practice patterns in many areas of medical care we inquired into the possible existence of similar variation among specialists treating ESRD patients. If specialists of fistula placements can be distinguished within ESRD Networks, it may be possible to increase referrals for such placements and increase fistula placement rates.

Methods: We reviewed over 600,000 part B Medicare claims for late stage CKD and ESRD patients who had hemodialysis access placements and/or access related diagnostic, maintenance or revision procedures during 2009.

Results: The variation in the ratio of surgeon AVF placement procedures over all procedures done by surgeons is over 2.5, from 13.4% to 34.1%. Which physician specialists are doing more AVF placements than others? General surgeons do 46% of all AVFs, followed by vascular surgeons, who do 38%. NW variation revealed rates from 33% to 64% done by general surgeons (See display), and rates from 20% to 54% done by vascular surgeons across regions. What variation exists in the availability of surgical specialists who perform permanent access placements across ESRD Networks? The presence of Ambulatory surgeons and Physician Assistants doing AVF procedures varies considerably with more of these specialties active in rural western regions.



Conclusions: What surgical specialists do varies considerably across the 18 ESRD Networks. Practice patterns and concentration within a practice vary over two fold within the same surgical specialty depending on regional distinctions.

Funding: Other U.S. Government Support

SA-PO2901

Arteriovenous Fistula for the 80 Years and Older Patients on Hemodialysis: Is it Worth It? Annie-Claire Nadeau-Fredette, Remi Goupil, Bernard Montreuil, Annie Carignan, Martine Leblanc. *Hôpital Maisonneuve-Rosemont, Université de Montréal.*

Background: Over the last years, the proportion of patients older than 80 years with end-stage renal disease has been constantly growing. Arteriovenous fistula (AVF) is known as the best vascular access in hemodialysis (HD) population but the evidence for its added value is lacking for older patients. We evaluated patients aged 80 years and older (>80 yo), in whom a new AVF had been installed and compared their outcome to a group of HD patients between 50 and 60 years.

Methods: For both groups, we identified every new vascular access (AVF and central venous catheter [CVC]) created or installed between June 2005 and June 2008. We collected demographic and clinical data as well as radiological and surgical interventions from June 2005 to April 2010. We calculated primary failure rates (AVF never used after 6 months from creation), primary patency (intervention-free survival) and secondary patency (survival until definitive failure) durations for every new AVF.

Results: In our study, 55 and 57 patients had a new vascular access (AVF or CVC) in the 50 to 60 yo and > 80 yo groups respectively. Among these, 41 and 26 were new AVF in the younger and older groups. As recognized in the literature, primary failure was significantly more frequent in the older group than in the younger group (40% vs 17%, p =0.0449). When excluding primary failure, the primary patency was not significantly different in both groups (median 8 vs 16 months, p=0.32). However, the secondary patency was significantly shorter for the older group (p=0.04). During the 58 months of observation, the mortality rate was 24% in the 50 to 60 yo group while it was 54% for the > 80 yo patients. Among the younger group, the presence of an AVF was associated with a significant lower rate of mortality (12% vs 43% p=0.008) but not in the older group (46% vs 60%, p=0.28).

Conclusions: In conclusion, knowing the advantages of AVFs in the HD population and keeping in mind the similar primary patency duration shown here between the older and younger groups, patients of 80 yo should probably be considered just as younger patients for AVF creation for hemodialysis.

SA-PO2902

Hemodialysis Patients' Preference for Thigh Grafts – A Cross-Sectional Survey Zulqarnain Abro,[1] Sunanda J. Ram,[1] Neville R. Dossabhoy.[1,2] *[1]Nephrology, LSUHSC, Shreveport, LA; [2]Nephrology, VA Medical Center, Shreveport, LA.*

Background: Recent studies have shown that survival and complication rates of thigh grafts are similar to or better than those of arm grafts and fistulas. However, there is little information in the literature regarding patients' preference for thigh grafts.

Methods: This IRB-approved cross-sectional survey was conducted on patients currently on hemodialysis (n=159), who were queried regarding their preference for access location (arm access *vs.* thigh graft) and details of their dialysis access history. Data on age, race, gender and educational level was collected. Data is presented as means or percentages and analyzed using unpaired t test or chi square test. Significance set at P< 0.05.

Results: 93% were African American, 52% were female, 94% had been on dialysis ≥1 year. Most patients (79%) had completed high school or higher education. Most patients' current access was arm fistula (54%), followed by catheter (30%) and arm graft (13%). Only 3% had thigh grafts currently; 5.7% had previously had thigh grafts. Overall, 90% patients preferred arm accesses, but 10% said they would go for a thigh graft if there was no arm site available. The patients' preference for access location was not influenced by their level of education, nor by a prior history of placement of arm access. Patients' age, gender, particular dialysis unit and dialysis vintage did not seem to influence patient preference.

Those patients with a current (3.1%) or prior thigh graft (5.7%) were more likely to prefer a thigh graft for their next access, when compared to those without (P<0.05). However, even in this subset, the majority still preferred arm access (approximately 60% to 40%).

Conclusions: The likelihood of preference for placement of a thigh graft is increased by patients having a current or prior thigh graft - apparently influenced by possibly favorable

experience with thigh grafts. Other demographic factors and dialysis history seemed not to influence patient preference. As thigh grafts are a better option than tunneled dialysis catheters in patients who have exhausted all arm access sites, more efforts at educating patients on their benefits in this setting are warranted.

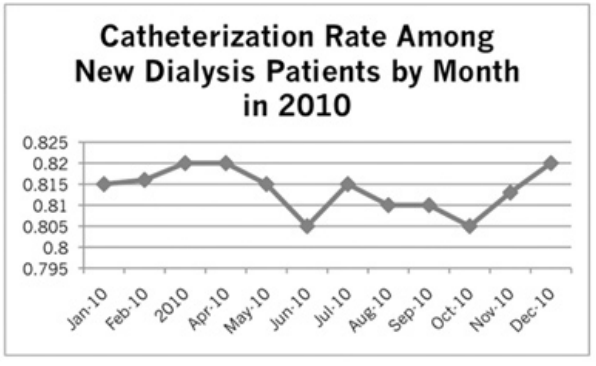
SA-PO2903

Contemporary Assessment of Incident Dialysis Patients Starting Dialysis with Catheters through 2010 Vincent Mor,[1] Franklin W. Maddux,[2] Mahesh Krishnan.[3] *[1]Brown Center for Gerontology & Health Care Research; [2]Fresenius Medical Care; [3]DaVita, Denver, CO.*

Background: The Performance Excellence and Accountability in Kidney Care (PEAK) initiative is a program sponsored by Kidney Care Partners. Its goal is to reduce first year patient mortality by 20% from 2007 to 2012. Since catheterization at dialysis initiated is associated with infections, we examined changes in incident patient vascular access for first outpatient dialysis sessions among new ESRD patients.

Methods: In order to get the most recent data, we used the Renal Management Information System (REMIS) available under a Data Use Agreement from CMS through December 2010. Beginning dates of entry of patients (from Medical Evidence form 2728), Patient demographic and clinical characteristics (2728/Patient Master file), and Date of death (From Patient Master file, Form 2746, Social Security Master Death File (SSMDF)) were obtained. Initial vascular access type was obtained through December 2010 from the completed 2728 forms for all new entrants initiating dialysis.

Results: The percentage of patients dialyzing with catheters as their primary access on first date of dialysis is recorded below.



Conclusions: Through 2010, over 80% of incident patients were documented to dialyze via a catheter on their first dialysis session. This was in spite of significant improvements in prevalent catheter rates as a result of efforts such as the Fistula First Breakthrough Initiative. While some of these patients may be dialyzing with a catheter as a temporary bridge access while a permanent access matures, their risks of infectious complications are directly correlated to catheter days. These data suggest that additional effort is needed to improve incident vascular access placement with sufficient time for maturation as a means to reduce a measure of incident patient risk.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

SA-PO2904

Differences in Vascular Access Use in Incident U.S. Hemodialysis Patients – Ethnic Disparities in the Optimal Delivery of Timely Nephrology Care Maria Cristina Arce, Aya Alice Mitani, Benjamin A. Goldstein, Wolfgang C. Winkelmayer. *Division of Nephrology, Stanford University, Palo Alto, CA.*

Background: Hispanics are the largest and fastest-growing minority in the U.S. and comprised 16.3% of the US population in 2010. Hispanics are at double the risk of end-stage renal disease (ESRD) compared with non-Hispanics. Numerous studies have demonstrated the poor access to health care and the low quality of the care received among Hispanic individuals; yet, relatively little is known about the preparation of Hispanic patients with progressive CKD for dialysis. We used a contemporary national dataset to investigate any differences by Hispanic ethnicity in the rate of incident arteriovenous access.

Methods: We used data from the Medical Evidence Report form CMS-2728-U3 (version 06/04) for the ascertainment of all patient characteristics and outcomes. All adult patients who initiated hemodialysis (HD) between 7/1/2005 and 12/31/08 were evaluated. Incident arteriovenous vascular access use was considered present if either an arteriovenous fistula (AVF) or graft (AVG) was noted. We used a log binomial model with state as a random effect to estimate crude and adjusted prevalence ratios (PR).

Results: Among 321,996 U.S. patients initiating HD, 13.1% were Hispanic. Fewer Hispanics than non-Hispanics received > 6 months of pre-dialysis nephrology care (40.1% vs. 45.5%). Use of incident AVF/AVG was reported in 14.5% of Hispanic and 17.6% in non-Hispanic patients. The unadjusted PR was 0.82 (95% CI: 0.80-0.84) indicating that Hispanics were 18% less likely to use AVF/AVG for their first HD session. Adjustment for age, gender, race, BMI, comorbidities, enrollment to Medicaid, and unemployment status attenuated the PR to 0.87 (95% CI: 0.85-0.90). After further adjustment for predialysis nephrology care, the PR was further attenuated to 0.95 (95% CI: 0.92-0.97).

Conclusions: Hispanics are less likely to use arteriovenous vascular access during the initiation of hemodialysis. While differences in clinical and socioeconomic characteristics explained some of the difference, access to adequate predialysis nephrology care explained more than half of the underuse of these more desirable access options.

Funding: NIDDK Support

SA-PO2905

Effect of Interventions in Hemodialysis Outcomes with Clinical Coordinated Care Model in Renal Therapy Service Colombia Network Angela S. Rivera. *Medical Director, Baxter, Bogota, Cundinamarca, Colombia.*

Background: Renal Therapy services (RTS) is a network of renal units in Colombia with national coverage in 49 renal unitswith a Clinical Coordinated Care Model focused on clinical quality assurance process (standardized clinical protocols, ongoing education and training), internal national and regional clinical audits for protocol compliance, disease management (anemia, hypertension, diabetes, nutrition, mineral & bone disorder, hepatitis B vaccination and Dialysis management including in center hemodialysis with single use dialyzers) and quality standards adherence monitored by nephrologists and nurses

Methods: A retrospective analysis of hemodialysis prevalent patients older than 18 years was performed from 2006 to 2010. There were analyzed trend of achievement of standards: Kt/V sp, hemoglobin, vascular access, adverse patient's occurrences (including clotted dialyzer rate) and outcomes as mortality rate. The data collection was made from clinical record.

The interventions implemented were:

On-site nephrologists

Monthly comprehensive evaluation by nephrologists

Dedicated and trained professional nurses to hemodialysis

Attention by nutrition, psychology and social work according to risk

Intensive and continuing education program in vascular access care for nurses

Strengthening quality process and define key performance indicators of care vascular access including monthly reports

Results: 23058 HD patients were attended. Mean age was 59.9 years, 38,5% were female and 34,95% were diabetic. Annual mortality rate decreased from 15.18% to 12.67%

Hemodialysis Standards Achievement

Standard (proportion of patients)	2006	2007	2008	2009	2010
Hb>=11gr%	58.7	52.8	52.8	74.4	75.5
Sp Kt/v >=1.2	79.8	80.2	84.2	88.8	91.4
Proportion Native AVF %	53	66	70	73.9	75
Adverse patient's occurrences (rate per 1000 sessions)	3.89	2.38	1.93	1.4	1.14
Clotted dialyzer (rate per 1000 sessions)	1.37	1.02	0.63	0.47	0.38

Standards Achievement Trend

Conclusions: The comprehensive model of RTS implemented in Colombia has resulted in consistently improvement of achievement of dialysis standards and a trend of reduction of mortality rate during 5 years of implementation

Funding: Pharmaceutical Company Support

SA-PO2906

Can a Nephrology Service Protect the Veins of Hospitalized Patients? Rita L. McGill, Jessica G. Lucas, Tomoki Tsukahara, Richard J. Marcus. *Division of Nephrology, West Penn Allegheny Health System, Pittsburgh, PA.*

Background: Patients with CKD should have no PICC lines or IV lines in their non-dominant arms. A prior survey of vein protection practices in our hospital suggested potential for improvement. Effects of a year-long educational initiative to improve compliance by increasing Nephrologist awareness of these guidelines were assessed, compared to prior results.

Methods: Estimated GFR (eGFR) was assessed on all hospital patients on a single day. Patients with eGFR <60 were examined for the locations of all intravenous devices and limb protection bracelets. Device rates were compared from before (2010) and after (2011) the initiative.

Results: For 415/420 patients, eGFR could be determined; 107 (26%) had eGFR<60. 41 had eGFR between 45-59, 29 had eGFR 30-44, and 37 had eGFR<30, of whom 15/37 were dialyzing. CKD rates differed widely within the hospital, present in more than 50% of patients in medical and thoracic ICU's and stepdown units, but <10% in Neurological, Neurosurgical, and Orthopedic areas. Med-Surg, Cardiology, and Oncology had CKD rates of 20-40%.

Limb protection bracelets were found on 19 patients, which represented a 9% improvement in the protection rate. 53% of dialysis patients had bracelets, and 10-12% of all other CKD groups. One PICC line and one peripheral IV were found in bracelet-protected arms. Among the 88/107 patients without protection bracelets, 86 had some IV device, of which 5 were central lines.

Unprotected CKD Patients (N=88)

	2010	2011
non-dominant PICC	12%	13%
dominant PICC	18%	16%
non-dominant IV	40%	41%
dominant IV	28%	38%

Conclusions: Raising awareness of vein protection among nephrologists resulted in a modest increase in the placement of limb bracelets, particularly among patients already on dialysis, but did not reduce the rate of PICC placement or prevent invasion of non-dominant arms in patients with CKD stages 3-5. Our results suggest that other factors may influence choice and placement of vascular access devices, which are now virtually universal in inpatients. Effective measures to protect veins in CKD must involve multiple stakeholders in the hospital community.

SA-PO2907

Fistula First Breakthrough Initiative (FFBI): Lessons about AVF Prevalence Goals Andrew D. Howard,[1,4] Robin S. Howard,[2] Stuart Goldstein,[4] Klemens B. Meyer.[3,4] *[1]Metropolitan Nephrology Asso, Alexandria, VA; [2]Clinical Investigation, Walter Reed Army Medical Center, Washington, DC; [3]Tufts Medical Center, Boston, MA; [4]Forum of ESRD Networks, Richmond, VA.*

Background: FFBI is a successful quality improvement collaboration between the Centers for Medicare & Medicaid Services (CMS) and ESRD Networks (NW). In 2005, CMS set a 2009 prevalent national arteriovenous fistula (pAVF) goal of 66%. 13/18 Networks failed to meet their individual 2009 pAVF goals. The Forum of ESRD NW analyzed these outcomes and proposed alternative approaches to goal-setting.

Methods: In 2006, CMS defined the Annual Improvement Target for each NW as (66%-NW's March pAVF rate) x 20%; with a maximum rate 4%, minimum 1%. We proposed a regression model based on 2004-09 data, relating pAVF changes to an annual average of each NW's pAVF rate, and compared model predictions to actual 2010 and 2011 pAVF changes for each of the 18 NW. Differences between achieved pAVF rates and model predictions vs CMS targets were compared using the Wilcoxon signed ranks test.

Results: The 2009 likelihood of failure was associated with pAVF rate. The CMS formula favored NW with pAVF ≥50% ($p<0.001$,Fisher's exact test): 12 NW with March 2008 pAVF rates <50% were unable to meet goal, and the 5 NW with pAVF rates >50% met their goals. The CMS formula assumes linear improvement, but the data suggest non-linear pAVF change. For 2010-11, the non-linear model more accurately predicted 26 of 36 pAVF rates (72%) than did the CMS formula ($p<0.001$, Wilcoxon signed ranks test).

Conclusions: CMS goals are based on March data only and do not reflect NW differences in prevalent and incident AVF rates, and in the number, demographics and characteristics of patients served. We suggest a more robust methodology using all data. Vascular access may become the next measure in the CMS Quality Incentive Program, and can be expected to incorporate goals for chronic venous catheters and pAVF rates for both facilities and NWs. We propose an adaptive approach to goal-setting, similar to that described by the FDA with reference to clinical trials, which includes prospectively planned opportunities for modification of one or more specified goals based on periodic analysis of data.

SA-PO2908

Reasons for Unavailability of AV Fistula at the Initiation of Hemodialysis in Pakistan Syed Rizwan Bokhari, Hafiz I. Ahmad. *Department of Nephrology, Allama Iqbal Medical College/Jinnah Hospital, Lahore, Pakistan.*

Background: Chronic kidney disease (CKD) is a challenging condition in the developing countries such as Pakistan. In our set up a great majority of patients presenting with CKD does not have an arteriovenous fistula (AVF) at the time of initiation of dialysis.

Methods: In this study, we investigated the reasons for unavailability of an AVF. One hundred consecutive CKD patients presenting for chronic hemodialysis through outpatients and emergency department during a period of three months (March to May, 2011) were included in this study. Patients were interviewed according to a standardized questionnaire.

Results: The demographic characteristics revealed that 52 were males (52%), with mean age 34.2 years. None of the patients had an AVF at the time of initiation of hemodialysis. 36% had received the advice to get AVF but refused the surgery. 31% were not aware of their pre-existing renal disease. Although 17% had prior knowledge of their kidney disease they were not referred to a nephrologist or a surgeon. 8% did not have the availability of surgeons trained in vascular access creation. 7% mentioned poverty/lack of resources. 6% had unsuccessful AVF surgery. Only 2% presented with presumed ARF. They received dialysis through temporary catheter and did not get AVF for 3 months in hope of recovery.

Conclusions: The two most frequent reasons for unavailability of AVF were the refusal to get permanent vascular access and the presence of advanced CKD (stage 5) without having a prior knowledge of renal disease. Both are modifiable risk factors and highlight the issue of lack of public awareness and health education and underscore the importance of robust efforts on part of medical community and health authorities to educate the masses about kidney diseases.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2909

Variability in Vascular Access Management for Canadian Home Hemodialysis Patients Undergoing Intensive Hemodialysis Deborah Lynn Zimmerman,[1] Robert P. Pauly,[2] Paul Komenda.[3] *[1]Medicine, University of Ottawa, ON, Canada; [2]Medicine, University of Alberta, Edmonton, AB, Canada; [3]Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: Despite growing interest in intensive hemodialysis (HD) (more frequent and/or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns.

Methods: Survey development was completed with the assistance of physician and allied health experts in intensive home HD. The survey underwent several iterations based on reviews of the expert panel prior to a face-to-face meeting with the physician experts before final instrument changes were made. The survey was completed on line. This abstract summarizes vascular access practice patterns.

Results: Of the 19 physicians contacted, 17 participated in survey development and provided program information. The preferred vascular access is the AVF in 14/16 programs. Lack of an AVF (6/16) might delay training but would not prevent training for any intensive HD program. The majority of programs are using the buttonhole technique for AVF needling (8, 100% of patients; 3, 81-99%; 4, 61-80%; 1, 21-40%; 1, 1-20%). One set of buttonholes is usually developed during training (12/17) but most programs have had to establish new buttonholes post training (14/16). Steel needles are used in 14/16 programs; 2/16 programs use angiocatheters. Routine monitoring of access flow is not done by the majority of programs. For patients on nocturnal HD, the majority of patients with an AVF or AVG use single needle HD in 3 and 5 programs respectively. For patients with CVCs, 5 programs do not have patients use any type of safety 'connectology'; 6 use lockboxes and 11 are using devices like the TEGO. Locking solutions were heparin and citrate for 6 and 11 programs respectively.

Conclusions: There is substantial variability among vascular access type, cannulation approaches, and utilization of access safety devices in Canadian home HD programs. Such variability may, in part, explain perceived differences in access complication rates, but further prospective study is necessary.

Funding: Clinical Revenue Support

SA-PO2910

Early Versus Late Arteriovenous Fistula Placement and Use in the First Year after an Acute Inpatient Start of Chronic Hemodialysis Stacy L. Andersen,[1] Yue-Fang Chang,[3] Patricia A. Seddon,[1] Susan C. Martin,[2] Kevin Ho.[1] *[1]Renal Electrolyte Division, University of Pittsburgh Medical Center, PA; [2]Donald D. Wolff, Jr. Center for Quality Improvement and Innovation, University of Pittsburgh Medical Center, PA; [3]University of Pittsburgh, PA.*

Background: Tunneled dialysis catheter (TDC) conversion to an AV fistula in incident hemodialysis (HD) is associated with a 30+% decrease in first-year mortality risk. But early AV fistula or graft (AVF/G) placement may not necessarily lead to successful use.

Methods: We studied if early AVF/G placement in adult incident HD inpatients is associated with less TDC use in the first 365 days (d) of outpatient HD. 84 such patients were discharged over 26 months(m) to 8 clinics affiliated with our academic hospital and transplant center. 30 had Early AVF/G, placed ≤90d after inpatient HD began; and 40 had Late AVF/G, placed >90d. 14 patients with <90d HD were excluded. To gauge AVF/G placement and function, we compared outpatient mean HD_{TDC} (HD_{TDC} = total HD treatments via TDC/total HD treatments) at 3, 6, and 12m for Early vs. Late AVF/G.

Results: 46% of patients (mean age 55 years) had diabetes, 36% were female; 16% had PD just before HD, and 19% had a failing renal allograft. 26% had other non-renal transplants. For 89% of patients, HD began via TDC, 10d (median) before hospital discharge. Those who eventually had an AVF/G placed took 101d (median) to do so. Of 59 non-ESRD patients, only 3 became dialysis-independent by 90d (5.1%); not one had prior CKD. Early AVF/G resulted in a mean HD_{TDC} = 57% from 3 to 6 m (n=29), compared with a HD_{TDC} = 91% for Late AVF/G (n=38) ($p=0.0004$). From 6 to 12 m, the HD_{TDC} for Early AVF/G was 33% (n=28) versus 74% for Late AVF/G (n=33) ($p=0.0004$). Early AVF/G is associated with progressively less TDC use. However, we were not able to correlate differences between Early and Late AVF/G HD_{TDC} with differences in hospital days or mortality.

Conclusions: Early AVF/G placement even in incident HD inpatients is associated with earlier AVF/G function and less TDC use in the first year of HD, providing a basis for inpatient vascular access planning.

SA-PO2911

Central Venous Catheter (CVC) Incidence at Hemodialysis Initiation: Is It the Right Metric for Provider Quality of Care? Karthik K. Tennankore, Steven D. Soroka, Bryce A. Kiberd. *Medicine, Dalhousie University, Halifax, NS, Canada.*

Background: High CVC rates for incident dialysis patients may indicate indicate poor quality of provider care. Nurse lead multi-disciplinary kidney clinics might reduce CVC starts.

Methods: We studied consecutive incident ESRD patients (hemo, PD and pre-emptive transplantation) over 36 months at a single center. Patients were excluded if their first presentation was to hospital with AKI.

Results: There are 3 nurse practitioners (NP) and 9 nephrologists (NEPH) involved with CKD multi-disciplinary clinic patients. NPs were less likely to start hemopatients

with a CVC (51% [20/39]) compared to NEPHs (65% [104/160]). However NPs were more likely (p=0.004) to see patients followed for greater than 2 years (80%) compared to physicians (62%) and 35 of the 36 CKD patients referred late (<0.5 year) were seen only by NEPHs. In a logistic model, the only predictor of CVC start was time of referral, not provider type. Patients referred <0.5 years were 8.3 (OR, 95% CI 3.1-23) more likely to start with a CVC.

Figure 1 shows the difference in CVC vs fistula rates considering hemodialysis alone. Rates among the 3 NPs differed (NP1 had highest and NP2 the lowest CVC rate) whereas the rates for the 2 selected NEPHs were similar and high. However figure 2 shows that when PD starts and pre-emptive transplants are included, all NPs were equivalent but the 2 physicians differed. NP1 and NEPH 8 had high pre-emptive transplant and peritoneal dialysis rates but only 40% of their starts were with a CVC when considering all patients.

Figure 1

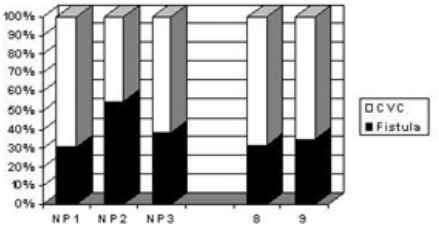
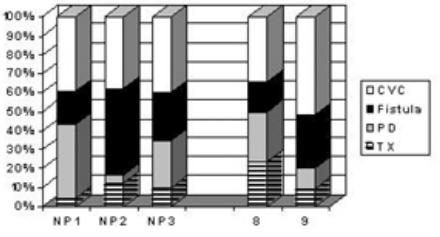


Figure 2



Conclusions: Since pre-emptive transplant and PD is a priority for some providers, CVC rates for incident hemodialysis patients may not truly reflect quality of provider care. After taking into account pre-dialysis provider exposure time, there is no difference in CVC rates for NPs versus NEPH care.

Funding: Clinical Revenue Support

SA-PO2912

Reducing Catheter Prevalence in a Dialysis Population by Using Early Intervention with a Multi-Disciplinary Vascular Access Team Vandana Niyyar, Jack Work, Jyothishree R. Pinnaka, Pankaj Manocha, James L. Bailey. *Nephrology/Internal Medicine, Emory University, Atlanta, GA.*

Background: Strategies to decrease central venous catheter (CVC) prevalence are required to minimize the complications associated with vascular access. Supported by an Emory University FAME grant, we implemented an aggressive approach to decrease the CVC prevalence in an inner-city Emory dialysis unit, by using a multidisciplinary vascular access team (VAT) including a nephrologist, an interventional nephrologist, a vascular surgeon and an access coordinator.

Methods: Of the 104 patients dialyzing at the start of the protocol, 42 patients had a CVC as their only access. Weekly access rounds were conducted by the interventional nephrologist and the access coordinator. A detailed vascular access history was obtained on all CVC-dependent patients, including venous mapping, as well as location and dates of prior accesses. This information was recorded in a catheter tracking spreadsheet and was used in monthly vascular access rounds with the entire team, which formulated long term plans for proposed future accesses. Every attempt was made to ensure placement of arteriovenous fistula (AVF). In those patients in whom an AVF was not possible due to poor vasculature, partial success was achieved by placing an arteriovenous graft (AVG).

Results: During the 9 month follow up (104 total patients, 42 CVC's); 11 were converted to functional AVF (success), 10 were converted to functional AVG (partial success), 5 patients have both a catheter and a permanent access in the process of maturation, and 9 are currently not candidates (comorbid conditions, illegal immigrants and patient refusal). There has been an attrition of 7 patients (1 died, 3 changed clinics and 3 recovered renal function). Thus the intervention was successful in decreasing CVC prevalence in this cohort from 40% to 14%.

Conclusions: Though there are multiple barriers (lack of patient education, transportation constraints resulting in missed appointments, failed access surgeries, multiple comorbidities), an aggressive, multidisciplinary approach is successful in decreasing CVC prevalence in an inner-city dialysis population.

Funding: Private Foundation Support

SA-PO2913

Nephrologist Care for 12 Months or More Increases Hemodialysis Initiation with Permanent Vascular Access Daijo Inaguma,[1] Masato Ikeda,[2] Nobuhiko Joki,[2] Takashi Shigematsu.[2] *[1]Nephrology, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan; [2]Nephrology, Study Group for Assessing Initiation of Renal Replacement Therapy, Tokyo, Japan.*

Background: The objective of this study was to evaluate the effect of early referral (ER) to nephrologists on the type of vascular access (VA) . In patients who have been followed by nephrologists for less than 3 months, management before the initiation of hemodialysis(HD) is often insufficient and urgent initiation of HD is often necessary; therefore, patients in this study were limited to those who had been followed for at least 3 months by nephrologists.

Methods: 940 patients at 9 institutions were enrolled in the study. The study was a retrospective observational study. We defined the ER group as patients followed up by nephrologists for at least 12 months, the late referral (LR) group as followed between 3 and 12 months, and the type of VA available was compared between the groups.

Results: ER was found to be significantly associated with the availability of a permanent VA at the time of initiation of HD (OR, 1.705; p=0.001). A multivariate analysis also revealed ER to be significantly associated with the availability of a permanent VA.

Logistic regression analysis of initiation of hemodialysis with permanent VA

Variables	95%CI	OR	p value
Crude			
ER	1.244-2.337	1.705	0.001
Model 1			
ER	1.241-2.354	1.709	0.001
Age (/1year)	0.980-1.001	0.991	0.084
Male gender	0.822-1.504	1.112	0.491
Model 2			
ER	1.058-2.153	1.509	0.023
Age (/1year)	0.981-1.006	0.994	0.303
Male gender	0.877-1.741	1.236	0.226
Diabetes	0.640-1.243	0.892	0.501
Heart failure	0.308-0.655	0.449	<0.0001
Use of ESA	1.730-3.797	2.563	<0.0001
eGFR (/1ml/min/1.73m2)	0.887-1.029	0.955	0.224
Albumin (/1g/dl)	1.039-1.853	1.387	0.027
Hemoglobin (/1g/dl)	1.050-1.310	1.172	0.005
C-reactive protein (/1mg/dl)	0.904-0.992	0.947	0.022

ER early referral, GFR glomelular filtration rate, ESA erythropoiesis stimulating agent, GFR glomerular filtration rate

Conclusions: ER is advantageous for increasing the likelihood of availability of a permanent VA even after patients who had been followed up for less than 3 months by nephrologists were excluded.

SA-PO2914

Low Renal Recovery in Inpatients Starting Hemodialysis and Transitioning to Outpatient Dialysis: Prior Chronic Kidney Disease and Vascular Access Planning Stacy L. Andersen,[1] Yue-Fang Chang,[3] Patricia A. Seddon,[1] Susan C. Martin,[2] Kevin Ho.[1] *[1]Renal Electrolyte Division, University of Pittsburgh Medical Center, PA; [2]Donald D. Wolff, Jr. Center for Quality Improvement and Innovation, University of Pittsburgh Medical Center, PA; [3]University of Pittsburgh, PA.*

Background: 81% of our local incident outpatient hemodialysis (HD) actually begins in hospital, yet little is known about renal recovery and vascular access (VA) planning for incident HD inpatients who then start outpatient HD. It is difficult to know which incident HD inpatients will need chronic HD and benefit from early VA planning.

Methods: We examined renal recovery (dialysis independence by 90d), AVF/G placement, and pre-ESRD renal care in 84 adult incident HD inpatients discharged from our tertiary care and transplant center to 8 affiliated HD units over 26 months (m). Inpatients were on HD neither at hospital admission nor in the preceding 12m. We defined Early AVF/G as access placement ≤90d and Late AVF/G as >90d after inpatient HD began. We calculated the Early Access Rate (EAR=Early AVF/G patients/All AVF/G patients) for each CKD stage.

Results: Of 59 non-ESRD incident HD inpatients discharged to outpatient HD, only 3 (5.1%) had renal recovery. All 48 patients with prior CKD remained dialysis-dependent at 3m: 1 CKD1, 7 CKD3A, 14 CKD3B, 19 CKD4, 7 CKD5. 5 patients had no CKD data. Of 6 patients with no prior CKD, 2 recovered. 89% of patients began HD via a TDC. Those without prior AVF/G who got one had it after 101d (median). 79% of CKD4 patients had prior renal care (mean=254d) with EAR=53%; 86% of CKD5 had renal care (mean=286d) with EAR=33%. 43% of CKD3B and 29% of CKD3A patients had renal care (mean=274d and 291d) with EAR=33% and 29%. For 15 ESRD patients with a failing renal graft, EAR=57%; and for 10 PD patients, EAR=50%. 95% of CKD3 patients had ≥1 and 53% ≥2 of the following: DM, age 65, AKI in the past 12m, or tacrolimus use.

Conclusions: Given these low renal recovery rates, CKD status may help to predict which incident HD inpatients will require chronic HD post-discharge and benefit from inpatient VA planning.

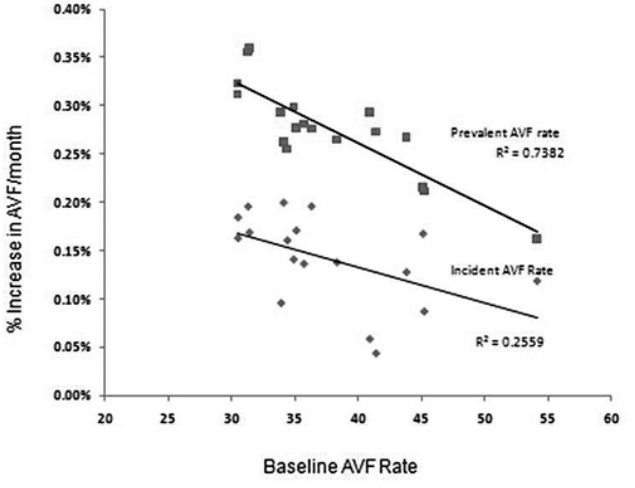
SA-PO2915

Geographic Variability in Rate of Improvement in Incident and Prevalent AVF Is Not Strongly Correlated Sumit Mohan, Edwin D. Huff, William M. McClellan, The FFBI Data Committee. *Fistula First Breakthrough Initiative.*

Background: Substantial regional variation in the rate of change of incident and prevalent AVF at the start of hemodialysis is noted in the United States. This report examines the degree to which the improvement in incident and prevalent AVF use are similar and correlate to the baseline AVF prevalence.

Methods: ESRD Networks collect a census of the vascular access currently in use of each patient within all treatment centers within their region. The date that hemodialysis for ESRD began is also recorded. We defined a patient as incident during the 91 days after the start of treatment and prevalent otherwise. Data aggregated at the network levels was used to calculate slopes of change in prevalent and incident AVF for the period from March 2007 to February 2011. The recorded AVF rates for March 2007 were taken as baseline AVF rate.

Results: Incident and prevalent mean (SD) and median rates for all Networks were 0.0578% /month (0.02), 0.0586% /month and 0.277% /month (0.05) and 0.277% /month (Figure). Network incident and prevalent AVF slopes were modestly correlated (Pearson correlation=0.2875). The Figure shows that the rates of incident and prevalent AVF improvement were inversely associated with baseline AVF rate.



Conclusions: Incident and prevalent AVF rates increased in all networks, with the largest increases in AVF prevalence occurring in networks with the lowest baseline AVF rates.

Funding: Other U.S. Government Support

SA-PO2916

The Majority of Autogenous Dialysis Access (AVF) Is Now Constructed with Advanced Surgical Techniques Michael P. Lilly,[1,2] Janet R. Lynch,[1] Nancy Jean Carlson,[2] Edwin D. Huff.[2] *[1]Mid-Atlantic Renal Coalition (NW 5), Richmond, VA; [2]Fistula First Breakthrough Initiative (FFBI) Data Committee, IPRO ESRD Network of NY, Lake Success, NY.*

Background: Concept 5 of the FFBI Change Package urges surgeons to "utilize current techniques for AVF placement including vein transposition." While there has been a progressive increase in the proportion of AVF construction among incident and prevalent HD patients, there are few data to show the contribution of changes in surgical practice to this result.

Methods: We reviewed CY 2009 claims submitted to the Centers for Medicare and Medicaid Services (CMS) for primary hemodialysis (HD) access procedures performed in NW5. Data were grouped by self-designated CMS surgical specialty codes (General Surgery 2 [GS], Thoracic Surgery 33 [TS] & Vascular Surgery 76+77[VS]) and primary access procedures were grouped into direct AVF (36821), complex AVF (36818, 36819, 36820, 36825) and AVG (36830).

Results: In CY 2009, 4747 long-term surgical HD accesses were constructed in NW5. Self-designated VS performed 62% of cases, and GS performed nearly one third. Surgeons constructed autogenous AVFs in 64% of cases and complex AVFs accounted for 52% of all AVFs. Although direct AVF as a proportion of all access was similar among GS and VS, the ratio of complex AVF to all AVF was higher among GS.

Procedure Description	GS	VS	TS	Total #	%
# HD primary access	1538	2955	264	4757	
% HD primary access	32.3%	62.1%	5.6%		
Direct AVF	480 (32%)	920 (31%)	67 (25%)	1467	30.8%
Complex AVF	596 (39%)	886 (30%)	90 (34%)	1572	33.1%
AVG	462 (30%)	1149 (39%)	107 (41%)	1718	36.1%
Complex AVF/ all AVF	55.4%	49.1%	57.3%	51.7%	

Conclusions: Although construction of AVG remains high, these data clearly show significant adoption of advanced AVF construction techniques by surgeons in NW5 and that these approaches contributed significantly to the rate of AVF construction in NW5. Subspecialty contributions must be interpreted with caution since this is a self-designated parameter. Claims data provide no information regarding the eventual maturation or function of these accesses. Regardless, these data support the success of the "spread" methodology of the FFBI as regards surgical practice.

Funding: Other U.S. Government Support

SA-PO2917

Starting Long-Term Hemodialysis with an Arterio-Venous Fistula: The Importance of a Formal Patient-Based Chronic Kidney Disease Course Kumar Pannagasayanam Dinesh, Jose A. Morfin, Andrew I. Chin. *Division of Nephrology, University of California, Davis School of Medicine, Sacramento, CA.*

Background: Starting long-term hemodialysis (HD) with an arterio-venous fistula (AVF) is critically important, but most patients in this country start with a catheter. We analyzed data from our renal clinics to determine which factors were signficant in predicting the initiation of HD with an AVF or having an AVF placed.

Methods: CQI data of all new patient HD starts within an urban academic medical center over a 12 month period were examined. We eliminated patients who had no prior renal care or were not seen in our Nephrology clinics. Data analyzed included: demographics, insurance, med history, creatinine levels, number of clinic visits, documents of physician counselling, Chronic Kidney Disease (CKD) education course participation, access vein mapping, and placement of AV shunt. Binary Logistic Regression modeling was used to find significant associations.

Results: 110 patients were started on HD. We excluded (67%) those who had no prior renal care, were seen in other clinics, or were prevalent to HD. 37 patients (33%) were cared for in our Nephrology clinics prior to HD - these were the subjects of our analyses. 10 patients initiated HD with an AVF. In 27 patients who started with a catheter, 6 had AVF's in place but not ready to use. Binary logistic regression for *start of HD with AVF* showed that attending our CKD education course was significantly associated with that outcome (0.021, 0.001 to 0.51; p=0.018). When we analyzed for the outcome of *having any AVF placed prior to HD*, regardless of whether it was used or not, having attended the CKD education course was also favorably associated (0.02, 0.00 to 0.86; p=0.042).

Conclusions: A large percentage of patients who started long-term HD presented with no prior renal care and started HD with a catheter. Of patients who were seen in the Nephrology clinics, those who had participated in a formal CKD education course that is led by a team consisting of an experienced Renal nurse specialist, an Access nurse coordinator and a Renal dietician, were more likely to have an AVF in place at the start of HD *and* to initiate HD with an AVF.

Funding: Clinical Revenue Support

SA-PO2918

Patients with Cardiac Failure and Female Gender Is Most Likely To Start Hemodialysis Using a Double Lumen Catheter Eirini Grapsa,[1] Anna Vourliotou,[2] Paraskevi Tseke,[1] Chrisoula Pipili,[1] Konstantinos Pantelias,[1] Edmond Deda,[1] Spiridon Moutafis,[2] Helen Tzanatos.[1] *[1]Nephrology, Aretaieion University Hospital, Athens, Greece; [2]Renal Unit, Henry Dunant Hospital, Athens, Greece.*

Background: Vascular access is the key step for a successful hemodialysis (HD) treatment. Early Nephrologists referral provide better options for a long term HD vascular access. Despite this important issue, a number of patients are without a permanent vascular access at HD initiation. The aim of the study was to assess whether age, gender and primary renal disease associate with differences in the type of first vascular access placement.

Methods: We reviewed the records of 145 patients on HD 44 female and 101 male with mean age 64±14.5 on HD for 46.9±41.2 months (range 1-252). The primary renal diseases were diabetes, (23.4%), hypertention (17.2%.) glomerulonephritis (25.7%), cardiac failure (6.9%) and others (26.8%).

Results: Double lumen catheter and arteriovenous fistula was the first vascular access in 109 (75%) and 35 patients (24) patients respectively. Graft was the first vascular access in 1 patient (0.5%). Arteriovenous fistula was the fist choice for 29.7% of the male patients and 9% of the female. Double lumen catheter was the first choice for 70.3% of the male (subclavian 50.5%, femoral 4.95% , and jugular 14.85%) and 86.4% of the female (subclavian 75%, femoral 2.3%, and jugular 9.1%) (p=0.04). Double lumen catheter was the first choice for the 79.7% of the patients older than 65 years old of age and 70.4% for the patients <65 years old (p=0.19). Double lumen catheter was the first choice for all the patients with cardiac failure as primary cause of renal disease.

Conclusions: Double lumen catheter was the first vascular access for the majority of our patients, probably due to late Nephrologists referral. Cardiac failure as primary cause of renal disease, Female gender, but not the age seems to influence this choice.

SA-PO2919

A Randomized, Controlled, Prospective, Double-Blinded Trial Investigating the Prevention of Catheter-Caused Infections Via a Coating Containing Bismuth Alexander Gontcharov,[1] Marc De Almeida,[1] Heike B. Lebsanft,[2] Birgit Doris Bader,[1] Karsten Schlieps,[1] Werner Beck,[2] Christiane M. Erley.[1] *[1]Nephrology, St. Joseph Krankenhaus (Hospital), Berlin, Germany; [2]Gambro Medical & Safety Office, Gambro Dialysatoren GmbH, Hechingen, Germany.*

Background: Hemodialysis (HD) catheter-related bacteremia is a major cause of increased morbidity and mortality of patients with acute renal failure (ARF) and chronic renal failure (CRF).

Methods: We conducted a randomized, controlled, prospective, double-blind clinical study investigating the efficacy of a new non tunnelled HD-catheter with a surface coating containing bismuth in patients in need of temporary short-term vascular access. Implanted was a standard catheter (GamCath™, SC) or a bismuth-containing surface coated catheter (GamCath™ Dolphin® Protect, BCC) both with identical standard design. After removal of the catheter due to medical indication, both arterial and venous lumina were rinsed and the fluid cultured for detection of bacterial colony-forming units (CFU). The catheter tip was placed in a tube containing sterile saline, ultrasonicated for 2 min at 35 kHz and shaken at 300 rpm for 10 min The filtrate was assayed for colonization.

Results: 88 patients received a catheter. 76 suffered from ARF, 12 from CRF (the catheter was implanted due to initiation of HD or shunt dysfunction). 58 (66%) catheters were removed due to no further need. 5 SC and 3 BCC were removed after assumed infection. The time to catheter removal for any reason was shorter for SC with a mean dwell time of 13 days vs 21 days for BCC.

Bacterial colonization over cut off 100 CFU/mL was not different, both for collected catheter tips as well as for rinsing fluids. However, 19 (50%) of collected SC were not colonized vs 23 (59%) of BCC. Mean predialytic CRP was significantly lower for BCC treatments (p<0,001) and procalcitonin values tended to be lower for BSC treatments. The last CRP value during catheter dwell time of each patient was significantly lower for BCC treatments (p<0,034).

Conclusions: Surface modification with bismuth film offers a new promising alternative to reduce HD catheter-related septicaemia in patients who need temporary non-tunnelled central venous catheters.

Funding: Pharmaceutical Company Support

SA-PO2920

Prevention of Tunneled Cuffed Hemodialysis (HD) Catheter-Related Thrombosis and Bacteremia by the TEGO® Connector: A Monocentric Randomized Controlled Study Florence Bonkain,[1] Judith Racape,[1] Isabelle Goncalvez,[1] Micheline Moerman,[1] Olivier Denis,[2] Nadia Gammar,[1] Karine Gastaldello,[1] Joelle L. Nortier.[1] *[1]Nephrology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium; [2]Microbiology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.*

Background: The TEGO® connector (ICU Medical) is a closed positive pressure system for tunneled cuffed catheter (TCC) attached on each hub flushed with 0.9% NaCl, and used during 3 consecutive HD sessions. We conducted a randomized controlled trial, approved by our Ethical Committee, comparing the incidence of TCC-related thrombosis and bacteremia in patients carrying a TEGO® connector *vs* controls receiving trisodium citrate 46.7 % as locking solution (Citralock®).

Methods: All adult HD patients of our Unit, prevalent or incident, were included, except those with a mature AV fistula and with a dysfunctional TCC (mean blood flow < 250 ml/min). The primary combined outcome was the incidence of thrombotic complications (defined by the need of a fibrinolytic agent and/or a mean blood flow < 250 ml/min during 2 consecutive HD sessions) and TCC-bloodstream infections (defined by positive blood cultures). Patients who developed TCC thrombosis or bacteremia were censured. The time period of TCC use was calculated and the incidence of complications was expressed per 1,000 TCC days.

Results: Sixty-six patients were followed during 9,194 days. Baseline characteristics were similar in both groups. The combined primary outcome was not significantly different in the TEGO® group *vs* in controls (3.15 *vs* 4.36 /1,000 TCC days, p=0.34). TCC-thrombosis rates were equivalent (2.96 *vs* 3.15 /1,000 TCC days, p=0.87). Only 6 TCC-bacteremia episodes were identified, 1 in the TEGO® group (0.19 *vs* 1.2 / 1,000 TCC days, p=0.06). The annual cost of the TEGO® procedure was significantly lower than the Citralock® one (560 *vs* 1,086 $ per patient).

Conclusions: This study confirms the non-inferiority of the TEGO® connector in preventing TCC-related complications compared to citrate locking solution. Strict nursing procedures and TCC hub manipulations can explain our low bacteremia rate. The TEGO® connector seems to be a promising and attractive device.

Funding: Clinical Revenue Support

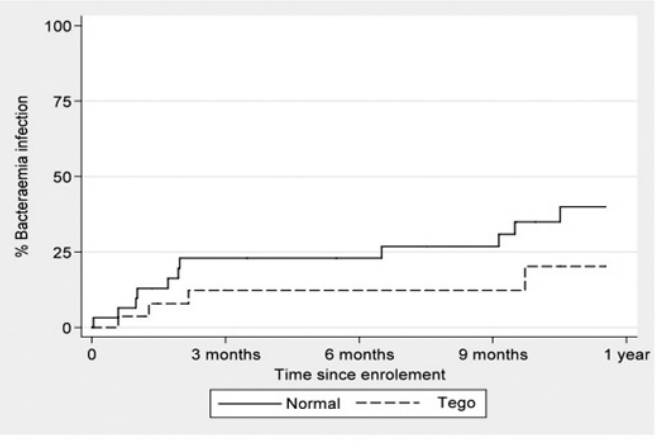
SA-PO2921

Randomised Control Trial Comparing Bacteraemia Rates in Closed Luer Lock Access Devices (TEGO) with Standard Access Devices in the Outpatient Haemodialysis Setting Frank J. O Brien, Claire Kennedy, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background: Catheter related bloodstream infections (CRBSI) are the second commonest cause of death in haemodialysis patients. Patients with permanent indwelling catheters have a 50% higher risk per year of developing systemic sepsis compared to patients with arteriovenous fistulae. The international rates of CRBSI's are 2.5-5 episodes per one hundred catheter days. This study hypothesizes that reduced handling of central catheters will reduce rates of CRBSI

Methods: A randomised controlled trial is being conducted from April 2010 to March 2012. This compares CRBSI rates in patients who have closed luer lock access devices (TEGO) placed at the end of their central catheters to those patients receiving current gold standard central catheter access techniques as per the current Beaumont Hospital protocol. All patients attending out patient haemodialysis units at Beaumont Hospital are eligible for inclusion in the study. This study highlights interim results at twelve months.

Results: 58 patients were recruited in the first twelve months, 27 in the TEGO group and 31 in the control group. 36 patients completed follow up at month 12. 11 episodes of CRBSI were noted in the control group, and four in the TEGO group, (p=0.159)



p=0.159

Conclusions: Preliminary twelve month follow up data on the use of closed luer lock access devices in reducing CRBSI shows a reduction in episodes of infection, but was not statistically significant. There was no difference in flow rates or thrombosis rates between the groups

Funding: Pharmaceutical Company Support

SA-PO2922

TEGO® Connectors Reduce Heparin Use without Affecting Blood Flow Rate Compared to Traditional Central Venous Catheter Locks Mahesh Krishnan, Tracy Jack Mayne, Carol Farthing, Shaun S. Collard, Allen R. Nissenson. *DaVita Inc, Denver, CO.*

Background: The TEGO® Connector is a heparin-free device developed to reduce catheter-related infections and clots associated with central venous catheters (CVCs). We compared the efficacy and cost effectiveness of the TEGO Connectors and saline CVC locks to traditional heparin CVC locks, and compared both to the costs of using rt-PA.

Methods: We conducted a pre/post study comparing conversion from traditional CVC locks to TEGO Connectors in hemodialysis patients. The pre-period was defined as 90 days before, and the post-period 90 days after TEGO Connector conversion. Outcomes included monthly blood flow rate and heparin use (efficacy analysis); and cost of heparin, connectors, syringes, activase, sodium citrate, medication and equipment (cost analysis). Both CVC locks and TEGO Connector costs were compared to the cost of using rt-PA.

Results: Blood flow rate remained unchanged over the course of the evaluation period (Table). Total heparin use decreased nearly 2000 units 3 months after TEGO conversion. The 6-month cost of traditional locks + caps + heparin + syringes was $127.92 per CVC patient compared to $111.54 for TEGO Connectors. Both are significantly less than rt-PA ($1834).

Blood Flow Rate and Heparin Use (before and after conversion to TEGO Connectors)

Variable	Days Prior to Conversion			Days After Conversion to TEGO Connectors		
	90-61	60-31	30-01	01-30	31-60	61-90
# Facilities	225	230	234	234	233	232
# Patients	2,040	2,302	2,656	2,656	2,447	2,256
Blood Flow Rate (mL/min) Mean ± SD	348.3 ± 41.5	348.2 ± 42.6	346.7 ± 40.3	345.5 ± 40.7	343.1 ± 41.4	341.4 ± 41.1
Total Heparin Units / Treatment	6,177	6,782	7,798	6,691	4,931	4,240

Note: the observed periods pivot around the date the dialysis facility switched to TEGO Connectors.

Conclusions: Use of TEGO Connectors decreased heparin use without affecting blood flow rate at a lower monthly cost. Cost of both TEGO and saline CVC locks were significantly lower than use of rt-PA. TEGO Connectors are a viable and cost-effective alternative to traditional locks.

Funding: Clinical Revenue Support

SA-PO2923

Comparison of Trisodium Citrate and Heparin as Catheter Locking Solution in Hemodialysis without Anticoagulant Xiaolei Chen, Ping Fu. *Department of Nephrology, West China Hospital, Sichuan University, Chengdu, Sichuan, China.*

Background: Bleeding complications are common in hemodialysis patients, especially for those with high risk. To prevent the hemorragic events, adjustment for the anticoagulant in catheter locking solution should be considered.Because of the property of local antigulation, trisodium citrate has recently become an ideal capping solution for hemodialysis.

Methods: The efficacy of different locking solutions were prospectively explored in a randomized, controlled, single blind trial. All enrolled patients used temporary central venous catheter as dialysis access. They were prescribed no-anticoagulant hemodialysis due to hemorrage tendency. These patients were randomly assigned to three groups according to the locking solution: Group A (routine heparin,4168U/ml),Group B (low heparin, 2084U/ml),Group C (4% trisodium citrate). The incidence of bleeding, index of coagulation function and catheter patency during one observational period were evaluated. The observational period indicated the interval between two consecutive hemodialysis sessions. Prothrobin time(PT) , activated partial thromboplastin time (APTT) were determined at 0.5 hour, 2 hour and 24 hour after the catheter locking procedure.

Results: Ninety patients were enrolled and thirty in each group. There were no significant differences in patient and catheter characteristics on inclusion. Both PT and APTT prolonged distinctly at 0.5 hour and 2h after the locking in Group A ($P<0.01$), then regressd to normal at 24h. In Group B, just APTT at 0.5h prolonged significantly ($P<0.05$) and it had quickly reversed to normal at 2h. No significant changes on coagulation index were observed in Group C. During the observational period, six hemorragic cases occurred in Group A (20%), just one in Group B (3.3%) and no bleeding in Group C. The incidence is apparently higher in Group A($P<0.05$). Besides, two cases of minor catheter clotting were observed in Group B.

Conclusions: To prevent post-hemodialysis bleeding, adjustment for the concentration or type of the locking antigulant should be considerd. Due to the better outcomes of local antigulation, trisodium citrate can be advocated as a safe and less expensive alternatinve to heparin.

Funding: Clinical Revenue Support

SA-PO2924

Peripherally Inserted Central Catheters Use in Patients with Acute and Chronic Kidney Disease: Single Center Experience Mireille El Ters,[#1] Andrew D. Rule,[#1] Amy Mahon,[#2] Bernice (Bonnie) M. Jenson,[#1] Amy W. Williams,[#1] Sanjay Misra,[#2] Robert C. Albright,[#1] Sandra J. Taler,[#1] Marie C. Hogan.[#1] *[1]Div of Nephrology, Mayo Clinic; [2]Dept of Radiology, Mayo Clinic, Rochester, MN.*

Background: Peripherally inserted central catheter(PICC) use has increased significantly due to many perceived advantages in acutely ill patients.Their potential to cause central venous thrombosis/stenosis led to efforts to limit their use in the CKD population.We implemented a new PICC line orderset in our institution in 2010 listing elevated creatinine (↑sCr) as relative contraindication for PICC placement and studied the impact of changes to this orderset before & after on physician prescribing patterns of PICCs at our center.

Methods: In a cross-sectional study, the medical records of a total of 550 patients undergoing PICC placement (equally divided before and after revised orderset implementation) were reviewed to determine sCr levels at the time of PICC request.The PICC RN is required to contact ordering physician to discuss contraindications before proceeding. Acute kidney injury (AKI) was defined as ↑sCr of 25% from baseline at the time of the PICC placement and CKD defined as ↑sCr ≥1.3mg/dL in women and ≥1.5mg/dL in men.

Results: Of 275 patients undergoing PICC placement before the orderset change, 52 met the criteria for ↑sCr (18.9%). After the revised orderset was implemented of 275 individuals 66 met the criteria for ↑sCr (26.4%). The percentage of patients with CKD in the sample prior to orderset implementation was 18/275(6.5 %)& increased to 36/275 (13.1 %). No statistically significant difference was noted between the two samples both for ↑sCr & CKD (p:0.145 and 0.09 respectively).

Conclusions: Despite efforts to increase awareness of the risks associated with PICC use in the CKD population, this venous access continues to be used frequently due to its ease and convenience. In fact we observed an increasing trend toward more CKD patients among those receiving PICC lines even after listing it as a relative contraindication.Effective processes should be developed to promote alternative venous access in our CKD population, including small bore internal jugular access, in order to preserve future venous access.

SA-PO2925

Improvements in Clinical and Operational Outcomes for a Cohort of Patients Converted from Central Venous Catheter Access Andrew Glowalla, Andrew Barba, Randy Smith, Chan Basho, Abbe Volz. *DaVita Inc, Denver, CO.*

Background: Improvements in mortality and morbidity related to the reduction of central venous catheters (CVCs) in end-stage renal disease (ESRD) patients have been reported, however, little has been described regarding improvements in surrogate biochemical outcomes and operational parameters. Given the economic incentives associated with the new ESRD bundled payment system, improvements in operational and surrogate biochemical outcomes may be an important ancillary driver to CVC reduction efforts.

Methods: We assessed operational and biochemical outcomes of patients after implementation of a CVC reduction program called Cathaway™ which significantly reduced CVC rates from 2008 to present. Data from 6 months pre and post CVC conversion were assessed in a cohort of patients who converted from CVCs between October 2008 and June 2010 (n=3235). The analysis had 2 components, (i) impact on relevant clinical parameters including albumin, Kt/V, hemoglobin (Hb), and average blood flow rate (BFR) and (ii) impact on operational dialysis parameters including heparin use, tissue plasminogen activator (tPA) use, and missed treatments. Three month averages for the cohort from months -6 to -4 were compared to months +4 to +6.

Results: Improvements were noted in most of the clinical parameters including an improvement in BFR. These clinical improvements were achieved with more efficient resource utilization, specifically heparin and tPA (Table).

Parameter	Months -6 to -4	Months +4 to +6	P-value	% Change
Albumin (mg/dL)	3.67±0.45	3.86±0.38	<0.0001	5.2%
Kt/V	1.61±0.39	1.70±0.34	<0.0001	5.6%
Hb (mg/dL)	11.63±1.08	11.59±0.87	0.10 (NS)	(0.34%)
BFR	359±41	414±51	<0.0001	15.3%
Heparin (U/tx)	11.44±6.02	4.92±2.70	<0.0001	(57.0%)
tPA (mg/tx)	0.072±0.24	0.0051±0.073	<0.0001	(92.9%)
Missed tx/month	0.92±1.72	0.85±1.66	0.10 (NS)	(7.6%)

Conclusions: We demonstrate that tangible benefits exist both in terms of patient outcomes and operational parameters with the successful conversion of patients from CVCs as a method of vascular access. These findings add to the compelling rationale for continuing to reduce the prevalence of CVC access in ESRD patients.

Funding: Clinical Revenue Support

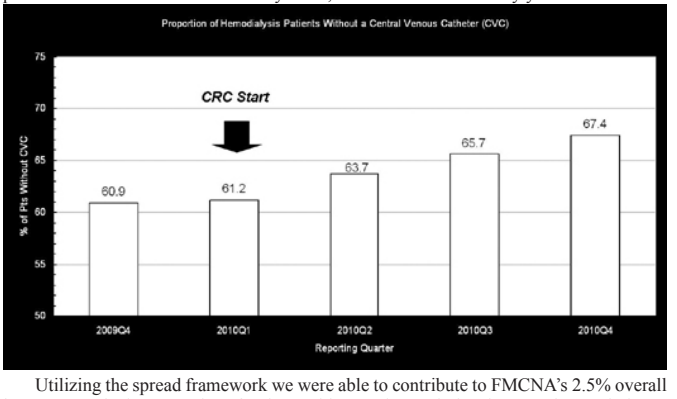
SA-PO2926

Outcomes from a Multi-Center Catheter Reduction Collaborative Program Alex J. Rosenblum, Eduardo K. Lacson, Shu-Fang Lin, Jill A. Hall, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

Background: In 2010, Fresenius Medical Care, North America (FMCNA) implemented a pilot Catheter Reduction Collaborative (CRC) based on the Institute for Healthcare Improvement (IHI) Framework for Spread Model to reduce prevalent hemodialysis catheter rates.

Methods: Medical Directors and their facility team from high-catheter facilities were invited to participate in the CRC. They were expected to adopt and implement a list of ten clinical and operational "best practices" and engage in six months of peer to peer learning meetings and calls. The proportion of patients dialyzing without a central venous catheter (CVC) was tracked. Overall rates of positive blood cultures are also being collected.

Results: 164 facilities volunteered to participate. The group improved percentage of patients without a catheter in 2010 by 6.2%, from 61.2% to 67.4% by year-end.



Utilizing the spread framework we were able to contribute to FMCNA's 2.5% overall improvement in the proportion of patients without catheters during the same time period. Top 5 best practices according to CRC participants included: 1) Physicians taking accountability and asserting leadership, 2) Use of a chronic kidney disease education program, 3) Selective referral to surgeons with best outcomes and timely appointments, 4) Use of vascular access manager/champion, and 5) Documented catheter removal plan. Preliminary trends from ongoing analyses show a decline in positive blood cultures.

Conclusions: Our CRC experience demonstrated that the IHI spread model with utilization of "best practices" along with ongoing facilitation and peer-to-peer sharing can effectively reduce catheter rates. Reduction in CVC may have contributed to early observations of declining rates of bloodstream infections.

SA-PO2927

Accuracy of Blood Culture Results from the Hemodialysis Circulation Based on Guidelines for Diagnosing Catheter-Related Blood Stream Infections Friederike S. Quittnat Pelletier, Mohammad Z.H. Joarder, Charmaine E. Lok. *Toronto General Hospital, Toronto, ON, Canada.*

Background: Most hemodialysis (HD) units diagnose HD catheter-related bloodstream infections (CRBSI) by obtaining blood cultures (BC) from the HD bloodline concurrent with clinical exclusion of other sources of infections.

The recently updated guidelines from the Infectious Diseases Society of America (IDSA) recommend making the diagnosis of CRBSI by cultivating the same organism from a peripheral vein and from the catheter hub meeting criteria of differential time to positivity. The IDSA criteria for CRBSI have not been validated in HD patients.

Our hypothesis is that a BC taken from a peripheral vein during HD will yield the same result as a BC taken from the dialysis circulation or the catheter hub.

We further hypothesize that obtaining peripheral vein BC will be challenging (<50%) and may limit application of IDSA guidelines.

Methods: Four adult sets of BC (from a peripheral vein, from both catheter hubs and from the dialysis line) were obtained from patients who were suspected to have a CRBSI. Data was collected on the challenges of obtaining peripheral BC.

Results: To date, 40 patients who presented with signs and symptoms of CRBSI have been enrolled in this study. Bacteremia was found in 45% of these patients and all BC consistently grew the same bacteria from all culture sites. In 55% with undetectable bacteremia, all BC were negative for bacterial growth.

Peripheral vein BC were obtained in 77.5% of the suspected CRBSI. 28.6% had > 1 attempt before successful blood flow; 10% required a second nurse to obtain the peripheral blood. 1 Patient refused peripheral blood sample drawing, and in one case the blood sample did not yield the appropriate amount for a BC set due to collapsing peripheral veins.

Conclusions: More than ¾ of HD patients had successful peripheral vein BC drawn. 100% of organisms identified in peripheral vein samples were identical to those obtained from the dialysis bloodline, suggesting that the current standard of practice of obtaining BC from the HD bloodline concurrent with clinical exclusion of any other sources of infections is a valid method of diagnosing CRBSI.

Funding: Clinical Revenue Support

SA-PO2928

Attributed Mortality of Catheter Use in Incident Dialysis Patients: The Impact of an Acute Dialysis Start Karthik K. Tennankore, Steven D. Soroka, Bryce A. Kiberd. *Nephrology, Dalhousie University, Halifax, NS, Canada.*

Background: Central venous catheter (CVC) use as incident dialysis access is associated with mortality. However, some patients unexpectedly progress to end stage renal disease prior to reaching a GFR at which one would expect to establish alternative access. The purpose of this study was to identify the impact of this "acute start" on attributed CVC mortality.

Methods: We studied 406 incident dialysis patients from 1/2006 to 12/2009. Patients were classified as "acute start" if the MDRD eGFR was >25 ml/min/1.73 m2[/sup], ≤3 months prior to dialysis initiation and declined after an acute event (n= 48). RPGN/myeloma patients without prior GFR measurements were also included (n= 10). All remaining patients were classified as "CKD start" (n=348).

Results: 58.7, 23.6 and 17.7% of patients initiated dialysis with a CVC, fistula and peritoneal dialysis catheter, respectively. 81 patients (20%) were referred late (within 90 days of dialysis start). At 2 years, there were 103 deaths. The mortality of acute start patients was significantly higher than CKD start patients (figure 1, p<0.001). In a univariate Cox survival analysis, acute start (HR 3.44, p<0.001), CVC use (HR 1.88, p=0.004), and late referral (HR 2.52, p<0.001) were associated with mortality. Adjusting for age, gender, Charlson Comorbidity Index and albumin, only acute start remained associated with mortality (HR 2.50, 95% CI 1.57-3.99, p<0.001). Restricting the analysis to CKD start patients on hemodialysis (n = 276), CVCs were not associated with mortality (HR 0.86, 95% CI 0.49-1.53, p=0.614).

Conclusions: A significant proportion of early dialysis mortality occurs after an "acute start". Most of this mortality is related to the acute event itself, as opposed to CVC use or late referral. Dialysis registry analyses may overestimate attributed CVC mortality without considering the impact of an "acute start".

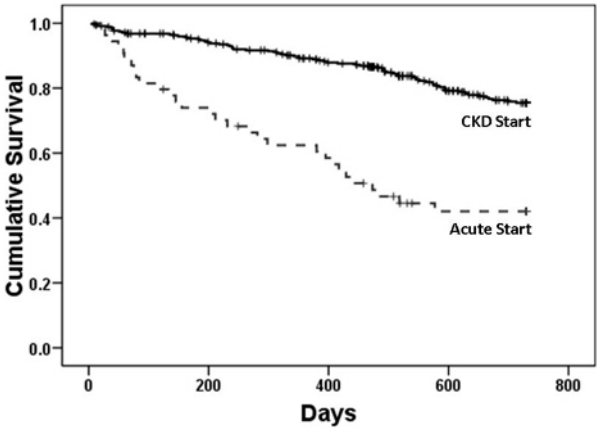


Figure 1. Cumulative survival for "Acute Start" patients, log rank p<0.001

Funding: Clinical Revenue Support

SA-PO2929

Catheter Related Infections with Sodium Citrate 4% Locks Compared to Heparin Locks in Hemodialysis Patients Calantha K. Yon, Eva Wong, Chai L. Low. *Pharmacy Services, VA San Diego Healthcare System, San Diego, CA.*

Background: This open, prospective cohort study aims to evaluate the difference in the incidence of catheter related infections (CRI) with sodium citrate 4% compared to heparin 5,000 units/ml as a catheter locking solution in hemodialysis (HD) patients. Secondary outcomes include hospitalization, mortality, and catheter patency.

Methods: The use of citrate locks were compared to a historical control using heparin locks before September 2009. Medical records of chronic HD patients with permanent catheters between the periods of July 2008 - July 2009 and September 2009 - December 2010 were reviewed. CRI incidence was calculated as the number of infections per total number of catheter days. Pertinent information on patient medical history, thrombosis, infections, hospitalization, and mortality were collected.

Results: Mean age was above 60 years, and more than 90% of the patients were male in both groups. The major causes for ESRD were diabetes and hypertension in the 2 cohorts. A total of 412 and 441 patient-months were included in the heparin and citrate groups respectively. There were 22 CRIs with heparin and 11 CRIs with citrate (p = 0.013). CRI for heparin was 1.76 infections per 1,000 catheter days compared to 0.82 infections per 1,000 catheter days in the citrate group. Hospitalization occurred in 17 of the 22 CRIs in the heparin group and 9 of 11 CRIs in the citrate group (p = 0.086). The heparin group had 41 incidents of thrombosis compared to 40 in the citrate group (p = 0.69). CRI and thrombosis led to catheter exchange or removal in 36 cases in the heparin group and 18 cases in the citrate group (p = 0.09). There was no statistical difference in 6-month mortality from CRI between the treatment groups (p = 0.27).

Conclusions: This study showed a statistically significant difference in the incidence of CRI using sodium citrate versus heparin as a locking solution. Sodium citrate is also as effective as heparin as an antithrombotic agent. Secondary outcomes were also comparable between sodium citrate and heparin. Results of this study support the use of sodium citrate as an alternative to heparin for the maintenance of catheter patency and prevention of CRI.

Funding: Veterans Administration Support

SA-PO2930

Hypertonic Trisodium Citrate Induces Protein Precipitation in Hemodialysis Catheters Gernot Schilcher,[1] Hubert Scharnagl,[2] Joerg H. Horina,[1] Werner Ribitsch,[1] Alexander R. Rosenkranz,[1] Tatjana Stojakovic,[2] Hans D. Polaschegg.[1] *[1]Department of Internal Medicine, Division of Nephrology and Hemodialysis, Medical University of Graz, Graz, Austria; [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria.*

Background: Between haemodialysis treatments catheters are locked with a locking anticoagulant. Because of its antimicrobial properties hypertonic trisodium citrate has become popular. This solution is not only spilled when injected, but is in part exchanged against whole blood due to its high density. Plasma proteins are therefore exposed to hypertonic trisodium citrate.

Methods: In vitro, whole blood and trisodium citrate (concentrations ranging from 4.7 to 46.7%) mixtures were used to assess protein precipitation. In vivo, listed filling volumes of hemodialysis catheters locked with trisodium citrate 4% (n=10) or 46.7% (n=10) were aspirated and then analyzed for protein precipitation.

Results: During in-vitro tests with hypertonic trisodium citrate protein precipitation was observed at concentrations exceeding 12%. When catheter locks were aspirated in-vivo, precipitated protein could only be separated and analysed in catheters locked with 46.7%. The main constituent was albumin.

Literature search revealed data confirming precipitation of proteins by different salts. 'Salting out of plasma proteins by sodiumcitrate' has become a common method for serum protein purification since the 19th century. Nevertheless, none of these papers has considered its relevance with respect to clinical application in hemodialysis patients.

Conclusions: Hypertonic trisodium citrate lock solution is exchanged against whole blood even up to the highest point in the catheter. During this process plasma proteins come into contact with hypertonic citrate and subsequently precipitate. Therefore, hypertonic trisodium citrate lock solutions are potentially dangerous and may be the underlying cause for reported embolic complications in patients with central venous catheters. Based on our findings we suggest that only commercially available trisodium citrate 4 % lock solution can be used safely.

SA-PO2931

Gentamicin Catheter Locks: Trends and Markers for Development of Resistance and Clinical Outcomes in One Urban Outpatient Dialysis Center Sudhir B. Vyakaranam,[1] Heather Duncan,[1,2] Karthikeyan Meganathan,[3] Kotagal Shashi Kant.[1,2] *[1]Int Med, Div Neph & HTN, Univ Cincinnati Coll Med, Cincinnati, OH; [2]DCI, Cincinnati, OH; [3]Public Health Science, Univ Cincinnati, Cincinnati, OH.*

Background: Catheter Related Bacteremia (CRB) and associated deep infections remain the second leading cause of hospitalization and mortality for patients dialyzing with tunneled catheters (TC). Antibiotic catheter lock solutions (CLS) reliably reduce CRBs. Several studies report development of resistance to gentamicin (GR). To address these concerns we examined CLS (gent 2.7mg/mL heparin) use in one large urban dialysis facility

Methods: We identified all TC patients over a three year period. Demographics, labs, ESA use and positive blood cultures were reviewed. Unlike in some past reports, ALL inpatient and outpatient CRBs were identified.

Results: There were a total of 27 separate CRBs- below 1.2 CRB per 1000 catheter days in each period of the study. There were three "deep" infections: endocarditis (period 2) and septic arthritis and osteomyelitis (period 3). Patients with and without CRBs are compared in Table 1; Diabetes was more prevalent in the group of patients who did get a CRB.

Comparison of no CRB patients with CRB patients

measure (sd)	no CRB	CRB
% Male	61	50
% Black	91	90
% diabetic	46	75*
Age (yrs)	56.9 (14.1)	57.3 (9.0)
ESRD Vintage (yrs)	4.4 (5.8)	2.9 (4.0)
avg monthly EPO/kg	124.2 (113.1)	104.1 (86.5)
Albumin	3.7 (0.4)	3.6 (0.5)
Hemoglobin	11.4 (0.7)	10.9 (1.5)
Kt/V	1.6 (0.3)	1.5 (0.3)

Fisher's exact p<0.05

There were 9 instances of GR in the three years. Mean days of exposure to the CLS prior to GR was 611; the earliest this appeared was 211 days. In no instance did the patient who had GR require gent for treatment of an infection. No patients with GR have died as a result of infection with a GR organism, with more than 37000 catheter days in approximately 200 patients. No increasing temporal trend for GR was obvious.

Conclusions: The practice of Gent CLS in this institution has been associated with a stable, low CRB rate, and no apparent increase in GR; in contrast to the study of Landry et al (2010). Continued study and observation is warranted for this important issue.

Funding: Clinical Revenue Support

SA-PO2932

Comparative Evaluation of Shower-Washing Technique and Aseptic Technique for Exit-Site Care of Tunneled Cuffed Venous Catheters Nami Shibahara,[1] Hiroshi Shibahara,[2] Susumu Takahashi. *[1]Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; [2]Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan.*

Background: We have reported a new care method designed to prevent infection by normalizing the skin condition at the exit site of a tunneled cuffed venous catheter (TCC): the exit site is directly cleaned using a shower without the use of antiseptic. In this study, we compared the shower-washing technique with the aseptic technique for exit-site care of TCC.

Methods: The subjects were 100 hemodialysis patients who had begun to use a TCC in our institutions from January 2005 to January 2010. Fifty subjects used shower-washing technique (Group S) and 50 used the aseptic technique (Group A). The shower-washing technique in Group S involved washing the exit site of the TCC with tap water immediately after TCC insertion and at every dialysis session. Moisture could be wiped away with non-sterile gauze. No antiseptic was applied. The aseptic technique in Group A was a conventional exit-site care method using povidone-iodine. In both groups we evaluated the skin conditions of the exit-site at the time of removal of TCC and the incidence of catheter-related infections, and compared the white blood cell (WBC) count and C-reactive protein (CRP) at the time of insertion with those at the time of removal of TCC.

Results: No significant difference was observed in the skin conditions of the exit site at the time of removal of TCC. Two exit-site infections (ESIs) and two catheter-related blood stream infections (CRBSIs) occurred in Group S, and three ESIs and two CRBSIs in Group A. There was no significant difference in WBC count and CRP between the time of insertion and at removal of TCC in both groups.

Conclusions: The shower-washing technique for exit-site care of TCC is simple, effective and as safe as the aseptic technique. Future studies are needed to compare these approaches in a larger number of subjects.

SA-PO2933

Comparative Evaluation of Tensile Strength between Cuff and Catheter of Soft Cell and Another Type of Tunneled Cuffed Catheter Hiroshi Shibahara,[1] Nami Shibahara,[2] Susumu Takahashi. *[1]Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan; [2]Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan.*

Background: A characteristic of tunneled cuffed venous catheters (TCC) is that the cuff promotes tissue in-growth, providing a barrier against infection and anchoring the catheter. However, we have reported 16 cases of catheter malfunction of a Soft Cell (Bard Access Systems, Salt Lake City, UT, USA), due to a poor connection between the cuff and the catheter since December 2008 (*Ther Apher Dial* 2011;15:213-215). Therefore, we evaluated the tensile strength (TS) between the cuff and the catheter in Soft Cells with a similar expiration date to those associated with malfunction (Group SC-1), Soft Cells with a later expiration date (Group SC-2), and another type of TCCs (Group SP).

Methods: Group SC-1 consisted of Soft Cells with an expiration date before February 2012; Group SC-2, Soft Cells with an expiration date after March 2012; and Group SP, Split Caths (Medcomp, Harleysville, PA, USA). TS between the cuff and the catheter of 10 unused TCC in each of the three groups was measured using a Universal Testing Machine (Imada Seisakusho Co., Ltd., Aichi, Japan) at a crosshead speed of 200 mm/min and a grip distance of 30 mm.

Results: Mean TS between the cuff and the catheter was 34.0±14.7 N in Group SC-1, 67.2±13.3 N in Group SC-2, and 56.0±11.1 N in Group SP. TS in Group SC-1 was significantly lower than that in Groups SC-2 and SP ($p<0.01$, $p<0.01$), without a significant difference in TS between groups SC-2 and SP.

Conclusions: It was revealed that the connection between the cuff and the catheter was poorer in Group SC-1, which might have been one of the reasons for catheter malfunction in 16 Soft Cells we experienced. It is of great concern that TS between the cuff and the catheter of Soft Cell significantly differed according to the expiration date. Currently, TS between the cuff and the catheter of TCC is determined by each manufacturer voluntarily. In order to ensure safe use of TCCs, it is necessary to establish an international standard.

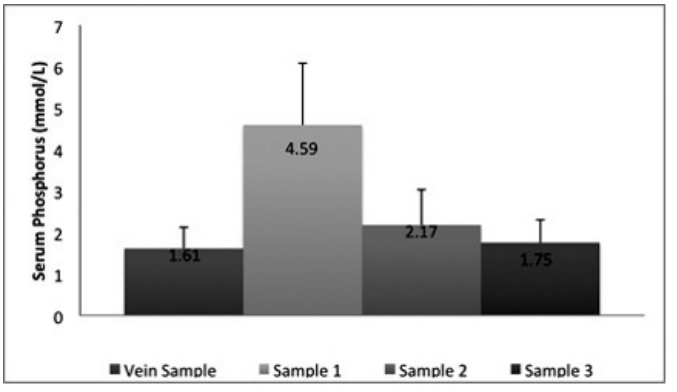
SA-PO2934

Use of Alteplase in Dialysis Catheters May Lead to Hyperphosphatemia Overtreatment Dimitrios Anestis Moutzouris, Panoraia Papadopoulou, Sotiris Mikros, Alexandra Fatourou, Fotios Makris. *Nephrology, "Mesogeios" Satellite Dialysis Unit, Palaio Faliro, Athens, Attica, Greece.*

Background: In dialysis catheters, heparin is usually used as locking solution with alteplase being administered in malfunctioning catheters. According to Clinical and Laboratory Standards Institute, when drawing blood from a vascular access device, after flushing, the first 5ml of blood or five times the dead space should be discarded. We aimed to investigate whether alteplase as locking solution can be the cause of increased serum phosphorus.

Methods: We examined serum phosphorus in 27 dialysis patients with catheters, with alteplase as locking solution. We used four blood samples: one sample was drawn from a peripheral vein before session (Vein Sample); the second sample was drawn from the arterial port after 4ml of blood was discarded (Sample 1); the third sample after 5ml was discarded (Sample 2) and the fourth after 7ml was discarded (Sample 3).

Results: The mean age of patients was 73.4 ± 11.3 years. There were significant differences regarding serum phosphorus among Vein Sample and Samples 1,2 and 3. Whereas the difference between Vein Sample and Sample 1 was anticipated (alteplase contains phosphoric acid), differences remained significant after 5ml or even 7ml of blood was discarded.



Columns represent mean serum phosphorus in the four samples (mmol/L). There were significant differences between Venous Sample and Sample 1 ($p=0.004$), Venous Sample and Sample 2 ($p<0.0001$), Venous sample and Sample 3 ($p<0.001$). Bars represent standard deviation.

Conclusions: Alteplase as locking solution may increase serum phosphorus. Even moderate increases of serum phosphorus might be due to blood contamination with alteplase. Specific guidelines should be established to address blood sampling. We recommend that at least 7ml of blood should be discarded before sampling to avoid spurious hyperphosphatemia.

SA-PO2935

Citrate (46%) Based Catheterlock Solution Reduces the Incidence of Catheter Related Sepsis in Dialysis Patients as Compared to Heparin, but Might Induce a Change in Pathogens Hans S. Brink,[1] Anniek Brink,[1] Ron M.G. Hendrix.[2] *[1]Internal Medicine, Medisch Spectrum Twente, Enschede, Netherlands; [2]Laboratorium voor Microbiologie Twente Achterhoek, Enschede, Netherlands.*

Background: Catheter related sepsis is a major cause of morbidity and mortality in haemodialysis patients. Citrate-based catheter lock solutions were reported to reduce its incidence.

Methods: We tested the effect of citrate (46%) on 7 species of pathogenic bacteria, using the Kirby-Bauer method. All tunnelled dialysis catheters used in our centre from 1-1-2000 until 7-1-2009 were reviewed. Episodes of sepsis were identified and responsible microorganisms were recorded. On 10-1-2003 citrate replaced heparin as a catheter lock solution. The incidence of sepsis before and after this switch was calculated. Sepsis free survival of the catheters was compared using Kaplan Meijer analysis.

Results: In vitro, citrate was bactericidal for Staph. aureus and S. pneumoniae. Enterococci and Gram-negative species were not affected. 438 tunnelled dialysis catheters in 330 patients were studied. Heparin was used in 175 catheters; citrate in 263 catheters. The number of catheter days per catheter in both cohorts was similar. The sepsis rate was

0.60 for heparin and 0.29 per 1000 days for citrate. The Kaplan Meijer analysis showed a statistically significant longer sepsis free survival of the dialysis catheters with citrate than with heparin ($p < 0.05$).While using heparin, 1 out of 20 episodes of catheter related sepsis was caused by Gram negative species. With citrate 3 out of 19 episodes of sepsis were caused by Gram negative species.

Conclusions: In our dialysis centre the rate of catheter related sepsis is low. After the switch to citrate as a catheter lock solution it further decreased. However, we also observed a change in the pathogens causing catheter related sepsis. Our data support the view that prevention of catheter related sepsis is not primarily dependent on the catheterlock solution. The effect of citrate is not due to its bactericidal effect. The shift in pathogens causing catheter related sepsis indicates the need for close surveillance in order to adjust primary treatment for catheter related sepsis.

Funding: Clinical Revenue Support

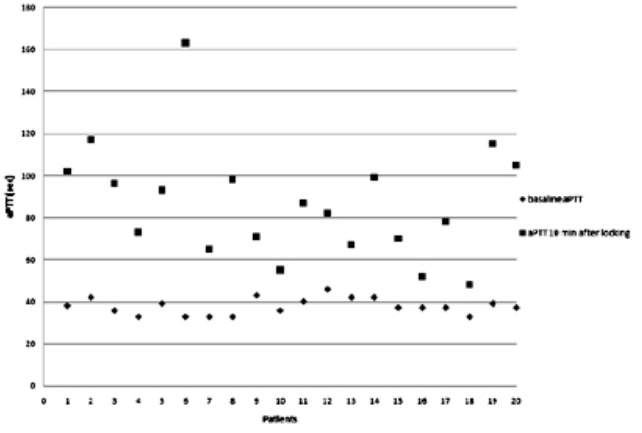
SA-PO2936

Systemic Anticoagulation as a Result of Heparin Locking of Temporary Hemodialysis Catheters Liza Di Monte,[1] Pablo Martin Jimenez,[1] Aldo J. Peixoto,[2] Marcelo Orias.[1] *[1]Sanatorio Allende, Argentina; [2]Yale University.*

Background: Bleeding is an important problem in critically ill patients with AKI. Temporary hemodialysis (HD) catheters require locking with heparin to maintain patency. In permanent tunelled catheters, there is evidence that heparin leaks into the circulation in concentrations that are high enough to produce anticoagulation. Impact of heparin locking on systemic coagulation has not been tested with temporary catheters.

Methods: We performed a prospective study of 20 patients requiring HD, and used a 11 Fr, 20 cm double lumen temporary catheter (Balton Co., Warsaw, Poland). Catheters were locked using unfractionated heparin 5,000 U/ml according to manufacturer's instructions. We obtained peripheral blood draws before catheter use for HD and 10 minutes after the catheter was locked post-HD. HD was performed without heparin. Coagulation parameters measured included activated partial thromboplastin time (aPTT), prothrombin time (PT) and platelet count.

Results: We recruited 17 men and 3 women aged 60±16 years (range 21-85). All patients had normal baseline coagulation parameters. Baseline aPTT was 38 ±3.8 seconds, whereas the mean aPTT 10 min after locking was 87±27 seconds. All patients had an increase in aPTT; the average increase was 130% from baseline ($p< 0.01$). Three of the 20 patients had bleeding complications in the 24h period following testing. All 3 had post-locking aPTT levels >100 seconds. Patients with femoral catheters were associated with significantly higher post-lock aPTT levels as compared with jugular catheters despite identical catheter size and heparin dose.



Conclusions: Temporary double lumen catheter locking with heparin increases aPTT to levels capable of inducing systemic anticoagulation . This must be considered an important risk factor for bleeding in patients undergoing HD through a catheter in the acute setting.

Funding: Private Foundation Support

SA-PO2937

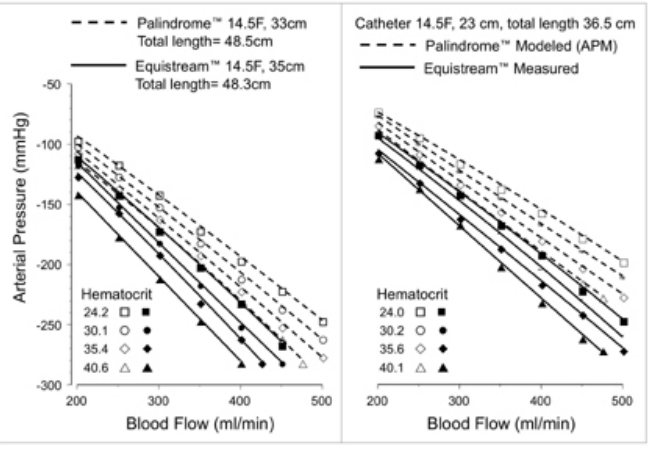
Mathematical Modeling of *In Vitro* Data Provides an Accurate Method for Comparing the Performance of Dialysis Catheters Stanley Frinak, Anatole Besarab, Lalathaksha Murthy Kumbar, Jerry Yee. *Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Background: Patient outcomes depend on catheter blood flow rate (Qb); therefore a method is needed to compare catheter performance since they vary in length and design. Modeling of *in vitro* data was used to calculate negative arterial pressure (AP) at any Qb or hematocrit (HCT), allowing comparison of catheters from different manufacturers.

Methods: Palindrome™ (PAL) (19, 33, 55 cm, 14.5 F) and Equistream™ (EQ) (23, 27, 35, 42 cm, 14.5 F) catheters were evaluated in an *in vitro* dialysis circuit. Blood was adjusted to HCTs of 40%, 35%, 30%, 24% using plasma. Height difference (ΔH) from AP transducer to reservoir blood level was -24cm. AP was recorded at each HCT starting at a Qb of 200 ml/min and increased by 50 ml/min until 500 ml/min. Total catheter length (TL) was recorded. Data was analyzed using multiple nonlinear regression (DataFit). EQ 23cm data was used in the PAL AP model (APM) to calculate AP at each Qb and HCT. Mean difference EQ AP - PAL APM was calculated at Qb = 200 to 500 ml/min for all TLs and HCTs.

Results: TLs were PAL (33.8, 48.5, 70.1 cm) and EQ (36.5, 40.7, 48.3, 55.8 cm). HCTs for all data were 24.2±0.1 (N=46), 30.2±0.1 (45), 35.4±0.3 (38), and 40.4±0.2 (36). Modeling of PAL data gave $APM = 0.00001(TL)^{0.57536}Qb^2 - 0.10262(TL)^{0.57536}(HCT^2 + 0.00796\ HCT + 0.49958)Qb - (\Delta H)(0.0518\ HCT + 0.74232)$ with R^2=0.989. Measured AP for PAL 33cm and EQ 35 cm are shown in Fig. left. EQ (23cm) measured AP and PAL values from APM are shown in Fig. right. Mean difference EQ AP - PAL APM for Qb = 200, 300, 400 and 500 was -25±4 (N=14), -33±6 (14), -46±7 (14), and -55±10 (5).

Conclusions: For all Qb and HCT values, EQ catheters had greater negative APs than PAL. Modeling of *in vitro* data provides an accurate method for comparison of catheter performance regardless of variations in catheter length and design.



SA-PO2938

***Staphylococcus Aureus* Invasive Infections and Vascular Access in a Hospital Setting: A Retrospective Database Analysis** Mário Raimundo, Fernando Neves, Antonio Gomes da Costa. *Nephrology and Renal Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

Background: *Staphylococcus aureus* (SA) is the most frequent bacteria responsible for bacteriemia in End-Stage Renal Disease (ESRD) patients undergoing hemodialysis (HD). In this group of patients vascular access type is the main risk factor for bacteriemia, hospitalization related to infection and mortality. Our aim was to evaluate the incidence and mortality of SA infection in patients treated with extracorporeal depuration techniques in a hospital unit and its relationship with the type of vascular access.

Methods: We retrospectively analyzed the databases of the hemodialysis and bacteriology departments of Hospital de Santa Maria (Lisbon) for a two-year period.

Results: There were a total of 511 SA invasive infections in the entire hospital, of which 40% were methicilin-resistant species. Patients who needed extracorporeal depuration techniques contributed to 25% of these infections. Eigthy percent of these had a central venous catheter (CVC) as vascular access, while less than 10% had an arterio-venous fistula (AVF). Infection rates (n/1000 dialysis treatments) in patients who needed extracorporeal depuration techniques were: overall 5.6; AVF 1.44; short-term CVC 7.94 (OR compared to AVF: 5.57 [2.81-11.02], $p<0.0005$); tunelized CVC 6.78 (OR compared to AVF: 4.75, [2.39-9.41], $p<0.0005$); PTFE grafts 9.44 (OR compared to AVF: 6.63, [3.006-14.40], $p<0.0005$). The overall SA invasive infection mortality in patients who needed extracorporeal depuration techniques was 32% (40% with methicilin-resistant species). There were no differences in mortality between the types of vascular access once the infection was established.

Conclusions: ESRD patients undergoing HD are a group at risk for SA invasive infections and its incidence is directly related to the type of vascular access. Strategies for solving this problem should focus on prevention, including early referral of ESRD patients to the Nephrologist and adoption of strict hygienic and barrier measures.

SA-PO2939

Impact of Co-Morbidity and Type of Access on Subsequent Hospitalisation Following a Bacteraemic Episode – Observational Study in a Hemodialysis Cohort Manivarma Kamalanathan, Josie Murphy, Subash Somalanka, Maggi Steele, David Makanjuola. *Nephrology, St. Helier Hospital, Surrey, United Kingdom.*

Background: Patients on hemodialysis (HD) are at risk of bacteraemia and this is a major cause of morbidity and mortality. The type of dialysis access is a major factor, with HD lines being more likely to become infected than AV fistulae. Co-morbidities may increase the risk of hospitalisation, and the length of stay (LOS) in hospital.

Objective: To assess the effect of a bacteraemic episode on morbidity (as defined by the need for hospitalisation), and to see whether this is influenced by the type of HD access or co-morbidity in HD patients.

Methods: Data were obtained on patients who had HD in 2009. We studied two groups - those who experienced a bacteraemia in 2009 (group A) and patients who did not (group B). Data on the source of the infection, HD access at the time of infection, Davis co-morbidity score, and diabetes status were analysed, as was the total in-patient days in the 12 months following the bacteraemic episode.

Results: 720 patients were included in the study. 61(8.5%) in group A and 659 (91.5%) in group B. 243 (34%) patients were diabetic, 30 (49%) in group A, and 213 (30%) in group B. In group A, 66% were dialysing via a line, and 34% were dialysing through an AVF. Mortality within 12 months: group A - 16 (26%), group B - 88 (13.3%). Co-morbidity scores were similar in both groups.

Hospitalisations: group A - 92% , group B - 50%. Average LOS - group A - 40.9 days, group B - 9.4 days.

Conclusions: Morbidity was higher in the subsequent 12 months in patients who had a bacteraemia, as compared with those who did not. The LOS in hospital was also greater in group A especially in diabetics. Patients in group A were more likely to be dialysing via lines. Co-morbidities were similar in both groups, but diabetics had an increased risk of bacteraemia and re-admissions.

Costs of treating bacteraemias, and the increased LOS in hospital add to the financial burden on the health service. One modifiable risk factor is the type of HD access, and we feel our study adds weight to the need to reduce the number of patients on HD via lines.

SA-PO2940

Factors Associated with Bacterial Endocarditis in Dialysis Patients Darren Green, Paul Dunne, Philip A. Kalra. *Department of Renal Medicine, Salford Royal Hospital, United Kingdom.*

Background: Following a cluster of sub-acute bacterial endocarditis (SBE) in a nephrology unit, we reviewed who was most at risk.

Methods: Most SBE patients were on hemodialysis via a catheter. We reviewed data from the prospectively collected audit of tunneled dialysis catheters inserted at this centre. We hypothesised that those most at risk had underlying structural heart disease or immunosuppression.

Results: 13 patients were treated for SBE over 2 years.

Table 1. Characteristics of SBE in dialysis patients

	Age	Organism	Vegetation	Duke	Access	Complication	Pre-existing valve changes
1	67	Staph Aureus	AV	Yes	Temporary	None	Metallic AVR
2	72	Culture -ve	AV	No	Tunneled	Death	No
3	60	Culture -ve	No	Yes	Tunneled	Death	MR MS AR
4	58	Culture -ve	No	Yes	AVF	Death	No
5	74	Enterococcus	AV	Yes	Tunneled	Death	MR MS AR
6	49	Staph Aureus	MV	Yes	Tunneled	Death	No
7	68	Staph Aureus	AV MV	Yes	Tunneled	MR AR	Unk
8	82	Pseudomonas	AV	Yes	Tunneled	Death	No
9	70	Staph Aureus	MV	Yes	Tunneled	MVR	No
10	63	Pseudomonas	MV	Yes	Tunneled	Embolic CVA	MR
11	78	Staph Aureus	MV	Yes	Tunneled	MVR	MR
12	64	Staph Aureus	MV	Yes	Tenckhoff	Embolic CVA	MR
13	57	Coag -ve staph	MV	Yes	Tunneled	Embolic CVA	MR

12 cases met Duke criteria. There was no difference in age, gender, diabetes, BMI or immunosupression between SBE and non-SBE cases. There were 156 tunneled catheters over 2 years. 9 of 34 patients (26.5%) with a previous catheter associated bacteremia developed SBE. Of the 156 tunneled catheter patients, 109 had a previous echocardiogram. 5 of 8 patients with underlying mitral regurgitation (MR) who had a previous catheter-related bacteremia developed SBE. This compared with 4 of 26 patients without MR (63% vs 15%, p=0.064). In total, 5 of 30 people with underlying moderate or severe MR developed SBE compared with 4 of 79 of people with no or mild MR (17% vs 5%, p=0.049).

Conclusions: More than half of dialysis patients who develop SBE die as a result. Tunneled catheters, previous bacteremia and MR appear to be associated with developing SBE. Screening for mitral valve disease may be worthwhile in dialysis patients, as such patients may need to undergo line removal in cases of bacteraemia, and be prioritized for vascular access surgery.

SA-PO2941

Abstract Withdrawn

SA-PO2942

The Risk of Peritonitis after Exit Site Infection in Peritoneal Dialysis: A Contemporary Analysis Alissa Lloyd,[1] Sharon Nessim,[2] Leigh Anne Shafer,[1] Jeffrey Perl,[3] Mauro Verrelli,[1] Claudio Rigatto,[1] Paul Komenda,[1] Manish M. Sood.[1] *[1]Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]Medicine, McGill University, Montreal, QC, Canada; [3]Medicine, University of Toronto, ON, Canada.*

Background: Peritonitis remains a leading cause of morbidity and technique failure among Peritoneal Dialysis (PD) patients. It is generally accepted that the development of PD catheter exit site infections (ESIs) may directly lead to peritonitis with the same organism, however, the evidence to support this conclusion is limited.

Methods: The cohort consisted of 991 incident adult patients (≥ 18 years of age) residing in the province of Manitoba, Canada who received PD during the period from 2000-2009. 975 (98%) of patients were included where complete data was available. All ESIs were treated with oral or local antibiotics. Patients who experienced an ESI were matched by time on dialysis to those with no ESI and the probability of subsequent peritonitis was determined using generalized estimating equations (GEE) at 3, 6, and 9 months.

Results: There were a total of 1,002 ESIs and 1,228 episodes of peritonitis among 692 individuals. Nine hundred and forty-seven matched pairs were analyzed. Patients with an ESI and peritonitis on the same day were excluded. When the organisms were classified as gram positive, gram negative, culture negative or fungal, the risk of peritonitis following ESI with the same class of organism was significantly increased at 3, 6, and 9 month intervals post-ESI (3 mths: 2.02 CI 1.18-3.48, 6 mths: OR 1.81 CI 1.20-2.75, 9 mths: 1.84 CI 1.30-2.64). When individual organisms were examined, the risk of peritonitis due to *Staphylococcus aureus* or *Pseudomonas* species was significantly increased up to 9 month post-ESI due to the same organism. In all analyses, the effects persisted after adjustment for diabetes, Aboriginal status and gender.

Conclusions: Patients with one or more ESI had an increased likelihood of developing peritonitis following the ESI. This increased risk occurred despite antibiotic treatment of the ESI suggesting biofilm formation, occult tunnel infection or poor technique.

SA-PO2943

Left Atrial Enlargement Is Associated with a Rapid Decline in Residual Renal Function in ESRD Patients on Peritoneal Dialysis Seung Jun Kim, Mi Jung Lee, Dong Ho Shin, Dong Eun Yoo, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. *Dept. of Int. Medicine, College of Medicine, BK 21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Left atrial volume index(LAVI) has been considered an indicator of diastolic dysfunction and an independent predictor of mortality in patients with end-stage renal disease(ESRD). Residual renal function(RRF) has also been recognized as a significant predictor of morbidity and mortality in these patients. However, little is known on the relationship between LA enlargement(LAE) and the changes in RRF in ESRD patients. Therefore, we conducted this prospective observational study to investigate the impact of LAVI on the decline in RRF in ESRD patients on peritoneal dialysis(PD).

Methods: One hundred and twenty-one incident PD patients were included. Within 2 months after PD initiation, LAE was determined by echocardiography and RRF by 24-hour urine collection. Subsequently, RRF was measured every 6 months. Patients were divided into 2 groups according to the presence of LAE(LAVI >32 ml/m^2), and the clinical and laboratory data, including the rates of decline in RRF, were compared between the two groups.

Results: Patients with LAE tended to have higher baseline RRF, but RRF at 24-month was significantly lower in patients with LAE(P=0.014). The overall rates of decline in RRF were significantly greater in patients with LAE compared to those without LAE(-0.17±0.18 vs. -0.07±0.16 ml/min/month/1.73m^2, P=0.002). Moreover, there was a significant inverse correlation between the slope of the decline in RRF and LAVI(P=0.036). Simple linear regression analysis revealed that male gender, diabetes, higher body mass index and baseline RRF, and enlarged LA were associated with a rapid decline in RRF. In multiple linear regression analysis adjusted for other risk factors, LAVI was found as an independent determinant of the rates of decline in RRF(β =-0.026, P=0.018) along with diabetes(β=-0.513, P=0.016) and baseline RRF(β=-0.129, P<0.001).

Conclusions: This study shows that a higher LAVI is independently associated with a more rapid decline in RRF in ESRD patients on PD, suggesting that volume and pressure control may help to preserve RRF in these patients.

SA-PO2944

Automated Wearable Artificial Kidney (AWAK): Feasibility of Removing Protein-Bound Toxins Martin Roberts,[1-3] John F. Collins,[4] Marjorie Wai Yin Foo,[5] Siti Noor Huda,[5] Christian G. Bluchel,[6] David B. Lee.[1-3] *[1]VAGLA Healthcare System, Los Angeles, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]AWAK Technologies, Singapore; [4]Auckland City Hospital, Auckland, New Zealand; [5]Renal Medicine, Singapore General Hospital, Singapore; [6]Temasek Engineering School, Temasek Polytechnic, Singapore.*

Background: Current dialytic modalities do not remove protein-bound (P-B) uremic toxins. Using digoxin as a surrogate P-B uremic toxin, we examined whether our peritoneal dialysis (PD)-based AWAK removes this ligand from spent peritoneal dialysate (SPDs).

Methods: From a CAPD patient taking 62.5 µg of digoxin orally every other day, the SPDs drained from an overnight dwell contained a [digoxin] and [protein] concentrations (per L) of 0.5 µg and 0.82 g, respectively. This SPDs was flowed through (single-pass) a miniature AWAK sorbent cartridge (SC), 1/30 in size and content of a regular SC and at a proportionately reduced dialysate flow rate of 200 mL/h.

Results: [Digoxin] dropped to undetectable levels (<0.2 µg/L) in the first effluent emerging from the SC and remained undetectable through a 6-hour study. Initial effluent [protein] dropped to 0.49 g/L, but returned to its pre-SC levels within 15 min and thereafter. These observations were duplicated in 3 additional studies using synthetic SPDs with the addition (to each L) of 6 µg digoxin and 1 g bovine serum albumin. Thus, the SC has the capacity to clear digoxin at a concentration 10-fold higher than that encountered in the SPDs of the patient treated with digoxin. At our projected dialysate flow rate of 1 – 4 L/h using AWAK, 144 – 576 µg of digoxin could be removed daily. Of the 3 sorbents in the SC, digoxin removal was accomplished mostly by the activated carbon with some contributions from zirconium phosphate and none by the hydrated zirconium oxide.

Conclusions: Our results indicate that the AWAK SC removes the protein-bound ligand, digoxin, but does not remove dialysate protein and thus has the potential of removing protein-bound uremic toxins. The toxin-free protein is returned to the patient where it can bind more toxins.

Funding: Private Foundation Support

SA-PO2945

Predictors of Peritoneal Dialysis (PD) Failure in Incident U.S. Patients Jenny Shen, Aya Alice Mitani, Benjamin A. Goldstein, Wolfgang C. Winkelmayer. *Stanford University, Palo Alto, CA.*

Background: Switching from PD to hemodialysis (HD) is undesirable, due to complications from temporary vascular access, disruption of daily routine, and higher costs. Little is known about the role social factors play in technique failure.

Methods: We identified and followed for 5 yrs a U.S. cohort of 1588 patients who initiated PD (1996-97). Modality failure was defined as any switch from PD to HD. Follow-up was censored at transplantation, death, loss to follow-up. We used Cox regression to examine associations among sociodemographic, medical, and healthcare related factors and the outcome. We estimated hazard ratios (HR) with 95% confidence intervals (CI).

Results: In a multivariate analysis, female sex (HR=0.78; 95%CI: 0.66-0.92) and older age (per 10 yrs; HR=0.89; 95%CI: 0.83-0.96) were associated with lower risks of technique failure. Coronary artery disease (HR=1.38; 95%CI: 1.15-1.65), heart failure (HR=2.20; 95%CI: 1.01-4.77), and receiving Medicaid (HR=1.50; 95%CI: 1.23-1.82) were associated with higher risks of failure. Subjects who completed high school were more likely to fail than those who did not complete high school (HR=1.33; 95%CI: 1.08-1.62). Separated, divorced, and widowed subjects also had a higher risk of failure than married subjects (HR=1.30; 95%CI: 1.03-1.60). Virtually all other levels of employment had a greater risk of failure than those who worked full time (Table 1). In an analysis restricted to subjects who had pre-dialysis care information, early referral to a nephrologist (> 3 months) and the primary decision maker of dialysis modality (physician vs. patient vs. shared) were not significantly associated with technique failure.

Adjusted hazard ratio for technique failure by level of employment

Level of Employment	Hazard ratio (95% confidence interval)
Full Time	1.0
Part Time	1.51 (1.05-2.17)
Homemaker	1.53 (1.07-2.20)
Retired	2.01 (1.52-2.65)
Unemployed	1.30 (0.94-1.81)
Disabled	1.50 (1.16-1.95)
Other	1.41 (1.02-1.95)

Conclusions: Our study confirms that in addition to demographic and medical factors, several social factors are also associated with technique failure. They emphasize the importance of social and financial support in maintaining peritoneal dialysis.

Funding: Private Foundation Support

SA-PO2946

Understanding the Variability in Ultrafiltration Obtained with Icodextrin – From Theory To Bedside Zanzhe Yu,[1,2] Mark Lambie,[1,2] Simon J. Davies.[1,2] *[1]Department of Nephrology, University Hospital of North Staffordshire, Stoke on Trent, Staffordshire, United Kingdom; [2]Institute of Science and Technology in Medicine, Stoke on Trent, Staffordshire, United Kingdom.*

Background: There is considerable between patient variability in ultrafiltration (UF) obtained with icodextrin (ICO) that is not fully understood. Modelling of individual patients using the 3-pore model suggests that both hydrostatic and oncotic pressure differences as well as membrane characteristics need to be considered. The purpose of this study was to elucidate clinical predictors of the variability in UF including indirect measures of these additional factors.

Methods: Net UF obtained during ICO dwell was recorded as well as membrane characteristics and clinical factors every 6 monthly. Multi-level analysis was used to identify the predictor of UF taking account of within subject correlations.

Results: 690 dwells in 202 patients were analysed, among which 280 were CAPD (typically 9 hours overnight dwell), 289 APD long day exchanges (typically 15 hours), and 126 in APD patients using an additional day-time exchange (typically 9 hours day time dwell). In multi-level mixed linear modelling, on CAPD predicted 160mls more UF compared with APD, no matter 9 hours or 15 hours. High input volume (2.5L) was related to an 111mls less UF compared with 2L. The UF negatively correlated to time on PD therapy and serum albumin. D/P creatinine, UF capacity (UF in PET) and BMI contributed positively to UF.

Conclusions: These observations fit with the theoretical modelling. They confirm the impact of membrane characteristics on UF that fast transport status and better UF capacity indicate more UF and prolonged time on PD decreases UF. They also clarify that factors which are likely to affect the oncotic pressure gradient (plasma albumin) and hydrostatic pressure gradient (input volume, patient position, BMI and gender) are more important than the dwell length in explaining UF variability. These observations have clear implications for dialysis prescription.

Funding: Government Support - Non-U.S.

SA-PO2947

Stability and Maintenance of Hemoglobin Levels with a Once Monthly Subcutaneous Administration of CERA in Chronic Kidney Disease Patients on Peritoneal Dialysis: The MISTRAL Study Raymond Azar,[1] Jean-Philippe Ryckelynck,[2] Philippe Rieu,[3] Pierre Yves Durand,[4] Bertrand Morel,[5] Robert Milongo,[6] Ali Aizel,[7] David Pau,[8] Christian Verger.[9] *[1]CH Dunkerque; [2]CHRU Caen; [3]CHU Reims; [4]CH Quimper; [5]CH Chambery; [6]Agduc Grenoble; [7]Aurar Reunion; [8]Roche, Neuilly sur Seine; [9]CH Pontoise, France.*

Background: Maintaining the stability of hemoglobin (Hb) level is a major goal of anemia treatment. The new European recommendations (ERBP 2009) indicate that Hb level should be in the target range of 11-12 g/dl without intentionally exceeding 13 g/dL. CERA, continuous erythropoietin receptor activator, corrects anemia and maintains the stability of Hb level with a once monthly administration. This French multicenter clinical trial aims to provide more data about CERA in peritoneal dialysis (PD).

Methods: Patients (pts) without any absolute iron deficiency and stable with darbepoetin alfa, epoetin alfa or beta, received subcutaneous once monthly CERA during 44 weeks. The dose of CERA was adjusted to maintain the Hb level within a range of ± 1 g/dl and in the target range 10-12 g/dL. The primary endpoint was the proportion of pts maintaining Hb level within [10–12] g/dL during the evaluation period (weeks 16, 20 and 24).

Results: 95 PD patients (men: 60%) were analyzed (age: 67±16 years). Main etiologies of chronic kidney disease were hypertension (32%), glomerular diseases (22%) and diabetes (20%). Mean duration of dialysis was 2.4±3.1 years. At baseline, mean dose of previous ESA was 3903±2502 IU/week. Mean Hb level was 11.3±0.6 g/dL at baseline and 11.6±1.0 g/dL during the evaluation period. 51% of pts were within [10-12] g/dL during the evaluation period and 71% of pts were within the target range newly recommended by the ERBP 2009. 74% of pts had a variation of Hb ≤1 g/dL since baseline or their Hb between 10-12 g/dL during the evaluation period. Mean monthly dose of CERA was 133±30 µg at baseline and 127±76 µg after 6 months. 71 serious adverse events were reported in 47 pts (49%) and none were related to CERA.

Conclusions: A once monthly administration of CERA is safe and effective in maintaining the stability of Hb level in PD patients.

Funding: Pharmaceutical Company Support

SA-PO2948

Further Development of an Efficient Dialysate Delivery Protocol for Application in a Peritoneal Dialysis-Based Automated Wearable Artificial Kidney (AWAK) Marjorie Wai Yin Foo,[1] John F. Collins,[2] Martin Roberts,[3-5] Siti Noor Huda,[1] Christian G. Bluchel,[6] Kok-Seng Wong,[1] David B. Lee.[3-5] *[1]Renal Medicine, Singapore General Hospital, Singapore; [2]Auckland City Hospital, Auckland, New Zealand; [3]VAGLA Healthcare System, Los Angeles, CA; [4]David Geffen School of Medicine at UCLA, Los Angeles, CA; [5]AWAK Technologies, Singapore; [6]Temasek Engineering School, Temasek Polytechnic, Singapore.*

Background: We have reported high dialysate (D) flow rate (FR, 4 L/h), using tidal PD (TPD) with low tidal volume (TV) and reserve volume (RV). We now report solute exchanges and ultrafiltration (UF) at several D FRs.

Methods: 5-hour TPD (TV 250, RV 500 mL) was conducted in 4 high transporters (HiT) and 4 low transporters (LoT), using Baxter Home Choice system and pH neutralized Dianeal 1.5%. Clearance (mL/min) for urea nitrogen (Cun), creatinine (Ccr), phosphorus (Cp) and beta-2 microglobulin (Cβ); glucose uptake (G-uptake, g/5h); and UF (mL/5h) were measured in each subject at D FR of 1, 2, 3 or 4 L/h, respectively.

Results: 1.Cun is FR-dependent (FR-D) and not membrane-dependent (M-D), i.e., similar C between HiT and LoT. Calculated Kt/V range is 2.5-4.3. 2.Ccr, Cp and Cβ are M-D and relatively FR-independent. Extrapolated peritoneal Cp (L/week/1.73m2) were 64 (at 1L/h, HiT & LoT) and 111 (HiT) and 95 (LoT) at 4L/h. 3. G-uptake was both FR-D and M-D and varied from 106-190 g/day. 4.UF in LoT is higher (500-690 vs 200-580 mL/5h in HiT) and is relatively FR-independent; while UF in HiT is FR-D.

D FR	1 (HiT/LoT)	2 (HiT/LoT)	3 (HiT/LoT)	4 (HiT/LoT)
Cun	8.8/9.7	12.4/12.2	14.5/12.8	15.2/15.5
Ccr	6.2 /6.1	8.4 /7.1	9.5 /7.5	9.3 /8.1
Cp	4.2/4.1	5.4 /4.8	5.7 /4.8	6.8/5.6
Cβ	1.2/0.9	1.6 /0.8	1.8/0.8	1.4/0.9
G-uptake	22.0/21.5	30.4/27.1	35.9/32.2	39.5/36.7
UF	200/500	320/620	480/550	580/690

Conclusions: 1.Dialysis adequacy is maintained with FR as low as 1L/h. A lower FR and lighter sorbent cartridge may be used during day time and a higher FR, heavier cartridge for night time. 2.Adequate UF is obtained at all FRs in both HiT and LoT using only 1.5% D. G-uptake, compared to CAPD (100-150 g/d) is not excessive. 3. Cp exceeds those reported (CJASN, 6:591. 2011) for CAPD, 43/34 (HiT/LoT), and APD, 44/28.

Funding: Private Foundation Support

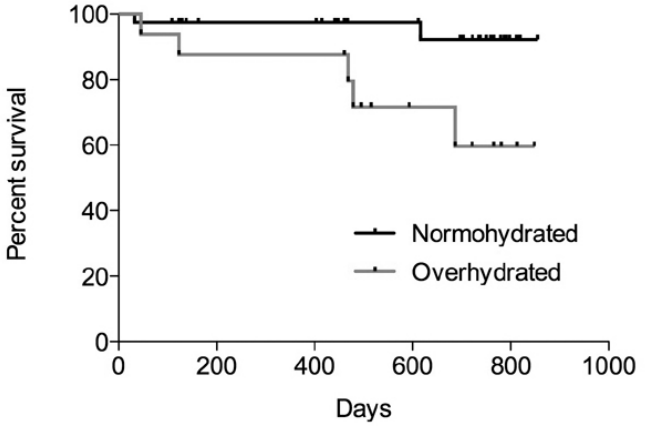
SA-PO2949

The Mortality Risk of Overhydration in Peritoneal Dialysis Lars Kihm, Joerg Seckinger, Christian Morath, Claudia Sommerer, Martin G. Zeier, Vedat Schwenger. *Nephrology, University of Heidelberg, Germany.*

Background: Maintaining euvolemia is an important goal in peritoneal dialysis (PD) patients. Adequate assessment of volume status in clinical practice is difficult. A new multifrequency bioimpedance tool has recently been validated for volume assessment. In a longitudinal observation, we evaluated the association of hypervolemia on cardiac biomarkers, morbidity and mortality in PD patients.

Methods: In 54 PD patients Body Composition Monitoring (BCM; Fresenius Medical Care, Bad Homburg, Germany) and measurement of plasma troponine T (cTNT) and N-terminal-pro brain natriuretic peptide (NT-proBNP) was performed. Clinical overhydration was defined as an overhydration-to-extracellular water ratio of >0.15. Mortality was assessed over a follow-up period of 24 months.

Results: Clinical overhydration was found in 29.6% of the PD patients. Patients with overhydration had significantly higher cTNT and NT-proBNP levels compared to euvolaemic patients (cTNT median 0.089 ng/mL, interquartile range 0.041-0.140 vs. 0.028 ng/mL, 0.010-0.067; p<0,001; NT-proBNP median 13976 pg/mL, interquartile range 4336-31095 vs. 2266 pg/mL, 361-9510; p<0,001). Chronic PD patients with clinical overhydration >0.15, cTNT >0,049 ng/mL and NT-proBNP >7266 pg/mL were more likely to die in the follow-up period.



Conclusions: Although much clinical attention is paid to volume status, almost one third of PD patients still have clinically relevant volume overload. Overhydration assessed by BCM, increased NT-proBNP and cTNT are strongly associated with adverse outcome in PD patients, and are useful tools for risk stratification.

SA-PO2950

A Reduced Ultrafiltration Rate in Children Undergoing Peritoneal Dialysis and a History of Peritonitis Depends on a High Intraperitoneal Pressure Rainer Buescher, Anja K. Büscher, Anika Von Gliszczynski, Peter F. Hoyer. *Pediatrics II, Pediatric Nephrology, University of Duisburg-Essen, Essen, Germany.*

Background: The adequacy and efficiency of peritoneal dialysis (PD) treatment in children is a dynamic process and depends on the administered intraperitoneal volume (IPV), dwell times, intraperitoneal pressure (IPP) and maintenance of peritoneal function following complications such as episodes of peritonitis. While optimal IPP-ranges are well defined for pediatric and adult PD patients under stable clinical conditions, little is known about changes of IPP and ultrafiltration rate (UFR) in children following one or more episodes of peritonitis.

Methods: IPP was measured in 19 children (10 males and 9 females, mean age 12.0 ± 1.8 years (range 2 - 18 years), mean weight 31.4 ± 19.4 kg, (range 8.1 - 60.2 kg) as standard procedure every six months and in addition one month following each peritonitis. Seventeen patients were on automated pertitoneal dialysis (APD) and two on continuous ambulatory peritoneal dialysis (CAPD). All children underwent one (n=14), two (n=3) or three (n=2) episodes of peritonitis. Patients were stratified into two groups according to their initial IPP pressure (IPP < 10 cm H_2O, n = 11 and IPP > 10 cm H_2O, n = 8) and UFR was monitored.

Results: Mean initial IPV was 959.7 ± 281.4 ml/m² BSA, mean UFR prior to the first peritonitis 620.8 ± 335.6 ml/m²BSA/24 h and mean IPP for an inflow volume of 0 ml was 9.1 ± 4.3 cm H_2O. IPP increased lineary by 1.8 ± 0.5 cm H_2O when the IPV was increased by 250 ml/m²BSA. One month after the first peritonitis, UFR did significantly drop in those patients with an initial IPP > 10 cm H_2O (IPP: < 10 cm H_2O: 657.3 ± 253.1 ml/m²BSA/24h vs. IPP > 10 cm H_2O: 364.6 ± 307.7 ml/m²BSA/24h; $p = 0.036$). Hernias were not observed within the study population.

Conclusions: We observed a drop of UFR after one peritonitis in those patients who already presented with a high IPP. Whether this effect is due to a irrversible fibrotic remodeling of the peritoneum or a transient phenomenon needs to be investigated in future studies.

Funding: Private Foundation Support

SA-PO2951

Post-Implantation Abdominal X-Ray Parameters Can Predict Functional Catheter Problems in Peritoneal Dialysis Bert Bammens, Domien Peeters, Yves Vanrenterghem. *Nephrology, Dialysis and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium.*

Background: Adequate peritoneal access is a prerequisite for good peritoneal dialysis (PD) treatment. Functional catheter problems constitute a major challenge for every PD program.

Methods: We reviewed the files of patients who received a PD catheter between 01/2005 and 08/2010. Only swan neck double cuffed curled catheters (SWAN NECK™ Missouri) were used and all were placed by open surgical technique. The "internal memory" of these catheters requires two features to be taken into account for good function: (1) swan neck angle and (2) correct inclination of catheter through the abdominal wall. Based on plain abdominal X-ray post-implantation, we defined the following categories: swan neck angle in PA view (<45°, 45-90° or >90°), inclination of catheter through abdominal wall in lateral view (angle between intramural part of catheter with imaginary horizontal line <30° or ≥30°). In addition, we evaluated the position of the internal cuff relative to the spine (L1-2, L3-4 or lower) and the position of the pigtail (hypogastric, umbilical or subcostal zone) in PA view. Endpoints: occurrence of clinically overt functional catheter problems and need for surgical reintervention.

Results: 110 patients (64 male, mean age 56±16 years) were studied. During follow-up (till October 2010), there was at least one documented episode of mechanical catheter dysfunction in 42 patients (38%). 21 patients (19%) needed surgical repositioning of the catheter. Swan neck angle (larger vs. smaller angle, HR 1.58 [1.10-2.28]) and inclination through abdominal wall (<30° vs. ≥30°, HR 3.99 [2.15-7.40]) were associated with clinically overt functional catheter problems. The inclination through abdominal wall (<30° vs. ≥30°, HR 2.80 [1.18-6.60]) was associated with need for surgical reintervention.

Conclusions: Respecting the so-called "internal catheter memory" is of utmost importance to avoid functional PD catheter problems. Plain abdominal X-ray post-implantation is an easy tool to evaluate these parameters and may serve for educational and internal audit purposes.

SA-PO2952

Different Influence of Peritoneal Dialysis Duration on Outcome of Peritonitis between First and Successive Episode Peritonitis Rong Xu, Yanjun Li, Jie Dong. *Institute of Nephrology, Peking University.*

Background: The leading cause for patients dropout from long-term PD therapy is peritonitis. We aim to explore whether there is difference between first and successive episode peritonitis in the influence of peritoneal dialysis duration on outcome of peritonitis.

Methods: A total of 248 episodes of peritonitis (124 first-episodes and 124 successive-episodes) were observed between 1st January 2008 and 30th April 2011. In first-episodes and successive-episodes respectively, patients were divided into "shorter duration" group and "longer duration" group according to the median of PD duration when peritonitis occurred. The baseline demographic data and latest biochemical parameters measured no more than 3 months before the peritonitis were recorded. Multivariate logistic regression analysis was used to find the predictor of peritonitis outcome.

Results: Both in first-episodes and successive-episodes, the age, serum albumin, hemoglobin, total Kt/v, bacterial distribution, dialysate white cell count on 3rd and 5th day were no different between "shorter duration" group and "longer duration" group, although the latter had more or marginally more female, less diabetes patients and lower residual renal function. "Longer duration" group had similar peritonitis dropout rate in first episode but higher dropout rate in successive episode than "shorter duration" group. Longer PD duration was independent predictor for outcome of successive-episode peritonitis (adjusted OR 6.80, 95% CI 1.36-33.96, p=0.02) but not for outcome of first-episode peritonitis.

Conclusions: Compared with peritonitis occurred at shorter PD duration, peritonitis occurred at longer PD duration had similar outcome in first-episode peritonitis, but poor outcome in successive episodes, which couldn't be explained by difference in demographic characteristics and biochemical parameters.

SA-PO2953

Evaluation of Diverticular Diseases by Computed Tomography Is Useful for Selection of Peritoneal Dialysis Modality and Asymptomatic Diverticulosis Is Not a Risk Factor for Peritonitis Yasuhiko Ito, Susumu Toda, Masashi Mizuno, Yasuhiro Suzuki, Isao Ito, Hideki Hiramatsu, Takenori Ozaki, Waichi Sato, Naotake Tsuboi, Shoichi Maruyama, Enyu Imai, Seiichi Matsuo. *Nephrology and Renal Replacement Therapy, Nagoya University, Nagoya, Japan.*

Background: Colonic diverticulitis is an important cause of polymicrobial peritonitis, which requires surgical treatment and cessation of peritoneal dialysis (PD). The aim of this study was to examine whether plain abdominal computed tomography (CT) is useful for evaluating colonic diverticulosis at renal replacement therapy (RRT) modality selection and to explore whether colonic diverticulosis is a risk factor for enteric peritonitis.

Methods: Subjects were 137 consecutive chronic kidney disease patients (stage 4 or 5) who were candidates for PD from February 2005 to November 2009. Abdominal CT without contrast media was performed in all PD candidates. Average individual peritonitis rate, enteric peritonitis rate, and cumulative survival and technical failure survival rate were assessed.

Results: Diverticula of the colon were detected by plain CT in 57 cases (41.6%). The number of diverticula tended to increase with age. The most common site of involvement of diverticulosis was the ascending colon (70.1%). In patients treated with PD, the incidence of peritonitis was higher in patients with diverticulosis than in those without diverticulosis (p=0.004). However, only one episode of enteric peritonitis was observed among patients with diverticulosis. The cumulative survival and technical failure survival rate was not different between the diverticulosis-positive and -negative groups. PD was not selected in 4 cases due to a high frequency of diverticula with episodes of abdominal pain. Two of these 4 cases developed severe diverticulitis with peritonitis and underwent resection of the colon.

Conclusions: Our study suggests that plain CT examination is useful for detecting diverticulosis at modality selection for RRT. Silent diverticulosis is not a risk factor for peritonitis. PD may be contraindicated in cases having frequent diverticulosis with episodes of lower abdominal pain.

SA-PO2954

The Use of Tidal Peritoneal Dialysis in Northern Ireland and the Effect on Residual Renal Function Agnes Masengu,[1] Ronan Cunningham,[2] Robert Mullan.[2] *[1]Nephrology, Belfast City Hospital, Belfast, United Kingdom; [2]Nephrology, Antrim Area Hospital, Antrim, United Kingdom.*

Background: In Northern Ireland (NI) there has been a steady increase in the percentage of patients performing automated peritoneal dialysis (APD). As a consequence of this more patients are being placed on tidal peritoneal dialysis (TPD), meaning that only a certain percentage of dialysis fluid is drained after each cycle. The aims of this study were to review the indications and prescription practices for TPD and determine if any important patient associations exist.

Methods: All NI patients on APD in December 2010 with at least one previous PD adequacy test had relevant data collected from clinical notes and electonic records. A questionnaire concerning TPD prescription and monitoring was also sent to each PD nurse specialist. Statistical analysis was performed using GraphPad Prism version 5.

Results: Of the 54 APD patients identified 93% were receiving TPD. In 68% the indication for TPD was to alleviate pain during drainage. All PD nurses reported prescribing TPD with no direct input from the patient's supervising nephrologist. There appeared to be a regional variation as to how the patient's dialysis prescription was altered after the initiation of TPD, with only some nursing staff making adjustments to account for alterations in dialysis dwell time that can occur with TPD. No association was detected between the amount of TPD prescribed versus membrane transporter status and dialysis adequacy. However there was a significant association between TPD and residual renal function (RRF). Patients on <75% TPD (i.e. >25% dialysis fluid not drained after each cycle) had a higher residual urine output compared to patients on >75% TPD (mean RRF 1530 mls +/- 222 mls vs 922.5 mls +/-112 mls respectively, p=0.02).

Conclusions: The vast majority of APD patients are now prescribed TPD by nursing staff mainly to alleviate drainage pain. Theoretically TPD may alter solute clearance but this was not demonstrated in our study. The amount of TPD prescribed did have a significant effect on urine output and therefore raises the intriguing possibility that TPD plays a role in the preservation of RRF, the mechanism for which remains unclear.

SA-PO2955

Characteristics of Eosinophilic Peritonitis in 19 Children Receiving Peritoneal Dialysis Masako Ikemiyagi, Yuko Hamasaki, Takeshi Yamada, Riku Hamada, Tomoyuki Sakai, Kenji Ishikura, Hiroshi Hataya, Masataka Honda. *Nephrology, Tokyo metropolitan Children's Medical Center, Fuchu, Tokyo, Japan.*

Background: Peritonitis is a common, yet potentially serious complication in patients who are receiving peritoneal dialysis (PD). Eosinophilic peritonitis (EP) is usually benign and often resolves without intervention. It is essential to distinguish EP from bacterial and fungal peritonitis to avoid unnecessary treatments. We retrospectively analyzed children with EP to better characterize the condition.

Methods: EP was defined as >100 white cells per milliliter of dialysate effluent, of which eosinophils constitute >10% of the all white cells.

Results: Between January 2000 and April 2011, 72 children commenced PD. During follow-up, we identified 27 episodes of EP in 19 children (median 2.7 years). One child had 4 episodes, which was the maximum. The study group comprised 11 boys and 8 girls, with a median age of 3.0 years (4 months-13 years). The median interval from the initiation of PD to the first episode was 12 days (0-232 days). The median leukocyte count of the dialysate was 640/μl, and the eosinophil count was 287/μl. The median leukocyte count of peripheral blood was 7554/μl, the median eosinophil count was 554 /μl, and the CRP concentration was 0.2 mg/ml. Twenty-four episodes of EP resolved without antibiotics, and 3 were treated until effluent cultures became negative. Four episodes were associated with physical symptoms (fever, 2 episodes; abdominal pain, 2 episodes). Other episodes were unassociated with physical symptoms. Seventeen of the 27 episodes were associated with mechanical trauma: 11, involved catheter intervention; 5, omental resection; and 1, herniorrhaphy. Five episodes were associated with intraperitoneal administration of antibiotics. Eighty-nine percent of episodes occurred within 14 days after the apparent cause of EP.

Conclusions: EP has an excellent prognosis even in children who are receiving PD. Among the 27 episodes, 24 resolved spontaneously within 1 week. The possibility of EP should be considered in children who present with cloudy dialysate within 14 days after mechanical trauma, even in the absence of physical symptoms.

SA-PO2956

Peritoneal Dialysis Is a Good Option for Patients with Polycystic Kidney Disease Patients Ana M. Tato,[1] Jose Portoles,[2] Paula López,[2] Maria Rosario Llopez Carratala,[2] Maite Rivera.[3] *[1]Nephrology, Hospital Universitario Fundacion Alcorcon, Alcorcon, Madrid, Spain; [2]Nephrology, Hospital Puerta de Hierrro, Majadahonda, Madrid, Spain; [3]Nephrology, Hospital Ramón y Cajal, Madrid, Spain.*

Background: Polycystic kidney disease (PKD) is considered a partial contraindication for peritoneal dialysis (PD) because of concern about hernias and peritonitis eventually would end in technique failure.

Methods: Multicentre observational study of patients starting PD between 2003 and 2007 and follow them up to January 2010.

Outcomes: Patient survival, technique survival; peritonitis rate.

Results: The PD-centre-group's data base (GCDP) includes 882 incident patients (2003-07) from 19 public hospitals. Patients are free to choose the renal replacement therapy, and they mainly opt for hemodialysis (HD) (regional- registry-data: 83.6% HD, 14.9% PD, and 1.5% pre-emptive-transplantation) . PKD patients seem to prefer PD rather than HD (12% vs 7.5%). They are younger and have less morbidity than nonPKD patients. Although the tranfer rate to hemodialysis is similar in both goups, in PKD patiens is more often due to abdominal space related complications. Evolution of PKD patients vs nonPKD patients is summarized in table 1.

	PKD	no PKD	p
Age (yr)	51.7	55.7	0.05<
Charlson index	3.9	5.5	0.05<
RRF (ml/min)	7.1	7.5	0.001<
Diabetes (%)	2.1	24.7	0.001<
KT/V	2.64	2.68	ns
CCr (l/w)	96.1	94.5	ns
Hb(g/dl)	12.7	12.1	ns
TAS mm Hg	126	135	0.05<
TAD	79.5	79.3	ns
peritonitis/yr	0.48	0.58	ns
mortality rate (%)	1.19	6.47	0.008
Hospital admisions (%)	0.46	0.62	0.02
transplant rate (%)	32.1	19.3	0.001<
transfer toHD	8.3	9.2	ns

Conclusions: In our cohort PD is a feasible kidney replacement therapy for most patients with PKD. Their peritonitis rate and technique survival is similar to those of non PKD patients.

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Funding: Pharmaceutical Company Support

SA-PO2957

Improved Technique Survival in Automated Peritoneal Dialysis (APD) Patients Compared to Continuous Ambulatory Peritoneal Dialysis (CAPD) Patients during the Initial 2 Years of Therapy Andrew C. Hayes, Kenneth Story, Steven Guest, Ira D. Davis. *Medical Products, Baxter Healthcare Corporation, McGaw Park, IL.*

Background: Peritoneal dialysis (PD) is an effective therapy for ESRD, with two modes: APD (Automated PD) and CAPD (Continuous ambulatory PD). Mujais et al (KI 70:S21-S26, 2006) assessed a patient cohort of initiating PD therapy between 2000 -2003, determining that technique success was higher for APD. This current study evaluates five subsequent years, evaluating cause for patients leaving PD therapy during the initial 24 months.

Methods: Baxter's U.S. database of PD patient starts from 2004-2008 was reviewed. Demographic data is limited to age, sex, diabetes. Patient status after 24 months was determined; 4 cause codes for leaving PD therapy were available: Transplant; Change to competitive PD product; Transfer to HD; other. The probability of leaving Baxter PD products was computed for both APD and CAPD patients. Hazard ratios were also computed comparing APD and CAPD patients for the risk of leaving Baxter PD products.

Results: A total of 35,968 patients initiated APD; 9,054 initiated CAPD. The APD population was younger (55.6 vs. 56.5), more likely to be male (55.8% vs. 51.0%), but less likely to be diabetic (41.2% vs. 42.0%). Of patients on PD therapy for at least 90 days, by 24 months, 51.7% of the APD patients were no longer performing APD versus 59.1% of those from the CAPD group (p<0.001). Median time to leaving Baxter PD products was 56.2 (APD) months vs. 48.7 (CAPD) months- difference of 7.5 months, p-value <0.0001, from Log Rank test. The hazard ratio comparing APD and CAPD patients for the risk of leaving Baxter PD products, after accounting for age, gender and diabetic status, was 0.872.

Conclusions: There is a 7.4% difference in 2 year technique survival between the CAPD and APD cohorts. Patient groups appear similar based on demographics (age, sex, diabetes, transplant rates), suggesting other factors led to the difference. It is postulated that CAPD, despite being a simpler, non-automated procedure, requires greater patient competencies such as performing four-times daily compared to APD with one start plus one end technique each day.

Funding: Pharmaceutical Company Support

SA-PO2958

Diastolic Dysfunction as a Cause of Rapid Decline of Residual Renal Function in Patients with Peritoneal Dialysis Jwa-Kyung Kim, Jung-Woo Noh, Ja-Ryong Koo, Young Rim Song. *Department of Internal Medicine & Kidney Research Institute, Hallym University Medical Center, Korea.*

Background: Diastolic dysfunction is frequently observed in dialyzed patients; however, the significance of diastolic dysfunction has not been clearly determined. We evaluated whether diastolic dysfunction could influence the rate of RRF loss in 82 ESRD patients starting peritoneal dialysis.

Methods: This study performed at the Hallym University Hospital between April 2005 and March 2009, and subjects with pre-existing systolic dysfunction were excluded. Timed urine collections were performed at baseline (within 1 month of start) and at 6-month intervals thereafter. Loss of renal function was defined as urine output less than 200mL/day.

Results: The mean follow-up period was 22.9±7.3 months. The median slope of RRF decline was -0.07mL/minute/month/1.73m^2 and the rate was significantly faster in diabetes compared to that of non-diabetes (-0.085 vs. -0.049). Multivariate analysis showed that the rate of RRF loss had the highest correlation with diabetes (r=-0.314, p=0.004), followed by LAVI (left atrial volume index) (r=-0.276, p=0.015), increasing aging (r=-0.256, p=0.020), LV hypertrophy (r=-0.236, p=0.032) and E/E' ratio (early peak transmitral inflow velocity to peak mitral annulus velocity) (r=-0.221, p=0.048). Forty-five patients (54.9%) with fast RRF decline (<-0.07mL/minute/month/1.73m^2) had higher prevalence of diabetes (68.9%) and showed significantly elevated baseline LAVI, LAD (left atrial diameter) and E/E' ratio (p<0.05). However, LV ejection fraction and other laboratory parameters were comparable between the two groups. In unadjusted logistic regression, the presence of diabetes (OR 4.15 95%CI 1.65-10.44), baseline RRF (OR 0.57 95%CI 0.39-0.82), LAVI ≥32 mL/m^2 (OR 3.94 95%CI 1.52-10.21) and eccentric LV hypertrophy (OR 5.50 95%CI 1.26-23.94) was closely associated with the increased risk of accelerated loss of RRF. After adjustment for other clinical variables, LAVI ≥32 mL/m^2 was an independent predictor for RRF loss.

Conclusions: In conclusion, left ventricular diastolic dysfunction could be the main determinant for loss of RRF in ESRD patients starting peritoneal dialysis.

SA-PO2959

Good Glycemic Control Is Associated with Better Survival in Diabetic Patients on Peritoneal Dialysis Dong Eun Yoo,[1] Tae Ik Chang,[2] Mi Jung Lee,[1] Dong Ho Shin,[1] Seung Jun Kim,[1] Hyung Jung Oh,[1] Seung Hyeok Han,[1] Tae-Hyun Yoo,[1] Shin-Wook Kang.[1] *[1]Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea; [2]Dept. of Int. Medicine, NHIC Ilsan Hospital, Goyang, Korea.*

Background: Previous studies have demonstrated that strict glycemic control was associated with slow progression of diabetic nephropathy, and with higher survival rates in diabetic patients on hemodialysis. However, the impact of glycemic control on the outcome of patients with diabetes(DM) on peritoneal dialysis(PD) has largely been unexplored. This prospective observational study was undertaken to clarify whether good glycemic control is associated with a better outcome in DM patients on PD.

Methods: We conducted a prospective observational study, in which 140 DM patients who started PD between Jan 2001 and Dec 2008 were recruited. Patients were divided into tertiles according to the means of quarterly HbA1C levels measured during the first year after the initiation of PD. Demographic and laboratory data were compared among the 3 groups. In addition, the associations between HbA1C levels and all-cause and cardiovascular mortalities were determined using Cox proportional hazards models.

Results: The mean age was 58.7 years, 59.3% were male, and the mean follow-up duration was 3.5 years. The mean HbA1C levels were 6.3±0.3, 7.1±0.3, and 8.5±1.1% in the 1st, 2nd, and 3rd tertiles, respectively. Compared to the 1st tertile, the overall mortality rates were higher in the 2nd tertile(HR, 2.70; 95% CI, 0.65-11.13; p=0.170) and significantly higher in the 3rd tertile(HR, 8.78; 95% CI, 1.92-40.13; p=0.005), after adjusting confounding factors(p for trend=0.015). Cardiovascular mortality, however, did not differ significantly among the tertiles. In contrast, non-cardiovascular deaths, most of which were caused by infection, were more frequent in the 2nd tertile(HR, 4.23; 95% CI, 0.45-40.01; p=0.208) and significantly more frequent in the 3rd tertile(HR, 27.05; 95% CI, 2.61-280.96; p=0.006) than the 1st tertile(p for trend=0.007).

Conclusions: Poor glycemic control is associated with high mortality rates in DM patients on PD, suggesting that better glycemic control may improve the outcomes of these patients.

SA-PO2960

Predictive Value of Red Blood Cell Distribution Width on Overall Mortality in ESRD Patients on Peritoneal Dialysis Shin-Wook Kang, Mi Jung Lee, Dong Ho Shin, Seung Jun Kim, Dong Eun Yoo, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo. *Dept. of Int. Medicine, Collge of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Red blood cell distribution width (RDW) has been found to be independently associated with adverse outcomes in patients with coronary heart disease. Although increased RDW levels are frequently observed in patients with end-stage renal disease, little is known on the relationship between RDW and outcomes in this population. This study was conducted to determine whether RDW values are associated with mortality in peritoneal dialysis(PD) patients.

Methods: We included 197 incident PD patients and classified into 2 groups according to the RDW levels at 3-month, and clinical and laboratory data, echocardiographic findings, and overall and cardiovascular mortalities were compared between the two groups. Cox proportional hazard analysis was performed to determine the independent prognostic power for overall mortality of RDW, and Kaplan-Meier curves were constructed to compare the differences in overall and cardiovascular mortalities between the two groups.

Results: The mean age was 55 years, 58.4% were male, and 51 patients(25.8%) had RDW above the upper limit of normal value(>14.5%). Patients with high RDW (H group) were significantly older (P<0.01) and had significantly more comorbidities (P<0.05) than patients with normal RDW(N group). The proportion of male patients was significantly higher, and serum albumin and total cholesterol concentrations were significantly lower in the H group (P<0.05). In addition, LVMI, LAVI, RVP, and E/E' were significantly higher in the H group compared to the N group (P<0.01). In contrast, there were no significant differences in Hb levels, iron profiles, and LVEF between the two groups. Both overall and cardiovascular mortality rates were also significantly higher in the H group compared to the N group (P<0.01). Cox proportional hazard analysis revealed that RDW was a significant independent predictor of overall mortality even after adjusting for other risk factors (HR, 1.29; 95% CI, 1.07-2.45; P<0.05).

Conclusions: This study shows that RDW provides a meaningful prognostic value on overall mortality in incident PD patients.

SA-PO2961

Statins Decrease Small, Dense Low-Density Lipoprotein in Patients Undergoing Peritoneal Dialysis Anna Clementi, Jeong Chul Kim, Matteo Floris, Dinna N. Cruz, Francesco Garzotto, Monica Zanella, Federico Nalesso, Alessandra Brendolan, Claudio Ronco. *Nephrology, St Bortolo Hosp, Vicenza, Italy.*

Background: Cardiovascular (CV) disease is a major cause of mortality among hemodialysis (HD) and peritoneal dialysis (PD) patients, and dyslipidemia plays an important role in its pathogenesis. Small dense LDL (sd-LDL) have been recently highlighted as an emerging CV risk factor. PD patients exhibit a more abnormal lipid profile than HD patients, likely due to peritoneal dialysate. Statins are able to decrease all LDL subclasses levels, but it is not known whether they can influence the proportion of sd- LDL. Few studies have been performed in HD patients and none in PD patients. We compared the lipid profile and the effect of statins on it in HD and PD patients.

Methods: We enrolled 101 and 79 patients undergoing HD and PD at St Bortolo Hospital in Vicenza. The mean age of HD and PD patients was 66±13.9 and 58±15.9 (yr). Men were 67.3% and 59.5% in the 2 groups. 83.2% of HD and 81% of PD patients had hypertension. 27.7% of HD and 19% of PD patients were diabetic. We divided HD and PD patients into 2 groups according to statin therapy.

Sd-LDL was measured by LDL-EX "SEIKEN" homogeneous Assay on ADVIA 2400 Clin Chemistry Syst. SPSS software version 17 was used for statistical analysis.

Results: HD patients presented lower levels of total cholesterol (TC)(p=0.001), triglyceride (p=0.007), LDL-C (p=0.001), Apo-B (p=0.001), absolute amount of sd-LDL and their proportion (p=0.001). Among HD patients the statin group presented lower levels of TC (p=0.002), LDL-C (p=0.002), HDL-C (p=0.001), Apo-AI (p=0.009), Apo-B (p=0.006). No differences in terms of sd-LDL and their proportion (p=0.058, p=0.745). Among PD patients the statin group presented lower levels of TC (p=0.011), LDL-C (p=0.01), Apo-B (p=0.001), absolute amount and proportion of sd-LDL (p=0.013, p=0.036).

Conclusions: Our study suggests that statins may reduce the absolute amount and the proportion of sd-LDL in patients with a more abnormal lipid profile, such as PD patient. Statins do not alter sd-LDL levels in HD patients due to the higher number of patients with hypertension and diabetes, which can influence their generation.

SA-PO2962

Left Atrium Volume Index as Useful Predictor for Decline of Residual Kidney Function in Peritoneal Dialysis Patients Takeshi Yokoyama,[1] Katsuomi Matsui,[1] Tsutomu Sakurada,[2] Yugo Shibagaki,[2] Yusuke Konno,[1] Takashi Yasuda,[2] Kenjiro Kimura.[2] *[1]Division of Nephrology and Hypertension, Department of Internal Medicine, Kawasaki Municipal Tama Hospital, Kawasaki, Kanagawa, Japan; [2]Division of Nephrology and Hypertension, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.*

Background: Preservation of residual kidney function (RKF) has been recognized as an independent predictor of survival in peritoneal dialysis (PD) patients. Although heart failure is known to associate with decline of RKF, little is known which parameters of ultrasound cardiography (UCG) are helpful to predict decline of RKF.

The aim of present study is to clarify the relationship between the rate of decline of RKF and the parameters of UCG.

Methods: UCG was performed in 15 PD patients at the initiation of PD, the following parameters such as left atrial dimension (LAD), left atrial volume index (LAVi), left ventricular end-diastolic dimension (LVDd), left ventricular internal dimension in systolic (LVDs) and left ventricular mass index (LVMi) were measured. We calculated RKF as the half of the sum of weekly urea and creatinine clearances normalized to 1.73m^2 BSA, and defined the rate of decline of RKF as [(the latest RKF - intial RKF) / duration of PD]. We examined the correlations between the rate of decline of RKF and the parameters of UCG.

Results: Average RKF at the initiation of PD was 36.11 ± 19.14 L/week/1.73m^2 and average rate of decline of RKF was -0.16 ± 2.07 L/week/1.73m^2/month. Rate of decline of RKF was significant correlated with LAD (r = -0.546), LVDd (r = -0.544), LVMi (r =

-0.562) and LAVi (r = -0.649). Multiple regression analysis revealed that only LAVi was an independent predictor for decline of RKF.

Conclusions: Among the parameters of UCG, LAVi was the most useful predictor of a decline of RKF in PD patients.

SA-PO2963

Use of Dressing with Polyurethane Absorptive Foam with Chlorhexidine Gluconate (BIOPATCH®) To Reduce Exit-Site Infection and Peritonitis in Patients Undergoing Peritoneal Dialysis

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Background: Peritoneal dialysis (PD) catheter insertion related infections cause significant morbidity and often force the removal of the catheter.

Aim of this study was to find the difference in incidence of exit site infections (ESI) and peritonitis in patients who had PD catheter inserted a year before (1/5/06 to 30/4/07) and after (1/5/07 to 30/4/08) introduction of regular use of Chlorhexidine Gluconate containing foam patch dressing Biopatch.

Methods: We gathered data on 59 patients who had a PD catheter inserted over a year before introduction of Biopatch and 70 patients who had PD catheter inserted over a year afterwards. Our practice is to use Biopatch dressing at the time of catheter insertion and change it weekly for 6 weeks. Data was recorded on ESI, tunnel infection,PD peritonitis, catheter loss and mortality during first 12 weeks after catheter insertion.

Results: 17 patients could not complete the study period in pre-Biopatch group (3-died, 8 - catheter malfunction, 1 - transplant, 2 - transferred out & 2 - lost due to infection).

10 patients could not complete the study period in post-Biopatch group (2-died, 5-catheter malfunction, 1-transferred out & 1 -lost due to infection).

Incidence of infection leading to catheter loss was not statistically different in 2 groups. (p=0.11)

ALL INFECTION EPISODES:

	0-7 days	8-28 days	29-42 days	42-84 days	TOTAL
PRE BIOPATCH	1 (1.7%)	4 (6.8%)	1 (1.7%)	5 (8.5%)	11 (18.6%)
POST BIOPATCH	0 (0%)	5 (7.1%)	1 (1.4%)	7 (10%)	13 (18.6%)
					p = 0.12

ESI

	0-7 days	8-28 days	29-42 days	42-84 days	Total
PRE BIOPATCH	1 (1.7%)	4 (6.8%)	1 (1.7%)	3(5.1%)	9(15.3%)
POST BIOPATCH	0 (0%)	5 (7.1%)	0 (0%)	6 (8.6%)	11(15.3%)
				p = 0.025	p = 0.12

PD peritonitis

	0-7 days	8-28 days	29-42 days	42-84 days	Total
PRE BIOPATCH	0	1 (1.7%)	0	2 (3.4%)	3 (5.1%)
POST BIOPATCH	0	0	1 (1.4%)	0	1 (1.4%)
				p = 0.044	p = 0.066

Conclusions: Introduction of Biopatch made no difference to overall incidence of exit site infection or PD peritonitis in first 12 weeks after PD catheter insertion. However, there was increased incidence of ESI in post Biopatch group after stopping Biopatch application (42 to 84 days after insertion) (p= 0.025). On the other hand incidence of Peritonitis was higher in pre-Biopatch group in period between 42 and 84 days after catheter insertion (p=0.044).

SA-PO2964

Urgent Peritoneal Dialysis Start for Late Referred End-Stage Renal Disease Patients – Single Center Experience

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Background: Despite improvement in the predialysis care, still 30–50% of the ESRD pts start dialysis urgently via acute central venous catheter. There is some positive experience with acute start on chronic peritoneal dialysis (PD) right after catheter implantation by open surgery. The aim of the study was to assess the impact of unplanned PD start on the outcomes and PD related complications.

Methods: This is a retrospective study of incident ESRD pts treated by PD at our center between Jan 2005 and Dec 2010. Tenckhoff catheter was inserted via open surgery. PD initiated < 9 days after catheter implantation was referred as an acute start. The groups of planned and unplanned start were compared for the outcomes and PD related complications.

Results: From 82 pts, aged 52±17 years, 21 (26%) started PD urgently (12 h overnight APD + dry day, dwell volume 1L). They had lower eGFR (6.4±3.3 vs 8.6±3.1ml/min, p<0.01), lower Hb(9.5±1.3 vs 10.5±1.3g/dL, p<0.01), and serum albumin (3.3±0,4 vs 3.5±0.5 g/dL, p<0.05), and the same Charlson Comorbidity Index (6±3). The pts were observed for 1621 pts-months, median follow-up was 16 (1-61) months. A percent of early (first 2 weeks) mechanical complications was slightly but not significantly higher in the unplanned pts with bleeding in 5 (24%) vs 3 (5%) pts, and leakage in 3 pts (14%) vs none (p=0.09). There was no exit site infection nor peritonitis. Late mechanical complications occurred in 3 (15%) of unplanned pts (leakage in 1, hernia in 2) and 10 (18%) of planned pts (leakage in 4, hernia in 7); p=0.9. PD catheter migration was noted in 1 (5%) urgent and 9 (15%) elective started pts; p=0.2. The incidence of peritonitis was 1/44 pts-months in the unplanned and 1/48 pts-months in the planned group; p=0.6. At the end of follow-up 43% of unplanned and 39% planned pts continued PD; 24% vs 28% were transplanted, 5% vs 21% moved to HD, and 28% vs 12% died; p=0.5.

Conclusions: The PD can be a safe alternative to HD in acute start allowing to avoid a temporary cannulation of large veins.

SA-PO2965

Does the Glomerular Filtration Rate at Initiation of Peritoneal Dialysis Affect the Nutritional Status and Survival of Patients?

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Background: The effect of initial GFR on clinical outcomes on follow-up in Indian PD patients has not been studied.We aimed to study the effect of baseline GFR on nutrition status and survival of PD patients.

Methods: We included 342 **PD** patients (age 51.5±14 yrs,250 male,179 diabetics) and followed for 21.6±14.4 patient-months. Nutrition status of patients was assessed by anthropometry, biochemical parameters, diet-diary and Subjective Global Assessment(SGA). GFR was calculated by using Cockcroft-Gault (CG) formula.Patients were classified into 3 groups(grp): Grp1: GFR<5ml/min, Grp II GFR 5-10 ml/min and Grp III GFR >10ml/min. The clinical outcome and nutritionparameters at basline and follow up were compared between three groups

Results: The variables between 3 grp are shown in the table.

Table 1:various parameters in three different groups

variables	Group 1	Group 2	Group 3	P value
Kt/V Urea	1.79 ±0.41	1.89 ±0.46	1.91 ±0.44	0.76
CrCl (L/week)	63.09 ±19.25	65.1±17.14	69.46±23.54	0.94
D/P Cr	0.67±0.10	0.70 ±0.11	0.69±0.11	0.58
Age (Years)	56.3±14.4	51.8±15.2	49.6±16.3	0.05
S. Albumin(g/dl)	3.0±0.67	3.20±0.5	3.5±0.5	0.04
SGA score	3.5±1.4	4.4±1.6	4.4 ±1.7	0.01
NRI score	86.7±11.5	90.1±7.6	91.3 ±9.1	0.02
Energy(Kcal/Kg/day)	17.9±5.8	19.0 ±6.5	19.1± 6.1	0.66
Protein (g/Kg/day)	0.77±0.30	0.81±0.29	0.84 ±0.27	0.53

D/P cr, dialysate/plasma ctreatinine ratio at 4 hours; NRI,Nutritional risk index;CrCl,creatinine clearance

The median survival (patient-months) in grp I [20 (95% CI 15.5-24.5)] was inferior to Grp II [45.3(95% CI 36.0-54.6) and Grp III [59 (95% CI 18.5-99.5, p-0.001)].The risk ratio of death in Grp I was higher than grp II [OR=2.2(95% CI 1.5-3.1), p<0.001] and grp III [OR 4.7 (95% CI 2.8-8.2), p<0.001] patients.The risk ratio of malnutrition was also higher in grp I compared to grp II [OR=1.3(95% CI 1.2-1.4) p=0.008] and grp III [OR=1.3 (95%CI 1.2-1.6), p=0.003] patients. There was a ↓ in serum albumin in grp I (-0.08±0.59) and ↑ in Grp II (0.06±60.46) and grp III (0.99±0.44). There was a significant ↑ in mean SGA score in grp III (0.66±0.94) compared to grp II (-0.12±0.88) and grp I (-0.41±0.49) patients.

Conclusions: Initial GFR is an important factor to affect the nutrition status and survival of PD patients.

Funding: Government Support - Non-U.S.

SA-PO2966

Assessment of Hydration State in Peritoneal Dialysis Patients by Two Different Bioimpedance Methods-Body Composition Monitor and Calf Bioimpedance

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Background: Our aims were to evaluate hydration state in peritoneal dialysis (PD) patients using body composition monitor (BCM) and calf bioimpedance (cBI), and compare both methods.

Methods: 58 PD patients studied. Overhydration (OH) was measured by BCM device (normal limits:-1.1 to1L). Patients were assigned to normo and hypervolemic groups according to OH value. Normalized calf resistivity (nRho) was calculated from resistance at 5 Khz using cBI. The mean value of 3 BP measured at different time on study day were used.

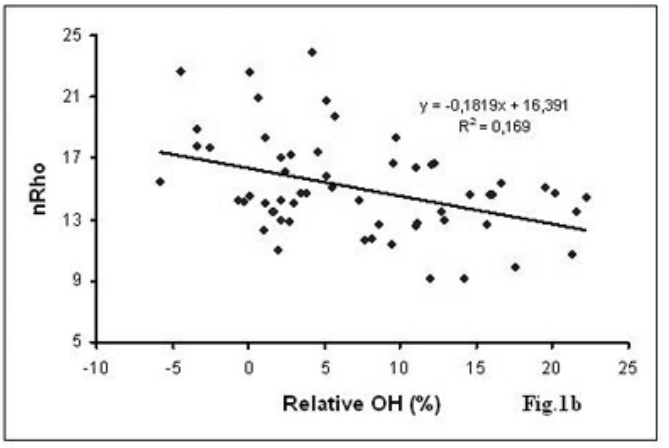
Results: Mean age was 47.2±0.12, 41 (70.7%) male. Clinical characteristics of OH groups were given in the table.

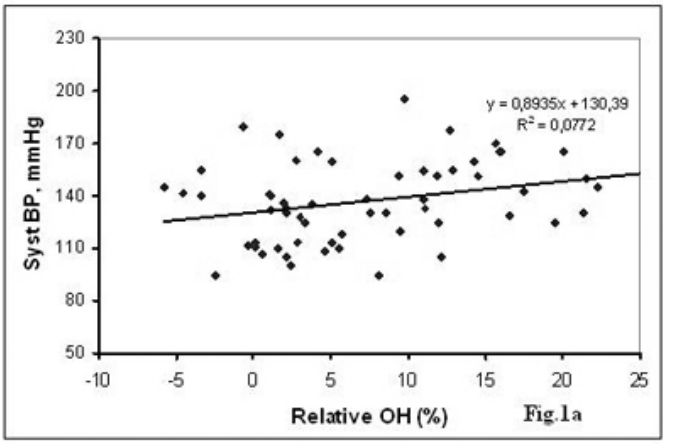
Table 1. Clinical Characteristics of Patients Grouped by BCM Data

Parameters	Hypervolemic (n=24)	Normovolemic (n:34)	P value
Age	51,9±14,9	44.0±12.6	0.034
OH (L)	2,58±0,92	0,312±0,45	0,001
nRho	13.7±2.4	16.0±3.3	0.005
eGFR (mL/min)	4.2±3.9	3.0±3.8	0.270
D/P creatinine	0.69±0.17	0.66±0.11	0.530
No.of antihypertensive	1.54±1.06	0.97±0.93	0.035
Edema + pts (n, %)	11 (45.8%)	6 (17.6%)	0.042

Frequency of edema, Anti-HT drug use were significantly higher in hypervolemic group. nRho was lower in hypervolemic group.Sys BP showed a significant correlation with Relative OH % (OH/ECV), but not with nRho (Figure 1a.)

There was an inverse relation between rel.OH and nRho (p=0.001 r:-0.41) (Fig1b).





28 of the patients who have been using anti-HT drugs didn't have edema. Of these 28 patients, 11 had hypervolemia (OH>1.1 L). In a sub-group analysis of these 28 pts, nRho was lower in hypervolemics (14.8±1.9 vs 16.1±3.4 p=0.517)

Conclusions: In PD patients, BCM may become a benefit tool to evaluate hydration state and determine occult hypervolemia. There was a moderate inverse correlation between BCM and cBIS techniques in terms of assesment of hydration state.

SA-PO2967

Effect of Intra-Peritoneal Dialysate on Evaluation of Volume Status by Bioelectrical Impedance Analysis in the Peritoneal Dialysis Patients Jung-Ju Seo,[1,2] Jang-Hee Cho,[1,2] Mi-Kyung Jin,[1,2] Owen Kwon,[1,2] Kyung-Deuk Hong,[1,2] Ji-Young Choi,[1,2] Se-Hee Yoon,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Yong-Lim Kim.[1,2] *[1]Department of Internal Medicine, Kyungpook National University Hospital, Dague, Republic of Korea; [2]Clinical Research Center for End Stage Renal Disease, Republic of Korea.*

Background: Direct segmental multi-frequency bioelectrical impedance analysis (DSM-BIA) has been proposed as a tool for adequate assessment of fluid status in continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods: We examined the influence of intra-peritoneal dialysate on total body water (TBW) and the accuracy of DSM-BIA to estimate TBW. We measured the change of TBW by DSM-BIA ($TBW_{DSM-BIA}$) and compared $TBW_{DSM-BIA}$ with TBW by the Watson formula (TBW_{Watson}) under influence of dialysate in 55 CAPD patients.

Results: After infusion of dialysate, $TBW_{DSM-BIA}$, the index of hydration (TBW/weight(BW), %), and obesity (fat mass(FM)/BW, %) were significantly increased ($p < 0.001$), but the index of edema (extracellular fluid(ECF)/TBW) was not changed. $TBW_{DSM-BIA}$ had fairly strong correlation with TBW_{Watson} before and after infusing dialysate ($r = 0.940$ or 0.946, $p < 0.001$), but $TBW_{DSM-BIA}$ was overestimated versus TBW_{Watson} ($p < 0.001$). The difference between two methods was correlated with index of hydration ($r = 0.529$ or 0.542, $p < 0.001$) and obesity ($r = -0.515$ or -0.538, $p < 0.001$).

Conclusions: The dialysate influences the evaluation of $TBW_{DSM-BIA}$, however, we were able to use the edema index to evaluate fluid status of CAPD patients without the effect of dialysate. DSM-BIA overestimated TBW in hydrated patients and underestimated it in obese patients when compared with the Watson formula.

Funding: Government Support - Non-U.S.

SA-PO2968

Better Technique Survival with an Alternative Treatment Strategy of PD Related Peritonitis Wim Rüger,[1] Pieter M. Ter Wee.[1] *[1]Nephrology, VU University Medical Center, Amsterdam, Netherlands; [2]Nephrology, University Medical Center, Utrecht, Netherlands.*

Background: ISPD PD-related infections recommendations suggest catheter removal in refractory, relapsing and enteric peritonitis. We compared the outcomes of all PD-peritonitis episodes (1998-2008) treated with different treatment strategies in two university hospitals.

Methods: *Hospital 1:* Initial treatment: intraperitoneal (i.p.) rifampicin and gentamicin, if need be adapted to culture results. Decision to remove PD-catheter guided by ISPD recommendations.

Hospital 2: Patients ≤50 yrs: i.p.cephalotin; Patients >50 yrs: considered at risk for enteral peritonitis. Assuming that PD hinders omental sealing of (micro)perforations, PD was discontinued and meropenem given intravenously and intracatheter. In cases of enteric peritonitis continued for 1 week, then restart of PD with 1 week i.p. meropenem. If cultures yielded non-enteral organisms, PD was resumed with appropriate i.p. antibiotics. Fungal peritonitis was treated with i.p. fluconazol, oral flucytosine and intracatheter amphotericin B. Enteral or fungal peritonitis were no indication for catheter removal.

Results: 323 peritonitis episodes in *Hospital 1* and 251 in *Hospital 2*. Patient and peritonitis episode characteristics were similar. Fungal episodes occurred in 7.4% vs. 3.2%.

Antibiotics alone resulted in cure in 79.9% of episodes in *Hospital 1* vs. 92.8% in *Hospital 2*. PD-catheter removed in 16.7% vs. 2.4% of episodes. Recuperation after catheter removal in 11.4% vs. 1.6%, with patient returning to PD in 3.4% vs. 0.0%, permanently switching to HD in 8.0% vs. 1.6% of episodes. Technique survival thus was 83.3% vs. 92.8% (OR 2.60; 95%CI 1.48-4.56).

Respectively in 5.0% and 0.8% of episodes patients died despite catheter removal and in 3.7% vs. 4.8% with catheter in place. Patient survival thus was 91.3% vs. 94.4% (OR 1.61; 95%CI 0.83-3.10).

Conclusions: A peritonitis treatment strategy with interrupting PD, intravenous and intracatheter meropenem, tailored to patient age and causative micro-organism and particular fungal peritonitis treatment resulted in better PD-technique survival than a strategy consisting of intraperitoneal antibiotics and catheter removal according to ISPD-recommendations.

SA-PO2969

Outcomes of Peritonitis in Incident Peritoneal Dialysis Patients Eduardo K. Lacson, Nien-Chen Li, Raymond M. Hakim, J. Michael Lazarus, Franklin W. Maddux, Joseph P. Pulliam. *Fresenius Medical Care, North America, Waltham, MA.*

Background: We evaluated the incidence and outcomes of peritonitis among peritoneal dialysis (PD) patients who begin home dialysis within 90-days of first (ever) dialysis.

Methods: All 1,984 incident PD patients admitted to Fresenius Medical Care North America facilities from January 1 to December 31, 2009 were followed for one year from their first home PD treatment. We tracked patients that developed peritonitis and investigated outcomes within 30 and 90 days thereafter.

Results: 557 patients (28.1%) developed peritonitis over a median time of 123 days (139±98 days). Compared to patients without peritonitis, mean ages were similar (56.8 vs. 56.3 years, p=0.58), male gender (59.1% vs. 55.2%, p=0.11), white race (69.1% vs 76.7%, p=0.001), diabetes (53.7% vs 53.5% p=0.93), mean BMI (33.3 vs 31.9, p<0.05, medians 30.5 vs 29.3). Overall technique failure rate in patients who had peritonitis was 28.4% compared to patients who did not have peritonitis at 18.4% (p<0.0001). Subsequent hospitalization rates were higher for peritonitis patients as well (72% vs. 52.7%, p<0.0001). Odds ratios for technique failure and hospitalization for patients with peritonitis were 2.31 and 1.75, both p<0.0001. Within 30 days following diagnosis of peritonitis, 140 were hospitalized (25.1%), 43 switched to HD (7.7%) and 5 died (0.9%). One patient who died and 22 that switched to HD did so post-hospitalization. Among 43 patients that switched to HD, only 8 returned to PD and 2 of them eventually had multiple modality switches and were on HD at year-end. By 90 days of diagnosis, 189 were hospitalized (33.9%), 89 switched to HD (16.0%) and 11 died (2.0%). Among 89 patients that switched to HD, only a total of 13 patients returned to PD including the same 2 patients having multiple modality switches as noted above.

Conclusions: Peritonitis episodes were associated with increased hospitalization and technique failure among incident PD patients within 30 and 90 days of the event. Further research is needed to determine interventions that can decrease peritonitis rates in this population.

SA-PO2970

Development and Evaluation of a Novel Semi-Long Peritoneal Dialysis Catheter for Upper Abdominal Exit-Sites Narutoshi Kabashima,[1] Tetsu Miyamoto,[2] Akihiro Kuma,[2] Nana Ishimatsu,[2] Yumi Furuno,[2] Kaori Kanegae,[1] Ryota Serino,[2] Yutaka Otsuji,[2] Masahito Tamura.[1] *[1]Kidney Center, University Hospital of Environmental and Occupational Health, Kitakyushu, Japan; [2]Second Department of Internal Medicine, University Hospital of Environmental and Occupational Health, Kitakyushu, Japan.*

Background: Conventional Swan-Neck peritoneal dialysis catheters (PDC, 43.5cm) have been hitherto used in Japan. Recently several ultra long PDC (80 cm) designed for the presternal or upper abdominal exit-site were established to avoid infectious complications. However, it is not easy to handle these long catheters at the PDC implantation procedure. Furthermore, the problem of dislocation and omental wrapping remain unsolved.

Methods: We developed a newly designed Flexible-Neck semi-long PDC (JBS-2) to reduce the incidence of catheter-related problems including dislocations and omental wrappings. JBS-2 consists of 65 cm of lengths (5 mm of diameter) with 34 smaller side-holes (0.5 mm) on the four longitudinal slit lines. The tube around the peritoneal cuff (8.0 cm between the peritoneal and subcutaneous cuffs) was thickened and reinforced to prevent dislocation. We implanted JBS-2 for 16 patients and assessed implantation procedure and inflow-/outflow- time with 1500 ml of peritoneal dialysis fluid (PDF). The incidence of dislocation and omental wrapping were also recorded.

Results: Owing to the length of PDC, JBS-2 made the implantation technique easier than the ultra long catheters. We could provide upper abdominal exit (UAE) without cutting PDC. It took 7.5 minutes of mean inflow time and 15 minutes of mean outflow time. No omental wrapping and no PDC dislocation has been observed in patients with JBS-2.

Conclusions: Our newly developed PDC (JBS-2) was appropriate for UAE without catheter-related problems including dislocations, omental wrappings and inflow-/outflow-delays.

SA-PO2971

Patient Recruitment to Home Hemodialysis (HHD) in Canada Robert P. Pauly,[1] Paul Komenda,[2] Deborah Lynn Zimmerman.[3] *[1]University of Alberta, Canada; [2]University of Manitoba, Canada; [3]University of Ottawa, Canada.*

Background: There is growing interest in HHD though little is published on establishing and maintaining an HHD program. Canada is a recognized leader in HHD delivery and the purpose of this study was to leverage Canadian expertise and survey HHD programs nationwide to describe practice patterns in a variety of domains. The current abstract focuses on patient recruitment to HHD.

Methods: A comprehensive questionnaire of HHD practice patterns was developed by an expert panel with input from allied health services and underwent multiple modifications. It was distributed and completed online between July and December 2010; data reflect practices during this time period.

Results: Seventeen of 19 (89%) programs responded. Thirteen of 17 (76%) have a specific recruitment strategy including special modality education classes (11/17 – 65%), a designated modality educator (12/17 – 71%), posters advertising HHD (11/17 – 65%), an information video on HHD (10/17 – 59%), discussion of potential HHD patients at patient care rounds (12/17 – 71%), and active recruitment of failing peritoneal dialysis patients (14/17 – 82%). Many programs restrict HHD access to patients expected to remain on HHD for at least 6 months (12/17 – 71%) or at least 12 months (6/17 – 35%), while other programs have no such policy and will train patients even if they are expected to exit the program sooner (eg. for planned living donor transplantation). Ten of 17 programs (59%) mandate an HHD assistant for selected patients; all programs allow family members to act as HHD helpers, and 4 of these 10 (40%) allow paid assistants. Ten of 17 (59%) estimate that <10% of pre-dialysis clinic patients choose HHD as their initial modality and 13/17 (76%) estimate <10% of unplanned (precipitated) dialysis starts will choose HHD.

Conclusions: Most Canadian renal programs use specific strategies to encourage uptake of HHD, though the majority of patients originate from the prevalent ESRD population. There is greater variability in policy concerning the need for HHD assistants and the minimum length of time a patient is expected to remain on HHD. Sharing successful recruitment strategies may facilitate increased uptake.

SA-PO2972

Comparison of Percutaneous and Open Surgical Techniques for First-Time Peritoneal Dialysis Catheter Placement in the Unbreached Peritoneum Samar A. Medani, Wael F. Hussein, Mohamed Shantier, Catherine A. Wall, George Mellotte. *Nephrology, Adelaide & Meath Hospital, Dublin, Ireland.*

Background: The percutaneous seldinger method of peritoneal dialysis catheter (PDC) insertion has gained favour over recent years whereas traditionally it was reserved for patients unfit for general anaesthesia. This blind technique is believed to be less safe in patients with previous abdominal surgery therefore a criticism of this method is selection bias. In those with no history of abdominal surgery the optimal method of insertion has not been established.

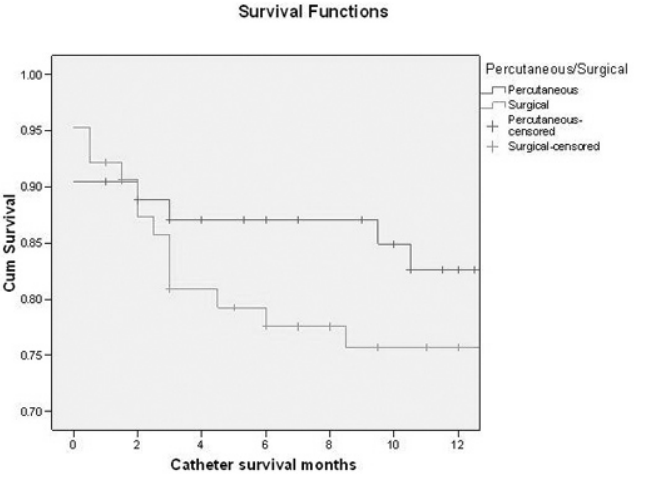
Methods: We retrospectively compared the outcomes of first-time percutaneous (group P) and surgical (group S) PDC placements in our centre between January 2003 and April 2010 in patients without a history of abdominal surgery. We reviewed outcomes and complications of PDCs until April 2011. Kaplan Meier curves were generated to represent technical survival of catheters for the first 12 months after insertion.

Results: 64 percutaneous and 63 surgical catheter insertions were analysed. PDC related complications are shown.

Complications of PDC insertions

Complications	Group S	Group P	P value
Peritonitis (patient-months per episode)	17.2	20	0.66
Exit leak	4 (6.3%)	10 (15.9%)	0.15
Poor initial drainage	7 (10.9%)	6 (9.5%)	0.34
Secondary drainage failure	12 (18.8%)	5 (7.9%)	0.09

There were no differences between the two groups in peritonitis rates, leaks, poor initial drainage and secondary drainage failure. Technical survival for group P catheters compared favourably with group S (P=0.35).



Conclusions: Our results emphasise the success and safety of percutaneous PDC insertion in experienced hands. Technical survival and complication rates were comparable with the surgical technique in this cohort of patients without previous abdominal surgery. The percutaneous technique offers the advantage of ease of insertion and can be done as a day case procedure.

SA-PO2973

Peritoneal Dialysis in Patients with Cancer; the Experience of One Centre in the United Kingdom Harsha Wodeyar Kagodu Surendranath, Muhammad Imran, Gordon M. Bell, Hameed Anijeet, Pearl Pai. *Nephrology, Royal Liverpool University Hospital, Liverpool, Merseyside, United Kingdom.*

Background: Peritoneal dialysis (PD) is often regarded as an inferior modality of renal replacement despite many advances in PD technology and solution formulation. There is also concern whether cancer patients should access chronic dialysis therapy. We report our experience of cancer patients on PD over the last 7 years in a well established PD unit in the north-west of England.

Methods: We reviewed the data of our PD population and the clinical details of those suffering from malignant disease. Our PD programme (n=80) includes 8 patients suffering from malignant disease in the last 7 years. Their details are below:

Age/Gender	Renal Diagnosis	Malignancy (diagnosis year)	PD vintage (months)	Current status
25/Female	Drug induced renal failure	Neuroectodermal tumour (2007)	17	Not on dialysis due to sufficient renal recovery
54/Female	Obstructive uropathy	Endometrial (2010)	26	Still on PD
62/Female	Membranous glomerulonephritis	Breast and bladder (2010)	8	Still on PD
40/Female	Diabetic nephropathy	Malignant melanoma with lymphnode metastasis (2005)	24	Still on PD
65/Female	Myeloma kidney	Multiple Myeloma (2007)	18	Deceased
70/Female	Myeloma kidney	Multiple Myeloma (2005)	24	Deceased
20/Male	IgA nephropathy	Osteosarcoma with lung metastasis (2004)	24	Deceased
54/Male	Myeloma kidney	Multiple Myeloma (2009)	15	Deceased

Results: Our cohort had good nutritional status (mean albumin= 38.5 g/L and range=37 to 45 g/L) and positive feedback with no patient withdrawal from treatment during the study period.

Conclusions: Many of these PD patients with cancer will never be on transplant waiting lists. In the United Kingdom, the use of a cycler (automated PD) frees restrictions and allows the patients to dialyse at their home. The quality of dialysis and life of cancer patients on PD would appear to be just as good as in non-cancer patients.

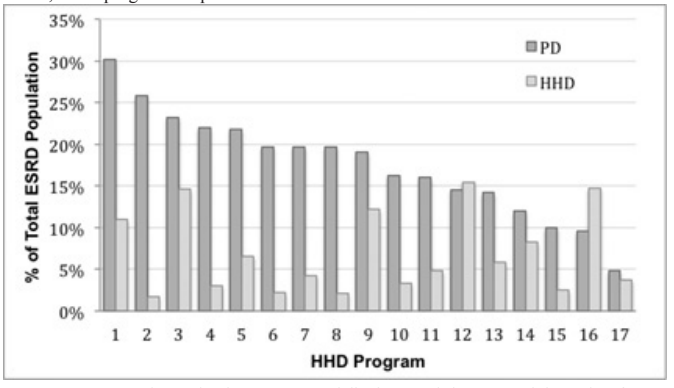
SA-PO2974

Home Hemodialysis (HHD) and Peritoneal Dialysis (PD) in Canada: The Status of Co-Existing Home Therapies in 2010 Robert P. Pauly,[1] Paul Komenda,[2] Deborah Lynn Zimmerman.[3] *[1]University of Alberta, Canada; [2]University of Manitoba, Canada; [3]University of Ottawa, Canada.*

Background: Canada has historically treated a relatively high proportion of ESRD patients with PD. Canada is also a leader in the delivery of HHD, particularly with respect to nocturnal hemodialysis (NHD), short-daily hemodialysis (SDHD), and variations on these prescriptions. Uncertain is the extent to which these two home modalities can successfully co-exist in individual renal programs.

Methods: Between Jul and Dec 2010, 19 dialysis programs (14 university-based centers and 5 community centres known to provide all options of home dialysis therapies) were surveyed to determine the relative proportion of HHD and PD.

Results: Seventeen of 19 programs responded. Uptake of home therapies (HHD and PD) averaged 24.8±7.2% (range 9-41%) of prevalent ESRD patients; the balance was conventional thrice weekly in-centre or satellite HD. One of 17 (6%) programs had a combined HHD and PD population ≥40%, 4 of 17 (24%) had a combined home therapies proportion ≥30%, and 15 of 17 (88%) had a combined population ≥20%. Of patients receiving HHD, the median proportion (and range) receiving some form of NHD (6-8 hours on ≥3 nights per week) was 74% (24-94%), while a median of 4% (0-41%) received SDHD (1.5-3 hours on 6-7 days per week); non-NHD, non-SDHD dialysis prescriptions were also common in the home setting. Program 1 (graph) represents a suburban community dialysis centre, while program 3 represents an urban academic centre.



Conclusions: The predominant HHD modality in Canada is NHD. High uptake of HHD does not necessarily equate to poor PD prevalence; in fact, several programs with HHD uptake >10% also have high PD uptake suggesting that both home dialysis paradigms can successfully co-exist in both academic and nonacademic, urban and suburban settings.

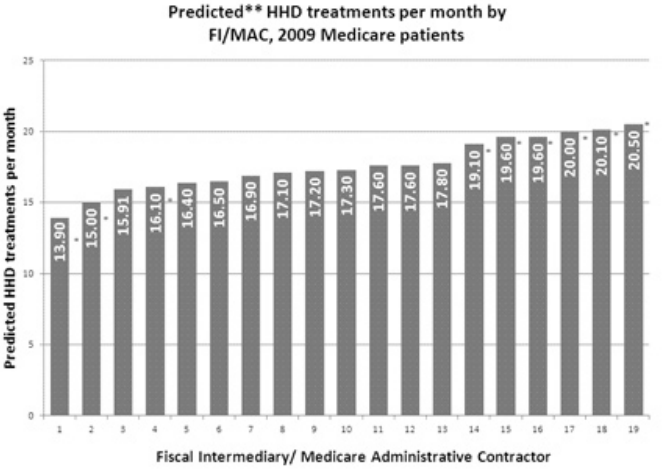
SA-PO2975

Frequency of Payments for More Than Thrice Weekly Dialysis for Home Hemodialysis (HHD) Richard Hirth,[1] Kathryn Sleeman,[1] John R.C. Wheeler,[1] Marc Turenne,[2] Wei Zhang,[1] Adam S. Wilk,[1] Joseph M. Messana.[1] *[1]Kidney Epidemiology & Cost Center, University of Michigan; [2]Arbor Research.*

Background: The limitation to three paid dialysis treatments weekly has been cited as a barrier to the diffusion of HHD, which is often performed on a daily basis. However, the regional fiscal intermediaries (FIs) and Medicare Administrative Contractors (MACs) that pay Medicare claims can authorize the payment of additional treatments based on medical necessity. It is unknown how often additional payments have been authorized and how this varies across FIs/MACs.

Methods: We identified all Medicare HHD patients in 2009 by FI/MAC. The average HHD patient had 17.3 paid treatments per month vs. 11.6 for In-center HD patients. Ordinary Least Square regression was used to define the association between HHD patient treatments per month and individual FIs/MACs active in 2009, adjusting for patient age, race, BMI and an extensive list of Medicare Claims derived comorbidities ,. FI/MACs with fewer than 100 HHD patient months were excluded.

Results: Most FIs/MACs already paid for more than the standard three weekly treatments for HHD in 2009. In addition, we observed substantial variation in average number of paid HHD treatments per month across the 19 FIs/MACs.



n=38,209 HHD patient months. * p<0.0001 vs FI/MAC#10. **OLS with patient HHD treatments/mo. as dependent variable and FI/MAC as independent variables, adjusted for patient demographics, and an extensive comorbidity list.. R. Sq. 0.246

Conclusions: Results of regression analysis suggest that only some of this variation in HHD treatment frequency is predicted by demographics and patient comorbidities. The FI or MAC responsible for administering dialysis facility claims is an independent predictor of number of paid HHD sessions, potentially contributing significantly to the variation in HHD practice.

Funding: Other U.S. Government Support

SA-PO2976

Survival of Patients Receiving Home Daily Hemodialysis: A Multinational Cohort Study Rita Suri,[1] Lihua Li,[1] Robert M. Lindsay,[1] Amit X. Garg,[1] Louise M. Moist,[1] Peter Austin,[2] Cécile Couchoud,[3] Ronald L. Pisoni,[4] Bruce M. Robinson,[4] Meaghan S. Cuerden,[1] Gihad E. Nesrallah.[1] *[1]U Western Ontario; [2]ICES; [3]Biomedicine Agency; [4]Arbor Research.*

Background: Increasing hemodialysis (HD) frequency from 3 to 6 times per week improves left ventricular mass and quality of life, but effects on survival are unknown.

Methods: We identified 177 patients from France, the US, and Canada in the International Quotidian Dialysis Registry, who received home daily HD ≥5 times/wk from 2001-2010. Using propensity-score based matching techniques, we matched 106 of these patients to 240 contemporaneous patients receiving in-center conventional, 3 times weekly HD in the Dialysis Outcomes and Practice Patterns Study. HD session times were <5 hrs. We compared mortality rates between groups using Cox proportional hazards regression. Because the proportional hazards assumption was not met, we divided the followup time into fixed periods and calculated hazard ratios (HR) for each.

Results: The daily group received 5.6±0.5 sessions/wk. Mean weekly treatment time was 16.1±4.8hrs (daily gp) and 11.5±1.3hrs (conv gp). After matching, there were no significant differences in baseline characteristics between groups, except more daily patients had fistulae (68%vs.55%), while more conventional patients had grafts (19%vs.5%). Mean age was 53±14, 71% were male, 32% had diabetes. During 668 patient-yrs, 59/346 patients died. There was a trend toward higher risk of death with home daily HD until 1 yr, after which time the risk was less than with in-center conventional HD.

HR of Death with Home Daily HD

Period	3ms	6ms	12ms	24ms	36ms
HR(95%CI)	2.0(1.0-4.2)	1.2(0.6-2.1)	0.67(0.3-1.3)	0.37(0.2-0.9)	0.27(0.1-0.8)

Conclusions: Compared to in-center conventional HD, the risk of death associated with home daily HD changed over time. Despite rigorous matching, it is possible that our results are affected by indication bias as we did not know reasons for daily HD initiation in this retrospective cohort. Previous studies have had similar limitations. As large RCTs of daily HD have not been feasible, well-conducted prospective studies are needed to better assess the effects of daily HD on survival.

SA-PO2977

Daily Hemodialysis (DHD) Improves Overall Quality of Life (QOL) and Physical Intimacy: Interim Results from the FREEDOM Study Michael A. Kraus,[1] Fredric O. Finkelstein,[2] Rachid Daoui,[3] Janice P. Lea,[4] Yoojin Lee,[5] Brigitte Schiller,[6] Isaac Teitelbaum,[7] Bertrand L. Jaber.[5] *[1]IU, IN; [2]Yale, CT; [3]Rubin, NY; [4]Emory, GA; [5]Tufts, MA; [6]Satellite, CA; [7]U Colorado, CO.*

Background: The FREEDOM study, an ongoing prospective cohort study investigating the clinical and economic benefits of DHD, has demonstrated improvements in various QOL measures following initiation of DHD, including SF-36, depressive symptoms, post-dialysis recovery time, sleep and restless leg symptoms.

Methods: In this *a priori* planned interim analysis, results of the following 3 special study questions (SSQ) from the QOL survey are reported on a 10-point Likert scale (0=worst, 10=best) for the Per Protocol (PP) and Intention-To-Treat (ITT) populations:

1) Considering all aspects of your life, physical, emotional, spiritual, and financial, how would you rate your overall QOL?

2) How satisfied are you with your degree of physical intimacy over the last 4 weeks?

3) If you were given a choice of changing back to the previous regimen you were receiving prior to DHD, how likely would you be to change? (0=extremely likely, 10=strongly opposed)

Results: Of the 299 enrolled pts, 165 completed 12-month f/u. Mean age was 53 yrs, 65% were male, 70% white, 58% used an AVF, 45% had diabetes and 26% CHF. Mean estimates (with 95% CI) of the PP analysis are shown below:

SSQ	Baseline score[1,2]	Month-4 score[1,3]	Month-12 score[2,3]	Global P value
Overall QOL	6.4 (6.0, 6.7)	7.3 (7.0, 7.6)	7.0 (6.6, 7.4)	<0.0001
Physical Intimacy	4.9 (4.3, 5.5)	6.1 (5.5, 6.7)	5.7 (5.1, 6.3)	0.0005
Likelihood of returning to prior dialysis regimen	8.1 (7.6, 8.7)	9.1 (8.7, 9.5)	9.1 (8.7, 9.5)	0.002

[1]All baseline vs. month-4 p≤0.003, [2]All baseline vs. month-12 p≤0.03, [3]All month-4 vs. 12 p>0.05 by ANOVA with Bonferroni correction.

Conclusions: In summary, pts initiating DHD reported sig. improvements in overall QOL and phys. intimacy at 4 & 12 mths. The ITT analyses demonstrated similar results. In the PP analysis, pts initiating DHD were clearly motivated and unlikely to consider returning to prior dialysis modality over 12 mths. These results support previous analyses demonstrating the QOL benefits of DHD.

Funding: Pharmaceutical Company Support

SA-PO2978

Continual Improvement in Anemia Control beyond 1 Year of Nocturnal Home Hemodialysis with an Alternate Night Schedule Hon-Lok Tang,[1] Samuel K.S. Fung,[1] Ho Sing Joseph Wong,[2] Clara Poon,[1] Kwok Hong Chu,[1] William Lee,[1] Au Cheuk,[1] Ka Fai Yim,[1] Ka-Foon Chau,[2] Matthew K.L. Tong.[1] *[1]Division of Nephrology, Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong, China; [2]Renal Unit, Department of Medicine, Queen Elizabeth Hospital, Hong Kong, China.*

Background: Nocturnal home hemodialysis (NHHD) offers superior uremia clearance in patients with ESRD. This increased clearance is associated with a rise in hemoglobin (Hb) and reduction of erythropoietin (EPO) requirement, possibly by removing toxins that inhibit erythropoiesis. We previously demonstrated improvement in anemia and reduction of EPO requirement after 1 year of NHHD. This study aims to investigate whether these benefits will extend beyond 1 year of NHHD treatment.

Methods: Forty-three consecutive patients were started on NHHD in 2 dialysis centers between 2006 and 2011 with an alternate night schedule for 6-9 hours (3.5x/week). Hb, EPO dose, weekly spKt/V, iron saturation and serum ferritin at baseline before NHHD, at 12 and 24 month were retrospectively reviewed.

Results: Sixteen patients had completed NHHD for 24 months. Results were:

	Hb,g/dL (n=16)	P value	EPO dose,U/kg/week (n=15)*	P value
Baseline vs 24month	9.4±1.5 vs 11.8±2.6	<0.01	116.9±45.1 vs 40.6±40.2	<0.01
Baseline vs 12month	9.4±1.5 vs 11.5±2.0	<0.01	116.9±45.1 vs 62.6±57.5	<0.01
12month vs 24month	11.5±2.0 vs 11.8±2.6	0.552	62.6±57.5 vs 40.6±40.2	<0.05

* 1 patient did not require EPO at baseline

Hb increased significantly at 12 month and maintained at 24 month. However, the EPO dose requirement continued to decrease beyond 12 month with dosage significantly lower at 24 month. Five patients (33%) were able to be taken off EPO. Two of them stopped EPO beyond 12 month: 1 at 20 month and 1 at 24 month. Weekly spKt/V increased from 3.60±0.91 at baseline to 9.86±3.98 at 24 month (p<0.001, n=16). Iron indexes remained unchanged. Iron saturation was 27±10% at baseline vs 28±8% at 24 month (p=0.798, n=15) and serum ferrritin 805±586 pmol/L vs 586±428 pmol/L at 24 month (p=0.140, n=15).

Conclusions: Enhanced uremia clearance in NHHD results in continual improvement in anemia control with reduction of EPO requirement or cessation of EPO therapy beyond 1 year of NHHD treatment.

SA-PO2979

Effect of Frequent or Extended Thrice-Weekly Hemodialysis on Cardiac and Blood Pressure (BP) Parameters: A Meta-Analysis Paweena Susantitaphong, Ioannis Koulouridis, Nicolaos E. Madias, Bertrand L. Jaber. *Medicine, St. Elizabeth's Medical Center, Boston, MA.*

Background: Left ventricular hypertrophy is a risk factor for cardiovascular mortality in patients with chronic kidney failure. We conducted a meta-analysis to examine the effects of frequent (>3 sessions) or extended (>4 hours) thrice-weekly HD on cardiac and BP parameters.

Methods: Sources used to identify eligible studies included MEDLINE, Clinicaltrials.gov, and the Cochrane Central Register of Trials. We selected randomized controlled trials and single-arm studies, which included within patient comparisons, and examined the efficacy of frequent or extended thrice-weekly HD on cardiac parameters measured by echocardiography or MRI, and BP parameters. Random-effects model meta-analyses were used to compute changes in the outcomes of interest.

Results: 46 single-arm studies (1,159 pts) and 2 randomized controlled trials (296 pts) were identified. In an analysis of 23 study arms that assessed LVMI, frequent or extended thrice-weekly HD resulted in a significant improvement in LVMI (-32.8 g/m^2, 95% CI -41.4,-24.2, P<0.001). However, the test for heterogeneity was significant (I^2=82%; P<0.001). A similar but less pronounced net improvement in LVMI was observed in the 2 randomized trials (-7.4 g/m^2, 95% CI -11.2,-3.6, P<0.001). Other cardiac parameters displayed similar improvements, including LVM, LVSD, LVEDD, LVPW, IVS, LAEDD, and LVEF (Table).

Summary effect on other cardiac and BP parameters

Outcomes	Change (95%CI)
LVM (g)	-64.0 (-95.3,-32.6)
LVSD (mm)	-3.9 (-6.2,-1.5)
LVEDD (mm)	-4.3 (-5.7,-3.0)
LVPW (mm)	-1.1 (-1.6,-0.7)
IVS (mm)	-1.7 (-2.1,-1.3)
LAEDD (mm)	-3.8 (-6.2,-1.3)
LVEF (%)	5.4 (1.3,9.5)
SBP (mmHg)	-14.0 (-17.1,-11.0)
DBP (mmHg)	-7.0 (-9.0,-5.0)
MAP (mmHg)	-11.6 (-13.5,-9.6)
Anti-hypertensive drugs (No.)	-0.8 (-1.2,-0.5)

Finally, there was a significant decrease in SBP, DBP, MAP, and mean number of anti-hypertensive drugs (Table), and 54% of pts discontinued anti-hypertensive drugs.

Conclusions: Conversion from conventional thrice-weekly to frequent or extended thrice-weekly HD is associated with an improvement in LVMI and other cardiac and BP parameters, which may have a potential longterm cardiovascular benefit.

SA-PO2980

Nephrocystin-4 Is a Negative Regulator of Hippo Signaling Sandra Habbig,[1,2] Malte P. Bartram,[1] Roman-Ulrich Mueller,[1] Ricarda Schwarz,[1] Max C. Liebau,[1,2] Thomas Benzing,[1] Bernhard Schermer.[1] *[1]Renal Division, Department of Medicine and Center for Molecular Medicine Cologne, University of Cologne, Germany; [2]Department of Pediatrics, University of Cologne.*

Background: Nephronophthisis (NPH) is the most common genetic cause for endstage renal disease in children and adolescents. NPH is characterized by renal fibrosis and cyst formation, however in contrast to ADPKD the size of the kidneys is normal or even reduced. Although 11 different disease causing genes have been identified recently, there is only little known about the function of the related NPH-proteins (NPHP1-11).

Here we report that NPHP4 negatively regulates Hippo signaling. The Hippo pathway has recently emerged as a potent regulator of cell proliferation and organ size. NPHP4 directly interacts with LATS1, the central kinase of the pathway, and prevents the inactivation of the downstream effectors YAP and TAZ. In the presence of NPHP4, TAZ is released from 14-3-3 binding and translocates to the nucleus thereby promoting TAZ/TEAD-dependent pro-proliferative transcriptional activity. Consistently, knockdown of NPHP4 results in reduced transcription of TAZ/TEAD target genes and diminishes cell proliferation in human kidney epithelial cells and several tumour cell lines. Currently, we are investigating the influence of other nephrocystins on Hippo signaling.

Our data suggest that NPHP4 promotes cell proliferation through the control of Hippo signaling. Loss of NPHP4 in NPH patients might result in hyperactive Hippo signaling and reduced pro-proliferative transcriptional activity. These changes might be critical in the pathogenesis of NPH characterized by small-sized kidneys and atrophic tubular epithelium. In contrast, loss of Hippo signaling activity might be associated with polycystic kidney diseases characterized by massive proliferation such as ADPKD.

Funding: Government Support - Non-U.S.

SA-PO2981

3-D-Spheroid Defects in NPHP Knockdown Cells Are Rescued by the Somatostatin Agonist Octreotide Amiya K. Ghosh,[1] Toby W. Hurd,[1] Friedhelm Hildebrandt.[1,2] *[1]Department of Pediatrics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.*

Background: Ciliopathies are a clinically and genetically heterogeneous group of diseases that exhibit shared clinical phenotypes, including renal cysts, retinal degeneration, mental retardation and obesity. Nephronophthisis (NPHP) is one such ciliopathy characterized by kidney cysts at the cortico-medullary border. Among the 10 different disease-causing genes (*NPHP1-NPHP10*), mutations in *NPHP3, NPHP6* or *NPHP8* cause the ciliopathy variants of NPHP, Joubert syndrome and Meckel Syndrome.

In this study we hypothesized that loss of function of *NPHP3, NPHP6* or *NPHP8* leads to morphological defects in a 3-dimentional (3-D) renal cell (IMCD3) culture model by either lack of cilia formation and/or cell polarity defects.

Methods: Stable shRNA knockdown cells of *NPHP3, NPHP6* and *NPHP8* were generated using a retroviral transduction method. Knockdown cell lines were examined in 3-D spheroid culture followed by rhodamine-phalloidin staining to assess spheroid architecture.

Results: We observed significantly higher percentages of abnormal spheroids for all three stable cell lines (NPHP3; 72%, NPHP6; 80% and NPHP8; 50%) compared to control shRNA cells (25%). Recent studies indicated that the somatostatin receptor agonist octreotide decreased cAMP level and inhibited cysts formation in PKD model systems. In our study, we observed that treatment of knockdown spheroids with octreotide (2mM) reduced the percentage of abnormal spheroids to 30% (*NPHP3*), 36% (*NPHP6*), and (*NPHP8*) 35% (*NPHP8*) respectively, whereas vehicle control abnormal spheroids were 66% (*NPHP3*), 91% (*NPHP6*), 60% (*NPHP8*).

Conclusions: This study reveals that the lack of NPHP3, NHPH6 or NPHP8 leads to cell polarity defects resulting in spheroid abnormalities which can be rescued by inhibiting cAMP levels with octreotide treatment. Our study confirms that manipulation of cAMP-pathway could be one of the therapeutic approaches in treating patients with kidney cysts.

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SA-PO2982

Ciliated Cell-Type Specific Functions of Cep290/NPHP6 Christina Austin,[1] Iain A. Drummond.[1,2] *[1]Department of Genetics, Harvard Medical School, Boston, MA; [2]Nephrology Division, Massachusetts General Hospital, Charlestown, MA.*

Background: Mutations in the centrosomal protein Nephrocystin 6 (NPHP6/Cep290) are implicated in a broad spectrum of genetic disorders featuring polycystic kidneys in addition to pleiotropic ciliopathy phenotypes. A recent study finds that Chlamydomonas reinhardtii Cep290 mutants have truncated cilia with abnormal protein content, which was attributed to defective protein gating at the basal body transition zone. Nevertheless, cilia structural defects have not been observed in vertebrate models of Cep290 loss of function.

Methods: We set out to determine whether the Cep290 protein is, in fact, required for vertebrate ciliogenesis using the zebrafish Danio rerio as a model system particularly amenable to the study of cilia in vivo. Cilia of the photoreceptors, pronephros, and Kupffer's vesicle (KV) were characterized after disruption of Cep290 expression by injection of antisense morpholino oligonucleotides.

Results: In contrast to IFT protein mutants, we find that a subset of single cilia, which includes sensory cilia, are truncated in Cep290 morphants, while the length of motile, multiciliated bundles is unchanged. Despite the apparent restriction of cilia structural changes to singly ciliated cells, immunostaining with anti-zebrafish Cep290 antibody revealed that the protein localizes to the basal bodies of all ciliated cell types in the zebrafish embryo. Furthermore, immunoelectron microscopy demonstrated that Cep290 localization is not restricted to the transition zone but also accumulates on the basal body and ciliary rootlet, implying that Cep290 may play roles independent of protein entry into the cilium.

Conclusions: In summary, we have characterized a vertebrate Cep290 loss-of-function model that implicates the basal body-localized protein, Cep290, in the structural maintenance of a select class of cilia.

Funding: NIDDK Support

SA-PO2983

Abelson Helper Integration Site-1 Knockdown in Zebrafish Models the Ciliopathy Joubert Syndrome and Is Associated with Altered Left-Right Axis Determination Roslyn Jane Simms, Lorraine Eley, Ann Marie Hynes, Colin Miles, Bill Chaudhry, John A. Sayer. *Nephrology, Institute of Genetic Medicine, International Centre for Life, Newcastle upon Tyne, United Kingdom.*

Background: Joubert syndrome and related disorders (JSRD) are autosomal recessive cerebello-oculo-renal syndromes, associated with cystic kidney disease such as nephronopthisis (NPHP) in over 20% of cases. Mutations in *Abelson helper integration site-1* (*AHI1*) are a leading cause of JSRD and similar to all other cystic kidney diseases, the protein product is expressed in the primary cilium/basal body. We were interested in evaluating the effect of *ahi1* knockdown on cilia in zebrafish (ZF) models of JSRD.

Methods: Antisense morpholino oligonucleotides (MO) designed to block ZF *ahi1* were injected into *cldnb*-lyn-GFP and *cmcl2*-GFP transgenic ZF embryos. *Cldnb-lyn*-GFP embryos were fixed at 8-10 somites for Kupffer's vesicle (KV) analysis and 72-96 hours post fertilisation (hpf) to evaluate for pronephric cysts and neuromasts of the posterior lateral line (PLL). Following immunofluorescence using anti-acetylated tubulin, cilia were studied. Left-right patterning (initiated and regulated by KV) was assessed by scoring cardiac looping in *cmcl2*-GFP embryos at 48-56 hpf.

Results: *ahi1* knockdown in ZF causes abnormal development of KV with an absence of cilia in 92% of embryos. Cardiac looping was reversed in 68% of a*hi1* injected embryos and there was a reduction in the number of neuromasts in the PLL, with loss of kinocilia. *ahi1* morphants display ciliopathy phenotypes including: curved body axis; hydrocephalus; developmental retinal and rudimentary ear anomalies. Cilia remain present in the distal pronephros, however there is dilatation of the cloaca in a*hi1* morphants.

Conclusions: In ZF models of human JSRD, a*hi1* knockdown is associated with several developmental cilial defects and subsequent aberrant organogenesis. The loss of cilia from KV and reduced formation of neuromasts and kinocilia in the PLL, suggests a role for *ahi1* in both ciliogenesis and planar cell polarity signalling.

SA-PO2984

The Deglutamylase ccp5 Is Essential for the Structural and Functional Integrity of Zebrafish Pronephric Cilia Narendra H. Pathak,[1] Christina Austin,[2] Iain A. Drummond.[1,2] *[1]Nephrology Division, Massachusetts General Hospital, Charlestown, MA; [2]Department of Genetics, Harvard Medical School, Boston, MA; [3]Department of Pathology, Massachusetts General Hospital, Charlestown, MA.*

Background: Cilia are microtubule-based organelles that mediate diverse functions in kidney epithelial cells and other organs. Ciliary axonemal microtubules are rich in posttranslational tubulin polymodifications, glutamylation and glycylation. Glutamylation is required for interactions of microtubules with their associated proteins. The equilibrium in microtubule glutamylation levels is dynamically maintained by opposing activities of Ttll (glutamylating) and recently discovered Ccp (deglutamylating) enzymes. Hypo as well as hyper glutamylation of axonemal microtubules disrupts structural integrity and motility of cilia. Our earlier work has highlighted an essential role for the glutamylating enzymes, Ttlls 1, 3 and 6 in cilia. However little is known about deglutamylases that function in cilia.

Methods: ccp5 expression was assessed by in situ hybridization. Morpholino knockdown of ccp5 was conducted in zebrafish embryos and larvae and effects on axoneme glutamylation and cystic phenotypes in ccp5 MO and fleer mutants were examined using confocal immunofluorescence and histology.

Results: We show here that the deglutamylase encoded by ccp5 is expressed in ciliated tissues and that its inactivation in wildtype zebrafish produces characteristic ciliopathic phenotypes and structurally defective B-tubules in microtubules of the ciliary axoneme. Our earlier work identified a role for the zebrafish TPR repeat protein Fleer in regulation of cilia tubulin glutamylation and glycylation. Interestingly, we show that knockdown of ccp5 in the fleer mutant partially restores glutamylation to otherwise hypoglutamylated axonemes and mitigates the severity of pronephric cysts.

Conclusions: Ccp5 is an essential regulator of axoneme tubulin glutamylation. Rescue of tubulin glutamylation and suppression of cyst formation by ccp5 knockdown in fleer mutants suggests that the Fleer protein may act as a negative regulator of Ccp5.

Funding: NIDDK Support

SA-PO2985

The Centriolar Satelite Protein Wtip Regulates Cilia Mediated Processes by Modulating Non-Canonical Wnt Signaling Tomoko Obara. *Cell Biology, University of Oklahoma Health Science Center, Oklahoma City, OK.*

Background: Defects in cilia and basal bodies/centrosomes are linked to human cystic kidney diseases (ciliopathies). However, pathological mechanisms leading to kidney cyst formation still remain unknown and no effective treatments are available.

Methods: We and others established zebrafish as a powerful model system to study the physiological roles of cilia, basal bodies/centrosomes related human ciliopathies genes in vivo.

Results: During our studies, we unexpectedly discovered that zebrafish Wtip protein localizes to the basal bodies/centrosomes. However, the precise in vivo function of the Wtip is still unknown. The wtip gene knock down in zebrafish resulted in pronephric cyst accompanied with cloaca occlusion, hydrocephalus, body axis curvature, and heart edema, a phenotype similar to pkd2 knockdown related to human ciliopathies. These phenotypes were specific since they could be completely rescued by co-injection of zebrafish wtip mRNA. We also showed that early defects in wtip knockdown could be explained by mitotic defects including centrosome, spindle formation and centrosome migration to the apical cell surface. Moreover, we discovered that phenotype caused by loss of Wtip implies a possible involvement of Wtip in planar cell polarity (PCP) modulating non-canonical Wnt signaling. Wtip is required to maintain PCP and cilia protein trafficking defects for both Polycystin-2 and Ift88 by interacting with core PCP Vangl2. To further understand the function of Wtip in zebrafish embryos, we performed a yeast two-hybrid screening with full-length zebrafish wtip as a bait and identified zebrafish lats2. Lats2 is a Serine/threonine-protein kinase, which is part of the Hippo signaling pathway. Intriguingly, our preliminary data showed that knockdown of lats2 in zebrafish resulted in same phenotypes as that seen in the wtip knockdown.

Conclusions: Based on our preliminary data, we hypothesized that Wtip is a new player in ciliopathies, which interacts with Lats2 and regulates PCP and cilia protein trafficking by interacting with core PCP Vangl2. Our studies in zebrafish demonstrated for the first time in a non-mammalian vertebrate the function of Wtip as a model of ciliopathies.

Funding: NIDDK Support

SA-PO2986

The von Hippel-Lindau Tumor Suppressor Is Required for Proximal Tubule/Glomerulus Integrity during Zebrafish Development and Regulates Renal Endosomal Trafficking Rachel H. Giles,[1,2] Ellen Van Rooijen,[2,3] Ive Logister,[2,3] Emile E. Voest,[2] Stefan Schulte-Merker.[3] *[1]Nephrology and Hypertension, University Medical Center Utrecht, Netherlands; [2]Medical Oncology, University Medical Center Utrecht, Netherlands; [3]Hubrecht Institute, Utrecht, Netherlands.*

Background: The von Hippel-Lindau (VHL) tumor suppressor gene is commonly mutated in hereditary and sporadic clear cell renal cell carcinoma (ccRCC).

Methods: We generated two stable zebrafish lines with null alleles of vhl and no detectable protein. Because the pronephros is externally visible in transparent zebrafish embryos, we could observe transformative events in situ over time. Analysis of the kidney phenotype was performed by immunohistochemistry, confocal microscopy in living fish and electronic microscopy. Human cell cultures from VHL-/- renal cell carcinoma and epithelial renal cells knocking down endogenous VHL by siRNA were recapitulated the in vivo phenotype.

Results: Here, we report that loss of vhl in zebrafish embryos results in severe pronephric abnormalities. In vhl mutants the glomerulus is enlarged, the Bowman space is widened and dilated cxcr4a-positive capillary loops are observed. While siblings exhibit a single layer of cuboidal cells comprising the proximal tubule, vhl-/- tubule cells are irregular with a alveolar appearance. Ultrastructural analysis revealed that mutant cells accumulate excessive amounts of vesicles that are variable in size and electron density. Since glomerular filtration and endocytosis in vhl-/- proximal tubule cells is not impaired, this might reflect a defect in exocytosis. VEGF receptor inhibition confirms that neovascularization of the vhl-/- proximal tubule does not obviously contribute to the aberrant cell morphology. Live cell imaging and vesicle tracking experiments suggest that human kidney epithelial cells lacking VHL, either derived from a renal cell carcinoma or upon acute siRNA or shRNA knock-down of VHL likewise accumulate Rab5, Rab7 and Rab11 endosomes and demonstrate defective transport.

Conclusions: Our data indicate that vhl is required to maintain pronephric tubule and glomerulus integrity during zebrafish development possibly through endosome homeostasis.

SA-PO2987

Functional Analysis of Renal Ciliopathy Disease Alleles in 3D Spheroid Culture Rachel H. Giles,[1] Liyun Sang,[2] Peter K. Jackson.[2] *[1]Nephrology and Hypertension, University Medical Center Utrecht, Netherlands; [2]Cell Regulation, Genentech, South San Francisco, CA.*

Background: Renal cystic diseases such as Bardet-Biedl, nephronophthisis (NPHP), Joubert, and Meckel-Gruber syndromes are phenotypically and genetically overlapping diseases displaying variable mental retardation, blindness, cerebellar ataxia, and renal anomalies. Genes known to be mutated in these disorders often code for proteins associated with cilia and/or basal bodies.

Methods: Standard 2D immunofluorescence often does not reveal clear effects on renal cilia when we reduced cellular levels of a panel of 21 ciliopathy-associated proteins by siRNA knockdown, which were validated by qPCR. To improve the sensitivity of our readout we generated a more physiological setting by growing siRNA-treated murine IMCD cells in a 3D matrix consisting of matrigel and collagen.

Results: We observe reduced apical-basal polarity, lumen irregularities, and small but significant changes in cilia numbers or length, depending on the target. Reconstitution experiments with a subset of the proteins using siRNA-insensitive alleles confirmed specificity of the effects in all cases tested. Live cell imaging of the vasopressin receptor in these 3D spheroids reveals functional transport of AQ2 to the apical membrane upon exposure to arginine vasopressin. Furthermore, IMCD3 stable lines expressing ciliary GFP- or RFP-labeled serotonin receptor 5-HT6 grown in 3D spheroids revealed ciliary dynamics including rubbing, membrane shedding, and extended contact with debris in the lumen. Chimeric GFP- and RFP-spheroids can be generated such that one cell type has been treated with siRNA, but is surrounded by wild-type sister cells. We demonstrate this principle by evaluating the cell-autonomous effects of siRNA of Nphp1, Nphp2, Bbs1, Bbs2, Kif3A, or Mks1 with real-time imaging of 3D spheroid cultures.

Conclusions: We conclude that this assay is a robust and sensitive read-out for loss of function or hypomorph mutations involving ciliopathy disease alleles.

Funding: Pharmaceutical Company Support

SA-PO2988

Kinesin Family Member 12 Localizes to Primary Apical Cilium and Correlates with Structural and Developmental Expression Patterns of Key Cystogenic Genes Michal Mrug,[1] Bruce Aronow,[2] Lisa M. Guay-Woodford.[1] *[1]University of Alabama at Birmingham, AL; [2]Cincinnati Children's Hospital, Cincinnati, OH.*

Background: We have mapped a major modifier of polycystic kidney disease (Mpkd) interval to mouse chromosome (Chr) 4 using a large [C57BL/6J (B6)-*Cys1*$^{cpk/+}$ x CAST/Ei] F1 intercross and determined that this interval contains at least three individual loci that modulate progression of cystic kidney disease (*Mpkd1-3*).

Methods: To validate cystogenesis-promoting effects of the *Mpkd1-3* loci, we crossed B6-*Cys1*$^{cpk/+}$ mice with congenic mouse lines carrying either the entire CAST/Ei-derived *Mpkd1-3* interval or its *Mpkd1-2* segment on a B6 background. Resulting positional candidate genes for these loci were further evaluated using analyses based on gene and protein molecular features, mouse strain SNP haplotypes, mutational analyses, NIDDK GUDMAP developing kidney gene expression atlas profiling, and candidate gene product immunolocalization.

Results: CAST/Ei-derived *Mpkd1-3* and a *Mpkd1-2* intervals strongly modified cystic disease progression. Relative strength of independent dominant cystogenic effects associated with CAST/Ei-derived *Mpkd1-2* interval allowed the use of congenic recombinants to fine map the *Mpkd2* locus to <14 Mbp interval with 69 RefSeq genes. Among them, 32 were expressed in kidney and liver. Of these, 16 contained non-synonymous single nucleotide polymorphisms. The latter subset included *Kif12*. Among the positional *Mpkd2* candidates only *Kif12* contained an apparently functional coding variation (a B6-associated deletion in catalytic domain of kinesin motor). In addition, only *Kif12*-encoded protein, kinesin family member 12, was present in polycystin-1 positive urinary exosome-like vesicles together with proteins encoded by *Cys1*, *Pkhd1* and *Pkd2*. Moreover, we have demonstrated that like most cystogenic proteins kinesin family member 12 localizes to primary apical cilium. Finally, we have shown that *Kif12* expression correlates with developmental and structural distribution profiles of *Cys1*, *Pkhd1* and *Hnf1b*.

Conclusions: Taken together, the above data point to *Kif12* (*Mpkd2*) as a novel component of a pathway capable of modifying the severity of recessive polycystic kidney disease.

Funding: Private Foundation Support

SA-PO2989

Renal Epithelial Cells Lacking Primary Cilia Have Reduced Sensitivity to a Hyperosmolal Microenvironment Bradley P. Dixon, Brian J. Siroky, Lu Lu, John J. Bissler. *Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Defects in primary cilia have been linked to cystic kidney diseases, and may play a role in certain cancers. Recent evidence suggests that cilia sense changes in osmolality through activation of transient receptor potential vanilloid (TRPV) channels. Activation of TRPV1, a sensor of hyperosmolality, also activates the ATM-chk2-p53 DNA damage response pathway, causing cell cycle checkpoint activation and arrest. Defective cilia may therefore lead to aberrant cellular responses to osmotic stress in the hyperosmolal renal microenvironment. We sought to characterize the effects of gradual adaptation to hyperosmolal microenvironments in renal epithelial cells that either have, or lack, functional cilia.

Methods: The 176-5 renal epithelial cell line contains a floxed Kif3a gene (a kinesin motor required for ciliogenesis) and a tamoxifen-inducible Cre recombinase, and was cultured both without tamoxifen, and with tamoxifen to conditionally delete Kif3a (176-5Δ cells). These cells were gradually adapted to 450mOsm/kg and 600mOsm/kg with NaCl or urea, or maintained under control conditions. Cell proliferation was measured by crystal violet DNA binding assay. Activation of signaling pathways was measured by western blot.

Results: Cilia-proficient 176-5 cells experienced arrest of cell proliferation upon adaptation to hyperosmolal NaCl or urea, whereas cilia-deficient 176-5Δ cells continued to proliferate in spite of hyperosmolal adaptation. Expression of PCNA, a marker of cell proliferation, was unchanged in osmotically adapted 176-5Δ cells, whereas 176-5 cells demonstrated a reduction in PCNA under hyperosmolal conditions. Interestingly, activation of p53 was not present in either cell following adaptation to hyperosmolal conditions.

Conclusions: These results indicate that cilia are required for reduction in cell proliferation following adaptation to hyperosmolal conditions, and this reduction appears to be ATM-independent. Further studies are needed to determine the role TRPV1 plays in the transduction of the hyperosmolal microenvironment to cell cycle arrest.

Funding: NIDDK Support

SA-PO2990

Loss of the Primary Cilia Leads to Elevated Activity of the Disintegrin Metalloenzyme ADAM17 Monika Gooz, May Y. Amria, Yujing Dang, Binlin Song, P. Darwin Bell. *Medicine, Medical University of South Carolina, Charleston, SC.*

Background: Epidermal growth factor receptor (EGFR)-dependent signaling pathways are among the most important regulatory circuits that induce and maintain cellular proliferation. In polycystic kidney disease (PKD) abnormal expression\function of EGFR and its substrate growth factors have been described. Since ADAM17 is the main sheddase enzyme responsible for activation of EGFR ligands, we investigated whether primary cilia dysfunction leads to increased ADAM17 activity, which in turn maintains an autocrine signaling loop resulting in the proliferative phenotype found in PKD.

Methods: We used a tamoxifen-inducible Cre recombinant adult mouse model of autosomal recessive PKD that had the conditional floxed allele for the *Ift88*/*Tg737* gene, a component of the primary cilia.

Results: We observed significant cyst development in cilia (-) animals compared to cilia (+) animals. There were no significant differences in overall ADAM17 expression using whole kidney lysates from cilia (+) compared to cilia (-) mice by Western blot. However, using immunohistology, there was intense ADAM17 staining in the epithelium that lined cystic structures in cilia (-) mice. This staining was localized mainly to the apical surface of these cells. In cilia (+) kidneys, ADAM17 staining was distributed more evenly across apical and basolateral membranes of epithelial cells. Interestingly, we observed strong ADAM17 staining in the primary cilia. Since cyst development is localized mainly to the collecting duct, we next used the cilia (-) and cilia (+) collecting duct cell lines PCDNA and BAP2 originating from the orpk mouse (*Itf88* hypomorph) to compare ADAM17 activity. Using a fluorogenic substrate we observed increased ADAM17 activity in cilia (-) cells. Phorbol ester (PMA) treatment elevated ADAM17 activity by 2-fold in cilia (+) cells but, interestingly, PMA-induced ADAM17 activity was attenuated in cilia (-) cells.

Conclusions: We conclude that in PKD, ciliary dysfunction induces localized ADAM17 activity in the kidney, resulting in continued up-regulation of EGFR activity and maintenance of the proliferative phenotype of cystic epithelium.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2991

Expression Patterns of Joubert Syndrome Genes during Development Ann Marie Hynes, Yuzhu Cheng, Jennifer Whitehead, Lorraine Eley, Roslyn Jane Simms, Colin Miles, Susan Lindsay, John A. Sayer. *Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*

Background: Joubert syndrome (JBTS) is an inherited ciliopathy leading to a cerebellar-retinal-renal syndrome. Recent genetic advances have allowed positional cloning and identification of several JBTS genes. AHI1 encodes Jouberin and CEP290 (alias NPHP6) encodes nephrocystin-6. Mutations in AHI1 account for ~12% of patients with JBTS and mutations in CEP290 account for ~7% of patients with JBTS.

Methods: Using human embryonic and fetal tissue from the MRC/Wellcome Trust-funded Human Developmental Biology Resource (www.hdbr.org), we reveal an early

onset neuronal / retinal / renal expression pattern for AHI1 and CEP290 which persists throughout development.

The expression profile of CEP290 during development was compared directly to that of Cep290 by making use of a β-geo reporter gene, being placed under transcriptional control in Cep290+/LacZ transgenic mice. X-gal staining of tissue in adult and embryos was performed.

Results: AHI1 and CEP290 expression was abundant in the developing forebrain, especially the ventricular zone and in the neural retina and throughout the developing hindbrain.

AHI1 and CEP290 expression during human nephrogenesis revealed that AHI1 and CEP290 transcripts are abundant in both the developing mesonephros and metanephros. Developing glomeruli, tubules and collecting ducts strongly expressed AHI1 and CEP290.

In Cep290+/- animals, specific X-gal staining representative of Cep290 expression was observed in the brain, retina, nasal epithelium and renal tissues.

Conclusions: These data support a role for AHI1 and CEP290 in multiple organs throughout development and explain the wide phenotypic spectrum of JBTS secondary to AHI1 and CEP290 mutations in man.

SA-PO2992

α-actinin4 Localizes to the Basal Body in Collecting Duct Cells Robert J. Kolb,[1] P. Darwin Bell.[2] *[1]Pediatrics, Medical University of South Carolina, Charleston, SC; [2]Medicine, Medical University of South Carolina, Charleston, SC.*

Background: Primary cilia are microtubule projections from basal body anchored below the surface of renal cells. Evidence suggests cilia along the nephron function as a flow sensor. Cilia signaling events are associated with the cell cycle and growth, events leading to the development of a polarized apical and basolateral membrane through the organization of the cytoskelton Numerous reports suggest a link between dysfunctional cilia of the collecting duct and Polycystic Kidney Disease.

Methods: Coimmunoprecipitation experiments with a specific antibody to γ-tubulin, a basal body component, helped identify potentially novel basal body components. To monitor changes in BB composition in a PKD cell model, Western Blot analysis was used to to determine cellular expression of proteins known to localize to the basal body in comparison to our candidate proteins in CD cell lines with normal length cilia and in a cell line with stunted cilia. We confirmed potential changes in localization and expression of these basal body proteins by immunofluorescence in both cell types.

Results: We provide novel evidence for α-actinin4 localization to the basal body in normal collecting duct cells. Coimmunopreciption of γ-tubulin revealed α-actinin4 and acetylated α-tubulin are in a complex. To determine if α-actinin4 was bound to a bona fide ciliary protein, a polaris antibody was used. This result shows acetylated α-tubulin but not α-actinin4 binds to polaris. Immunofluorescent labeling of the basal body revealed colocalization of α-actinin4 and acetylated α-tubulin, confirming our co-IP study.

Conclusions: Our data suggests α-actinin4 binds to the basal body but not to the protein polaris. To our knowledge, this is the first report of α-actinin4 binding to basal bodies belonging to non-motile cilia. Our current hypothesis is α-actinin4 links the sub-apical actin ring to the microtubule cytoskelton and the basal body. Considering the role for calcium in cilia signaling events, plus membrane vessicle trafficking and insertion, the calcium binding protein α-actinin4 is a potential candidate for future studies on cellular differentiation, cilia and PKD.

Funding: NIDDK Support

SA-PO2993

PAR6γ, a New Interaction Partner of PLCε1/NPHS3, Is Required for Renal Glomerulogenesis in Zebrafish Embryos Weibin Zhou,[1] Shazia Ashraf,[1] Friedhelm Hildebrandt.[1,2] *[1]Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute.*

Background: Steroid resistant nephrotic syndrome (NS) is a malfunction of the kidney glomerular filter that leads to proteinuria, hypoalbuminemia, edema, and renal failure. Mutations in the *phospholipase C epsilon 1* (*PLCE1*) have been associated with SRNS manifesting diffuse mesangial sclerosis (DMS) and focal segmental glomerulosclerosis (FSGS). To understand the pathogenic role of PLCE1 in NS, we have identified a number of new interaction partners of PLCE1, including PAR6 through proteomic analyses following GST pulldown.

Methods: To test whether PAR6γ plays a role in normal glomerular development, we characterized a zebrafish mutant of *pard6gb*, which contains a non-sense mutation at AA position 63 in the zebrafish ortholog of PAR6gb (2). The mutated protein partially lacks the PB1 domain (aPKC-interating) and completely deletes the C domain (cdc42-interating) and the PDZ domain (Par3-interacting).

Results: Using a transgenic zebrafish line expressing GFP under the control of zebrafish podocin promoter, we showed by confocal microscopy that the pronephric glomeruli in pard6gb$^{-/-}$ embryos are morphologically abnormal. Examination of the developing glomeruli using *podocin* and *wt1a* as markers revealed that the developing primordia in pard6gb$^{-/-}$ embryos failed to reach the midline of the embryo during the morphogenesis of the pronephric glomeruli.

Conclusions: Our study of the loss-of-function *pard6gb* mutant in zebrafish demonstrated that PAR6gb plays an essential role in normal glomerular development. Given its interaction with PLCE1, loss of cell polarity may be underlying the DMS phenotypes that result from loss of PLCE1 function.

Funding: NIDDK Support, Private Foundation Support

SA-PO2994

Generation and Analyses of AQP11 BAC Transgenic Mice Yuichi Inoue,[1] Eisei Sohara,[1] Katsuki Kobayashi,[2] Tatemitsu Rai,[1] Kenichi Ishibashi,[3] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Nephrology, Tokyo Medical and Dental University, Bunkyoku, Tokyo, Japan; [2]Division of Molecular Genetics, Clinical Research Center, Chiba-East National Hospital, Chiba, Japan; [3]Medical Physiology, Meiji Pharmaceutical University, Kiyose, Tokyo, Japan.*

Background: Aquaporin 11 (AQP11) belongs to superaquaporins since it has Asn-Pro-Cys (NPC) motif instead of the typical NPA motif. We previously reported that the disruption of AQP11 gene in mice resulted in polycystic kidneys following vaculolization of the proximal tubular cells. However, mechanism of the cyst formation and the vaculolization has not yet been clarified partly because of lack of an antibody good for detecting endogenous AQP11 in mouse tissues.

Methods: To enable the analyses of AQP11 in mice at the protein level, we decided to generate AQP11 BAC transgenic mice that expresses AQP11 tagged with 3xHA sequences at its N-terminus. By injecting the transgene containing whole exons of mouse AQP11 with its promoter region into fertilized eggs of C57BL/6J mice, we could obtain seven lines of transgenic mice with different copy numbers.

Results: In two lines carrying three and ten copies of the transgene respectively, we first performed immunofluorescence in the kidney, and found that 3xHA-AQP11 was mainly localized in the cytoplasm of proximal tubules. Double immunofluorescence with organelle markers revealed that 3xHA-AQP11 was partially co-localized with KDEL, an ER marker, suggesting that AQP11 is an aquaporin of intracellular organelles. In addition to kidney, we investigated the expression of AQP11 in other organs. Immunoblots of various mouse organs with anti-HA antibody revealed a single 27kDa band in brain, liver, and testis, which is consistent with the previous Northern blot of AQP11. Moreover, we detected abundant 3xHA-AQP11 in thymus, spleen, stomach, small intestine and colon, which has not been identified before.

Conclusions: Thus, we clarified in vivo intracellular localization and tissue distribution of AQP11. The AQP11 transgenic mice will be a useful tool to clarify the physiological role of AQP11 as well as the pathogenesis of polycystic kidney in the AQP11 knockout mice.

Funding: Government Support - Non-U.S.

SA-PO2995

CFTR Is Highly Expressed in the Cyst-Lininng Epithelial Cells of the AQP11 Knockout Mouse Kidney Katsuki Kobayashi,[1] Shinichi Uchida,[2] Sei Sasaki.[2] *[1]Division of Molecular Genetics, Clinical Research Center, Chiba-East National Hospital, Chiba City, Chiba, Japan; [2]Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

Background: AQP11 knockout mouse suffers from polycystic kidney disease and dies of end-stage renal failure at the weaning stage of the neonatal development. A large number of studies have established that the aberrant cell proliferation of the cyst epithelial cells and the abnormal secretion of fluid into the cyst cavity are the essential mechanisms of the cyst formation and growth in the polycystic kidney disease. We have previously reported that cell proliferation induced by ER stress is involved in the cystogenesis of the AQP11 knockout mouse. In this study we examined the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel, supposed to be the channel playing the central role in the fluid secretion, in the AQP11-null mouse kidney.

Methods: To elucidate the expression we stained the wild-type mouse and AQP11 knockout mouse kidneys with the anti-CFTR antibody, produced in rabbit, affinity-isolated (HPA021939, Sigma-Aldrich, St. Louis, MO). For the functional test we subcutaneously administered the CFTR inhibitor (CFTR(inh)-172, Sigma-Aldrich, St. Louis, MO) to the AQP11-deficient mouse for one week starting at the postnatal day 14 (5 mg/kg of body weight/day).

Results: Immunohistochemistry using the specific antibody against the CFTR protein displayed that CFTR was expressed in the proximal tubule cells deeply located in the cortex of the wild type mouse and in the mutant mouse CFTR was markedly expressed in the cyst-lining epithelial cells. This result prompted us to administer the CFTR inhibitor to AQP11 knockout mouse in order to investigate its effect on the cyst growth. Subcutaneous administration of the CFTR inhibitor, however, produced no significant effect on the cyst growth in the AQP11 knockout mouse.

Conclusions: These results collectively suggest that CFTR could function as the main channel via which chloride ion is secreted into the cyst cavity in the AQP11 knockout mouse, but further experiments are needed to determine its significance in the cystogenensis.

Funding: Government Support - Non-U.S.

SA-PO2996

P-Glycoprotein Expression and Function in Cystinotic Proximal Tubular Cells Elena N. Levtchenko,[1] Karen Peeters,[1] Martijn J. Wilmer,[2] Rosalinde Masereeuw.[2] *[1]Dept of Pediatric Nephrology/Laboratory for Pediatrics, KULeuven, Leuven, Belgium; [2]Dept. of Pharmacology and Toxicology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

Background: P-glycoprotein (P-gp) is an ATP-dependent organic cation transporter localized on the apical membrane of the proximal tubules, that plays a role in the efflux of endogenous waste products and xenobiotics into urine. Studies in mice deficient for P-gp showed proximal tubular dysfunction and ATP deficiency combined with swollen mitochondria, resembling the phenotype of patients with cystinosis. We now have investigated whether the proximal tubular efflux transporter P-gp is affected in cystinosis.

Methods: We used conditionally immortalized (ci) proximal tubular cells (ciPTEC) obtained from urine from cystinotic patients and controls. ciPTEC were cultured to determine P-gp expression by Western blot and P-gp gene expression was assessed by qPCR. P-gp-mediated transport was measured by using the fluorescent P-gp substrate calcein in the presence and absence of the P-gp-inhibitor PSC833. Additionally, the effect of the cystine depleting drug cysteamine on transport activity was determined by the calcein-assay.

Results: The activity of P-pg, in presence or absence of PSC833, in control cell lines is comparable to the activity in cell lines of cystinotic patients. Cysteamine depletes cystine acculumation in cystinotic cell lines and had a stimulatory effect on P-gp activity. PO_4 uptake was determined in cystinotic and control ciPTEC and was decreased in cystinotic cells. The uptake was sodium-dependent and in the majority of the cells and conditions tested, both PSC833 and cysteamine (pre)incubation resulted in significant decreased uptake of phosphate.

Conclusions: Inhibition of P-gp activity in ciPTEC resulted in decreased uptake of phosphate comparable to the phenotype of P-gp deficient mice and to that of patients with cystinosis. Although cysteamine depleted cystine accumulation in cystinotic cells and increased P-gp activity, it inhibited phosphate uptake in ciPTEC. This observation is compatible to the persistence of renal Fanconi syndrome in vivo under cysteamine therapy and requires further study.

Funding: Private Foundation Support

SA-PO2997

Pathology and Clinical Manifestations in Patients with Uremic Tumoral Calcinosis Undergoing Hemodialysis Rikako Hiramatsu, Keiichi Sumida, Masayuki Yamanouchi, Yoshifumi Ubara. *Nephrology Center, Toranomon Hospital, Tokyo, Minato-ku, Japan.*

Background: Uremic tumoral calcinosis (UTC) is a rare complication in patients undergoing hemodialysis (HD) and its clinical manifestations are poorly understood. This study aimed to elucidate clinical manifestations and pathological features in cases with UTC.

Methods: The subjects were 8 HD patients (1 male and 7 females; age, 41 - 75 years) with UTC, who visited our hospital between 1999 and 2011. HD duration was 6 years or shorter in 87.5%.

Results: Calcification was observed in about 75% of large joints, such as hips, wrist and shoulders. Mean serum adjusted Ca and P were 10.7±0.6 mg/dl and 7.0±1.1 mg/dl, and the mean Ca x P was 72± 11.7 mg^2/dl^2. Mean serum CRP was 1.46 ± 2.1 mg/dl. Intact-PTH (iPTH) was 247±325 pg/ml, and iPTH higher than 500 pg/ml (high iPTH) was observed in 37.5% suggesting secondary hyperparathyroidism, while low iPTH (iPTH < 100 pg/ml) was seen in 37.5%. All cases with high iPTH showed positive CRP. The aortic calcification index (ACI) was 16.7% on average and 0% in three cases with high iPTH. In cases with high iPTH, parathyroidectomy with Ca x P control eliminated UTC and CRP became negative. Biopsy was performed in all cases of UTC and only calcium depositions were observed in 75%, while the remaining two cases showed ectopic bone tissue with positive CRP and fibrous bone. Of the 5 cases undergoing right iliac bone biopsy, there were 2 high iPTH cases with osteitis and 3 low iPTH cases with adynamic bone.

Conclusions: UTC was observed around large joints in cases with short HD duration and high Ca x P with hyperphosphatemia. Parathyroid hyperfunction may contribute to large UTC, while hypoparathyroidism may lead to small UTC.Serum CRP was positive in cases with secondary hyperparathyroidism, which suggested that tumoral calcinosis induced an inflammatory reaction that in turn led to ectopic bone formation. Vascular calcification was mild and not always present in UTC. It is conceivable that different onset mechanisms and factors other than Ca x P and iPTH are involved in UTC.

Funding: Private Foundation Support

SA-PO2998

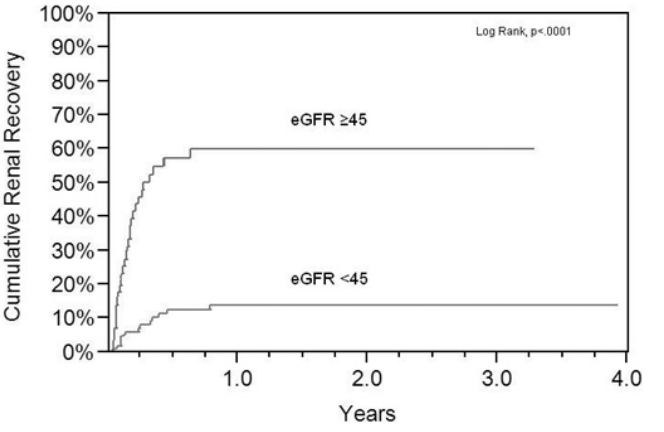
Recovery of Renal Function in Incident Hemodialysis Patients Initiating Dialysis Therapy in the Hospital Sanjay Chaudhary, LaTonya J. Hickson, Andrew D. Rule, John J. Dillon, Robert C. Albright, Amy W. Williams. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Patients initiating renal replacement therapy (RRT) may have acute or chronic renal failure. The rate at which renal function recovers in patients dismissed to a chronic hemodialysis unit following RRT initiation in the hospital is not well known.

Objective: To determine rate and clinical predictors of renal recovery in incident hemodialysis patients beginning RRT in the hospital and continuing chronic outpatient therapy.

Methods: Incident hemodialysis patients initiating RRT in a tertiary care setting from 2007-2009 were included in the study and followed for recovery of renal function with complete discontinuation of dialysis.

Results: Of 173 incident patients: mean age 64 years, 64% males, 87% Caucasian, 51% diabetic, 47% coronary artery disease (CAD), 47% congestive heart failure (CHF). Baseline estimated GFR (eGFR) was 34±33 ml/min with eGFR≥45 in 36%. Over a mean period of 1.1±1.1 years, 46 recovered renal function. Kaplan-Meier estimate of cumulative renal recovery rate at 6 months was 27%. Baseline eGFR (HR=1.01 per ml/min, CI 1.01-1.02, p<.0001) was a strong positive predictor of renal recovery. eGFR≥45 was also associated with renal recovery, HR=6.3, CI 3.4-12.6, p<0.0001 (see figure). Also in univariate analysis, diabetes (HR=0.56, CI 0.30-0.998, p=0.049), CAD (HR=0.54, CI 0.29-0.98, p=0.044) and CHF (HR=0.40, CI 0.21-0.75, p=0.003) were negative predictors of renal recovery. In multivariate analysis, only eGFR was associated with renal recovery (HR=1.01, CI 1.00-1.02, p=0.001), while diabetes, CAD, and CHF were not.



Conclusions: Recovery of renal function in the chronic hemodialysis unit setting following dialysis initiation in the hospital is not infrequent. Patients with normal to even moderately impaired function at baseline are more likely to recover.

SA-PO2999

Long Term Use of Trisodium Citrate for Regional Anticoagulation in Chronic Intermittent Hemodialysis in Patients with Risk of Bleeding Birgit Doris Bader, Anna Weber, Christiane M. Erley. *Department of Medicine II, St. Joseph Hospital Berlin-Tempelhof, Berlin, Germany.*

Background: Hemodialysis patients suffer often from bleeding complications. Their management is difficult. Frequently the bleeding is associated with heparin anticoagulation during dialysis treatment. An alternative to heparin is the regional citrate anticoagulation (RCA), described first in 1961 and in wider clinical use since 1983. Normally RCA is used in short term and in continuous renal replacement therapy. This retrospective study documents the chronic use of RCA (trisodium citrate) in intermittent hemodialysis.

Methods: 6 patients (5 women, 1 man; aged 46-80 years) with bleeding complications were followed up from 12/2008 over 10-28 months. They received >1000 intermittent hemodialysis treatments with RCA. Initially the patients got a trisodium citrate 4% solution by an infusion rate of 250-280 ml/min with a blood flow of 200-350 ml/min, since 09/2010 a trisodium citrate 30% solution was used (infusion rates of 50-70 ml/min with a blood flow of 200-250 ml/min). Citrate flow was adapted to achieve post-filter ionized calcium of 0.5-0.7 mmol/L. Calcium substitution was adapted to maintain the patients' serum calcium levels within the physiological range. Low calcium dialysis fluid (1.00 – 1.25 mmol/L) was used.

Results: Within the extracorporal blood circuit a successful regional anticoagulation was achieved while the coagulation of all 6 patients remained within physiological ranges. Only one patient showed clotting problems with RCA, solved by increasing the citrate dose and changing the filter from high to low flux. Adverse events didn't occur, safety laboratory measurements stayed within normal ranges. In all 6 patients the bleeding complications decreased or stopped during dialysis with RCA. The rate of transfusion was reduced markedly.

Conclusions: The long term use of trisodium citrate (4% or 30%) for regional anticoagulation in chronic intermittent hemodialysis in patients with risk of bleeding is feasible, safe and effective.

SA-PO3000

A Quality Audit Analysis of Hemodialysis Circuit Clotting in an Inpatient Dialysis Unit Merfake Semret,[1] Mary Ann Ryan,[2] Robert C. Albright,[1] John J. Dillon,[1] Sandra J. Taler,[1] Marie C. Hogan.[1] *[1]Nephrology Division, Dept of Internal Medicine; [2]Dept of Nursing, Mayo Clinic, Rochester, MN.*

Background: Quality of delivery of inpatient hemodialysis (HD) in the acutely ill patient has received little attention & no clinical performance measure guidelines exist. HD circuit clotting has adverse effects on the process and quality of dialysis. Past studies reveal clotting can occur in up to a quarter of heparin-free dialysis treatments. The purpose of this audit was to evaluate HD circuit clotting rates in acutely hospitalized patients, & to determine if heparin use rates could be safely increased.

Methods: We audited heparin use & dialyzer clotting rate during HD before & following a practice intervention in an inpatient dialysis unit. The intervention was to notify nephrologist to prescribe heparin on all dialysis patients, if not contraindicated. The dialysis unit was located in a 1500 bed acute surgical & medical hospital which runs >6000 HD treatments yearly. All non-ICU inpatient HD runs were included. Clotting and heparin use rates were recorded for 2 weeks pre-intervention & 4 weeks post intervention & medical records reviewed.

Results: Of 178 pre-intervention HD treatments, 111 (62%) were in a non ICU setting. Heparin was used in 17 (15%) these HD treatments. Clotting of the HD circuit occurred in 12 (10.8%) treatments. Of those whose HD circuit clotted, 58% were on chronic HD, 83% had a tunneled dialysis catheter & 42% had a documented reason for not using heparin. Of the 549 post intervention HD treatments, 317 (58%) were in a non ICU setting. Heparin use increased to 23.2% (p=0.08) & HD circuit clotting decreased to 2.2% (p=0.0005) of treatments.

Conclusions: At our center, heparin use in acutely ill surgical & medical inpatients in the sampled cohort was low & dialyzer clotting rates were higher than anticipated. We demonstrated that heparin prescribing can be modified when the prescribing nephrologist is made aware of the extent of the problem of HD clotting rates in their patients. Following this quality intervention, we increased heparin prescribing rates without negative effects and further audits are ongoing.

SA-PO3001

Heparin-Free Hemodialysis: A Multi-Center, Prospective, Randomized, Crossover Study between Two Hemodialyzers VIE Versus EVODIAL Mohamed Shariful Islam,[1] Zarih A. Hassan,[2] Olivier Moranne,[1] Sandor Vido,[1] Mohamed Berrada,[1] David Verhelst,[2] Patrick Donnadieu,[2] Vincent L.M. Esnault.[1] *[1]Nephrology, CHU, Nice; [2]CH, Avignon, France.*

Background: Several strategies of Heparin-free hemodialysis (HFH) were performed in patients with high risk bleeding problems. In this study, we tested the non-inferiority of the hemodialyzer VIE (vitamin E coated) having anti-oxidative and anti-thrombotic activities compared with EVODIAL (heparin coated) having anti clotting properties in a HFH strategy.

Methods: (NCT01221337): 32 patients aged 68±18, with well functioning fistulas or long-term catheters and no major hemodynamic or inflammatory disorders, were assigned to be dialyzed in random order with VIE or EVODIAL. The two study periods consisted of 4 hemodialysis (HD) sessions of 4-hours, separated by a washout period of two HD sessions with the patient's usual hemodialyzer. During each study period, the usual heparin dose was reduced to 50% for the 1st HD session, to 25% for the 2nd, followed by 2 successive HFH sessions. The primary endpoint was the success rate with no clotting event leading to a premature end of the HD session. Secondary endpoints included total HD duration without clotting, the number of saline flashes, the bubble trap and the hemodialyzer restitution qualities (1-4 grades), eKt/V (Daugirdas) and hemoglobin levels.

Results: The success rate with VIE (81 %) was not inferior to EVODIAL (78 %, p = NS). Restitution quality was better with VIE compared to EVODIAL (p = 0,002).

Secondary endpoints		VIE	EVODIAL	P
Total HD duration without clotting (min/patient)		900	907	NS
Saline flash (number)		0.31±0.68	0.26±0.67	NS
Blood flow rate (ml/min)		340±26	344±23	NS
End Transmembrane pressure (mmHg)		23.5±20	24.2±21	NS
Kt/V		1.48±0.3	1.43±0.3	0.07
Hemoglobin level (g/dL)		11.2±1.4	11.2±1.5	NS
Bubble trap quality	1	34(28)	32(27)	
n (%)	2	19(16)	26(22)	
	3	63(52)	57(47)	
	4	5(4)	5(4)	NS
Hemodialyzer restitution quality	1	25(21)	2(2)	
n (%)	2	72(59)	30(25)	
	3	22(18)	83(67)	
	4	2(2)	5(4)	0.002

Conclusions: Both VIE and EVODIAL permitted to perform 4-hour HD sessions with reduced dose or no heparin. As the mechanisms of clot prevention are different with these two hemodialyzers, further large-scale studies are required.

Funding: Pharmaceutical Company Support

SA-PO3002

Effects of Hydration Status and Relative Blood Volume Changes on Peripheral Skin Blood Flow during Hemodialysis Sylvie Sulkova, Roman Safranek, Michaela Kubisova, Lydia Habanova, Petr Moucka, Erik Mistrik, Katerina Petranova, Lubos Sobotka. *Department of Nephrology, Gerontology and Metabolic Care, Medical Faculty and Teaching Hospital, Hradec Kralove, Czech Republic.*

Background: In chronic hemodialysis patients, disorders of perfusion of peripheral tissues are very common, clinically important and may for example aggravate healing of frequent skin defects. The aim of our study was to investigate effect of hemodialysis procedure (HD) on peripheral skin blood flow.

Methods: Forty-one clinically and hemodynamically stable hemodialysis patients (22 males, 19 females, 64 (29-84) years) underwent routine HD (4h isotermic HD, low-flux dialyzer FX10 1.8m^2, QB 300ml/min, ultrafiltration to "dry weight" set by clinician). Skin blood flow was measured noninvasively at 1-1.5mm depth on the principle of backscattered light from moving red blood cells using Laser Doppler Line Scanner. Skin blood flow was estimated on hands and feet in every patient before HD, at 30, 90, 240min of HD, and 30 min postHD. Using Crit-line we assessed relative blood volume changes during HD. Bioimpedance spectroscopy (Body Composition Monitor) was used to assess hydration status.

Results: Skin perfusion progressively declined during HD ($p<0.001$), being decreased by 39% in toes, and 35% in fingers at the end of HD. 30 minutes after the end of HD, skin perfusion was still decreased; by 35 and 30% in toes and fingers, respectively. Skin perfusion decreased in patients with observed either decrease or increase in blood pressure during HD. Lower hydration at the end of HD was associated with more pronounced decrease in peripheral perfusion (correlation coefficient 0.51-0.65 for different areas of perfusion measurement).

Conclusions: HD significantly reduces peripheral skin blood flow. Drop in peripheral perfusion is dependent on relative blood volume decrease during HD and hydration status. Change in blood pressure during HD cannot be used as a simple tool to assess peripheral perfusion.

Funding: Government Support - Non-U.S.

SA-PO3003

Serum Sodium Concentration and Interdialytic Weight Gain in a Cohort of Hemodialysis Patients Prince Mohan, Lingpin Hung, Sayed Husain, Manaf Alroumoh, Gabriel EL-Kass, Marilyn Galler, Chaim Charytan, Bruce S. Spinowitz. *Nephrology, New York Hospital Queens, Flushing, NY.*

Background: Patients on hemodialysis (HD) have high rates of cardiovascular mortality and morbidity. Important risk factors include hypertension and fluid retention, measured as interdialytic weight gain (IDWG). This study aimed to describe the distribution of pre-dialysis serum sodium(SS) concentrations and to investigate the relationships between SS and IDWG.

Methods: This retrospective analysis determined the relationship with monthly SS levels and 48 hour subsequent IDWG. We reviewed the records of 260 patients on HD over a period of 12 months. IDWG, SS concentration, age and gender were tabulated. The mixed procedure method in SAS was utilized with a mixed effect model to capture the individual differences, and the fixed effect model to see the relationship between SS and IDWG.

Results: 260 patients, 106 male and 154 female, with total of 5054 dialysis sessions in 12 months period were analyzed. Average SS for the group was 137.2 + 2.1 and average IDWG was 2.42 + 1.42 KG. Data showed that IDWG and SS level are inversely related to each other in the entire male cohort. Subgroup analysis shows a significant relationship in all patients >80 years. SS expressed as mean mEq/L + SD and IDWG as mean Kg+ SD.

table1

	<60 Years	60-70 Years	71-80 Years	>80 Years
Female	Na 137.5±2.7 IDWG 2.42±1.08 P= 0.293	Na 137.3±2.8 IDWG 2.25±0.92 P = 0.904	Na 136.2±3.6 IDWG 1.80±0.95 P = 0.759	Na 137.5±3.5 IDWG 1.63±0.80 P = 0.011
Male	Na 137.0±2.5 IDWG 3.23±1.21 P = 0.800	Na 137.4±3.1IDWG 2.87±1.18 P = 0.318	Na 136.7±3.3 IDWG 2.53± 0.99 P = 0.130	Na 137.8± 2.7 IDWG 2.09±0.84 P = 0.003

Conclusions: For a given patient, a decreasing sodium level is predictive of a greater subsequent IDWG. This is most prominent in male patients and in all patients above the age of 80. This finding, if confirmed, will have implications for guiding patient treatment and dietary counseling. Further evaluation as to mechanism is needed.

SA-PO3004

Dialysate Sodium and Mortality in Hemodialysis Finnian R. McCausland, Steven M. Brunelli, Sushrut S. Waikar. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Dialysate sodium concentration has increased over many years in response to controlled ultrafiltration and shorter treatment times. However, higher dialysate sodium has been associated with increased thirst, weight gain and higher blood pressure. The relationship between dialysate sodium and outcome, and influence on serum sodium, has not yet been thoroughly investigated.

Methods: We studied a cohort of 2272 subjects from a medium-sized dialysis provider. Available data included demographic, laboratory and clinical parameters, detailed information of the dialysis prescription and 2.5 year follow-up for all subjects. Patterns of dialysate sodium prescription were examined within and between centers. Cox regression models, stratified on clinical center, were used to compare all-cause mortality according to dialysate sodium concentrations. Interaction was examined for in relation to serum sodium, inter-dialytic weight gain and pre-dialysis systolic blood pressure.

Results: The mean pre-dialysis serum sodium was 136.1mmol/L, without significant difference across dialysate sodium concentrations. There was evidence for interaction between serum and dialysate sodium and their relationship with all-cause mortality (p=0.04). The hazard ratio for death for higher dialysate sodium (>140mmol/L or sodium modeling) versus lower dialysate sodium (≤140mmol/L) was 1.05 (0.85, 1.30) at serum sodium of 134mmol/L; 1.15 (0.94, 1.40) at serum sodium of 136mmol/L; and 1.26 (1.01, 1.58) at serum sodium of 138mmol/L.

Conclusions: The dialysate sodium varies within and between centers; does not appear to effect the pre-dialysis serum sodium concentration; but has a variable relationship with mortality, according to the pre-dialysis serum sodium. Future research should focus on changes in total body sodium during hemodialysis and potential biological mechanisms for it's association with mortality.

Funding: Private Foundation Support

SA-PO3005

The Effect of Isonatremic Sodium Dialysate Prescription in Conventional In-Center Hemodialysis Patients: A Case Series Rohini Arramreddy,[1,2] Sumi J. Sun,[2] Jair Munoz Mendoza,[1,2] Glenn M. Chertow,[1,2] Brigitte Schiller.[1,2] *[1]Medicine, Division of Nephrology, Stanford University, School of Medicine, Stanford, CA; [2]Medical Affairs, Satellite Healthcare, Inc., San Jose, CA.*

Background: Recent studies have focused on the association between sodium (Na^+) dialysate prescriptions and interdialytic weight gain (IDWG). We report on a case series of 13 patients undergoing thrice weekly, conventional, in-center hemodialysis with an individualized, isonatremic dialysate prescription.

Methods: Isonatremia was achieved in all patients through a stepwise weekly reduction of the standard Na^+ dialysate prescription (140 mEq/L) by 2mEq/L until reaching a Na^+ gradient of -2 mEq/L (Na^+ dialysate minus average plasma Na^+ over the preceding 3 months). Dialysis logs from six consecutive treatments with the two different Na^+ prescriptions (standard and isonatremic) were reviewed. Changes were assessed for the following measures: interdialytic weight gain corrected for dry weight (IDWG%), blood pressure, and proportion of treatments with cramps, hypotension (drop in systolic blood pressure > 30 mmHg) and hypotension requiring intervention.

Results: The average age of the patients was 62 years; 10 of 13 were male. The pre-dialysis plasma Na^+ concentration ranged from 132 to 141 mEq/L. The mean pre-dialysis plasma Na^+ was unchanged (135.5 ± 3.7 mEq/L vs. 134.9 ± 3.9 mEq/L; p=0.3) but the mean post-HD plasma Na^+ concentration was significantly reduced (137 ± 3.1 mEq/L vs. 134.3 ± 3.4 mEq/L; p=0.03). IDWG% was decreased with isonatremic dialysate (3.4 ± 1.6% vs. 2.5 ± 1.0%; p=0.003) without affecting pre- or post-HD blood pressure (all p>0.05). No significant changes in the proportion of treatments with cramps (6% vs. 13%), hypotension (62% vs. 65%) or hypotension requiring an intervention (29% vs. 33%) were noted.

Conclusions: Individualized isonatremic dialysate prescriptions reduced IDWG without increasing the incidence of cramps or hypotension.

SA-PO3006

Interaction of Potassium, Sodium with Higher Magnesium Dialysate on Muscle Cramps in Chronic Hemodialysis Patients Sudhir Movva, Patrick Gerard Lynch, Nand K. Wadhwa. *Nephrology, Stony Brook University, Stony Brook, NY.*

Background: We reported an inverse relationship between predialysis serum magnesium (Mg) and muscle cramps in chronic hemodialysis (HD) patients (JASN 21:436A, 2010). The interaction of potassium and sodium with different dialysate Mg concentrations and muscle cramps in ESRD patients is scarce.

Methods: 62 ESRD patients (Mean age 60, range 25-87 years; 36 males, 26 females) on HD were studied. The patients were hemodialyzed initially with a dialysate Mg of 0.75 mEq/L and then with a dialysate Mg of 1.00 mEq/L. The patients received HD with each dialysate for at least 3 months. Monthly pre-HD laboratory data, before and after the change in dialysate Mg, were used for analysis. A single nephrology fellow conducted an in-person questionnaire on 62 patients twice. The severity of cramps was evaluated on a 0-10 scale, with 10 rated as maximal severity. We analyzed the frequency of muscle cramps in patients with serum potassium of <4 mEq/L or ≥4 mEq/L and serum sodium of <135 mEq/L or ≥135 mEq/L before and after change in dialysate Mg.

Results: 48 out of the total 62 ESRD patients had muscle cramps with dialysate Mg of 0.75 mEq/L, vs only 35 had muscle cramps with dialysate Mg of 1.00 mEq/L (χ^2=6.16, p =0.01). The frequency of muscle cramps significantly decreased with higher dialysate Mg (1.00 mEq/L) in patients with serum potassium of ≥4 mEq/L (χ^2= 4.74, p=0.02) or serum sodium of ≥135 mEq/L (χ^2= 4.59, p=0.03). The frequency of muscle cramps were not significantly decreased in patients with serum potassium of <4 mEq/L or serum sodium of <135 mEq/L with higher dialysate Mg.

Data are summarized below (mean ± SD)

Variable	Dialysate Mg 0.75 mEq/L	Dialysate Mg 1.00 mEq/L	p value
Cramp severity	5.36±3.63	3.95±3.93	0.004
Serum Magnesium mg/dL	1.89±0.26	2.15±0.31	0.002
Serum Potassium mEq/L	4.97±0.81	4.99±0.75	NS
Serum Sodium mEq/L	138.32±3.47	137.24±3.34	0.02

Conclusions: These results suggest that frequency of muscle cramps, when hemodialyzed with dialysate Mg concentration of 1.00 mEq/L, decreased significanly in ESRD patients with relatively higher serum potassium or sodium level. Further studies are needed to understand this interaction.

SA-PO3007

Fluid Distribution in the Chest and Calf in Hemodialysis Patients Samer Rateb Abbas,[1,2] Fansan Zhu,[1] Stephan Thijssen,[1] Rakesh Malhotra,[1] Peter Kotanko,[1] Nathan W. Levin.[1] *[1]Nephrology, RRI, NY; [2]Nephrology, BIMC, NY.*

Background: Knowledge of body fluid status is essential in the management of hemodialysis (HD), but excess fluid volume may not be uniformly distributed in the body. This study compares fluid removal from the chest and calf during HD.

Methods: Chronic HD patients were studied pre- and post-HD after the long (LINT) and short (SINT) interdialytic interval using 2 bioimpedance techniques, the ZOE fluid status monitor (NMT) for chest impedance (Zo), and Hydra 4200 for calf bioimpedance spectroscopy (cBIS) (Xitron Technologies). Calf and chest resistivities were determined and normalized to body mass index (ρ_{Ncalf}, ρ_{Nchest}).

Results: We studied 15 patients. During HD weight decreased in both LINT and SINT groups, the decrease was greater in LINT than SINT (LINT: -2.9±1.3; SINT -2.1±1.3: P<0.01). In LINT, ρ_{Ncalf} , chest Zo, and ρ_{Nchest} increased during HD.

		LINT			SINT	
	Pre HD	Post HD	P	Pre HD	Post HD	P
Weight, kg	94.2±23.6	91.3±22.9	<0.001	93.0±23.5	90.9±22.6	<0.001
cBIS_R, Ω	30.7±6.0	38.3±8.3	<0.001	32.1±8.3	39.6±9.1	<0.001
ρN_cBIS, [Ωm³/kg]/100	10.9±2.5	13.1±3.2	<0.001	11.5±3.5	13.7±3.6	<0.001
ZOE_Zo, Ω	21.9±2.8	24.2±3.3	<0.01	23.6±3.8	24.7±3.6	NS
ρN_ZOE, [Ωm³/kg]/100	28.0±8.8	31.9±10.8	<0.01	31.7±11.3	33.5±12.2	NS

In SINT, ρ_{Ncalf} increased but Zo and ρ_{Nchest} did not. Although pre-HD weight was lower after SINT (LINT: 94.2±23.6 kg; SINT 93.0±23.5 kg), no differences in pre-HD calf or chest measurements were observed between LINT and SINT.

Conclusions: Intradialytic fluid removal can be detected by both calf and chest bioimpedance measurements after the long interdialytic period, but after the short dialysis interval, calf BIS is more sensitive than chest impedance measurements in detecting fluid volume changes. Pre-HD ρ_N did not differ significantly between LINT and SINT, suggesting that measurement variability is too high to detect a significant effect of a 1.2 kg average weight reduction in a sample of 15 subjects. These findings indicate that the calf is the preferable location for measuring intradialytic body fluid volume changes.

SA-PO3008

Effect of Lowering Dialysate Temperature in Chronic Hemodialysis: A Systematic Review and a Meta-Analysis Reem Mustafa,[1] Fadi Bdair,[2] Elie Akl,[1,2] Gihad E. Nesrallah,[1,4,5] Amit X. Garg,[1,4] Holger Schunemann.[1,3] *[1]Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, ON, Canada; [2]Department of Medicine, State University of New York at Buffalo, Buffalo, NY; [3]Department of Medicine, McMaster University, Hamilton, ON, Canada; [4]Department of Medicine, University of Western Ontario, London, ON, Canada; [5]Department of Nephrology, Humber River Regional Hospital, Toronto, ON, Canada.*

Background: Lowering dialysate temperature may improve outcomes for patients undergoing maintenance chronic hemodialysis.

Objectives: To evaluate the effect of cooling dialysate compared to normal temperature dialysate on important outcomes in chronic hemodialysis patients

Methods: We searched the Cochrane Central Register of Controlled Trials, OVID MEDLINE, EMBASE and Pubmed (from inception to January 2011). We included all prospective randomized controlled trials that evaluated the effect of any method of cooling dialysate in adult patients receiving chronic hemodialysis. Two authors independently assessed trial quality and extracted data in duplicate. The GRADE methodology was followed to assess bias in individual studies and across studies for each outcome. We pooled data using a random effects model. We measured heterogeneity using the $\chi 2$ and I2 statistics. We registered this systematic review at PROSPERO: International prospective register of systematic reviews. 2011 (CRD420111104)

Results: We included twenty-six cross-over studies. Cool dialysis significantly reduced the rate of intradialytic hypotension (IDH) by 70% (95% CI 49%-89%; I2 =0%). Also it significantly increased mean arterial pressure (MAP) 14 mm Hg (95% CI 10 -18 mm Hg; I2 =89%).

Conclusions: Limitations: The overall quality of studies was poor. The effect on symptoms of discomfort were poorly studied. None of the studies reported long-term patient important outcomes

Cooling dialysate is an effective intervention to reduce IDH and to increase MAP in chronic hemodialysis patients. The effect on symptoms of discomfort were poorly studied. High quality, large, multicenter studies with long follow up are needed to assess the effect of cool dialysis on long-term patient important outcome.

SA-PO3009

Intradialytic Hypotension (IH): Variation in Dialysis Facilities Jeffrey J. Sands,[1] Len A. Usvyat,[2] Sandi Moore,[1] Mary T. Sullivan,[2] Jonathan H. Segal,[3] Paul M. Zabetakis,[1,2] Jose A. Diaz-Buxo.[1] *[1]Fresenius Medical Care NA; [2]Renal Research Institute; [3]U Michigan.*

Background: Intradialytic hypotension (IH) occurs in 10-30% of HD treatments (Tx) but is not routinely reported or aggregated to facilitate evaluation and prevention.

Methods: We studied IH (systolic blood pressure (SBP) < 90mmHg and decreased >30mmHg from Tx maximum) in 313 HD patients, (2788 Tx) in 4 facilities in an ongoing Quality Improvement Initiative. Blood pressure was captured by machine download and IH risk factors assessed by logistic regression.

Results: Patients are 58.1% white, 39.0% black, 52.7% diabetic, 62.0% AVF; 19.8% catheter, mean age 60.9±15.7 years, vintage 3.7±3.8 years. Mean pre-HD SBP is 138.8±22.1 mmHg, EDW 83.8±25.2 kg, Albumin 3.9±0.4 g/dL and Na gradient (dialysate-patient Na)-1.3±2.8 mEq/L. IH was common (20.8% Tx) and highly variable by patient (0 to 100% Tx), facility (5.3% to 27.4% Tx) and Tx date (0% to 43% Tx). 43.4% of patients had ≥1 IH (facility range: 8.6% to 86.3%) and 10.5% (facility range: 3.4% to 15.7%) had IH on ≥50% of Tx.

	Odds Ratio	95% CI		p-value
Vintage (yrs)	1.051	1.017	1.087	0.003
Pre-HD SBP (mmHg)	0.976	0.972	0.980	<0.0001
Male	0.349	0.279	0.436	<0.0001
Age (yrs)	1.030	1.021	1.038	<0.0001
Race (white)	1.256	0.997	1.583	0.054
Diabetes	1.314	1.037	1.666	0.024
Ultrafiltration (UFV) (L)	1.599	1.425	1.795	<0.0001
Shift (Mid)	1.078	0.806	1.442	0.614
Shift (PM)	0.800	0.564	1.136	0.212
Tx T,Th,Sat	1.122	0.793	1.587	0.515
Catheter	0.889	0.671	1.177	0.412
BMI (kg/m)²	1.001	0.989	1.013	0.883
Albumin (g/dL)	1.864	1.275	2.723	0.001
Dialysate-patient Na (mEq/L)	0.990	0.939	1.044	0.707
Post Weigh -EDW (kg)	1.112	1.059	1.116	<0.0001
Clinic 1	0.748	0.577	0.970	0.028
Clinic 2	0.448	0.259	0.773	0.004
Clinic 3	0.181	0.070	0.469	<0.001

Reference: black; female; non-diabetic; AM shift; M W F; AVF/AVG; Tx in clinic 4

Conclusions: IH is common and highly variable by patient and facility. Increased risk of IH was associated with older age, diabetes, higher UFV, albumin and post HD weight-EDW difference, lower pre-HD SBP and being dialyzed in clinic 4. Additional evaluation of facility practice patterns and modifiable risk factors is needed to decrease the frequency of IH.

SA-PO3010

Dialysate Calcium Concentration and Intradialysis Hemodynamic Stability Carlo Basile, Pasquale Libutti, Francesco Casucci, Piero Lisi, Carlo Lomonte. *Division of Nephrology, Miulli General Hospital, Acquaviva delle Fonti, Italy.*

Background: The interplay of correct sodium (Na+), potassium (K+) and total calcium (tCa) mass balances (respectively, Na+MB, K+MB and tCaMB) with adequate ultrafiltration volumes (V_{UF}) is crucial for the hemodynamic stability during hemodialysis (HD). This study was aimed to investigate the effects that three dialysate tCa concentrations (1.25, 1.375 and 1.50 mmol/l) may exert on the latter.

Methods: Twenty-two stable anuric uremic patients underwent three 4h-bicarbonate HD sessions, each with one of the three dialysate tCa concentrations. V_{UF} and dialysate Na+, K+ and bicarbonate concentrations were prescribed to be the same (respectively, 140, 2.0 and 35 mmol/l). Hourly measurements of plasma water (pw) ionized Ca (Ca++), Na+ and K+, blood pH and bicarbonate concentrations were effected. tCaMBs, Na+MBs and K+MBs were measured from the dialysate side (GENIUS batch dialysis system, FMC, Germany). Intradialysis systolic, diastolic and mean arterial pressure (SBP, DBP and MAP, respectively) and heart rate (HR) trends were analyzed.

Results: A statistically significant difference was observed among the mean hourly pwCa++ concentrations, being significantly higher with a dialysate tCa concentration of 1.50 ($P < 0.0001$). Mean tCaMBs were positive (diffusion gradient from the dialysate to the patient), being more and more higher by increasing dialysate tCa concentrations (+ 75 ± 122 mg, + 182 ± 125 mg, + 293 ± 228 mg, respectively) ($P < 0.0005$). No statistically significant difference was observed when comparing pw concentrations of Na+ and K+, blood pH and bicarbonate levels during the three different sessions (repeated measures ANOVA stratified for treatments). Mean Na+MBs and K+MBs were not statistically significantly different among the three treatments. No statistically significant difference was observed when comparing SBP, DBP, MAP and HR during the three different sessions (repeated measures ANOVA stratified for treatments).

Conclusions: These highly controlled experiments show that hemodynamic stability does not appear to be statistically significantly influenced by any specific dialysate tCa concentration in this peculiar subset of patients.

Funding: Clinical Revenue Support

SA-PO3011

The Effect of Pneumatic Compression Devices on Hemodynamic Parameters in Hemodialysis Patients: A Randomized Crossover Trial Davina J. Tai, Sofia B. Ahmed, Brenda Hemmelgarn, Jennifer M. MacRae. *University of Calgary.*

Background: Intradialytic hypotension (IDH) is the most common complication of chronic hemodialysis (HD) therapy, leading to increased morbidity and mortality. The pathophysiology of IDH is multifactorial and poorly understood. Studies have shown that central blood volume (CBV) is maintained in stable HD patients. Pneumatic compression devices (PCDs) are thought to improve venous return by preventing pooling of blood in the lower extremities. Thus, PCDs could potentially improve CBV and intradialytic blood pressure (BP) in HD patients.

Methods: We performed a randomized, two-period crossover trial to determine the effect of PCDs, compared with control, on central blood volume (CBV) in HD patients. Patients on intermittent HD ≥ 3 times per week for ≥ 3 months were eligible for study inclusion. The study period consisted of 2 consecutive mid-week HD sessions. Patients were randomized to begin with either 1 mid-week HD session with PCDs (the intervention), or 1 mid-week HD session without PCDs (the control), stratified by whether or not they were IDH-prone.

Results: 51 patients (75% male, 49% diabetic) with a mean age of 65 ± 14 years and mean HD vintage of 44 ± 37 months were randomized. Forty-six patients completed the study. During HD, the mean change in CBV in the control and intervention sessions was -0.077L vs -0.085L ($p=0.78$). Similarly, comparing control and intervention sessions, there were no differences in change in cardiac output (-0.49L/min vs -0.63L/min, $p=0.78$) and systemic vascular resistance (+1.55 mmHg/L/min vs +1.30 mmHg/L/min, $p=0.67$). Post HD systolic blood pressures (SBP) and minimum intradialytic SBPs were similar in the control and intervention sessions [(126 mmHg vs 128 mmHg, $p=0.08$), and (112 mmHg vs 108.5 mmHg, $p=0.19$), respectively].

Conclusions: Compared with standard of care, PCDs have no effect on hemodynamic parameters, including CBV, during HD. Further studies are required to better understand the physiological and hemodynamic changes in patients during HD, so that superior preventative strategies for IDH can be uncovered.

SA-PO3012

The Use of Crit-Line® To Monitor and Quantify Fluid Removal Rates in Dialysis Patients during a Baseline Observational Period Linda H. Ficociello,[1] Len A. Usvyat,[2] Michael Black,[1] Patrice B. Taylor,[2] Heather J. Ansede,[2] Kay D. Cuaton-Maier,[2] Antoinette M. Ordish,[2] Paul Balter,[2] Paul M. Zabetakis,[2] Claudy Mullon,[1] Jose A. Diaz-Buxo.[1] *[1]Fresenius Medical Care-NA, Waltham, MA; [2]Renal Research Institute (RRI), New York, NY.*

Background: Real-time blood volume (BV) monitoring during hemodialysis sessions (HD) using a Crit-Line® Blood Volume Monitor may help to quantify fluid removal for better fluid management.

Methods: As part of a quality improvement project, two RRI clinics monitored BV changes via Crit-Line during an observational period in 88 HDD-CKD patients. Crit-line monitors reporting BV changes every 1 minute were installed at all 14 stations in one center and 10 randomly selected stations in the second center. Patients with BV decreases of ≥ 3%/hour (hr) were considered to have an adequate fluid removal rate, patients with BV decrease of <3%/hr and BV decrease by HD end of ≥8% had a slower removal rate/hr but adequate BV decrease by HD end, and patients with BV decrease <3%/hr and a BV decrease <8% by HD end were considered to have inadequate fluid removal rates.

Results: On average, patients were 60 years old with a vintage of 4 years and HD time of 223 minutes. 66% of patients had a BV decrease of <3%/hr and <8% by HD end, 20% had a BV decrease of <3%/hr, but ≥8% by HD end, and 14% BV decreases ≥3%/hr. A BV decrease of <3%/hr and <8% by HD end was associated with documented hypertension (86%) or fluid overload/CHF (40%) when compared to patients with more adequate fluid removal. The amount of fluid removed during HD was highest for those with ≥3%/hr BV decrease and lowest for those with <3%/hr and <8% by HD end. The difference between post-treatment weight (Wt) and estimated dry Wt was similar between groups.

Conclusions: Monitoring of real-time BV changes using Crit-Line may be used to better determine appropriate fluid removal and develop new algorithms for fluid management.

Characteristic	BV decrease <3%/hr, <8% by HD end (n=58, 66%)	BV decrease <3%/hr, ≥8% by HD end (n=18, 20%)	BV decrease ≥3%/hr (n=12, 14%)	pvalue
Pre-Post HD Wt (kg)	-2.1	-2.8	-4.0	0.005
Post HD - Estimated Dry Wt (kg)	0.22	0.03	-0.10	0.37
% Hypertension	86	67	67	0.10
% FO/CHF	40	22	8	0.07

Funding: Pharmaceutical Company Support

SA-PO3013

Continuous Recording Reveals Extreme Blood-Pressure Variability in Nominally Stable Dialysis Scott Wilson, Gavin J. Becker. *Royal Melbourne Hospital, Australia.*

Background: The prediction and avoidance of haemodynamic instability during haemodialysis (HD) is important to minimize circulatory stress and maintain efficacy. Intradialytic hypo- or hypertension is defined as a symptomatic change in systolic blood pressure (SBP) of ≥20 mmHg and associated with increased mortality and morbidity. In clinical practice, intradialytic blood pressure records are used to adjust HD, ultrafiltration, and antihypertensive prescription. Clinicians assume this record captures a true haemodynamic profile and the descriptor "stable dialysis" is routinely drawn from such observation. We sought to investigate the actual SBP variability during HD using continuous recordings and compare these with standard clinical measures and changes in relative blood-volume (RBV).

Methods: Continuous measure of SBP by plethysmograph using the Finometer system and RBV were recorded in 6 "stable" midweek HD outpatients. In parallel, 5 arm-cuff SBP measures were made by HD staff blind to the continuous record. Time-series data from beat-to-beat recording (~17,000 data points/HD) was analysed using a heart-rate dependent median-hybrid filter.

Results: Concordance between the plethysmograph and simultaneous arm measures of SBP was excellent (mean difference <5mmHg). The time-series record revealed a slow increase in SBP through HD with a mean change from start (127mmHg) to end (167mmHg) of 40mmHg. Continuous recordings revealed significant asymptomatic fluctuations in SBP that weren't associated with variation in RBV. The mean difference between minimum (94mmHg) and maximum (199mmHg) SBP during HD was 105mmHg. SBP was beyond 2 standard deviations from baseline for an average of 18% of HD time, typically in the hypertensive range. Among the 6 dialyses only a single symptomatic event was observed.

Conclusions: Clinically silent SBP variability during "stable" HD is common and unrecognised. Such variation might be relevant to dialytic efficiency, interdialytic blood pressure control and long-term cardiovascular outcomes.

Funding: Private Foundation Support

SA-PO3014

Effect of Oxygen Therapy on Hemodynamic Stability during Hemodialysis with Continuous Blood Volume and O2 Saturation Monitoring Nupur Jhawar, Kiran M. Goli, Paul F. Visintainer, Jason D. Cooney, Joy Whitbeck, Michael J. Germain. *Baystate Medical Center/Tufts University School of Medicine., Springfield, MA.*

Background: Intradialytic hypotension (IDH) is the most common complication of hemodialysis (HD). The effects of oxygen administration on IDH during HD are currently unknown. To address this question, a randomized-controlled clinical trial (RCT) of the effects of oxygen(O2) administration on hemodynamic stability during HD is planned. Prior to initiating the clinical trial, we present results of a preliminary pilot study.

Methods: This pilot study was a non-randomized, prospective and two group study that included 24 patients receiving HD in a inpatient HD unit with either an AV fistula or a venous catheter. The study compared hemodynamic stability in 12 patients receiving 2 liters of O2 via nasal cannula during HD to 12 patients not receiving O2 during HD. BP was recorded every 30 minutes. RBV, Hct and O2 saturation were recorded continuously by Crit-line III monitor.

Results: Results showed that O2 administration during HD significantly stabilized systolic blood pressure (SBP) over time (average change in SBP = 1.10 mmHg every 30 minutes) compared to the placebo group (average change in SBP = -1.62 mmHg; p = 0.037). Furthermore, O2 administration resulted in substantially lower variability in the individual readings in the treatment group compared to the placebo group (SD_{tx} = 8.9 v. SD_{pbo} = 17.2; p < 0.001), suggesting that O2 may stabilize SBP over time during HD.

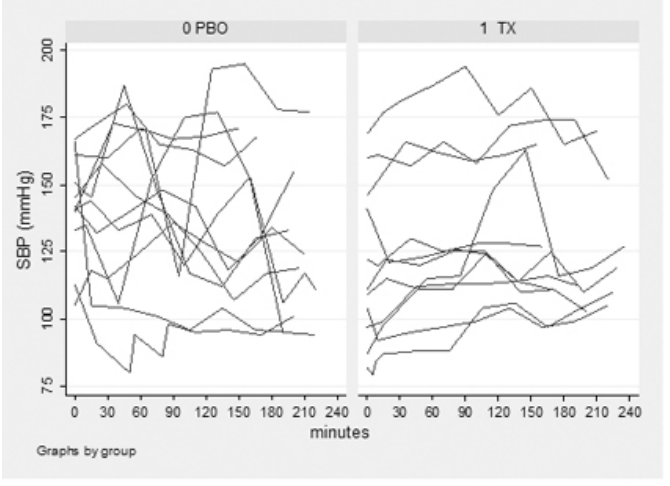


Figure 1. Oxygen administration resulted in substantially lower variability in the individual readings of SBP in the treatment group compared to the placebo group (p < 0.001), suggesting that oxygen may stabilize SBP over time during HD. 0 PBO refers to the placebo group. 1 TX refers to the treatment group. SBP refers to systolic blood pressure.

Finally, log-RBV slope showed a significantly steeper decline compared with placebo (average change in log-RBV every 30 minutes for treatment vs. placebo: -0.018 vs. -0.013, p = 0.005).

Conclusions: Greater hemodynamic stability and fewer episodes of IDH are achieved in the treatment group compared to the control group. These results need to be validated in a subsequent RCT.

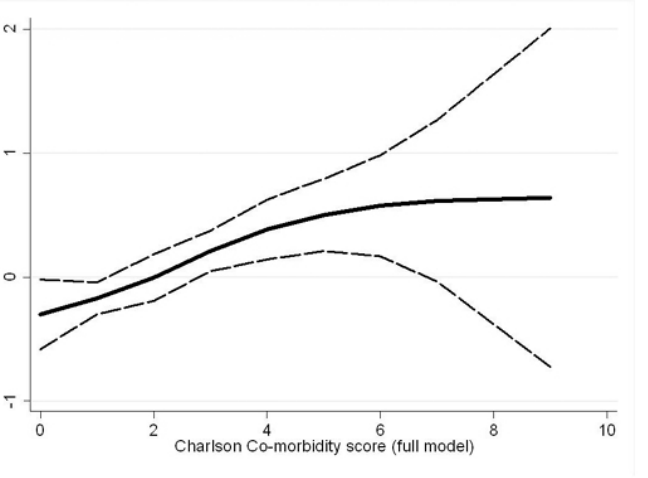
SA-PO3015

Mortality-Predictability of Charlson Comorbidity Score in Maintenance Hemodialysis Patients and the Role of Race Usama Feroze,[1] Miklos Z. Molnar,[1] Ramanath B. Dukkipati,[2] Csaba P. Kovesdy,[3] Allen R. Nissenson,[4] Keith C. Norris,[2] Joel D. Kopple,[2] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA; [4]DaVita, Inc, Denver, CO.*

Background: The Charlson co-morbidity index (CCI) is a commonly used scale for assessing morbidity. Maintenance hemodialysis dialysis (MHD) patients (pts) are a unique cohort with significant cardiovascular risk & marked variations in mortality characteristics by race/ethnicity.

Methods: A 6-year cohort of 893 MHD pts was examined for an association between a modified (without age and kidney disease) CCI (mCCI) & mortality.

Results: Pts were 53±15 years old(mean±SD), and included 47% women, 31% African-Americans & 55% diabetics. In our fully adjusted model, 2nd (mCCI: 1-2), 3rd (mCCI=3), and 4th (mCCI: 4-9) quartiles compared to 1st (mCCI=0) quartiles showed death hazard ratios (HR) (and 95% confidence intervals) of 1.43(0.92-2.23), 1.70(1.06-2.72), and 2.33(1.43-3.78), respectively.



The mCCI was a significant mortality predictor either in non-African Americans(1.40 {1.29-1.51}) or in African Americans(1.12 {1.01-1.23}) in our unadjusted model. After adjustment for nutritional and inflammation markers, mCCI was a significant mortality predictor in non-African Americans(1.27{1.12-1.44}), but not in African Americans(1.07 {0.94-1.21}).

Conclusions: The mCCI is a robust and linear predictor of mortality in MHD pts, in particular in non-African Americans. The mCCI scale for co-morbidities does not appear to be a significant factor for mortality risk in African American MHD pts after adjusting for nutritional & inflammatory markers. Studies to examine race-specific comorbidity scales as effective predictors for mortality risk in dialysis populations are warranted.

Funding: NIDDK Support

SA-PO3016

Associations of the Malnutrition-Inflammation Score with Depressive Symptoms and Kidney-Disease Targeted Health-Related Quality of Life Measures in a Brazilian Sample of Hemodialysis Patients Gustavo Behrens Pinto, Luiz Fernando Catto, Larissa Moura Silva, Raissa Araujo, Rafaela Lima, Camila Joau, Marcelio Piccolo, Gildete Barreto Lopes, Antonio Alberto Lopes. *Núcleo de Epidemiologia Clínica do Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia.*

Background: The malnutrition-inflammation score (MIS) has been largely used to evaluate nutritional status in maintenance dialysis (MHD) patients. There is a lack of studies, however, to assess associations of the MIS with kidney-disease targeted health-related quality of life (KDT-HRQOL) measures and depression symptoms in MHD patients. This study assessed associations of MIS with scores of KDT-HRQOL and depressive symptoms in MHD patients.

Methods: Cross-sectional study of 632 prevalent MHD patients enrolled in the Prospective Study of the Prognosis of Chronic Hemodialysis Patients (PROHEMO) developed in Salvador, Brazil. MIS≥6 defined worse nutrition status. The Kidney Disease Quality of Life Short-Form was used to determine scores (range: 0 to 100) for the eleven KDT-HRQOL scales. The Center for Epidemiological Studies Depression scale was used for depression symptoms scores (range: 0-60). Linear regression with adjustments for numerous covariates were used to determine differences in scores of HRQOL and depression symptoms by MIS categories.

Results: Patients with MIS≥6 (compared with MIS<6) were found to have significantly (P<0.05) lower adjusted scores for the following KDT-HRQOL: symptoms/problems (difference=-5.35), effects of kidney disease (difference=-4.28), burden of kidney disease (difference=-6.45), cognitive function (difference=-4.87), social interaction (difference=-4.37) and sleep (difference=-4.08). Depression symptoms scores were significantly (P<0.05) higher for patients with MIS ≥6. Gastrointestinal symptoms and functional capacity were the MIS components more strongly associated with poorer HRQOL and higher depression symptoms.

Conclusions: The data suggest that MHD patients with higher MIS have clinically significantly lower scores for several HRQOL components (both generic and kidney-disease targeted) and higher probability of depression.

SA-PO3017

Depression and Its Management in Maintenance Haemodialysis Patients Marguerite McCloskey, Ronan Cunningham, Robert Mullan, Agnes Masengu, Camille Harron. *Renal Unit, Antrim Area Hospital, Antrim, Northern Ireland, United Kingdom.*

Background: The purpose of this audit was to determine the prevalence of depression within our haemodialysis population and review treatment strategies in accordance with National Institute of Clinical Excellence (NICE) guidance.

Methods: A total of 126 haemodialysis patients (71 male, 55 female, median age 66.9) were asked to complete a voluntary depression questionnaire, namely the patient health questionnaire 9 (PHQ-9), which has been validated for use by general practitioners.

We also identified those patients on antidepressant medication using our computerised database.

Results: 76.2% (96/126) completed the questionnaire, 15.9% (20/126) refused, 0.8% (1/126) were unable to complete secondary to language barrier, and 6.3% (8/126) were unable to complete secondary to cognitive impairment. 42.7% (41/96) were identified as being depressed. 29.2% (28/96) were classified as mildly depressed, 35.7% (10/28) of whom were on an antidepressant. 8.3% (8/96) were classified as moderately depressed, 25% (2/8) of whom were on an antidepressant. 4.2% (4/96) were classified as moderately severely depressed, 75% (3/4) of whom were on an antidepressant, and one patient was classified as severely depressed and was already on an antidepressant.

32.5% (41/126) were already on an antidepressant at the time of assessment, 80.5% (33/41) of whom were on a serotonin selective reuptake inhibitor (SSRI), 12.2% (5/41) were on amytriptyline, 4.9% (2/41) were on mirtazepine, and 2.4% (1/41) were on a selective noradrenaline reuptake inhibitor (SNRI).

Conclusions: Depression is common in the haemodialysis population and the majority of those with moderately severe and severe symptoms were appropriately on medication. The majority of those treated were on low dose SSRIs. Those who were mildly/moderately depressed did not appear to be identified and treated for depressive symptoms as readily.

This audit also highlights the use of antidepressant medication in this population group, and the difficulties in management that may be related to side effects.

The results were reviewed and consideration made to the use of alternative approaches, such as counselling and art therapy.

SA-PO3018

Buddhist Intrahemodialytic-Insight Meditation Improves Depression in Hemodialysis Patients Kriengsak Vareesangthip. *Renal Division, Department of Medicine, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand.*

Background: End stage renal disease (ESRD) patients can be lived longer with renal replacement therapy, including hemodialysis, continuous ambulatory peritoneal dialysis and kidney transplantation. Living on hemodialysis is a perpetual challenge, due to the demanding treatment schedule, dietary restrictions, and changes in function. The life expectancy of dialysis patients is 1/3 to 1/6 that of general population. Depression has long been identified as the primary mental health problem of patients with ESRD. It has been clearly shown that Buddhist meditation can improve the depression state of chronic illness patients. We, therefore, hypothesized that buddhist intradialytic-Insight Meditation for 10 weeks could calm down the severity of depression in hemodialysis patients.

Methods: Twenty stable hemodialysis patients who have been dialysed three times a week and all have Kt/V > 1.2. All patients were trained to practice Insight Meditation schedule in the term of Anapanasati during hemodialysis for 30 minutes in each hemodialysis session for 10 weeks. The severity of depression was assessed at pre and post period of insight meditation schedule by using Thai Depression Inventory. The Thai Depression Inventory was developed as a self-rating instrument for measuring the severity of depression. The scale was tested with the Hamilton Rating Scale for Depression.

Results: The depression score in hemodialysed patients was significant improved after practicing the buddhist insight meditation (pre and post depression score, 15.0 ± 7.2 vs. 10.3 ± 7.8, p = 0.01).

Conclusions: The buddhist insight meditation can be used to improve the depression and would provide good quality of life in hemodialysis patients.

Funding: Private Foundation Support

SA-PO3019

Anxiety during Dialysis in Maintenance Dialysis (MD) Patients: A Highly Prevalent Co-Morbidity Usama Feroze,[1,2] David J. Martin,[1,3] Astrid Reina-Patton,[1,3] Jun Chul Kim,[1,2] Kamyar Kalantar-Zadeh,[1,2,3,4] Joel D. Kopple.[1,2,3,4] *[1]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; [2]Harold Simmons Center for Chronic Disease Research and Epidemiology, Torrance, CA; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]UCLA School of Public Health, Los Angeles, CA.*

Background: Maintenance dialysis (MD) patients have a high prevalence of anxiety and depression. We have begun to study the frequency of anxiety episodes related to regular dialysis sessions and factors which may engender anxiety in MD patients.

Methods: 170 patients, 155 undergoing Maintenance hemodialysis (MHD)patients and 15 undergoing chronic peritoneal dialysis, were examined.Inclusion criteria included dialysis vintage of at least six months.Patients completed the Beck Anxiety Inventory and Beck Depression Inventory and questionnaires that examined their feelings of anxiety related to individual dialysis sessions.

Results: Patients' age was 56±SD 16 years, dialysis vintage, 55±48 months, 46% were female. Anxiety and depression were found in 53% and 36 % of patients, respectively. Patients frequently experienced episodes of anxiety associated with a number of the events that commonly occur in dialysis centers. As examples, 30% of MHD patients described moderate to extreme anxiety when they come for their regular MHD sessions, 24% showed moderate to extreme anxiety when a new or unknown staff person connected them to the dialyzer machine, and 18% reported moderate to extreme anxiety if the alarm sounded on the dialysis machine while they were undergoing dialysis.

Anxiety Level

Activity	None	Mild	Moderate	Quite a Bit	Extreme
Coming for a MHD treatment	50%	20%	13%	9%	8%
Connected to dialyzer by a new person	51%	25%	8%	6%	10%
Alarm sounds on dialysis machine	59%	23%	8%	6%	4%

Conclusions: Our study indicates that even well-established MD patients become anxious, often substantially so, in response to a number of the events that they commonly experience in during a chronic hemodialysis treatment. Research to examine the causes and methods of prevention and treatment of these anxieties would seem to be strongly indicated.

SA-PO3020

Recovery after a Hemodialysis Session: Associations with Patient's Characteristics, Quality of Life and Depression Symptoms in a Sample of Brazilian Patients Camila Joau, Marcelio Piccolo, Raissa Araujo, Larissa Moura Silva, Gustavo Behrens Pinto, Luiz Fernando Catto, Rafaela Lima, Gildete Barreto Lopes, Antonio Alberto Lopes. *Núcleo de Epidemiologia Clínica e Medicina Baseada em Evidências, Universidade Federal da Bahia, Salvador, BA, Brazil.*

Background: Many patients do not feel well after a hemodialysis (HD) session and need some time to recover. The study investigated associations of recovery after a HD session with patient's characteristics, health-related quality of life (HRQOL) and depression symptoms.

Methods: Cross-sectional study of 718 patients enrolled in the ongoing Prospective Study of the Prognosis of Hemodialysis Patients (PROHEMO) in Salvador, Brazil. The SF-36 was used to determine HRQOL scores for physical component summary (PCS) and mental component summary (MCS). The Center for Epidemiologic Studies Depression (CESD) scale was used for depression symptoms. Multivariable logistic regression was used to identify characteristics associated with recovery after HD and multivariable linear regression to estimate differences in scores with adjustments for age, gender, vintage, Kt/V, vascular access, albumin, creatinine, hemoglobin, diabetes, heart failure, cerebrovascular disease, peripheral vascular disease and intra-HD hypotension requiring IV saline.

Results: Approximately 30% of the patients (213/718) reported some time to recover after a HD session. Intra-HD hypotension was the variable more strongly associated with higher odds of reporting some time to recover (adjusted odds ratio=2.68, P=0.003). Compared with patients who reported feeling well after a HD session, those who reported some time to recover had significantly lower PCS score (adjusted difference (AD)= -4.77), lower MCS score (AD= -4.48) and higher CESD score (AD= +4.66); each P<0.001.

Conclusions: The results suggest that patients experiencing intra-HD hypotension are more likely to need some time to recover after a HD session, independently of age, Kt/V and comorbidities. It is also suggested that patients who need some time to recover have, in general, poorer HRQOL and higher depression probability, independently of intra-HD hypotension and numerous other covariates.

SA-PO3021

Under-Recognised and Undertreated? Symptoms in Haemodialysis Patients Catherine Susanna Vinen,[1] Chris Jones,[1] Kate A. Shepherd,[2] Heather Jane Brown,[3] Katherine Bristowe,[2] Beverley Matthews,[4] Donal O'Donoghue,[5] Fliss E. Murtagh.[2] *[1]King's College Hospital, London, United Kingdom; [2]King's College London, United Kingdom; [3]Guy's and St Thomas', United Kingdom; [4]NHS Kidney Care, London, United Kingdom; [5]Department of Health, United Kingdom.*

Background: Symptoms in haemodialysis patients are under-researched and under –treated. We identified the prevalence and severity of key symptoms using a validated assessment tool.

Methods: We undertook a cross-sectional study of haemodialysis patients in 2 UK renal units. We used the patient-reported Palliative Outcome Scale (POS-S renal) – a symptom module adapted for renal patients to capture prevalence and severity of 17 physical and psychological symptoms.

Results: 330 patients were included, mean age 61 years (range 22-89), mean time on dialysis 4.3 years (range 0–31 years). Most prevalent symptoms were: weakness (79%), pain (69%), poor mobility (66%), pruritis (63%), insomnia (61%) and dyspnoea (57%). 33% of all participants had moderate and 19% had severe/overwhelming weakness, 30% had moderate and 17% severe/overwhelming pain, and 29% had moderate and 20% severe/overwhelming poor mobility The mean global symptom score was 14/68 (range 0-56). There was no correlation between global symptom score and: age, dialysis vintage, gender, or ethnic group. Diabetes mellitus (as primary cause of end-stage kidney disease) was associated with significantly higher global symptom score compared to reno-vascular disease (17.4 vs 11.9, p=0.01). Analysis of symptoms by age tertiles (22-57, 58-72, 73-89 years)

showed insomnia was more common and decreased mobility less common in the youngest age group (p=0.01 & p<0.001, respectively). Global symptom score showed significant but weak negative correlation with urea reduction ratio (coeff -0.13, p=0.016).

Conclusions: Data reveal symptoms are highly prevalent among dialysis patients. Global symptom score does not correlate with age or dialysis vintage, and specific symptoms are more prevalent in certain age and diagnostic groups. Aggressive symptom management will improve quality of life and could be targeted by age and diagnostic group.

This work is part of a project led by NHS Kidney Care.

Funding: Government Support - Non-U.S.

SA-PO3022

Daytime Intradialytic Sleep, Nocturnal Sleep, and Mortality Risk among Hemodialysis Patients in the CDS Nancy G. Kutner,[1] Rebecca H. Zhang,[1] Kirsten L. Johansen,[1,2] Donald L. Bliwise.[1] *[1]USRDS Rehabilitation/QoL Special Studies Center, Emory University, Atlanta, GA; [2]Nephrology Section, San Francisco VA Medical Center, San Francisco, CA.*

Background: Increased sleepiness during HD may reflect treatment-induced alterations in arousal status and/or thermoregulatory processes (*Sleep* 23:887-891, 2000). We explored intradialytic sleep, nighttime sleep, and survival among HD participants in the Comprehensive Dialysis Study (CDS).

Methods: Incident dialysis patients aged >18 from 296 randomly selected clinics were surveyed. 1,439 HD patients reported how long they typically dozed off/slept during HD, usual nighttime sleep hours, and trouble with waking up at night (nocturnal sleep fragmentation). These variables, and age, gender, race, education, employment status, diabetes, cardiovascular comorbidity, BMI, early nephrology care, SF-12 PCS score, and reported RLS, were included in a Cox proportional hazards model predicting mortality from dialysis start date to September 30, 2009. Patients were censored at change to PD or transplant.

Results: Younger age and diabetes were associated with greater doze/sleep time. 684 patients (47.5%) slept 6 or fewer hours at night, and 755 patients (52.5%) slept >6 hours at night. More patients who slept 6 or fewer hours reported always/sometimes experiencing nocturnal sleep fragmentation (73% vs. 47%; p < 0.0001), and they reported more intradialytic doze/sleep time than patients who slept >6 hours at night (0.97 [0.94] vs. 0.85 [0.90] hour; p = 0.01). In the multivariable Cox model, mortality risk increased as HD doze/sleep time increased among patients with 6 or fewer nighttime sleep hours (HR 1.23 [95% CI 1.06-1.44]; p = 0.008), but among patients with >6 hours nighttime sleep, greater HD doze/sleep time was not associated with increased mortality risk (HR 1.02 [95% CI 0.86-1.20]; p = 0.85).

Conclusions: Consistent with evidence that subjective sleepiness increases during HD treatment, intradialytic sleep was common. Patient and treatment factors that may be associated with different intradialytic sleep and nighttime sleep quantity/quality patterns may have important clinical implications.

Funding: NIDDK Support

SA-PO3023

John Henryism Active Coping, Perceived Health, and Depression Symptoms in Brazilian Hemodialysis Patients: The PROHEMO Study Gildete Barreto Lopes,[1] Carolina Cartaxo Penalva,[2] Marcelio Piccolo,[1] Camila Joau,[1] Sherman A. James,[3] Antonio Alberto Lopes.[1] *[1]Universidade Federal da Bahia (UFBA), Brazil; [2]Clinica NEPHRON; [3]Duke University.*

Background: John Henryism refers to a strong behavioral predisposition to actively cope with difficult psychosocial stressors. It is measured by the 12 item John Henryism Active Coping Scale (JHAC). Sample JHAC questions are: 1) In the past, even when things got really tough, I never lost sight of my goals; 2) Once I make up my mind to do something, I stay with it until the job is completely done. Persons who score high on the JHAC believe they can overcome difficult problems through determination and hard work. This study assessed if JHAC scores were associated with perceived general health (HRQOL) and depressive symptoms in maintenance hemodialysis (MHD) patients.

Methods: Cross-sectional analyses were conducted on 585 patients from phase II of the PROHEMO Study developed in Salvador, Brazil. John Henryism was assessed by the scores of the 12-item JHAC (range 12-60). The SF-36 was used to determine general health score (range 0-100); and the Center for Epidemiologic Studies Depression (CESD) scale to depression symptoms score (range 0-60). Linear regression was used to test associations of JHAC score above the median with general health and depression symptoms scores, adjusting for several sociodemographic, treatment and comorbid factors.

Results: Mean age was 48.29±13.66 yr, 61.3% males, 19.4% diabetics and 88.5% non-whites. Median JHAC=52. Patients with JHAC score above the median had higher mean general health score (difference= +6.36 points, P=0.002) and lower mean depressive symptoms score (difference= -2.43, P=0.013). The differences in scores after adjustments for covariates were +6.75 (P=0.002) for general health and -2.55 (P=0.011) for depression symptoms. Similar patterns of associations were observed by subgroups of age (<60 and ≥60 yr), race, gender and diabetic status.

Conclusions: These results suggest that MHD patients with high JHAC scores are more likely to report better general health and lower depression symptoms than patients with low JHAC scores, independently of sociodemographic factors and comorbidities.

Funding: Government Support - Non-U.S.

SA-PO3024

Predictive Characteristics of Successful Hemodialysis Patient Survival in the First 120 Days of Treatment Len A. Usvyat, Penny Faith Palmiero, Lizette Borges, Lisa A. Pacelli, Mary T. Sullivan, Paul Balter, Peter Kotanko, Paul M. Zabetakis. *Renal Research Institute, NY, NY.*

Background: In 2007, mortality rate in the first 3 months of dialysis was more than double compared to the overall annual mortality rate of 19% of prevalent hemodialysis patients (pts) [USRDS]. We aim to understand some of the specific factors that impact success during this period of care.

Methods: We reviewed data of all HD pts who were admitted to the RRI clinics within 30 days from their first date of dialysis b-n 2001 and 2010. Survival was assessed in the end of the incident dialysis period at 120 days.

2 analyses were conducted:

--Analysis 1 (pre-dialysis "PRE-D" factors) looked at success factors at the initiation of dialysis

--Analysis 2 (incident dialysis "INC-D" factors) looked at success factors present at the end of the incident period

Cox proportional hazards models were constructed for PRE-D and INC-D adjusted for: gender, race, ethnicity, age, comorbid conditions, facility effect, access type, BMI, presence of cardiac medication [beta blockers, ACEs, ARBs] and nutritional supplements, EPO dose, blood pressure, albumin, online clearance [OLC], neutrophil to lymphocyte ratio [NLR] and target weight.

Results: We analyzed data of 11,727 pts for PRE-D.

Table 1 summarizes the outcomes of Cox models for PRE-D and INC-D. Only significant predictors are shown (p<0.05). Other covariates described above were not significant.

PRE-D

Associated with better outcomes	Associated with poorer outcomes
Higher systolic blood pressure: 1% greater chance of survival for every 1 extra mmHg of BP	Being Male: 26% greater risk of death
Higher diastolic blood pressure: 1% greater chance of survival for every 1 extra mmHg of BP	Higher Age: 3.6% greater risk of death for every 1 year of age
	Presence of catheter as first access: 158% greater risk of death
	Presence of the following co-morbids: hepatitis and history of myocardial infraction suggest poorer survival
	Higher EPO dose: 2.5% greater risk of death for every 1000 additional units of EPO
	Larger size: 0.8% greater risk of death for every 1 unit of BMI

INC-D

Associated with better outcomes	Associated with poorer outcomes
Higher systolic blood pressure: 1% greater chance of survival for every 1 extra mmHg of BP	Higher Age: 2.7% greater risk of death for every 1 year of age
Higher diastolic blood pressure: 4% greater chance of survival for every 1 extra mmHg of BP	Presence of catheter as the access at the end of 120 days (or death): 251% greater risk of death
Higher albumin: 63% greater chance of survival for every 1 g/dL higher albumin	Higher neutrophil lymphocyte ratio (NLR): 0.3% greater risk of death for every 1 unit increase in NLR
Higher online clearance (LOC): 56% greater chance of survival for every 1 unit higher OLC	Presence of the following co-morbids: hepatitis suggest poorer survival
Presence of cardio-protective drugs: 30% greater chance of survival for patients who are on cardio-protective therapy	

Conclusions: Incident success as measured by survival in the first 120 days of dialysis is being closely watched for all dialysis patients. We observed that the following were associated with improved survival: AVG/AVF, higher blood pressure, utilization of cardio-protective drugs, and higher online clearance. Factors associated with poorer outcomes are lower albumin, higher NLR, being male, higher age, higher EPO dose, and various comorbid conditions.

SA-PO3025

The Effect of the Bundled Payment System on the Utilization of Erythropoiesis Stimulating Agent Therapy Mahmoud T. El-Khatib,[1,2] Heather Duncan,[1,2] Kotagal Shashi Kant.[1,2] *[1]Int Med, Div Neph and HTN, University of Cincinnati Coll Med, Cincinnati, OH; [2]DCI, Cincinnati, OH.*

Background: In 2011, CMS implemented a "Bundled" payment system, in which dialysis treatment payments include dialysis procedures and drug therapies. It is widely believed that this system will change treatment practices, especially dosing of ESA, the most expensive drug therapy. To decrease ESA usage, changes in IV iron use and route of ESA administration were implemented. The aim of the study is to assess effectiveness and consequences for patients.

Methods: Records for 90 patients from a single dialysis unit were examined. Period A (July 2010) and Period B (April 2011) were compared as 2 months with complete data for the measures of interest, and stable "before" and "after" time points. The changes to ESA management included: brands of IV iron, ESA route and frequency. The level of ferritin at which IV iron was stopped increased to 1200 ng/mL, while TSAT limit remained at 50%, and hemoglobin (Hb) target remained 10-12 g/dL.We examined ESA and IV iron dosing, Hb, Ferritin, and TSAT. Paired T and Wilcoxin Signed Rank Tests were used for analysis.

Results: In comparing Period A to Period B, ESA dose decreased, but IV iron doses did not change. Hb and TSAT remained the same. Ferritin levels were significantly higher after the change in IV iron product used.

ESA dosing and outcomes

Measure mean (sem)	Period A	Period B
Hb g/dL	11.0 (0.12)	11.2 (0.11)
TSAT %	28.1 (1.47)	27.3 (1.35)
Ferritin ng/mL	958.9 (200.67)	1157.0* (165.59)
ESA units per week per kg	177.5 (13.53)	126.0* (5.72)
IV iron mg per month	175.6 (21.74)	214.9 (27.0)

* p<.01

Conclusions: The strategies implemented in the dialysis unit successfully decreased ESA use and thus expense, yet maintained Hb level within target. IV iron dosing and TSAT remained the same, yet ferritin levels increased. It is difficult to identify if one factor (brand of IV iron, IV vs. SC dosing, better dosing protocols) boosted cost-effectiveness more than another, but the combined effect appears beneficial. Because of the limitation of the short period of observation, we will continue to monitor this strategy and the possible long-term effects of these increased ferritin levels.

Funding: Clinical Revenue Support

SA-PO3026

Drop-Out Rates in Home Hemodialysis (HHD): Understanding the Challenges Rohini Arramreddy,[1,2] Sumi J. Sun,[2] Brigitte Schiller.[1,2] *[1]Medicine, Division of Nephrology, Stanford University, School of Medicine, Stanford, CA; [2]Medical Clinical Affairs, Satellite Healthcare, Inc., San Jose, CA.*

Background: Advanced technology has led to the re-emergence of HHD in the US. However, more frequent dialysis schedules may limit acceptance of HHD due to the increased burden of therapy on patient and/or partner. Limited data exist on modality failure with HHD. We report HHD drop-out rates from a dialysis provider with dedicated home training centers caring for about 200 HHD patients.

Methods: We performed a retrospective analysis of patients undergoing HHD with the NxStage System One (NxStage Medical Inc., Lawrence, MA) who dropped out during a 3 year period (2008-2010) to further understand reasons for drop out.

Results: The annual drop-out rate for HHD patients was 48% over the 3 years. 186 events of discontinuation were noted in a total of 182 patients. Reasons are shown below.

Transferred to center hemodialysis (CHD)	n = 84 (45%)
Died	n = 31 (17%)
Transplanted	n = 34 (18%)
Transferred to peritoneal dialysis	n = 15 (8%)
Transferred to another home program	n = 13 (7%)
Failed to complete training	n = 9 (5%)

Reasons for modality switch to CHD were documented for 72/84 (86%) events; 12 were unknown. Primary reason for modality switch to CHD was found to be "burn-out" in 34 patients (47%). Either HHD became overwhelming, strained the patient-partner relationship or resulted in care partner's fatigue. Medical, non-dialysis related reasons caused drop-out in 16 patients (22%), while access issues were responsible in 12 patients (17%). Acute medical issues of the care partner resulted in 4 patients (6%) transferring to CHD. Lack of access to a home dialysis provider after moving resulted in 4 patients (6%) changing to CHD. Non-adherence and lifestyle issues resulted in transfer of 2 patients (3%).One third of the transfer to CHD occurred within the first 90 days after starting HHD.

Conclusions: Drop-out rate in HHD was found to be substantial and caused by a variety of reasons. The primary cause was the perceived burden of HHD and subsequent fatigue of both patient and partner. The impact of close attention to possible "burn-out" symptoms and respite care options needs to be evaluated.

SA-PO3027

Which Dialysis Facilities Chose to Transition into the Expanded Prospective Payment System (PPS)? Implications for Access to Care Richard Hirth,[1] Tammie A. Nahra,[1] Marc Turenne,[2] John R.C. Wheeler,[1] Joseph M. Messana.[1] *[1]Kidney Epidemiology & Cost Center, Univ. of MI; [2]Arbor Research.*

Background: January 1, 2011, Medicare implemented an expanded PPS under which drugs and tests formerly billed on a fee-for-service basis were added to a bundle of dialysis-related services. Dialysis facilities were given the opportunity to transition into the new payment system over 4 years. Those choosing to transition have payments based 75% on prior system ,and 25% on the expanded PPS in 2011, with an increasing percentage based on the PPS until the phase-in is complete in 2014.

Methods: We examined determinants of facilities' choice to transition rather than going directly to the PPS.

Large-dialysis organizations (LDOs) did not opt for the transition. Our analysis focused on non-LDO facilities, 22.3% of which opted for the transition. We estimated a logit model predicting the choice to transition.

Results: Opting for the transition was predicted by being hospital-based (vs. free-standing; adjusted odds ratio (AOR)=1.88), % Black patients (AOR 1.08 per 10% increase), and being in certain Census regions. Membership in a regional chain (vs. independent; AOR 0.36) and having >5% of patients on peritoneal dialysis (PD) (AOR 0.67) predicted lower likelihood of choosing the transition. Facility size, rural location, % pediatric, or having an exception to the prior payment system did not significantly predict the choice to transition.

Conclusions: Overall, a minority of facilities opted for the transitional payment, suggesting that they anticipate being worse off financially under the new system. Knowing which types of facilities were most likely to choose the transition can serve as an early warning system to identify possible access to care barriers (e.g., for Black patients) or financial challenges (e.g., for hospital-based or independent facilities), allowing CMS and others to monitor facility closures and other measures of access to high quality care. Even though LDOs entered the new payment system as a block, monitoring could extend to LDO facilities with similar characteristics. The lower likelihood of opting for the transition by PD facilities suggests that the PPS may be favorable to home dialysis.

Funding: Other U.S. Government Support

SA-PO3028

Economic Impact of Reduced HBV Testing among Hemodialysis Patients in the New Environment of Bundling: Results of a Study of De Novo HBV Infection Rates in a Mayo Clinic Hemodialysis Population – A Call for Changes in Current US CDC Guidelines on HBV Testing Protocols Macaulay A. Onuigbo,[1,2] Nnonyelum T. Onuigbo.[3] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology/Hypertension/Transplant, Mayo Clinic Health System, Eau Claire, WI; [3]Information Technology, NT Systems, Eau Claire, WI.*

Background: Hepatitis B Virus (HBV) infection is a serious public health issue. Hemodialysis (HD) exposes ESRD patients to significantly higher HBV risks. Therefore, current US CDC guidelines call for monthly HBsAg tests for all HD patients. The charge to Medicare per HBsAg test is about $100 per patient per month. In the new environment of Medicare Bundling, this is unwise and wasteful if the de novo HBV infection is rare.

The aim of this study is to determine de novo HBV infection rates among HD patients in a Mayo Clinic HD population between July 2000 to July 2010.

Methods: A retrospective analysis of electronic databases of all relevant HBV serology and clinical data from patients attending five Mayo Clinic HD units between July 2000 and July 2010 was carried out to identify de novo HBV infection.

Results: A total of 965 HD patients were studied. There was one de novo HBV infection - a case incidence rate of 0.1%. He was a 54-year old Caucasian male with a known history of IV drug abuse, and previous hepatitis C carrier. Transient asymptomatic Recombinant post-vaccination HBsAgenemia (false positive HBsAg) was identified in another patient, two days following an Engerix B HBV booster vaccination.

Conclusions: Our study demonstrated that de novo HBV infection among HD patients in the US is very rare. We recommend 3-monthly HBsAg testing for all HD patients, but to continue current monthly testing for IV drug users and other high-risk groups. There exists no solid evidence-base for the efficacy of monthly HBsAg testing. At the national level, with over 500,000 HD patients, this would translate to a mind-boggling $40 billion annual savings in Medicare charges. The US CDC current guidelines on HBV serology testing among US HD patients, last revised in 2001, are outdated, and must be revised to fall in line with current clinical realities on the ground.

SA-PO3029

Overview of Regular Hemodialysis Treatment in China Guangyan Cai,[1] Dong Zhang,[1] Xiang-Mei Chen.[1] *[1]Nephrology, PLA Genaral Hospital, State Key Laboratory of Kidney Disease, Beijing, China; [2]Nephrology, PLA Genaral Hospital, State Key Laboratory of Kidney Disease, Beijing, China.*

Background: The China Renal Data System (CRDS) is built to understand the situation of hemodialysis in China.

Methods: The data system collected information of dialysis center, dialysis equipment, demographic and treatment of hemodialysis.

Results: There were 3583 hemodialysis centers in China registered in CRDS. The total number of hemodialysis patients at the end of 2010 was 221 628. The mean age of patients was 53 and the male to female ratio was 1.44:1. The mean duration of hemodialysis was 3.03 years. Primary diseases were primary glomerular diseases (58.76%), diabetic nephropathy (16.83%), hypertensive nephropathy (10.7%), polycystic kidney disease (3.54%), renal calculus (2.39%) and others (7.78%). The standard-achieving rate of blood pressure in predialysis (<=140/90 mmHg) was 39.08%. The average dose of erythropoietin was 7498.33IU/week and the standard-achieving rate of hemoglobin in predialysis (>=110g/L) was 18.56%. The level of serum calcium was 2.1 to 2.37mmol/L in 36.73 percent of patients. There were 13.08% patients whose serum phosphorus was 1.13 to 1.78mmol/L. The standard-achieving rate of calcium-phosphorus product (<55%) was 51.08%. There were 24.22% patients whose serum phosphorus was 1.13 to 1.78mmol/L. The HBsAg positive rate was 7.81% and the HCV antibody positive rate was 33.64%.

The new hemodialysis patients in 2010 was 58732. The mean age was 52.18 and the male to female ratio was 1.5:1. The diseases were primary glomerular diseases (55.74%), diabetic nephropathy (17.29%), hypertensive nephropathy (9.62%), renal calculus (2.96%), polycystic kidney disease (2.67%) and others (4.36%). 6424 hemodialysis patients died in 2010, the mean age was 59. The mean duration of hemodialysis was 3.4 years. The causes of death were cardiovascular events (45.78%), cerebrovascular events (18.05%), infection (7.37%). In 2010, 1137 hemodialysis patients received kidney transplant treatment, and 653 patients changed to peritoneal dialysis.

Conclusions: This is the first survey on the treatment of hemodialysis in China. We have had an overview of situation of hemodialysis in China.

Funding: Government Support - Non-U.S.

SA-PO3030

The Changing Pattern of Primary Renal Disease in the Haemodialysis Population: A Forty Year Retrospective Review Siddhesh Mukund Prabhavalkar, Aisling E. Courtney. *Nephrology, Belfast City Hospital, Belfast, United Kingdom.*

Background: There is established geographical variation in the epidemiology of primary renal disease worldwide, but less is known about the temporal change in patterns of primary renal diagnoses over time. The aim of this study is to identify the variation in the pattern of primary renal diagnoses among incident chronic haemodialysis (HD) patients over last four decades.

Methods: All 4142 patients that were established on the chronic haemodialysis programme in Northern Ireland (NI) between 1970 and 2009 were included in this study. Clinical data was obtained from a prospectively recorded database. Each patient had a primary renal diagnosis classified according to the European Dialysis and Transplant Association (EDTA) coding system. The study period was divided into four decades: A (1970-79), B (1980-89), C (1990-99), and D (2000-09) in order to assess the change in patterns over time. Statistical analysis was performed using SPSS software and Cochran-Armitage Chi-square test was used for analysing trends.

Results: The greatest change was the proportion of people with diabetic nephropathy (type 1 diabetes rising from 0% in the 1970s to 9.5% in 2000s, $p<0.001$ and type 2 diabetes from 0.5% to 9.4%, $p<0.001$). There was also significant increases in the number of patients with chronic renal failure of unknown aetiology (from 17.8% to 29.1%, $p<0.001$), and renovascular disease (from 2.7% to 10.6%, $p=0.003$).

Conversely, there was a significant decrease in the proportion of patients with non-IgA glomerulonephritis (37% to 5.7%, $p<0.001$), interstitial nephritis (25.6% to 13.7%, $p<0.001$), and congenital nephropathy (2.3% to 0.8%, $p<0.001$).

Conclusions: There has been a significant change in the primary renal disease diagnosis in the HD population over past 40 years. The reasons for this are probably multifactorial including restricted acceptance policies in the early dialysis era, and advances in the management of diabetes mellitus and cardiovascular disease with increased numbers surviving to reach end-stage kidney disease. This is of relevance in planning HD provision in the future.

SA-PO3031

Emergent Support for Dialysis Patients Evacuated from the Northeast Japan Earthquake Disaster-Lesson from Previous Earthquakes Junichiro J. Kazama, Yoshikatsu Kaneko, Noriaki Iino, Shin Goto, Ichiei Narita. *Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.*

Background: Dialysis patients need to receive regular hemodialysis therapy, which requires a large amount of water and electricity supply. Therefore, they have less chance to survive under disastrous condition. In 2004 and 2007, magnitude 6.8 earthquakes attacked Niigata, a prefecture located at northwest coast of mainland Japan. One of the earthquakes even caused a minor accident in a nuclear power plant. We have overcome the crises by sending patients from destroyed dialysis facilities to unaffected ones.

Results: A magnitude 9.0 earthquake and subsequent tsunami struck northeast mainland of Japan on March 11, 2011. Dialysis patients had to evacuate from the affected area, because water and electricity supplies were severely destroyed. Within a week from the earthquake, Niigata University Hospital and its related facilities accepted 181 of those evacuated dialysis patients. None of them brought their own medical records. Upon arriving at Niigata, they immediately received triage decision by expert nephrologists, and those who diagnosed as in critical condition immediately received emergent hemodialysis. Severely exhausted patients were directly admitted to hospitals. Some of patients received decontamination before the hemodialysis session, because they had been exposed by radioactive substances by the Fukushima-Daiichi nuclear power plant accident. The incoming patients were given tags recording their name, age, blood access side, and dry weight, so that all the medical staff could view and share basic information regarding large numbers of first-time visiting patients in the dialysis facilities. Thus, the emergent rescue and subsequent maintenance hemodialysis therapies were successfully performed without any remarkable troubles.

Conclusions: The experience of previous two earthquakes was definitely helpful when we accepted evacuated hemodialysis patients on this present great disaster. The concrete plans for saving hemodialysis patients under disastrous conditions must be drawn up before it comes into reality.

Funding: Government Support - Non-U.S.

SA-PO3032

Aggravation of Blood Pressure Control in Patients with CKD5D after the March 11, 2011 Disaster in Japan and Its Potential Therapeutic Role as an α-Blocker Yoshihiro Tani, Kimio Watanabe, Yuki Kusano, Kenichi Tanaka, Yoshimitsu Hayashi, Koichi Asahi, Masaaki Nakayama, Tsuyoshi Watanabe. *Nephrology and Hypertension, Fukushima Medical University, Fukushima, Japan.*

Background: Control of blood pressure (BP) is aggravated by natural disasters such as earthquakes and it usually resolves within 4 weeks to the basal level. We examined the time course of BP variation and its influencing factors in patients with CKD 5D in areas of Japan affected by the earthquake that occurred on March 11, 2011.

Methods: We recruited 98 patients on regular hemodialysis (HD) from 3 HD centers in the Fukushima City area between February 28 and April 9, 2011. The profiles of the patients were as follows: mean age, 64.8 ± 13.9 years; male, 63%; HD vintage, 9.5 ± 12.6 years. The patients were administered with the following anti-hypertensive agents: calcium-channel blockers, 62.2%; ARB, 64.3%; ACE-I, 25.5%; diuretics, 27.6%; α-blockers, 29.6%; β-blockers, 21.4%; and DRI, 12.2%. Systolic and diastolic BP, heart rate (HR) and body weight (BW) at 4 weeks after the earthquake were retrospectively determined from medical records.

Results: Predialysis systolic and diastolic BP did not significantly change during the study period despite a significant reduction in increases in BW between dialysis sessions during the first week. However, postdialysis systolic and diastolic BP were significantly elevated (both $P < 0.01$; one-way repeated measures ANOVA), whereas HR and postdialysis BW did not change. The BP elevation after dialysis was sustained for 4 weeks even after correcting for the dry weight of each patient. Multiple logistic regression analysis revealed that α-blockers comprised the only independent factor for better post-dialysis blood pressure during the survey period: e.g., systolic and diastolic BP at week 4: OR, 0.25 (95%CI, 0.09 - 0.73) and 0.27, (0.07 - 0.65), respectively.

Conclusions: Blood pressure was aggravated at 4 weeks after the earthquake in patients with CKD 5D, and α-blockers comprised an independent influential factor for better BP. These findings indicate that α-blockers play a role in aggravated BP control after natural disasters in patients with CKD.

SA-PO3033

Advantage of HD/PD Combination Therapy in Disaster: Lessons from the Experience of the Japan Earthquake and the Fukushima Nuclear Accident Keita Kimura,[1] Makoto Ogura,[1] Akihiko Hamaguchi,[1] Tatsuo Hosoya,[1] Hiroyuki Terawaki,[1] Yasuo Kimura.[2] *[1]Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan; [2]Shinkasiwa Clinic, Chiba, Japan.*

Background: The HD (hemodialysis) / PD (peritoneal dialysis) combination therapy, which comprises five or six days of PD combined with one HD session per week, is performed in Japan to better control body fluid and remove solute. After the Japan earthquake and the Fukushima nuclear accident, medical resources for dialysis therapy were temporarily limited because of many transferred patients from the destroyed area to our hospital, shortage of a dialyzer and dialysate and failure of power supply. In addition, dialysis patients had difficulty in visiting the hospital because of transportation systems failure. In such extraordinary situations, in Kashiwa Hospital of Jikei University School of Medicine, ten patients on the combination therapy were obliged to stop HD session for two weeks. To evaluate the safety and validity of it, we assessed the condition of the patients before and after the earthquake.

Methods: Respiratory condition, body weight gain, blood urea nitrogen, creatinine and potassium level were evaluated before and after the earthquake at the beginning of each HD sessions.

Results: After the earthquake, ten patients visited the hospital two weeks after the last HD session skipping one HD session. No one showed symptom of respiratory failure after the earthquake. Body weight gain(2.0±0.6 vs1.9±0.5% of dry weight), blood urea nitrogen (55.9±10.8 vs 59.1±18.4 mg/dl), creatinine (14.9±2.1 vs 16.2±2.6 mg/dl) and potassium (4.6±0.8 vs 4.6±0.6 mEq/l) level were not significantly different before and after the earthquake.

Conclusions: The HD/PD combination therapy is a modality which allows patient to choose from both PD and HD after disaster safely depending on the situation. This is a large merit of the HD/PD combination therapy.

SA-PO3034

Gene Therapy with Indoleamine 2,3-dioxygenase Ameliorates Development of Chronic Rejection Changes in the Rat Allotransplantation Diana Vavrincova-Yaghi,[1] Maria Sandovici,[1] Marc Seelen,[2] Harry Van Goor,[3] Robert H. Henning,[1] L.E. Deelman.[1] *[1]Dep. of Clinical Pharmacology, UMCG, Groningen, Netherlands; [2]Dep. of Internal Medicine, UMCG, Groningen, Netherlands; [3]Dep. of Pathology and Medical Biology, UMCG, Groningen, Netherlands.*

Background: Chronic transplant dysfunction (CTD) is the primary reason for late allograft loss in kidney transplantation. Because there is no effective treatment available, improvement of long-term graft survival remains the major challenge in the kidney transplantation field. Indoleamine 2, 3-dioxygenase (IDO) is crucially involved in foeto-maternal tolerance, and prevents allograft rejection. In our previous experiment, we showed that gene therapy with IDO inhibits acute rejection of renal allograft. The aim of current experiment is to show whether IDO is also able to improve CTD.

Methods: Kidney transplantation was performed in a rat Dark-Agouti (DA) to Wistar-Furth (WF) CTD model. RGD modified adenovirus carrying IDO gene (RGD-AdTIDO, n=4) or RGD modified adenovirus carrying gene for GFP (RGD-AdTL, n=4) were injected into the renal artery of the donor kidney before transplantation. Recipients were immunosuppressed with cyclosporine for 10 days. After 10 days, the contra lateral kidney was removed. Body weight, serum creatinine and blood pressure (BP) were measured and 24 hour urine was collected every two weeks. Rats were sacrificed after 12 weeks.

Results: Local gene therapy with IDO significantly improved body weight during the whole experiment in comparison with RGD-AdTL treated rats. It also decreased elevated plasma creatinine (40 ± 4.8 μmol/l) compared to treatment with RGD-AdTL (55 ± 8.5 μmol/l, 12nd week) and elevated proteinuria (10.3 ± 3.1 mg/24 h for RGD-AdTIDO and 32.2 ± 9.3 mg/24 h for RGD-AdTL, 12nd week). The therapy did not affect blood pressure, except for the second week (121 ± 5 mmHg for RGD-AdTIDO and 144 ± 4 mmHg for RGD-AdTL). Moreover, IDO therapy significantly decreased the incidence of focal glomerulosclerosis (10.5 ± 1.5 %) compared to AdTL therapy (33.1 ± 3.37 %).

Conclusions: Here we show for first time the beneficial effect of local IDO gene therapy in a model of CTD.

SA-PO3035

Y-box Protein-1 Induces Collagen I Production in Mesangial Cells Following CsA Treatment Lydia Hanßen,[1] Bjoern C. Frye,[1] Tammo Ostendorf,[1] Christina Alidousty,[1] Sonja Djudjaj,[1,2] Peter Boor,[1] Thomas Rauen,[1] Jurgen Floege,[1] Peter R. Mertens,[2] Ute Raffetseder.[1] *[1]Department of Clinical Immunology and Nephrology, University Hospital RWTH Aachen, Aachen, Germany; [2]Department of Renal Medicine and Hypertonia, University Hospital Magdeburg, Magdeburg, Germany.*

Background: The immunosuppressants cyclosporine A (CsA) and tacrolimus (Tac) are part of the standard therapy to prevent allograft transplant rejection, however they can cause tubulointerstitial/mesangial fibrosis. The fibrogenic effect of these calcineurin inhibitors (CNIs) is induced amongst others by transforming growth factor (TGF)-β with reactive oxygen species (ROS) as mediators..

Methods: The YB-1 content of CsA and Tac treated rat mesangial cells (rMCs) was determined via immunoblot. YB-1 was immunoprecipitated from cytoplasm of CsA-challenged rMCs and coprecipitated mRNA was detected by RT-PCR. *In vivo* experiment was performed in C57BL/6 mice (single dose CsA s.c., 100 mg/kg).

Results: We investigated the role of Y-box-binding protein-1 (YB-1), a highly conserved transcription and translation factor, in the CNI-triggered renal fibrogenesis. Upon treatment with therapeutic relevant doses of CsA and Tac, the intracellular content of YB-1 protein rose up to 10-fold in rMCs depending on time and dose. Both hindering translation through cycloheximide as well as blocking the ERK/Akt phosphorylation pathways prevented CsA-triggered YB-1 activation in contrast to inhibition of transcription via actinomycin D. Furthermore, degradation rate of YB-1 protein was significantly reduced in response to CsA. Thus, rapid accumulation of YB-1 following CsA-challenge is due to translation of YB-1 mRNA stores and enhanced protein stability. ROS, especially hydrogen peroxide, mediate YB-1 upregulation under CsA. Notably, this process is independent of TGF-b. The enhanced expression of collagen I (Col I) in rMCs following CsA application is caused by mRNA-stabilization due to physical interaction with YB-1 protein and consequently, absent in YB-1-depleted cells. The CsA-induced accumulation of YB-1 in the mesangium could be confirmed *in vivo*.

Conclusions: YB-1 induces profibrotic effects of CNI in the kidney via regulation of Col I translation.

Funding: Private Foundation Support

SA-PO3036

Tacrolimus Induces a Myofibroblast-Like Phenotype in Human Kidney Fibroblasts by Ligand-Independent Activation of TGF-Beta Receptor Christian Koppelstaetter,[1] Susie-Jane Noppert,[1] Gerhard A. Mueller,[3] Michael Rudnicki,[1] Herbert Schramek,[1] Gert J. Mayer.[1] *[1]Department of Internal Medicine VI, Nephrology and Hypertension, Medical University of Innsbruck, Austria; [2]Department of Internal Medicine I, Clinical Immunology and Infectious Diseases, Medical University of Innsbruck, Austria; [3]Nephrology & Rheumatology Medical Center, Georg-August University Goettingen, Germany.*

Background: TGF-b is considered a strong inducer of renal interstitial fibrosis, which is a dominant factor in long-term outcome of kidney transplant recipients. Here we demonstrate the TGF-b-like effects of tacrolimus on kidney fibroblasts *in vitro* and the modulatory effect of NAD(P)H-oxidase 4 on this process.

Methods: The human renal fibroblast cell line TK-173 was treated with varying doses of tacrolimus (FK506, Prograf®) for three days. mRNA expression levels for NAD(P)H-oxidase 4, transgelin (a myofibroblast marker), TGF-b1, tropomyosin 1, and the collagen chain alpha-1(V) were determined by real-time qPCR. NOX4 protein expression and intracellular peroxide concentration were also determined.

Results: Tacrolimus-treated renal fibroblasts showed increased expression of NOX4, transgelin, tropomyosin 1, TGF-b1, and collagen mRNA. NOX4 up-regulation lead to a 20 % (max.) increase in intracellular hydrogen peroxide levels. TGF-b1 treatment duplicated the effects of tacrolimus. Specific inhibition of the TGF-b pathway repressed the effects of both tacrolimus and TGF-b1. Neutralization of extracellular TGF-b by specific antibodies almost completely abolished the reaction to TGF-b1, but left the response to tacrolimus unchanged. Si-RNA mediated knock-down of NOX4 had no effect on the tacrolimus-induced effects.

Conclusions: Tacrolimus at low nanomolar concentrations had TGF-b-like effects on cultured human renal fibroblasts. The binding of tacrolimus to FK506 binding protein 12 (FKBP12) leads to increased TGF-b receptor activity, even in the complete absence of ligand. This effect was sufficient to induce a myofibroblast-like phenotype and might thereby contribute to the induction of interstitial fibrosis in immunosuppressed kidney transplant patients.

SA-PO3037

Protection from Kidney Injury during Hibernation Is Associated with Increased Expression of X-Linked Inhibitor of Apoptosis Proteins (XIAP) Alkesh Jani, Swati Jain, Kameswaran Ravichandran, Charles L. Edelstein. *U of Colorado.*

Background: The 13-lined ground squirrel is a hibernating mammal that cycles through alternating phases of extreme cold ischemia (CI) for several days during torpor (T) followed by re-warming during interbout arousal (IBA). Hibernation is a natural model of DGF as evidenced by repeated prolonged CI during T followed by warm reperfusion during IBA. We hypothesized that hibernating squirrel kidneys are protected from apoptosis and necrosis.

Methods: Kidneys of C57BL6 mice and 1-2 year old summer (S), IBA and T squirrels were perfused with cold UW solution. The contralateral right kidney was immediately used as a control and the left kidney was stored in UW for 72 hours. Caspase-3/7 activity was measured using the fluorescent substrate DEVD-AMC. Apoptotic cells and the % of tubules displaying BBI were counted and scored by a pathologist. XIAP was detected by immunoblot and quantitated by densitometry.

Results: Tubular apoptosis, Brush Border Injury (BBI) and caspase-3 activity were significantly increased in mice vs. squirrels. To investigate the mechanism of protection against apoptosis in hibernating squirrels kidneys, we examined the protein expression of X-linked inhibitor of apoptosis proteins (XIAP). XIAP is a naturally occurring inhibitor of caspase-3. XIAP was significantly increased in hibernating squirrel kidneys, especially during IBA, whereas it was undetectable in mouse kidneys.

Table 1

Stage	Caspase-3 activity (nmol/min/mg)	Apoptosis (cells/hpf)	BBI score	XIAP protein
S (0 hr)	14	0.2	0	++
S (72 hr)	27	0.1	< 10%	++
T (0 hr)	6.5	0.1	0	+
T (72 hr)	13.6	0.0	0	+
IBA (0 hr)	23	0.1	<10%	+++
IBA (72 hr)	51	0	< 10 %	+++
Mouse (0 hr)	0	0	0	ND
Mouse (72 hr)	800*	1*	> 50%*	ND

n = 3; * p <0.05 vs mouse 0 hr, S, LT, IBA. ND = not detected.

Conclusions: Protection against CI in hibernating squirrel kidneys is associated with increases of XIAP. Mouse kidneys that are not protected against CI have undetectable XIAP. IBA kidneys that are most susceptible to CI and WR have the highest levels of XIAP.

Funding: NIDDK Support

SA-PO3038

Indoleamine 2,3-dioxygenase Inhibition Improves Renal Blood Flow Following 30 Minutes of Ischemia Todd D. Merchen,[1] John R. Gardner,[1] Rachel Harbarger,[1] A. Mellor,[1] James J. Wynn,[1] N. Stanley Nahman,[2,3] David M. Pollock.[1] *[1]Surgery, Georgia Health Sciences University, Augusta, GA; [2]Medicine, Georgia Health Sciences University, Augusta, GA; [3]Medicine, Charlie Norwood VAMC, Augusta, GA.*

Background: Ischemia reperfusion injury (IRI) is associated with delayed function and chronic allograft injury after renal transplant. Indoleamine 1, 2-dioxygenase (IDO) is an immunodulatory enzyme that mediates degradation of tryptophan. In a mouse renal ischemia model, IDO inhibition with 1-methyl-D-tryptophan (1-MT) improved serum creatinine (Cr) and decreased tubular epithelial cell apoptosis. To assess whether IDO improves post ischemia hemodynamics, we measured bilateral renal blood flow (RBF) in a rat ischemia model.

Methods: Three groups of SD rats (N=5) had bilateral renal artery probe placement and 1 hr of recovery. Shams (S) then had 30 mins of observation. Control (C) and 1-MT treated had 30 mins of renal artery clamp time, followed by 1 hr of recovery (POST). All rats then underwent recovery of serum and kidneys. 1-MT were pre-treated with 140 mg/kg of 1-MT 24 hrs prior to surgery and 1 hr prior to clamping.

Results: The serum kynurenine/tryptophan ratio was significantly reduced by 1-MT (0.052±.01 vs 0.103±.01uM, mean±SEM for 1-MT vs C, $p < 0.05$) indicating suppression of IDO. There were no differences in mean arterial pressure (101±2.1, 104±5, and 106±2.7mmHg, for S, C and 1-MT) or pre-ischemia renal blood flow (PRE RBF) (9±0.7, 11±0.7, and 14.4±1 ml/min for and 1-MT). However, 1-MT had significantly higher POST RBF: 11.6±1 vs 7.5±0.6 ml/min for 1-MT vs C ($p < 0.05$). Also, recovery of RBF in 1-MT was significantly increased: 83.2±3.5 vs 68.2±2.8% of PRE RBF levels for 1-MT vs C ($p < 0.05$). No differences were noted in post ischemia serum Cr(1.22±0.15, 1.21±0.10,1.21±0.04 mg/dl for S, C and 1-MT). These data suggest that IDO inhibition with 1-MT prior to renal artery cross clamping improves RBF 1 hr post ischemia.

Conclusions: IDO inhibition with 1-MT may help preserve renal function via an improvement in post ischemia hemodynamics. These findings also suggest that IDO may be a useful target to protect donor kidneys from the effects of IRI.

Funding: Private Foundation Support, Clinical Revenue Support

SA-PO3039

Early Outcome in Renal Transplantation Using Size Mismatched Recipients and Donors – A Porcine Experimental Model Tashi Chhoden,[1] Kristian Ravlo,[1] Peter Søndergaard,[1] Niels Secher,[2] Anna Krarup Keller,[4] Michael Pedersen,[3] Ulla Moeldrup,[5] Ernst Oeyvind Oestraat,[5] Rikke Norregaard,[4] Henrik Birn,[1] Bente Jespersen.[1] *[1]Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Anaesthesiology, Aarhus, Denmark; [3]MR Research Center, Aarhus, Denmark; [4]Inst. of Clinical Medicine, Aarhus, Denmark; [5]Dept. of Urology, Aarhus, Denmark.*

Background: Transplantation of a kidney from a large donor to a small recipient, as in pediatric transplantation, is associated with marginal renal blood flow, shut down of GFR and risk of thrombosis. To study the mechanisms regulating early graft function we established a porcine model of size mismatched renal transplantation. GFR, renal blood flow (RBF) and markers of kidney injury were studied within 10 h after transplantation.

Methods: After induction of brain death, kidneys were removed from 60 kg donor pigs and kept in cold storage for 22h until transplanted into small (15 kg, n= 8) or size-matched (60 kg, n=8) recipients. The first 10 h after transplantation we measured GFR as urinary clearance of 51Cr-EDTA with constant infusion, RBF by MRI-technique, urinary NGAL-excretion by ELISA, and renal expression of hemeoxygenase (HO)-1 by qRT-PCR.

Results: In small recipients mean GFR was reduced within 30 min after reperfusion compared to size-matched recipients. This was associated with a significant reduction in medullary(M)-RBF, which decreased steadily up to 9 h after reperfusion. No difference was observed in cortical RBF. While no significant difference was observed in urinary NGAL excretion, increased HO-1 mRNA levels were observed in the cortex of small recipients compared to size-matched recipients.

Conclusions: After transplantation of kidneys from large donors to small recipients in a model with high risk of delayed graft function, a very early reduction in GFR was observed when compared to size matched recipients. This may in part be due to a reduction in M-RBF, although other mechanisms are likely to be involved as M-RBF continued to decrease while GFR remained stable. Increased expression of HO-1 in kidney cortex may reflect ischemic injury, although cortical RBF was not reduced in the small recipients.

SA-PO3040

Association of Transforming Growth Factor-beta (*TGF*-β) Gene Polymorphism with Acute Rejection in Korean Kidney Transplantation Recipients Hyun Ju Kim, Tae Hee Kim, Sunwoo Kang. *Department of Nephrology, College of Medicine, Pusan Paik Hospital, Pusan.*

Background: Acute renal graft rejection influences the results of kidney transplantation. Acute rejection (AR) is mainly caused by T-cell immune responses activated by cytokines, included transforming growth factor-β (*TGF*-β). *TGF*-β inhibits the inflammatory response of T helper cells. Recent investigations based on epidemiologic and genetic studies have defined several single nucleotide polymorphisms (SNPs) in regulatory sequences of cytokines have been associated with allograft survival. In this study, we examined whether polymorphisms of the TGF-β gene were associated with susceptibility to kidney transplantation rejection.

Methods: A total of 342 patients who had received kidney transplants were included. We extracted genomic DNA from blood samples and amplified the genomic DNA using the primers for each SNP. Three SNPs of *TGF*-β gene were genotyped from genomic DNA with direct sequencing. We analyzed 3 SNPs of *TGF*-β gene (rs2228048, rs764522, rs3087465).

Results: Acute rejection developed in 62 patients (18%). There is no significant differences in age, sex, number of HLA mismatches, cause of renal failure, immunosuppressant regimen between the AR and non-AR group. The one SNP (rs3087465) of the *TGF*-β gene were significantly associated with the fewer episode of acute rejection in the recessive model (odds ratio 0.0 ; 95% confidence interval 0.00-NA, P=0.042).

Conclusions: Our results show that one *TGF*-β gene polymorphism was associated with acute rejection in Korean kidney transplantation recipients.

SA-PO3041

Investigating the Mechanisms That Prevent Antibody-Mediated Injury in Patients with Donor-Specific Anti-HLA Antibodies by Microarrays Nicole A. Hayde,[1] Yi Bao,[2] Robert Brent Calder,[3] Bin Ye,[3] Enver Akalin.[2] *[1]Pediatric Nephrology; [2]Einstein/Montefiore Kidney Transplant Program; [3]Computational Genomics Facility, Albert Einstein College of Medicine, Bronx, NY.*

Background: Although, most patients with donor-specific anti-HLA antibodies (DSA) develope acute or chronic antibody-mediated rejection (AMR), some demonstrate normal allograft biopsies. We aimed to investigate the mechanisms involved in protection of the allograft from antibody-mediated injury by microarrays.

Methods: We retrospectively reviewed biopsies of 221 patients performed in 2009 and 2010. The gene expression profiles of transplant kidney biopsies were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: 77 patients had DSAs at the time of the biopsy and 43 showed antibody-mediated injury (9 acute, 34 chronic AMR) and 24 were normal or demonstrated minimal injury. There was no difference in frequency or mean fluoresence intensity (MFI) values of DSAs between 2 groups. 12 chronic AMR samples and 5 with minimal injury were available for microarray analysis and demonstrated differential gene expression profiles, mostly related to immune responses. 16 of the top 50 differentially expressed genes were non-coding RNAs. Gene set enrichment analysis using Pathogenesis Based Transcripts created by Edmonton Group demonstrated cAMR biopsies had upregulated interferon-gamma-dependent rejection-induced ($p<0.01$) and cytotoxic T cell associated transcripts ($p=0.03$).

	Normal or Minimal Allograft Injury (n=24)	Antibody-mediated injury (n=43)	p-value
Age	47 (33-62)	45 (30-52)	0.51
Male sex (%)	58	56	0.84
African-American Race (%)	38	37	0.98
Deceased-donor transplant (%)	71	67	0.77
Previous transplant (%)	17	12	0.56
Time to biopsy (days)	118 (56-500)	1110 (447-2483)	< 0.001
Class I DSA (%)	67	74	0.5
Class II DSA (%)	46	63	0.18
Median Peak Class I DSA MFI	2334 (2041-4196)	3560 (2437-5245)	0.22
Median Peak Class II DSA MFI	7704 (1675-12279)	4980 (1904-8524)	0.62

Conclusions: The strength of DSAs could not determine the development of antibody-mediated injury. Microarray analysis of biopsy samples suggested that the control of immune response for prevention of antibody-mediated injury may be mediated through non-coding RNAs.

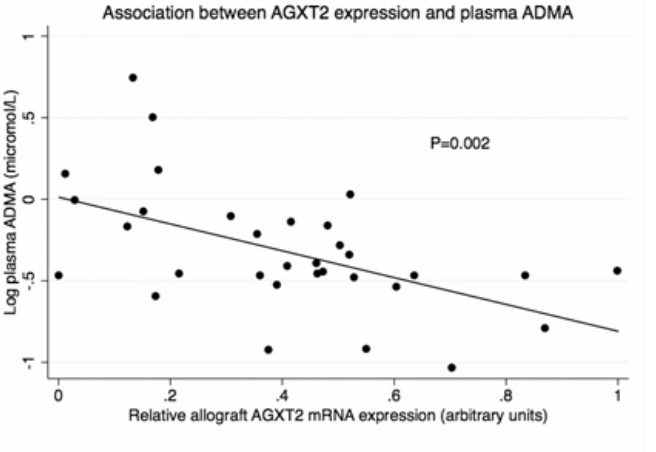
SA-PO3042

Allograft Expression of Alanine Glyoxylate Aminotransferase-2 in Protocol Biopsies Is Associated with Asymmetric Dimethylarginine Levels and Rate of Decline of Renal Function Ben Caplin,[1] Dorothea Nitsch,[2] Valeria Mas,[3] Jill T. Norman,[1] James M. Leiper,[4] David C. Wheeler.[1] *[1]UCL Medical School; [2]London School of Hygiene and Tropical Medicine; [3]Virginia Commonwealth University; [4]MRC Clinical Sciences Centre.*

Background: Asymmetric dimethylarginine (ADMA), inhibits nitric oxide synthesis, is metabolised by dimethylarginine dimethylaminohydrolase-1 (DDAH1) and alanine glyoxylate aminotransferase-2 (AGXT2), and is raised in kidney transplant reicipients. DDAH1 has been reported to influence decline in kidney function so we investigated the association between allograft AGXT2 gene expression, change in glomerular filtration rate (eGFR) and ADMA levels in renal transplant patients.

Methods: AGXT2 and DDAH1 mRNA were quantified by RTqPCR in 3-month protocol biopsies and plasma ADMA measured by LCMS. Associations between gene expression and decline in renal function were examined using a multilevel model of eGFR over the next year.

Results: Normalised mRNA levels were generated for 36 first kidney transplants, mean eGFR 52.9mL/min/1.73m^2 at biopsy. AGXT2 and DDAH1 expression were correlated. AGXT2 levels were inversely associated with plasma ADMA (Figure) and this persisted after adjustment for DDAH1 expression and eGFR. There was also a strong association between AGXT2 mRNA and eGFR decline: -0.17mL/min/1.73m^2/week (95% CI -0.32 to -0.02) for allograft AGXT2 expression above versus below the median (adjusted for donor and recipient age, sex and ethnicity and DDAH1 expression).



Conclusions: Independent replication of these observations is being pursued in a US cohort but these data raise the possibility that renal AGXT2 regulates plasma ADMA in transplant recipients. Taken with data on DDAH1 these findings suggest increased renal methylarginine metabolism associates with early decline in allograft function and that AGXT2 may be a potential therapeutic target.

Funding: Government Support - Non-U.S.

SA-PO3043

Renal Transplant Genome Wide Association Study (GWAS) Demonstrates Genetic Indicator of Long-Term Allograft Function/Survival Paul J. Phelan,[1] Robert P. O'Brien,[2] Judith Conroy,[3] Sean Ennis,[3] Mary T. Keogan,[4] Gianpiero Cavalleri,[2] Susan Jennings,[4] Derk O'Neill,[4] Peter J. Conlon.[1] *[1]Nephrology and Transplantation, Beaumont Hospital, Dublin, Ireland; [2]Molecular and Cellular Therapeutics, Royal College of Surgeons of Ireland, Dublin, Ireland; [3]School of Medicine and Medical Science, University College Dublin, Dublin, Ireland; [4]Histocompatibility and Immunogenetics, Beaumont Hospital, Dublin, Ireland.*

Background: Genetic interaction between donor and recipient genomes in renal transplantation is known to contribute to allograft rejection and overall outcome, although the nature of the genetic component remains largely uncharacterized. In this study we aimed to examine genetic variation in the recipient genome, with respect to allograft function at 5 years, as a pilot study to a wider donor/recipient genome-wide association study (GWAS).

Methods: We performed GWAS on a primary cohort of 326 Irish transplant recipients using the Illumina 610-Quad chip. Patients were first time, kidney-only, deceased donor transplants between 1993 and 2002. All patients were on calcineurin inhibitor based immunosuppression.

Results: Using linear regression, we found a significant signal (p=6.9 x 10-08) in Chromosme 14 in the TRA locus (TRA@) with respect to allograft function at 5 years post transplantation, as measured by serum creatinine. Serum creatinine at 5 years showed a statistically significant difference between genotypes (p=0.0006; mean 153.3 µmol/L V. 255.5 µmol/L). The TRA locus codes for T cell receptor alpha chains which have a key function in immune recognition.

Conclusions: These findings are interesting given our relatively small yet homogenous patient population and the potential biological mechanism at play. It emphasizes the importance of studying genetic variation in transplant outcome. We aim to investigate this further by replication in a new recipient cohort.

Funding: Private Foundation Support

SA-PO3044

Renal Allograft Biopsies Classified as AIN Display a CTL Molecular Signature Michelle L. Lubetzky, Darshana Dadhania, Janani Rangaswami, Thangamani Muthukumar, Kenar D. Jhaveri, Surya V. Seshan, Manikkam Suthanthiran. *Nephrology, Cornell University, NY, NY.*

Background: The molecular signature of post transplant allergic interstitial nephritis (AIN) has not been well described. Characterized by prominent eosinophils on biopsy, it is also known to share pathologic features with acute rejection (AR).

Methods: We identified 19 biopsies classified by pathology as AIN from a cohort of 512 for-cause biopsies from Jan 2008 to Feb 2011 (3.7%). From this cohort 8 patients had urinary specimens available for gene expression profiling. Immunohisotchemical staining (IHC) was done on corresponding biopsy specimens. We compared these urinary profiles and IHC to 5 patients with AR.

Results: Table 1 shows patient characteristics and treatments. Urinary profiling for Granzyme B (GB) revealed evidence of cytotoxic T cell response similar to that in AR (Figure 1A). IHC demonstrated significant staining for GB positive T cells (range of 5%-35%) in both AIN and AR biopsy groups (P=0.28). Graft survival was better when AIN occurred early in the post transplant course (<1 month, n=13) as compared with late (n=6), P=0.005, Figure 1B.

Table 1 AIN Patient Characteristics and Treatment Course (n=19)

Days post transplant	27 (5-3643)
Common offending agents	Bactrim (n=11), PPI (n=6)
Cre at biopsy (mean±SD)	2.99±1.88
% Peripheral Eosinophilia (mean±SD)	4.64±0.03
Eosinophils on biopsy	89% (17/19)
Treatment with Steroids (oral or IV Pulse)	89% (17/19)
Treatment by withdrawal of offending agent	100% (19/19)
Response to Therapy (return of cre to within 15% of baseline at 4 weeks)	79% (15/19)
Graft Loss	26% (5/19)

Figure 1A

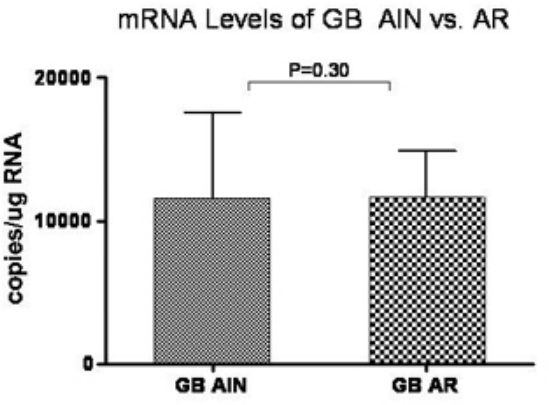
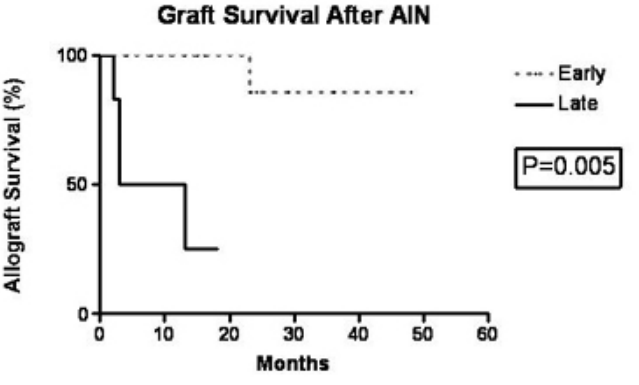


Figure 1 B



Conclusions: Our data suggests that AIN is associated with heightened expression of cytotoxic attack molecules similar to that seen in AR. The molecular response seen may have implications for the management of AIN.

SA-PO3045

KIM-1 Expression in Pre-Transplant Donor Kidney Biopsies as a Predictor of 1 Year Post Transplant Renal Function and Incidence of Acute Rejection Dwight M. Matthew,[1] Rachel E. Musial,[2] Sandeep Aggarwal,[1] Suganthi Soundararajan,[2] Gregory Malat,[3] Karthik M. Ranganna.[1] *[1]Nephrology, Drexel University College of Medicine, Philadelphia, PA; [2]Pathology, Drexel University College of Medicine, Philadelphia, PA; [3]Pharmacy, Hahnemann University Hospital, Philadelphia, PA.*

Background: Kidney injury molecule-1 (KIM-1), a type 1 transmembrane glycoprotein, is believed to be a specific histological biomarker for the diagnosis of early kidney allograft injury. However, its usefulness as a predictor of allograft function is uncertain.

Methods: The purpose of our study is to evaluate KIM-1 expression in pre-transplant donor biopsies as a predictor of 1 year post transplant renal function and acute rejection in the immediate post transplant period. In this study we included 44 patients who underwent deceased-donor kidney transplant from May 2008 to April 2010. We stained pre-transplant donor wedge biopsies for KIM-1 using immunohistochemistry.

Results: 23 out of 44 patients who had pre-transplant wedge biopsy for KIM-1 had a 1 month post transplant biopsy. Two out of 13 KIM-1 negative patients had evidence of acute rejection in 1 month post transplant biopsy while, 1 out 10 KIM-1 positive patients had acute rejection. The difference was not statistically significant (p=0.696). The 1 year post transplant eGFR (by MDRD) was 59.4 ±20.2 in KIM-1 positive and 60.5±23.7 in KIM-1 negative groups respectively (p=0.880).

	Biopsy 1 Month Post Transplant		
Donor Wedge Biopsies	AR	IFTA 0-2	IFTA 2-4
Positive KIM-1 staining: Total - 10 patients	1/10	9/10	1/10
Negative KIM-1 staining: Total - 13 patients	2/13	11/13	2/13

IFTA: Interstitial Fibrosis and Tubular Atrophy;

AR: Acute Rejection (both cellular and anti-body mediated)

Conclusions: Our results suggest that KIM-1 positivity in pre-transplant biopsies did not influence the 1 year post transplant renal function in patients who receive a deceased donor renal allograft. KIM-1 positivity did not predict an increased risk of developing acute rejection in the immediate post transplant period. The IFTA score, a marker of chronic allograft nephropathy, was also not different between the two groups.

SA-PO3046

Glomerular mRNA Expression of Pro- and Antithrombotic Factors in Thrombotic Microangiopathy in Renal Transplants Clemens L. Bockmeyer,[1] Putri Andina Agustian,[1] Svjetlana Lovric,[2] Anke Schwarz,[2] Mario Schiffer,[2] Maximilian Ernst Daemmrich,[1] Friedrich Modde,[1] Thorsten Feldkamp,[4] Udo Vester,[3] Verena Broecker,[1] Jan U. Becker.[1] *[1]Institute of Pathology, Hannover Medical School, Germany; [2]Clinic for Nephrology and Hypertension, Hannover Medical School, Germany; [3]Clinic for Pediatrics II, University Hospital Essen, Germany; [4]Clinic for Nephrology, University Hospital Essen, Germany.*

Background: Thrombotic microangiopathy (TMA) occurs in renal transplants as recurrent TMA or as de novo TMA with the two subforms CNI-toxicity TMA (CNI-TMA) and acute humoral rejection TMA (AHR-TMA). Since little is known about the contribution of the renal microvessels to thrombus formation, we analyzed the glomerular expression of pro- and antithrombotic genes.

Methods: Biopsies from 17 patients with TMA after transplantation (12 de novo and 5 recurrent TMA) were compared to 8 transplant biopsies without signs of TMA or humoral rejection. RNA was isolated from microdissected glomeruli of paraffin-embedded biopsies. The relative expressions of ADAMTS13, von Willebrand Factor (VWF), PAI-1, tPA, uPA, membrane cofactor protein (MCP), tissue factor and thrombomodulin were determined by quantitative RT-PCR after preamplification.

Results: Glomerular ADAMTS13 was higher in recurrent TMA and lower in de novo TMA compared to controls. Glomerular tPA was lower in de novo TMA, CNI-TMA and AHR-TMA compared to controls. Glomerular uPA was higher in recurrent and de novo TMA compared to the controls. Glomerular PAI-1 was higher in recurrent TMA, de novo TMA and AHR-TMA compared to controls. Glomerular MCP was higher in recurrent and de novo TMA than in controls. VWF, tissue factor and thrombomodulin were not found to be different between the cohorts.

Conclusions: These data provide insight into the contribution of the glomerular capillary bed to microthrombus formation in renal transplants. While all TMA forms seem to share partial defects in fibrinolysis and compensatory upregulation of MCP, different etiologies are reflected in differential expression of ADAMTS13. The increase of glomerular ADAMTS13 expression in recurrent and decrease in de novo TMA could be diagnostically and therapeutically relevant.

SA-PO3047

The Impact of Living Kidney Donation on Peripheral Blood Micro-RNAs Sophie Domhan,[1] Claudia Sommerer,[1] Martin G. Zeier,[1] Amir Abdollahi.[2] *[1]Medicine/Nephrology, University of Heidelberg Medical School, Heidelberg, Germany; [2]Radiation Oncology, University of Heidelberg Medical School, Germany.*

Background: The molecular effects of kidney donation by living donors are poorly investigated. Micro-RNAs (miRs) are considered as the masterregulators of transcriptome. We aimed to detect differential expression of peripheral blood microRNAs (miRs) as the function of unilateral nephrectomy in living donors.

Methods: So far, 16 patients consented to participate in this study. Whole blood total RNA including miRs were collected prior and post nephrectomy using PAXgene tubes. MiR isolation was performed using Qiagen's PAXgene Blood miRNA Kit and the QIAcube system. RNA quality control and quantitation was performed using total- and small RNA Agilent Chips (Bioanalyzer, Agilent) and NanoDrop spectrophotometer. Genome-wide miR profiling was performed with 12 samples representing 6 patients using Illumina's microRNA DASL assay. Clustering and statistics were performed using SUMO software package.

Results: We found 39 unique miRs to be differentially regulated post nephrectomy ($p<0.01$).The prevailing pattern was downregulation of miRs post nephrectomy (25 miRs) as compared to upregulation (14 miRs). In addition the extent of regulation was stronger among downregulated miRs. Given the inhibitory function of miRs on post/transcriptional regulation, our data suggest a global activation of the trancriptome via downregulation of miRs in response to nephrectomy. Interestingly, we found an enrichment of certain miR families among the downregulated miRs e.g., miR18a/18b, miR20/20* or miR27a/27b. Real-time quantitative RT-PCR using Taqman probes are performed to confirm the regulation of miRs in the entire collective. We further attempt to correlate the miR expression with renal function, urinary protein excretion and development of hypertension in kidney donors.

Conclusions: Our data demonstrate the feasibility of peripheral blood based miRNAs as sentinel organ to detect nephrectomy related effects in living kidney donors. This promising data might be instrumental towards identifying kidney donors at risk for post-nephrectomy associated deteriorations of health status via novel blood miR based biomarkers.

SA-PO3048

Urinary Angiotensin-Converting Enzyme 2 (ACE2) Is Increased in Renal Transplant Recipients Fengxia Xiao, Joe A. Zimpelmann, Valerie Catherine Cronin, Dean Fergusson, Greg A. Knoll, Kevin D. Burns. *Kidney Research Centre, Division of Nephrology, Dept. of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada.*

Background: Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the kidney, and may protect against kidney disease progression by degrading angiotensin (Ang) II to Ang-(1-7). ACE2 has been identified in human urine, and may be shed from tubular epithelial cells, where it is localized to the apical membrane. We tested the hypothesis that urinary ACE2 is increased in renal transplant recipients.

Methods: Spot urine samples were collected from healthy subjects (C: age: 43.6 yrs, eGFR 81.0 mls/min, urine alb/Cr 9.3±1.9 μg/mg, n=50), renal transplant recipients without albuminuria (Tx: age: 52.3 yrs, 30% diabetic, eGFR 48.6 mls/min, urine alb/Cr 5.3±0.6 μg/mg, n=50), and renal transplant patients with significant albuminuria (Tx+A: age 52.0 yrs, 48% diabetic, eGFR 49.5 mls/min, urine alb/Cr 246.8±33.1 μg/mg, n=50). No subjects were being treated with inhibitors of the renin-angiotensin system.

Results: Levels of urinary ACE2 mRNA did not differ amongst the 3 groups, while ACE mRNA was increased in Tx+A, compared to C ($p<0.03$). In contrast, urinary ACE2 activity was significantly increased in both groups of renal transplant recipients, compared to healthy subjects (C: 2.35±0.32 vs Tx: 5.94±0.82 vs Tx+A: 6.01±1.39 pg eq/mg Cr x 10^5; $p<0.03$ vs C), while ACE activity did not differ amongst the groups. By immunoblot, ACE2 was detected in urine specimens as a protein of ~120 kDa, with a smaller band at ~ 90 kDa. Transplant patients had significantly higher levels of urinary ACE2 protein on blot analysis, compared to controls ($p<0.04$). Urinary levels of Ang-(1-7) were significantly increased in transplant patients (C: 0.55±0.14 vs Tx: 1.38±0.25 vs Tx+A: 1.33±0.24 ng/mg Cr; n=50, $p<0.04$ vs C).

Conclusions: These data indicate that urinary ACE2 protein is increased in renal transplant recipients, independent of albuminuria. The higher urinary levels of Ang-(1-7) in these subjects suggest increased cleavage of Ang II by urinary ACE2 in this setting. Although its source is unclear, urinary ACE2 may be a marker of renal injury in transplant recipients.

Funding: Private Foundation Support, Clinical Revenue Support, Government Support - Non-U.S.

SA-PO3049

Effect of Immunosuppressive Drugs on DNA Repair in Human Peripheral Mononuclear Blood Cells Uzi Gafter,[1,2] Michal Herman-Edelstein,[1,2] Yaacov Ori,[1,2] Tsipora Malachi.[1] *[1]Nephrology and Hypertension, Rabin Medical Center, Petah Tikva, Israel; [2]Sackler School of Medcine, Tel Aviv University, Tel Aviv, Israel.*

Background: Cancer is a major cause of mortality among transplant recipients. Immunosuppressive treatment is one of the modifiable factors contributing to this phenomenon. Cyclosporine treatment in kidney transplant recipients was associated with reduced UV-induced DNA repair by peripheral blood mononuclear cells (PBMC) and increased cancer rate.

Aim: To investigate the effect of currently used immunosuppressive drugs on DNA repair.

Methods: H_2O_2-induced DNA repair by human PBMC was tested in-vitro in the presence of cyclosporine, tacrolimus, mycophenolic acid (MPA), sirolimus and everolimus at low to high non-toxic concentrations. The effect of combination therapy at maintenance levels was also tested.

Results: Cyclosporine and tacrolimus suppressed DNA repair throughout the tested dose range. In contrast, MPA, sirolimuis and everolimus did so only at the high doses. Maintenance doses of a combination of tacrolimus and MPA, the most frequent treatment regimen, reduced DNA repair, while MPA with sirolimus or everolimus did not.

Conclusions: Regarding risk of post-transplant malignancy, long-term treatment with mycophenolate mofetil or mycophenolate sodium combined with an mTOR inhibitor could be the treatment of choice.

SA-PO3050

Urinary Biomarkers in Cyclosporine A-Induced Nephrotoxicity Carla P. Carlos,[1] Nathalia M. Sonehara,[2] Sonia M. Oliani,[2] Emmanuel A. Burdmann.[1,3] *[1]Sao Jose do Rio Preto Medical School; [2]IBILCE, UNESP; [3]University of Sao Paulo Medical School, Brazil.*

Background: The aim of this study was to assess potential urinary biomarkers (Elisa assays) for cyclosporine A (CsA)-induced acute and chronic renal injury and compare them to renal tissue immunohistochemistry markers.

Methods: Male rats on low salt-diet (n=6/group) were studied 7, 14 and 21 d after CsA (15 mg/kg/day) or vehicle (V) treatment.

Results: CsA increased urinary fibronectin (ng/mL) after 7 (40.7±10.4 vs. 17.8±3.8 in V, mean ± SD) and 14 d of treatment (33.4±7.9 vs. 24.8±4.9 in V, $p<0.05$). Urinary TNF-alpha (pg/ml) presented the same pattern: 866±535 in CsA vs. 159±184 in V at day 7 and 836±243 in CsA vs. 401±349 in V at day 14, $p<0.05$. CsA increased urinary TGF-beta after 21d (746±1097 vs. 88±40 in V, $p<0.05$). CsA treatment increased urinary osteopontin (ng/ml) after 14 (404±73 vs. 167±99 in V) and 21d (467±139 vs. 120±59 in V, $p<0.01$). CsA increased urinary KIM-1 (pg/ml) in all periods: 1134±47 vs. 184±82 in V at day 7; 1152±50 vs. 264±87 in V at day 14 and 1013±186 vs. 372±224 in V at day 21, $p<0.01$). Renal immunohistochemistry showed an increased ED-1 infiltration (positive cells) in

CsA-treated rats at 14 (3.9±2.4 vs. 0.5±0.5 in V) and 21 d (15.4±13.1 vs. 0.8±0.8 in V, p<0.05). In the same way, increased tubulointerstitial alpha-actin expression (densitometry, arbitrary units) was observed in CsA groups after 14 (113±27 vs. 80±6 in V, p<0.05) and 21 d (142±16 vs. 69±10 in V, p<0.0001). Vimentin expression was increased only in CsA 21d (158±18 vs. 61±17 in V, p<0.0001), as well as periglomerular alpha-actin (145±12 vs. 70±11 in V, p<0.0001). Collagen IV expression was increased in all CsA-treated groups (137±10 vs. 88±10 in V at 7d, p<0.0001; 133±11 vs. 108±12 in V at 14d, p<0.01 and 135±7 vs. 111±17 in V at 21d, p<0.05).

Conclusions: We conclude that urinary fibronectin and TNF-alpha were indicators of the early phase of CsA-induced nephrotoxicity and urinary TGF-beta and osteopontin were observed in the later phase of the lesion. Urinary KIM-1 was elevated in all nephrotoxicity stages, suggesting that tubular damage is a continuous process during CsA exposure. Financial support: FAPESP 09/17100-2 and CNPq 150954/2009-3.

Funding: Government Support - Non-U.S.

SA-PO3051

Urinary NGAL Allows for Differential Diagnosis of Acute Kidney Injury in Renal Allograft Recipients Martina Guthoff,[1] Stephan Kemmner,[1] Silvio Nadalin,[2] Hans-Ulrich Haering,[1] Nils Heyne.[1] *[1]Dept. of Endocrinology and Diabetes, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Germany; [2]Dept. of General, Visceral and Transplantation Surgery, University of Tuebingen, Germany.*

Background: Neutrophil gelatinase-associated lipocalin (NGAL) regulates growth and differentiation in renal epithelia. In the absence of systemic inflammation (SIRS), urinary NGAL is of renal origin and an early and specific marker of acute kidney injury. Here, we demonstrate urinary NGAL at respective cut-off to accurately predict acute rejection among other causes of acute kidney injury in renal allograft recipients.

Methods: Spot urine specimen were prospectively assessed in 182 consecutive renal allograft recipients on maintenance immunosuppression upon presentation at our outpatient clinic. Samples were blinded and NGAL concentrations determined by ELISA. Patient data were classed according to allograft function and AKIN criteria into stable allograft function or acute kidney injury (AKI) and according to underlying condition into control, chronic allograft nephropathy (IFTA), bacterial- or viral infection, allograft rejection or other.

Results: In stable allograft recipients, median urinary NGAL [interquartile range] was 6.9 [3 - 13] ng/ml, or 10.8 [5 - 27] µg/g creatinine. A moderate increase was seen in IFTA, CMV and BKV infection. Urinary tract infection was associated with a significant increase in urinary NGAL, yet highest values were observed in acute allograft rejection. With a cutoff at 30 ng/ml, urinary NGAL discerned stable allograft function from AKI. At cutoff 100 ng/ml, elevated urinary NGAL accurately predicted acute allograft rejection within our cohort (AUC-ROC 0.98, sensitivity 1.0, specificity 0.93), even in the presence of urinary tract infection.

Conclusions: Urinary NGAL, at respective cutoff, accurately predicted acute allograft rejection among all other causes of acute kidney injury in kidney transplant recipients. As a readily available parameter, urinary NGAL facilitates to quickly delineate the pathogenesis of renal functional deterioration in allograft recipients presenting with a rise in serum creatinine.

SA-PO3052

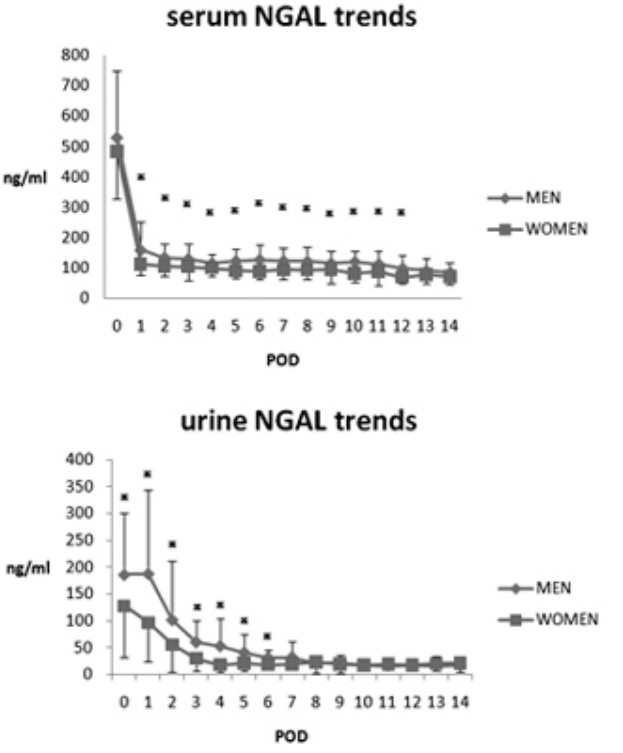
NGAL Trends in Living Donated Kidney Transplant Recipients during the Postoperative Period: Gender Differences Junko Kohei, Kosaku Nitta, Ken Tsuchiya, Takumi Yoshida, Michihiro Mitobe, Hidekazu Sugiura, Shunji Shiohira. *Fourth Department of Internal Medicine, Kidney Center, Shinjuku, Tokyo, Japan.*

Background: Neutrophil gelatinase-associated lipocalin (N-GAL) variations have previously been associated with acute kidney injury in postoperative period, and there are some reports about the NGAL trends after kidney transplantation. However gender difference in the NGAL trends after living donated kidney transplant recipients has not been studied in depth. This study's objective is to examine the gender differences in the NGAL trends during recovery after surgery.

Methods: We included only adult recipients undergoing living donated kidney transplantation. For NGAL detection the serum and urine samples were collected everyday after operation. The samples were analyzed using an immunoassay (ARCHITECT).

Results: In the results, 38 male cases and 20 female cases without delayed graft function were examined (mean age of male was 47±15, female was 47±13). In the results the male was changing highly intentionally rather than the females in the both serum NGAL and urine NGAL level. Gender difference in the urine NGAL trends became less clear on the POD7, in the serum NGAL, it was on the POD13 similarly.

[figure1]



Conclusions: This is a first report of gender differences in the serum and urine NGAL trends in the recovery process after renal transplantation. Renal dysfunction after kidney transplantation is difficult to diagnose with only serum creatinine, therefore using of the biomarker is expected as auxiliary diagnosis. This research has a high possibility of helping to diagnose delayed graft function in the postoperative early stage.

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-PO3053

Peripheral Blood CD19+ Lymphocytes Predict Kidney Allograft Interstitial Fibrosis and Tubular Atrophy on 1-Year Protocol Biopsies Karlo M. Mihovilovic,[1] Stela B. Bulimbasic,[2] Zoran S. Siftar,[3] Danica Galesic Ljubanovic,[2] Mladen Knotek.[1] *[1]Department of Medicine, University of Zagreb Medical School, Clinical Hospital Merkur, Zagreb, HR, Croatia; [2]Department of Pathology, University of Zagreb Medical School, Clinical Hospital Dubrava, Zagreb, HR, Croatia; [3]Department of Clinical Chemistry, Clinical Hospital Merkur, Zagreb, Croatia.*

Background: Noninvasive tests of cellular or humoral immunity may be helpful in prediction of late kidney allograft function and progression of chronic histology changes. Aim of this study was to assess whether specific subset of peripheral blood lymphocytes could predict 1-year renal function and renal histology changes over 1 year.

Methods: A cohort of 46 kidney transplant patients was analyzed. Immunophenotyping of peripheral blood lymphocytes (CD4, CD8, CD127, CD25 and CD19) was done by flow cytometry at kidney transplantation. Protocol kidney biopsies were done on day 0 and at 1 year after transplant and were scored according to the Banff 07 classification. Δci, Δct and Δah were calculated by subtracting 0 scores from 1 year scores. Univariate and multiple regression analyses were done to test correlations.

Results: In a univariate analysis recipient and donor age were positively correlated with 1-year Δci, Δct and Δah. The number of CD19+ and CD8+ cells was negatively correlated with 1-year Δci, Δct and Δah. Δah was also in a positive correlation with CD4/CD8 ratio. In a multiple regression analysis Δci (b=-0,54 ± 0.20, p=0,007) and Δct (b=-0,50 ± 0,20, p=0,018) were independently associated only with number of CD19+ lymphocytes. Δah remained significantly correlated only with value of CD4/CD8 (b=0,50 ± 0,17, p=0,007). In a univariate analysis 1-year creatinine clearence was negatively correlated with recipient and donor age and positively with CD19+ and CD8+ lymphocyte counts. However, recipient and donor age were the only variables that remained significantly correlated with 1-year kidney function in multiple regression analysis.

Conclusions: CD19+ cells in peripheral blood may be a useful biomarker for prediction of 1-year kidney allograft histology changes. Higher CD19+ cell count at kidney transplantation may be associated with slower progression of IF/TA.

Funding: Government Support - Non-U.S.

SA-PO3054

The Clinical and Molecular Significance of Glomerular C4d Staining Nicole A. Hayde,[1] Yi Bao,[2] Robert Brent Calder,[3] Bin Ye,[3] Enver Akalin.[2] *[1]Pediatric Nephrology; [2]Einstein/Montefiore Kidney Transplant Program; [3]Computational Genomics Facility, Albert Einstein College of Medicine, Bronx, NY.*

Background: Diffuse C4d staining, defined as involving more than 50% of the peritubular capillaries (PTC), is accepted as a footprint of antibody-mediated rejection (AMR). However, the clinical and molecular significance of isolated glomerular C4d staining is not known.

Methods: We retrospectively reviewed biopsies of 221 patients performed in 2009 and 2010. The gene expression profiles of transplant kidney biopsies were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: 115 biopsies (52%) were C4d negative, 40 (18.1%) were PTC C4d+ (22 diffuse and 18 focal) and 66 (29.9%) were isolated focal glomerular C4d+. While positive PTC C4d staining is significantly associated with acute or chronic AMR, circulating DSAs and higher class I DSA MFI values, higher spot urine protein/creatinine ratio, there were no statistical significant differences between isolated glomerular C4d+ patients compared to C4d negative patients. Comparison of gene expression profiles of 12 chronic AMR biopsies to 6 isolated glomerular C4d+ biopsies without DSAs and 4 with DSAs, revealed 2630 and 717 differentially expressed genes, respectively ($p < 0.01$), where 618 genes were mutual in both comparison. Differentially expressed genes were mostly related to cellular immune response activation in chronic AMR.

	C4d neg (n=115)	Isolated Glomerular C4d+ (n=66)	PTC C4d+	p-value
Age	50±15	45±15	39±13	< 0.001
Male Sex (%)	65	41	43	0.66
African-American race (%)	35	35	40	0.85
Deceased-donor tx (%)	73	71	63	0.45
History of previous acute rejection (%)	16	5	33	0.001
DSA frequency (%)	24	26	83	< 0.001
Peak Class I DSA MFI	2882 (1980-5551)	3603 (2041-7266)	5106 (3622-8034)	0.05
Peak Class II DSA MFI	4596 (1904-73170	3603 (2041-7266)	5106 (3622-8034)	0.16
Pathologic diagnosis (%)				< 0.001
Acute cellular rejection	7	3	2	
Acute AMR	0	0	15	
Chronic AMR/TGP	19	18	65	
Serum creatinine (mg/dL)	2.5	2.5	2.5	
Spot urine pr/cr	0.3 (0.1-1.2)	0.4 (0.1-1.2)	1.1 (0.2-2.4)	0.04
History of Previous Tx (%)	9	15	10	0.4

Conclusions: Isolated glomerular C4d staining does not have clinical significance and the gene expression profiles do not demonstrate immune activation.

SA-PO3055

Proximal Tubular Expression of Activated Ask1 Is Up-Regulated in Recipients with Early New-Onset Diabetes, Pre-Existing Diabetes Mellitus, and Impaired Fasting Glucose Post-Renal Transplantation Zoltan G. Laszik,[1] Sindhu Chandran,[2] David G. Breckenridge,[3] Flavio G. Vincenti.[2] *[1]Department of Pathology, University of California San Francisco, San Francisco, CA; [2]Division of Nephrology, University of California San Francisco, San Francisco, CA; [3]Gilead Sciences, Palo Alto, CA.*

Background: Apoptosis signal-regulating kinase 1 (ASK1) activates c-Jun N-terminal kinase and p38 in response to various stimuli such as oxidative stress and cytokines. Activation of ASK1 results in diverse cellular reactions including cell differentiation, inflammation, and apoptosis. New-onset diabetes after transplantation (NODAT) is an independent risk factor for graft loss of unclear etiology. The aim of our study was to assess the proximal tubular expression of activated Thr845 autophosphorylated ASK1 (pASK1) in 6-month post-transplantation biopsies with normal morphology of recipients with NODAT, pre-existing type 2 diabetes mellitus (DM), or impaired fasting glucose (IFG).

Methods: Renal transplant recipients with NODAT, pre-existing type 2 DM, and IFG who underwent a 6-month surveillance biopsy at UCSF were identified. Quantitative immunofluorescence analysis of pASK1 expression was compared in groups of recipients with NODAT (n=6), pre-existing type 2 DM (n=9), IFG (n=9), or non-diabetic recipients (n=7). ImageJ software was used to quantify nuclear expression of pASK1 in the proximal tubules as visualized by double pASK1 and Lotus stain on frozen sections.

Results: pASK1 nuclear expression in the proximal tubules was significantly up-regulated in recipients with NODAT (0.0930 +/-0.046, p=0.0001), pre-existing type 2 DM (0.0937 +/-0.050, p=0.0001), and those with IFG (0.0721 +/-0.035, p=0.0006) compared to non-diabetic recipients (0.0333 +/-0.023). No significant differences were detected between recipients with NODAT and those with pre-existing type 2 DM (p=0.48) and IFG (p=0.086).

Conclusions: Increased nuclear expression of pASK1 in the proximal tubules in 6 month surveillance biopsies of recipients with NODAT, pre-existing type 2 DM and IFG indicates a potential role of ASK1 in the pathogenesis of graft dysfunction in the diabetic milieu.

Funding: Pharmaceutical Company Support

SA-PO3056

Significance of Isolated Intimal Arteritis (v1) in Kidney Transplants: A Multicenter Observational Study Banu Sis, S. Bagnasco, Belinda Lategan, Mark Haas, Parmjeet S. Randhawa, Lynn D. Cornell, A. Magil, A. Herzenberg, Ian W. Gibson, Philip F. Halloran, Edward S. Kraus. *University of Alberta.*

Background: Microarray studies of biopsies from kidney transplants identified few isolated v-lesions (intimal arteritis) with no/minimal tubulointerstitial inflammation and low T cell transcripts, questioning whether these cases reflect true rejection (AJT 2007:7:2712-22).

We investigated the clinical significance of isolated v1 lesions in 266 conventional kidney transplants performed between 1999 and 2010 in seven transplant centers in North America.

Methods: We studied clinical parameters and graft survival (median follow-up after biopsy 44 months) in 100 isolated v1 biopsies (v1 and i<2 and t<2; group 1), in comparison to 90 biopsies with v1 plus high tubulointerstitial inflammation (v1 and i≥2 and t≥2; group 2) and 91 biopsies with v0 with minimal tubulointerstitial inflammation (v0 and i<2 and t<2; group 3). Biopsies with C4d positivity or biopsies from ABOi or cross-match positive kidneys were excluded. In selection of controls, no clinical parameter was matched (not to introduce bias). The biopsies were reviewed by a central pathology committee.

Results: The median post transplant time was 29 days in isolated v1, 33 days in group 2, and 21 days in group 3 biopsies (p>0.05). Indication for biopsies differed among groups: delayed or slow graft function triggered biopsies in 24% of isolated v1 group, but was uncommon in control groups (5% in group 2, 11% in group 3) (p<0.05). Serum creatinine at biopsy, 1 month and 6 month post biopsy did not differ among groups (p>0.05). Graft survival also did not differ among groups.

Conclusions: We conclude that, 1) isolated v1 biopsies are seen early and associated with increased delayed graft function; 2) v1 with or without high tubulointerstitial inflammation is not related to increased graft failure compared to v0. Thus, isolated v1 lesions, after the exclusion of antibody-mediated rejection, are of two types: T cell-mediated rejection and endothelial injury, and have no independent prognostic significance following anti-rejection treatment.

SA-PO3057

Pharmacodynamic Immune Monitoring in Pediatric Renal Allograft Recipients – Analysis of NFAT-Regulated Gene Expression and Immuknow® Yo Han Ahn,[1] Kyoung Hee Han,[2] Se Eun Lee,[2] Seong Heon Kim,[2] IL-Soo Ha,[2] Hae Il Cheong,[2] Hee Gyung Kang.[2] *[1]Center for Pediatric Oncology, National Cancer Center, Goyang, Republic of Korea; [2]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea.*

Background: Introduction of calcineurin inhibitor (CNI) as immunosuppressant has markedly improved the outcome of kidney transplantation. While therapeutic drug monitoring (TDM) is used to adjust the dosage of CNI, some patients, especially children, still suffer from rejection or infection and CNI toxicity. This study was to assess the adequacy of immunosuppression using pharmacodynamic monitoring.

Methods: Pharmacodynamic monitoring was done for 37 pediatric kidney allograft recipients. Expression of nuclear factor of activated T lymphocytes (NFAT)-regulated genes in patients' mononuclear cells was measured after activation, by qPCR of IL-2, IFN-g, and GM-CSF before (trough) and 1.5hr (peak) after ingestion of tacrolimus and the residual gene expression (RGE) was calculated. Global immune response was assessed by Cylex-ImmuKnow® assay. Trough and peak levels of tacrolimus were measured and clinical findings of rejection episodes and infectious complications were reviewed retrospectively.

Results: REG of IFN-γ and mean REG of the three NFAT-regulated genes showed negative correlation with tacrolimus peak levels. In patients with acute rejection episodes, REGs of IL-2 (35 ± 9 vs. 18 ± 24%) and IFN-γ (97 ± 58 vs. 40 ± 20%) were higher and tacrolimus peak levels were lower (15.3 ± 5.4 vs. 8.4 ± 3.0 ng/ml), compared to those in children without rejection. REG of GM-CSF (28 ± 33 vs. 47 ± 21%) was lower and tacrolimus trough level was higher (5.2 ± 1.8 vs. 4.0 ± 2.1 ng/ml) in patients with infectiouscomplication than in those without infectious complications. On the other hand, immune response measured by ImmuKnow® was not correlated with tacrolimus levels or clinical manifestations.

Conclusions: REG of NFAT-regulated genes showed correlation with clinical manifestation of under- or over-suppression of immune function in pediatric kidney allograft recipients. Further studies are required to assess whether pharmacodynamic monitoring as such would be more relevant than TDM.

Funding: Private Foundation Support

SA-PO3058

Subclinical Rejection Management and Transplanted Kidney Function in Children Undergoing Kidney Transplantation Yuko Hamasaki,[1] Masaki Muramatsu,[2] Riku Hamada,[1] Tomoyuki Sakai,[1] Kenji Ishikura,[1] Hiroshi Hataya,[1] Hiroyuki Satoh,[2] Seiichirou Shishido,[3] Masataka Honda.[1] *[1]Nephrology, Tokyo Metropolitan Children's Medical Center, Fuchu, Tokyo, Japan; [2]Urology, Tokyo Metropolitan Children's Medical Center, Fuchu, Tokyo, Japan; [3]Pediatric Nephrology, Toho University Omori Medical Center, Ota-ku, Tokyo, Japan.*

Background: The introduction of basiliximab and other chimeric monoclonal antibodies has decreased the incidence of early clinical rejection after transplantation. However, subclinical rejection may occur, and its treatment remains controversial.

Methods: We analyzed 81 children (45 boys) without clinical rejection after ABO-compatible living donor kidney transplantation. Their mean age was 8.5 years. Routine kidney biopsies were performed 4 months and 1 year after transplantation. Histopathological findings were evaluated according to the Banff classification (2007). Borderline (BL) or severer changes were defined as subclinical rejection. Rejection was treated by methylprednisolone or deoxyspergualin. Kidney function was assessed on the basis of glomerular filtration rates (GFR), calculated with the Schwartz formula. Changes in histopathological findings and transplanted kidney function at 1 year were examined according to the presence or absence of treatment 4 months after transplantation.

Results: Histopathological examination 4 months after transplantation showed BL in 42 patients (51.9%) and acute T cell-mediated rejection of grade IA or higher (AR) in 13 (16.0%). Anti-rejection therapy was given to 6 patients with BL and all patients with AR. Histopathological findings at 1 year were normal in 5 and BL in 1 of the 6 patients with BL who received therapy. The GFR improved slightly from 73.9 ± 9.5 mL/min at 4 months to 77.8 ± 9.6 mL/min at 1 year. In 8 of the 13 patients with AR, histopathological findings at 1 year were BL. Among the patients with BL untreated 4 months after transplantation, histopathological findings at 1 year were BL in 22 (61.1%) and AR in 8 (22.2%). The GFR decreased significantly from 80.0 ± 13.3 mL/min to 77.0 ± 14.4 mL/min ($p<0.05$).

Conclusions: BL or severer subclinical rejection should be treated to improve outcomes after kidney transplantation.

SA-PO3059

Effect of Treatment of Subclinical Borderline Rejection on Early Renal Allograft Function Grace Chabala Chibesakunda,[1] Ignatius Yun-Sang Tang,[1] Suman Setty,[2] Sanjeev Akkina.[1] *[1]Medicine, University of Illinois at Chicago, IL; [2]Pathology, University of Illinois at Chicago, IL.*

Background: The management of subclinical borderline rejection on protocol biopsy and its impact on renal allograft function is unclear. We evaluated the effects of two treatment strategies on early allograft function

Methods: All patients who underwent protocol biopsy within the first year of transplant were evaluated. Patients with subclinical borderline rejection were divided into two groups according to their treatment. Group 1 received methyprednisolone 1500 mg over two to three days and an increase in baseline immunosuppresion while Group 2 was treated with an increase in baseline immunosuppresion only. The baseline demographics, renal allograft function as assessed by MDRD eGFR at baseline, time of biopsy and 1, 3, and 6 months after treatment were evaluated. The change in eGFR from the time of biopsy at 1, 3, and 6 months after treatment was compared between the two groups.

Results: Twenty three patients had subclinical acute cellular rejection: Banff 1A (3), Banff 1B (2) and borderline rejection (18). There were 7 patients in Group 1 and 11 in Group 2. Group 1 had a lower eGFR than Group 2 at the time of biopsy (42.4+/- 13.3 vs. 63.4 +/- 22.5 mL/min/1/73m^2, p = 0.041).The eGFR at the time of biopsy and at 1, 3 and 6 months after treatment are shown in the table. There was a greater percent improvement in eGFR at one month in Group 1 than Group2 (134.2% +/- 33.8 vs. 103.1%+/-18.9, p = 0.027) but there was no difference between the two groups at 3 and 6 months.

Renal Allograft Function

	Group 1 (n =7)	Group 2 (n =11)	
Time	eGFR mL/min/1.73m²		P Value
At Biopsy	42.4±13.2	63.4±22.4	0.04
1 Month	55.7±18.8	60.2±21.8	0.67
3 Months	48.3±21.8	63.0±25.4	0.21
6 Months	47.8±18.7	76.0±27.6	0.05

Conclusions: In patients with subclinical borderline rejection, steroid treatment resulted in improvement of 1- month eGFR. However there was no difference at 3 and 6 months. Further studies are needed to assess the impact of steroid treatment on long term graft function

Funding: NIDDK Support

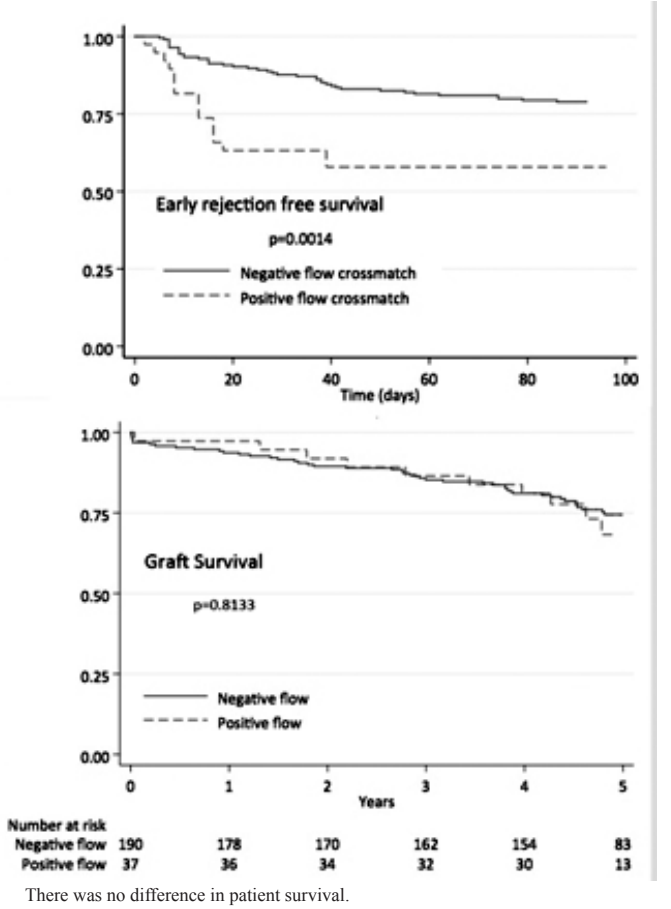
SA-PO3060

The Impact of a Positive Flow Cytometric Crossmatch on Long-Term Outcomes Following Deceased Donor Renal Transplant Geoffrey K. Dube, Sumit Mohan, David J. Cohen. *Medicine, Columbia University Medical Center, New York, NY.*

Background: Flow crossmatch (FCM) is often done at the time of deceased donor renal transplant (DDRT), and a positive FCM may be used either to exclude the recipient from receiving that transplant or to alter post-transplant management. Data are conflicting regarding whether a positive FCM at the time of DDRT is associated with an increased risk of acute rejection or graft loss. We analyzed the effect of a positive FXM at the time of DDRT on long-term outcomes.

Methods: We reviewed all adult recipients of a DDRT who were transplanted at a single center from 1/01/05-1/31/07. All patients had a FCM performed. 233 patients were transplanted, 38 had a positive FCM. All patients were on a rapid steroid withdrawal protocol. Maintenance immunosuppression consisted of tacrolimus and mycophenolate. All patients received induction therapy.

Results: Recipient demographics are shown in table 1. Rejection-free survival and graft-survival are shown in figure 1.



There was no difference in patient survival.

	Positive FCM (n=38)	Negative FCM (n=195)	p-value
Mean follow up (yrs)	4.27	4.35	NS
Age (yrs)	45.3	52.4	0.001
% female	42.1	45.1	NS
% African-American	23.7	31.8	NS
% diabetes	13.2	29.2	0.04
% retransplant	34.2	17.2	0.018
% thymoglobulin	77.8	92.1	0.044
% delayed graft function	42.1	48.2	NS
% ECD	18.4	30.2	NS
Cold ischemia (hrs)	30.5	31.6	NS
Creatinine 1-yr	1.8	1.8	NS
Creatinine 2-yrs	1.74	1.73	NS
Creatinine 3-yrs	1.61	1.74	NS

Conclusions: A positive FCM at the time of DDRT is associated with an increased risk of acute rejection. Despite a higher rate of acute rejection, especially in the first month post-transplant, there is no difference in patient survival, graft survival, or renal function at 3 years. A pre-tranplant positive FCM should not be used to exclude patients from receiving a DDRT.

SA-PO3061

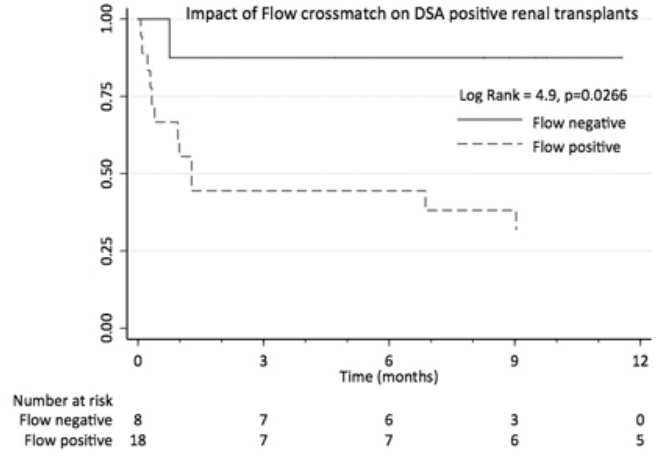
Flow Crossmatch Is a Better Predictor in Patients with Preformed Donor Specific Antibodies Demetra Tsapepas, Amudha Palanisamy, Sumit Mohan, Elena Rodica Vasilescu, Russell J. Crew, Bekir Tanriover, Geoffrey K. Dube, Nicole M. Ali, Lloyd Ratner, David J. Cohen. *Columbia University.*

Background: The impact of flow crossmatch results on transplant outcomes remains unclear, particularly in patients with donor specific antibodies (DSA). We attempt to determine if patients with a positive flow crossmatch (flow-xm) were more likely to have acute rejections than those who had a negative flow-xm despite the presence of DSA.

Methods: We performed a retrospective review of all adult DDRT recipients from 1/2009-12/2010. All patients had a negative CDC crossmatch at the time of transplantation and had solid phase assay measurement of HLA antibodies. A flow-xm was subsequently done if HLA antibodies (donor specific or otherwise) were detected by Luminex. We identified 26 patients with DSA who did not receive any additional treatment other than our standard induction regimen at the time of transplantation followed by our steroid free immunosuppression protocol of tacrolimus and mpa. In this cohort, 18 had a positive flow-xm and 8 had a negative flow-xm.

Results: Patients with positive flow-xm had a similar age (50.9 ± 14 v 57.5 ± 12.9, p=0.27), gender distribution (female 62.5 v 37.5%, p=0.35), race (white 50 v 62.5%, p=0.27) prevalence of pts with previous transplants (50 v 50%, p=ns) and cold ischemia time (28.2 ± 10.1 v 30.9 ± 9.6, p=0.55) to patients with a negative flow-xm. All patients with a negative flow-xm had MFI < 4500 by luminex. Patients with positive flow-xm have a significantly

higher risk of rejection (14.3 v 62.5%) and the 6 cases of early antibody mediated rejection (AMR) seen in our cohort were all present in the positive flow-xm group.



Conclusions: Among patients with preformed DSA, the presence of a positive flow-xm appears to predict a significantly higher risk of early acute rejection and in particular acute AMR.

SA-PO3062

Pre-Transplant Immunologic Risk Assessment in Patients with Donor-Specific Anti-HLA Antibodies Kwaku Marfo, Min Ling, Daniel G. Glicklich, Graciela De Boccardo, Enver Akalin. *Einstein/Montefiore Kidney Transplant Program, Bronx, NY.*

Background: We developed an algorithm for pretransplant immunologic risk assessment based on mean fluorescence intensity (MFI) values of Luminex single antigen beads and channel shift values of flow-cytometry cross-match results in patients with donor-specific anti-HLA antibodies (DSA) to decrease antibody-mediated rejection (AMR), which has been reported as 30-40%.

Methods: Patients' Anti-HLA antibodies with MFI values more than 5,000 were reported to UNET as unacceptable antigens. Kidney transplants were performed only if the MFI values of DSAs were less than 5,000 and flow T and B cell cross-match channel shift values were less than 150 and 250, respectively. Patients received anti-thymocyte globulin induction and IVIG (2 gram/kg) induction treatment.

Results: Cohort of 22 patients with pre-transplant DSAs received a transplant with this protocol. There were 15 female, 7 male, 15 African-American, 17 deceased-donor and 5 living-donor recipients with a median age of 50. 12 patients had class I, 8 class II, 2 both class I and II DSAs with a mean number of DSAs 1.68 ± 0.72, DSA MFI 3766 ± 3752, and peak Luminex PRA 70 ± 30 prior to transplant. During a mean follow-up of 373 ± 225 days, patient and graft survival was 95% (one patient died due to cardiovascular disease with a functioning allograft). 16 patients underwent 22 clinically indicated kidney biopsies and 2 showed (9%) AMR (both responded to treatment) and there was no acute cellular rejection or transplant glomerulopathy. 8 patients (36%) lost their DSAs during follow-up and the remaining patients' DSA MFI was 3471 ± 4783 at the last clinic visit. Patients have stable kidney function with a median serum creatinine level of 1.25 mg/dL. Patients were screened monthly for BK viremia and 2 patients (9%) developed BK viremia without polyoma nephropathy.

Conclusions: We demonstrate that pretransplant immunologic risk assessment based on MFI values of Luminex single antigen beads and channel shift values of flow-cytometry cross-match and transplant with anti-thymocyte globulin and IVIG significantly decreases AMR rates in kidney transplant recipients with DSAs.

SA-PO3063

The Effectiveness of Rituximab/Intravenous Immunoglobulin Therapy with Chronic Active Antibody Mediated Rejection in Renal Transplant Recipients Yu Ah Hong, Hyun Gyung Kim, In O Sun, Sun Ryoung Choi, Hoon Suk Park, Byung Ha Chung, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Nephrology, Internal Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea.*

Background: Chronic active antibody mediated rejection (cAMR) is one of the causes of chronic allograft kidney dysfunction. Until now, there is no established treatment protocol of cAMR. We report on our experience of a combination of rituximab and intravenous immunoglobulin (IVIG) treatment in patients with cAMR.

Methods: Six renal transplant recipients who showed progressive deterioration of graft function were included. The diagnosis of cAMR was confirmed with graft biopsy based on Banff' 05 classification. The patients received single dose of rituximab (375mg/m^2), followed by intravenous immunoglobulin (0.4g/Kg) once daily for 4 days. Steroid pulse was given concurrently at first 3 days. Human leukocyte antigen-donor specific antibodies (DSA) were detected by Luminex solid-phase assays before and after 2 weeks. The effect of a combination of rituximab and IVIG was assessed by graft function, amount of proteinuria, and titer of DSAs. The responder group was defined as glomeular filtration rate improved or stabilized after treatment.

Results: The response rate was 50% (3/6). GFR increased or maintained in three patients after Rituximab/IVIG treatment, but three patients did not respond to therapy. One patient of responder group showed a marked reduction of proteinuria after treatment. But, amount of proteinuria increased in all three non-responders. Follow up donor specific antibodies after treatment were checked in two responder patients. DSA titer dropped significantly in response to the therapy in one patient, whereas unchanged donor specific antibody titer was observed the other patients.

Non-responders had the high degree of transplant glomerulopathy, severely deteriorated allograft function, and heavy proteinuria at the time of diagnosis of cAMR. They had a longer duration of post-transplant time in comparison with responder patients. The treatment regimen was well tolerated.

Conclusions: A combination of Rituximab and IVIG may be effective for the treatment of cAMR at earlier stage.

SA-PO3064

Treatment of Vascular Rejection with ATG by CD3 Monitored Dosing Is Safe and Effective in Renal Transplantation Scott R. Henderson, Lucy A. Galloway, Robert Vaughan, David S. Game. *Nephrology & Transplantation, Guy's Hospital, London, United Kingdom.*

Background: Acute vascular rejection carries a poor prognosis for graft survival and proposed treatments have been associated with morbidity and mortality. Reports of ATG to treat vascular rejection are prior to the routine use of anti-CD25 antibody induction and include steroid resistant rejection. ATG dose guiding is most often performed according to total white cell count. We use ATG as first line treatment for Banff acute T cell mediated rejection type 2 and adjust dose according to CD3 count. This study reviews the efficacy and safety of this approach.

Methods: All patients treated at our centre with ATG between 2007 and 2009 were reviewed.

Results: 16 kidney transplant recipients and 4 simultaneous kidney-pancreas transplant recipients received ATG. 15 cases were classified as Banff 2A and 5 cases as 2B. Humoral component to the rejection was noted in 4/20. 17/20 patients received anti-CD25 antibody induction and all were receiving calcineurin inhibition, mycophenolate mofetil and prednisolone during acute rejection. 11/16 kidney transplant recipients had baseline immunosuppression switched from ciclosporin to tacrolimus; all other patients continued on tacrolimus. After an average of 642 days follow-up, 3 patients lost their grafts (average 8 months; 2/3 had persistent humoral component treated with plasma exchange and IVIg) and 1 patient died with a functioning graft. The mean creatinine censored for graft survival was 151 umol/l (n=17, SEM 44), representing an average 37% improvement from pre-treatment creatinine in patients not requiring pre-treatment dialysis (n=15). Bacterial infection was uncommon (n=1) although 6 patients were treated empirically for bacterial chest infections. CMV viraemia was common (n=12) and often within 1 week (n=7). There were no reports of fungal infection or malignancy.

Conclusions: ATG treatment for Banff type 2 acute cellular rejection in the modern era of immunosuppression is effective and safe when dose adjusted for CD3 count. CMV viraemia is common early after ATG treatment if a CMV surveillance strategy is used. A randomised controlled trial is required to further confirm the efficacy of this approach.

SA-PO3065

Evolving Therapies for Antibody Mediated Rejection: Is Bortezomib Better Than Rituximab? Michelle L. Lubetzky, Jennifer K. Walker, Marie Matignon, Choli Hartono, Thangamani Muthukumar, Surya V. Seshan, Manikkam Suthanthiran, Darshana Dadhania. *Nephrology, Cornell University, NY, NY.*

Background: It is not known if addition of Rituximab (Ritux) or Bortexomib (Bort) therapy (Rx) has added value in treating ABMR. We compared outcomes of ABMR in 26 renal allograft recipients treated with Ritux (n=11) or Bort (n=15) based Rx.

Methods: We reviewed for-cause renal allograft biopsies from 1/08 to 3/11. Inclusion criteria were ABMR by Banff'07, presence of DSA, and use of Bort or Ritux as a key component of Rx. Primary endpoint was response to Rx (return of Cre to within 15% of baseline at 4 wks). Secondary endpoints were reduction in DSA, proteinuria, and graft survival.

Results: Table 1 shows baseline data and details of ABMR. Response to Rx at 6 mths was 54% vs. 71% in Ritux and Bort groups, respectively. Bort was associated with a greater reduction in Class I DSA and proteinuria in patients with >1g/day of protein at time of ABMR diagnosis (Fig 1). 6 mth graft survival was 73% & 93% in Ritux and Bort groups respectively.

	Ritux	Bort	P
Post transplant days (median)	779 (26-2889)	38 (3-1853)	0.03
Biopsy Creatinine (mg/dl) (mean±SD)	3.55±2.2	3.92±2.8	0.94
Biopsy Protein (g/d) (mean±SD)	2.13±2.4	1.84±1.9	0.98
Rx IVIG ±TPE	73%	100%	0.06
Rx r-ATG	55%	73%	0.68
Response to Therapy	55%	73%	0.41
Post Rx Creatinine (3mths, mg/dl) (mean±SD)	2.9±1.9	2.1±1.1	0.23
Reduction of Class I iDSA	11.3±19.6% (n=3)	83.0±21.9% (n=9)	0.02
Reduction of Class II iDSA	14.2±15.0% (n=6)	38.2±34.5% (n=10)	0.43
Post Rx Proteinuria (3-6mths, g/day) (mean±SD)	7.3±11.4	1.29±1.7	0.05
6 month graft suvival	73%	92%	0.28

Figure 1A

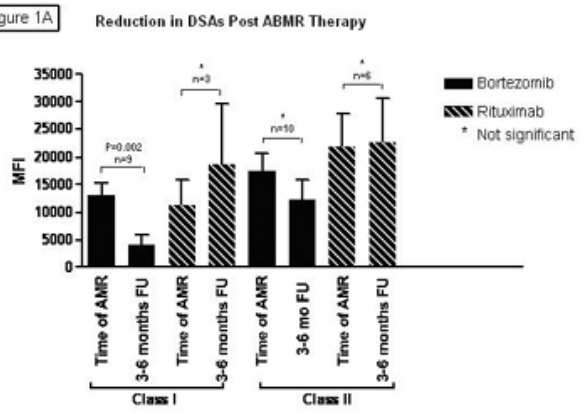
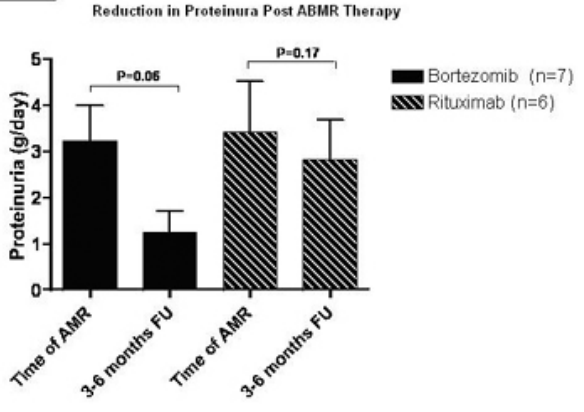


Figure 1B



Conclusions: Bort was associated with a higher rate of ABMR reversal and significantly greater reductions in DSA and proteinuria compared to Ritux. Validation of our observations favoring Bort over Ritux may help develop a standardized protocol for ABMR.

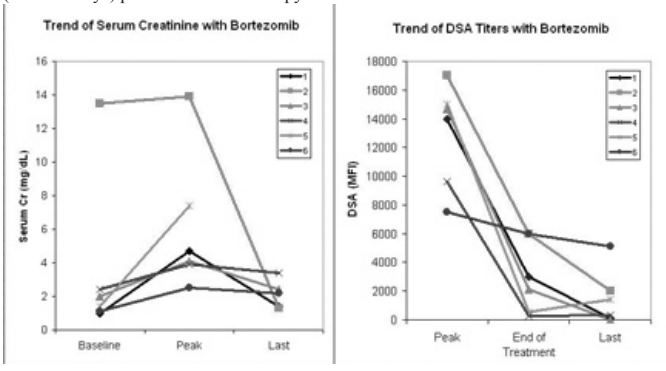
SA-PO3066

Treatment of Antibody Mediated Rejection in Kidney Transplantation: A Single Center Experience with a Bortezomib-Based Regimen Swati Arora,[1] Janice G. Nigos,[1] Parineesha Nath,[1] Ngoc L. Thai,[2] Akhtar Khan,[2] Kusum B. Tom,[2] Richard J. Marcus,[1] Sabiha M. Hussain,[1] Tina Y. Ko,[1] Rita L. McGill,[1] Kalathil K. Sureshkumar.[1] *[1]Nephrology and Hypertension, Allegheny General Hospital; [2]Abdominal Transplantation, Allegheny General Hospital, Pittsburgh, PA.*

Background: Antibody mediated rejection (AMR) following kidney transplantation (KTx) responds poorly to conventional anti-rejection therapies. The proteasome inhibitor bortezomib has activity against mature plasma cells that produce damaging donor-specific antibodies (DSA). We present our experience of using a bortezomib-based regimen in patients with severe AMR.

Methods: A retrospective chart review was performed on patients with biopsy proven AMR after KTx at our institution over 12-months. Diagnosis of AMR was made on the basis of positive peritubular capillary C4d staining along with either histological evidence of acute rejection or positive DSA titers. Treatment for AMR included plasmapheresis (1- 1.5 Volume), IVIG (cumulative dose of 1-2 g/kg), steroids, single dose rituximab (375 mg/m^2) along with bortezomib (1.3 mg/m^2) on days 1, 4, 8 and 11.

Results: AMR was diagnosed in 6 patients, aged 43±13 years, with PRA range 0-80%, and 4±1 HLA mismatches. There were 5 females, 2/6 had living donors, 5/6 had first KTx, 4/6 had alemtuzumab and 2/6 had rabbit-antithymocyte globulin induction; all were maintained on a tacrolimus/MMF/early steroid withdrawal protocol. Figure 1 shows serum creatinine and DSA titer trends. Five patients responded to treatment; one (#5) became dialysis dependent. Responders had stable kidney function during the follow-up (223±143days) post-bortezomib therapy.



Conclusions: This series demonstrates effectiveness of a bortezomib-based regimen in achieving reduction of DSA titers and stabilizing allograft function in patients experiencing severe AMR following kidney transplantation.

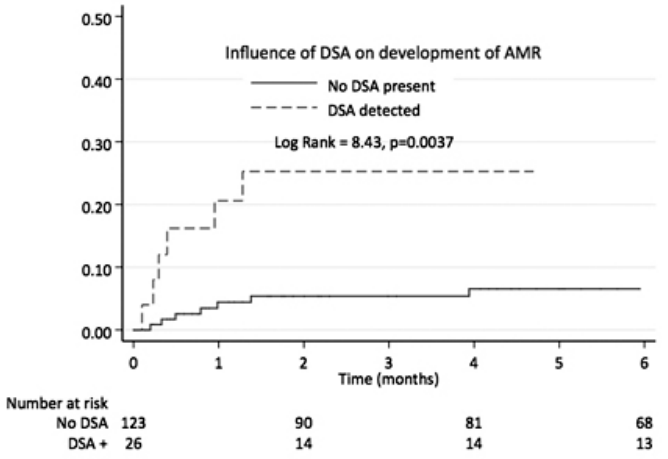
SA-PO3067

Presence of Donor Specific Antibodies Increases the Risk of Antibody Mediated Rejection Amudha Palanisamy, Demetra Tsapepas, Sumit Mohan, Bekir Tanriover, Russell J. Crew, Geoffrey K. Dube, Nicole M. Ali, Lloyd Ratner, David J. Cohen, Elena Rodica Vasilescu. *Columbia University.*

Background: The introduction of solid phase methods provides increased sensitivity and specificity for the detection of HLA antibodies. The clinical impact of the detection of preformed donor specific antibodies (DSA)on early rejections is unclear.

Methods: We performed a retrospective review of all adult DDRT recipients at our center from 1/2009 to 12/2010. All patients had a negative CDC crossmatch at the time of transplantation and had solid phase assay measurement of HLA antibodies. A flow crossmatch was subsequently done if HLA antibodies (donor specific or otherwise) were detected by luminex. We identified 149 patients with a negative flow crossmatch, 26 had DSA by Luminex and received no additional treatment other than our standard induction regimen at the time of transplantation followed by our steroid free immunosuppression protocol of tacrolimus and mycophenolic acid.

Results: In our cohort, patients with DSA were of similar age (53±13.8 v 55.8±14.1 yrs, p=0.35) and cold ischemia time (28.9±9.8 v 29.6±9.8, p=0.75) but were more frequently female (61.5 v 32.3%, p=0.005) and had a previous transplant (50 v 17%, p<0.001) than patients without DSA. Overall rejection rates (cellular and AMR) rates were higher in pts with DSA (50% v 22.6%, p=0.004) as were rates of antibody mediated rejection (23.1 v 6.5%, p=0.004). Additionally, the lowest MFI of preformed DSA among pts experiencing AMR was 6200.



Conclusions: Our center data demonstrates a higher overall risk of rejections and specifically a higher risk of antibody mediated rejections in patients with preformed DSA.

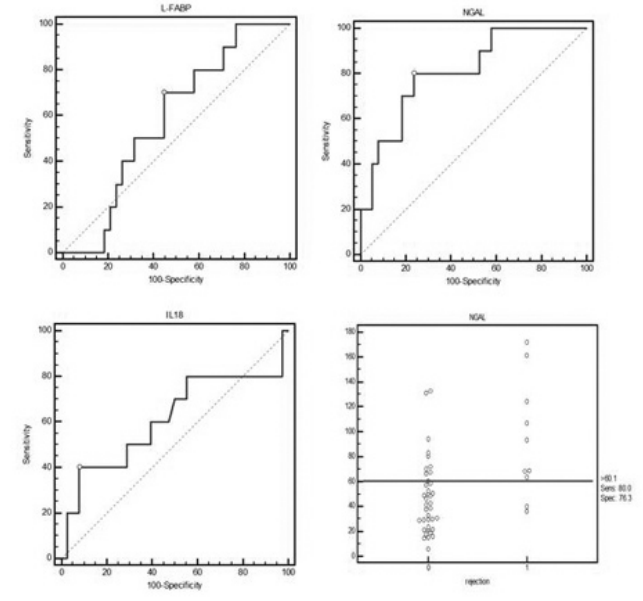
SA-PO3068

Neutrophil Gelatinase-Associated Lipocalin Is Most Sensitive to the Acute Rejection after Living Donated Kidney Transplantation Junko Kohei, Kosaku Nitta, Ken Tsuchiya, Takumi Yoshida, Michihiro Mitobe, Hidekazu Sugiura, Shunji Shiohira. *Fourth Department of Internal Medicine, Kidney Center, Shinjuku, Tokyo, Japan.*

Background: Delayed graft function (DGF) after kidney transplantation is difficult to diagnose with only serum creatinine, therefore using of the biomarker is expected as auxiliary diagnosis. Identification and validation of biomarkers of kidney injury has currently reported in this area. We hypothesized that these markers predicted rejection after living donated kidney transplantation.

Methods: This study was prospective, single-center study of living-donor kidney transplant patients to evaluate urinary NGAL, L-FABP and IL-18 including consecutive 48 (male=32) patients. We collected serial urine samples of 3 days after transplantation and analyzed using an immunoassay (ARCHITECT) for NGAL and standard commercial ELISA kits for LFABP and IL18. For diagnostic sensitivity of these biomarkers, receiver operating characteristic curve (ROC) was plotted and area under the curve (AUC) was calculated to quantify the accuracy of the parameter.

Results: Ten cases were clinically diagnosed or noted by biopsy as acute rejection and DGF was not observed in other 38 cases. NGAL was the most sensitive to detect an acute rejection in these markers. The best cut-off value of NGAL was 60.1ng/ml, with an Area under the ROC curve of 0.811 (95% CI 0.671-0.909). AUC of L-FABP, IL-18 were 0.584 (95% CI 0.433-0.725), 0.612 (95% CI 0.460-0.749).



Conclusions: Current methods for predicting graft recovery after kidney transplantation are not reliable. This research has a high possibility of helping to diagnose rejection in the postoperative early stage.

Funding: Pharmaceutical Company Support

SA-PO3069

Correlation of Pretransplant Trough Tacrolimus Level with Early Acute Rejection in Live Donor Renal Transplantation Kalpesh D. Gohel, Umapati Hegde, Sishir D. Gang, Mohan M. Rajapurkar. *Department of Nephrology, Muljibhai Patel Urological Hospital Society for Research in Nephro-Urology, Nadiad, Gujarat, India.*

Background: The risk of acute rejection is greater in the first week post transplant and progressively decreases after the first month. Thus, the concentration of immunosuppression must be maximal initially and tapered during subsequent months.The information of pretransplant administration of immunosuppression and acute rejection is minimal. So we aimed the present study to assess the correlation of baseline pretransplant trough tacrolimus level with early rejection.

Methods: We prospectively analyzed the trough tacrolimus level on pretransplant day of 179 patients transplanted from September 2007 to September 2009. We divided them into three groups according to the trough levels: Group I - < 5 ng/ml (n=34), Group II - 5-15 ng/ml(n=112) and Group III - 15ng/ml(n=33). Their demography, rejections, NOD, infections and biopsy proven CNI toxicity were studied.

Results: Their demography was comparable.Incidence of biopsy proven acute rejections were highest in the Group I and lowest in the Group III. Incidence of post transplant infection, new onset diabetes were comparable. Incidence of biopsy proven CNI toxicity was higher from group I to Group III.

Table.1 Results

Parameters	Group I (Tac Level< 5)	Group II (Tac Level 5-15)	Group III (Tac Level >15)
Mean Age (years)	49.06 + 10.15	47.38 + 9.6	49.42 + 10.2
Gender Male: Female	24:10	94:18	27:6
Relation: Related	28	76	22
Relation: Other than related	6	36	11
Donor Age	49.05 + 10.15	47.7 + 10.14	46.42 + 10.2
Induction	15 (44.1%)	35 (31.2%)	17(51.5%)
Anti proliferative -Azathioprine: MMF	20:14	50:62	11:22
Biopsy proven CNI toxicity	2(5.8%)	9(8%)	5(15%)
New Onset Diabetes	17(50%)	42(37.5%)	14(42.4%)
Rejection Episodes	12(35.3%)	27(24.1%)	05(15.2%)
Banff > 2 A	3	8	0
Post Tx Infections	12(35.3%)	33(29.5%)	15(45.4%)
Graft Survival % (1 year)	97.1	98.2	100

Tac level - Tacrolimus trough level expressed in ng/ml, CNI - calcineurine inhibitor

Conclusions: Incidences as well as severity of early rejection reduces as the pretransplant trough level increases. We did not encounter any higher grade TIR or antibody mediated rejection when trough level was > 15 ng/ml.

SA-PO3070

Assessment of Renal Allograft Fibrosis by Transient Elastography: Possibilities and Limitations Claudia Sommerer,[1] Michael Scharf,[1] Christoph Seitz,[1] Gunda Millonig,[2] Sebastian Mueller,[2] Martin G. Zeier.[1] *[1]Nephrology, University Hospital Heidelberg, Heidelberg, Germany; [2]Internal Medicine, Salem Medical Center, Heidelberg, Germany.*

Background: Chronic allograft dysfunction remains the major reason for late allograft loss. To date renal biopsy has been the only option for an early diagnosis. Throughout the last couple of years Transient Elastography (TE) has become a valid non-invasive alternative for diagnosing hepatic fibrosis. The purpose of the present study was to evaluate the possibility of identifying renal allograft fibrosis by using TE as well as analyzing its inherent limitations.

Methods: The tissue stiffness of 164 patients with renal allograft was measured twice, at the pole and the pars media of the allograft, using TE (FibroScan®). Clinical and anatomical data were collected for each patient.

Results: The measurement was successful in 310 out of 328 cases (94.5%). The mean elasticity was 34.8 ± 19.6 kPa and 33.6 ± 19.1 kPa for the pole and pars media respectively. There was no evidence for a correlation between allograft elasticity and renal function (estimated glomerular filtration rate: $r_{sp\text{-}Pole}$: -0.02; $p_{\text{-}Pol}$: 0.76 and $r_{sp\text{-}PM}$:-0.03; $p_{\text{-}PM}$: 0.68). Body-mass-index (BMI) and distance between skin and allograft have an impact on the success rate of the examination (BMI: $r_{sp\text{-}Pol}$: -0.31; $p_{\text{-}Pol}$: < 0.001 and $r_{sp\text{-}PM}$:-0.27; $p_{\text{-}PM}$: <0,001; distance: $r_{sp\text{-}Pol}$: -0.50; $p_{\text{-}Pol}$: < 0.001 and $r_{sp\text{-}PM}$:-0.56; $p_{\text{-}PM}$: <0.001). Sonographic proof of peri- or intrarenal accumulated fluid (cysts, lymphoceles) was accompanied by a drop in the success rate of 12.2% at the pole and 17.3% at the pars media.

Conclusions: Altogether using TE for the examination of renal allografts might be possible. Taking into account certain confounders, TE gains clinical significance. A requirement for a broader application, however, is the implementation of several technical modifications.

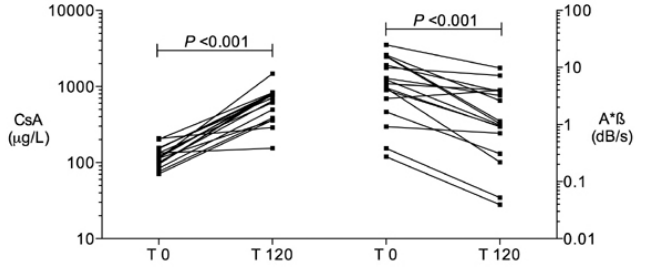
SA-PO3071

Acute Effects of Cyclosporine A on Kidney Allograft Microperfusion Visualized by Contrast-Enhanced Sonography Lars Kihm, Joerg Seckinger, Christian Morath, Martin G. Zeier, Vedat Schwenger. *Nephrology, University of Heidelberg, Germany.*

Background: Cyclosporine A (CsA) induces detrimental vascular remodeling, which is a leading cause of chronic allograft failure. Real-time contrast-enhanced sonography (CES) is a relatively new technique in providing quantitative information on microvascular tissue perfusion in kidney allografts in more detail. The purpose of the study is to assess acute effects of CsA to kidney allograft perfusion.

Methods: In an explorative single-center clinical trial, renal parenchymal tissue perfusion of 17 stable kidney allograft recipients was evaluated with CES prior to and two hours after the intake of CsA. In addition to laboratory and clinical parameters, Doppler indices and estimated glomerular filtration rate were measured.

Results: Systolic and diastolic blood pressure and color Doppler indices (RI and PI) did not significantly differ prior to and after the administration of CsA. There was a significant decrease of renal blood flow two hours after the intake of CsA compared to baseline. Kidney allograft microperfusion was reduced by 5.27 ± 4.08 dB/s.



Furthermore, there was a significant correlation between renal blood flow obtained prior to CsA administration and kidney function.

Conclusions: Acute effects of CsA on kidney allograft microperfusion visualized by CES revealed a 54 % reduction 2 h after the intake of CsA. Kidney allograft microperfusion correlates with kidney allograft function.

SA-PO3072

Combination of Blood Oxygen Level-Dependent Magnetic Resonance Imaging and Doppler Ultrasonography in Prediction of Acute Renal Rejection Ying Xu, Fei Han, Jianghua Chen. *Kidney Disease Center, First affiliated hospital, Zhejiang University, Hangzhou, Zhejiang, China.*

Background: Our previous study has shown that blood oxygen level-dependent magnetic resonance imaging (BOLD MRI) could discriminate between acute rejection (AR) and acute tubular necrosis. Other studies have demonstrated that Doppler ultrasonography could predict renal allograft outcome. This study aims to establish a mathematic model for predicting AR through the analysis of the data obtained from the imaging modalities.

Methods: 103 patients took BOLD MRI and Doppler ultrasound within 6 months after transplantation. 82 patients with normal functioning grafts took the examinations within 3 weeks post-surgery, 21 patients with biopsy-proven AR took the examination within 6 days before or after biopsy. Four parameters were recorded: medullary R2* value (MR2*), cortical R2* value (CR2*), segmental arterial resistance index (RI) and segmental arterial pulsatility index (PI). Support Vector Machine (SVM) was applied to separating the 4-dimensional data set (CR2*, MR2*, RI and PI) into two categories, AR and normal. A SVM model was tuned with 3-fold cross validation, the parameters were regularized to calibrate the classification and improve the training accuracy, which is the ratio of number of correct classification/total number of training samples.

Results: After performing the 3-fold cross validation, the average SVM accuracy on training data was 100%, which means the sensitivity and specificity are 100%, and the average training accuracy (MR2*+CR2*, MR2*+RI, MR2*+PI, CR2*+RI, CR2*+PI, RI+PI) was respectively 74%, 79.10%, 81.60%, 79.10%, 81.60%. After training the SVM model, a kernel function was produced for prediction. Due to the over-fitting of the classifier, the average test accuracy (the ratio of number of correct classification using the trained kernel function/total number of test samples) for four parameters is not high enough (around 65%) for AR.

Conclusions: The combination of BOLD-MRI and Doppler ultrasonography could be useful for the prediction of AR posttransplant by the SVM model and kernel method, larger sample should be tested to verify the accuracy, sensitivity and specificity of this mathematic model.

SA-PO3073

Clinical Usefulness of 3-Dimension Computed Tomography Angiography for Detection of Transplant Renal Artery Stenosis In O Sun, Yu Ah Hong, Hyun Gyung Kim, Hoon Suk Park, Sun Ryoung Choi, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: The aim of this study is to evaluate whether 3-Dimension computed tomography angiography (3D-CTA) is useful to detect transplant renal artery stenosis (TRAS).

Methods: In our transplant center, 9 of 514 (1.8 %) renal transplant recipients were diagnosed with TRAS according to renal angiography from 2004 to 2010. These patients received color doppler ultrasonography (CDU) and 3D-CTA ahead of renal angiography. A usefulness of 3D-CTA was compared to CDU on the basis of angiography. To investigate the safety of 3D-CTA, estimated GFR (eGFR) were measured before and after the 3D-CTA examinations.

Results: The patients included six men and three women, 27-56 years old (mean age, 43). The TRAS occurred from 2.6 to 24 months after transplantation (average 9.6 months). The median eGFR was 72.4 ml/min/1.73m^2 (range, 57-92). Three out of the nine patients diagnosed with TRAS were not detected by CDU. Two of them had TRAS with end-to-side (ES) arterial anastomosis, in which it can be difficult to detect the stenosis by CDU compared with the cases of EE. That is because the PSV at the stenosis in ES could be lower than that in EE due to anatomic and hemodynamic differences. However, 3D-CTA detected the significant stenosis in all patients with TRAS. Moreover, the stenotic area in 3D-CTA was similar to that of renal angiography (69.2±8.0 vs 69.5±6.5, p=0.88). There was no difference in eGFR before and after 3D-CTA examinations (77 ± 13.2 vs. 72 ± 14.1 ml/min/1.73 m^2, p = 0.54). All nine patients with TRAS were treated successfully by percutaneous transluminal renal angioplasty (PTA). After PTA, allograft function (72 ± 14.1 vs. 80 ± 11.0 ml/min/1.73 m^2, p = 0.028) and hypertension (mean arterial pressure, 111 ± 10.1 vs 98± 7.0, p = 0.011) improved.

Conclusions: The 3D-CTA is effective and safe method to screen the renal artery stenosis in renal transplant recipients with eGFR > 60 ml/min/1.73 m^2.

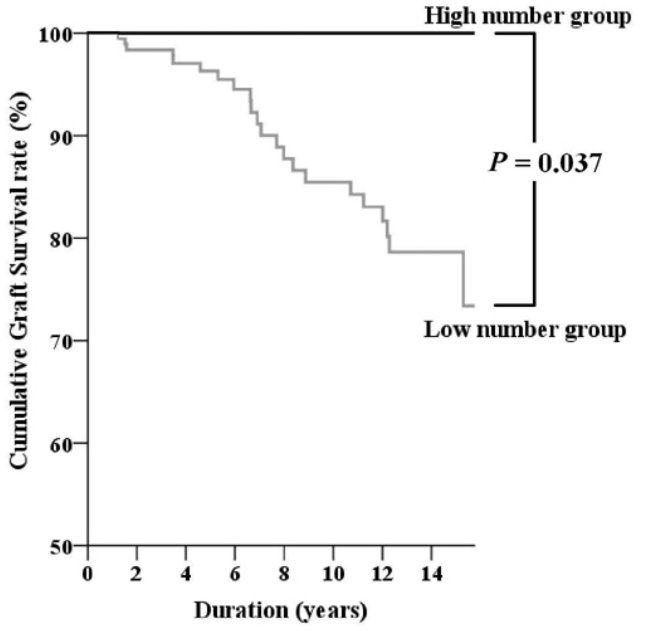
SA-PO3074

Combined Effect of Recipient's and Donor's Matrix Metalloproteinase Gene Polymorphisms on the Process of Kidney Aging after Kidney Transplantation Seung Seok Han,[1] Seung Hee Yang,[1] Jin Suk Han,[1] Suhnggwon Kim,[1] Yon Su Kim.[1,2] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Clinical Research Center for End Stage Renal Disease, Seoul, Korea.*

Background: The process of kidney aging is different in each individual, but the factors in the variation are largely unknown. Matrix metallopeptidase (MMP) 7 and 20 genes are associated with kidney aging, and thus we assessed the correlation between the MMP gene polymorphisms (rs880197 in MMP 7 (A > T) and rs1711437 in MMP 20 (G > A) and transplant outcomes in 235 recipients.

Methods: The transplant outcomes were assessed according to the number of A alleles combining those from both the recipient and the donor: the high-number group of A alleles (≥ 3) and the low-number group (< 3).

Results: During the follow-up period (mean 92.3 months), the high-number group of MMP 7 had a lower risk of chronic tubulointerstitial lesion than the low-number group (P = 0.015), while the high-number group of MMP 20 had greater risks of chronic tubulointerstitial lesion (P = 0.055) and glomerulonephritis (P = 0.025) than the low-number group. However, in the long-term (after 9 years of transplantation), the high-number group of MMP 20 had a greater transplant function (eGFR) than the low-number group.The high-number groups of MMP 20 showed a trend toward better graft survival rate than the low-number groups, especially when we analyzed the recipients who were followed for more than 1 year (P = 0.037).



Conclusions: The polymorphism of MMP 7 and 20 genes may be a surrogate marker that may be used to predict the long-term outcome after kidney transplantation.

SA-PO3075

Genetic Polymorphisms of Interleukines Associated with PTDM in Korean Renal Allograft Recipients Yang Gyun Kim,[1] Sang-Ho Lee,[1] Chun-Gyoo Ihm,[1] Tae Won Lee,[1] Kyung-Hwan Jeong,[1] Ju-Young Moon,[1] Sul-Ra Lee,[1] Eun Young Kim,[1] Yeong Hoon Kim,[2] Sunwoo Kang.[2] *[1]Nephrology, Kyung Hee University College of Medicine, Seoul, Republic of Korea; [2]Nephrology, Inje University Paik Hospital, Busan, Republic of Korea.*

Background: Posttransplantation diabetes mellitus (PTDM) is a serious metabolic complication after renal transplantation. Although β-cell dysfunction is considered the main contributing factor for the development of PTDM, precise pathogenesis was not identified. There are several studies about various cytokines that induce inflammation and destruction of islet beta cells in diabetes mellitus. But there is rare study about the cytokines associated with β-cell dysfunction in PTDM. So, was examined the association between PTDM and 18 single nucleotide polymorphisms (SNPs) located within the genes of 10 interleukines or their receptors, which were related to diabetes mellitus in Korean renal allograft recipients.

Methods: A total of 305 renal transplants recipients between 2000 and 2009 were included at 3 transplants centers, without a history of diabetes. We analyzed the association between the PTDM development and the 18 IL genes.

Results: The prevalence of PTDM was 18% (52/305 patients). Patients with PTDM group were older than those in non-PTDM (44.91±1.33 vs 38.34±0.71, p<0.001) Ten SNPs in five genes were significantly associated with PTDM development after adjusting with age, sex : IL1B (rs3136558), IL4 (rs2243250, rs2070874), IL7R (rs1494558, rs2172749) ,IL17R (rs2229151, rs4819554), IL17RB (rs1043261, rs1025689, rs3733075). Among haplotypes, the frequency of GGT in IL7R gene and TT in IL2 gene were significantly different between the patients with PTDM and those without PTDM. (p=0.044, 0.041,respectively)

Conclusions: These data suggest that genomic variations in IL1B, IL4, IL7R, IL17R and IL17RB are significantly associated with PTDM in Korea. Especially, significant variations of IL7R and IL17R, which was recently reported to be associated with type 1 DM, could elucidate the pathogenesis of PTDM in renal transplant recipients.

SA-PO3076

Significant Associations between Angiotensin Converting Enzyme and Angiotensinogen Genes Polymorphisms and Post Transplantational Diabetes Mellitus in Renal Allograft Recipients Sul-Ra Lee,[1] Kyung-Hwan Jeong,[1] Yang Gyun Kim,[2] Ju-Young Moon,[2] Sang-Ho Lee,[2] Chun-Gyoo Ihm,[1] Tae Won Lee.[1] *[1]Division of Nephrology, School of Medicine, Kyung Hee University, Seoul, Korea; [2]Kohwang Medical Research Institute, School of Medicine, Kyung Hee University, Sroul, Korea.*

Background: Post-transplant diabetes mellitus (PTDM) is a frequent and serious complication after kidney transplantation. Genetic polymorphisms of the angiotensin-converting enzyme (ACE) and angiotensinogen (AGT) genes have been reported to be related to diabetes mellitus and insulin sensitivity. But the role of these genes on the development of PTDM is not known. For this purpose, we investigated the association of polymorphisms in the genes for ACE and AGT genes with development of PTDM in Korean patients who had undergone renal transplants.

Methods: A total of 312 patients who had received kidney transplants without a prior history of diabetes were included. One ACE SNP (rs4291 in promoter –262) and two AGT SNPs (rs 699 in inton_1 and rs 4762 in intron 2) were genotyped from genomic DNA with direct sequencing.

Results: Of 312 patients, PTDM developed in 56 patients (17.9%). Patients in the PTDM group were older than those in the non- PTDM group (43.18±9.51years vs. 36.75±11.58). There were significant difference between two subjects in the percentage of tacrolimus use (n=18 vs. n=76; p=0.01).

Of three SNPs, the rs699 in intron_1 of the *AGT* gene was significantly associated with the development of PTDM in the codominant 1 (p = 0.009) and dominant models (p = 0.02).

In haplotypes of the rs699 in intron-1 of AGT gene, the frequency of the TCA haplotype was significantly higher in patients with PTDM than in those without PTDM.

Conclusions: AGT gene rs699 polymorphisms may act as genetic markers for the development of PTDM. Angiotensinogen may help identify patients who are at risk for PTDM. The exact molecular mechanisms still need to be clarified.

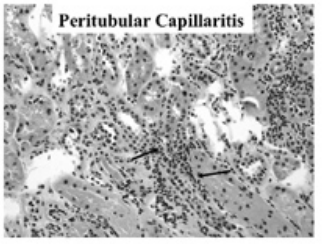
SA-PO3077

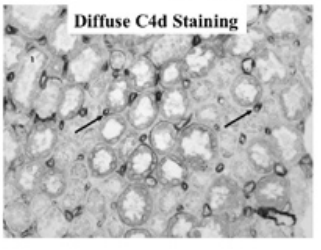
Concurrent Peritubular Capillary C4d Staining and BK Virus Nephropathy: Long-Term Outcomes Vijay Vidyasagar, Sanjeev R. Shah, Maha A. Mohamed, Brenda L. Muth, Jose R. Torrealba, John D. Pirsch, Dixon Kaufman, Hans Sollinger, Arjang Djamali. *Medicine and Surgery, UW-SMPH, Madison, WI.*

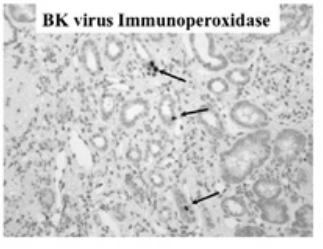
Background: BK virus nephropathy (BKVN) results from over-immunosuppression while peritubular capillary deposition of C4d is associated with under-immunosuppression. There is a paucity of information on long-term outcomes in patients who present with concurrent BKVN and C4d staining.

Methods: In a retrospective analyses of 69 consecutive kidney allograft recipients with biopsy confirmed BKVN between 2005 and 2011, we examined patient and graft outcomes in the presence or absence of peritubular capillary C4d staining (focal or diffuse). BKVN alone was treated with a reduction in immunosuppression ± cidofovir and IVIG while combined BKVN and C4d staining was treated with a reduction in immunosuppression + IVIG ± plasma exchange.

Results: There were 57 (82.6%) patients with BKVN alone and 12 (17.3%) patients with concurrent BKVN and focal or diffuse C4 staining.







The majority of patients were male (76%). Mean age at biopsy was 46.8 years. Mean follow-up after biopsy was 5.6 years. There were 6 deaths (8.7%) and 17 graft losses (24.6%). These differences were not statistically significant between the 2 groups. Similarly, there was no significant difference between Scr levels at biopsy and last follow-up suggesting that concurrent C4d staining in patients with BKVN is not associated with worse outcomes.

	C4d (+)	C4d (-)	p
N	12	57	
Age at Bx	42	47	0.19
Follow-up interval (m)	50	71	0.24
Scr at Bx	2.73	2.67	0.73
Last Scr	3.38	3.38	0.98
Patient Death	0	6	0.54
Graft Loss	4	13	0.68

Conclusions: These studies demonstrate that

(1) approximately 1/4 of allografts with BKVN may be lost within 5.6 years of diagnosis

(2) concurrent BKVN and peritubular C4d is not an uncommon feature on biopsy samples (17%)

(3) the presence of C4d is not a predictor of worse outcomes in patients with BKVN

SA-PO3078

Urinary Haufen Testing for Diagnosing Polyomavirus Nephropathy & Assessing Intrarenal Viral Load Levels: Comparative Analysis with Current Screening Tests Harsharan Singh,[1] Tomasz Kozlowski,[2] Vimal K. Derebail,[3] Volker Nickeleit.[1] *[1]Div. of Nephropathology, University of North Carolina, Chapel Hill, NC; [2]Transplant and Abdominal Surgery, University of North Carolina, Chapel Hill, NC; [3]Div. of Nephrology, University of North Carolina, Chapel Hill, NC.*

Background: PVN, a common complication post-renal-transplant, has an incidence of 4% (BANFF-PVN working group). Early non-invasive diagnosis facilitates patient management and graft survival. We developed a new qualitative test to detect three-dimensional viral casts, called "Haufen", in voided urine samples to accurately diagnose PVN and previously reported that urinary Haufen shedding is tightly correlated with PVN (positive and negative predictive values >95%). Hypothesis: The degree of urine Haufen shedding most accurately reflects extent of intrarenal PVN and can help distinguish PVN disease stage A (early) from stages B and C.

Methods: Quantitative analysis of urine Haufen shedding (#Haufen/mL urine), quantitative PCR assays on serum/urine,and urine decoy cells on 40 samples from 31 patients with concurrent biopsy proven PVN. Comparative analysis with biopsy findings (PVN disease stage, #cells/tubules expressing viruses (manual & morphometric counts, i.e.SV40-T, capsid proteins). Statistical analysis using Kruskal Wallis testing with ties.

Results: A) PVN disease stage: Only Haufen shedding showed a statistically significant difference between PVN stage A versus B or C ($p<0.0001$). Neither degree of viremia or viruria predicted any disease stage. B) Degree of intrarenal polyomavirus replication: Only Haufen shedding accurately reflected extent of polyomavirus replication in renal tubules, i.e.correlated with number of virus expressing cells or tubules. No significant correlation was found between degree of viremia and viruria.

Conclusions: Compared to other common laboratory tests, only quantitation of urinary Haufen shedding is an accurate reflection of the intrarenal burden of polyomavirus and can help predict PVN disease stages. This observation makes "Haufen-testing" the most accurate non-invasive technique not only to initially diagnose PVN but to also monitor therapeutic effects during follow-up.

SA-PO3079

Center-Specific Plasma BK Virus Levels in the Presumptive Diagnosis of BK Virus Allograft Nephropathy Amit Arora,[1] Sanjeev Akkina,[1] Suman Setty,[2] Shrihari Kadkol,[2] Ignatius Yun-Sang Tang.[1] *[1]Medicine, University of Illinois at Chicago, IL; [2]Pathology, University of Illinois at Chicago, IL.*

Background: The KDIGO clinical practice guidelines for the care of kidney transplant recipients suggest a threshold plasma BK virus (BKV) PCR level of >10,000 copies/ml for presumptive diagnosis of BKV allograft nephropathy (BKVAN). Determination of BK viremia by real time PCR varies with the methodology, the target gene (VP-1, 2, 3 or large T antigen) and the primers used. We evaluated the performance of an in-house developed PCR assay in the diagnosis of BKVAN.

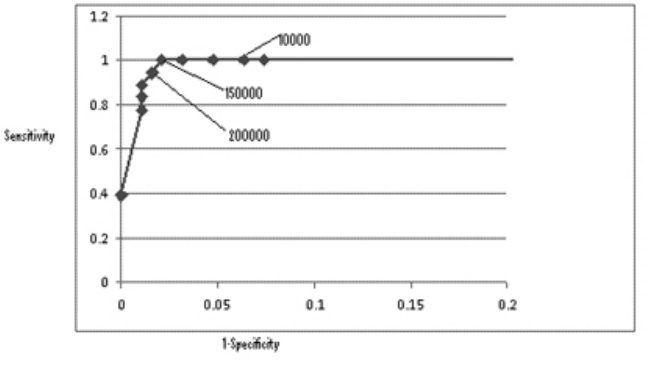
Methods: All transplant recipients with renal allograft biopsies and plasma BK virus PCR measurements during 2010 were evaluated. Viremia was measured using a primer set against the large T antigen. BKVAN was diagnosed with cytopathic changes and positive SV40 immunohistochemical stain. The sensitivity, specificity, positive and negative predictive values, positive likelihood ratio, and ROC curve were computed.

Results: Two hundred eighteen transplant recipients were evaluated: 39 had BK viremia and 18 were diagnosed with BKVAN. The performance characteristics are shown in Table.

Performance Characteristics

Plasma BKV (copies/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+Likelihood ratio
10000	100	93.7	60	100	15.9
50000	100	96.8	75	100	31.3
100000	100	96.8	75	100	31.3
150000	100	97.9	81.8	100	47.6
200000	94	98.4	85	99.5	58.8

The ROC analysis is depicted in Figure.



Compared to a plasma BKV PCR of 10,000 copies/ml, values between 50,000 and 150,000 copies/ml were associated with better positive predictive value and positive likelihood ratio.

Conclusions: The plasma BKV PCR levels for presumptive diagnosis of BKVAN should be validated for each individual assay. Nephrologists caring for transplant recipients should be aware of the differences in the performance of BKV PCR assays to optimize management.

Funding: NIDDK Support

SA-PO3080

Impact of Polyoma Virus in Blood Only Taken at Week 6 after Kidney Transplantation on Inflammation and Fibrosis/Atrophy in Biopsies Taken at Week 52 Willy Aasebo. *Akershus University Hospital.*

Background: Polyoma virus associated nephropathy (PVAN) is associated with graft loss due to fibrosis in kidney transplant recipients and is defined as the presents of BK virus (BKv) in biopsies. The aim of this study is to assess if early presence of BKv in blood only, PVAN excluded, affects histological markers of inflammation and fibrosis after one year.

Methods: Protocol biopsies were taken at weeks 6 and 52 in patiens transplanted during 2009. BKv-PCR was measured in weeks 10 and 52. Biopsies were stained for BKv if BKv-PCR in blood was positive, or if any histological or clinical suspicion of PVAN were present. Recipients with PVAN at week 6 were excluded.

Immunosuppressive treatment consisted of induction therapy with Basiliximab (two doses), thereafter calcineunone inhibitor, mycophenolate and steroids.

Results: After excluding 3 patients with PVAN at week 6, 156 patients were included.

At week 6 BKv were positive in 14 patients (9%), of whom 7 (50%) had BKv-PCR >5 x 10^3 copies/ml (median: 1.8 x 10^4 copies/ml).

At one year 12 recipients had positive BKv (8%), of whom 6 (50%) had >5 x 10^3 copies/ml.

Four patients were BKv positive both at week 6 and week 52.

Inflammation, fibrosis and graft function at one year according to Polyoma in blood at 6 weeks.

	Polyoma neg	Polyoma pos	P	BKv >5000	P
	N = 157	N = 14		N = 7	(neg vs.>5000)
Interst inflam	0.36 (0.77)	0.69 (0.95)	0.15	0.86 (1.07)	0.046
Tubulitis	0.50 (0.80)	0.92 (0.86)	0.074	1.00 (1.10)	0.061
Interst fibrosis	0.81 (0.63)	0.93 (0.83)	0.53	0.71 (0.76)	0.76
Tub atrophy	0.99 (0.59)	1.12 (0.86)	0.41	0.86 (0.69)	0.62
Creatinine (µmol/l)	116 (44)	103 (29)	0.27	98 (14)	0.33
Prot/Creat (mg/mmol)	27 (79)	21 (40)	0.95	30 (54)	0.89

Mean (SD)

The 4 recipients with BKv positive at both week 6 and 52 had mean interstitial fibrosis of 0.5, tubular atrophy of 0.75, tubulitis: 1.00 and interst. inflam: 1.00. Mean creatinine was 95 µmol/l (SD 13.2) and Prot/creat 8,5 mg/mmol (12.8) (NS vs. one positive BKv).

Conclusions: The presence of BKv in blood only early after transplantation does not increase fibrosis/atrophy one year after transplantation.

However, they had a tendency towards increased inflammation, especially in patients with BKv-PCR >5 x 10^3 and in patients with viremia both early and at one year.

SA-PO3081

Incidence of BK Virus Infection in Renal Transplant Recipients Induced with Alemtuzumab Versus Anti-Thymocyte Globulin Shahrouz Shadrou,[1] Bishal B. Rawal,[1] Kevin C. Roe,[1] Riaz Ali Shah,[2] Tadahiro Uemura,[2] Nasrollah Ghahramani.[1] *[1]Nephrology, Penn State Hershey Medical Center, Hershey, PA; [2]Transplant surgery, Penn State Hershey Medical Center, Hershey, PA.*

Background: BK virus associated nephropathy (BKVAN) is a major cause of graft failure. Treatment with lymphocyte depleting agents has been associated with increased incidence of BKVAN. Our primary aim was to compare the incidence of BK virus infection in renal transplant recipients based on the type of initial induction agent.

Methods: In a retrospective case-control study, 99 patients who underwent kidney alone transplant at our center between 8/2006 and 6/2010 were selected. We compared the incidence of BK virus infection in the first year among the alemtuzumab (AL) (n=50) and anti-thymocye globulin (ATG) groups (n=49). Maintenance immunosuppression for the AL group was steroid-free; for the ATG group, a 6-month steroid taper was used. Both groups also received tacrolimus and mycophenolate mofetil.

Results: In the AL group, 19 (38%) had living donors, 37 (74%) were Caucasian, 27 (54%) were male and age range was 53.6 ± 15.7. In the ATG group, 23 (46.9%) had living donors, 40(81.6%) were Caucasian, 34(69.3%) were male and age range was 49.4 ± 16.1.

The incidence of elevated BK PCR (>4000 copies) within the first year was not statistically different between the AL (n=12) and ATG (n=10) groups. Biopsy proven BKVAN was also not significantly different (AL: 2 patients; ATG: 4 patients). The incidence of biopsy proven acute rejection (AR) was not statistically different between the groups (AL: 21 episodes in 16 patients; ATG: 26 episodes in 18 patients). However, the mean time of onset of AR was significantly delayed in the AL group compared with the ATG group (157± 125 days vs. 73 ± 82 days; p=0.01).

Conclusions: Incidence of BKVAN and elevation of BK PCR is similar in induction with alemtuzumab and ATG. The incidence of acute rejection is similar between the two groups, but occurs later with alemtuzumab induction.

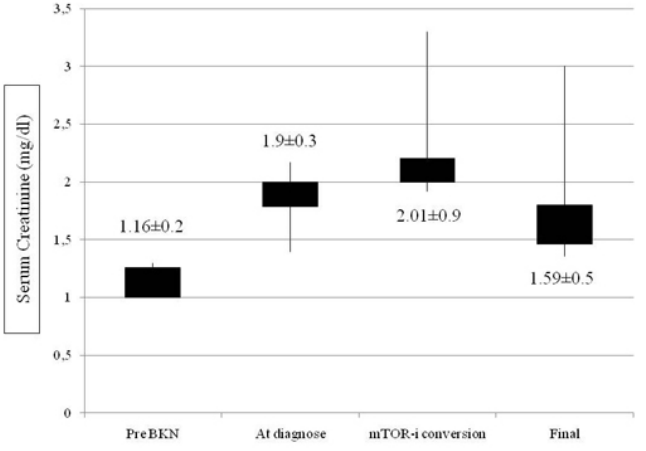
SA-PO3082

Successful Evolution of BK Virus Nephropathy Treated with Everolimus-Based Immunosuppression Natalia I. Polanco Fernandez, Laura Garcia-Puente Suarez, Esther Gonzalez Monte, Enrique Morales, Ignacio Bengoa, Jose M. Morales, Manuel Praga, Amado Andres. *Nephrology, H. U. 12 de Octubre, Madrid, Spain.*

Background: The incidence of renal allograft dysfunction due to BK virus nephropathy (BKVN) has increased over the last decade.Over the last few years mTOR-inhibitors (mTOR-i) have gained strength as a plausible treatment option.Based on this data, we initiated in 2007 an anticalcineurinic-free regime based on Everolimus (EVE) conversion protocol in patients with BKVN.Aims of the study were to evaluate renal allograft survival and BK viral load after conversion.

Methods: Diagnostic criteria for BKVN were:biopsy proven infection and/or positive serum viral load≥10000 copies demonstrated by PCR on at least two consecutive measurements.Of the 647 renal transplants done in our center between 2007 and 2010, 13 cases of BKVN were diagnosed.

Results: Of the 13 BKVN diagnosed cases, 8(4m/4f, aged 34.7±13.3 years) were converted to EVE-based regime, being the rest excluded because of proteinuria >0.8 gr/d.Follow up was 25±7 months.Maintenance immunosuppressive therapy consisted of tacrolimus(T), mycophenolate mofetil(MMF) and corticosteroids. Post-transplant renal function was optimal achieving a serum creatinine (sCr) of 1.16±0.2 mg/dl. Evolution is shown in the figure.



Treatment with MMF was stopped in all the patients and conversion from T to EVE was performed.Renal function improved in all the patients and the viral load became negative in 6 of them and diminished over 80% in the other two.None of them developed acute rejection after conversion to EVE.At the end of follow-up all renal allografts were functioning and viral load was under 10000 BK copies in every case.

Conclusions: BKVN treatment is still controversial. Our data suggest that treatment with mTOR-i could provide additional benefits in selected patients, preserving renal allograft function and lowering BK viral load.

SA-PO3083

Obstructive Uropathy May Be a Risk Factor for Polyomavirus Progression in Pediatric Kidney Transplant Recipients Debora Matossian, Farah N. Ali, Richard A. Cohn, Craig B. Langman. *Pediatric Kidney Disease, Childrens Memorial Hospital, McGaw Medical Center, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Background: Polyomavirus–associated nephropathy (BKVN) is recognized increasingly as a disease that may lead to progressive allograft dysfunction in pediatric kidney transplant (KTX) recipients. Published pediatric surveys reveal an incidence of BK-viruria from 18%-33%, BK-viremia 6%-16%, and BKVN 2%-8%.

The purpose of the study is identify patients with a KTX who have BK viruria, and evaluate for predictive risk factors for progression to BK viremia.

Methods: Retrospective chart review was performed of pediatric KTX recipients from January 2005 - December 2009. Analysis used unpaired t-test for normally distributed data, Mann-Whitney rank sum test for data not normally distributed, and Fisher exact test for qualitative data.

Results: 93 patients received a KTX in the 5 year period. 22 patients developed viruria (24%) and 12 of the latter group developed viremia (13% of total KTX). One patient with BK viremia had confirmed BKVN. No patient had graft loss due to BK disease during this period. Obstructive uropathy (OU) was the cause of end stage kidney disease in 22% of our total KTX population. 24% of such patients developed viremia, while only 7/71 patients (9.7%) of patients without OU had BK viremia (p=0.001). In patients who developed viruria but not viremia (n= 10), BK presented later in the transplant course (median 13.5 vs. 6 months post-KTX), in a higher proportion of preemptive KTX (50% vs.17%), in those with living donors (60 % vs. 33%) and in those with a younger age at the time KTX (median 6.5 vs. 13 years).

Conclusions: We found expected rates of viruria, viremia, and BKVN. However, BK viremia was more frequent in patients with obstructive uropathy. This supports the "two-hit" hypothesis in which immunosuppression and viral reactivation alone are insufficient to lead to BKVN; a damaged uroepithelium from a high-pressure system may be the predisposing factor. Ideal KTX scenarios (living donor, preemptive KTX, and younger age) favored resolution of viruria, decreased progression to viremia and consequently, a lower risk of BKVN.

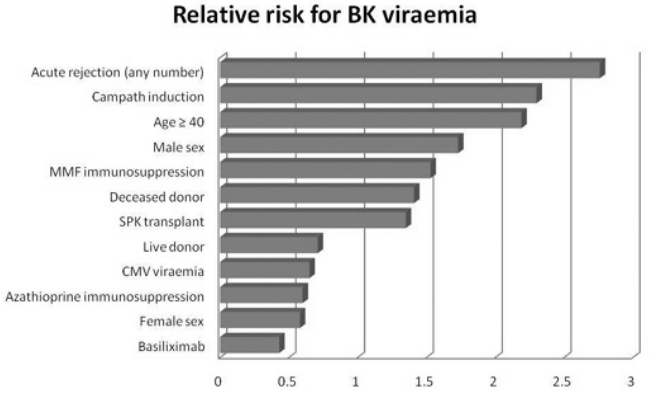
SA-PO3084

Risk Stratification To Improve Screening for BK Viraemia and Nephropathy in Kidney Transplant Recipients Vivian W. Yiu, Rui Gao, Miriam Rose Berry, Yisu Yisu Gu, Afzal N. Chaudhry, Sharon Mulroy. *Nephrology, Addenbrookes Hospital, Cambridge, United Kingdom.*

Background: BK viraemia occurs in 10-15% patients after renal transplantation. It can lead to graft dysfunction and loss, hence an effective screening programme can lead to early treatment and better outcomes. A screening protocol was introduced at our hospital in 2009 and we report on a risk stratification strategy developed after an audit of compliance.

Methods: A database was set up to collect data on all patients receiving a kidney transplant or simultaneous kidney-pancreas (SPK) transplant from May 2009 to January 2011. Data were collected from electronic and paper records.

Results: Acute rejection is the most predictive factor for developing subsequent BK viraemia, with a relative risk (RR) of 2.76. There was no significant association between the severity of the histological grade of acute rejection with BK viraemia. Other significant risk factors include Campath induction (RR=2.30), age ≥40 years (RR= 2.19), male gender (RR= 1.73), the use of Mycophenolate mofetil (MMF) in immunosuppression regimes (RR= 1.53), a deceased donor (RR= 1.41) and receiving a SPK transplant (RR= 1.35). Factors associated with lower risk of viraemia include Basiliximab induction (RR=0.43), the incidence of CMV viraemia (RR=0.65), use of Azathioprine (RR= 0.6) and female gender (RR=0.58).



Conclusions: Patients with more risk factors for BK viraemia should be screened more intensely in order to identify viraemia early and allow prompt treatment. SPK patients (receiving Campath and MMF) and those treated for acute rejection should prompt more frequent screening. We propose to develop a scoring system based upon these risk factors to streamline the screening protocol and target monitoring more effectively.

SA-PO3085

Factors Influencing the Course of Polyoma BK-Virus Nephropathy after Renal Transplantation Anke Schwarz,[1] Albrecht Heim,[2] Theodor Framke,[3] Hermann G. Haller.[1] *[1]Medicine, Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Virology, Hannover Medical School, Hannover, Germany; [3]Statistics, Hannover Medical School, Hannover, Germany.*

Background: The course of polyoma BK-virus nephropathy (BKVN) is still difficult to predict.

Methods: We evaluated factors influencing renal function and viral clearing during BKV infection.

Results: BKVN was diagnosed in 46 patients since 2008 by routinely done quantitative PCR (qPCR) and SV40 staining in all biopsies (done by protocol 6/ 12/ 26 weeks post-transplantation and for cause at any time). Either immunosuppression was generally reduced (calcineurin inhibitor=CNI by 30%, mycophenolate-mofetil=MMF by 30 to 50%, n=23), or CNI was switched primarily to mTOR inhibitor (n=7), or CNI was switched to mTOR inhibitor as a secondary step in case of delayed viral clearing (n=16). The influence of dichotomous variables on the response variables eGFR (≥0 or <0) and the time in weeks for qPCR reduction by 1 log (≤13 or >13 weeks) were measured as well as that of continuous variables. Tacrolimus (65%) and cyclosporine treatment (35%) always was combined with MMF and steroids. - 15% of cases suffered graft failure after 30±13 weeks. eGFR was stable or increased in 63% and decreased in 37%. BKV viral clearing was fast in 54% (reduction by 1 log ≤13 weeks) and slow in 46% (>13 weeks). Fast viral clearing was associated with stable or increasing eGFR (84%) compared to slow viral clearing (33%; p=0.001). Univariate logistic regression revealed that factors with a negative influence on renal function and viral reduction time were maximal viral load, tacrolimus treatment and late diagnosis by biopsy for cause. Using multiple logistic regressions, maximal viral load was the most important factor. In Cox regression analysis defining viral reduction by 1 log as event, tacrolimus compared to cyclosporine had a delaying influence on viral

reduction. In 88% of patients with delayed viral clearing the switch of CNI to mTOR inhibitor effected viral reduction.

Conclusions: We conclude that maximal viral load, tacrolimus treatment, and late diagnosis are influencing renal function and viral reduction time; and viral reduction time is important for renal function.

Funding: Government Support - Non-U.S.

SA-PO3086

A Vitamin D Metabolism Signature Predictive of BK Viremia and Acute Rejection: Implications for Vitamin D Repletion John R. Lee, Thangamani Muthukumar, Darshana Dadhania, Jun B. Lee, Manikkam Suthanthiran. *Nephrology and Hypertension, Weill Cornell Medical College, New York, NY.*

Background: Vitamin D may play a crucial role in the regulation of the immune system. In this study, we examined whether levels of 25-hydroxyvitamin D (25-Vit D) or 25-dihydroxyvitamin D (1,25(OH)2 Vit D) are associated with allograft dysfunction, BK viremia (BKV), or acute rejection (AR).

Methods: We identified 171 renal transplant recipients who had both 25-Vit D and 1,25(OH)2 Vit D measured within the first 3 months of transplantation. We examined whether levels of 25-Vit D or 1,25(OH)2 Vit D were associated with serum creatinine (Cr), with AR, or with BKV during 24 month follow up.

Results: The high 25-Vit D group (≥35ng/ml,n=26) was associated with significantly worse allograft function at 24 months than the low 25-Vit D group (<35ng/mL,n=145)(Cr 2.10 mg/dL vs. 1.59 mg/dL,p=.049). As a possible explanation, the high 25-Vit D group was associated with more BKV than the low 25-Vit D group (30.8% vs. 11.7%, p=.029);Cox Proportion Hazard model revealed a high 25-Vit D level as an independent risk factor for BKV (HR=3.1,p=.019)(Fig 1A). Interestingly, treatment with a 25-Vit D analog was an independent risk factor for BKV (HR=11.4,p =.019).

The low 1,25(OH)2 Vit D group (<29pg/ml,n=119) was associated with significantly worse allograft function at 24 months than the high 1,25(OH)2 Vit D group (≥29pg/ml,n=52) (Cr 1.80 mg/dL vs. 1.37 mg/dL,p=.035). As a possible explanation, the low 1,25(OH)2 Vit D group was associated with more AR than the high 1,25(OH)2 Vit D group (16.8% vs. 5.8%,p=.055). Cox Proportion Hazard model showed that a low 1,25(OH)2 Vit D level was an independent risk factor for AR (HR=3.89,p=.040)(Fig 1B).

Figure 1A: Cox Proportion Hazard Model for BK Viremia

	B	SE	HR	p
HIGH 25-Vit D	**1.13**	**0.48**	**3.10**	**0.019**
High Risk Tx	0.36	0.52	1.44	0.483
Deceased Donor Tx	0.19	0.42	1.20	0.661
Sex (F)	-0.22	0.44	0.81	0.622
Race (AA)	-0.22	0.58	0.80	0.706
Tx 25-Vit D analog	**2.43**	**1.03**	**11.36**	**0.019**

Figure 1B: Cox Proportion Hazard Model for Acute Rejection

	B	SE	HR	p
LOW 1,25(OH)2 Vit D	**1.36**	**0.66**	**3.89**	**0.040**
High Risk Tx	1.21	0.52	3.34	0.020
Deceased Donor Tx	0.35	0.45	1.42	0.442
Sex (F)	0.41	0.46	1.51	0.368
Race (AA)	-0.76	0.64	0.47	0.230
Tx 1,25(OH)2 Vit D analog	-0.02	0.44	0.98	0.963

Conclusions: We have identified that a high 25-Vit D level is an independent risk factor for BKV whereas a low 1,25(OH)2 Vit D level is an independent risk factor for AR.

SA-PO3087

Polyoma Virus in Patients Admitted for Indication Biopsy Long Time after Kidney Transplantation Willy Aasebo. *Akershus University Hospital, Norway.*

Background: Once Polyoma virus associated nephropathy (PVAN) is established long-term graft outcome is poor due to development of graft fibrosis. PVAN is probably related to use and dosages of immunosuppressive drugs. The aim of this report is to find out how Polyoma virus affects kidney grafts in patients that were transplanted several years ago and were using maintenance immunosuppressive treatment (low or standard dosages).

Methods: All kidney recipients that were transplanted >12 months before they were admitted to indication biopsies during the years 2008, 2009 and 2010 were included. Biopsies were graded according to Banff criteria.

PVAN was defined as the presence of BK virus (BKv) in biopsies.

Results: A total of 308 kidney transplant recipients were included. Median time after transplantation was 66 months (range: 13 - 415 months).

Nine recipients (2.9 %) had detectable Polyoma virus in blood (all had BKv), of whom 5 had PVAN (1.6%).

Five patients had BKv-PCR >5.0 x 10^3 copies/ml (1.6 %)(median: 5.3 x 10^5. Range: 8.2 x 10^3 - 8.2 x 10^7), of whom 4 (80%) had PVAN. One recipient had PVAN with 2,1 X 10^2 copies/ml.

Inflammation, fibrosis and graft function in recipients transplanted >12 months before biopsy.

	Polyoma neg	Polyoma pos	P	BKv-PCR >5000	P
	N = 299	N = 9		N = 5	neg vs. BKv >5000
Interst fibrosis	1.66 (0.94)	1.89 (0.78)	0.48	2.00 (071)	0.42
Tubular atrophy	1.69 (0.90)	1.78 (0.67)	0.77	1.80 (0.45)	0.78
Interst inflam	0.68 (0.93)	1.25 (0.71)	0.089	1.40 (0.89)	0.095
Tubulitis	0.69 (0.81)	1.33 (0.71)	0.019	1.60 (0.55)	0.016
Creatinine (μμmol/l)	215.1 (85.7)	212.8 (114.2)	0.95	199.3 (25.9)	0.71
Prot/Creat (mg/mmol)	122.7 (188.6)	140.4 (286.3)	0.81	31.3 (28.5)	0.41

Mean (SD)

Conclusions: PVAN is rare in kidney recipients with "old" transplants (1,6%). However, 80% of the recipients with BKv-PCR in blood >5.0 X 10^3 had PVAN.

Recipients with polyoma viremia had more tubulitis, and a tendency towards more interstitial inflammation compared to other patients with indication biopsies and they had considerable amount of interstitial fibrosis and tubular atrophy.

SA-PO3088

A Systematic Review and Meta-Analysis of Prophylactic *Versus* Pre-Emptive Approach for Preventing Cytomegalovirus (CMV) Infection in Renal Transplant Recipients Bishal B. Rawal,[1] Shahrouz Shadrou,[1] Feroz Abubacker,[2] Nasrollah Ghahramani.[1] *[1]Nephrology, Penn State Hershey Medical Center, Hershey, PA; [2]Internal Medicine, York Hospital, York, PA.*

Background: In kidney transplant (KT) recipients, CMV infection poses significant morbidity and mortality. Both prophylactic and pre-emptive approaches for preventing CMV infection have been utilized.

Methods: We conducted a systematic review and meta-analysis comparing the effectiveness of routine prophylaxis vs. pre-emptive treatment for preventing CMV disease after KT. Combining 4 comprehensive search themes (CMV, renal transplant, prophylaxis, pre-emptive), we searched MEDLINE, EMBASE, ISI Web of Science, and Cochrane Central Register from inception through January 2011. We also evaluated studies referenced in review articles and abstracts from meetings of major nephrology and transplant societies (2009-2011). Two authors independently extracted data and assessed methodological criteria. The primary outcome was the pooled estimate of the odds ratio (OR) of developing CMV infection. Secondary outcomes included OR of acute rejection (AR), OR of graft loss and OR of death within first year of KT. Comprehensive Meta-analysis V2 software was used for data analysis.

Results: Analysis of 9 randomized controlled trials (893 patients; Ganciclovir=5, Valganciclovir=4) with CMV infection as an outcome revealed the OR of CMV infection to be 0.34 [0.25-0.46, 95% CI, p=0.008] for the prophylactic vs. the pre-emptive groups. The risks of AR (7 studies; OR 0.65 [0.34-1.23, 95% CI, p=0.12]), graft loss (7 studies; OR 0.52 [0.34-1.12; p=0.32] and mortality (6 studies; OR 0.84 [0.62-1.23; p=0.23]) were similar between the two groups.

Conclusions: Prophylactic approach is superior to pre-emptive approach in preventing CMV infection within the first year of kidney transplant. There is no significant difference in the risk of developing AR, graft loss and mortality with either approach.

SA-PO3089

Trimethoprim/Sulfamethoxazole vs. Atovaquone Prophylaxis: Respective Roles in Risk of Urinary Tract Infection Post-Kidney Transplantation Gaurav Gupta,[1] Dionissios Neofytos,[2] Kavita M. Kakkad,[1] Robert Avery Montgomery,[3] Edward S. Kraus,[1] Nada Alachkar.[1] *[1]Division of Nephrology, Johns Hopkins Hospital; [2]Division of Infectious Diseases, Johns Hopkins Hospital; [3]Division of Transplant Surgery, Johns Hopkins Hospital, Baltimore, MD.*

Background: Primary prophylaxis with trimethoprim/sulfamethoxazole (TMP/SMX) for *Pneumocystis carinii* is administered for the first six months post-kidney transplant (KTx). In some patients, TMP/SMX may be substituted to an alternative agent (AA; e.g. atovaquone) due to allergy, cytopenias etc. At our center we do not provide additional antibiotic coverage for urinary tract infection (UTI) prophylaxis to these patients. We hypothesized that TMP/SMX administration during first 6 months post-KTx may decrease the rate of UTIs.

Methods: All adult KTx recipients transplanted between 01/09 and 06/10 were identified. UTIs up till 6 months post-KTx were reviewed. UTI was defined as either, a positive urine culture for a pathogen at >10^4 colonies/mL with urinalysis (UA) positive for >5 WBCs/HPF and/or nitrite and leukocyte esterase; or a pathogen at >10^5 colonies/mL, regardless of UA.

Results: 65% (209/323) received TMP/SMX and 35% (114/323) received an AA. Overall, 22%(72/323) patients had at least one UTI. Patients with UTI were more likely to be female (53% vs. 35%; p=0.01) and diabetic (36% vs. 14%; p<0.01). Compared to TMP/SMX group (37/209; 18%), a greater number of patients on AA (35/114; 31%) developed UTI (p=0.01). This association was preserved on multivariate analysis(RR=0.45, p=0.005) after controlling for age, gender and diabetes. TMP/SMX group did not differ from AA group in terms of presence of ureteric stent or urinary tract obstruction at time of UTI. Most UTIs (50/72; 69%) were caused by the Enterobacteriaceae, of which, most were resistant to TMP/SMX (72%).

Conclusions: KTx patients who received prophylaxis with an AA had a higher incidence of UTIs within 6 months post-KTx, compared to those on TMP/SMX. Further studies will define which patients might benefit from additional UTI prophylaxis and the effect of UTI prevention on allograft function.

SA-PO3090

Acute Tubular Necrosis (ATN) on Implant Biopsy of Living Kidney Donors Is Associated with Lower Recipient Graft Function Amr El Toukhy, Richard A. Fatica, Milen Amde, Emilio D. Poggio, Titte Srinivas. *Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

Background: Donor kidneys removed by laparoscopy may exhibit slow graft function likely reflecting acute kidney injury. This acute kidney injury may manifest in implant kidney biopsies (ImBx) as histologic acute tubular necrosis (ATN). We examined the association between histologic ATN in IMBx and recipient graft function

Methods: We studied relationships between IMBx ATN, metabolic syndrome (MS) and 1 yr recipient eGFR (CKD-EPI equation) by multiple linear regression in 161 living donor-recipient pairs transplanted 2005-09. In a logistic model we studied associations of IMBx ATN and the proportion of patients with eGFR<45 ml/min/1.73 m². (MS: BMI>25, SBP >130 mm Hg, Triglyceride >150, Low HDL/sex and FBS>100mg/dl)

Results: ATN was observed 21.4 % of ImBx. Donor factors associated with increased incidence of IMBx ATN were 1) Donor MS: Of those with IMBx ATN 21.2 % % (7/33) had MS vs. 6.5 % (8/124) of those with no IMBx ATN; p=.01) and 2) Donor uric acid levels, (p=0.05). Donor age, gender and race were not significantly related to IMBx ATN. Overall, adjusted transplanted iothalamate GFR (donor GFR x Proportion of total kidney volume transplanted and adjusted for recipient BSA) averaged 53.2 ml/min/1.73m²(sd 11.86) and mean recipient eGFR at 1 year was 55.23 ml/min/1.73m² (sd 17.5). Recipient eGFR at 1 year was 56.5 ml/min/1.73m² for recipients with IMBx ATN vs 49.7 ml/min/1.73m² (p= 0.010) in those with no ATN by univariable analysis.

In a multivariable linear regression model, IMBx ATN was a significant correlate of 1 yr recipient eGFR.

Table

Parameter	Value mL/min/1.73 m²	p-value
Intercept	48.5	<0.0001
No IMBx ATN	3.34	0.0361
No acute rejection	2.7	0.0264
Recipient Age/yr	-0.24	0.0160
Donor age	-0.32	0.0008
Donor Race (Black)	-0.74	0.0160
Donor Sex (Male)	-0.74	0.6428

Conclusions: ATN in the implant biopsy is associated with a significantly lower 1 year recipient eGFR . The impact of IMBx ATN on recipent eGFR is similar in to that of acute rejection. Minimal surgical manipulation is thus desirable to reduce IMBx ATN. The long-term impact of IMBx ATN on transplant outcomes merits further study.

SA-PO3091

Thymoglobulin Attenuates Renal Ischemia Reperfusion Injury Prabir Roy-Chaudhury, Yang Wang, Kamyar A. Zahedi, Meenakshi J. Mistry, Virgilius Cornea, Manoocher Soleimani. *Division of Nephrology and Hypertension, University of Cincinnati, OH.*

Background: Ischemia reperfusion injury (IRI) resulting in delayed graft function (DGF) is an important cause of morbidity in renal allograft recipients. Despite its clinical significance there are no effective interventions for the prevention of renal allograft IRI. Thymoglobulin (Thymo) is a potent T cell depleting polyclonal antibody which also reduces the expression of adhesion molecules which play an important role in the causation of renal IRI. The aim of the current study, was to assess the role of a murine surrogate of Thymo (rabbit anti-mouse thymocyte globulin), in attenuating renal IRI in a mouse model of bilateral renal artery ligation.

Methods: 20 C57Bl/6 mice were administered either mouse Thymo (T) 500 mcg IV through the tail vein, control mouse IgG (I) in a similar dose, or no therapy (C = Control); 30 minutes prior to clamping the renal arteries bilaterally (for 30 minutes). Animals were sacrificed at the 24 hr, 3d and 14d time points. Both kidneys were assessed for changes of acute tubular necrosis (tubular dilation, cast formation, brush border loss, necrosis and epithelial vacuolization) using a semi-quantitative scoring scale from 0 to 3+ (0 = normal, 1+ = <25% renal parenchyma involved, 2+ = 25-50%, 3+ = > 50%). An ANOVA test was used for the statistical analysis.

Results: Table 1 describes the composite injury score for the three groups. Note the significant reductions in histologic injury (at both early and late time points) in animals treated with mouse Thymo as compared to controls, and also in most cases IgG.

Conclusions: Our experience suggests that murine Thymo might have important beneficial effects in the setting of mouse renal IRI. Further clinical studies are needed to ascertain whether Thymo should be preferentially used prior to clinical reperfusion in renal transplant patients at increased risk of DGF.

Composite Injury Score

Time	Control (C)	Mouse IgG (I)	Mouse Thymo. (T)
24 Hrs	1.5+/-0.3	1.0+/-0.3	0.17+/-0.2*#
3 Days	2.0+/-0.6	0.25+/-0.25*	0.25+/-0.25*
14 Days	1.75+/-0.5	1.75+/-0.5	0*#

*=p<0.05 compared to Control; #=p<0.05 compared to IgG

Funding: Pharmaceutical Company Support

SA-PO3092

Serum Erythropoietin-Levels of Deceased Organ Donors Are Predictive of Delayed Graft Function after Renal Transplantation Teresa Kauke,[4,5] Thomas Breidenbach,[6] Detlef Boesebeck,[6] Ulf Schoenermarck,[1] Bernhard Banas,[2] Manfred J. Stangl,[4] Peter Schnuelle,[3] Bernhard K. Krämer,[3] Michael Fischereder.[1] *[1]Nephrology, Klinikum der LMU, Munich, Germany; [2]Nephrology, Universityhospital, Regensburg, Germany; [3]Nephrology, UMMH, Mannheim, Germany; [4]Surgery, Klinikum der LMU, Munich, Germany; [5]Laboratory of Immunogenetics, Klinikum der LMU, Munich, Germany; [6]DSO, Munich, Germany.*

Background: Delayed graft function (DGF) affects acute and long-term function of renal transplantats. As experimental data suggest a benefit from epoetin (EPO) administration to the donor, we examined the effect of endogenous donor EPO (dEPO) levels on DGF incidence.

Methods: We examined dEPO levels in 55 deceased organ donors. DGF was defined as the need for ≥ dialysis treatment during the first week post transplantation. Demographic data, DGF incidence and transplant function were obtained from the patient record.

Results: Mean donor age was 52.9 years (SD ± 16.0), median donor age was 53.0 years (range 15 – 83), 20 donors were 60 years or older. Mean recipient age was 50.7 years (± 12.3). Mean dEPO level was 57.8 mU/ml (± 86.4), median dEPO level was 33.7 mU/ml (range 4.6 – 502). DGF was significantly less in recipients with dEPO > 70 mU/ml than in recipients with dEPO < 70 ng/ml, i.e. 1 of 16 vs. 23 of 58 patients (p=0.038). Recipients of dEPO > 70 mU/ml kidneys required on average 0.3 dialysis treatments (± 1.0) vs. 2.7 dialysis treatments for patients with dEPO < 70 mU/ml (p=0.0447). dEPO > 70 mU/ml resulted in serum-creatinine of 1.7 mg/dl (± 0.4) vs. 2.4 mg/dl (± 1.9; p=0.14) on day 28 and shorter hospitalization 22.7 days (± 6.4) vs. 29.0 days (± 12.8; p=0.0809). Cold ischemia time, donor and recipient age, PRA and immunosuppressive regimens were not different for dEPO< 70 or > 70 mU/ml.

Conclusions: dEPO levels < 70 mU/ml have a sensitivity of 95% for prediction of DGF with a specificity of 30%. The negative predictive value of dEPO > 70 mU/ml for DGF is 93.8%.

dEPO levels > 70 mU/ml appear protective in deceased kidney donors and allow prediction of DGF. Modification of dEPO levels to reduce DGF deserves further study.

SA-PO3093

Comparison of Chronic Kidney Disease Complications Management in Kidney Transplanted and Non-Transplanted Patients Saleh Kaysi, Julien Aniort, Carole Philipponnet, Patrice M. Deteix, Anne-Elisabeth Heng. *Nephrology, CHU Gabreil Montpied, Clermont-Ferrand, France.*

Background: Chronic kidney disease (CKD) complications are common in kidney transplanted patients (KT). We compared retrospectively the management of CKD complications according to the K/DOQI guidelines in KT and non-transplanted CKD patients (NT).

Methods: Files of all KT and NT patients with CKD stages 4 and 5 (GFR obtained by MDRD equation) seen in consultations in the department of nephrology at the University Hospital of Clermont-Ferrand between May 2009 and June 2010 were screened. Inclusion criteria were; more than 6 months of follow up, and a complete file concerning the data being collected. Exclusion criteria were; admission to the hospital or the emergency or infection in the 3 months before data collection date, cancer or primary hyperparathyroidism. The proportion of patients who achieved K/DOQI guidelines was analyzed (*: p<0.05).

Results: 58 KT were compared to 85 NT CKD patients matched by CKD stage (age 57y±13* vs 67y±17, diabetes: 21 vs 27%, GFR: 21.3±6* vs 18.3±6 ml/min respectively). Results after data analyzing (mean ± standard derivation) and proportions of patients achieving K/DOQI guidelines targets are shown in the following table 1.

	KT n=58	KDOQI %	NT n=85	KDOQI %
Hemoglobin (g/dl)	10,9 ± 1,3	41% *	11,6 1,5	51%
Ferritin (ng/ml)	225 [62-1650]	80% *	121[31-593]	52%
Cholesterol (g/l)	1,97 ± 0,4	35% *	1,94 ±0,28	60%
LDL (g/l)	1,1 ± 0,24	38% *	0,99 ± 0,25	64%
Ca (mmol/l)	2,3 ± 0,14	67% *	2,22 ± 0,17	60%
Ph (mmol/l)	1,3 ±0,32	79% *	1,43 ± 0,32	62%
PTH (ng/ml)	156 ± 108	50% *	140 ± 144	65%
Vit D (ng/ml)	20 ± 11	35% *	17 ± 10	20%
Albumin (g/l)	35 ± 5	22%	22%	22%
sBP (mmHg)	135 ± 20	52% *	140 ± 20	41%

table 1

Conclusions: More attention has to be given to achieve a better control of anemia, dyslipidemia and hyperparathyroidism in KT CKD patients. Immunosuppressive treatment, inflammation, weight gain, may explain the difference of CKD complications management observed between the KT and NT patients.

Prospective studies are needed to confirm the beneficial effect of treating CKD complications on graft function and patient outcomes

Funding: Government Support - Non-U.S.

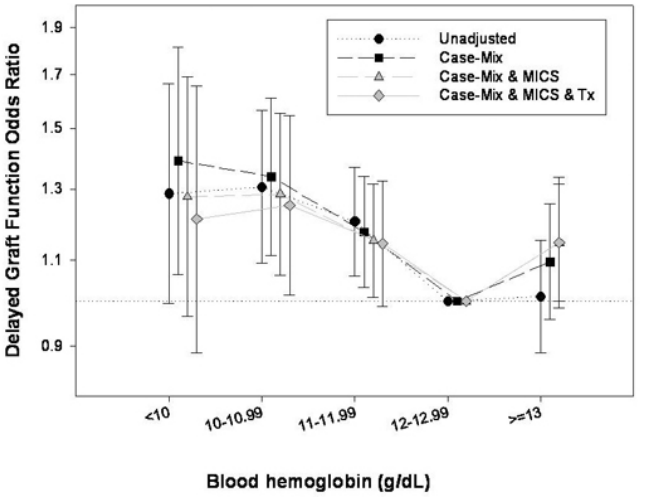
SA-PO3094

Associations of Pre-Transplant Hemoglobin and Iron Deficiency with Post-Transplant Delayed Graft Function in Kidney Transplant Recipients Miklos Z. Molnar,[1,2] Csaba P. Kovesdy,[3] Laszlo Rosivall,[2] Suphamai Bunnapradist,[4] Junichi Hoshino,[1] Elani Streja,[1] Mahesh Krishnan,[5] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]Salem VA Medical Center, Salem, VA; [4]David Geffen School of Medicine at UCLA, Los Angeles, CA; [5]DaVita, Inc, Denver, CO.*

Background: Delayed graft function(DGF) complicates kidney allograft outcomes in the immediate post-transplantation period. We hypothesized that in hemodialysis patients (pts) more severe anemia & iron deficiency are associated with higher risk of DGF.

Methods: Linking 5-year hemodialysis pts data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 11836 hemodialysis pts. Using logistic regression analyses we examined the association between pre-transplant parameters and post-transplant DGF.

Results: Pts were 49±14(mean±SD) years old and included 38% women, 27% Blacks and 26% diabetics. Compared to pre-transplant hemoglobin of 12-<13 g/dL, there was 25% higher risk of DGF with blood hemoglobin 10-<11 g/dL (OR=1.25;95%CI: 1.01-1.55), whereas blood hemoglobin ≥13 g/dL exhibited 15% higher risk of DGF (OR=1.15;95%CI: 0.98-1.34).



After adjustment for confounders, no association was found between iron parameters & DGF.

Association hemoglobin, iron parameters and DGF

	Unadjusted	Case-mix adjusted	Case-mix&MICS adjusted	Case-mix&MICS&Transplant adjusted
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Hb (+1 g/dL increase)	0.62 (0.40-0.96)	0.51 (0.33-0.79)	0.55 (0.35-0.86)	0.58 (0.35-0.96)
Serum ferritin (+100 ng/mL increase)	1.05 (1.03-1.06)	1.02 (1.01-1.04)	1.02 (1.00-1.03)	1.01 (0.99-1.03)
Iron saturation ratio (+10 increase)	1.47 (1.05-2.06)	1.38 (0.98-1.94)	1.39 (0.98-1.97)	1.39 (0.97-2.00)

Conclusions: Pre-transplant either high or low blood hemoglobin but not iron markers are associated with higher risk of DGF.

Funding: NIDDK Support

SA-PO3095

Associations of Pre-Transplant Anemia Management with Post-Transplant Delayed Graft Function in Kidney Transplant Recipients Miklos Z. Molnar,[1,2] Csaba P. Kovesdy,[3] Junichi Hoshino,[4] Suphamai Bunnapradist,[5] Laszlo Rosivall,[2] Elani Streja,[1] Mahesh Krishnan,[6] Kamyar Kalantar-Zadeh.[1,4,5] *[1]Harold Simmons Center, Torrance, CA; [2]Semmelweis University, Budapest, Hungary; [3]Salem VA Medical Center, Salem, VA; [4]UCLA School of Public Health, Los Angeles, CA; [5]David Geffen School of Medicine at UCLA, Los Angeles, CA; [6]DaVita, Inc, Denver, CO.*

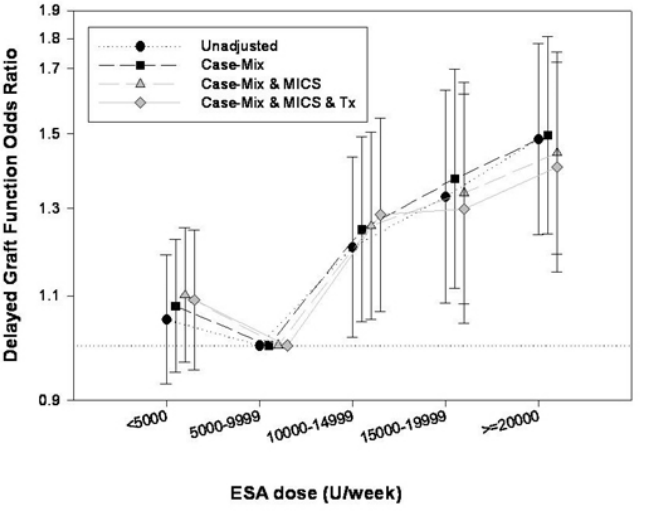
Background: Delayed graft function (DGF) is an important complication in early post-transplant period. We hypothesized that in hemodialysis patients the requirement for higher doses of erythropoietin stimulating agents (ESA) or blood transfusions prior to transplantation is associated with higher risk of DGF.

Methods: Linking 5-year hemodialysis patient data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 11,836 hemodialysis patients. Using logistic regression analyses we examined the association between pre-transplant anemia management and post-transplant DGF.

Results: Patients were 49±14 (mean±SD) years old and included 38% women, 27% Blacks and 26% diabetics. After adjusting for relevant covariates, pre-transplant blood transfusion was associated with 33% higher DGF risk (odds Ratio [OR]=1.33; 95% confidence interval[CI]: 1.19-1.48).

	Unadjusted	Case-mix adjusted	Case-mix & MICS adjusted	Fully adjusted
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Pretransplant transfusion	1.30 (1.18-1.44)	1.29 (1.16-1.43)	1.31 (1.18-1.45)	1.33 (1.19-1.48)
ESA dose (+5000 units/wk increase)	1.07 (1.04-1.10)	1.07 (1.03-1.10)	1.05 (1.02-1.09)	1.05 (1.02-1.09)

Each 5000 U/week increase of pre-transplant ESA dose was associated with 5% higher DGF (OR=1.05; 95%CI: 1.02-1.09).



Conclusions: Pre-transplant blood transfusion and higher ESA dose are associated with higher risk of DGF.

Funding: NIDDK Support

SA-PO3096

Alemtuzamab Induction and Tacrolimus Monotherapy Maintenance Eliminates Cellular Rejection in Kidney Allografts: Five Year Follow Up Milap Pokharel, Prajwol R. Pant, Michael C. Chobanian. *Medicine, Dartmouth Medical School, Hanover, NH.*

Background: Alemtuzumab is increasingly used as induction therapy for renal transplants to minimize post transplant immunosuppression. Biopsy confirmed acute rejection (BCAR) has been shown to be less frequent with alemtuzumab compared to conventional induction therapies and standard immunosuppressive regimens. We investigated the rate of BCAR in patients receiving alemtuzumab induction followed by tacrolimus monotherapy and compared the results to those receiving alternative induction and maintenance therapies.

Methods: We retrospectively reviewed data from all kidney transplant recipients between 1992 and 2010 with BCAR. Demographics, type of kidney transplant, type and cause of rejection, graft survival and patient survival times were determined.

Results: 647 renal transplants were performed, 345 patients in the pre and 302 patients in the post alemtuzumab era. 36 patients had 44 episodes of BCAR, 32 (9.3%) were pre and 12 (3.9%) were post alemtuzumab. Mean age was 34.2 years, 56.8% were male and 91.7% were Caucasians. 16 (36.3%) rejected allografts were from living and 28 (63.4%) were from deceased donors. Of the pre alemtuzumab rejections, 13 (40.6%) were antibody mediated (AMR) while 19 (59.4%) were ACR. In the post alemtuzumab era, 5 (41.7%) were AMR and 7 (58.3%) were ACR. No patients in the post alemtuzumab era died at the end of the study period, whereas 5 year survival rate was 93.7% for patients in the pre alemtuzumab era. Mean time of graft survival was 3.7 versus 10.6 months in the pre vs post alemtuzumab group. Incidence of both ACR and AMR were significantly lower in patients who received alemtuzumab induction (9.3% vs 3.9%). Of the 7 cases of ACR in the post alemtuzumab era, 5 had non compliance to medications and 2 were taking cyclosporine, prednisone, and MMF, not tacrolimus monotherapy.

Conclusions: Alemtuzumab induction plus tacrolimus monotherapy maintenance eliminates ACR up to 5 yrs post kidney transplant in compliant patients. Alemtuzamab induction appears to confer greater longevity in both patients and grafts undergoing rejections than other induction modalities.

SA-PO3097

Acute Rejection and Long Term Graft Survival in Renal Transplant Recipients Using Campath 1-H Induction Mekdess Abebe, Diane Triolo, Heesuck Suh, Frank Darras, Mersema Abate, Edward P. Nord. *Nephrology and Transplantation, SUNY Stony Brook, Stony Brook, NY.*

Background: Acute rejection (AR) remains a determinant of long term graft survival.

Methods: We report on the AR rate and its impact on long term graft survival following alemtuzumab (campath-1H) induction (30 mg) and rapid steroid withdrawal (methylprednisolone 500 mg , 250 mg, and 125 mg on days 1-3 respectively) in 487 consecutive renal transplant patients from July 2003 through January 2011. Maintenance immunosuppression consisted of tacrolimus and mycophenolate mofetil with target level of 8-10 ng/ml and 1.5-3.0 ng/ml respectively. A renal biopsy was prompted by an unexplained rise in serum creatinine.

Results: AR was biopsy proven in 55/487 (11.3%) patients and an additional 10 patients had borderline changes (see table). By Banff criteria, 31 (6.4%) showed acute cellular rejection (ACR), 16 (3.3%) showed acute humoral rejection (AHR), and 8 (1.6%) displayed both ACR and AHR. The mean age of these 65 patients was 53 ± 14 yrs, 35 (54%) were male, 19 (29%) were African American, 11(17%) were Hispanic, 48 (74%) received a deceased donor kidney, and 14 (21%) were retransplants. AHR was treated with plasmapharesis, IVIG and rituximab and ACR with steroid and/or thymoglobulin or OKT3. Mean follow up was 43 ± 22 months post AR.

Banff category	ACR (Type 4)	AHR (Type 2)	ACR+AHR	Borderline
Number of patients(%)	31 (6.4%)	16 (3.3%)	8 (1.6%)	10 (2.1%)
PRA (%), (mean±SD)	10 ± 26	56 ± 44	41 ± 46	28 ± 44
HLA match (mean±SD)	2.4 ± 2.4	1.1 ± 1.2	1.8 ± 0.8	1.1 ± 1.6
Days to AR(mean±SD)	209 ± 172	15 ± 23	221± 269	385 ± 300
Outcome				
Graft survival	18	10	4	9
Dialysis dependent	13	6	4	1
Death	4	2	0	2

At 43 ± 22 months post AR patient survival was 88% (57/65) and graft survival was 63% (41/66), with a mean serum creatinine of 2.1 ± 0.97 mg/dL. Eight of 65 patients died, 4 with a functioning graft. The cause of death was sepsis 3, CAD 2, PTLD 1, lung cancer 1, and stroke 1.

Conclusions: 1. Using campath-1H induction the incidence of ACR was 6.4%, AHR 3.3% and ACR+AHR 1.6%. **2.** AHR occurs at an earlier time point than ACR or ACR+AHR and is associated with a better outcome.

SA-PO3098

Independent Predictors of Renal Allograft Failure in Patients with Acute Antibody-Mediated Rejection Marie Matignon, Thangamani Muthukumar, Michelle L. Lubetzky, Darshana Dadhania, Surya V. Seshan, Manikkam Suthanthiran, Choli Hartono. *Cornell University.*

Background: The impact of co-existing histological findings in renal allograft biopsies showing acute antibody-mediated rejection (AMR) on graft outcome has not been well defined.

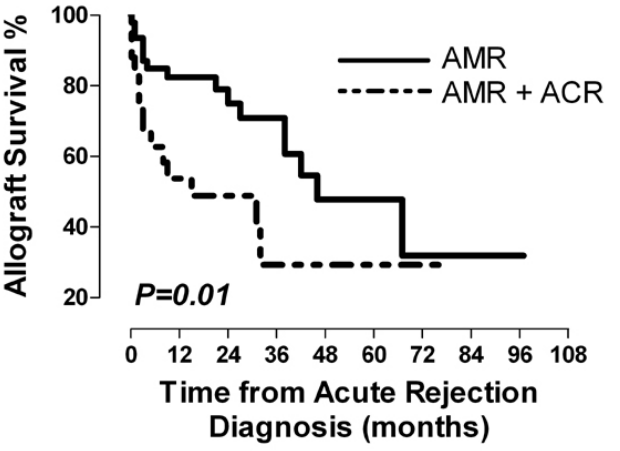
Methods: We reviewed all for-cause kidney allograft biopsies between 12/2003 and 12/2010. We identified AMR as defined by Banff 2009. Primary outcome was graft loss. Significant baseline variables were entered into a Cox model to identify independent predictors of outcome. Robustness of the model was verified by analysis with a logistic model.

Results: 76 of 1386 (5.5%) kidney recipients had AMR. Median follow up was 21(0-97) months (mo) after diagnosis. 31 (40%)lost their graft, 48% of them within 3 mo of AMR.

Concomitant acute cellular rejection (c-ACR) on biopsy and eGFR at the time of AMR were the only predictors of graft loss. 26 (34%) of the 76 with AMR had c-ACR.

	Cox Model for Graft Loss After AMR			**Logistic Regression Model for Graft Loss at 1-year After AMR**		
VARIABLE	**Hazard Ratio**	**95%CI**	**P**	**Odds Ratio**	**95%CI**	**P**
Concomitant ACR	2.7	1.2 to 6.3	0.02	5.1	1.02 to 25.6	0.04
eGFR at AMR	0.95	0.92 to 0.99	0.016	0.93	0.86 to 0.99	0.03

Median graft survival for AMR with c-ACR was 15 mo (vs. 46 mo for AMR alone) from the diagnosis of rejection.



These predictors were independent of (i) time to AMR, (ii) presence of concomitant (a) chronic active AMR, (b) IF/TA, (c) g/ptc inflammation, (d) arteriolar hyalinosis, and (iii) addition of (a) Rituximab, (b) anti-thymocyte globulin or (c) bortezomib for the treatment of AMR, to the standard treatment (steroids, plasmapheresis or intravenous immunoglobulin).

By logistic regression, the same two variables predicted graft loss at 1-year after AMR.

Conclusions: In biopsies showing AMR, the concomitant presence of ACR, independent of any other allograft pathology or rejection therapy, is a risk factor for graft loss.

SA-PO3099

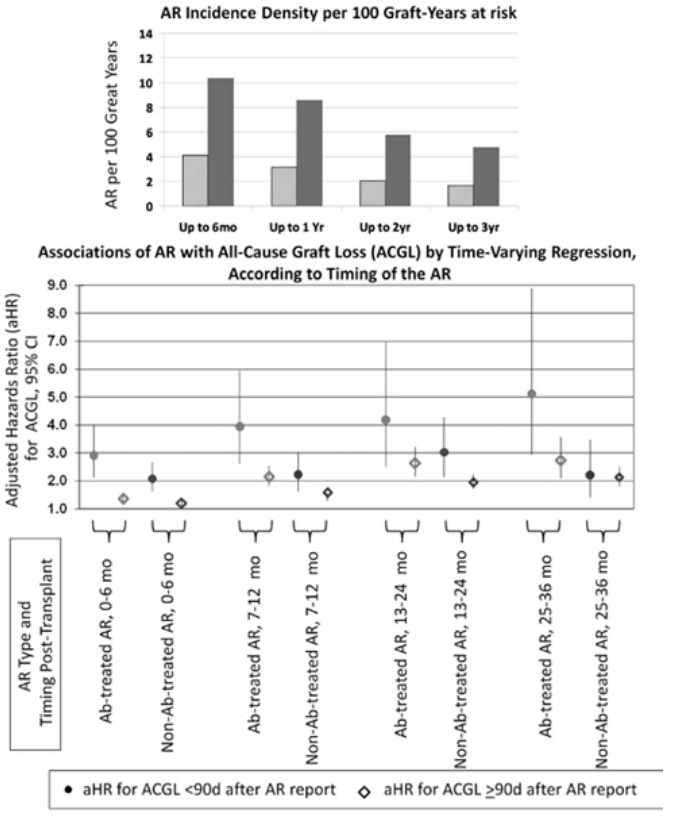
The Outcomes of Acute Rejection in Contemporary Kidney Transplantation: Importance of Timing and Risk Window Krista L. Lentine,[1] Adrian Gheorghian,[1] Gilbert Litalien,[2] Anupama Kalsekar,[2] Mark Schnitzler.[1] *[1]Saint Louis Univ; [2]Bristol-Myers Squibb.*

Background: We examined the frequency of acute rejection (AR) in contemporary U.S. kidney transplantation (KT) and the clinical impact of AR based on: 1) AR timing after KT, 2) risk period after the AR event.

Methods: Data for Medicare-insured KT recipients in 2000-2007 (n=44,831) were drawn from the USRDS. AR events were ascertained from OPTN reports covering 0-6, 7-12, 13-24, and 25-36 mo post-transplant. AR was classified as antibody-treated AR (Ab-AR) or other management (non-Ab-AR). Associations of AR with subsequent all-cause graft loss (adjusted hazards ratio, aHR) were estimated with time-varying Cox regression. Risk associated with AR was partitioned within the first 90d after AR or ≥90d after the AR events. Covariates included factors in the UNOS Kidney Allocation Review Committee survival model.

Results: AR was more common when evaluated early after KT (e.g., within 6 mo or 1yr) compared to over longer intervals. Non-Ab-treated AR was more than twice as common as Ab-treated AR by period and donor type. In time-varying multivariate regression, development of Ab-AR predicted greater risk of graft loss than non-Ab-AR.

The aHR for graft loss from Ab-AR increased with later timing of AR after transplant, while risk associated with non-Ab AR peaked for events reported in mo 13-24 after KT. Regardless of the diagnosis time, the relative risk of graft loss was higher in the first 90d after a given AR report compared to beyond 90d.



Conclusions: While AR after KT is less common than in the past, AR is associated with increased graft loss risk when it occurs. AR events recognized later after transplant have more serious graft loss implications, especially within the first 90d following an AR event. This observation may reflect reduced monitoring, delays in diagnosis, or clinico-pathological features of late AR.

Funding: Pharmaceutical Company Support

SA-PO3100

Posttransplant Glomerulonephritis; Incidence and Impact on Graft Outcome

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Background: Recently, the significance of posttransplant glomerulonephritis (PTGN) is revisited as it may induce the allograft failure. The purpose of this study is to identify the incidence of PTGN and the association between PTGN and allograft failure, and to analyze the risk factors of PTGN.

Methods: In this cohort study, among 910 Korean patients who underwent renal transplantation from 1995 to 2009, a total of 681 patients were enrolled, excluding patients under age 18 and received second or multi-organ transplantation. The medical records were reviewed.

Results: During the follow-up period, a total of 70 patients (10.3%) were diagnosed as PTGN. The incidence increased with time after transplantation, from 10.7% at 5year to 18% at 10year. PTGN was diagnosed in 14.6% of biopsy-proven or clinically diagnosed chronic GN, compared to 10.6% in unknown etiology group, and 4.1% in other etiology group such as diabetes and hypertension. In patients with PTGN, allograft survival rate was significantly decreased (p<0.001). As PTGN developed, the incidence of graft failure increased 3.86 times (95%CI 1.67-8.93), and in case of occurrence of both acute rejection and PTGN, the odds ratio (OR) of graft failure was 7.03 (95%CI 2.93-16.89) after adjusting for other risk factors. In univariate analysis for probability of PTGN, underlying diseases, era of transplantation, preemptive transplantation, and the use of tacrolimus and simulect were significant risk factors. However, donor type, timing difference of referral, and HLA antigen mismatch were not associated. In addition, through multivariate analysis, the use of tacrolimus (OR 2.44, 95%CI 1.49-4.00, p<0.001) and basiliximab (OR 2.29, 95%CI 1.36-3.87, p=0.002) increased the development of PTGN significantly.

Conclusions: The development of PTGN was strongly associated with the poor kidney allograft survival. Therefore, the main focus should aim for the management of recurrent or de-novo GN after kidney transplantation.

SA-PO3101

Rituximab in Pediatric Recurrent Focal Segmental Glomerulosclerosis

Juhi Kumar,[1] Ibrahim F. Shatat,[4] Amy L. Skversky,[3] Eduardo M. Perelstein,[1] Valerie L. Johnson,[1] Shefali Mahesh.[2] *[1]Pediatrics, Weill Cornell Medical Center, New York, NY; [2]Pediatrics, Akron Childrens Hospital, Akron, OH; [3]Pediatrics, Children's Hospital at Montefiore, Bronx, NY; [4]Pediatrics, MUSC Children's Hospital, Charleston, SC.*

Background: FSGS recurs in 35-50% of allografts. Plasmapheresis (TPE) has been one of the mainstays of treatment but results are variable. Rituximab (RTX), a anti CD20 antibody is being used for its treatment but pediatric experience is very limited.

Methods: We report 8 cases of recurrent FSGS treated with Rituximab.

Results: See Table 1 for details.

8 children, age range 7 to 17 years had recurrence of FSGS within 2 weeks after transplant. All were on TPE for an average of 7 days to 5 years with persistent nephrotic range proteinuria. They received 1 to 4 doses of RTX.

Complete response, urine protein creatinine (u p/c) ratio < 0.2 was seen in 2/8 patients. Partial decrease in u p/c ratio was seen in 3/8 patients. 3 patients had no response. Those who responded did so within the first month of RTX.

One patient received RTX for biopsy proven severe recurrence with allograft dysfunction requiring hemodialysis. Proteinuria diminished and serum creatinine improved significantly post RTX. The patient developed severe respiratory failure 4 weeks post Rituximab and died. No infectious agent was identified on bronchoscopy. Autopsy revealed thrombotic microangiopathy in multiple organs.

One patient developed CNS malignancy 2 years post Rituximab and one had ATN after receiving Rituximab.

Table 1

	Age/Sex/ Race	Transplant Type	Pre Transplant Pheresis	Nephrectomy	CD19%pre/ post	Scr pre/post	Urine P/C pre/post
Case 1	15/m/w	LRD	y	n	23/0	1.2/0.9	2.3/1.2
Case 2	12/m/w	DD	y	n	17/0	1.25/1.13	8.1/3.3
Case 3	8/m/b	LURD	n	n	11/0	0.74/1.24	4.4/7.5
Case 4	16/m/b	LURD	y	n	67/0	4.22/1.57	5/2.2
Case 5	17/f/w	DD	n	u/l	12/1	2.2/2.8	10/8
Case 6	14/f/w	LRD	n	n	-/0	1.8/1.7	25/21
Case 7	7/m/w	LRD	y	y, b/l	1.2/0.5	1/0.8	23/0.1
Case 8	15/m/w	LRD	y	y, b/l	1/0	1.2/1.0	4.19/0.14

Post :1 mo after RTX

Conclusions: Rituximab can be used as a treatment for recurrent FSGS. Efficacy is variable. Larger multicenter studies are needed to prove its sustained efficacy in those who respond. Long term follow up is required to monitor for adverse effects.

SA-PO3102

Successful Treatment of Recurrent Focal Segmental Glomerulosclerosis after Kidney Transplantation Results in Recovery of Podocyte Injury

Kavita M. Kakkad,[1] Michelle M. Estrella,[1] Rachel Marino,[2] Hamid Rabb,[1] Nada Alachkar.[1] *[1]Nephrology, Johns Hopkins University, Baltimore, MD; [2]Surgery, Johns Hopkins University.*

Background: Focal segmental glomerulosclerosis (FSGS) commonly recurs after kidney transplantation (Tx). We describe the clinical course of individuals with recurrent FSGS treated with plasmapheresis (PP)±rituximab.

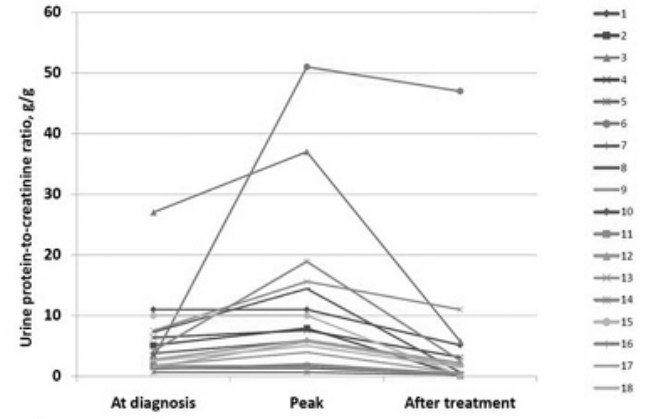
Methods: Eighteen patients with recurrent FSGS after Tx between 2005 and 2011 were followed for up to 2 years. All received PP (median 19 exchanges [IQR: 10-24]); 8 who were refractory to PP received 1 dose of rituximab. 17 patients underwent kidney biopsy (Bx) at the time of recurrence and at least 1 Bx after treatment. Response to therapy was defined by the decline in proteinuria to sub-nephrotic range or complete resolution.

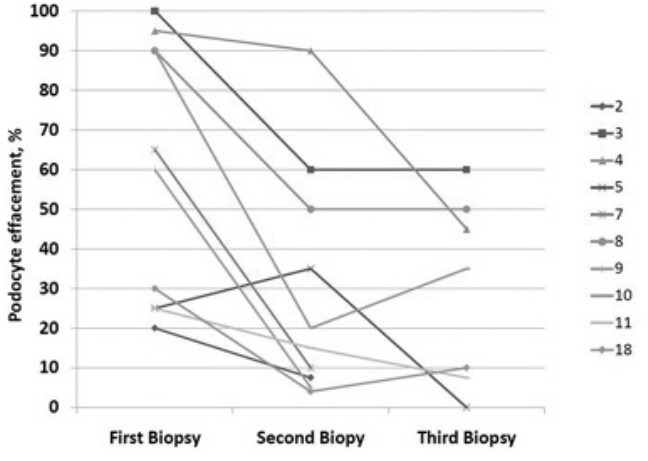
Results: Clinical characteristics are shown in the table.

Clinical Characteristics

Mean age at Txp, y(SD)	41(13)
Black, %	55
Male, %	55
Mean years on dilaysis, y(SD)	2.8(3.1)
Per Txp Nephrectomy, %	28
Tx Type, %	
Deceased	44
Living related	28
Living unrelated	28
Serum Cr at recurrence mg/dl, mean(SD)	2.7(1.7)
Pr/Cr at recurrence g/g, mean(SD)	5.5(6.2)

Median time to recurrence was 20 days (IQR 5-90) after Tx. On initial Bx, individuals had variable degree of podocyte effacement (PE) on EM; only 2 had FSGS changes on light microscopy (LM). Mean serum creatinine improved from 2.7 to 2 mg/dL (P=0.07) and mean urine protein/creatinine ratio (Pr/Cr) declined from 11.7 (peak) to 4.9 g/g (P<0.01) after treatment. Receipt of rituximab improved Pr/Cr by a mean of 3.3 g/g (P=0.03). 10 patients had improvement in PE after treatment; 8 of whom did not develop FSGS changes on LM and 2 had persistent changes. Treatment Failure did not result in improvement of PE (7 patients).





Conclusions: Decreased proteinuria was accompanied by improvement in podocyte effacement in patients treated with PP ± rituximab for recurrent FSGS after Tx.

SA-PO3103

High-Dose Corticosteroid Therapy (HDCS) for Recurrent idiopathic Membranous Nephropathy (IMN) after Renal Transplantation Maria Lopez Picasso,[1] Esther Gonzalez Monte,[2] Alberto De Lorenzo,[2] Maria Moya,[1] Laura Garcia-Puente Suarez,[2] Jose M. Morales,[2] Amado Andres,[2] Manuel Praga.[2] *[1]Nephrology, H.U. La Princesa, Madrid, Spain; [2]Nephrology, H. U. 12 de Octubre, Madrid, Spain.*

Background: IMN can recur in 40% of renal allografts or appear de novo. Corticosteroids as a single therapy for IMN is not recommended.No studies have been performed in IMN recurring or presenting de novo after renal transplantation.We observed a beneficial effect of HDCS in transplant patients with renal biopsy showed an IMN.In this study, we collect our experience with HDCS in IMN of renal transplant patients.

Methods: Single-center,observational study of patients with biopsy-proven IMN after renal transplantation.Patients were divided into two groups according to whether they received HDCS in addition to their immunosuppressive regimen(group 1) or not(group 2).

Results: Twenty-one patients(66.7% male, mean age 51.3 ± 13.3 years)were collected.47.6% were HCV+.

Fourteen patients (group 1) received HDCS, 6 of them with oral steroids (0.7 mg/kg/day) and 8 with intravenous pulses (mean dose 1,25gr).Seven patients (group 2) received no corticosteroid treatment. The baseline characteristics, clinical presentation and outcome are summarized in the table.

Characteristics and outcome of group 1 vs group 2

	Group 1 (HDCS) (14p)	Group 2 (7p)	p
Age (years)	49,3±12	52,7±7,6	NS
Time from Tx to diagnosis (months)	39,3±32,2	35,5±23	NS
sCr at diagnosis (mg/dl)	1,4±0,3	1,7±0,46	NS
eGFR at diagnosis (ml/min)	46,9±17,4	42,5±24,4	NS
Proteinuria at diagnosis (gr/d)	9,1±4,6	6,2±5,1	0,06
Patients receiving CSA or TAC at the time of diagnosis (%)	100	100	NS
Patients receiving ACEI / ARB(%)	100	42,8	0,006
Patients with CR or PR (%)	12(85,7)	2(28,6)	0,009
Time to reach complete or partial remission (months)	5±3,4	12±3	0,001
Final sCr (mg/dl)	1,8±0,9	3,2±2	0,04
Final proteinuria (gr/d)	2,1(r0,1-10)	5,9(r0,2-20)	NS

Group 1 patients achieved a higher rate of complete (CR) or partial (PR) remission, as compared to those of group 2 (85.7 vs 28.6%). Final sCr was significantly better among group1.

Conclusions: A majority of patients with recurrent or de novo IMN achieved CR or PR of nephrotic syndrome after receiving oral or i.v. high-dose corticosteroid therapy.

SA-PO3104

The Graft Outcome of HLA Non-Mismatch Kidney Transplantation; the Impact of Recurrent Glomerulonephritis and Rejection Hee Jung Jeon,[1] Ran-Hui Cha,[2,3] Jung Nam An,[1] Jung Pyo Lee,[3,4] Curie Ahn,[1] Suhnggwon Kim,[1] Yon Su Kim.[1,3] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Korea; [2]Department of Internal Medicine, National Medical Center, Korea; [3]Clinical Research Center for End Stage Renal Disease, Korea; [4]Department of Internal Medicine, Boramae Hospital, Korea.*

Background: Although histocompatibility leukocyte antigen (HLA)-identical renal transplantation reveals superior graft outcome, the graft survival has not been indefinite. Original disease recurrence and effect of acute rejection (AR) may preclude indefinite survival. Here, we analyzed the factors that affected the graft outcomes in HLA non-mismatch condition.

Methods: We have studied the effect of recurrent glomerulonephritis (GN) and AR on graft outcomes in HLA non-mismatch (n=122), 3-4 mismatch (n=317), and 5-6-mismatch (n=102) renal allograft of Seoul National University Hospital. And 41% patients had GN as underlying disease.

Results: Overall graft survival was 93.4% at 5 years, 83.3% at 10 years, and 55.5% at 20 years. Surprisingly, HLA compatibility did not affect the graft survival (0 vs. 3-4 vs. 5-6 mismatch; 92.8% vs. 92.7% vs. 95.8% at 5 years, 82.5% vs. 77.6% vs. 88.9% at 10 years, 42.0% vs. 73.1% vs. 37.2% at 20 years, respectively, p=0.602). In GN subgroup, male recipients (p=0.041), acute rejection (p=0.001), and recurrent GN (p=0.003) were the risk factors for graft loss, whereas living donor graft showed the protective effect (p=0.029). The acute rejection was more prevalent as more HLA incompatibility [0 (ref.) < 3-4 < 5-6 mismatch; p=0.047 and p=0.014]. But the recurrence of GN showed the opposite trend, i.e., the less HLA mismatch, the more recurrence of GN [0 (ref.) > 3-4 > 5-6 mismatch; p=0.106 and p=0.022]. Furthermore, the graft loss due to recurrent GN was significant in HLA non-mismatch group compared with 3-4 mismatch group (p=0.047).

Conclusions: Although HLA non-mismatch group experienced less AR, the graft survival was not different from others, which was mainly due to the recurrence of underlying disease. Therefore, the main focus should aim for the management of recurrence, especially in HLA-identical renal transplantation.

SA-PO3105

Proteinuria as a Marker of Renal Injury in Children after Kidney Transplantation Tanya E. Pereira, Jayanthi Chandar, Chryso P. Katsoufis, Wacharee Seeherunvong, Carolyn L. Abitbol, Michael Freundlich, Gaston E. Zilleruelo. *Division of Pediatric Nephrology, Department of Pediatrics, University of Miami, Miller School of Medicine, FL.*

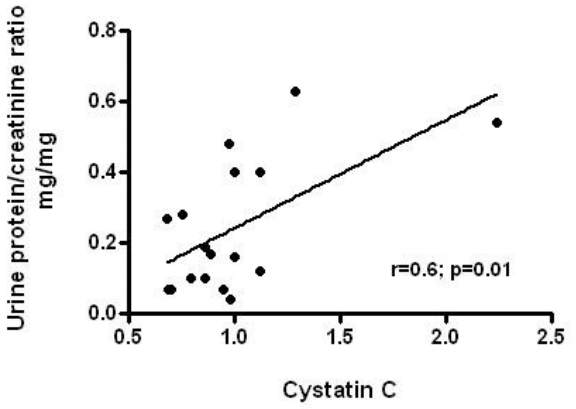
Background: Acute kidney injury occurs at the time of kidney transplantation as a consequence of ischemia-reperfusion injury.

The purpose of this study is to sequentially and quantitatively measure proteinuria in children with kidney transplants and assess the correlation between cystatin C and proteinuria at the end of the first post-transplant year.

Methods: The cohort consists of 25 children with kidney transplants followed at the University of Miami between the years 2008 to 2010. Proteinuria was assessed by urine protein/creatinine (Upr/cr -mg/mg) ratio at 2 days, 1 week, 1 month and 1 year post-transplant. Comparisons were made between living donor (LD) and deceased donor (DD) transplants. Children with FSGS were analyzed separately.

Results: The mean age was 12± 4.9 years with 11 males. Seventeen of 25 had DD.transplants. At one month post-transplant, there was a significant decrease in proteinuria in both DD and LD. The urine pr/cr ratio was 1.35±1.45, 0.27±0.16, 0.19± 0.17 in DD versus 0.2 ± 0.5, 0.38± 0.5 and 0.27± 0.16 in LD at 1 week, 1 month and 1 year post-transplant respectively. Those with FSGS had a mean urine pr/cr ratio of 8.3±6.4 versus those without

FSGS who had a pr/cr ratio of 1.6± 1.5 2 at 2 days post transplant (p< 0.02). Forty percent of the initial proteinuria consisted of albumin. There was a significant correlation between cyststain C and urine pr/cr ratio at the end of the first post- transplant year.



Conclusions: Proteinuria is a useful marker of post-transplant renal injury, and when used in conjunction with serum cystatin c is a good predictor of renal parenchymal integrity. Sequential assessment of urine pr/cr ratio helps determine recovery from acute kidney injury.

Funding: Clinical Revenue Support

SA-PO3106

Altered mTOR Pathway in Glomerular Epithelial Cells May Not Be Associated with Proteinuria Ping L. Zhang, Wei Li. *Department of Anatomic Pathology, William Beaumont Hosptial, Royal Oak, MI.*

Background: Several studies report that transplant patients treated with Sirolimus, an inhibitor of mammalian target of rapamycin pathway (mTOR), have a high chance to develop a reversible proteinuria. In this study, we evaluated phosphorylated (p) form of mTOR and its downstream signals 70S6K and 4EBP in glomerular epithelium in both native and transplant biopsies from patients with proteinuria, and correlated the expression of these markers with proteinuria levels.

Methods: The first study included 17 control cases, 18 focal segmental glomerulosclerosis (FSGS) and 22 immune complex mediated glomerulopathy (ICMGN). The second study consisted of 30 unremarkable control kidney sections (removed for renal tumors), 30 transplant cases with Sirolimus treatment (Siro), 59 cases with both Sirolimus and Cyclosporine therapy (Siro+Cyclo) and 39 transplant cases with only cyclosporine treatment (Cyclo). All sections were stained for p-mTOR, p-p70S6K and p-4EBP. Nuclear staining was assessed and categorized into four grades: 0 - 3+ based on intensity of staining in proximal tubules (PT), glomerular parietal epithelium (PE) and visceral epithelium (VE).

Results: In the first study, nephrotic range of proteinuria was found in FSGS and IMCMG groups. In the second study, proteinuria was significantly higher in Siro and Siro+Cyclo groups than Cyclo group. In both native and transplant biopsies, study groups all showed 1- 2 fold higher staining for all 3 markers in PT, PE and VE when compared to controls. In both studies, proteinuria was not associated with expression of any marker in any location but the proteinuria was significantly associated with interstitial fibrosis in the transplant study. Expression of p-70S6K in proximal tubules was significantly related to either serum creatinine (native study) or acute cellular rejection (transplant study).

Conclusions: Our data demonstrated a low activity of mTOR pathway in PT, PE and VE in control kidneys. The extent of proteinuria was not significantly associated glomerular expression of the mTOR pathway activity in both native and transplant studies, as activated mTOR pathway in glomerular epithelium appeared related to the high mTOR pathway activity in the PT.

Funding: Pharmaceutical Company Support

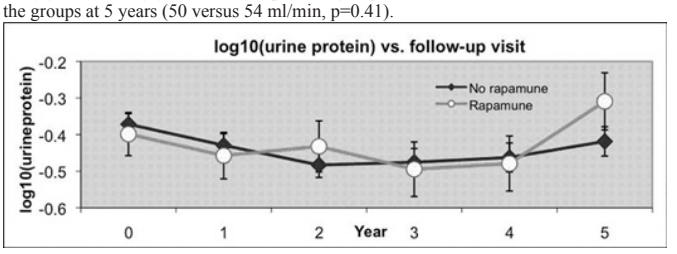
SA-PO3107

De Novo Rapamune Use Is Not Associated with the Development of Proteinuria Allyson Hart,[1] Liangxing Zou,[2] James Hodges,[2] Hassan N. Ibrahim.[1] *[1]Medicine - Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]Coordinating Center for Biometric Research - Biostatistics, University of Minnesota, Minneapolis, MN.*

Background: Rapamune has been associated with the development of proteinuria when used to replace calcineurin inhibitors for allograft dysfunction. The long-term effect of de novo Rapamune use on proteinuria is less well studied. We evaluated the effects of Rapamune use on proteinuria and GFR in kidney transplant recipients followed up for five years.

Methods: 153 kidney transplant recipients in the Angiotensin II Blockade for Chronic Allograft Nephropathy trial (NCT00067990) received Rapamune plus CNI (n=29) or Cellcept plus CNI (n=124). Iothalamate GFR and 24-hour urine albumin and protein were obtained at baseline and annually for 5 years. Statistical analyses used mixed linear models with a random effect for subjects and AR(1) errors within subject, and adjusted for age, sex, donor source, episodes of acute rejection, and systolic blood pressure.

Results: The Rapamune group was 44% female; mean age was 49 and 75% were live donor recipients. The non-Rapamune group did not differ with respect to sex, age, donor source, or systolic blood pressure at any follow-up time. GFR was higher at baseline in the Rapamune group (54 vs. 65 ml/min, p=0.002). At annual follow-up visits, GFR, proteinuria, albuminuria, and systolic blood pressure did not differ between Rapamune and non-Rapamune groups. At 5 years, adjusted mean urine protein in the Rapamune group was 0.5 g/g versus 0.4 g/g in the non-Rapamune group (P=0.21). GFR did not differ between the groups at 5 years (50 versus 54 ml/min, p=0.41).



Conclusions: De novo Rapamune use was not associated with increased proteinuria or a difference in measured GFR. The noted association of Rapamune with proteinuria may be due to the removal of the vasoconstrictive effects of CNIs.

Funding: NIDDK Support

SA-PO3108

Safety and Efficacy of Administering the Maximal Dose of Candesartan in Renal Transplant Recipients Noritaka Kawada,[1] Toshiki Moriyama,[2] Masayoshi Okumi,[3] Naotsugu Ichimaru,[3] Harumi Kitamura,[1] Jun-Ya Kaimori,[1] Norio Nonomura,[3] Shiro Takahara,[4] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Div. of Nephrol, Osaka Univ. Graduate School of Med., Suita, Osaka, Japan; [2]Health Care Center, Osaka university, Suita, Osaka, Japan; [3]Dep. of Urology, Osaka Univ. Graduate School of Med., Suita, Osaka, Japan; [4]Advanced Technology for Transplantation, Osaka Univ. Graduate School of Med., Suita, Osaka, Japan.*

Background: The regular dose of angiotensin II type-1 receptor blocker (ARB) used in renal transplant patients for hypertension is shown to be safe and effective. However, information on the appropriate dosing of ARB in renal transplant patients is limited. We evaluate the efficacy and safety of the maximal dose of candesartan administered to renal transplant patients.

Methods: Sixty-nine recipients were enrolled in this study. Patients were divided into three groups based on the basal dose of candesartan, patients not taking candesartan (Group A), taking low to medium dose candesartan (2-4mg/day; Group B), and taking high dose candesartan (8mg/day; Group C). During the course of the study, patients were treated with a gradual increase of candesartan to a final dose of 12 mg/day. Physiological and biochemical parameters were acquired before and after the twelve-month study period.

Results: Ninety-one percent of patients succeeded in continuing their administration of candesartan for one year and 75% tolerated the administration of the maximal dose of candesartan. Significant differences in proteinuria, albuminuria, serum creatinine, and eGFR level among the groups were detected. In group A, candesartan reduced systolic blood pressure, decreased the levels of proteinuria, albuminuria, eGFR, and hemoglobin and increased plasma potassium, creatinine level, and plasma renin activity.

Conclusions: Gradual increase of ARB to its maximal dose in renal transplant patients is safe when carefully monitored. We could demonstrate the impact of maximal RAS blockade on both proteinuria and albuminuria, which indicates the need for future, long-term randomized prospective trials to further establish the impact of maximal RAS blockade on renal and cardiovascular protection in transplant patients.

Funding: Government Support - Non-U.S.

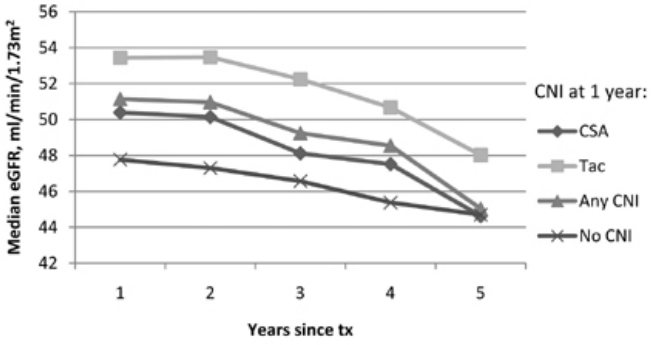
SA-PO3109

Estimated Glomerular Filtration Rate during Years 1 to 5 Post-Transplant by CNI Use, Data from the Patient Outcomes in Renal Transplant (PORT) International Data Collaboration Jon J. Snyder,[1] Melissa Skeans,[1] Ajay K. Israni,[1,2] Gilbert Litalien,[3] Bertram L. Kasiske.[1,2] *[1]Chronic Disease Research Group, Minneapolis, MN; [2]Hennepin County Medical Center, Minneapolis, MN; [3]Bristol-Myers Squibb, New Haven, CT.*

Background: Serum creatinine-based estimation of glomerular filtration rate (eGFR) is the standard marker for monitoring graft function. Monitoring changes in eGFR can aid clinicians in anticipating long-term prognoses.

Methods: We explored the association between MDRD eGFR during years 1 through 5 post-transplant and CNI use at 1 year post-transplant to see if differences were evident by CNI and would aid inference based on eGFR measurements. We studied 7,594 patients from 11 transplant centers in the Patient Outcomes in Renal Transplantation (PORT) international database transplanted 1999-2006 whose allografts survived 1 year post-transplant. Patients who returned to dialysis were assumed to have an eGFR of 0 from the time of graft failure until the earliest of death, loss-to-follow-up, or end of study.

Results: Use of any CNI at 1 year post transplant was reported for 88% of patients. Cyclosporine (CSA) was more common, reported in 56% of patients; Tacroliumus (Tac) was reported in 28% of patients. Median eGFR for was highest in Tac users at every post-transplant time point, from 53.5-48.0 ml/min/1.73 m2 in years 1 to 5 (Figure). Median eGFR in CSA users was 3-4 ml/min/1.73 m2 lower than in Tac users (p<0.001), and 3-6 ml/min/1.73 m2 lower in people in whom no CNI was reported (p<0.001). Median eGFR declined over time in all groups, regardless of CNI use or type of CNI.



Conclusions: In summary, median eGFR between years 1 and 5 post-transplant was highest in patients who were on Tac 12-months post-transplant; however, median eGFR declined over time in all groups.

Funding: Pharmaceutical Company Support

SA-PO3110

Over Ten Years Kidney Graft Survival Determinants Anabela Malho, Jorge Malheiro, Isabel Fonseca, La Salete Martins, Sofia Pedroso, Manuela Almeida, Leonidio Dias, Antonio Andresen Henriques, António Manuel Nunes Cabrita. *Serviço de Nefrologia, Hospital Santo António, Porto, Portugal.*

Background: Kidney graft survival has been mainly evaluated using an up to 10-years threshold. Instead, in this study our aim was to evaluate predictive variables that impact long term kidney graft survival (≥10 years).

Methods: We analyzed data from 996 kidney transplants performed between 1983 and the end of June 2000. Inclusion criteria: graft survival > 3 months and patient survival > 1 year post-transplant (PT). Exclusion criteria: simultaneous multiple grafts recipients. We enrolled 892 patients in our analysis: 638 patients with functioning graft at 10-years PT and 254 patients with graft failure at 10-years PT (considering patient death with a functioning graft <10 years PT as graft failure). Between groups comparisons were done using Mann-Whitney and chi-square test. To determine independent predictive variables for long-term graft survival a multivariate-adjusted logistic regression was performed.

Results: Patients with long-term graft survival had significant lower donor age, 12-month PT creatinine, HLA-B mismatches, panel of reactive antibodies (PRA) level (<30%) and number of transfusions (<3); higher frequency of immediate graft function, absence of acute rejections (AR) episodes, positive CMV IgG and induction with anti-thymocyte immunoglobulin (ATG). Significant predictors of long term graft survival were 12-month PT creatinine (OR=0.26, P<0.001), donor age (OR=0.98, P=0.004), time on dialysis (OR=0.93, P=0.044), recipient positive CMV IgG (OR=1.59, P=0.040), absence of AR episodes (OR=1.57, P=0.047), 0 to 1 HLA-B mismatch (OR=1.80, P=0.004) and recipients male gender (OR=1.84, P=0.005). Recipients age at transplant, number of transfusions (< vs ≥ 3), PRA level (< vs ≥ 30%), immediate graft function and induction with ATG were not significant predictors.

Conclusions: Recipient gender, IgG CMV status, dialysis vintage, absence of AR episodes, lower number of HLA-B mismatches and donor age were all significant predictors, while a lower 12-month PT creatinine remained the stronger determinant for long term kidney graft survival.

SA-PO3111

Favorable Effect of Systemic Insulin Delivery on Lipid Profiles in Simultaneous Kidney Pancreas Transplantation George A. Osuchukwu,[1] Ijeoma C. Nwelue,[1] Bruns A. Watts,[1] Tina Kochar,[1] Horacio E. Adrogue.[2] *[1]Department of Nephrology and Hypertension, University of Texas Medical Branch, Galveston, TX; [2]Methodist J.C Walter Jr Transplant Center, Department of Internal Medicine Methodist Hospital, Houston, TX.*

Background: Simultaneous kidney pancreas transplant (SPK-t) is the treatment of choice for carefully selected patients with diabetes mellitus and end stage renal disease. Due to the ease of the surgery a systemic delivery of the exocrine drainage is favored. Systemic insulin delivery in SPK-t has been demonstrated to lead to hyperinsulinism. Hyperinsulinism and insulin resistance in other populations are associated with an unfavourable lipid profile, one factor in their overall cardiovascular risk profile. Here we examine the effect of a systemic delivery of insulin on lipid profile in SPK-t patients.

Methods: Fasting glucose, insulin, c-peptide and lipid profile levels were obtained from 27 (SPK-t) patients with systemic exocrine drainage during routine post transplantation follow up. Homeostasis model of Assessment 2 calculations were used to estimate the beta cell secretory capacity HOMA-B, insulin resistance HOMA-IR and insulin sensitivity HOMA-S. The Fasting lipid profile of SPK-t patients was compared to 53 age, sex and immunosupresion protocol matched nondiabetic kidney transplant (K-t) patients using the unpaired student's t-test.

Results: HOMA scores for the SPK-t group were (mean ± SD): HOMA-B 203 ± 54, HOMA-IR 2.5 ± 1.2 and HOMA-S 48.8 ± 21. These results suggest hyperinsulinism, insulin resistance and an increased beta cell mass, which are consistent with previous studies. The SPK-t group had a lower total cholesterol (155 ± 31 SPK-t vs 183 ± 36 K-t; p< 0.0001), lower LDL (89.3 ± 27 vs. 105.5 ± 30.4 ; p<0.01) and a higher HDL (50.6 ± 10.7 vs. 40.6 ± 10.2 ; p<0.01) levels than the K-t group, indicating a more favorable lipid profile in SPK-t than in K-t.

Conclusions: Previous studies have shown that Systemic delivery of insulin is associated with hyperinsulinism and insulin resistance in SPK –t. Our findings show that this type of hyperinsulinism, due to a systemic delivery of insulin in SPK-t patients is associated with a significantly more favorable lipid profile.

SA-PO3112

Anemia in Adult Renal Allograft Recipients: Prevalence and Predictors Jorge Malheiro, La Salete Martins, Isabel Fonseca, Manuela Almeida, Josefina Santos Lascasas, Antonio Andresen Henriques, António Manuel Nunes Cabrita. *Nephrology Unit, CH Porto, Porto, Portugal.*

Background: Posttransplantation anemia (PTA) is multifactorial. Besides being associated with kidney dysfunction, other factors play a role, namely immunosuppressant regimens (IsR). Our purpose was to investigate an adult kidney graft recipient's population to elucidate the prevalence of PTA and its predictors.

Methods: Clinical data recorded throughout 2010 of adult kidney graft recipients with > 1-year postengraftment and no use of erythropoiesis stimulating agents was randomly selected from our unit, weighted for gender, age and time since transplantation (TsT). We aimed to examine clinical characteristics of the enrolled population and determine the prevalence of PTA. Multivariate adjusted linear regression was undertaken to detect hemoglobin (Hb) predictors: age, gender, estimated glomerular filtration rate (eGFR) by MDRD equation, log-body mass index (BMI), diabetic status, log-ferritin, log-transferrin saturation and serum albumin, mycophenolate mofetil (MMF), azathioprine (AZA), calcineurin inhibitor (CNI) and angiotensin converting enzyme inhibitors (ACEI) use. WHO anemia criteria were employed.

Results: A total of 302 (119 females) patients were studied with a mean age of 49.6±13.4 years, a median TsT of 7.6 years and a mean eGFR of 51.9±18.5 ml/min. The prevalence of anemia was 39.4% with a mean Hb of 13.2±1.6 g/dl. CNI was used in 97.7% and 77.8% took prednisolone (all with ≤10 mg). The most common IsR were CNI plus MMF (73.5%), CNI plus AZA (10.6%) and CNI alone (11.9%). No significant difference in Hb was found between these regimens. Multivariate adjusted linear regression (R^2=0.41) showed as significant predictors of lower Hb: female gender (*P*<0.001); lower eGFR (*P*<0.001), log-BMI (*P*=0.01), albumin (*P*= 0.02), log-transferrin saturation (*P*=0.01); higher log-ferritin (*P*=0.03); MMF (*P*=0.002) and ACEI (*P*=0.01) use.

Conclusions: Prevalence of PTA was high. Expectable demographical (gender) and clinical (eGFR) variables were strong predictors of Hb. Inflammation markers (lower albumin, higher ferritin) and drugs with reported negative effect in erythropoiesis (MMF and ACEI) were also associated with lower Hb.

SA-PO3113

Undiagnosed Glucose Metabolism Disorders in Dialysis Patients: An Analysis Using Oral Glucose Tolerance Tests in German Dialysis Centers Bernhard K. Krämer,[1] Bernhard Banas,[2] Miriam C. Banas,[2] Bernd Krüger.[1] *[1]Medizinische Klinik V, Mannheim University Hospital, Mannheim, Germany; [2]Nephrology, Regensburg University Hospital, Regensburg, Germany.*

Background: Post-transplant diabetes mellitus (PTDM) or new-onset diabetes mellitus (NODM) after renal transplantation is considered a major health threat for renal transplant recipients, that goes along with decreased patient and graft survival.

Methods: Screening for undiagnosed diabetes mellitus was done with the use of oral glucose tolerance test (oGTT) in 4 dialysis centers in Germany according to ADA criteria. Impaired glucose metabolism disorders were defined as a fasting glucose level ≥ 100 - 125 mg/dL (impaired fasting Glucose IFG) and/or a 2 h glucose level 140-199 mg/dL (impaired glucose tolerance IGT). Overt diabetes mellitus was defined as a fasting glucose level ≥ 126 mg/dL and/or a 2 h glucose level ≥ 200 mg/dL.

Results: 237 adult hemodialysis patients were considered for inclusion in this trial. 91 patients (=38.4%), that were known to be diabetic were excluded from the trial leaving 146 non-diabetics. Since oGTT was not performed in 40 of these nondiabetics due to refusal to participate or inability to give informed consent or to participate or nonadherence, 106 patients underwent oGTT. From these 106 patients, 12.3% had an abnormal fasting glucose (≥ 100 and < 126 mg/dL), 18.9% had an impaired glucose tolerance (2 h glucose level ≥ 140 and < 200 mg/dL), and 9.4% had overt diabetes mellitus.

Conclusions: There is a considerable number of undiagnosed glucose metabolism disorders including overt diabetes mellitus in German hemodialysis patients. These patients may (eronneously) be classified as PTDM or NODM after renal transplantation.

Funding: Clinical Revenue Support

SA-PO3114

Risk Factors for Vitamin D Deficiency Post Transplant and Role in PTH Regulation Temitope Ojo,[1] Priyanka Jain,[1] Carmen Castaneda-Sceppa,[2] Vaidyanathapura S. Balakrishnan,[1] Madhumathi Rao.[1] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Health Sciences, Northeastern University, Boston, MA.*

Background: 25-OH Vitamin D (VitD) deficiency is common in kidney transplant (KTX) recipients, secondary to insufficient intake and sun exposure, and increased catabolism by medications and FGF-23 hypersecretion. We examined risk factors for VitD deficiency and its association with PTH levels post-transplant.

Methods: In 106 clinically stable KTX recipients enrolled between 6 months and 5 years from transplant,VitD levels were measured by liquid chromatography and tandem mass spectroscopy.Intact PTH in serum was measured by non-competitive chemiluminescent immunoassay and intact FGF-23in plasma by ELISA (Immutopics, San Clemente, CA).

Results: The mean (SD) age was 47(11) years, 63% were male and 12% African American; 54% had CKD stage 1T and 2T, 41% stage 3T and 5% stage 4T; 62% had low VitD levels, 73% had hypophosphatemia, and 66% had elevated PTH levels. VitD levels were lower in African American subjects and within the first year from transplant; higher levels were seen after pre-emptive transplantation, and subjects with lower eGFR and using of VitD supplements. A significant inverse correlation was seen between PTH and VitD levels (r=-0.31; p=0.001). Indeed, PTH levels did not vary with eGFR or serum calcium, suggesting impaired normal feedback regulation. Median (95% CI) PTH levels were higher by 0.85(0.74-0.98) pg/mL for a 10ng/mL lower VitD level (p=0.04). Higher PTH levels were associated with longer duration on dialysis pre-transplant (mean, 95%CI: 56, 39-81 pg/mL among pre-emptive transplant, 85, 61-119 pg/mL with up to 3 years of dialysis, and 145, 106-199 pg/mL with > 3 years of dialysis pre-transplant; p<0.001). VitD supplementation was used in 62% of the study population; however, 45% of individuals with low VitD were not receiving supplementation.

Conclusions: In summary, low VitD levels are common after transplantation, likely secondary to depletion while on dialysis and could potentially play a role in PTH elevation after transplant. Pre-transplant repletion may be a relevant therapeutic option for post-transplant mineral bone disorder.

Funding: NIDDK Support

SA-PO3115

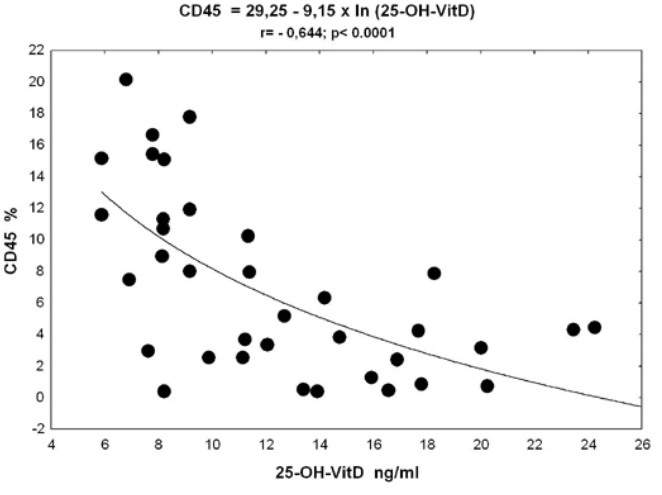
Mineral Metabolism, Parenchymal Calcification and Inflammatory Indices in Kidney Transplant Biopsies Ofelia Jercan, Carlo Maria Alfieri, Donata Cresseri, Gabriella Moroni, Maria Teresa Gandolfo, Maria Daniela Croci, Maria Pia Rastaldi, Piergiorgio Messa. *Nephrology and Dialysis, IRCCS Fondazione Ca' Granda Ospedale Policlinico, Milan, Italy.*

Background: Some studies suggested that parenchymal calcifications (PC), inflammation (I), epithelial-mesenchymal transition (EMT) and fibrosis (F) might contribute to chronic graft dysfunction in transplanted kidneys (KTx). A role for mineral metabolism (MM) derangement in these pathologic changes has been suggested. The aims of our study were to evaluate: a) the relationships between MM and PC, I, EMT and F indices in KTx biopsies (Bx) ; b) their possible relationship with graft outcome.

Methods: In the Bx of 87 KTx pts (46 M; aged 48 ±12 yrs), stainings for leukocytes (CD45: I marker), vimentin (EMT marker), alizarin red (PC marker), and Sirius red (F marker) were quantitatively evaluated by electronic image analysis on 20 low power fields (% of the total area). PC was estimated as present (+) or absent (-). MM (PTH, Ca, phosphate), biochemical and clinical parameters were recorded at Bx and 1 year after Bx. 25-(OH)-VitD levels were assessed in 35 patients.

Results: In the 76% of PC+ Bx, I and EMT markers were lower than in PC- (4.8±6.54 vs 8.6±7.3 % p=0.03; 7.75±5.1 vs 10.5±6.09 p=0.05, resp). MM parameters were not related with either PC or any other marker.

I and EMT were negatively related with MDRD (p=0.007, p=0.01 respectively). A neg correlation was found between 25OH-VitD and both I (p=0.0002)[figure 1] and EMT (p=0.01), but not with MDRD.



In the 14 patients restarting dialysis during the follow-up, I (12.91±7.9 vs 7.0±6.8 % p=0.004) and EMT were significantly higher(12.6±7.0 vs 9.3±5.6 % p=0.05).

Conclusions: Our results suggest: a) CD45 and Vimentin as good prognostic markers of graft outcome; b) no major effects of MM parameters on injury markers, apart from a possible protective role for VitD status.

SA-PO3116

Correlates of FGF23 Levels in a Population of Stable Kidney Transplant Recipients with Hypovitaminosis D and Hyperparathyroidism Mariana S. Markell, Sima Terebelo. *Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: FGF-23 interacts with Vitamin D and parathyroid metabolism in a complex fashion that has not been fully delineated, resulting in conflicting results when patients with CKD are studied. FGF23 is elevated early post-transplant but has not been carefully studied at longer time periods.

Methods: 41 stable kidney transplant recipients (KTRs) with hyperparathyroidism and hypovitaminosis D were studied. Patients were not receiving supplementation with any form of vitamin D. Full length FGF23 was measured by ELISA.

Results: The population included 22 men (51%), 34 Blacks (83%), mean age 49.2±13.5; 55.5±47 months since last transplant, creatinine 1.5±0.4 mg/dl, phosphorus, 2.97±0.56, magnesium, 1.72±0.3, 25-OH Vit D 16.2±0.7, 1,25 (OH)2 vit D3, 49.1±18.2, PTH, 187±78.3, GFR by MDRD, 56.9±19.2 m;/min (range 21.7-103.4 ml/min). The mean FGF23 value was 103.6±64.8 pg/ml (range 33.8-388.6 pg/ml). 90% of the patients had FGF23 levels > 55 pg/ml. FGF-23 levels positively correlated with gender p=.039, r=.324, months since last transplant, r=.322, p=.045, BUN r=0.484, p=0.001, creatinine r=0.369, p=.017, serum phosphorus r=.428, p=.006, and serum magnesium r=.374, p=.016. FGF-23 levels correlated inversely with 1,25 vit D r=-.351, p=.026, and GFR by MDRD r= -.469, p=.002, although 1,25 Vit D levels did not correlate with GFR. By T-test Black pts had lower FGF23 values than Non-black, mean 94.3 pg/ml vs. 148.7 pg/ml, p=.042 although GFR by MDRD did not differ. There were no correlations between FGF-23 and age, tacrolimus dosage or level, prednisone daily dose, diuretic use, BP, calcium level, 25-OH vitamin D level, or PTH.

Conclusions: 1) FGF23 maintains an inverse relationship with 1,25 Vitamin D in the pts with Vitamin D deficiency despite elevated PTH levels and abnormal kidney function, suggesting that FGF23 may be more important in regulating 1,25 Vit D than PTH in this setting . 2) Black pts have lower FGF23 levels than Non-Blacks despite similar GFR, 3) FGF23 maintains a strong correlation with gender, GFR by MDRD, PO4, Mg++ and time since transplant, but as these factors are interrelated, a larger population will be needed to examine independent relationships.

Funding: Pharmaceutical Company Support

SA-PO3117

Serum Adipokines and Coronary Artery Calcification in Renal Transplant Recipients with Metabolic Syndrome Kuo-Hsiung Shu,[1,4] I-Chen Tsai,[2] Hao-Chung Ho,[3] Chi-Hung Cheng,[1,4] Ming-Ju Wu,[1,4] Cheng-Hsu Chen,[1] Tung-Min Yu,[1] Ya-Wen Chuang,[1] Shih-Tin Huang.[1] *[1]Div. of Nephrology, Dept. of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; [2]Radiology, Taichung Veterans General Hospital, Taichung, Taiwan; [3]Div. of Urology, Dept. of Surgery, Taichung Veterans General Hospital, Taichung, Taiwan; [4]School of Medicine, Chung-Shan Medical University, Taichung, Taiwan.*

Background: Renal transplant (RTX) recipients are associated with several metabolic disorders, of which metabolic syndrome (MetS) is relatively common. The current study aims at elucidation of the alterations of adipokines in RTX patients with MetS.

Methods: A cross-sectional study was carried out in our prevalent RTX patients. A modified Asian version of the ATP III criteria was adopted for the definition of MetS, in which the abdominal obesity was defined as a waist circumference >90 cm for male and >80 cm for female. Fasting blood samples were obtained for adiponectin, leptin and resistin by using commercial available ELISA kits. A multidetector computed tomography was performed to access coronary artery calcium score (CACS).

Results: A total of 271 RTX patients (male:female=133:138) were enrolled for the study. MetS was diagnosed in 72 (26.6%). MetS patients tend to be older (54.8±9.6 vs 51.4±13.4, p=0.055), had higher body mass index (26.1±3.9 vs 27.4±51.0, p<0.0001), serum creatinine (1.61±0.74 vs 1.41±0.55 mg/dl, p=0.009), hemoglobin A1c (6.84±1.44 vs 6.03±4.38% p<0.0001), CACS (848.9±1224.9 vs 286.4±720.7, p=0.016) and number of antihypertensive agents used (1.69±0.97 vs 0.82±1.02, p<0.0001). There was no significant difference in terms of gender, age and drugs used for immunosuppression. MetS patients had significantly lower serum adiponectin level (21.8±13.9 vs 28.7±18.7 mg/ml, p=0.002), higher leptin level (29.6±24.3 vs 15.5±20.0 ng/ml, p<0.0001) but comparable resistin level (2.6±1.7 vs 2.6±2.1 ng/ml, p=0.364).

Conclusions: RTX recipients with MetS were associated with alterations of adipokines that favor the development of atherosclerosis. Together with the finding of a significantly higher CACS, these patients are at high risk of acquiring coronary artery disease.

Funding: Government Support - Non-U.S.

SA-PO3118

Value of Adenosine Stress Cardiac Magnetic Resonance Imaging for Coronary Artery Disease Screening before Renal Transplantation Francois Glowacki,[1] Alexandra Botte,[1] Sébastien Homs,[1] Marco Midulla,[2] Christian Noël,[1] Jean-Paul Beregi.[2, 3] *[1]Nephrology, University Hospital, Lille, France; [2]Radiology, University Hospital, Lille, France; [3]Radiology, University Hospital, Nimes, France.*

Background: Coronary heart disease remains a major cause of morbidity and mortality after renal transplantation, also some practice guidelines recommend to evaluate ischemic heart disease before renal transplantation. We studied cardiac stress-magnetic resonance imaging (MRI) in this indication.

Methods: Candidates for kidney transplantation with risk factors for coronary disease underwent a cardiac stress-MRI scan (injection of gadoterate and coronary stress induced by adenosine). When the MRI scan revealed positive results, a coronary angiography was carried out. The incidence of major cardiovascular events and gadoterate toxicity were recorded during follow-up.

Results: Since January 2008, 114 patients have undergone a cardiac stress-MRI scan. These patients had an average of 2.7±1.4 cardiovascular risk factors (age 55.6±10.4 years, diabetes: 35.1 %). Coronary lesions were suspected on 22 MRI scans and confirmed in 85 % by systematic coronary angiography. Thus, we diagnosed 9.4% of coronary disease in patients with no previous history of coronary artery disease, leading to specific treatment. In the population, sensitivity of cardiac MRI was 89.5 % and specificity 62.5 %. During follow-up (1.67±0.7 years), 35 patients received a kidney transplant and 5 patients had a major cardiac event. Negative predictive value of cardiac MRI for major coronary events is 96.7 %. No patient developed nephrogenic systemic fibrosis.

Conclusions: With its high negative predictive value, stress-cardiac MRI appears to be a valuable tool for excluding ischemic heart disease before kidney transplantation.

Funding: Government Support - Non-U.S.

SA-PO3119

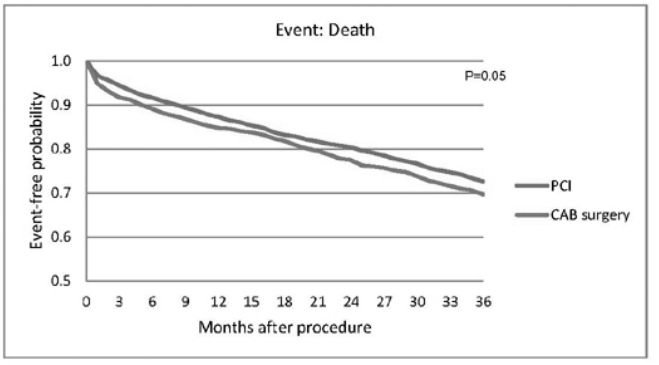
Risks of Death and Graft Failure Following Percutaneous Compared with Surgical Coronary Revascularization in Renal Transplant Patients David M. Charytan,[1] Yang Qiu,[2] Shuling Li,[2] Charles A. Herzog.[2] *[1]Medicine, Brigham and Women's Hospital, Boston, MA; [2]CVSSC, USRDS, Minneapolis, MN.*

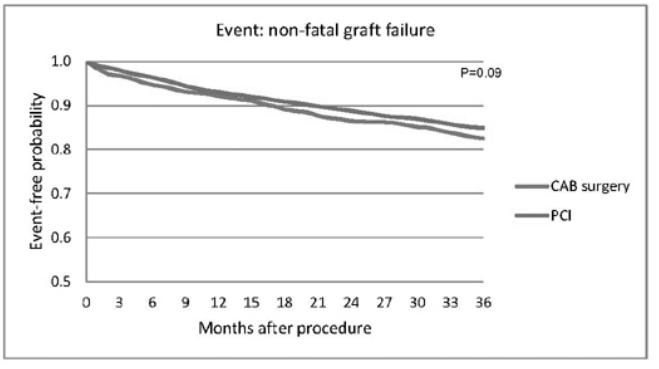
Background: Surgical (CABG) or percutaneous (PCI) intervention may precipitate graft failure (GF) in patients with renal transplants, but reliable data on the absolute and relative risks of death and GF following PCI and CABG are unavailable.

Methods: Renal transplant patients undergoing PCI (3,531) or CABG (1,003) were identified using the USRDS. Comorbidity adjusted Cox models were used to assess the risks of death and non-fatal GF.

Results: Age race and sex were similarly distributed in the PCI and CABG groups. In the PCI group, 36% received bare metal stents, 36% received drug eluting stents, and 17% had intervention on ≥ 2 vessels. Internal mammary grafts were used in 84% of CABG patients, 95% had ≥2 vessels bypassed, and 83% had on-pump procedures. In-hospital death (2.3% vs. 5.3%) was more frequent after CABG. Overall survival was non-significantly lower with CABG (HR 1.14, P=0.05) in crude models [figure 1], but revascularization type was not associated with overall survival after adjustment for comorbidities (HR 0.89, P=0.12). At 3 years, freedom from non-fatal GF was lower with CABG vs. PCI (83% vs. 85%). However, the difference in non-fatal GF did not achieve significance in crude (P=0.09) or adjusted models (P=0.72). The risk of GF was not different following off-pump vs. on-pump CABG (HR 0.78, P=0.34).

Conclusions: CABG does not appear to be associated with improved survival compared with PCI in the renal transplant population. Non-fatal GF occurs more frequently following CABG, but the risk of non-fatal GF is similar with both PCI vs. CABG and off-pump vs. on-pump CABG following adjustment for baseline comorbidity.





Funding: NIDDK Support

SA-PO3120

Renal Cell Carcinoma in Allograft Kidneys: Use of Short Tandem Repeat Analysis To Determine Donor Origin of Cancer Kumarpal C. Shrishrimal, Eric P. Cohen, Ehab R. Saad, Lauren N. Parsons, Min Le, Alexander C. Mackinnon. *Medical College of Wisconsin.*

Background: Kidney transplant recipients have a high risk for renal cell carcinoma (RCC) in their native kidneys. We describe 3 cases of RCC in allograft kidneys. Short tandem repeat (STR) DNA was used to determine donor origin of the RCC.

Methods: RCC tissue was obtained in all cases. Donor DNA was obtained from cancer-free regions of the kidney (cases 1 and 2); Donor DNA was not available for case 3. Host DNA was obtained from fresh leukocytes (cases 1 and 3) or a non-cancerous lymph node (case 2). Penta C and Penta D genotypes were determined using GeneMapper.

Renal Cell Carcinoma in Allograft Kidneys

Case	Age at Transplant (years)	Time to cancer (years)	Histology of RCC	RCC recurrence
1	31	15	Clear Cell	None
2	54	8	Clear Cell	None
3	16	19	Clear Cell & Papillary	6 years with metastases

Results: Penta C and Penta D alleles were robustly amplified in all samples. Each case had two or more informative Penta C or Penta D alleles. In the two cases with donor DNA samples (cases 1 and 2), the Penta C and Penta D alleles from the cancer exactly matched the Penta C and Penta D alleles derived from the uninvolved donor kidney. Furthermore, one or more Penta C or Penta D alleles from the host were absent from the cancer. This shows that the cancer DNA is donor-derived, being genetically identical to the donor DNA. In case 3 donor DNA was not available for analysis, so cancer and host alleles were compared. In this instance, none of the Penta D alleles matched in the two specimens, and each specimen had a unique Penta C allele indicating that the cancer is genetically distinct from the host.

Conclusions: Determination of tumor cell origin is potentially significant for treating metastatic cancers, as in case 3. Reduction of immunosuppression may cause cancer rejection for cancers derived from the donor. But host derived cancers would not respond to reduction of immunosuppression. The Penta C and Penta D method is informative of sample identity over 99% of the time. Our data show that STR analysis is a simple strategy to determine donor versus recipient origin of cancers in kidney transplant patients.

SA-PO3121

Clinical Features and Outcomes of Tuberculosis among Kidney Tranplant Recipients in Brazil: A Report of the Last Decade Igor Marques,[1] Renato Antunes Caires,[2] Victor Sato,[2] Lilian P.F. Carmo,[1] Gustavo Ferreira,[1] Ligia C. Pierrotti.[1] *[1]Renal Transplant Service, University of Sao Paulo School of Medicine, SP, Brazil; [2]Nephrology Division, University of Sao Paulo School of Medicine, SP, Brazil.*

Background: It is necessary to clarify the incidence of tuberculosis (TB) among kidney transplant recipients (KTR) as well as changes in the chronology, clinical presentation and prognosis of the disease, especially in highly endemic areas, in order to develop prophylaxis strategies.

Methods: Retrospective single-center observational study involving all cases of TB that occurred in KTR between 2000 and 2010 confirmed by culture, isolation of *M. tuberculosis* DNA by polymerase chain reaction or histopathology, according to WHO criteria.

Results: Among the 1549 kidney transplantations performed during the study period, 43 (2.8%) developed TB, with an incidence of 803 cases per 10^5 patients per year, which was very higher than in general population in Brazil (45 cases per 10^5 inhabitants per year; RR 17.8). Of the TB cases, 84% occurred within the first 2 years posttransplant, and 72% were pulmonary forms. Previous TB infection was present in 3 (7%) patients. No chemoprophylaxis was applied. The most common symptoms were fever (79%), cough (35%) and dyspnea (16%). Time elapsed from the onset of symptoms to the start of treatment was 28 days (range, 2 - 138 days). Median length of antituberculous therapy was 196 days. Immunosuppressive therapy was reduced in 15 (35%) patients and incidence of acute rejection was higher in TB than in non-TB group (44% vs. 28%, p=0.03, respectively). Crude mortality was 14%, and attributable mortality was 12%. Ten year death-censored graft survival (44.3% vs. 56.3%, p=0.64) and patient survival (69.1% vs. 72.4%, p=0.67) were similar between TB and non-TB groups, respectively.

Conclusions: Kidney transplantation increases the risk of TB. Symptoms of infection are often attenuated, leading to delayed diagnosis. TB-attributable mortality is still high. Clinicians should consider chemoprophylaxis for high risk patients, as the residents of endemic areas. However, randomized controlled trials are needed to confirm the benefits of such approach.

SA-PO3122

The Effect of Interferon Therapy on HLA Alloantibodies in Waitlisted Patients with Hepatitis C Infection Yasmin G. Brahmbhatt, Bernd Schroppel, Michele Witlieb, Vinay Nair. *Kidney Transplantation, Mount Sinai Medical Center, NY, NY.*

Background: Interferon (IFN) therapy is recommended for dialysis patients with chronic hepatitis C (HCV) awaiting kidney transplantation. IFN is known to cause autoimmunity and increase alloimmunity. We aimed to examine the effect of IFN on HLA antibodies (Ab) in waitlisted chronic HCV patients.

Methods: All current kidney waitlisted patients and patients that received a kidney transplant from 1/2008 to 1/2011 were screened for HCV serostatus. Patients with a positive HCV pcr and pre-existing HLA antibodies determined by Luminex single antigen (LSA) beads were considered for study. Patients without LSA testing 3 months to 12 months apart were excluded. We identified 2 groups of patient, those treated with IFN (treatment group) and those not treated (control group) in-between LSA testing. Antibody strength (Ab) was quantified by mean fluorescence intensity (MFI). We used a threshold of 10,000 MFI to determine cPRA

Results: We identified 7 patients who were treated with IFN and 23 patients who were not IFN treated that had 2 sets of LSA testing. In the treatment group 3 patients had a prior kidney transplant of which 1 had the graft present at time of IFN. In the control group, 3 patients had a prior transplant of which 2 had the graft present at the time of LSA testing.

43% (3/7) IFN treated patients and 26% (6/23) of patients in the control group had a significant rise in median MFI (p= 0.64, Fishers exact test). The median change in MFI was 239 (IQR -377 to 2053) in the IFN group and 4 (IQR -3226 to 940) in the control group. The change in Ab strength was not significantly different between groups (p=0.14, Mann-Whitney test). Class I and class II Ab strength was not significantly different between groups. The median cPRA (MFI >10,000) was 94% (range 0-100) in the IFN group and did not change after IFN therapy (median cPRA 94% pre-IFN vs. 94% post-IFN).

Conclusions: Compared to a matched control group IFN did not change the strength or breadth of preexisting HLA antibodies. Our data suggests that the decision to treat chronic HCV with IFN should not be altered out of concern for increasing sensitization in kidney transplant waitlisted patients.

SA-PO3123

Tacrolimus Percent Coefficient of Variation as a Superior Marker of Late Acute Rejection Associated with Nonadherence in Adolescent Renal Transplant Recipients Hilda E. Fernandez, Robert B. Ettenger, Eileen W. Tsai. *Pediatric Nephrology, UCLA, Los Angeles, CA.*

Background: We have previously shown that tacrolimus (TAC) percent coefficient of variation (CV%) is a superior marker compared to TAC standard deviation (SD) for detecting the likelihood of acute rejection (rej) associated with medication nonadherence (NA) in pediatric renal transplantation (Tx). We aimed to identify a significant threshold and time interval for which TAC CV% is predictive of late acute rej associated with NA in adolescents.

Methods: TAC SD and CV% were measured in 31 adolescents (ages 11-21) who underwent Tx from 2004 - 2008. Patients were maintained on immunosuppression with TAC and MMF ± steroids. All rej was confirmed by biopsy (Bx). Bxs were classified by Banff 2007 criteria. SD and CV% was calculated from trough TAC levels over various time intervals post-Tx. Receiver operator curve (ROC) analysis was used to analyze TAC CV% values.

Results: We found that the significant time interval for TAC CV% measurement was 6 mos prior to the first rej or, in non-rejectors, 6 mos prior to the last clinic follow-up. TAC CV% was higher in rejectors vs. non-rejectors (58.2% vs. 26.9%, p = 0.021).

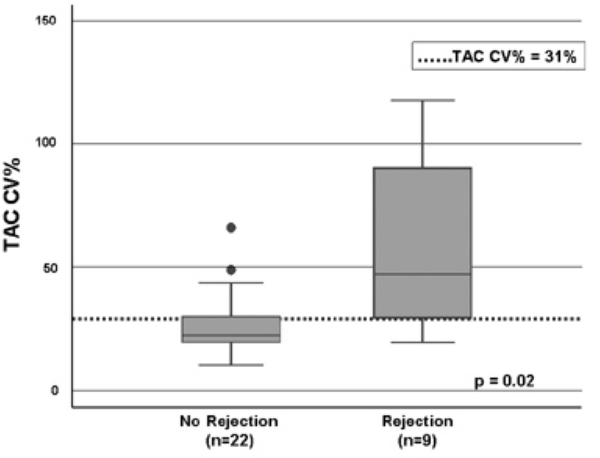


Figure 1: TAC CV% by rejection status 6 months prior to last clinical follow-up or biopsy-proven rejection.

The mean TAC SD was not different (2.52 vs. 1.79, p = 0.098). Using ROC analysis with an AUC of 0.77, TAC CV% > 31% had an OR=6.8 (p=0.04).

Conclusions: TAC CV% is superior to TAC SD in detecting late acute rej 6 months prior to allograft rej. This is the first time that a clinically relevant TAC CV% of > 31% has been demonstrated to be associated with an increased risk of allograft rej. Frequent monitoring with TAC CV% for adolescents may decrease acute rej due to medication NA in adolescent Tx recipients.

Funding: Private Foundation Support

SA-PO3124

Cardio-Renal Interaction in Living Kidney Donors: Correlation between Glomerulosclerosis in 0-Hour Biopsy and Left Ventricular Mass Index (LVMI) Naoki Haruyama,[1] Akihiro Tsuchimoto,[1] Toshiaki Nakano,[1] Masatomo Taniguchi,[1] Hidehisa Kitada,[2] Kazuhiko Tsuruya,[1] Takanari Kitazono.[1] *[1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: Although living kidney donors are almost normal healthy individuals, the "0-hour" biopsy from them often reveal glomerulosclerosis (GS) to various degrees. Recently, cardiorenal interaction has been a major clinical implication and it is well known about high prevalence of left ventricular hypertrophy (LVH) in CKD patients. However, this interaction in the subjects with GS despite normal kidney function has not been previously reported.

Methods: We examined the association between the proportion of GS (%GS) in the 0-hour biopsy and LVMI determined by the echocardiogram before surgery. In the 206 subjects who donated a kidney in our hospital from March 2006 to May 2011, 50 donors were excluded because of lack of clinical data or inadequate kidney samples (less than 10 glomeruli). We divided the remaining 156 donors into the three groups according to %GS; the subjects without GS (Group I, n=41), the subjects with %GS of 0.1~9.9% (Group II, n=58), and the subjects with %GS above 10% (Group III, n=57). We compared baseline characteristics and LVMI among the groups. Moreover, we investigated correlation between %GS and LVMI by multivariate analysis. Data are expressed as mean ± SD.

Results: LVMI in Group III was significantly higher than those in the other two groups (80 ± 3, 81 ± 3, and 91 ± 3 g/m^2 in Group I, II, and III, respectively; $p < 0.05$). The multivariate linear regression analysis showed that %GS was significantly associated with LVMI even after adjusting for the other confounders like age, renal function, and hypertension (standardized $\beta = 0.16$, $p < 0.05$).

Conclusions: In the present study, the association between GS and LVMI was observed in the living kidney donors. This association was independent of blood pressure, renal function and age, suggesting that cardio-renal interaction might exist even before renal function declines.

SA-PO3125

Expression of FGF23/KLOTHO System in Human Vascular Tissue Javier Donate,[3] Mercedes Muros,[2] Carmen Mora,[2] Violeta Cazaña,[2] Javier García-Pérez,[1] Rafael Martinez Sanz,[4] Juan F. Navarro Gonzalez.[1,2] *[1]Nephrology, University Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [2]Clinical Biochemistry, University Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [3]Research Unit, University Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [4]Cardiac Surgery, Hospital Universitario de Canarias, La Laguna, Spain.*

Background: Fibroblast growth factor (FGF)-23 levels have been independently associated with impaired vasoreactivity and increased arterial stiffness, as well as cardiovascular events and mortality, whereas a protective function of KLOTHO against endothelial dysfunction has been reported, suggesting the potential participation of FGF23-KLOTHO axis in human vascular pathophysiology. Since expression of members of the FGF23-KLOTHO system in human vascular tissue remains unproved, we aimed to study the expression of FGF23, FGF receptors (FGFR) and KLOTHO in human aorta.

Methods: Thoracic aorta specimens from 44 patients with coronary artery disease who underwent elective coronary artery bypass surgery were tested for expression of FGF23-KLOTHO system, including FGF receptors.

Results: Expression of KLOTHO (mean expression level 4.85±5.43, arbitrary units) and two of the three cognate FGFR (FGFR-1 and -3) were detected and confirmed by RT-PCR, sequencing and qRT-PCR. However, expression of FGF23 and FGFR4 was not observed. We also detected the expression of membrane–anchored A Desintegrin and Metalloproteinases (ADAM)-17, the enzyme responsible for the shedding of KLOTHO from the cell surface, and the anti-inflammatory cytokine interleukin (IL)-10. Interestingly, there was a direct association between the KLOTHO mRNA expression levels and those of ADAM-17 and IL-10 (r=0.54, $P<0.001$; r=0.51, $P<0.01$, respectively).

Conclusions: Human vascular tissue expresses members of the FGF23-KLOTHO system, indicating that it can be a direct target organ for FGF23. These findings suggest a putative role of FGF23-KLOTHO axis in human vascular pathophysiology and cardiovascular disease.

Funding: Government Support - Non-U.S.

SA-PO3126

Niacin Protects Against Renal but Aggravates Cardiac Damage in Rats with the Metabolic Syndrome Praveen N. Chander,[1] Youn-Jung Kang,[2] Charles T. Stier.[2] *[1]Pathology, New York Medical College, Valhalla, NY; [2]Pharmacology, New York Medical College, Valhalla, NY.*

Background: Studies were conducted to examine the end-organ protective effects of niacin in obese spontaneously hypertensive rats (SHROB/cp); a model of the metabolic syndrome.

Methods: Animals were chronically treated with either a low dose (1% in chow; n=5) or a high dose (5% in chow; n=5) of niacin starting at 7.14 weeks of age. SHROB/cp (n=9) and lean hypertensive control animals (SHROB/Kol) received Purina 5008 chow to which no niacin was added. Urinary protein excretion and pretermial systolic blood pressure (SBP) were measured and the animals sacrificed at 15 weeks of age. Hearts and kidneys were harvested for histology.

Results:

	SHROB/kol	SHROB/cp	SHROB/cp & Low Niacin	SHROB/cp & HighNiacin
SBP (mm Hg)	215±4	200±2**	185±4**††	193±4*
UPE (mg/day)	20±2	221±21**	158±25**†	48±12††
Insulin (ng/mL)	7.6±1.8	64.0±7.4**	60.9±3.5**	29.8±7.4*††♠♠
FSGS (%)	0.8±0.3	12.4±3.3**	6.9±1.4	1.3±0.4††
Cardiac infarcts (#)	0.9±0.4	17.9±3.3**	35.6±5.8**†	12.4±3.0*♠

*P<0.05, **P<0.01 v SHROB/kol; † P<0.05, †† P<0.01 v SHROB/cp; ♠ P<0.05, ♠♠ P<0.01 v SHROB/cp low niacin

SBP was slightly but significantly lower in SHROB/cp vs SHROB/Kol and was not affected by niacin treatment. Consistent with the metabolic syndrome, SHROB/cp showed hyperinsulinemia which was lowered by high dose but not low dose niacin. However, both dose levels of niacin significantly decreased urinary protein excretion and the degree of focal segmental glomerular sclerosis (FSGS) in SHROB/cp. Despite lower SBP in SHROB/cp, these animals had significantly more myocardial microinfarcts frequently undergoing organization. Additionally, there were hypereosinophillic myocardial fibers suggesting ongoing acute necrosis in a few animals. Unlike kidney damage, cardiac injury was not reduced by niacin treatment and, with low dose niacin, was instead significantly increased.

Conclusions: These results suggest that 1) cardiac and renal damage in SHROB/cp are independent of hypertension; the latter considered secondary to hyperperfusion injury of the metabolic syndrome and 2) niacin causes target-organ protection in the kidney, but may further aggravate cardiac injury.

Funding: Private Foundation Support

SA-PO3127

Loss of TDAG51 Inhibits Vascular Medial Calcification Induced by High Doses of 1,25 Dihydroxycholecalciferol Imtisal Al-Bondokji, Jeffrey G. Dickhout, Alistair J. Ingram, Joan C. Krepinsky, Richard Austin. *Medicine, McMaster University, Hamilton, ON, Canada.*

Background: Vascular calcification (VC) is a progressive disorder that increases stiffening in the aorta and large capacitative arteries. This process is associated with uraemia produced by end stage renal disease (ESRD) and increases the cardiovascular morbidity and mortality in these patients. However, the underlying molecular mechanism of vascular calcification in uraemia remains unclear. We hypothesized that an imbalance between genes that promote and inhibit osteoblast differentiation drives this process. Thus, we tested the effects of T-cell death associated gene 51 (TDAG51), a novel osteoblast differentiation promoting gene, which modulates and induces smooth muscle cell calcification and differentiation to osteoblast-like cell phenotype.

Methods: We induced vascular medial calcification in mice by administration of super-physiological doses of 1,25 dihydroxycholecalciferol (Vitamin D_3). This VC model increases bone resorption leading to elevated plasma Ca^{2+} and PO_4 levels inducing genes that promote osteoblast differentiation and calcification. C57BL/6 and TDAG51 knockout male mice (8 weeks of age) were administered subcutaneous injection of Vitamin D_3 (50,000IU) for three consecutive days. Four days post-treatment, tissues were harvested for protein and mRNA analysis.

Results: Our results indicate C57BL/6 male mice receiving Vitamin D_3 had increased TDAG51 mRNA and protein levels in the aorta. TDAG51 knockout mice aorta and aortic smooth muscle cells (ASMC) have significantly less Ca^{2+} deposition/mg protein, reduced mineralization observed by von Kossa, xylenol orange staining and decreased alkaline phosphatase activity. Furthermore, peroxisome proliferator-activated receptor (PPAR) gamma mRNA and protein levels are up-regulated in TDAG51$^{-/-}$ ASMC, whereas osteoblast differentiation marker RUNX2/Cbfa1, Osterix, and phospho-SMAD 1/5 are down-regulated.

Conclusions: This data suggests, the loss of TDAG51 gene may confer resistance to smooth muscle calcification and differentiation to an osteoblast-like phenotype, thereby decreasing the risk of cardiovascular disease in ESRD.

Funding: Government Support - Non-U.S.

SA-PO3128

The Interaction of Uraemic Toxins and Endothelial Progenitor Cells in the Progression of Cardiovascular Disease Shaundeep Sen,[1,2,3] Claudine Sharon Bonder,[1,3] Stephen P. McDonald.[1,2] *[1]Department of Medicine, University of Adelaide, Adelaide, South Australia, Australia; [2]Central & Northern Adelaide Renal & Transplantation Service, Royal Adelaide Hospital, Adelaide, South Australia, Australia; [3]Division of Human Immunology, Centre for Cancer Biology, SA Pathology, Adelaide, South Australia, Australia.*

Background: Bone marrow (BM) derived endothelial progenitor cells (EPC) have a role in both blood vessel formation and regulation of function. How EPCs are affected by uraemic toxins p-cresol (PC) and indoxyl sulfate (IS), and how this relates to cardiovascular disease (CVD) risk is not clear.

Methods: Peripheral blood (PB) concentrations of EPCs, IS and PC were measured in control (Cx), haemodialysis (HDx) and kidney transplant (KTx) subjects, and compared to markers of CVD. In vitro, functional assays were performed on cultured HUVEC and EPC in the setting of increasing physiological concentrations of PC and IS.

Results: PB EPC concentration (% of mononuclear cells, MNCs) was decreased in KTx (0.006±0.006%, 95%CI) and HDx (0.006±0.005) compared to Cx (0.03±0.02, n=9/group, $p<0.01$). Both PC sulfate (Cx 2.1±0.8mg/L, HDx 23.6±12.4, KTx 3.6±2.5) and IS (Cx 1.9±1.7mg/L, HDx 60±26, KTx 1.9±1.3) were increased in HDx ($p<0.001$). There was no association between EPC and toxin concentrations (p=ns). Low EPC counts were associated with history of CVD (p=0.002). Carotid intima medial thickness, aortic pulse wave velocity and augmentation index did not correlate with toxin or EPC concentration.

In vitro, PC inhibited tube-forming capacity of HUVEC (n=5, one-way ANOVA $p<0.01$), with no benefit of addition of EPC (n=5, p=ns). Increased IS concentration inhibited HUVEC tube formation (n=5, $p<0.05$), however EPC addition prevented IS reduction in HUVEC tubes (n=4, p=ns). No difference was seen in EPC migration with toxins. Vascular cell adhesion molecule-1 expression increased on HUVEC with increased PC/IS concentration ($p<0.05$), but with no effect of EPC addition.

Conclusions: EPC PB concentration does not correlate with toxin concentration, however reduced EPC function, as shown in vitro to PC and IS, may be more clinically relevant.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO3129

Effect of Different Vitamin D Receptor Activators on Left Ventricular Hypertrophy and Myocardial Fibrosis in Subtotal Nephrectomised Rats
Sara Panizo,[1] Sara Barrio-Vazquez,[1] Natalia Carrillo-Lopez,[1] Amalia Fernandez-Vazquez,[2] Socorro Braga,[3] Ana Rodriguez-Rebollar,[1] Manuel Naves,[1] Jorge B. Cannata-Andia.[1] *[1]Bone and Mineral Research Unit, Hospital Universitario Central de Asturias. Instituto Reina Sofía de Investigación. REDinREN del ISCIII. Universidad de Oviedo, Oviedo, Asturias, Spain; [2]Pathological Anatomy Unit, Centro Médico de Asturias, Oviedo, Asturias, Spain; [3]Biochemistry Unit, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain.*

Background: Observational data suggest that increased survival in CKD patients treated with vitamin D receptor activators (VDRAs) could be in part due to their positive cardiac effect. The aim of this study was to analyze the effect of different VDRAs on left ventricular hypertrophy (LVH) and myocardial fibrosis in uremic rats.

Methods: Rats (n=22) with 7/8 nephrectomy were treated with equivalent doses of VDRAs (calcitriol 10 ng/kg/day, alfacalcidol 20 ng/kg/day and paricalcitol 30 ng/kg/day, 5 days per week) for 4 weeks. Placebo (n=7) and Sham (n=7) groups were included for comparison. Blood and tissues were collected at sacrifice. Histological and molecular parameters of LVH and fibrosis were evaluated.

Results: All VDRAs prevented LVH, the values of heart/body weight ratio, wall and septum thickness and cardiomyocites size were similar to those observed in the Sham group. In addition, all VDRAs showed a significant decrease in atrial and brain natriuretic peptides (ANP and BNP) expression and they were able to decrease the phosphorilation of ERK 1/2, this effect was more marked in the paricalcitol group. Paricalcitol was the only VDRA able to reduce myocardial fibrosis compared to Placebo (2.6±0.6 % vs. 10.5±3.0 %, p=0.027) (Masson staining), showing similar values than the Sham group (2.8±1.1 %). Paricalcitol and alfacalcidol reduced collagen I expression, but only paricalcitol showed significant increases of collagenase MMP1 expression, a finding that suggest a high rate of collagen degradation.

Conclusions: In summary, the use of VDRAs prevents LVH in uremic rats. Paricalcitol was the most effective preventing myocardial fibrosis.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

SA-PO3130

Differential Expression of MicroRNA-126 Contributes to Renal Microvascular Heterogeneity in VCAM-1 Protein Expression in Inflammation
Coen van Solingen,[2,3] Sigridur Anna Asgeirsdottir,[1] Neng Fisheri Kurniati,[1] Peter Heeringa,[1] J. Kamps,[1] Peter Zwiers,[1] Ton J. Rabelink,[2,3] Anton Jan Van Zonneveld,[2,3] Grietje Molema.[1] *[1]Department of Pathology & Medical Biology, UMCG, Groningen, Netherlands; [2]Department of Nephrology, LUMC, Leiden, Netherlands; [3]Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, Netherlands.*

Background: Endothelial cells (EC) in different microvascular segments of the kidney have different functions and exhibit differential responsiveness to disease stimuli. Microvascular-segment specific responses to anti-glomerular basement membrane (anti-GBM) glomerulonephritis (GN)-induction as well as TNF-α, LPS, and anti-MPO induced GN showed that glomerular E-selectin expression was transcriptionally regulated. In contrast, VCAM-1 mRNA expression was highly increased in both arterioles and glomeruli, while the protein was only expressed to a limited extent in the glomerular compartment suggesting post-transcriptional regulation.

Methods: To assess a role for microRNA-126 (miR-126) in microvascular-segment specific regulation of VCAM-1 we employed mouse models of inflammation and isolated seven hundred glomeruli and arteriolar vascular segments and quantified both the expression of mRNAs and miR-126. Using an antagomir we silenced miR-126 in mice to identify the role of this miR in regional VCAM-1 expression.

Results: We validated the regulation of VCAM-1 by miR-126 in glomerular EC *in vitro*. In mice with experimental anti-GBM glomerulonephritis VCAM-1 mRNA expression was highly increased in both arterioles and glomeruli, while VCAM-1 protein was expressed to a limited extent in the glomeruli. These high VCAM-1 mRNA - low VCAM-1 protein levels were associated with high local miR-126 levels. *In vivo* silencing of miR-126 unleashed VCAM-1 protein expression in the glomerular EC upon inflammatory challenge with TNF-α. Heterogenic expression of the transcription factor Ets-1 with preferential expression in the glomerular compartment likely underlies the spatial expression pattern of miR-126.

Conclusions: These data imply that miR-126 has a major role in the segmental, heterogenic response of microvascular EC to systemic inflammatory stimuli.

Funding: Other NIH Support - Netherlands Heart Foundation: NHS2006B145

SA-PO3131

Uremia Regulates Tissue Factor Stability and Ubiquitylation and Predisposes to Stent Thrombosis
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Background: Coronary stent thrombosis (ST) is a fatal complication that occurs in 1.3% of patients and results in 50% mortality. CRF has emerged as one of the strongest predictors with a 6-fold higher risk. Tissue factor (TF) is a crucial mediator of injury-related thrombosis. We investigated effects and mechanism of uremic serum and solutes on thrombosis and TF regulation.

Methods: Pooled sera from 23 ESRD patients and healthy individuals were used. As a model of the de-endothelialitzed, post-interventional state, we exposed primary human vascular smooth muscle cells (vSMC) pretreated with uremic or control sera in an ex vivo flow-loop system. TF abundance, activity and mRNA in vSMC were examined. vSMCs were treated with individual uremic solutes at a concentration observed in ESRD patients.

Results: vSMCs pretreated with uremic serum showed significantly greater clot formation in flow-loop model. Uremic serum induced higher TF expression and activity in vSMCs. This effect was partially recapitulated by isolated uremic solutes including indole-3-acetic acid (IA), indoxyl sulfate (IS) and uric acid (UA). We further demonstrate that TF is ubiquitylated at baseline and IA and IS significantly prolong TF half-life by reducing its ubiquitylation. Consistently, vSMCs treated with IA, IS and UA were more thrombogenic in flow-loop model.

Conclusions: Uremia significantly increases thrombogenicty by unpregulating TF in vSMCs. Uremic solutes partially account for this via regulating TF stability by decreasing ubiquitylation. Together, these data demonstrate for the first time, the importance of post- translational regulation of TF in uremia and provides a mechanistic link between uremia and ST.

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SA-PO3132

Calcineurin Inhibitors Stimulate MAPK Signaling in Endothelial Cells: Implications for Novel Therapeutics
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Background: The calcineurin inhibitors (CNI), cyclosporine A (CsA) and FK506, are potent immunosuppressive agents used to treat a broad range of renal diseases. While the rationale for these strategies is based on their ability to inhibit lymphocyte activation, we propose that these agents alter additional molecular signals in non-immune cells that serve to attenuate progression of the underlying disease process. We recently observed that CNIs have effects on the activity of Ras family GTPases within vascular endothelial cells (EC). Here, we tested the hypothesis that the pro-activational effects of CNIs in EC involve Raf/MAPK and/or PI-3K/Akt signaling.

Methods: Purified human EC (HUVEC) were cultured in the absence or presence of CsA or FK506 (1 to 1000ng/ml), and the temporal phosphorylation of Akt, mTOR, p70S6K and p44/42 were evaluated by Western blot analysis.

Results: In multiple experiments, we found little effect of each CNI on the activity of the PI-3K/Akt signaling pathway, but we consistently found a marked increase in the expression of phospho-p44/42 after ~ 2.5hrs of treatment. We also assessed the interaction between each CNI and VEGF, which is known to induce activity of these intracellular signals. VEGF (5-20ng/ml) enhanced MAPK activity; and we found that CNIs have an additive effect to further augment expression of phospho-p44/42 in EC. Finally, to determine the functional implications of these observations, HUVEC were cultured with CsA (0.1-1μg/ml) or FK506 (0.01-1μg/ml) +/- VEGF for 72hrs and the degree of proliferation was assessed by 3[H]-thymidine incorporation. CNIs alone were found to augment EC proliferation from basal levels, but were potent to augment the VEGF-induced proliferative response.

Conclusions: Collectively, these observations demonstrate that CNIs have direct and/or indirect effects on the activation of the MAPK intracellular signaling pathway in ECs. Our studies have implications for therapeutic uses of CNIs to promote vasculoprotection or vascular repair; and they provide insight into how these agents may protect against immune-mediated renal injury.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO3133

Neurohumoral and Renal Mechanisms in the Pathogenesis of Hypertension in Polycystic Kidney Disease Benjamin F. Wyse,[1] Joanne Harrison,[2] Jacqueline K. Phillips.[1] *[1]Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia; [2]School of Veterinary and Biomedical Sciences, Murdoch University, Perth, WA, Australia.*

Background: Hypertension is a common presenting factor in polycystic kidney disease (PKD) patients prior to the onset of renal failure. Neurohumoral systems such as the sympathetic nervous system (SNS) and renin angiotensin system (RAS) are key regulators of blood pressure and may contribute to hypertension in these patients. We have shown that AngII levels and PRA are reduced in the Lewis Polycystic Kidney (LPK) model of PKD at 12 wks of age. In this study we examined AngII at different ages, and baseline levels of SNS activation by determination of circulating catecholamines. Activation of an intra-renal RAS as a local mediator of hypertension was assessed by renal RAS gene expression.

Methods: LPK & age matched Lewis controls were assessed at 6, 10 and 16 wks old. Systolic blood pressure was measured via tail cuff and upon euthanasia, serum collected. Kidneys were collected for quantitative RT-PCR for RAS genes (angiotensinogen, renin, angiotensin converting enzyme (ACE) I/II and the ATR1A. Plasma AngII levels were determined by radioimmunoassay and catecholamines by HPLC.

Results: In LPK of all age groups, intra-renal expression of RAS genes were greater in the LPK ($p<0.05$, n=32). ATR1A receptor and ACE displayed 8 fold increases, whilst ACE2, Renin and Angio displayed 2, 4 and 6 fold increases, respectively, in comparison to Lewis. Ang II levels were decreased in the LPK across all ages groups (LPK = 24.1 v Lewis = 54pg/mL) whilst catecholamine levels were greater for noradrenaline (4.9 v. 0.54nmol/mL) & adrenaline (4.7 v. 0.87nmol/mL) in 10 wk old LPK ($p<0.05$).

Conclusions: Increased expression of intra-renal RAS genes, in concert with a concomitant decrease in serum AngII levels provide evidence for intra-renal RAS as a local mechanism driving hypertension in PKD. However, increased circulating catecholamines suggest an additive role for the SNS, which in addition to direct vascular effects may modulate RAS gene expression in the kidney.

SA-PO3134

Impact of Vitamin D on Cachexia, Cardiovascular Disease and Mortality in Chronic Kidney Disease Robert H. Mak,[1] Wai W. Cheung,[1] Yusu Gu,[2] Nancy Dalton,[2] Erika Alvarez,[2] Kirk L. Peterson.[2] *[1]Pediatrics, University of California San Diego, La Jolla, CA; [2]Medicine, Cardiovascular Institute, La Jolla, CA.*

Background: Patients with chronic kidney disease (CKD) have increased mortality and morbidity. Known risk factors include vitamin D insufficiency, cardiovascular (CV) disease, and cachexia. We studied the effect of vitamin D supplementation on nutrition, CV system, and mortality in a mouse model of CKD.

Methods: 9-month old c57BL/6J mice underwent 5/6 nephrectomy (N). We previously showed decreased serum levels of 25-VitD and 1,25-VitD in N mice. N mice received 25-VitD (N-25VitD) (0.08mg/kg, ip, 3x/week), paracalcitol (N-PC) (0.15mg/kg, ip, 3x/week) or vehicle (N-V). Sham-operated mice received vehicle (S-V). N-V mice were fed ad libitum. S-V, N-25VitD, N-PC mice were pair-fed to N-V mice. The study period was 90 days.

Results: Serum BUN and creatinine was significantly higher in N-V, N-25VitD and N-PC compared with S-V mice ($p<0.01$). The mortality rate in N-V mice (57.5%) was significantly higher than N-25VitD (40.0%) or N-PC (30.0%) mice. N-25VitD and N-PC mice gained more weight than N-V mice ($p<0.01$) despite equal food consumption. Basal metabolic rate was significantly higher in N-V compared with N-VitD25 and N-PC mice ($p<0.01$). N-V mice lost lean body mass and fat mass whereas N-25VitD and N-PC mice gained lean body mass and fat mass. Muscle strength was assessed. Rotarod activity and grip strength were significantly improved in N-25VitD, N-PC compared with N-V mice ($p<0.001$). The mean systolic blood pressure in N/V was significantly higher than S-V, N-25VitD or N-PC (145.7±8.2 mmHg vs 99.8±3.7 mmHg, 113.5±5.6 mmHg and 123.2±3.5 mmHg, respectively, $p<0.001$) mice. Left ventricular mass adjusted for body weight was significantly elevated in N-V mice than S-V, N-25VitD and N-PC (26.4±7.8 mg/10g vs 20.9±3.1 mg/10g, 23.4±2.1 mg/10g and 21.6±3.2 mg/10g, respectively, $p<0.01$).

Conclusions: 25-VitD and PC ameliorated cachexia, muscle wasting, hypertension, left ventricular hypertrophy and mortality in mice with CKD. Correction of vitamin D insufficiency is important in preventing nutritional and CV complications of CKD, leading to improvement in survival.

Funding: Pharmaceutical Company Support

SA-PO3135

Direct Renin Inhibitor Is Better Than Angiotensin II Receptor Blocker for Intrarenal Arterioles Kazushige Nakanishi,[1] Yohko Nagai,[1,2] Nobuaki Yamanaka.[2] *[1]Department of General Medicine and Emergency Care, Faculty of Medicine,Toho University, Tokyo, Japan; [2]Tokyo Kidney Research Institute, Tokyo, Japan.*

Background: We have reported that long-term administration of angiotensin II receptor blockers (ARBs) on rats induced unusual proliferative changes of smooth muscle cells (SMCs) in renal afferent arteriolar walls, and the changes were suspected to be induced by the overproduction of renin in JG cells. We examined the effect of the long-term administration of a direct renin inhibitor (DRI) on intrarenal arterioles in spontaneous hypertensive rats (SHR).

Methods: Sixteen 6-week-old male SHR were divided into the following three groups: DRI group (n=5) fed a standard diet containing aliskiren 30mg/kg/day, ARB group (n=6) fed a standard diet containing varsartan 10mg/kg/day, and control group (n=5) fed a standard diet, respectively. Blood pressure and proteinuria were measured every three weeks. After 12 weeks, blood samples were drawn; afterward, lightmicroscopic, electronmicroscopic and immonohistochemical examinations were performed.

Results: Blood pressure in the DRI group and the control group was significantly high compared to the ARB group (188.4±4.2, 185.8±19.3, 124.5±7.3mmHg, respectively).. Remarkable proliferative changes in the afferent arteriolar SMCs were more frequently observed in the ARB group than in the control group (48.9%±6.7% versus 5.1%±2.9% of total observed arterioles in each rat, p=0.0061), but the SMC changes were rarely seen in the DRI group. In the DRI group, glomerular abnormalities and tubulointerstitial changes were very mild. Urinary protein excretion significantly decreased in the DRI group (11.0±3.6mg/day) compared to the ARB group and the control group (30.1±7.6 and 37.5±8.5mg/day, p=0.0487 and p=0.0043, respectively). The serum creatinine level was higher in the DRI group than in the control group (0.46±0.40 versus 0.16±0.03mg/dl, p=0.0158).

Conclusions: The long-term ARB administration induces unusual proliferative changes of SMCs in afferent arterioles; however, DRI does not induce such proliferative changes in SHR. It is indicated that DRI is better than ARB for intrarenal arterioles to suppress the renin-angiotensin system.

SA-PO3136

Chronic Kidney Disease in a Rat Model Modifies Tissue Concentrations of Vitamin K Kristin M. McCabe,[1] Xueyan Fu,[2] Michael A. Adams,[1] Rachel M. Holden.[1] *[1]Queen's University, Kingston, ON, Canada; [2]Tufts University, Boston, MA.*

Background: Patients with chronic kidney disease (CKD) develop vascular calcification (VC) and serum phosphorus is considered a putative signalling molecule in this process. Matrix-Gla protein (MGP) is a vitamin-K dependent protein that inhibits calcification. The major form of vitamin K consumed in the diet and preferentially found in the liver is Phylloquinone (K1). Menaquinone-4 (MK-4), although present in the diet in small quantities, is preferentially found in extra-hepatic tissues. In the present study we assessed tissue concentrations of K1 and MK-4 in the presence of CKD.

Methods: Sprague Dawley rats were fed a diet containing either 0.25% adenine (CKD; n=24) or 0% adenine (control; n=18), both diets containing high phosphate (1%) and low vitamin K1 (0.2 mg/kg) for 7 weeks. Kidney function was assessed by measuring serum creatinine. The concentrations of K1 and MK-4 in tissue and serum were determined by reversed phase HPLC and expressed relative to wet weight.

Results: Serum creatinine was elevated in the CKD group (352 ± 107 μM) compared to controls (50 ± 10 μM, $p<0.001$). CKD animals had significantly higher levels of K1 in the serum (1.4±0.1 vs 0.7±0.5 pmol/g, p=0.019) and lower levels in the liver (33.6±20 vs 57.3±31pmol/g, $p<0.001$) compared to control animals. The kidney medulla in CKD animals had significantly higher MK-4 levels (58.3±21 vs 36.9±8.8 pmol/g, $p<0.001$) and lower K1 levels (5.4±0.4 vs 11.2±8.8 pmol/g, p=0.015) compared to controls. The thoracic aorta accumulated MK-4 and K1 in CKD animals (104.5±46.3 and 152.3±234.9 pmol/g, n=9). MK-4 concentration in the thoracic aorta had a positive correlation with serum phosphorus ($r^2= 0.8$, p=0.001).

Conclusions: These results indicate that CKD modifies tissue concentrations of K1 and MK-4 under low dietary conditions. The presence of CKD attenuates K1 storage in the liver but enhances MK-4 accumulation in non-hepatic tissues. In the thoracic aorta, we found a significant correlation between serum phosphorus and MK-4. We hypothesize that there are, as yet undefined, factors associated with CKD that increase peripheral conversion of K1 to MK-4.

Funding: Government Support - Non-U.S.

SA-PO3137

Lipopolysaccharide and TNF-α Aggravate Calcification in Vascular Smooth Muscle Cells by High Inorganic Phosphate and High Calcium Concentration Media Matsuhiko Hayashi,[1] Ichiro Takamatsu,[1] Chihiro Horimai,[1] Tadashi Yoshida,[1] Toshifumi Wakamatsu.[2] *[1]Apheresis and Dialysis Center, Keio University, School of Medicine, Shinjuku-ku, Tokyo, Japan; [2]Institute of Molecular Design, Bunkyo-ku, Tokyo, Japan.*

Background: Uremia and disturbances in calcium/phosphate metabolism induce vascular calcification (VC), which contributes to cardiovascular diseases in the patients on chronic hemodialysis (HD), and inflammation also plays important roles in VC. To examine the roles of pro-inflammatory factors, TNF-α and lipopolysaccharide (LPS), which are related to chronic inflammation in HD patients, we performed the in vitro studies with cultured human vascular smooth muscle cells (VSMC).

Methods: VSMC was cultured in control (C, phosphate concentration; Pi 1.0 mM, calcium concentration; Ca 1.8 mM), high inorganic phosphate (HPi, Pi 3.6 mM, Ca 1.8 mM), and high calcium (HCa, Pi 1.0 mM, Ca 3.6 mM) media. After 7 to 10 days of cell culture, calcium contents of the cells were measured and RNA was extracted for real time PCR.

Results: HPi and HCa media significantly increased calcium contents of VSMC, and TNF-α (50 ng/ml) and LPS (500ng/ml) further increased calcium contents in either HPi (2.25 and 2.31 fold increases, respectively) or HCa (1.92 and 1.27 fold increases, respectively), significantly, as previously reported for TNF-α. Since recent reports suggested involvement of endochondral transformation in vascular calcification by uremia, mRNA expressions of BMP2, an osteoblast marker, and RUNX3/cbfa3, a chondrocyte marker, were determined by real-time PCR, showing that TNF-α and LPS significantly increased BMP2 but not RUNX3/cbfa3. Furthermore, we examined roles of nuclear factor κB (NFκB), which plays key roles in inflammation and bone metabolism, by specific inhibitor for IKKβ activation, IMD-0354, and inhibition of NFκB activation by IMD-0354 did not prevent VSMC calcification by either TNF-α or LPS.

Conclusions: TNF-α and LPS aggravate VC, involving osteoblastic but not chondrocytic transformation via NFκB-independent pathway. Since LPS is often contaminated in water for dialysate, it is suggested that water purification is important to prevent progression of VC in the patients on chronic HD.

Funding: Government Support - Non-U.S.

SA-PO3138

(Pro)renin Receptor-Like Immunoreactivity in Patients with Chronic Renal Failure Kazuhito Totsune,[1] Takuo Hirose,[2] Nobuyoshi Mori,[3] Hirohito Metoki,[4] Kei Asayama,[2] Masahiro Kikuya,[2] Osamu Murakami,[5] Yutaka Imai,[2] Kazuhiro Takahashi.[6] *[1]Faculty of Synthetic, Tohoku Fukushi University, Sendai, Japan; [2]Planning for Drug Development and Clinical Evaluation, Tohoku Univ Grad Sch of Pharm Sci and Med; [3]Department of Internal Medicine and Rehabilitation, Tohoku Univ Grad Sch of Med; [4]Obstetrics and Gynecology, Tohoku Univ Grad Sch of Med; [5]Department of Medicine, Tohoku Univ Grad Sch of Med; [6]Endocrinology and Applied Med Sci, Tohoku Univ Grad Sch of Med, Sendai, Japan.*

Background: (Pro)renin receptor ((P)RR) is a new member of the renin-angiotensin system. The presence of soluble type of (P)RR with 28 kDa has been reported in the human and rat blood. However, the characteristics of the soluble type of (P)RR in the plasma are unclear to date.

Methods: We therefore examined the (P)RR-like immunoreactivity ((P)RR-LI) in the human plasma by a specific enzyme immunoassay (EIA) which we newly developed, and Western blot analysis. The antiserum against (P)RR was raised in a rabbit by injecting the peptide fragment of human (P)RR corresponding to 224-237 amino acid (human (P)$RR_{224-237}$) conjugated with bovine serum albumin. The EIA has a sensitivity limit of 7.8 fmol/tube and no cross-reactivity to other peptides tested such as endothelin-1 and urotensin II. 50 µl of plasma was directly assayed. Plasma (P)RR-LI levels were measured in 51 hemodialysis patients.

Results: In the EIA, a 2-fold dilution curve of human plasma paralleled with a standard curve. (P)RR-LI levels were significantly elevated during HD session by 1.2-fold ($p<0.001$ by paired t-test). Western blot analysis showed a band with a size over 100 kDa, but no band at the position of 28 kDa, where a previously reported soluble type of (P)RR was found. In contrast, rat plasma showed a 28 kDa band in addition to a band over 100 kDa.

Conclusions: The present study has shown for the first time the changes of (P)RR-LI levels in human plasma and that the major component of (P)RR-LI has a higher molecular size, possibly an assembly of several (P)RR molecules. Our results suggest that (P)RR-LI may act as a circulating receptor and play an important role in the cardiovascular regulation of renal failure patients.

Funding: Government Support - Non-U.S.

SA-PO3139

Effect of Lower Calcium Dialysate on Laboratory Parameters in Chronic Kidney Disease Associated Mineral and Bone Disorder (CKD-MBD) Anip Bansal,[1] Gagangeet S. Sandhu,[1] Rohit Chitale,[2] Rushi K. Nayak,[1] Shriharsha Kallahalli,[1] James P. Jones,[1] Ira S. Meisels.[1] *[1]Nephrology, St. Luke's-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, NY; [2]International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Background: Increased vascular calcification is a concern as a risk factor for cardiovascular disease in patients with ESRD. The incidence of vascular calcification may be decreased by use of a lower dialysate calcium(Ca). However, this effect may be mitigated by increased use of Ca based phosphate binders and increased requirement for active Vitamin D analogues(VDA).

Methods: We retrospectively reviewed the charts of 141 eligible patients who were changed from a higher to a lower Ca dialysate and who were on maintenance hemodialysis (HD) for at least 3 or more months to determine the effect of lower Ca dialysate (2.25 mEq/L) on biochemical variables associated with CKD-MBD and on the use of phosphate binders and VDAs. Student paired t-test was used.

Results: There was a statistically significant decrease in the mean serum Ca and an increase in the mean serum P. The change did not lead to a statistically significant difference in the mean serum intact PTH or Ca-P product, nor in requirement of calcium-based phosphate binders (CPB), non-calcium based phosphate binders, or VDAs.

Table 1

	High Ca Dialysate (2.50 mEq/L)	Low Ca dialysate (2.25 mEq/L)	Mean difference	P value
Total Serum Ca (mEq/L)	9.26±0.06	8.97±0.06	0.29±0.08	<0.0001
Serum P (mg/dL)	5.04±0.12	5.48±0.14	0.44±0.14	0.002
Ca-P product	46.36±1.19	48.88±1.33	2.52±1.35	0.06
Intact PTH	514.95±45.17	567.49±46.58	52.54±38.69	0.18
Paricalcitol dose per HD (mcg)	4.68±0.25	4.91±0.26	0.23±0.27	0.39
No. of Ca acetate pills per meal	0.49±0.08	0.45±0.08	0.05±0.09	0.58
No. of sevelamer pills per meal	1.62±0.14	1.78±0.14	0.16±0.13	0.21

Comparison of mean values of biochemical variables and drug dosage with high vs. lower Ca dialysate

Conclusions: The use of lower Ca dialysate in maintenance hemodialysis patients leads to reduction in mean serum Ca concentrations without significantly increasing requirement for VDAs and CPBs. The long term impact of this intervention needs further study.

SA-PO3140

The Effect of Kidney Function on Intimal Neovascularization and Intraplaque Hemorrhage in Coronary Atherosclerosis: The Hisayama Study Toshiaki Nakano,[1,2] Toshiharu Ninomiya,[1] Kazuhiko Tsuruya,[1] Yutaka Kiyohara,[3] Takanari Kitazono.[1] *[1]Department of Medicine and Clinical Science, Kyushu University, Fukuoka, Japan; [2]Pathophysiological and Experimental Pathology, Kyushu University, Fukuoka, Japan; [3]Department of Environmental Medicine, Kyushu University, Fukuoka, Japan.*

Background: People with chronic kidney disease (CKD) are at the increased risk of coronary heart disease. The aim of this study is to investigate the relationships of CKD with neovascularization and intraplaque hemorrhage in coronary atherosclerosis.

Methods: We randomly selected 126 subjects from 844 consecutive autopsy samples of residents of the town of Hisayama, Japan and examined the relationship of estimated glomerular filtration rate (eGFR) with the severity of coronary atherosclerosis. The subjects were classified into four categories based on eGFR. We estimated the numbers of intimal neovascularization and intraplaque hemorrhages in the vessels. The expressions of oxidized low-density lipoprotein (oxLDL) and vascular endothelial growth factor (VEGF) in the vessels were examined immunohistochemically.

Results: Lower eGFR was associated with increased numbers of neovascularization (10.7, 11.8, 18.7, and 23.5 per vessel by eGFR levels) and intraplaque hemorrhages. The positive areas of oxLDL and VEGF increased significantly with reducing eGFR levels. Likewise, the positive areas of oxLDL and VEGF were correlated significantly with the numbers of neovascularization and intraplaque hemorrhages (all $p<0.01$).

Conclusions: People with CKD have higher risks of intimal neovascularization and intraplaque hemorrhage in coronary arteries. Greater expressions of oxLDL and VEGF may be attributed to neovascularization and consequent intraplaque hemorrhage in coronary atherosclerosis.

SA-PO3141

High Concentration Ucid Acid Trigger Oxidative Stress in Endothelial Cell Via Aldose Reductase Pathway Di Wu, Zhiyong Huang, Quan Hong, Liyuan Wang, Shaoyuan Cui. *Department of Nephrology, Chinese General Hospital of PLA, State Key Laboratory of Kidney Diease, Beijing, China.*

Background: Hyperuricemia is an independent risk factor for cardiovascular and kidney disease. Uric acid is an antioxidant but high level uric acid(UA) become a prooxidant. In this study, we tried to what causes the switch.

Results: The human umbilical vein endothelial cell line (HUVEC) were cultured with 600µmol/L UA and no UA was added in the control group. Using stable isotope labeling by amino acids in cell culture (SILAC) combined with liquid chromatography-mass spectrometry, we selected 39 proteins with significant difference. Two interesting proteins relating to oxidative stress, increased aldose reductase (AR) and decreased mitochondrial superoxide dismutase (Mn-SOD), attracted our attention.

We detected the changes of total ROS and its components (O2•-, H_2O_2, $1O_2$, •OH) by laser scanning confocal microscope. The total intracellular ROS, O2•- and H_2O_2 was upregulated, but 1O2, and•OH was decreased, which suggested that high UA level keeps parts of its antioxidant property but lose the capability of decreasing the O2•- and / or H_2O_2. Because the O2•- quickly transforms to H_2O the H_2O_2 might be the major contributor of UA-mediated endothelial dysfunction. The supernatant NO level significantly upregulated but it may be a sign of inflammation. Removing the H_2O_2 with catalase can attenuate the injury.

When the HUVEC were pretreated with AR inhibitor, the intracellular total ROS, as well as the NO level significantly decreased compared with the high level UA group. The AR inhibitor also inhibited the NADPH oxidase (NOX) activation which suggested that high level UA may activate the AR pathway first, then increase the generation of ROS by NOX, finally promote the inflammatory response.To verify the results in vivo, hyperuricemia mouse model was established in male C57B6 mice. After given the AR inhibitor or PEG-SOD, the endothelium function recovered.

Conclusions: Our results demonstrated that high level UA actives the AR then increase the H_2O_2 level which causing endothelial dysfunction. But UA keeps its capability of decreasing other ROS components.

Funding: Government Support - Non-U.S.

PUB001

Modulation of Tubular Cell Injury by Mesenchymal Stromal Bone Marrow Cells in a Single Kidney Partial Protection Model Dileep Kumar, Kang Cheng, Partab Rai, Rivka Lederman, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Cisplatimum has been demonstrated to induce acute tubular necrosis both in human and animal experimental models. Mesenchymal stromal bone marrow cells (MS-BMCs) have been demonstrated to provide cytoprotection by the modulation of cytokine production in several cytotoxic models. In the present study, we evaluated the effectof MS-BMCs on cisplatinum -induced tubular cell injury.

Methods: MS-BMCs were harvested from bone marrows of mice and their profile was characterized. Mice in groups of six were administered either buffer (group A), cisplatinum alone (group B, 12.5 mg/Kg, intraperitoneal), intracapsular instillation of MS-BMCs in left kidney 24 hours prior to cisplatinum administration (group C). All mice were sacrificed on day 3; urine and blood samples were collected for BUN and albumin: creatinine ratio. Kidneys were harvested for renal histology and TUNEL staining. Immunohistochemical studies were carried out to study interstitial inflammatory milieu. In parallel sets of experiments, conditioned media of MS-BMC was collected. The effect of conditioned media of MS-BMC was evaluated on cisplatinum-induced tubular cell apoptosis in in vitro studies.

Results: Group B mice showed elevated BUN when compared to group A mice (84.5 ± 12 vs. 40.9 ± 2 mg/dl, P<0.5). However, group C mice displayed only mild elevation of BUN (56.3 ± 4 mg/dl). Group C (5,495 ± 716 mg/gm creatinine) mice displayed decrease (P<0.05) in albumin: creatinine ratio vs. Group B (9,615 ± 2306 mg/gm creatinine) mice. Left kidneys from the group C displayed decreased number for TUNEL +ve tubular cells when compared to the contralateral kidneys of the same group and kidneys from the group B. In in vitro studies, conditioned media from MS-BMC provided partial protection to tubular cells against cisplatinum-induced injury

Conclusions: These findings indicate that MS-BMCs provide protection from injurious effect of cisplatinum by modulating apoptotic signaling in renal cells.

Funding: NIDDK Support

PUB002

Erythropoietin Effects on Renal Ischemia/Reperfusion Injury Vitor Maciel de Sousa Pinto, Vicente de Paulo Castro Teixeira, Waldemar S. Almeida, Elisa M.S Higa, Nestor Schor. *Medicine - Division of Nephrology, Universidade Federal de São Paulo- Escola Paulista de Medicina, São Paulo, Brazil.*

Background: Recently has been suggested a protective action of rHuEPO (recombinant human erythropoietin) in ischemia/reperfusion (I/R) injury in several tissues, including the kidney. However, the mechanism of action of rHuEPO in the model of ischemia/reperfusion AKI is not clear. We evaluated the effects of rHuEPO in rats subjected to I/R and the potential role of rHuEPO in the inflammatory process as a consequent reaction of the injury.

Methods: Males wistar rats received i.v. infusion of a high dose of rHuEPO (3,000UI/kg) or saline 15 minutes prior the bilateral clamping of renal artery during 45 minutes. Serum creatinine (sCr), urea (sUr) and nitric oxide (sNO) levels were evaluated before and up to 24, 48 and 72 h after I/R injury. Tissue samples were evaluated after 72 h I /R via a semi-quantitative scale.

Results: After 24h and 48h sUr from saline group increased more than the rHuEPO group, and sCr, sNO did not change up to 72h. However, a striking differences were observed with the histological evaluation since rHuEPO (N=8) group I/R presented a significantly less tubular damage (TD) in comparison to saline group (N=5); p<0.05. Data are expressed as distribution frequency of renal TD severity score. Scores: 0= until 10%; 1= 10 to 25%; 2= 25 to 50%; 3= 50 to 75%; 4= above 75%. The group rHuEPO had predominance in low scores, while the group saline had predominance in high scores.

Histology - Tubular damage

Group	Score 0	Score 1	Score 2	Score 3	Score 4
Sham	100%	0	0	0	0
Saline	0	0	20%	20%	60%
rHuEPO	0	25%	50%	25%	0

Conclusions: Our preliminary results suggest that administration of rHuEPO as a single and high dose before the onset of I/R produced a significant reduction in tubular injury. Further studies are underway in order to identifying mechanisms involved in this mechanism.

PUB003

Podocyte, a Type of Newly Discovered Target Cell for Aristolochic Acid in Aristolochic Acid Nephropathy Xueqin Bian, Junwei Yang, Chunsun Dai. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jinagsu, China.*

Background: Aristolochic acid nephropathy (AAN), a rapidly progressive tubulo-interstitial nephropathy, is manifested as proteinuria in most of the cases. Whether Aristolochic acid (AA) can directly impair podocyte, one of the most important parts of glomerular filtration barrier, remains completely unknown. Here we reported that Aristolochic acid could direct damage podocyte and lead to albuminuria in mice.

Results: In this study, to induce AAN, we injected male ICR/CD-1 mice intraperitoneally with AA (6mg/kg) twice during the first week. Mice were sacrificed at day 3, 7 and 30 after first injection and urine and kidney samples were collected at each time point respectively. At day 3 after AA injection, all of the mice developed apparently albuminuria according to urinary protein SDS-PAGE gel electrophoresis and albumin ELISA assay. Immunofluorescent staining results showed that nephrin staining pattern was changed from linear to granular. Both Wilms tumor 1 (WT1) and podocin expression level in isolated glomeruli were suppressed significantly compared to those in control. In cultured mouse podocytes, AA could dose-dependently induce cell death and F-actin distribution alteration, from initial trabs-shape to surrounding along the cell membrane.

Conclusions: In conclusion, AA can directly induce podocyte damage and albuminuria, which provides a new aspect for the therapy of AA nephropathy.

Funding: Government Support - Non-U.S.

PUB004

Baselining Drug-Induced Kidney Injury Urinary Biomarkers in Rats within an Automated Blood Sampling and Telemetry System Yafei Chen, Harriet Kamendi, David Brott, Patricia Bentley, Herbert Barthlow, James Fikes, Lewis B. Kinter, Russell Bialecki. *Global Safety Assessment, AstraZeneca Pharmaceuticals, Wilmington, DE.*

Background: Drug-induced kidney injury (DIKI) is routinely monitored in preclinical safety testing using clinical chemistry, urinalysis and histopathology. However, limitations with these traditional approaches are increasingly recognized, especially for prediction of DIKI in early drug discovery. Nephron site specific DIKI biomarkers recently have shown better sensitivity and specificity than regulatory standards (serum creatinine and blood urea nitrogen) for detection or prediction of kidney injury following acute drug exposure in rats. However, baseline values for these biomarkers under normal physiological conditions are not well described.

Methods: In this study, we evaluated eight urinary biomarkers (αGST, GSTYb1, albumin, clusterin, KIM-1, RPA-1, lipocalin and osteopontin) from rats in an integrated automated blood sampling and telemetry (ABST) system for DIKI detection (Kamendi, et al. J. Pharmacol Toxicol Methods. 62(1):30-9, 2010). To establish the reference range for these DIKI biomarkers, urine samples were collected overnight (16-18 hrs) from surgically cannulated and telemetered control animals (n≥4 rats/study, 8 studies) within ABST system using a quantitative urine collection unit.

Results: Urine basal levels of DIKI biomarkers were measured using Multiplex Meso Scale Discovery® assay kits. Baseline values were determined for all biomarkers and normalized to urinary creatinine (biomarker/creatinine: α-GST, 25.4±6.3; GSTYb1, 7.7±1.6; Clusterin, 2.8±0.4; Albumin, 3.5±0.4; KIM-1, 0.15±0.03; Lipocalin, 0.3±0.1; Osteopontin, 0.9±0.3; RPA-1, 0.3±0.03).

Conclusions: These preliminary baseline findings provide valuable background information on DIKI urinary biomarkers for subsequent qualification and validation studies using benchmark compounds within ABST platform.

Funding: Pharmaceutical Company Support

PUB005

The Change of Urinary Kidney Injury Molecule-1 after Cardiac Catheterization in Children with Congenital Heart Disease Min Hyun Cho. *Pediatrics, Kyungpook National University Hospital, Daegu, Korea.*

Background: Contrast-induced nephropathy (CIN) is an important cause of acute kidney injury (AKI) in hospitalized patients. The aim of this study was to investigate the frequency of CIN caused by the contrast material used for cardiac catheterization in pediatric patients with congenital heart disease, and to evaluate the clinical usefulness of the Kidney Injury Molecule-1 (KIM-1) for detecting AKI in pediatric group.

Methods: A variety of clinical findings were analyzed in 26 children that received cardiac catheterization for congenital heart disease; including the amount of contrast agent used and the level of serum creatinine before and after the catheterization. No patient had a prior history of renal disease. In addition, the level of urine KIM-1 was evaluated and compared as a biomarker for AKI, by ELISA, before and after catheterization.

Results: The mean age of the patients was 7.1 years and the male: female ratio was 12:14. Although only one patient had cyanosis caused by pulmonary atresia (oxygen saturation 89%), the others had no cyanosis or congestive heart failure. Ultravist®, a low osmolar dye, was used as the contrast media in all 26 cases; the amount of contrast agent used was on average 31.2±16.7 mL (2.5±2.8 mL/kg of weight). The levels of serum creatinine were checked before, 6h, and 24h after catheterization and showed little change within the normal range as: 0.4±0.19, 0.41±0.19, 0.41±0.22 mg/dL, respectively. However, the average levels of the urine KIM-1 evaluated before, at 6h and 24h after catheterization were: 70.94±80.83, 78.33±52.5, and 107.98±94.2 pg/mL respectively, showing a progressive increase after catheterization. There was significant difference between the level of KIM-1 before and at 24h after catheterization.

Conclusions: Although typical CIN may be rare, contrast agents are a potential cause of AKI in children with congenital heart disease. Further long-term prospective research is needed.

PUB006

Acyclovir Induced Rhabdomyolysis Vince Faridani, Adil Akthar, Ahmed M. Awad. *Nephrology, University of Missouri - Kansas City, Kansas City, MO.*

Background: Acyclovir is an acyclic guanosine analogue used in the treatment of herpes simplex virus and varicella-zoster virus. Although generally well tolerated, usage of acyclovir may be complicated by development of acute renal failure (ARF) in select patients. The following case demonstrates a rare example of ARF secondary to acyclovir-induced rhabdomyolysis.

Case: A 29 year old female with a known history of Type I Diabetes and ESRD on peritoneal dialysis presented to the hospital with altered mental status, weakness, and vomiting. Four days prior she was started on oral acyclovir therapy for treatment of genital herpes. Upon initial presentation, afebrile, vital signs stable. Physical examination revealed the patient to be alert but not orientated x 0, no focal neurological deficits. Initial evaluation, CT head was negative for acute injury and lumbar puncture revealed CSF to be normal. Laboratory studies revealed Na 122, K 3.7, BUN 101, Creatinine 12.3, and Phosphorus 11.2, creatine kinase (CK) 20,199. Serological work and HIV viral load was negative. The patient was immediately discontinued from her acyclovir, given IV fluids, and underwent emergent dialysis for two consecutive days given her acute on chronic renal failure, altered mental status, and elevated CK. Nearly 48 hours later her CK decreased and menation returned to baseline. Hemodialysis was discontinued and she resumed her peritoneal dialysis.

Discussion:

The mechanism of acyclovir-induced ARF classically involves the administration of intravenous acyclovir leading to precipitation of crystals within the nephron tubules and renal parenchyma. However, the above case highlights a different physiologic mechanism whereby oral acyclovir can lead to reversible rhabdomyolysis-induced uremic encephalopathy and oliguric acute kidney injury. Clinicians should also be aware that acyclovir-related ARF can occur with lower doses of oral therapy as well as rapid IV infusions. This consideration is especially important when dealing with patients who have underlying chronic kidney disease as seen here. These patients warrant lower doses of acyclovir treatment given their greater predisposition to renal damage.

PUB007

Effects of Pretreatment with High Doses of Methylprednisolone (MP) on Renal Ischemia/Reperfusion (I/R) Injury Ida M. Fernandes,[1] Gloria Mendes,[1] Emmanuel A. Burdmann.[1,2] *[1]Medical School of Sao Jose do Rio Preto, Sao Paulo, Brazil; [2]University of Sao Paulo Medical School, Brazil.*

Background: Renal ischemia is the most important cause of acute kidney injury.

Methods: To assess a possible protective role of MP in renal I/R male rats were treated with MP 30mg/kg or saline (S) iv 1 h before 30 min renal ischemia (RI). They were divided into 3 groups (n=8 each): sham control (C, sham surgery, no RI), I/R (saline infusion before RI), and MP (MP infusion before RI). Inulin clearance (GFR, ml/min/100g), sodium fractional excretion (FENa, %), urinary volume, osmolality and renal histological analysis were assessed 2 d after I/R. Immunohistochemical staining was performed to measure macrophages (ED-1 positive cells), neutrophils (No), and lymphocytes (Lo) and nuclear factor-kappa- B (NFκ-B), scored according to the extent of tubulointerstitial (TI) immunostaining in the cortex and outer medulla (OM) areas. Results are mean±SD (ANOVA, followed by Bonferroni test, $p<0.05$).

Results: GFR was 0.92±0.3 in MP, 0.90±0.3ml in C, and 0.47±0.2 in I/R ($p<0.05$ vs. MP and C). FENa was similar in the MP (0.19) and C groups (0.35), and higher in the I/R group (0.57, $p<0.05$ vs. MP and C). Urinary volume and osmolality were similar among the 3 groups. Acute epithelial degenerative changes and tubular dilatation were significantly more intense in the I/R group than the MP and C groups, with focal acute tubular necrosis. TI staining for Lo was significantly higher in I/R as compared to the C and MP groups in the cortex (14.4±3 vs. 6.7±1 and 5.3±1.6, respectively, $p<0.05$ I/R vs C and MP) and OM areas (10.6±3 vs. 4.3±1 and 3.7±0.6, $p<0.05$ I/R vs. C and MP). Similar results were seem for OM staining of ED1 (9.8±3 in I/R vs. 4.6±1 in C and 4.1±2 in MP, $p<0.05$) and No (3.1±2 in I/R vs. 1.1±0.3 in C and 1.4±1 in MP, $p<0.05$). NFκ-B staining was more intense in the OM of I/R compared with the C and the MP (0.6±0.3 in I/R vs. 0.03±0.03 in C and 0.1±0.1 in MP, $p<0.05$).

Conclusions: Pretreatment with high doses of MP conferred striking protection against renal I/R. This effect is probably related to the modulation of I/R-induced inflammatory mechanisms by MP.

PUB008

Acute Kidney Injury Associated with Intravenous Vancomycin Administration Carlos R. Franco-Palacios,[1] Qi Qian.[1] *[1]Nephrology, Mayo Clinic; [2]Nephrology, Mayo Clinic.*

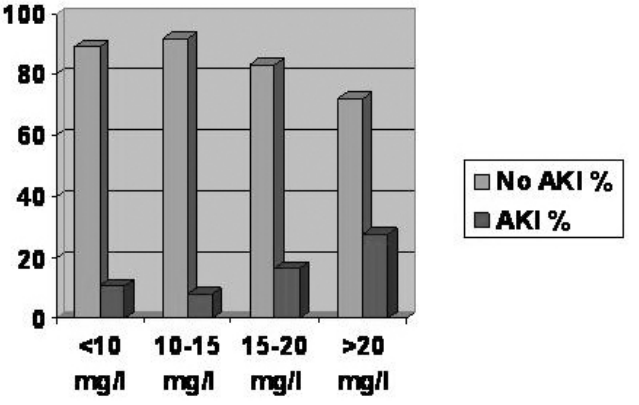
Background: Studies suggest an association of vancomycin and nephrotoxicity. The temporal relationship between the two remains unclear.

Methods: Retrospective study to determine the relationship between incidence of AKI and IV vancomycin. 547 patients with trough vancomycin level in our institution in 2009 were selected,428 were entered for analysis after excluding chronic dialysis patients, patients less than 18years, and patients with incomplete clinical data. Serum Cr levels before, during and after vancomycin treatment were examined.

Results: Baseline patient characteristics: Mean (SEM) age 59.7-±18.1, 56.7 % male, baseline serum Cr 0.92±0.53, diabetes 27.5%, hypertension 50.4%, CHF 17%, ICU admission 50.4%, pressor use 24.5%, aminoglycoside use 5.8%, loop diuretics 48.3%, thiazide diuretics 10.3%.

56 patients (13%) developed AKI. AKI patients had higher trough vancomycin level, 16.1±6.5 vs 13.1±5.7 (P=0.02); higher baseline Cr 1.32±1.05 vs 0.86±0.37 (P=0.02); more ICU admission (66% vs 47.5% P = 0.009), more vasopressor use (40% vs19.9%, P=0.009).

Higher vancomycin trough levels were associated with AKI incidence.



In multivariate regression analysis Vancomycin trough level remained an independent predictor for AKI (**Table 1**). After adjusting for NSAIDs, ACEI/ARBs, diuretics and aminoglycoside use, Vancomycin remained associated with AKI. (OR=1.06, 95% CI 1.01-1.10, p=0.01).

Table1. Predictors of AKI.

Variable	Odds Ratio (95% CI)	P value
Vancomycin trough level (mg/l)	1.05 (1.002, 1.10)	0.03
Hypotension	1.45 (1.12, 2.08)	0.01
CHF	1.08 (0.76, 1.63)	0.67
Baseline serum creatinine (mg/dl)	2.54 (1.47, 4.62)	0.001
HTN	1.14 (0.89, 1.66)	0.41
DM	0.94 (0.69, 1.37)	0.74

Longer duration was associated with a higher incidence of AKI (P=0.02), after adjusting all potential confounding factors.

Conclusions: High dosage and long-duration vancomycin use is an independent risk factor for AKI.

PUB009

Longitudinal Changes in Biomarkers Associated with Acute Kidney Injury in Rats Treated with Doxorubicin or Cisplatin for 15 Days Ryan M. Fryer, Suzanne Nodop Mazurek, James D. Smith, Rong Rhonda Chen, Akalushi C. Muthukumarana, Amy Hudak, Anthony Mineo, Kathleen A. Lincoln, Franklin D. Pack, Paul Harrison, Jonathan Phillips. *Boehringer-Ingelheim Pharmaceuticals Inc.*

Background: Numerous biomarkers of AKI have been proposed in pre-clinical research and some validated for clinical use.

Methods: We report temporal changes in functional and tissue-injury urinary biomarkers of AKI in rats treated weekly for 15D with doxorubicin (5 or 10 mg/kg i.v.) with confirmed renal pathology; the 10 mg/kg group was sacrificed at D11.

Results: Creatinine clearance (CrCL; D7, D15) was unaffected at D7 but was reduced at D15 (5 mg/kg). Albuminuria (mg Alb/Cr) was first detected on D4 at 10 mg/kg (0.29 *vs.* 0.02 in veh); at 5 mg/kg the onset of albuminuria was delayed (D7; 0.49 *vs.* 0.02) and peaked at D15 (34.79 *vs.* 0.03). Other functional biomarkers, including total protein, β_2 microglobulin, Cystatin C, and RBP4 generally increased in parallel with, but not before, that of albumin. Tissue-injury leakage biomarkers such as β-NAG increased on D7 at 10 mg/kg (0.09 *vs.* 0.05) and peaked at D11 (0.25 *vs.* 0.02) whereas values were not increased until D15 at 5 mg/kg (0.12 *vs.* 0.05); RPA-1 increased in both groups immediately before study-end. However, other putative leakage biomarkers including GST-α and GST-μ were unaffected; urinary LDH, γGT, ALP, and ALT were also unaffected although urinary AST increased in parallel with that of β-NAG. As expected, tissue-injury response biomarkers including clusterin and NGAL tended to increase slightly later than the functional biomarkers. Interestingly, KIM-1 increased only immediately before study-end and not to a magnitude typically observed with classic tubular toxicity.

Conclusions: Results suggest that renal damage was primarily glomerular leading to secondary protein overload in the tubules. While many of the biomarkers could be detected prior to changes in CrCL, none demonstrated the ability for earlier detection than more classically-accepted endpoints of AKI (e.g. albumin). A separate study in rats treated for 15D with Cisplatin that elicits direct tubular injury was in-progress at the time of abstract submission and will also be presented.

Funding: Pharmaceutical Company Support

PUB010

The Role and Mechanism of CHOP in Inflammation Induced by Hypoxia Reoxygenation in Renal Tubular Epithelial Cells Yani He, Yuna Tong, Wei-Wei Zhang, Jurong Yang, BenGang Huo. *Department of Nephrology, Daping Hospital, the Third Military Medical University, Chongqing, China.*

Background: Inflammation is the key pathophysiology basis in the development of acute kidney injury. Our study focuses on the role and mechanism of CHOP in inflammation induced by hypoxia reoxygenation in renal tubular epithelial cells.

Methods: Rat renal tubular epithelial cells (NRK-52E) were exposed to hypoxia for 4 hours and subsequently reoxygenation for 1, 3, 6, 12 and 24 hours, respectively. LDH in culture supernatant was detected with automatic biochemistry analyzer. ELISA was adopted to evaluate the level of IL-1β. RT-PCR and Western blot were applied to detect the expression of GRP78, CHOP, caspase-11, caspase-1 and IL-1β. Double immunofluorescent staining was performed to study the expression of CHOP and caspase-11.

Results: LDH leakage and the expression of IL-1β in culture supernatant were increased after H/R. H/R induced the expression of mRNAs and proteins of GRP78, CHOP, caspase-11, caspase-1 and IL-1β. Immunofluorescent study showed that most CHOP nuclear positive NRK-52E cells were co-stained with caspase-11 positive in cytoplasm. LDH, IL-1β in supernatant and caspase-11, active caspase-1, active IL-1β were decreased after knockdown of CHOP in NRK-52E cells by siRNA at H/R12h.

Conclusions: Our results indicated that H/R can activate ERS and CHOP to amplify inflammation cascade in renal tubular epithelial cells. CHOP-caspase pathway may regulate IL-1β activation in H/R induced inflammation in renal tubular epithelial cells, which may be an important mechanism of acute ischemic renal injury.

PUB011

Preconsultation Evaluation and Outcome in Acute Kidney Injury Patients

Naing L. Htike, Adeel A. Siddiqui, Geoffrey S. Teehan, Robert L. Benz. *Nephrology, Lankenau Medical Center and LIMR, Wynnewood, PA.*

Background: Upon renal consultation for Acute Kidney Injury (AKI), diagnostic data such as urine creatinine/lytes and urinalyses (UA) are often not available. We compared outcomes among those who did or did not have preconsult urine dx'ic studies (UDS).

Methods: Six month retrospective cohort study of AKI in a community teaching hospital. We defined AKI as creatinine >/= 0.3 mg/dl vs. baseline. Those with at least UA performed were Grp 1, and those without UA were Grp 2. Fisher's Exact Test and T-Test were employed.

Results: 116 patient charts were reviewed (Grp 1: N = 67, Grp 2: N = 49). The groups did not differ with respect to overall comorbidities (P = NS). There was a higher likelihood of having a full set of UDS in Grp 1 vs. Grp 2 (P = 0.001). Twice as many patients had renal imaging in Grp 1 (28%) vs. Grp 2 (14%) (P = 0.11). At least a doubling of baseline serum creatinine occurred in 41% of the cohort (48% in Grp 1 vs. 33% Grp 2) (P = 0.13). Death and the need for renal replacement therapy (RRT) occured more in Grp 1 (3% vs. 0% and 6% vs. 2%) but did not reach statistical significance (P = 0.5, 0.39). Returned to baseline creatinine was similar between groups (P = 0.62).

Characteristics and Outcomes

N = 116	Grp 1 (UA); n=67 (58%)	Grp 2 (non-UA); n=49 (42%)
Age (yr)	26-97	41-97
Female	31 (46%)	24 (49%)
Hispanic	0	2 (4%)
White	30 (45%)	23 (47%)
Black	37 (55%)	23 (45%)
CKD	47 (70%)	41 (84%)
HTN	61 (91%)	45 (92%)
DM	31 (46%)	28 (57%)
CAD	20 (30%)	30 (61%)
Primary called nephrologist	34 (51%)	15 (32%)
ICU	12 (18%)	10 (20%)
FeUr/ FeNa	36 (54%)	0
Renal Imaging	19 (28%)	7 (14%)
Cr doubled fr. Bl	32 (48%)	16 (33%)
ATN	16 (24%)	9 (18%)
RRT	4 (6%)	1 (2%)
Death	2 (3%)	0
Discharge Cr (P = 0.84)		
At Bl	14 (21%)	10 (20%)
<0.3mg/dl above Bl	24 (36%)	22 (45%)

Cr = Serum Creatinine; Bl = Baseline

Conclusions: While we support obtaining UDS prior to renal consultation, this study of 116 patients could not support the hypothesis that such studies preconsultation resulted in improved outcome vs. obtaining them upon consultation. We are unaware of evidence based studies showing otherwise. A larger study is indicated to verify whether preconsult UDS, while empirically making sense, actually alters outcomes.

PUB012

Acute Kidney Injury in Sepsis Is More Influenced by Shock, Than by Sepsis

Karin Janssen van Doorn,[1] Hilde Jansens,[2] Vanessa De Wit,[3] Philippe Jorens.[4] *[1]Nephrol-Hypert, Univ Hosp Antwerp; [2]Microbiology, Univ Hosp Antwerp; [3]i-ICT, Biomina, Univ Hosp Antwerp, Univ of Antwerp; [4]ICU, Univ Hosp Antwerp, Edegem, Belgium.*

Background: AKI can be defined by the RIFLE-classification with 3 different severity stages: Risk (R), Injury (I) or Failure (F). In this retrospective single centre study we looked for the influence of sepsis and hemodynamic instability on the evolution of AKI and the need for hemodialysis (HD).

Methods: For a 19 mo period, 82 pts with blood-cultures positive for pathogenic bacteria were evaluated. AKI was assessed starting from 1 day before blood-cultures were taken, until the end of the ICU stay. Pts with pre-existing renal failure were excluded. Pts were divided in the groups with stage R, I or F. The influence of the duration of (severe) hypotension (mean blood pressure <65 mmHg regardless of vasopressor necessity or dose) was evaluated on the evolution for AKI. Pts were evaluated for demographic and infectious parameters; SAPS 3 was used as predictor of the disease severity.

Results: Equal age, gender and diabetes distribution was present in the groups with or without HD. Infectious parameters were not significantly different. Pts requiring HD were sicker (higher SAPS score), needed higher doses of vasopressors and had a higher mortality rate.

Characteristics (mean)	HD	No-HD	P value HD vs No-HD
Number (n)	33	49	
Outcome (mortality)	78.8%	34.7%	<0.0001*
Days in ICU since positive blood culture	7.5	1	=0.0125*
SAPS 3-score	66.6	57.4	=0.0055*
Cumulative vasopressor load (y/day)	17.7	2.44	<0.0001*

*p<0.05 is considered significant

Multiple regression showed the influence of the hemodynamic parameters on the tendency to develop stage R, I or F. Within each stage the duration of hypotension was highly predictive for the occurrence of AKI (p=0,01 for stage I, p<0001 for stage R and F).

Conclusions: Critically ill pts with AKI due to sepsis have a worse outcome when they need HD. In pts requiring HD, a significantly higher percentage also needed vasopressors. The increasing need for vasopressors and the duration of hypotension (septic shock) are, besides changes in serum creatinine and/or changes in urinary output (RIFLE criteria), important as determinants for stage R and F.

PUB013

Metformin Reduces Renal Proximal Tubule Epithelial Cell Detachment Due to Ischemia by Maintaining Actin Stress Fiber Structure

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Background: Interruption of bloodflow to the kidney can cause ischemic damage, potentially including detachment of proximal tubule epithelial cells from the basement membrane, which leads to loss of resorptive surface area as well as tubule obstruction. Prior studies of drugs for preventing ischemic damage have focused on inflammation, but it is possible that preventing cell detachment may also provide effective protection. Metformin is a safe, inexpensive and widely used diabetes drug that we have found enhances *in vitro* renal epithelial cell attachment during ischemia.

Methods: Cultured murine S3 proximal tubule epithelial cells were used for all experiments. A previously established *in vitro* protocol of nucleotide triphosphate depletion ("depletion") was used to simulate ischemia. Cells were placed in glucose-free media and treated with Antimycin A, an inhibitor of oxidative phosphorylation, for 30 minutes. Metformin treatment was 1 uM concentration for four hours prior to depletion. Detached cells were counted with a hemocytometer. Total protein was measured using bicinchoninic acid assay. Phosphonucleotide ratios were calculated using high-performance liquid chromatography. Cell structures were examined via confocal microscopy.

Results: Cells treated with metformin prior to depletion had no significant difference in phosphonucleotide ratios from depleted control cells. Metformin-treated renal epithelial cells had higher protein concentration than depleted controls, and fewer detached cells were counted in cultures treated with metformin prior to depletion. When visualized microscopically, individual metformin-treated cells showed more basal F-actin than untreated control cells, although the F-actin did not have the appearance of normal stress fibers.

Conclusions: Metformin enables individual renal epithelial cells to remain attached to substrate during depletion by enabling individual epithelial cells to retain basal F-actin structures, unlike cells that are not treated with metformin.

Funding: NIDDK Support

PUB014

Differentiation, Division and Proliferation of Cultured Mesenchymal Stem Cells under Acute Kidney Injury Microenvironment

Nanmei Liu, Weiwei Wang, Jinyuan Zhang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: To establish acute kidney ischemia-reperfusion(I/R) injury model of mice, make kidney homogenate with renal cortex, culture mouse mesenchymal stem cells(mMSCs) with kidney homogenate,observe mMSCs' differentiation and replication.

Methods: To make acute kidney injury(AKI) mice models by clamping bilateral renal pedicles 30 minutes and reopening 30 minutes. Then immediately drew both renal cortex to make I/R kidney homogenate supernatant. C57BL/6 mice's mMSCs had been successfully isolated by Percoll density gradient centrifugation and adherence cultivation, surface markers were identified by flow cytometry. Then P3-mMSCs were treated with different group: Control group(low glucose DMEM medium with 10% fetal bovine serum);Intervention group(low glucose DMEM medium with 10% fetal bovine serum plus I/R kidney homogenate supernatant).Each group was incubated for 1d,3d,5d,7d. Use inverted microscope to observe morphological changes of these cells, ultrastructure changes were observed by transmission electron microscope. Cytokeratin-18 was detected by flow cytometry.Proliferation of mMSCs was detected by CCK-8, apoptosis was detected by TUNEL.

Results: The cells that we got highly expressed CD29,CD44,lowly expressed CD34,CD45.Compared with the control group, the cells of intervention group present oval shape, short fusiform on the third day, round shape, oval shape, short-pang fusiform on the seventh day. At the same time, it appeared much rough endoplasmic, lysosome, mitochondria.There was trace expression of CK18 in control group, after intervention of

I/R kidney homogenate supernatant,the expression rate increased significantly. The I/R kidney homogenate supernatant could alleviate the proliferation ability of mMSCs, while the apoptoic percentage increased significantly(P<0.01).

Conclusions: The simulation AKI microenvironment can induce mMSCs partially to transfer to renal tubular epithelial-shaping cells. But this kind of microenvironment can also make mMSCs' apoptosis, weaken their proliferation ability, as a result, decreased the number of mMSCs that can transdifferentiation.It may be that renal repair ability of MSCs'transplantation is limited.

Funding: Government Support - Non-U.S.

PUB015

Hypokalemia in Adult Patients with Acute Kidney Injury Due to Severe Leptospirosis: Association with Patient Characteristics and Case-Fatality Rate Marcelo Lopes,[1] Daniela Lopes,[1] Antonio Alberto Lopes.[2] *[1]Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil; [2]Núcleo de Epidemiologia Clínica e Medicina Baseada em Evidências, Universidade Federal da Bahia, Salvador, BA, Brazil.*

Background: The acute kidney injury due to leptospirosis is characterized by a predisposition to hypokalemia presumably due to an inhibition of Na, K-ATPase activity in proximal tubule. The study investigated patient characteristics associated with hypokalemia, i.e., serum potassium (sK) <3.5 mEq/L, and associations of hypokalemia with the case-fatality rate of leptospirosis.

Methods: A prospective study of 253 patients hospitalized with leptospirosis. All patients reached ≥26 points in the WHO probability score for leptospirosis and had a positive macroscopic agglutination test or a microscopic agglutination test (MAT) for leptospirosis. Results of serum potassium (sK) and other laboratory exams were obtained at admission.

Results: Mean age was 35.5±13.4 yr, 89.7% males, all patients were icteric. The distribution by sK concentration was 64.4% for <3.5 mEq/L, 34.8% for 3.5-5.5 mEq/L and 0.8% for >5.5 mEq/L. Compared with sK ≥3.5 mEqL, patients with sK <3.5 mEq/L had significanly (P<0.05) lower means of age (32.37±11.38 vs 38.10±14.47 yr), serum creatinine (4.14±1.77 vs 4.96±2.42 mg/dL), serum urea (167.40±97.23 vs 202.56±129.76 mg/dL) and serum sodium (132.59±5.95 vs 134.94±6.23 mEq/L) The frequency of non-oliguric (urinary output≥500 ml/dia) patients was 85.5% for sK <3.5 mEq/L and 72.2% for sK ≥3.5 mEq/L (P=0.021). The case fatality rate of leptospirosis was 4.5% in patients with sK<3.5 mEq/L and 12.2% with sK≥3.5 mEq/L (P=0.046). The association between sK and case fatality rate was virtually eliminated after logistic regression adjustment for age, renal function and urinary output by (odds ratio=0.80; P=0.768).

Conclusions: More than half of leptospirosis patients had hypokalemia (sK <3.5 mEqL). Results indicate that leptospirosis patients with hypokalemia are more often younger and non-oliguric. The case fatality rate of leptospirosis was lower for patients with sK<3.5 mEq/L, a finding explained by the younger age, better renal function and higher urinary output.

PUB016

AMPK Signaling in Wound Healing of High Glucose Treated Renal Proximal Tubular Cells Jianping Peng, Zheng Dong. *Department of Cellular Biology and Anatomy, Georgia Health Sciences University and Charlie Norwood VA Medical Center, Augusta, GA.*

Background: Recovery from acute kidney injury (AKI) is harder and slower in the patients with chronic kidney diseases including diabetic nephropathy, leading to higher risk of progression to end stage renal disease and higher mortality. We hypothesize that the diminished recovery is related to impaired wound healing capacity in kidney tissues of these patients.

Methods: We used a scratch wound healing model to examine the effect of high glucose in cultured renal proximal tubular cells. The cells were cultured for two weeks in media with 5.5 mM glucose, 30 mM glucose, or 30 mM mannitol, followed by scratch wounding.

Results: It was shown that wound healing was significantly slower in high glucose conditioned cells. To explore the signaling pathway, we initially focused on AMPK signaling pathway. It was shown that phosphorylated AMPK (p-AMPK) was significantly higher in high glucose conditioned cells. Following scratch wounding, p-AMPK increased in both low and high glucose cells. Pharmacologic activation of AMPK with metformin inhibited wound healing in low glucose and mannitol treated cells, but the inhibitory effect was markedly lower in high glucose RPTC cells.

Conclusions: These results suggest that up-regulation of AMPK signaling may contribute to the defective wound healing in high glucose treated renal cells and hyperglycemic tissues.

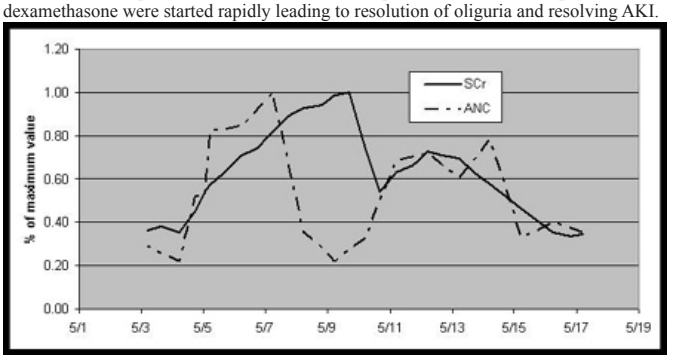
Funding: NIDDK Support, Veterans Administration Support

PUB017

Acute Kidney Injury Associated with Retinoic Acid Syndrome Jason Prosek, Udayan Y. Bhatt. *Division of Nephrology, Ohio State University, Columbus, OH.*

Background: Acute promyelocytic leukemia (APL), a variant of AML, has a high rate of mortality. Untreated, survival is less than one month. Treatment with all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO) induces a complete remission in 85% of cases. These agents therapeutically induce a sudden increase in differentiated myelocytes and neutrophils. This rapid maturation is accompanied by increased expression of Cathepsin G, the adhesion molecule LFA-1, and in growth factors IL-1b, TNF-a, and IL-6. This pro-inflammatory environment may result in retinoic acid syndrome (RAS). This potentially fatal complication occurs in approximately 25% of patients receiving ATRA/ATO. Clinical findings include capillary leak, pulmonary infiltrates, hypotension and fever. It is typically treated with dexamethasone. Renal involvement with RAS has not been fully described. We describe a patient whose first manifestation of RAS was AKI in the absence of hypotension and responded quickly with steroid therapy.

Results: The patient is an elderly female who began ATRA/ATO for APL. She developed oliguria with AKI and coarse granular casts on the fifth day of ATO therapy which coincided with a rapid increase in her absolute neutrophil count (ANC). Therapeutic doses of dexamethasone were started rapidly leading to resolution of oliguria and resolving AKI.



Conclusions: We previously described AKI complicating bone marrow engraftment. This is believed to be related to tubulitis during the pro-inflammatory period. Also, RAS has been described to include organ infiltration with newly maturated leukocytes. Similarly, the AKI in this report presented as oliguric ATN following a rise in ANC and resolved with dexamethasone treatment. We hypothesize that direct tubulitis from neutrophil migration and cytokine release into the renal parenchyma may be an important cause of reversible AKI during RAS, supporting the need for prompt therapy.

PUB018

Polymyxin: Renewed Antibiotic and the Old Nephrotoxicity Maria De Fatima Vattimo,[1] Cassiane Dezoti Fonseca,[1] Mirian Watanabe,[1] Fernanda Teixeira Borges.[2] *[1]School of Nursing, University of Sao Paulo, SP, Brazil; [2]Nephrology Division, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Polymyxin (Pmx) is a polypeptide cationic antibiotic with activity against multidrug resistant gram-negative bacteria and it re-emerged in clinical practice to be used in immunessupressed patients. Its nephrotoxicity consists of direct damage to the renal tubules, with the consequent release of reactive oxygen species (ROS) and inflammatory processes. The present study investigated the renoprotective effect of heme oxygenase -1 (HO-1) against toxicity caused by PmxB in rats.

Methods: Adult male Wistar rats, 250-300g, were submitted to the following treatments: Control, PmxB (rats that received 40,000 U/kg/day PmxB, i.p., for 5 successive days); PmxB preconditioning rats received HO-inducer or HO-inhibitor. Renal function (Jaffe method), urinary peroxides (FOX-2), renal tissue thiols, catalase activity, urinary TBARS and renal tissue histology (interstitial area fraction, IAF)were analyzed in vivo.

Results:

Table1: Renal function, oxidative profile and IAF.

Group (n)	crCl/100g (ml/min)	UP (nmol/g Creatinine)	Thiols (nmol / mg total protein)	CA (noml/ H2O2 / mg total protein)	TBARS (nmol/g creatinine)	IAF (%)
Control (6)	0.70±0.08	5.0±1.3	28.9±6.1	5.6±1.2	34.4±12.0	7.5±0.5
PmxB	0.30±0.33a	35.6±9.9a	15.7±1.3	1.2±0.4	98.1±35.7a	10,0±1.0a
PmxB+Hemin (6)	0.50±0.10ab	10.7±2.0b	28.0±11.9	3.4±1.0	46.6±7.9b	8.7±0.5
PmxB+ZnPP (6)	0.28±0.14ac	24.4±12.5ac	21.0±9.4	1.4±0.3	60.0±22.6b	10.4±1,.a

Results are reported as the mean ± standard deviation. a p < 0.05 vs Control. b p < 0.05 vs PmxB. c p < 0.05 vs PmxB + Hemin.

Conclusions: The disturbances in renal function, oxidative stress, tubular necrosis confirmed the nephrotoxicity of PmxB. The protective antioxidant role of the heme oxygenase inducer contributed to the amelioration of renal function and histology after PmxB treatment, suggesting that the heme oxigenase system can be involved in this model of acute kidney injury.

Funding: Government Support - Non-U.S.

PUB019

Clinical Characteristics and Risk Factors of Acute Kidney Injury in Patients with Acute Alcohol Intoxication Yu-Seon Yun,[1] Hyun Gyung Kim,[2] Young Ok Kim,[1] Jihan Yu.[1] *[1]Nephrology, Uijeongbu St. Mary's Hospital, Geumo-dong, Uijeongbu-si, Gyeonggi-do, Korea; [2]Nephrology, Seoul St. Mary's Hospital, Banpo-dong, Seocho-gu, Seoul, Korea.*

Background: Acute alcohol intoxication (AAI) causes various complications such as electrolyte imbalance, alcoholic ketoacidosis (AKA), rhabdomyolysis, and acute kidney injury (AKI). This study was performed to evaluate the clinical course and prognostic factors of patients with AKI due to AAI.

Methods: We retrospectively evaluated the medical records of 371 patients with AAI between January 2004 and May 2010 in our institute. We investigated the incidence and clinical courses of AKI and compared the clinical findings, laboratory results, morbidity and mortality rate between AKI and normal kidney function (NKF) groups.

Results: Of the total 371 patients with AAI, AKI occurred in 107 patients (28.8%). The peak serum creatinine level in AKI was 2.9±1.9 mg/dL. Thirteen of the 107 patients (12.1%) received renal replacement therapy. AKI group had higher incidence of decreased mentality (29.0% vs 16.3%, p=0.006), dyspnea (11.2% vs 1.9%, p=0.029), and hypotension (66.0% vs 41.7%, p<0.001), and lower incidence of gastrointestinal bleeding (22.4% vs 34.8%, p=0.019), compared to NKF group. The AKI group also had higher incidence of ketoacidosis (78.5% vs 28.8%, p<0.001), rhabdomyolysis (19.6% vs 4.2%, p<0.001), and pneumonia (22.4% vs 8.0%, p<0.001), and the independent risk factors of AKI were ketoacidosis (OR 4.484, 95% CI 1.498-13.416, p=0.007) and increased serum osmolality (OR 3.792, 95% CI 1.280-11.232, p=0.016). The length of ICU stay was longer (7.4 ± 10.8 vs 4.1 ± 6.1 days, p=0.003) and the mortality rate was higher (17.8% vs 2.3%, p<0.001) in AKI group. multivariate analysis confirmed that AKI, increased serum CPK level and pulmonary edema were independent predictors of mortality.

Conclusions: This study demonstrated that incidence of AKI in patients with AAI was 28.8%, and the independent risk factors of AKI were ketoacidosis and increased serum osmolality. AKI was associated with high morbidity and mortality, so in patients with AAI, early aggressive management is needed to prevent AKI in high risk patients.

Funding: Private Foundation Support

PUB020

The Influence of Age on the Effect of Dietary Supplementation with Reduced Glutathione (GSH) on GSH Levels in Mitochondria from Rat Kidney Cortex and Medulla

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Background: The purpose of dietary supplementation with antioxidants is to provide protection from oxidative stress inside cells by increasing the level of GSH, the principal antioxidant found in cells. A previous study reported that mitochondrial GSH levels in kidneys from old rats, but not young rats, increased following exogenous dietary supplementation with the antioxidant alpha lipoic acid. The present study was undertaken to determine whether rat age affects the influence of exogenous supplementation with GSH on cytosolic and mitochondrial GSH levels in kidney cortex and medulla.

Methods: Young (i.e., 3 months of age) and Old (i.e., 22 months of age) female Lewis rats were given GSH (250 mg/Kg body wt) via intraperitoneal injection for one week. Age-matched control rats were not given any exogenous supplementation. The kidneys were harvested at the end of the treatment period and separated into cortical and medullary sections. The sections were then further separated into cytosolic and mitochondrial fractions by differential centrifugation. GSH levels in the fractions were measured using a spectrophotometric assay. There were 4 to 6 rats in each group, and statistical comparisons between similar aged rats were done using a Student's t test.

Results: There was a significant increase in mitochondrial and cytosolic GSH levels in kidney cortex and medulla from both Young and Old rats.

		Young			Old	
		Control	Experimental		Control	Experimental
Cytosol						
umol/g kid wet wt	Cortex	5.7±0.3	8.3±0.5*		5.8±0.2	8.7±0.8*
	Medulla	3.5±0.1	6.0±0.9*		3.0±0.1	4.5±0.5*
Mitochondria						
nmol/g kid wet wt	Cortex	122±2	196±12*		139±3	164±5*
	Medulla	116±5	156±12*		141±4	155±3*

All data expressed as X +/- SEM. * significantly different from age-matched Control.

Conclusions: Age does not affect the increase in mitochondrial or cytosolic GSH levels in rat kidneys observed with exogenous dietary supplementation with GSH.

PUB021

Transfusion Related Acute Kidney Injury: Result of a Prospective Cohort Study

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Background: Acute kidney injury (AKI) occurs in up to two third of intensive care unit (ICU) patients. It has been shown that blood product transfusion increases risk of acute lung injury (TRALI). Considering strong association between ALI and AKI, we evaluated the association between transfusion and AKI in ICU patients.

Methods: We performed a retrospective analysis of a prospectively collected cohort of consecutive adults (>18 years of age) who were admitted to the ICU and received blood product transfusion from March 2004 to December 2005. We excluded those who developed AKI prior to receiving transfusion or who were on chronic hemodialysis at time of admission.

Results: A total of 127 patients met the inclusion criteria. The median age was 64 (IQR, 53-77), 65 were male (51%). A total of 49 (38%) patients developed AKI based on the AKIN criteria. In univariate analysis, there was no statistical significant difference in development of AKI based on the type of blood product that the patients received (cryoprecipitate, packed red blood cells, platelets or fresh frozen plasma). After adjustment for age, gender, baseline creatinine and presence of shock upon ICU admission, the transfusion of blood products was not independently associated with development of AKI (Odds ratio 1.06, 95% CI 0.95-1.18, p=0.25).

Conclusions: In a cohort of heterogenous group of ICU patient who received blood product transfusion, there was no increased risk of AKI as it is defined by AKIN criteria. However, this study does not rule out that subtle kidney injury might be detected with more sensitive novel biomarkers.

Funding: Private Foundation Support

PUB022

Renoprotective Effects of Long Term Oral Nicotine in a Rat Model of Proteinuria

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Background: Many proteinuric renal diseases are accompanied by renal inflammation. Reduction of inflammation might be crucial for long term preservation of renal function. Nicotine is known to have anti-inflammatory properties. A potential anti-inflammatory role of oral nicotine in proteinuric renal diseases is not known yet. Therefore we evaluated the effects of oral nicotine in a rat model of proteinuria-induced renal inflammation.

Methods: 24 wk old spontaneously proteinuric male Munich-Wistar-Fromter rats (n=40) were used. Four groups (n=10 each) were given either no (placebo), 20 (N20), 60 (N60) or 100 (N100) mg/l nicotine in drinking water, till 52 weeks of age. Body weight, blood pressure and water intake were measured weekly. Collection of blood and 24h urine were performed at baseline and monthly thereafter. At 52 weeks, histology and mRNA expression of inflammatory markers were assessed in renal tissue. ANOVA followed by Tukey post-hoc test was performed.

Results: Nicotine treatment in N60 and N100 vs placebo improved creatinine clearance (ml/min) (0.72±0.06, 0.71±0.05 vs 0.43±0.06 resp., both p<0.05) and reduced glomerulosclerosis score (scale of 0 till 400) (49±8, 54±12 vs 108±16 resp., both p<0.05). Percentage area of ED-1 positive macrophages (0.29±0.07, 0.28±0.06 vs 0.72±0.14 resp., both p<0.01) and myofibroblasts (0.68±0.19, 0.89±0.26 vs 2.1±0.60 resp., both p<0.01) were also reduced in N60 and N100 vs placebo. Nicotine treatment in N60 and N100 vs placebo furthermore reduced mRNA of MCP-1 (0.60±0.15, 0.63±0.08 vs 1.10±0.12 resp., both p<0.01) and VCAM-1 (0.66±0.12, 0.70±0.11 vs 1.10±0.16 resp., both p<0.05). Results of N20 did not differ from placebo. Other physiological parameters were similar among the groups throughout the experiment.

Conclusions: Long term oral nicotine is renoprotective by reducing renal inflammation and glomerulosclerosis in a rat model of proteinuria. We suggest to evaluate cholinergic agonists as a potential therapeutic option for treating proteinuric /inflammatory kidney diseases.

Funding: Government Support - Non-U.S.

PUB023

A Novel U-STAT3-Dependent Regulation of TGFβ1-Induced Renal Tubular Epithelial-Mesenchymal Transition by Chronic Nicotine Exposure

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Background: Smoking-associated damage of renal proximal tubules may contribute to tubulointerstitial injury and progression to chronic renal disease. Epithelial-mesenchymal-transition (EMT) could be mediated by the tobacco smoke component nicotine (NIC). We previously found that chronic exposure to NIC exacerbates severity of acute renal ischemic injury (AKI) in mice. Since STAT3 may be involved in interstitial fibrosis we tested the hypothesis that chronic NIC may augment renal tubular EMT in a STAT3-dependent manner.

Methods: Kidneys from postischemic (24 hours) mice were analyzed for pro-fibrotic markers (αSMA, TGFβ1) and STAT3 activation. Cultured renal proximal tubule cells (LLC-PK1) were used to study effects of chronic NIC treatment as well as STAT3 activation on TGFβ1-mediated tubular EMT.

Results: Renal TGFβ1 content as well as αSMA expression were augmented in the ischemic kidneys upon chronic NIC exposure. AKI-induced tyrosine phosphorylation of STAT3 (pTyrSTAT3) was attenuated while expression of unphosphorylated STAT3 (U-STAT3) was increased by NIC exposure. TGFβ1 treatment significantly increased αSMA expression and activity of the αSMA promoter along with concomitant F-actin reorganization in LLC-PK1 cells that were further enhanced by chronic NIC pretreatment. Adenoviral infection of LLC-PK1 cells with a U-STAT3 mimetic dramatically amplified both TGFβ1- and NIC+TGFβ1-induced αSMA expression and promoter activity.

Conclusions: Our results reveal a novel, U-STAT3-dependent mechanism that mediates a sustained αSMA transcription that is related to remodeling in the kidney. Hence, chronic NIC exposure may facilitate progression of AKI to CKD by sustained upregulation of αSMA transcription via a novel U-STAT3-dependent transcriptional pathway.

Funding: NIDDK Support, Other NIH Support - R01HL088101-04 and R01HL088101-02S1

PUB024

Lower Blood Pressure and Better Renal Function Despite Reduced Nephron Numbers in Caspase-3 Deficient Mice Midori Awazu,[1] Yoshifumi Yamaguchi,[2] Masayuki Miura,[2] Mariko Hida.[1] *[1]Department of Pediatrics, School of Medicine, Keio University, Tokyo, Japan; [2]Department of Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.*

Background: Pharmacological blockade of caspase-3 in organ-cultured metanephroi inhibits nephron formation and ureteric bud branching. In the present study, we examined whether caspase-3 deficiency leads to low nephron number *in vivo*, and if so, whether it results in elevated blood pressure and compromised renal function.

Methods: Homozygous caspase-3-deficient mice (Casp3-/-) were examined in comparison with heterozygous mice (Casp3+/-) and wild type mice (WT).

Results: The glomerular number, counted at 3 weeks postnatally using the acid maceration technique, was significantly reduced in Casp3-/- (n=7) compared with Casp+/- (n=8) (10127±357 vs 14174±314 per kidney). The body weight (11.1±1.1 vs 13.3±0.4 g) and kidney weight (143±13 vs 160±4 mg) were numerically lower in Casp3-/-. We next examined the consequences of low nephron number at 8 months of age. Since there was no difference between Casp+/- and WT in all parameters examined, data were combined. As shown in the Table, blood pressure measured by tail-cuff methods, urine protein/creatinine ratio, serum creatinine, and blood urea nitrogen were significantly lower in Casp3-/- compared with Casp3+/- and WT. Body weight and urine creatinine excretion were not different between the two groups.

Blood pressure and renal function

	Casp3+/- and WT (n=6)	Casp3-/- (n=4)
Blood pressure mmHg	111±1/77±2	87±7*/57±3*
Urine protein/creatinine ratio	11.4±1.7	3.0±0.8*
Serum creatinine mg/dl	0.78±0.07	0.23±0.03*
Blood urea nitrogen mg/dl	35.9±0.9	27.3±3.0*
Body weight g	29.9±0.9	28.0±1.0
Urine creatinine excretion mg/day	0.23±0.04	0.17±0.03

*, P<0.05 vs Casp+/- and WT

Conclusions: Caspase-3 deficiency reduces nephron number but prevents high blood pressure and renal function deterioration in later life. The mechanisms remain to be determined but may be related to apoptosis, cell motility, differentiation, or proliferation, functions associated with caspase-3. Superior renal function despite low nephron number indicates a possibility of a novel approach for prevention of chronic kidney disease.

Funding: Government Support - Non-U.S.

PUB025

Role of Cdc42 Interacting Protein-4 in TGF-β1-Induced Epithelial-Mesenchymal Transition of Renal Proximal Tubular Epithelial Cells Shoujun Bai, Rui Zeng, Chuou Xu, Qiaodan Zhou, Gang Xu. *Division of Nephrology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, HUST, Wuhan, Hubei, China.*

Background: Cdc42 interacting protein-4 (CIP4) is a membrane-binding protein which plays a key role in cancer cell invasion. The migration and invasion of cancer cells is a process analogous to that observed during epithelial-mesenchymal transition (EMT). In kidneys, EMT promotes the pathogenesis of renal fibrosis. Thus, the role of CIP4 in EMT and renal fibrosis was explored.

Methods: Western blotting and Immunofluorescence analysis for CIP4, E-cadherin and α-SMA; Immunohistochemistry and Masson staining for Rat kidney tissue

Results: The expression of CIP4 was increased in the tubular epithelia of 5/6-nephrectomized rats and in TGF-β1 treated HK-2 cells. Endogenous CIP4 had a polarized distribution at the cell surface of HK-2 cells. After TGF-β1 treatment, the CIP4 expression was gradually increased and migrated to the cytoplasm, and simultaneously the cells was confirmed to induce EMT by morphological change, loss of E-cadherin and gain in α-SMA expression. Overexpression of CIP4 promoted similar characteristics of EMT. Using small interfering RNA (siRNA) to knockdown CIP4, we demonstrated the reversed EMT.

Conclusions: Taken together, our results indicate the importance of CIP4 as a downstream target of TGF-β1 signal in the development and progression of renal fibrosis, and thus suggest that this is a novel therapeutic target for the treatment of renal fibrosis.

PUB026

In Vitro Study To Evaluate the Effect of Fructose Induced Metabolic Syndrome and Chronic Kidney Disease on Bone Marrow Mesenchymal Stem Cells Proliferation Potential Anna Bajek,[1] Mariusz Flisinski,[2] Andrzej Brymora,[2] Arkadiusz Jundzill,[1] Pawel Strozecki,[2] Tomasz Drewa,[1,3] Jacek Manitius.[2] *[1]Tissue Engineering Department, Nicolaus Copernicus University, Bydgoszcz, Poland; [2]Nephrology, Hypertension and Internal Diseases, Nicolaus Copernicus University, Bydgoszcz, Poland; [3]Urologic Oncology, Oncology Center, Bydgoszcz, Poland.*

Background: Fructose consumption deranges many metabolic processes and contributes to development of metabolic syndrome (MS).MS and direct effect of consumed fructose modulate function of many organs and cells. The aim of study was to evaluate the high- fructose intake on proliferation capacity of Bone Marrow Mesenchymal Stem Cells (MSCs) in rats with surgically induced CKD.

Methods: Male Wistar rats were divided into groups: control (CON) (n=12) was sham-operated, CKD ½ (n=12) - uninephrectomy; CKD 5/6 (n=12) uninephrectomy plus 1/3 of contra-lateral kidney cortex mass resection. Rats received regular diet containing <3% of fructose – F3; 60% fructose diet (Harlan Teklad) – F60 or regular diet with 10% solution of fructose in drinking water – F10. After 8 weeks creatinine in serum were measured and MSCs were isolated from femur. Cells were cultivated in Dulbecco's modified essential medium supplemented with 10% of fetal bovine serum. Cultures were observed for six passages. MSCs were counted with trypan blue test.

Results: We indicate statistically significant relationship between increasing fructose content in diet and deteriorating renal function. The number of MSCs are presented as mean ± SD (x10^3) in table 1.

Table 1

Diet	F3	F10	F60	p - ANOVA
CON	39.9±6.8	22±7.8	16.2±11.5	<0,01
CKD1/2	30.2±19.5	25.5±11.2	10.1±9.8	<0,05
CKD5/6	33.3±9.6	27.2±8.4	36.5±9.5	NS
p - ANOVA	NS	NS	<0,01	

Increasing fructose consumption was associated with continuous fall of capacity of MSCs to proliferate in CON and CKD1/2. But opposite tendency was observed in CKD 5/6 fed with F60.

Conclusions: Our study suggest that high fructose intake may adversely affect proliferation capacity of MSCs in healthy and CKD rats. But further study is demanded to elucidate whether fructose affects MSCs function directly or indirectly eg.: hyperuricemia or metabolic syndrome.

Funding: Other NIH Support - Nicolaus Copernicus University grants no. 2/2010, 3/2010

PUB027

Epithelial-to-Mesenchymal Transition was Induced by Indoxyl Sulfate in Rat Kidneys and Human Proximal Tubular Cells Dilinaer Bolati, Hidehisa Shimizu, Toshimitsu Niwa. *Advanced Medicine for Uremia, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: Indoxyl sulfate (IS) is a uremic toxin that accelerates the progression of chronic kidney disease (CKD). Hypertension is a consequence of renal disease and one of the most important risk factors for progressive CKD. This study aimed to determine if IS induces epithelial-to-mesenchymal transition (EMT) in the kidneys of hypertensive rats, and human cultured proximal tubular cells (HK-2).

Methods: EMT was evaluated by immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR) and immunoblotting of the epithelial makers E-cadherin and zonula occludens-1 (ZO-1), and the mesenchymal maker a-smooth muscle actin (a-SMA) in rat kidneys and HK-2 cells. The rat groups consisted of (1) Dahl salt-resistant normotensive rats (DN), (2) Dahl salt-resistant normotensive indoxyl sulfate-administered rats (DN + IS), (3) Dahl salt-sensitive hypertensive rats (DH), and (4) Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats (DH + IS). HK-2 cells were incubated with or without IS (250 mM).

Results: In the kidneys, DH rats showed reduced expression of E-cadherin and ZO-1, and enhanced expression of a-SMA compared with DN rats. Further, IS-administered rats, DN+IS and DH+IS rats, showed reduced expression of E-cadherin and ZO-1, and enhanced expression of a-SMA compared with DN and DH rats, respectively. IS-treated HK-2 cells showed reduced expression of E-cadherin and ZO-1, and enhanced expression of a-SMA compared with untreated cells.

Conclusions: IS induced EMT in the kidneys of hypertensive as well as normotensive rats, and in HK-2 cells accompanied by reduced expression of E-cadherin and ZO-1, and enhanced expression of a-SMA. IS-induced EMT might be involved in the progression of CKD.

PUB028

AST-120 Ameliorates Kidney Fibrosis by Inhibiting Epithelial-to-Mesenchymal Transition in CKD Rats Dilinaer Bolati, Hidehisa Shimizu, Toshimitsu Niwa. *Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: Indoxyl sulfate (IS), a uremic toxin, is a risk factor for progression of chronic kidney disease (CKD). AST-120 reduces serum IS, and delays the progression of CKD. This study aimed to examine if AST-120 inhibits epithelial-to-mesenchymal transition (EMT) in the kidneys of CKD rats.

Methods: CKD rats were produced by 5/6-nephrectomy, and divided into 2 groups: (1) CKD rats and (2) AST-120-treated CKD rats at a dose of 4 g/kg body weight/day. After 16 weeks, their kidneys were excised for histological and immunohistochemical analysis. EMT was evaluated by immunohistochemistry of zonula occludens (ZO-1), an epithelial marker, and a-smooth muscle actin (a-SMA), a mesenchymal marker. Interstitial fibrosis was evaluated by Masson's trichrome (MT) staining.

Results: CKD rats showed reduced expression of ZO-1, and enhanced expression of a-SMA as compared with normal rats. Administration of AST-120 to CKD rats increased expression of ZO-1, and decreased expression of a-SMA. Further, CKD rats showed enhanced extent of interstitial fibrosis as compared with normal rats. Administration of AST-120 to CKD rats ameliorated interstitial fibrosis.

Conclusions: AST-120 inhibited EMT, and ameliorated fibrosis in the kidneys of CKD rats.

PUB029

Effect of Iron Chelator, Deferiprone, on the Progression of Chronic Kidney Disease in Mouse Model Chhanda X. Bose,[1] Neriman Gokden,[2] Sudhir V. Shah,[1] Sundararaman Swaminathan.[1] *[1]Nephrology, University of Arkansas for Medical Sciences and Central Arkansas VA Medical Center, Little Rock, AR; [2]Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: There are no effective treatments for halting progression of chronic kidney disease (CKD). Catalytic iron capable of catalyzing free radical reactions has been implicated in tissue injury in various organ systems. We examined the effect of deferiprone, an oral iron chelator in a mouse model of CKD.

Methods: We utilized one 5/6 nephrectomy mice for our studies. After one week of 5/6 nephrectomy mice developed renal insufficiency with serum creatinine values of 0.49±0.0.02 mg/dl compared to sham operated controls (0.11±0.04). Average body weights were 25.72±0.13 g (sham) and 20.632±0.66 g (CKD). The CKD Mice were divided in to two groups with similar serum creatinine and body weights. Deferiprone was started at 125 mg/kg body weight in one group of CKD mice in drinking water and continued up to 12 weeks (n=7). Sham (n=5) and CKD groups (n=12) were given water. At 13 weeks, serum creatinine levels were significantly ($p>0.05$), lower in treated group of mice than CKD group, 0.58±0.08 and 0.38±0.025 mg/dl respectively. Histopathological changes in kidneys were evaluated and a quantitative scoring system was employed to account for histological changes. Scoring and validation was done by an expert kidney pathologist in a blinded manner. Sham operated controls showed no pathologic changes. CKD Kidneys showed interstitial fibrosis and tubular atrophy (Average score, 13.5±5.83%), interstitial inflammation (6.571±2.04%) and global sclerosis (3.875±2.08%). In contrast, these pathological changes were significantly attenuated in the kidneys of deferiprone treated mice. The average score was for interstitial fibrosis and tubular atrophy (6.66±1.1), interstitial inflammation (1) and global sclerosis (0.5±0.29).

Conclusions: Our preliminary data suggest a role of iron in the progression of CKD.

Funding: Veterans Administration Support

PUB030

Abstract Withdrawn

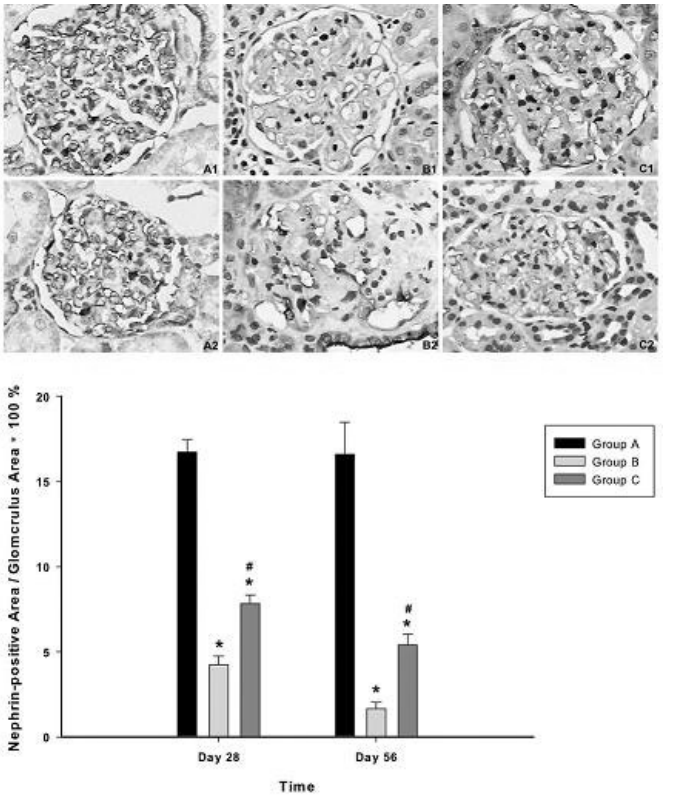
PUB031

Effect of Shen-Qi-Di-Huang Decoction on Reducing Proteinuria by Preserving Nephrin in Adriamycin-Induced Nephropathy Rats Hongyu Chen,[1] Qin Zhu.[2] *[1]Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China; [2]Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China.*

Background: The aim of this study is to investigate the effect of Shen-qi-di-huang decoction on reducing proteinuria and to discuss the mechanism of its action in Adriamycin (ADR)-induced nephropathy rats.

Methods: The rats were randomly divided into three groups (n=12 each group): normal control (group A); ADR model control (group B); ADR + Shen-qi-di-huang decoction (group C). In group B and C, the rats were intravenously injected with ADR (6.5mg/kg). The rats in group C were orally administrated with Shen-qi-di-huang decoction after the injection of ADR. On day 7, 14, 28, 56 after ADR injection, 24h urine protein was detected. On day 28, 56 after ADR injection, ALB, ALT, serum creatinine (Scr) and BUN were examined. The morphological changes of the kidneys were observed by light microscope and electron microscope on day 28, 56 after ADR injection. The expression of nephrin was determined by immunohistochemistry and RT-PCR on day 28, 56 after ADR injection.

Results: Compared with group B, 24h urine protein and Scr decreased in group C on day 56 ($P<0.05$). The expression of nephrin determined by immunohistochemistry and RT-PCR increased in group C on day 28, 56 ($P<0.05$). The morphology observed by light microscope and electron microscope improved in group C on day 28, 56.



Conclusions: Shen-qi-di-huang decoction decreases proteinuria, protects kidney function, and ameliorates histopathology in ADR-induced rats by preserving nephrin expression.

PUB032

The Epithelial-to-Mesenchymal Transition and ECM Accumulation of Human Peritoneal Mesothelial Cell in Response to LDL and Glucose In Vitro Yanhui Fang, Limeng Chen, Xuewang Li. *Department of Nephrology, Chinese Academy of Medical Science & Peking Union Medical College Hospital, Beijing, China.*

Background: Human Peritoneal mesothelial cells(HPMCs) play an important role in fibrogenesis and vasculopathy that underlie peritoneal membrane dysfunction. New and extensively studied aspects of peritoneal mesothelial cell biology include epithelial-to-mesenchymal transition (EMT) and cellular senescence.The objective of this study was to investigate the effect of LDL on EMT and ECM accumulation in HPMCs and study the role of the LDL receptor and PPARγsignaling pathway in these processes.

Methods: After co-cultured with the LDL for 24h, the LDL receptor was observed to expression on the cell membrane of HPMCs by immuno-fluorescence method. HPMCs morphological and cytoplasm immuno-fluorescence intensity of α-SMA changes were observed after stimulated with different concentration of LDL (0, 25, 50, 100μg/ml).PPARγ mRNA and protein expression were detected by realtime-PCR and Western blot.

Results: Oil red staining results showed that LDL could be up-taken into the cells and abolished by LDL receptor blocker. The increasing expression of α-SMA mRNA and protein, lower expression of E-cadherin mRNA were showed in HG + LDL group(glucose 120mmol/l, LDL 100μg/ml). ELISA assay showed that the COL-I protein was significantly increased. The difference COL-I and FNmRNA expression was not observed with or without LDL by realtime-PCR. The plasminogen inhibitor (PAI-1) mRNA and protein level of the cell culture supernatant of HG+LDL group were significantly increased than the control.HG+LDL significantly increased the expression of PPARγ mRNA. Added different concentrations of GW9662 (5μg/ml, 10μg/ml), a kind of PPARγ ligand blocker, the effects of HG+LDL showed above were abolished partly.

Conclusions: Human peritoneal mesothelial cells could uptake LDL into cells via LDL receptor. The PPARγ signaling pathway may be play a role in these processes of HPMCs EMT and ECM accumulation induced by LDL in the context of high glucose.

Funding: Government Support - Non-U.S.

PUB033

New Applications of the Mouse Electrocautery Model of Chronic Kidney Disease Raymonde Gagnon. *Internal Medicine, Division of Nephrology, McGill University Health Centre, Montreal, QC, Canada.*

Background: Arteriosclerotic vascular disease and vascular calcifications are common manifestations of chronic kidney disease (CKD) in man.

Methods: A model of CKD induced surgically in the mouse was described in 1983 (the model requires 2 surgical procedures i.e. electrocautery-induced damage to the surface of the right kidney followed by left nephrectomy 2 weeks later). The right kidney was chosen to undergo electrocautery-induced surface damage because it lies lower than the left kidney in the abdominal cavity (due to the presence of the liver) and therefore its entire surface is better visualized during the induction of the electrocautery-induced damage. Of note, this first surgical step is an essentially bloodless procedure which is another important consideration in young mice with a small blood volume. In this surgical model, the depth of the electrocautery-induced surface damage to the right kidney determines the degree of renal impairment observed following the left nephrectomy performed 2 weeks later i.e. mild, moderate or severe CKD. An interval of 2 weeks between the 2 surgical procedures is required to allow sufficient time for edema resorption and tissue regeneration to take place following the electrocautery-induced damage to the right renal parenchyma.

Results: During the initial 20 years following its description, this mouse model was used successfully in experiments conducted in genetically defined inbred mice only (usually young female mice of the C57BL/6 strain). Since the availability of knockout mice, the mouse electrocautery model of CKD has been used successfully to increase our understanding of the cellular and biochemical contributors to various serious vascular conditions observed in man with CKD i.e. arteriosclerosis and vascular calcifications.

Conclusions: It is impossible to predict at the outset the nature of the main future applications of a novel animal model. In the case of the mouse electrocautery model of CKD, the recent availability of genetically-modified mouse strains has contributed to make this animal model of CKD a prime model for research in vascular complications of CKD more than 2 decades after its original description.

PUB034

Inulin Immunoassay for Kidney Function Ernest V. Groman, Dennis E. Vaccaro, Christopher P. Reinhardt, James S. Weinberg. *BioPAL.*

Background: The absence of a widely accepted method to measure GFR in animal models, human research and clinical practice has had profoundly negative effects on developing methods and drugs for treating kidney disease, accurately assessing kidney transplant recipients and donors, calibrating drug dosing in the young and elderly, minimizing nephrotoxicity in the drug development process, and accurately assessing nephrotoxicity in the clinical setting. To correct this deficiency we have developed a sensitive, specific ELISA for inulin.

Methods: NZW rabbits were injected with inulin conjugate and responded with high titer anti-inulin antiserum. ELISA components include an inulin-coated-microtiter plate, inulin standards, inulin in sodium chloride, anti-inulin polyclonal antiserum, and HRP-goat anti-rabbit antiserum. The assay is completed in 70 minutes.

Results: Anti-inulin antiserum binds to polyfructans with a degree of polymerization greater than 4. The antibody has exquisite specificity, does not bind to fructose, glucose or glucose polymers, or other disaccharides and polysaccharides. The sensitivity of the assay is 10ng inulin/mL. The concentration required to inhibit 50% of the maximum signal is about 100ng inulin/mL. Reproducibility measurements for inulin in rat or human serum had CVs of less than 12%. Spike and recovery experiments gave recovery values between 85% and 112%. The sensitivity of the antiserum allows inulin doses 10 to 100 times lower than doses used in previously reported GFR protocols. A correlation coefficient between the inulin immunoassay and a fluorescent inulin assay in human serum of 0.98 was obtained. The high sensitivity of the anti-inulin antiserum allowed the detection of dietary inulin in human urine.

Conclusions: Inulin is widely used in foods, has beneficial effects on cancer treatment in animal models, and is the gold standard for assessing kidney function. Nutritional dogma regarding dietary inulin states that inulin is not absorbed from the gastrointestinal tract into blood; all dietary inulin is quantitatively eliminated in the feces. Demonstration that this dogma is not true may have significant implications for nephrology, gastroenterology, cancer therapy, nutritional studies, and food chemistry.

Funding: NIDDK Support

PUB035

Microarray Analysis of Gene Expression Profiles in Rat Kidney Fibroblasts Exposed to Advanced Lycation End Products Xuezhu Li,[1] Shougang Zhuang,[1,2] Haidong Yan.[1] *[1]Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai; [2]Department of Medicine, Brown University School of Medicine, Providence, RI.*

Background: Diabetic nephropathy (DN) is the most important cause of end-stage renal disease worldwide, and renal interstitial fibroblasts play a critical role in the development of diabetic nephropathy. Although advanced glycation end products (AGEs) have been showed to contribute to renal fibroblast activation and proliferation, mechanisms involved remain incompletely understood.

Methods: In this study, we examined the profile of gene expression in normal rat kidney fibroblast (NRK-49F) cells exposed to AGEs, and assessed the effect of ginkgo biloba extract (EGb), an antioxidant, on this response by using microarray analysis. Biological functions of the differentially expressed genes were analyzed using Gene Ontology (GO), and the up-regulated or down-regulated genes were further validated using real time-PCR.

Results: Compared with non-treated NRK-49F cells, 61 genes were up-regulated and 67 genes were down-regulated in cells exposed to AGEs. GO analysis showed that these differentially expressed genes were primarily related to the regulation of the biosynthetic and protein kinase activity, cell proliferation, gene transcription, cell cycle, apoptosis as well as cellular response to reactive oxygen species. Treatment with EGb dramatically altered expression of some genes including transforming growth factor-beta1, monocyte chemotactic protein-1, angiopoietin-1, superoxidase dismutase, matrix Gla protein, G protein-coupled receptor and inhibitor protein-kappa B. The real time-PCR results were consistent with the results obtained using gene chip analysis. AGEs exposure resulted in changes of multiple genes in NRK-49F cells, and EGb treatment altered the profile of gene expression.

Conclusions: These results lay the groundwork for further identification of novel molecules involved in the pathogenesis of diabetic nephropathy and elucidation of mechanisms of anti-fibrotic therapies in DN and other fibrotic kidney diseases.

Funding: Government Support - Non-U.S.

PUB036

Use of ACEI/ARB in Diabetic Patients with Late Stage Chronic Kidney Disease (CKD) Reduces the Risk for Mortality and Progression to Dialysis Jia-Sin Liu,[1] Ta-Wei Hsu,[2] Yu-Kang Chang,[1] Chih-Cheng Hsu,[1] Der-Cherng Tarng.[3] *[1]Institute of Population Health Sciences, NHRI, Miaoli County, Taiwan; [2]Division of Nephrology, Department of Internal Medicine, National Yang-Ming University Hospital, Yilan, Taiwan; [3]Department and Institute of Physiology, National Yang-Ming University, Taipei, Taiwan.*

Background: Whether the use of ACEI/ARB in diabetic patients with late stage CKD could reduce the risk for ESRD and/or mortality, since this issue lock of population-based evidence.

Methods: We conducted a nationwide, prospective cohort study in Taiwan using the national insurance database. There were 10,619 diabetic patients with CKD and the eGFR less than 12 ml/min/1.73m^2 at the beginning of the study in 2000-2005. All patients never staring dialysis before the recruitment were followed up until December 31, 2007. Cox proportional hazards model was applied to analyze the risk for progression to dialysis and/or death.

Results: The cohort had mean age of 65 years, 51% of female, 91% of hypertension prevalence. The median follow-up period was 4 years. In the first 2 years of follow up, 60% and more of the patients progressed to ESRD necessitating dialysis therapy. After adjustment for age, gender and hypertension, ACEI/ARB nonuser had higher risk for progression to dialysis and/or death than ACEI/ARB user with a hazard ratio of 1.26 (95% CI: 1.21-1.31, P <0.001).

The risk of ESRD or death in late stage CKD patients with diabetes

	number	incidence case	incidence rate #	crude HR	adjusted HR
Age					
20-44	408	400	147.1	1.13 (1.03-1.26)*	1.19 (1.07-1.31)*
45-64	4,663	4,595	132.4	ref.	ref.
65-74	3,400	3296	106	0.85 (0.81-0.89)*	0.85 (0.82-0.89)*
75 and more	2,148	2,056	105.2	0.85 (0.81-0.89)*	0.85 (0.80-0.89)*
Gender					
male	5,243	5,114	128.5	1.13 (1.09-1.18)*	1.11 (1.06-1.15)*
female	5,376	5,233	108.5	ref.	ref.
Hypertension					
prevalence	9,681	9,476	123.6	1.37 (1.28-1.47)*	1.38 (1.29-1.49)*
none	938	871	76.4	ref.	ref.
ACEI/ARB during the following					
nonuser	4,784	4,730	134.2	1.2 (1.16-1.25)*	1.26 (1.21-1.31)*
user	5,835	5,617	106.4	ref.	ref.

#: per 1000 person years, * :p<0.05, ref.:reference

Conclusions: Diabetic patients with late stage CKD treated with ACEI/ARB could decrease the risk for progression to dialysis and/or all-cause mortality.

PUB037

Glomerular and Interstitial Injury and Effect of Losartan (L) Treatment in Adenine-Induced Chronic Kidney Disease (CKD) Cristiene Okabe, Denise M. Malheiros, Flavia G. Machado, Simone R. Costa, Camilla Fanelli, Claudia R. Sena, Grasiela P. Barlette, Vivian L. Viana, Niels O.S. Camara, Roberto Zatz, Clarice K. Fujihara. *Univ Sao Paulo.*

Background: Chronic oral adenine (ADE) promotes interstitial (INT) inflammation due to tubular obstruction, being used as a model of CKD. We investigated whether ADE-induced CKD: 1. still progresses after ADE is discontinued. 2. responds to treatment with L, known to ameliorate CKD progression.

Methods: Adult male Munich-Wistar rats received ADE in the chow, at 0.75% for 1 week, then at 0.53% for 2 weeks. Two weeks after ADE was ceased, tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/d), serum creatinine (S_{cr}, mg/dL), glomerular volume (V_G, x 10^6 μm^3), % glomerular luminal area (%Lum), tubulointerstitial macrophage density (MØ, cells/mm^2, and % cortical INT (%INT) were measured in 5 rats, used as pretreatment controls (Group ADE_{prc}). The remaining rats were divided into groups ADE+V, untreated, and ADE+L, receiving oral L, 50 mg/kg/d, and followed for an additional 3 months. Age-matched normal controls (C) were also studied. Results (Mean±SE, ^{a}p<0.05 vs. C, ^{b}p<0.05 vs. ADE_{prc}, ^{c}p<0.05 vs. ADE):

Results:

	TCP	ALB	S_{cr}	V_G	%Lum	MØ	%INT
C	136±1	5±1	0.6±0.1	0.96±0.1	28.6±1.9	18±2	1±1
ADE_{pre}	145±4	4±1	1.4±0.1[a]	0.58±0.1[a]	27.8±2.1	196±18[a]	29±1[a]
ADE+V	194±3[ab]	14±5[ab]	1.3±0.2[a]	0.60±0.1[a]	21.3±1.0[ab]	179±33[a]	19±2[ab]
ADE+L	139±3[c]	4±1[c]	1.2±0.1[a]	0.54±0.1[a]	23.9±1.1	214±22[a]	18±3[ab]

Group ADEpre showed marked S_{cr} elevation, along with INT expansion and MØ infiltration. Although glomerulosclerosis was not observed, V_G was markedly reduced. Three months later, ALB and hypertension were associated with persistence of glomerular hypotrophy, now with partial loop collapse. INT expansion, but not MØ, regressed, in all likelihood due to partial release of tubular obstruction, suggested by a linear correlation between %INT and the number of crystals. L treatment normalized TCP and ALB, but failed to ameliorate any of the structural abnormalities.

Conclusions: ADE-induced INT expansion is only partially reversible after ADE cessation, while other parameters of renal injury persist, responding poorly to L. Further studies will clarify the injurious mechanisms involved in this model.

Funding: Government Support - Non-U.S.

PUB038

Renal Parenchymal Thickness and Maximum Interpolar Diameter in Ultrasound Investigation of Patients with Chronic Kidney Disease Roberto Palumbo,[1] Annalisa Noce,[2] Fulvio Fiorini,[3] Olga Durante,[2] Nicola Di Daniele.[2] *[1]Nephrolgy and Dialysis Unit, S. Eugenio Hospital, Rome, Italy; [2]Internal Medicine Department, Tor Vergata University, Rome, Italy; [3]Nephrology and Dialysis Unit, S. Maria della Misericordia Hospital, Rovigo, Italy.*

Background: To investigate the relationship between renal dimensions on US, i.e. renal parenchymal thickness (PT) and maximum interpolar diameter (MD) and GFR in chronic kidney disease.

Methods: Four-hundred seventy–one Caucasian subjects (235 males and 236 females, aged 60.54±9.80 years) with no history of nephropathy were investigated. An Esaote MyLab 70 X-vision gold (Esaote spa, Genoa, Italy) US system with a multi-frequence 2,5-5MHz curved-array transducer was used. Serum creatinine was dosed 30 days before and after US, with no variation between the two determinations. GFR was calculated in all subjects by Cockroft-Gault (CG), MDRD and CKD-EPI formulas, separately. According to GFR subjects were stratified into 1). controls (GFR>90ml/min/m^2, 111 subjects); 2). CKD St. II (GFR 60-90ml/min/m^2, 180 patients); 3). CKD St. III (GFR <60ml/min/m^2, 180 patients).

Results: There was no relationship between serum creatinine and renal MD or PT either in controls and in CKD patients. Conversely, there was a significant difference in renal PT among controls compared to CKD St. II and III patients ($p<0.0001$). Also, there was a significant difference in renal PT among CKD ST. II patients compared to CKD St. III (16,53±1,28 vs. 15,23±1,23mm, $p<0,0001$). A renal PT ≤15mm is always significantly related to a reduced GFR regardless the formula employed.

Conclusions: Our results suggest that renal PT rather than MD may be related to GFR in CKD patients.

PUB039

Impact of High-Fructose Feeding on Pancreatic Islets Insulin Secretion in Experimental Model of Chronic Kidney Disease (CKD) in Rats Marta Pokrywczynska,[1,] Arkadiusz Jundzill,[1,5] Mariusz Flisinski,[4] Andrzej Brymora,[4] Aleksander Deptula,[2] Magdalena Bodnar,[3] Tomasz Kloskowski,[1] Sandra Krzyzanowska,[1] Pawel Strozecki,[4] Andrzej Marszalek,[3] Eugenia Gospodarek,[2] Tomasz Drewa,[1] Jacek Manitius.[4] *[1]Tissue Engineering Department, Nicolaus Copernicus University, Bydgoszcz, Poland; [2]Microbiology, Nicolaus Copernicus University, Bydgoszcz, Poland; [3]Clinical Pathomorphology, Nicolaus Copernicus University, Bydgoszcz, Poland; [4]Nephrology, Hypertension and Internal Diseases, Nicolaus Copernicus University, Bydgoszcz, Poland; [5]General, Vascular and Endocrinology Surgery, Nicolaus Copernicus University, Bygoszcz, Poland.*

Background: The aim of the study was to evaluate the impact of fructose intake on insulin islets *in vitro* secretion in rats with CKD

Methods: Rats were divided into groups (each n=12): CON was sham-operated, CKD½ - uninephrectomy; CKD5/6 uninephrectomy + 1/3 of contraletral kidney cortex mass resection. Animals from each groups were further assigned to 3 different diets protocol: regular diet (RD), RD with 10% solution of fructose in drinking water - F10, 60% fructose diet – F60. After 8 weeks glucose, insulin and creatinine were measured. The pancreas islets were evaluated in *in vitro* static glucose insulin stimulation test (IST). Pancreatic tissue was stained for collagen formation.

Results: Glucose did not differ significantly between groups. Serum insulin and IST test are presented in table 1.

	CON	CKD1/2	CKD5/6	P
Diet	Insulin[ng/ml]	Insulin[ng/ml]	Insulin[ng/ml]	
RD	4,07±1,86	4,02±1,99	4,81±1,83	NS
F10	4,61±1,5	6,0±1,73	6,89±1,15	NS
F60	5,59±0,75	5,03±2,05	3,48±2,01	< 0,05
P	NS	NS	< 0,01	
	IST	IST	IST	
RD	0,63±0,33	0,19±0,07	3,89±4,73	NS
F10	0,46±0,17	1,80±2,11	4,76±3,15	<0,05
F60	2,24±3,08	1,46±1,78	0,98±1,18	NS
P	NS	NS	NS	

Mean±SD

These results confirmed that 10% fructose could stimulate while 60% fructose down-regulate pancreas islet insulin *in vitro* secretion in CKD rats. Extensive collagen formation was observed in CKD 5/6 and F60 group.

Conclusions: Our results suggest that overfiding with fructose may tremendous influence on insulin in vitro secretion in very advanced CKD but clinical meaning of this finding need further study.

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PUB040

Abstract Withdrawn

PUB041

HIV Enhances Tubular Cell Angiotensinogen Expression and Angiotensin II Production through Phosphorylation of p66ShcA Divya Salhan,[1] Himanshu Vashistha,[2] Shabina Rehman,[1] Mohammad Husain,[1] Ashwani Malhotra,[1] Leonard G. Meggs,[2] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Medicine, Ochsner Clinic, New Orleans, LA.*

Background: Renin angiotensin system (RAS) has been demonstrated to play an important role in the development of HIV-associated nephropathy (HIVAN). Moreover, modalities which either inhibit the production or block the effect of Ang II have been demonstrated to retard the progresson of HIVAN. We recently demonstrated that HIV also enhances phosphorylation of p66ShcA in kidney cells (J BC 284:16648-58, 2009). We hypothesized that HIV would enhance renal cell angiotensin (Ang) II production through phsophorylation of p66ShcA.

Methods: To have an in vitro model of tubular cell HIV infection, human tubular cells (HK2) were transduced with either vector only or HIV (NL4-3) constructs. Vector/HK2s and HIV/HK2s were incubated in serum-free media (SFM) for 24 hours and lysates were prepared for protein electrophoresis. Western studies were conducted for protein expression for phospho-p66ShcA, total p66ShcA, and angiotensinogen (Agt). Ang II ELISA was carried out on cells prepared under similar conditions. To establish a causal relationship between Agt and p66ShcA, EV/HK2s and HIV/HK2s were transduced with mu36-p66ShcA and then evaluated for Agt expression. Similarly, p66ShcA silenced tubualr cells (siRNA-p66ShcA/HK2s) were assayed for Agt expression.

Results: HIV/HK2s displayed 2.5 fold increased ($P<0.01$) expression of Agt when compared with EV/HK2s. Similarly, HIV/HK2 showed 3-fold increased ($P<0.01$) Ang II production when compared to EV/HK2s. HIV/HK2s also displayed increased ($P<0.01$) expression of phospho-p66ShcA vs EV/HK2s. On the other hand, mu36-p66ShcA transduced HK2s showed diminished expression of Agt. Similarly, si-RNA-p66ShcA/HK2 showed diminished Agt expression.

Conclusions: These findings indicated HIV-induced phosphorylation of p66ShcA promotes RAS activation in tubular cells. The present study provides an insight into the RAS activation in HIVAN patients.

Funding: NIDDK Support

PUB042

HIV-Associated Nephropathy: Role of Angiotensin Type 2 Receptor (AT_2R) Divya Salhan,[1] Rungwasee Rattanavich,[1] Subani Maheshwari,[1] Madhuri Adabala,[1] Mohammad Husain,[1] Ashwani Malhotra,[1] Guohua Ding,[2] Praveen N. Chander,[3] Pravin C. Singhal.[1] *[1]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; [2]Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [3]Pathology, New York Medical College, Valhalla, NY.*

Background: Activation of AT_1R and AT_2R contributes to opposite outcomes in several kidney disease models. AT_1R has been demonstrated to play a role in the progression of HIV-associated nephropathy (HIVAN). We evaluated the role of AT_2R in a mouse model of HIVAN (Tg26).

Methods: Age and sex matched control (FVB/N) and Tg26 mice aged 4, 8, and 16 weeks (n=4) were studied for renal tissue expression of AT1R and AT2R (Protocol A). In protocol B, 4 weeks old Tg26 mice were treated with either saline, telmisartan (TEL, TAT1 blocker), PD123319 (PD, AT_2R blocker), or TEL + PD for two weeks. Renal tissues and cells were evaluated or renal molecular and biomarkers.

Results: Renal tissue expression of AT2R was lower in Tg26 mice when compared with control mice. Eight weeks old Tg26 mice displayed HIVAN phenotype in the form glomerulosclerosis and formation of microcysts. TEL-receiving Tg26 (TRTg) displayed less advanced glomerular and tubular lesions when compared with saline-receiving Tg26 (SRTg). Although renal tissues of SRTgs displayed diminished expression of both AT_1R and

AT_2R when compared with saline-receiving FVB/N mice (SRF), but diminution in AT_2R expression was more pronounced. On the other hand, TRTgs displayed enhanced renal tissue AT_2R expression. Diminution of renal tissue AT_2R expression was associated with advanced renal lesions in SRTgs; whereas, upregulation of AT_2R expression in TRTgs was associated with attenuated renal lesions. PD-receiving Tg 26 mice (PDRTg) did not show any alteration in the course of HIVAN; whereas, PD + TEL-receiving Tg26 (PD-TRTg) showed worsening of renal lesions when compared with TRTgs. Interestingly, plasma as well as renal tissues of Tg26 mice displayed enhanced expression of Ang III, a metabolite of Ang II and a ligand of AT_2R.

Conclusions: These findings suggest that in TRTgs, besides AT_1R blockade, enhanced renal tissue AT_2R expression might have contributed to slowed progression of HIVAN.

Funding: NIDDK Support

PUB043

Novel Experimental Model of CKD with Uremia in Mice: Involvement of Inflammatory Mechanisms

Alexandre Santana,[1] Humberto Dellê,[1] Cleonice Silva,[1] Sergio Catanozi,[2] Sabrina Degaspari,[3] Cristoforo Scavone,[3] Paula Blanco,[4] Irene L. Noronha,[1] Kim Solez.[4] *[1]Nephrology, Univ Sao Paulo, Brazil; [2]Lipids, Univ Sao Paulo, Brazil; [3]Pharmacology, Univ Sao Paulo, Brazil; [4]Pathology, Univ Alberta, Canada.*

Background: Dietary adenine leads to the accumulation of 2.8-dihydroxyadenine in the kidney and causes progressive renal dysfunction that resembles stage CKD with uremic manifestations. Here, we developed this model of CKD in mice and analyzed the role of inflammatory mechanisms using thalidomide (Thalid) as an anti-inflammatory drug.

Methods: CKD was induced in C57/BL-6 mice (n=48) by adenine-containing diet for 6 weeks. Mice were divided into 3 groups: **Control**, receiving normal diet, **Adenine**, receiving adenine to develop CKD, and **Adenine+Thalid**, Adenine mice treated with Thalid. The following parameters were analyzed: biochemical, histological, and IL-1β, TNF-α, IL-6 serum and kidney mRNA levels (real time PCR).

Results: Adenine-fed mice developed CKD and resulted in significantly higher serum urea and creatinine levels compared with controls. Histological analyses showed dilated tubules, loss of tubular epithelial cells, crystalline deposits in tubular lumens, cortical scarring, and macrophage infiltration ($Mac\text{-}2^+$) in the renal tissue of Adenine mice. A significant increase of serum and relative mRNA levels of IL-1β, TNF-α and IL-6 was observed in mice with CKD. Thalidomide treatment significantly reduced all these parameters.

Parameters	Control	Adenine	Adenine+Thalid
Urea(mg/dl)	46.0±3.3	287±10.2#	102±3.9#*
Creatinine(mg/dl)	0.34±0.0	0.87±0.03#	0.50±0.03#*
Serum IL-1β(pg/ml)	13.2±5.1	57.8±23.5#	7.2±3.0#*
Serum TNF-α(pg/ml)	0.8±0.1	21.9±8.0#	1.3±0.5#*
Serum IL-6(pg/ml)	14.0±4.3	145.8±19.7#	4.8±2.7#*
$Mac\text{-}2^+$(cells/mm^2)	1.28±0.4	228.1±5.2#	104.7±6.4#*
IL-1β(mRNA level)	1.0±0.2	16.4±0.7#	5.0±0.7#*
TNF-α(mRNA level)	1.0±0.3	24.2±0.8#	15.8±0.3#*
IL-6(mRNA level)	1.0±0.7	24.7±1.0#	9.0±0.5#*

#p<0.05 vs Control; *p<0.05 vs Adenine

Conclusions: Dietary adenine caused advanced CKD in mice, providing a useful experimental model to study changes associated with uremia. Although crystalline precipitates in tubular lumens are a major change observed in this model, inflammatory mediated mechanisms play an important role.

Funding: Government Support - Non-U.S.

PUB044

Intra-Renal Angiotensin System Activation, Oxidative Stress, Inflammation and Impaired Nrf2 Activity in the Progression of Focal Glomerulosclerosis

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Background: The Imai rat is a model of spontaneous focal glomerulosclerosis (FGS) which leads to heavy proteinuria, hyperlipidemia and progressive renal failure. Treatment with AT-1 blockers (ARB) ameliorates proteinuria, hyperlipidemia, and nephropathy in this model. We hypothesized that progressive nephropathy in the Imai rat is accompanied by oxidative stress, inflammation and impaired activity of the nuclear factor-erythroid-2-related factor 2 (Nrf2), the master regulator of genes encoding antioxidant molecules activation and that amelioration of nephropathy with AT1 receptor blockade in this model may be associated with the reversal of these abnormalities.

Methods: Ten week-old Imai rats were randomized to the ARB-treated (olmesartan, 10 mg/kg/day) or vehicle-treated groups. Sprague-Dawley rats served as controls.

Results: At 34 weeks of age Imai rats showed heavy proteinuria, glomerulosclerosis and tubulointerstitial inflammation, increased number of angiotensin II expressing cells and AT1 and AT2 receptor expression, up regulation of NAD(P)H oxidase and inflammatory mediators, activation of nuclear factor kappa B (NF-kB) and reduction of Nrf2 activity and expression of its downstream gene products in the renal cortex. ARB therapy prevented nephropathy, suppressed oxidative stress and inflammation and restored Nrf2 activation and expression of the antioxidant enzymes.

Conclusions: These abnormalities are accompanied by activation of intra-renal angiotensin system and can be prevented by ARB administration.

Funding: Private Foundation Support

PUB045

Is Decrease Rate in ANCA Titer a Predictor of the Inopportunity Infection?

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Background: Without therpy renal vasuculitis in ANCA vasculitides will usually progress end-stage renal disease. The toxicity of high corticosteroid doses and immunosuppressive agent has been accepted to achieve this aim. Consequently elderly patients have a higher rate of severe infection and infective death. We found that some patients who had immunosuppresion treatment occurred infection disease, their ANCA titer was rapidly decrease .we hypothesized that ANCA titer is used to the incident of infectious disease.

Methods: We evaluated 10 (male:11 female:9 mean age:73.8) MPO-ANCA-associated vasculitis presenting with rapidly progressive glomerulonephritis undergoing steroid and immunosuppressive agent therapy in 2008-2010. We measured ANCA titers before treatment and after 2 months later.

We calculated the ANCA decrease rate. We defined rapidly decrease ANCA titers group (RD-ANCA) that more than 90% reduction in ANCA titers between before and after 2 months. And we examined in RD-ANCA group (n= 10) and non-RD group (n=10) about Creatinine, hemogulobin,infectous disease event and death.

Results: RD group are no difference in age, ANCA titer. albumin,and Hb. However significant difference in Cr (4.25mg/dl vs 2.38mg/dal, p =0.005) between RD group and NRD group. 3 patients had pnumonia due to cytomegalovirus and 2 patients died. They were all RD group.

Conclusions: In our study, Rapidly decrease ANCA titer might be related to opportunity infection disease. Their renal function were decreased before immunosuppuresive therapy. We suggest that ANCA titer is not only RPGN acitivity but also immune compromised condition.

PUB046

Inhibition of Obesity-Related Lomerulopathy by Grape Seed Roanthocyanidin Extract

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Background: Obesity-related glomerulopathy (ORG) is increasingly a cause of end-stage renal disease, and there is no effective treatment. It has been reported that oxidative stress may play a primary role in the pathogenesis of ORG. and grape seed proanthocyanidin extract (GSPE), an antioxidant agent, provides superior antioxidant efficacy. However, the therapeutic effect of GSPE on ORG has not yet been investigated.

Methods: In this study, we examined the effect of GSPE on ORG and explored mechanisms involved in a rat model of ORG. A rat model of ORG was established by feeding adult Sprague-Dawley (SD) rats with high-fat diet for 24 weeks. GSPE (200mg/kg) was then given daily for 12 weeks. Serum biochemical and oxidation indexes, urinary protein, renal pathology, and expression of UCP2, a candidate gene of obesity, were assessed.

Results: Our results showed that the body weight, body mass index, and 24-hour urinary protein were increased in rats with ORG. Administration of GSPE significantly reduced those parameters. Rats with ORG had increased serum cholesterol and low-density lipoprotein cholesterol (LDL-C) and decreased HDL-C. GSPE treatment reversed these responses. Compared with normal control group, there were increased serum levels of oxidant MD and decreased antioxidant enzyme, GSH-Px, in ORG group. GSPE treatment resulted in an increase in GSH-Px and decrease in serum oxidant malondialdehyde. Pathological analysis showed obesity-associated glomerulomegaly and increased mesangial matrix and glomerular sclerosis in rats with ORG. All those pathological changes were attenuated in animal subjected to GSPE treatment. Further, renal gene expression of mitochondrial uncoupling protein-2 (UCP2) was increased in rats with ORG and declined after GESP treatment.

Conclusions: These data indicate that development of ORG is associated with increased oxidative stress. Antioxidant GSPE supplementation can prevent obesity, ameliorate renal damage and reduce proteinuria. Thus, GSPE holds a therapeutic potential for treatment of ORG.

Funding: Government Support - Non-U.S.

PUB047

Erythropoietin Inhibited Complement 3-Mediated Renal Tubular Epithelial to Mesenchymal Transition

Jian-Xin Wan, Feng-Xia Zhang. *Nephrology, First Affiliated Hospital of Fujian Medical University, Fuzhou, China.*

Background: Our previous research had suggested that complement 3 (C3) involved in renal tubular epithelial to mesenchymal transition (EMT). Sun showed erythropoietin (EPO) could decrease renal fibrosis in mice with ureteral obstruction through inhibiting TGF-β induced EMT. This study should investigate EPO inhibit C3-mediatiated renal tubular EMT.

Methods: HK-2 cells were cultured respectively as follows: Control group, 10U/ml EPO group, 3ng/ml TGF-β1 group, 3ng/ml TGF-β1 + 10U/ml EPO group, 0.1μM C3a group and 10U/ml EPO + 0.1μM C3a group. Expressions of α-SMA, E-cadherin and C3 mRNA and protein of HK-2 cells was respectively detected by RT-PCR, western blot and cell immunofluorescence. And then model of UUO rats was established. Experimental animals were randomly divided into four groups: Control group (sham operation group), UUO group, UUO rats treated with low-dosage EPO (100U/Kg EPO) and with high-dosage EPO (1000U/Kg EPO). EPO was injected in intraperitoneal every other day from 3th to 14th day after estibilished UUO. All experimental rats were executed after anesthesia in

the 14th day after estibilished UUO. Morphological changes of renal tissue by HE stain, collagen deposition by Masson trichrome stain, expressions of α-SMA, E-cadherin and C3 by immunohistochemical stain were observed.

Results: The expression of α-SMA mRNA and protein had increased, the expression of E-cadherin mRNA and protein had decreased, the expression of complement 3 had increased after HK-2 cells stimulating with C3a or TGFβ1. After HK-2 cells stimulating with C3a or TGFβ1 combining with EPO, the expressions of α-SMA, E-cadherin and C3 mRNA and protein had occurred conspicuous changing (P<0.05). The expressions of α-SMA of UUO rats was significantly increased, and the expression of E-cadherin of UUO rats was significantly reduced. Simultaneously expressions of C3 of UUO rats was significantly reduced. After UUO rats treated with EPO, the expressions of α-SMA was significantly reduced, and the expression of E-cadherin was significantly increased. and the expressions of C3 was significantly increased.

Conclusions: EPO can inhibit C3-mediated renal tubular epithelial to mesenchymal transition.

PUB048

Generation of a Renal Fibroblast Cell Line To Study Modulation of Mineralocorticoid Receptor Hong Wang, Cheng-Kon Shih, Steven S. Pullen. *Cardiometabolic Diseases Research, Boehringer Ingelheim Parmaceuticals, Ridegfield, CT.*

Background: The renal protection provided by mineralocorticoid receptor (MR) antagonists has been ascribed to the blockage pro-inflammatory and pro-fibrotic genes in non-epithelial cells in the kidney. The NRK49F rat cell line is derived from kidney tubular fibroblasts and thus represents a renal non-epithelial cell type which is potentially suitable for in vitro studies. However, these cells were not responsive to aldosterone at physiological concentrations in preliminary studies, suggesting a lack of MR expression which was confirmed by RT-PCR quantification.

Methods: Cloned NRK49F cell lines stably expressing rat MR were generated for cellular studies. Expression was confirmed by RT-PCR quantification. A panel of genes previously described to be regulated by MR including PAI-1, Col1a1, Col4a1, OPN, and ORM-1 were quantified to characterize the response of the stable cell lines to aldosterone. Subsequently, the effect of the MR antagonist Spironolactore on the response of the cells was determined.

Results: Of the genes studied, ORM-1 was the most highly regulated in response to aldosterone. Using cell lines that express different levels of MR, it was demonstrated that the level of ORM-1 induction by aldosterone correlated with MR expression. Aldosterone induced ORM-1 expression was demonstrated to be inhibited by spironolactone in a dose responsive manner.

Conclusions: An aldosterone responsive, stably transfected rat kidney fibroblast cell line was generated and characterized. This cell line will be useful for further studies to investigate the effect of MR antagonism in fibroblast cells.

Funding: Pharmaceutical Company Support

PUB049

Prolonged Hypertension and Renal Impairment in Old Age of Neonatally Overfed Rats Hyung Eun Yim, Kee Hwan Yoo, Seong Woo Nam, In Sun Bae, Joo Won Lee. *Pediatrics, Korea University Medical Center, Seoul, Republic of Korea.*

Background: Neonatal growth plays a key role in "developmentally programmed" adult diseases. The objective was to evaluate the long-term influence of early postnatal overnutrition on the renal pathophysiological changes in aging rats.

Methods: Three or 10 male pups per mother were assigned to either the small litter (SL) or normal litter (NL) control groups during the first 21 days of life. The effects of early postnatal nutrition excess on body weight, blood pressure, blood glucose, and renal changes were determined at 12 months.

Results: Pups in the SL group weighed more than controls between 4 days and 6 months of age ($P < 0.05$). However, there was no difference of body weights between the two groups at 12 months. In the SL group, at 12 months of age, systolic blood pressure levels were higher than those of the controls ($P < 0.05$). The numbers of ED-1 positive macrophages and cortical apoptotic cells increased in the SL group ($P < 0.05$). The index scores for glomerulosclerosis and tubulointerstitial fibrosis were also increased in the SL group ($P < 0.05$). Immunoblotting and immunohistochemistry showed that intra-renal renin expression was decreased in the SL group ($P < 0.05$). However, angiotensin II type 1 receptor (AT1), AT2, matrix metalloproteinase-9, plasminogen activator inhibitor-1, tumor necrosis factor-α, and osteopontin expressions were unaffected in the SL group.

Conclusions: Our data suggests that early postnatal overfeeding increases systolic blood pressure, cortical macrophage infiltration and apoptosis, glomerulosclerosis, and tubulointerstitial fibrosis and suppresses intra-renal renin in aging male rats. Early postnatal overnutrition even following an appropriate birth weight can have long-term renal sequelae in late adulthood.

PUB050

Cell Surface Biliverdin Reductase Help To Sustain E-Cadherin Expression Level in Proximal Renal Tubular Cell Rui Zeng, Guangchang Pei, Qiaodan Zhou, Min Han, Gang Xu. *Division of Nephrology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College,HUST, Wuhan, Hubei, China.*

Background: Biliverdin reductase(BVR) was solely considered as a reductase that catalyzes biliverdin to bilirudin. Recent studies reveal that BVR functions as a serine/threonine/tyrosine kinase, a transcription factor, or an antioxidant. BVR can be induced by reactive oxygen species(ROS) and was identified to have PKB/Akt-like activity.

Methods: We detected expression of BVR,HIF1α,E-cadherin,CD34,P-AKT,Snail expression in a 5/6 nephrectomized rats model and renal biopsy specimens from patients with sclerosing glomerulonephritis or obstructive nephropathy. Nomal kidney tiusse and uninjured areas of pathologic specimens were used as control. We also examined the BVR expression in HKC and NRK52E cell in normoxic and hypoxia conditions.

Results: In normoxic renal sections of rats or human beings, BVR expressed cell surface of tight junction and basolateral membrane of proximal renal tubular cell, the expression of HIF1α,P-AKT,Snail tubules in normal rats was bare. In fibrotic kidney from either rats or human beings, along with a discrease of CD34 and E-cadherin and an increase of HIF1α, P-AKT,Snail, BVR is located in cytoplasm and nuclear in the renal epithelia, and the distribution patterns of HIF1α and BVR were highly congruent in the renal tubules on serial sections. The phenomenon of BVR transferred from cell surface to cytoplasm and nuclear was also confirmed in cell line. In hypoxia conditions, BVR which transferred into nuclear activated AKT and Snail,then reduced the expression of E-cadherin.When BVR in cytoplasm and nuclear was downreglated by SiRNA, E-cadherin restored to a level near to the normoxic condition.

Conclusions: Our findings suggest that BVR on cell surface play a role in sustaining E-cadherin expression level in proximal renal tubular cell.

PUB051

Conditional Knockout of E-Cadherin in Renal Proximal Tubular Epithelial Cells Aggravates Kidney Fibrosis in Murine Model of Unilateral Ureteral Obstruction (UUO) Guoping Zheng,[1] So Ra Lee,[1] Jianlin Zhang,[1] Tim Tzu-Ting Hsu,[1] Thian Kui Tan,[1] Ye Zhao,[1] David A.F. Loebel,[2] Isabelle Rubera,[3] Michel Tauc,[3] Ya Wang,[1] Qi Cao,[1] Yiping Wang,[1] Patrick P.l. Tam,[2] David C. Harris.[1] *[1]Centre for Transplantation and Renal Research, Westmead Millennium Institute, University of Sydney, Australia; [2]Children's Medical Research Institute, Australia; [3]University of Nice-Sophia Antipolis, France.*

Background: Epithelial-mesenchymal transition (EMT) has been proven to play an important role in kidney fibrosis. E-cadherin was found not only to be a cell adhesion molecule, but may also be involved actively in signal transduction in EMT. Our previous study showed that disruption of E-cadherin by MMPs caused EMT where E-cadherin passes on extra-cellular signals to nucleus through β-catenin and slug in murine models of kidney fibrosis. This study is to examine the critical role of E-cadherin in renal tubular EMT using a novel conditional knockout of E-cadherin in mouse proximal tubular epithelial cells (PTEC).

Methods: Conditional knockout of E-cadherin in PTEC was generated by crossing cdh1flox/flox mice sequentially with CMV Cre, Sglt2Cre mice and then backcrossed to cdh1flox/flox mice. UUO was performed to the knockout and control mice. Primary culture of PTEC from knockout mice was treated with TGF-β1 (3ng/ml) to examine the role of E-cadherin in EMT in vitro.

Results: Depletion of E-cadherin in PTEC did not result in histological changes to proximal tubules under light-microscope. However, an upregulation of α3 integrin was found in PTEC.There were no significant changes to other adhesion molecules such as ZO-1. Kidney fibrosis after UUO was worse in conditional knockout mice compared to control mice demonstrated by Gomori Trichrome and Sirius red staining, and by immunohistological staining of α-SMA. EMT was significantly up-regulated in proximal tubular cells from E-cadherin conditional knockout mice compared to control.

Conclusions: Conditional depletion of E-cadherin in proximal tubule does not directly cause kidney fibrosis, but aggravates kidney fibrosis in mouse UUO model, likely through increased level of α3 integrin and EMT in PTEC upon stimulation.

Funding: Government Support - Non-U.S.

PUB052

Uric Acid Induces Renal Inflammation Via Activating Tubular NF-kB Signaling Pathway Yang Zhou, Chunsun Dai, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Renal Inflammation is a pathologic feature of hyperuricemia in clinical settings. However, the underlying mechanisms remain unknown.

Results: Here, we found a significantly increased T cells and macrophages infiltration in kidneys from mice with hyperuricemia. This increased inflammatory cell infiltration was accompanied by upregulated kidney TNF-a, MCP-1 and RANTES expression. Immunohistochemical staining showed that the induction of RANTES was primarily localized to tubular epithelial cells, suggesting that tubular cells may play a role in uric acid-induced renal inflammation. Evidences of uric acid-induced NF-kB signaling activation were obtained *in vivo*. In cultured rat renal tubular epithelial cells (NRK-52E), uric acid treatment could induce TNF-a, MCP-1 and RANTES mRNA as well as RANTES protein

expression. Moreover, uric acid-induced TNF-a, MCP-1 and RANTES expression were largely abolished by a specific NF-kB signaling inhibitor, SN 50.

Conclusions: Taken together, these results suggest that tubular cell NF-kB signaling activation may play an important role in uric acid-induced renal inflammation.

Funding: Government Support - Non-U.S.

PUB053

Diabetes and High Protein Concentrations Alter Nrf2 Phosphorylation in Renal Tubule Cells Michelle T. Barati, Susan M. Isaacs, Jon B. Klein. *Nephrology, University of Louisville, KY.*

Background: Renal tubule cell exposure to high glucose and protein concentrations in diabetes increases formation of reactive oxygen species (ROS). A cellular mechanism to alleviate oxidative stress is activation of the transcription factor, nuclear factor erythroid-derived 2-related factor 2 (Nrf2). Nrf2 expression increases in glomeruli of diabetic patients and diabetic rat kidneys, suggesting a compensatory response to oxidative stress, yet the effect of diabetes on Nrf2 in tubules is not clear. The goal of this study was to define Nrf2 regulation in cortical renal tubules of diabetic mice and proximal tubule cells exposed to high protein concentrations.

Methods: Cortical tubules were isolated from 3 and 7 month old OVE26 diabetic and FVB non-diabetic mice and Nrf2 expression analyzed by immunoblotting (IB). Phospho-Ser40 Nrf2 (P-Nrf2) was analyzed by immunohistochemistry of mouse kidney sections with image analysis of cortical regions. Serine 40 Nrf2 phosphorylation occurs during its activation and nuclear import. For *in vitro* studies, proximal tubules cells (HK2) were treated with 1mg/ml human serum albumin (HSA) for various time points and cell extracts fractionated to determine total and P-Nrf2 in the cytosol and nuclei by IB.

Results: Total Nrf2 expression was not different between isolated tubules of 3 and 7 month old diabetic and non-diabetic mice. Nuclear P-Nrf2 was decreased in cortical tubules of 7 month old mice compared to 3 month old mice within the same group. Furthermore, cortical tubules of 7 month old diabetic mice had less nuclear P-Nrf2 compared to age-matched non-diabetic mice. HSA increased P-Nrf2 and Nrf2 nuclear import in HK2 cells, suggesting activation.

Conclusions: Together, decreased P-Nrf2 in cortical tubules of older mice with unaltered Nrf2 protein expression suggests dysregulation of this compensatory mechanism with age. Additional attenuation of this system in the older diabetic mice may be a mechanism to exacerbate oxidative stress. The ability of HK2 cells to activate Nrf2 following HSA exposure alone, suggests that additional factors in the diabetic milieu may be responsible for decreased nuclear Nrf2 localization in tubules of older diabetic mice.

Funding: NIDDK Support

PUB054

Diabetic Nephropathy in FVB/NJ Akita Mice: Temporal Pattern of Kidney Injury and Urinary Nephrin Excretion Jae-Hyung Chang,[1] Susan B. Gurley,[2] Robert F. Spurney.[2] *[1]Duke University, Durham, NC; [2]Duke University and Durham VAMC, Durham, NC.*

Background: Glomerular podocytes are terminally differentiated cells with little potential for proliferation. As a result, a sufficient loss of podocytes may lead to instability of the glomerular tuft and glomerulosclerosis. Increasing evidence suggests that a decrease in the number of glomerular podocytes is a characteristic feature of both animals and humans with diabetic kidney disease. Early detection of podocyte injury before podocyte loss might permit intensification of medical therapies to delay or prevent diabetic nephropathy (DN). In this regard, the podocyte protein nephrin can be detected in urine of both animals and patients with diabetes mellitus, perhaps reflecting early podocyte damage. In the present study, we investigated the relationship between hyperglycemia, podocyte number and apoptosis, albuminuria and urinary nephrin excretion during the course of renal disease in a genetic model of type 1 diabetes (FVB/NJ Akita mice).

Methods: Akita mice have phenotypic characteristics useful for studying the mechanisms of DN in humans including sustained hyperglycemia, progressive albuminuria and mesangial matrix expansion. The goal of this study was to examine the role of urinary nephrin excretion as an early marker of diabetic kidney injury.

Results: In 4-week old male Akita mice, the onset of hyperglycemia was accompanied by increased podocyte apoptosis and enhanced excretion of nephrin in urine before the development of albuminuria. After 4 weeks of age, Akita mice developed albuminuria, which increased progressively over time. By 20 weeks of age, Akita mice developed a 10-fold increase in albuminuria and a decrease in the number of glomerular podocytes. Urinary nephrin excretion was also significantly increased at 16 and 20 weeks of age and correlated with the urinary albumin excretion rate.

Conclusions: In summary, enhanced urinary nephrin excretion was associated with podocyte apoptosis in normoalbuminuric 4-week old diabetic mice as well as increased albuminuria in older diabetic animals. These data suggest that urinary nephrin excretion may be a useful marker of early glomerular injury in diabetes mellitus.

Funding: NIDDK Support

PUB055

Astragalus Modulates Advanced Glycation End Products-Induced Microinflammation of Macrophage Yong Gu,[1,2] Qiaojing Qin,[1] Jianying Niu.[1] *[1]Division of Nephrology, Fifth People's Hospital of Shanghai, Fudan University, Shanghai, China; [2]Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China.*

Background: Microinflammation induced by advanced glycation end products (AGEs)-activated macrophages is a cardinal mechanism in diabetic nephropathy(DN). Adverse effects from immunosuppressant limit immunosuppressive therapy apply for microflammation in DN. Astragalus is an herb and possess bidirectional immunological regulation properties in many diseases. So we evaluate the effects and mechanism of astagalus on macrophage secretion of the inflammatory cytokines, IL-1β and TNF-α, in AGEs and normal vitro environment to determine the value of astragalus treatment in DN.

Methods: The viability of Ana-1 murine macrophages on treatment with various concentrations of astragalus or AGEs was evaluated by MTT method. The cells were treated with astragalus, and/or AGEs, and receptor for advanced glycation end products (RAGE) antibody, and scavenger receptor A (SR-A) antibody. The cells were pretreated with RAGE or SR-A antibody for 1h before astragalus addition and were pretreated with astragalus for 1h before AGEs addition. The protein secretion of TNF-α and IL-1β was measured by ELISA and mRNA expression by RT-PCR. The activity of nuclear factor (NF)-κB was assayed using EMSA. The expression of Phospho-P38 mitogen-activated protein kinase (MAPK) was assessed by western blotting.

Results: We not found astragalus cytotoxicity in macrophage. Astragalus increased the protein secretion and mRNA expression of IL-1β and TNF-α and suppressed the AGEs-stimulated expression of IL-1β and TNF-α in macrophage. All the effects were seemed to occur via activation or inhibition NF-κB and Phospho-P38 MAPK pathway. Additionally, RAGE or SR-A antibody did not affect the secretion of IL-1β and TNF-α induced by astragalus in macrophage significantly.

Conclusions: Base on these data, we suggested that astragalus may decrease AGEs-stimulated microinflammation in macrophage while maintaining macrophage benefit of body protection. Astragalus may be efficacious and promising remedy in the microinflammation treatment of DN. The effects of astragalus on macrophage may associate multi-targets but the special receptor.

PUB056

Chondrogenic Phenotypic Change Contribute to the Irreversible Progression of the Diabetic Nephropathy Seiji Kishi, Hideharu Abe, Tatsuya Tominaga, Kojiro Nagai, Toshio Doi. *Nephrology, Univ of Tokushima, Tokushima, Japan.*

Background: We reported that BMP4 -Smad1 signaling pathway played a critical role for mesangial expansion and phenotypic alteration in diabetic nephropathy (DN) and Sox9 was also involved in the glomerulosclerosis. We hypothesized that MCs acquire a chondrocyte-like phenotype which was mediated by BMP signaling pathway and SOX9 during glomerulosclerosis progression.

Methods: Cell Culture, Alcian blue staining or immunocytochemistry, Transient transfection of MCs, Western blotting, Histological Examination, Animals; #transgenic mouse expressing inducible nitric oxide synthase under control of insulin promoter (iNOS Tgm) #BMP4 knock-in transgenic mice(BMP4 Tgm)

Results: MCs showed chondrogenic potential in a micromass culture and BMP4 induced the expressions of chondrocyte markers (SOX9 and COL2) in MCs.

AGEs induced the expression of chondrocyte markers downstream of BMP4-Smad1 signaling pathway in MCs. In addition, hypoxia also induced the expression of BMP4, HIF1α, and chondrocyte markers. Overexpression of SOX9 caused ectopic expression of proteoglycans and COL2 in MCs.Furthermore, overexpression of Smad1 induced expressions of chondrocyte markers. Dorsomorphin inhibited these inductions.

By using iNOS Tgm which exhibited severe DN, glomerular expressions of HIF1α,BMP4, and chondrocyte markers were observed.SOX9 was partially colocalized with HIF-1 and BMP4.

BMP4 Tgm showed not only similar pathological lesions to DN, but also the induction of chondrocyte markers in the sclerotic lesions.

Conclusions: Chondrogenic potential of MCs provide new aspects in the progression of glomerular injury. BMP4-Smad1 signaling and SOX9 are candidate regulators of phenotypic change in DN. Chondrocytes are the only cells found in cartilage, an avascular and hypoxic mesenchymal tissue. Therefore, it makes sense that this transdifferentiation of MCs is an adaptation to chronic pathological hypoxia but cartilage related ECM production caused irreversible structural change. HIF-1, which are known to be upstream molecules of SOX9, also have important an influence on the phenotypic change of MC in DN.

Funding: Government Support - Non-U.S.

PUB057

Reduced Beta Cell Mass and Function by High Glucose Feeding in Rats Anil K. Mandal,[5812] Linda M. Hiebert.[0980] *[1]Dept of Medicine, Univ of Florida, Gainesville, FL; [2]Veterinary Biomedical Science, Univ of Saskatchewan, Saskatoon, SK, Canada.*

Background: People with high normal blood glucose double the risk of diabetes (DM). We asked; does overeating reduce beta cell (BC) mass or function, causing DM? We designed an experiment to answer the question.

Methods: Wistar rats 250 g treated with 25% glucose (G) in water for 2 weeks (wk.) (G), then tap water (W) for 2 wk. (GW), G and N insulin (I) 2-4 units s.c. daily for 2 wk. (GI), then W for 2 wk. (GIW). Control given W for 2 and 4 wk. (C). Samples for G and creatinine (cr) drawn prior to treating, and at 2 wk. for groups GW and GIW. Samples for G, cr and I drawn from abdominal aorta at end of experiment (2 wk. for C, G, GI and 4 wk. for C, GW and GIW). Islet tissue extracted from pancreas and fixed for immunohistochemical staining (IHC). IHC staining run on Bond II immunostainer using guinea pig antiswine insulin primary antibody (1:150) and biotinylated rabbit antiguinea pig secondary antibody (1:100; both DAKO). 5 slides (C, G, GW, GI, GIW, 2 sections/slide) imaged on Olympus BX61 microscope using a Q Imaging Retega 4000R color camera and Velocity software. IHC stain area measured and lighter stained BC selected using intensity segmentation. BC areas measured and results pasted to a spreadsheet where the % of BC area to whole IHC area computed. Also computed sum, mean and standard deviation of each of the 5 ratios (IHC/BC) for each slide. Serum G and cr measured by standard procedures, I measured by ELISA (Mercodia). Mean ± SEM of data in all groups analyzed.

Results: G in G group (290.52 ± 23.22 mg/dL) was higher than C (196.9 ± 29.1 mg/dL, p=0.023) and GW (203.4 ± 20.5 mg/dL, p=0.014). IHC/BC ratio mean ± SD in G (5.83 ± 1.03) and GW (6.29 ± 1.54) was higher than C (3.74 ± 0.77). IHC/BC ratio in GIW (4.26 ± 0.84) was lower than GW. Significant reduction of BC mass was noted in all treated groups compared to C. I (ug/L) was higher in both G (2.89 ± 0.72) and GI (3.95 ± 0.84) groups, compared to C but two wk. W lowered I to control levels (GW 0.24 ± 0.04, GIW 0.41 ± 0.17). No difference in cr levels among groups.

Conclusions: G feeding reduces BC mass. Study highlights that overeating may shrink BC mass and cause DM in humans.

Funding: Private Foundation Support

PUB058

Effectiveness and Safety of Bicarbonate in the Prevention of Contrast Induced Nephropathies in Chronic Nephropathic Diabetic Patients Undergoing to Interventional Radiology of the Lower Limbs Filippo Mariano,[1] Luca Monge,[2] Valter Verna,[3] Mario Boffano,[2] Giorgio Triolo.[1] *[1]Dpt of Medicine Area, Nephrology and Dialysis Unit, CTO Hospital, Turin, Italy; [2]Dpt of Medicine Area, Diabetology Unit, CTO Hospital, Turin, Italy; [3]Dpt of Radiology, CTO Hospital, Turin, Italy.*

Background: The risk score for contrast-induced acute nephropathy (CI-AKI) can increase up to 50% in patients with chronic nephropathy and diabetes mellitus. The purpose of this study is to evaluate the effectiveness and the tolerability of a bicarbonate protocol infusion (BPI) in chronic nephropathic diabetics submitted to interventional radiology (IR) for lower limb revascularization, by comparing the incidence of CI-AKI with the pre-procedure risk score.

Methods: 98 diabetic chronic nephropathic patients (68M/30F, age 73.4±9.7 years old (average ± SD)) were prospectively studied between January 2010 and January 2011 for lower limb revascularization. Patients were treated as prophylaxis with BPI 1.4% (1ml/Kg/hour bw for 180' pre procedure followed in post procedure of 1ml/Kg/hour bw for 6 hours) and e n-Acetilcisteina per os (600 mg 2 times a day the day before and the day of the exam). The CI-AKI has been defined with a serum creatinine increase of 0.5 mg/dl or of 25% with respect to the basal value (post procedural control at 24-48 hours) and risk score of CI-AKI was calculated according to Mehran

Results: The average eGFR of nephropathic patients stage >=2 K-DOQI and not in dialysis (n 62, formula CKD-EPI) has been of 56.1.2±16.5 in the pre procedure and 62.5.2±20.2 ml/min/1.73 m^2 in the post procedure (pre vs. post procedure p 0.003). The average volume of contrast medium has been of 96.1±30.0 ml. Face to an average global score of risk of CI-AKI of 17.6% and of dialysis of 0.5%, the real incidence of CI-AKI with BPI has been of 6.4%. No hypervolemic complications or significant electrolytic alterations were observed. In patients with advanced nephropathy (level IV-V K-DOQI) no cases of CI-AKI were registered.

Conclusions: These results indicate that the applied BPI is an effective and safe measure for CI-AKI prevention in nephropathic diabetic patients submitted to IR for lower limbs revascularization.

Funding: Government Support - Non-U.S.

PUB059

Oxidative Stress in the Kidneys of the Fetus and Pre-Adolescent Offspring of Diabetic Rats Arwa Nada,[1,4] Lawrence Fordjour,[2] Charles Cai,[2] Dharmendra Kumar,[2] Morris J. Schoeneman,[1] Anil K. Mongia,[1] Bandana Paudyal,[1] Kelly Benedict,[1] Gloria Valencia,[2] Jacob Aranda,[2] Kay Beharry.[3] *[1]Department of Pediatrics, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY; [2]Department of Pediatrics. Division of Neonatology, SUNY Downstate Medical Center, Brooklyn, NY; [3]Department of Pediatrics. Division of Neonatology, University of California Irvine Medical Center, Orange, CA; [4]Department of Pediatrics, Division of Nephrology, Alexandria University Children's Hospoital, Alexandria, Egypt.*

Background: Oxidative stress (OS) plays an important role in the development of fetal complications of diabetic pregnancies. This role is not well studied in the kidney of embryos and offspring of diabetic mothers.

Methods: Objectives:To determine the effect of DM on OS in the kidneys of fetuses and the pre-adolescent offspring of diabetic mothers.

Results: Fetuses at embryonic age 20 (E20) and adolescent pups at postnatal age 14 (P14) were studied: 1) Non-diabetic, non-treated (E20-CTL); 2) diabetic, non-treated (E20-DNT); 3) diabetic insulin-treated (E20-DIT); 4) P14-CTL; and 5) P14-DIT. Prior to mating, DM was induced in rats using streptozocin (65 mg/kg IV). Once DM was confirmed , either insulin or placebo pellets subcutaneously were started. Markers of OS (8-isoprostane); DNA damage (OHdG); reactive oxygen species (H_2O_2); antioxidant activity (SOD, GPX, and catalase); and genes regulating OS were studied.

Results: Markers of OS had significant higher concentration in the kidney samples of E20NT group , 8-iso-PGF2a (P< 0.001), and 8-OHdG (P<0.01). E20IT group showed significant higher concentration of SOD (P<0.001). E20NT group showed upregulation of the gene expression of the antioxidative enzymes. E20IT group showed downregulation of gene expression of the antioxidative enzymes.P14-IT group showed upregulation of the gene expression of the antioxidative enzymes.

Conclusions: DM increases the oxidative stress in the kidneys of embryos and offspring of diabetic mothers. This effect extends to the postnatal period. These findings may suggest long term effect of diabetes on the kidneys of children of diabetic mothers, and their need to antioxidant therapy.

Funding: Government Support - Non-U.S.

PUB060

High Glucose Induces Human Glomerular Endothelial-to-Mesenchymal Transition by RhoA Activation Hui Peng, Yuanqing Li, Canming Li, Cheng Wang, Pengli Luo, Tan-Qi Lou. *Division of Nephrology, Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.*

Background: Emerging evidence suggests that endothelial-to-mesenchymal transition (EndMT) contributes to kidney fibrosis including STZ-induced diabetic nephropathy (DN). Researches including us have showed that hyperglycemia plays an important role in the development and progression of DN. Our aim is to investigate whether high glucose induces EndMT in glomerular endothelial cells through RhoA activation.

Methods: Primary human glomerular endothelial cells (hGEnCs) were exposed for 24 hours to culture medium containing: (1) no additions (control), (2) high glucose medium 1 (HG1, 15mM), (3) high glucose medium 2 (HG2, 30mM), (4) high glucose medium2 with ROCK1 inhibitor Y27632 (1mM), (5) mannital control. Immunofluorescent staining was performed to detect the expression of CD31, fibroblast specific protein-1 (FSP-1), and α -smooth muscle actin (α-SMA) in hGEnCs of different groups. Phenotypic changes of hGEnCs were observed using phase contrast microscope. Protein and mRNA expression of FSP-1, α-SMA and CD31 were measured by Western blotting and real-time PCR respectively. RhoA activity was detected by pull-down assay.

Results: It was showed hGEnCs changed their phenotype from cobble-like to fibroblast-like cells in high glucose, which did not happen in mannitol control groups. It also showed that α-SMA with CD31 double positive percentage was significantly increased in HG groups with a dose dependent manner when comparing with control group and mannitol group. However when pre-incubated with ROCK1 inhibitor Y27632 in high glucose, α-SMA with CD31 double positive stained cells did not increase (P<0.05, n=3). Real-time PCR and Western blotting results indicated FSP-1 and α-SMA mRNA and protein significantly increased in HG group compared with control group and mannitol group, but not in Y27632 group (P<0.05, n=3). RhoA activity was significantly increased in HG group compared with control group (P<0.01, n=3).

Conclusions: Our results indicate that high glucose may contribute to the EndMT of glomerular endothelial cells through RhoA signaling pathway.

Funding: Government Support - Non-U.S.

PUB061

Value of the Neutrophil to Lymphocyte Ratio as a Predictive Tool of Worsening Renal Function in Diabetic Population Jennifer Ross, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: The role of inflammation and inflammatory cytokines in diabetic nephropathy was studied over the last few years. Neutrophil to lymphocyte ratio rather than other white cell parameters was found to be a useful inflammatory marker which predicts adverse outcomes in acute and chronic medical and surgical conditions. Nevertheless, the use of Neutrophil to lymphocyte ratio (NLR) as an inflammatory marker for diabetic nephropathy has not been elucidated.

Methods: A338 diabetic patients were followed at our clinic between 2007 and 2009. The variables were total WBC, neutrophil, lymphocyte, monocyte and NLR, serum levels (creatinine, BUN, hemoglobin, hemoglobin A1C, LDL, and HDL), urine albumin creatinine ratio and GFR. Demographic variables (race, gender, age and BMI), presence of risk factors (e.g. hypertension, smoking, CAD, CHF) and medication were retrieved for each patient index. We arranged our patients into tertiles according to the initial NLR. Dropping of GFR ≥ 12 over a 3 year follow-up period with the last GFR ≤ 60 was considered a positive primary endpoint.

Results: The lowest NLR tertile had fewer patients (2.7%) with primary outcome(i.e. worsening renal functions) compared to middle and highest NLR tertile, which had more patients with primary outcomes (8.7% and 11.5% respectively) with a significant P value 0.01. Higher prevalence of stage ≥ 3 of CKD was found in higher two NLR tertiles(32% and 25%) versus 12% in the lowest NLR tertile group.

Conclusions: NLR as an inflammatory marker predicted the worsening of the renal function in the diabetic population. Further studies over a longer follow-up period are needed to confirm this result

PUB062

Urine pH as a Predictor of Developing Type 2 Diabetes Majed Samarneh, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: The role of urine pH in predicting the risk for developing diabetes mellitus remains unclear. We hypothesize that as urine pH increases, the risk of developing diabetes mellitus decreases.

Methods: We performed a retrospective cohort study of patients admitted to Staten Island University Hospital during the period from June 2008 to June 2009. Patients with conditions that are known to influence the urine pH were excluded: CKD (defined as GFR< 60 for over 3 months), urinary tract infection, acute kidney injury, and chronic indwelling bladder catheter.

Results: The final study cohort included 92 subjects who had an appropriate database to use the Atlanta diabetes risk prediction model for estimation of the diabetes risk score. Urine pH was inversely correlated with the diabetes risk prediction score but this was not statistically significant (r= -0.14, p=0.20). Univariate analysis showed that BMI ≥ 30 kg/m^2 was associated with a lower urine pH but this was not statistically significant. There was no association between urine pH and hypertension, low HDL or elevated TG. Our study had a few limitations.

Conclusions: First, it is a retrospective cohort and we have no long term follow-up on the patients to determine the exact incidence of diabetes in the population. Another limitation is the limited number of participants in the final cohort. Moreover, we have not validated the accuracy of a urine dip in calculating the urine pH for this study. Our study is the first to study the relationship between urine pH as a marker of insulin resistance and the risk of developing diabetes. We found an inverse relationship between urine pH and the risk of developing diabetes although not statistically significant. We also found an inverse correlation between BMI and urine pH, similar to the existing literature. Urine pH is a promising marker of insulin resistance. Further studies are necessary to determine whether urine pH is a true predictor of diabetes mellitus. If validated, urine pH has the potential to become one of the screening tools for diabetes in the primary care setting.

PUB063

Renal Function in Diabetic eNOS Knockout Mice Assessed by Dynamic ^{99m}Tc-MAG3 SPECT Imaging Mohammed Noor Tantawy,[1] Rosie T. Jiang,[2] Keiko Takahashi,[2] Christopher Chad Quarles,[1] Raymond C. Harris,[2] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt University Medical Center, Nashville, TN.*

Background: Endothelial dysfunction is a hallmark of diabetic vascular complications. Recent studies have shown that deficiency of endothelial nitric oxide synthase (eNOS) significantly advances diabetic renal injury in mice. The finding indicates a crucial role of eNOS in the pathogenesis of diabetic kidney disease. However, the underlying mechanisms are incompletely understood. In this study, we assessed renal function in diabetic eNOS knockout mice using ^{99m}Tc-MAG3 SPECT imaging.

Methods: Diabetes was induced in C57BL6 strain eNOS knockout (eNOSKO) and wild type males (n=5 per group) at the age of 8 wks by multiple low-dose STZ injections (50 mg/kg, i.p., 5 days). Citrate buffer-injected eNOSKO and wild type males (n=5 per group) were used as non-diabetic controls. Renal function was assessed by planar dynamic mode ^{99m}Tc-MAG3 NanoSPECT imaging [~37 MBq, retroorbital injection, 30min scan] at 6 and 14 wks post STZ injection. Time-activity curves (TACs) of each kidney were recorded over the duration of the scans. Renal perfusion was assessed by the peak activity, time-to-peak (TTP), and the slope of first influx (RBF).

Results: At 6 wks post STZ injection, the perfusion parameters (peak activity, TTP, and RBF) showed no differences among the four groups of mice. However, all diabetic eNOSKO mice showed a remarkable delay in renal ^{99m}Tc-MAG3 clearance, indicating impaired tubular function, while other groups of mice did not. At 14 wks post STZ injection, significant differences were also not observed in the perfusion parameters among the four groups of mice. Surprisingly, delayed ^{99m}Tc-MAG3 clearance was not observed in diabetic eNOSKO mice at this time point.

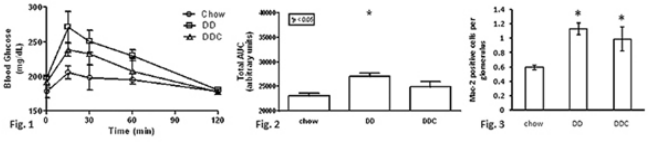
Conclusions: In STZ-eNOSKO (C57BL6) model, eNOS deficiency causes acute tubular dysfunction in the setting of diabetes. However, this disorder is restored in the following period. Significant changes in renal perfusion are not observed by ^{99m}Tc-MAG3 scintigraphy in STZ-eNOS KO mice up to 14 wks post STZ injection. Further follow-up study is currently underway.

Funding: NIDDK Support

PUB064

A Murine Model of Diet-Induced Insulin Resistance and Metabolic Syndrome Tomasz A. Wietecha,[1] Jinkyu Kim,[2] Antonio Haw Sta. Teresa,[2] Kelly L. Hudkins,[1] Ewa M. Skarbek,[1] Takahisa Kobayashi,[1] Bardia Askari,[1] Kevin D. O'Brien,[2] Charles E. Alpers.[1] *[1]Pathology, UW, Seattle, WA; [2]Cardiology, UW, Seattle, WA.*

Background: BTBR is an inbred mouse strain that is susceptible to development of diabetes and advanced complications including diabetic nephropathy(DN) (JASN. 2010; 21:1533), particularly in the presence of the leptin-deficiency mutation (*ob/ob*). Wild-type BTBR mice are not hyperglycemic on Chow diet, but have insulin resistance (high insulin levels). We sought to determine whether dietary modification could induce diabetes and DN in the presence of an intact leptin/leptin receptor axis. At 4 weeks of age, female BTBR mice were assigned to Chow, DD (diet high in fat and refined sugar) or DDC (DD diet with 0.15% cholesterol) groups. Oral glucose tolerance tests (OGTT) were performed at Baseline and after 8 and 16 weeks on diet, when all mice (n=7/group) were sacrificed. At 8 and 16 weeks, both DD and DDC groups had significantly impaired glucose responses, consistent with increased insulin resistance, represented as total area under the curve (Fig. 1, 2), but no significant increases in fasting glucose. Body weights were modestly increased in DD and DDC mice at 16 weeks. Histologically, a significantly increased glomerular influx of Mac-2 positive cells (monocytes) was detected in both DD and DDC compared to Chow group (1.1 and 1.0 vs. 0.6 Mac-2 positive cells per glom respectively, P<0.05, Fig.3). In all groups, there was no significant glomerular hypertrophy, mesangial expansion, glomerular cell activation or proliferation. Increased proteinuria in DD and DDC diet groups did not achieve significance. These results characterize early inflammatory changes of glomeruli in diet-induced insulin resistance and metabolic syndrome, which may be a first step towards development of structurally advanced DN.



PUB065

ACE2 Deficiency Is Not Sufficient To Aggravate Kidney Damage in Diabetic Mice on a C57BL Genetic Background Resistant to Kidney Disease Jan A. Wysocki,[1] Susan B. Gurley,[2] Minghao Ye,[1] Yashpal S. Kanwar,[1] Thomas M. Coffman,[2] Daniel Batlle.[1] *[1]Division of Nephrology & Hypertension, Northwestern University, Chicago, IL; [2]Duke University Medical Center, Durham, NC.*

Background: Angiotensin II overactivity is believed to be a major factor in diabetic kidney injury. ACE2 is an enzyme highly expressed in the kidney that dissipates Ang II and its expression has been shown to be altered in diabetic mouse models. We reasoned that ACE2 deficiency especially in the context of diabetes could lead to local Ang II overactivity due to a decreased Ang II degradation causing increased kidney damage.

Methods: We compared male WT and ACE2 KO mice (ace2$^{+/y}$ and ace2$^{-/y}$, respectively) on C57BL6/J background which were rendered diabetic using low (5x40mg/kg at week 0 and 7 of diabetes) and high-dose STZ (2x150mg/kg).

Results: ACE2 deficiency alone was not associated with albuminuria or any gross pathological kidney changes except for a mild mesangial expansion in glomeruli from ace2$^{-/y}$ mice as compared to WT (0.49±0.05 vs. 0.24±0.07, respectively, p<0.05). After low-dose STZ, mesangial score was about equally increased in ace2$^{-/y}$ or ace2$^{+/y}$ mice (0.74±0.08 vs. 0.78±0.08, respectively). Because low dose STZ did not cause albuminuria in either of the groups despite severe hyperglycemia, we used high dose STZ. High dose STZ was associated with a significant increase in albumin/creatinine ratio (ACR) in both groups. Initially, ACR was higher in ace2$^{-/y}$ than in ace2$^{+/y}$ mice but the trend was abolished later on. Mesangial score in STZ-treated ace2$^{-/y}$ mice was higher than in ace2$^{+/y}$ mice (0.89±0.29 vs. 0.56±0.13, respectively) but the difference was not statistically significant. Other indicators of kidney injury, such as glomerular fibronectin and MCP-1 staining as well as collagen expression were equally increased in diabetic ace2$^{-/y}$ and ace2$^{+/y}$ mice.

Conclusions: Genetic ACE2 ablation in itself does not accelerate STZ-induced diabetic injury in mice on the C57BL background and further demonstrates the resistance of this strain to chronic kidney injury. Studies in more injury-prone genetic backgrounds are needed to better understand the role of ACE2 in diabetic kidney disease.

Funding: NIDDK Support

PUB066

Urinary ACE2 as a Biological Marker of ACE2 Activity Jan A. Wysocki, Minghao Ye, Laura Garcia-Halpin, Daniel Batlle. *Division of Nephrology & Hypertension, Northwestern University, Chicago, IL.*

Background: Urine is an easily accessible biological fluid that, as it is formed, stays in a direct contact to kidney apical membranes from which proteins are instantly shed. We have shown that ACE-related carboxypeptidase (ACE2) is excreted in the urine in large quantity particularly in diabetic mice. Here we describe a method of distinguishing between ACE2-deficient and ACE2-replete mice using a simple, single-step approach that detects urinary enzymatic activity of a protein such as ACE2 which is expressed abundantly in the apical membrane of renal epithelial cells.

Methods: The procedure is based on a cleavage of an ACE2-specific fluorogenic substrate, Mca-APK-Dnp. Four uL of urine sample was used in two reaction wells (2 uL/per well) where one of wells constituted a blank containing a specific ACE2 inhibitor, MLN-4760 (10^{-5}M).

Results: Using purified recombinant mouse ACE2 it was established that the linear detection range of the assay captures ACE2 protein levels of normal mouse urines. To further validate the method we used WT mice carrying two alleles of the ace2 gene (ace2$^{+/y}$), ACE2 KO mice (ace2$^{-/-}$ or ace2$^{-/y}$) as well as heterozygous (HT) mice carrying only one allele of the ace2 gene (ace2$^{+/-}$) that exhibit an intermediate ACE2 phenotype between WT and KO. The results obtained by measuring urinary ACE2 activity, were confronted with the results of ACE2 expression in post-mortem kidney tissue. In urines from ACE2KO (n=45) the assay yielded median value of -0.05 (range -0.17 - 0.30), in HT mice (n=23) 1.22 (range 0.51-2.14) and in WT (n=29) 4.11 (range 1.55-8.3) providing an excellent discrimination between ACE2-replete and ACE2-deficient mice.

Conclusions: Measuring urinary enzymatic activity of proteins such as ACE2 which normally are expressed in kidney apical membranes provides a convenient tool for identification of mice with deficiency of the enzyme in question or its over-expression.

Using sensitive assays to measure urinary enzyme activity can be useful for non-invasive phenotyping and determining successfulness of time-restricted kidney-specific gene knock-out or over-expression attempts.

Funding: NIDDK Support

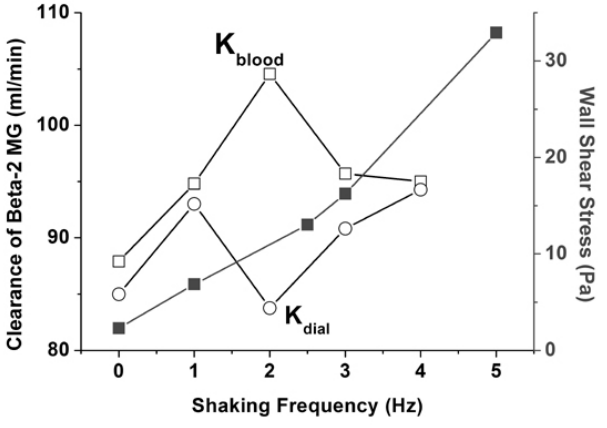
PUB067

Enhancement of Beta-2 Microglobulin Clearance by Shaking Hemodialyzer: Numerical and Experimental Studies Jeong Chul Kim,[1,2] Francesco Garzotto,[1] Mauro Neri,[1,2] Massimo de Cal,[1] Dinna N. Cruz,[1,2] Alessandra Brendolan,[1] Claudio Ronco,[1,2] Federico Nalesso.[1] *[1]Department of Nephrology, San Bortolo Hospital, Vicenza, Italy; [2]International Renal Research Institute Vicenza, Italy.*

Background: Clearances of middle-molecular-weight solutes in hemodialysis are limited by blood-membrane interaction such as protein gel layer and concentration polarization. To increase the clearances of middle molecules, shaking hemodialyzer can be useful because it increases wall shear stress at the surfaces of dialysis membrane and reduce resistances layers. We numerically predicted the effects of hemodialyzer shaking on hemodynamics inside hollow fiber and experimentally measured clearances of solutes at different shaking conditions.

Methods: Using a numerical package we analyzed hemodynamics of single hollow fiber according to the shaking profiles (longitudinal, transverse and rotational directions). To validate numerical model we developed a dialyzer shaking instrument and measured clearances of urea and beta-2 microglobulin in blood side and dialysate side and calculated mass balance errors.

Results: Numerical results showed that transverse shaking is optimal for clinical application because it provide uniform enhancement of effective blood flow rate and wall shear stress during the hemodialyzer shaking. These hemodynamic parameters were linearly proportional to the vibration amplitude or frequency. In experimental results, the effects of hemodialyzer shaking were negligible in urea diffusion while beta-2 microglobulin clearance increased with shaking hemodialyzer. However, the linear relationship between shaking parameters and beta-2 microglobulin clearance was not observed.



Conclusions: Shaking hemodialyzer could improve middle-molecular-weight solute clearances during hemodialysis.

PUB068

Profiling Cardiovascular Phenotype by Continuous Measurement Using FIR Median-Hybrid Preprocessing Scott Wilson, Gavin J. Becker. *Royal Melbourne Hospital, Australia.*

Results: The prediction and avoidance of significant haemodynamic instability during haemodialysis (HD) is a challenge in clinical monitoring. A 'normal' response to HD, and subsequently pathological variants, is not described. It is likely that particularly complex patterns are missed by routine arm-cuff measurement. Haemodynamic parameters would be ideally captured by continuous recording and trend-based analysis; however monitoring typical HD creates a massive data-set, typically yielding over 16,000 samples per session. Standard hardware and software can find usefully manipulating such a data load difficult. Separately, the sensitivity of research devices can create signal distortion and measurement artifacts when used in the clinical HD setting, obscuring true trend signals. Preprocessing is an attractive solution to these problems. Using real patient plethysmography data (Blood Pressure, Heart Rate, Peripheral Resistance) collected during HD treatments we have experimented with single and bidirectional, linear and nonlinear algorithms to reduce artifact whilst preserving the edges and direction of the underlying trends and subtrends. Linear temporal filters perform poorly through delay and sustained signal distortions depending upon the length of the observational window. Increasing filter complexity requires predefined assumptions about the character and quality of the underlying dataset. Single filter processes are inflexible and behave inconsistently across different haemodynamic parameters and individual patients. They do not alleviate the computational load of a large dataset.

We report the design and first known application of a 5 window Heart-Rate Dependent Finite-Impulse-Response Median Hybrid Filter (FirMHF) algorithm to cardiovascular monitoring. This filter reduces measurement artifact and preserves signal edge to create a robust time-series estimate of haemodynamic variables. No predefined tolerance thresholds or assumptions about the original dataset are required. The character of the physiologic trend is retained for analysis whilst the data-point burden is condensed to approximately 15% of the original continuous sample.

Funding: Private Foundation Support

PUB069

Relationship between Fluid Overload and Serum Sodium Concentration in Hemodialysis Patients Fansan Zhu,[1] Li Liu,[1,2] Jochen G. Raimann,[1] Peter Kotanko,[1] Stephan Thijssen,[1] Nathan W. Levin.[1] *[1]Renal Research Institute, New York, NY; [2]Dept. of Nephrology, Peking University, Beijing, China.*

Background: It has been proposed that fluid overload may be the cause of low serum sodium concentration [Na+] in hemodialysis (HD) patients. The aim of this study was to investigate the relationship of changes in [Na+] and fluid status in chronic HD patients.

Methods: In HD patients post HD weight was gradually reduced until dry weight as defined by calf bioimpedance spectroscopy (DW_{cBIS}) was reached.(Zhu, Physiol Meas 2008). Body weight (Wt), serum [Na+], and normalized calf resistivity (nRho; using Hydra 4200, Xitron Technologies) were measured pre and post HD at baseline (BL) and at DW_{cBIS}, respectively.

Results: From BL to DW_{cBIS} pre and post HD Wt decreased and nRho increased significantly. Serum [Na+] increased from pre to post HD both at BL and DW_{cBIS}, however, pre-HD serum [Na+] did not change between BL and DW_{cBIS} (Table 1).

Table 1

	Wt [kg]	nRho [Ω*m³/kg]/100	[Na+] [g/ml]
BL Pre HD	76.4±16.8**¤	14.9±2.8**¤	136.9±5.8*
BL Post HD	73.8±16.4¤	18.5±3.8¤	139.1±4.9
DW Pre HD	74.4±16.7**	17.1±3.2**	136.6±5.4**
DW Post HD	72.0±16.3	22.4±4.5	138.5±5

* and ** represent difference between pre and post HD.¤ represents difference between BL and DW (DWcBIS)

Conclusions: In this prospective study pre HD serum [Na+] was not affected by achieving dry weight. This finding argues against the notion that low pre-HD serum Na+ is due to fluid overload.

Funding: Private Foundation Support

PUB070

Iron and Ultrasound--A Case Report of High Ferritin Levels and Increased Renal Echogenicity James Paul Rogers,[1] Pran M. Kar,[2] Kevin B. Patel,[3] Sunny Kar.[4] *[1]Operative IT, Inc., Kennewick, WA; [2]Orlando Nephrology, Orlando, FL; [3]Florida State University; [4]LECOM, Bradenton, FL.*

Background: The lack of ionizing radiation in ultrasound imaging has made it a powerful yet safe diagnostic tool. When interpreting renal ultrasound images, echogenicity of the cortex and medulla has been used to identify changes in kidney function. In fact, where chronic kidney disease is concerned, a quantification of echogenicity has been suggested as a means of more accurately identifying abnormalities. But what happens when ferritin levels are high, such as in patients with hemochromatosis? What affect does this have on the echogenicity of a patient's organs? These cases bring to light the possibility that high ferritin levels can confound interpretations of renal ultrasound images by increasing the kidney's echogenicity.

Methods: Patient records were retrospectively examined for patients who presented with an acute kidney injury, increased kidney echogenicity on ultrasound, and high ferritin levels.

Results: Two patients in this case study displayed increased renal echogenicity and concomitantly high ferritin levels. Our first patient's acute kidney injury was probably medicinally induced. Therefore, the increased renal echogenicity observed was not expected. In our second patient, a renal ultrasound changed from a highly echogenic cortex to a normal cortex within ten days. This was also unexpected. Furthermore, she had normal renal function prior to her second admission. Therefore, it is worth considering that high ferritin levels contributed to the renal echogenicity we observed in both patients.

Conclusions: We postulate that high ferritin concentrations in the kidneys, as indicated by high serum ferritin levels, may contribute to increased renal echogenicity. Therefore, when ferritin levels are high, we advise caution when interpreting echogenic changes in renal ultrasounds as evidence of chronic disease.

PUB071

Reversion of High Glucose-Induced EMT in HPMC by the SARA/SBD Fusion Protein Containing Protein Transduction Domain Huang Chen,[1] Rui Du.[2] *[1]Nephrology, Xijing Hospital, Xi'an, China; [2]FMMU, Xi'an, China.*

Background: In this study, we constructed a SARA peptide aptamer based on the sequence of SBD(SARA/SBD). The recombinant SARA/SBD fused with a protein transduction domain (PTD-SARA) was cloned. We express and purify the PTD-SARA/SBD fusion protein in prokaryotic cells and determine its effect on the epithelial-to-mesenchymal transition of human peritoneal mesothelial cells treated with high glucose and possible mechanisms.

Methods: PTD-SARA/SBD gene was cloned into prokaryotic expression vector pET-44a(+) with His tag. Then the constructed recombinant plasmid was transformed to E.coli BL21 for expression under induction of IPTG. The expressed product was purified by Ni^{2+}-NTA affinity chromatography and identified by SDS-PAGE and Western

blot; Immunocytochemistry methods was used to test the transmembrane efficiency and colocalization of the fusion protein with Smad2 which was one of factors in TGF-β_1 signal transduction pathway in HPMC. Western Blot was used to test the level of E-cadherin, α-SMA, Smad2/3 and pSmad2/3 protein in HPMC to confirm whether the recombinant fusion protein can inhibit the epithelial-to-mesenchymal transition of HPMC treated with high glucose and its possible mechanisms.

Results: The PTD-SARA/SBD fusion protein was expressed and purified using the methods of genetic engineering. It was indicated that the expressed product contained 20% of total somatic protein and existed in a soluble form. The purity quotient was beyond 95% after being purified by Ni^{2+}-NTA affinity chromatography. The results of function experiments indicated that the PTD-SARA/SBD fusion protein could transduce into HPMC efficiently which mainly located in nucleolus and could colocalize with Smad2. The fusion protein could increase the level of E-cadherin protein and decrease the level of α-SMA protein in HPMC treated with high glucose. It could decrease the level of pSmad2 protein, which had no effect on the Smad3 phosphorylation level.

Conclusions: PTD-SARA/SBD fusion protein could prevent the epithelial-to-mesenchymal transition of HPMC treated with high glucose. The possible mechanism was that the fusion protein could prevent the phosphorylation of Smad2.

PUB072

Short-Term Impact of Sevelamer Hydrochloride on Hyperphosphatemia in Chinese Patients on Maintenance Hemodialysis Therapy Yi Fang, Jianzhou Zou, Jie Teng, Xiaoyan Zhang, Xiaoqiang Ding. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: This open-lable, self-control study was aimed to evaluate the safety and efficacy of Sevelamer Hydrochloride in treating Chinese maintenance hemodialysis (MHD) patients with hyperphosphemia.

Methods: Phosphate binders were discontinued during a two-week washout period. Patients whose serum phosphorus was more than 1.78mmol/L after two-week washout period were eligible for treatment. The dose could be adjusted every two weeks as necessary to achieve serum phosphorus control. Sevelamer Hydrochloride was administered to 138 MHD patients for 10 weeks and a second two-week washout period followed.

Results: 111 of the 138 patients fulfilled the whole 14-week study. Mean serum phosphorus and calcium-phosphate products started to declined after two-week Sevelamer Hydrochloride treatment. By the end of 10-week sevelamer hydrochloride treatment, mean serum level of phosphorus (1.85±0.5 *vs* 2.57±0.54mmol/L, p<0.01), calcium-phosphate product (4.16±1.72 *vs* 5.79±1.50 $mmol^2/L^2$, p<0.01) and low density lipoprotein (1.64 ±0.76 *vs* 2.31±0.87 mmol/L, p<0.01) were significantly lowered while the adjusted serum level of calcium and serum intact parathyroid hormone kept steady. Both serum phosphorus and calcium-phosphrus increased after the second washout period, but the levels were still lower than their pre-treatment ones (2.26±0.71vs 2.57±0.54 mmol/L; 5.12±1.63 vs 5.79±1.50 $mmol^2/L^2$, p<0.01). Of the 138 patients involved, 214 cases in 106 patients and 121 cases in 121 patients were reported as adverse events and adverse drug reaction respectively. Gastrointestinal symptoms, of which most were mild or moderate, happened to 68.12% patients.

Conclusions: Sevelamer Hydrochloride can control serum phosphorus and reduce the level of calcium-phosphorus product and cholesterol. Slight gastrointestinal symptoms like constipation were common during the treatment.

PUB073

Facility-Level Phosphorus (P) Control of Peritoneal Dialysis Patients in a Large Dialysis Organization Jamie Heise,[1] Kamyar Kalantar-Zadeh,[2] John Brian Copley,[1] Moshe Fridman,[3] Rajnish Mehrotra.[2] *[1]Medical Affairs, Shire Pharmaceuticals, Chesterbrook, PA; [2]Medicine, Harbor-UCLA Medical Center, Torrance, CA; [3]Statistics, AMF Consulting, Los Angeles, CA.*

Background: Increased serum P is a robust predictor of all-cause and cardiovascular mortality in dialysis patients. Though P control in the last decade has improved overall, performance varies widely among facilities.

Methods: 10, 177 patients treated with peritoneal dialysis in January of 2011 were selected for the study and aggregated by facility. The facilities with at least 5 patients (N=480) were then ranked by the proportion of patients with serum P < 5.5 mg/dl for the calendar month. The top and bottom 5% of facilities were compared for the achievement of targets for other laboratory measures (calcium, Ca; parathyroid hormone, PTH, and albumin), P-binder and vitamin D use, geographic region, and use of a central pharmacy.

Results: For the population studied, 67% of peritoneal dialysis patients achieved serum P < 5.5 mg/dl. The top 5% of facilities (n=24) treated 306 patients and bottom 5% (n=31) treated 248 patients. P control was achieved in 97% of patients of top-performing facilities compared to 34% of the bottom-ranked facilities. There was no significant difference in the proportion of patients achieving targets for serum albumin (< 3.5 g.dl; 69% vs. 73%, p=0.416), and calcium (< 9.5 mg/dl; 98% vs, 94%, p < 0.089) but top-performing facilities were more likely to achieve the PTH target (150-600 pg/ml; 75% vs. 60%, p=0.0004). Furthermore, top-performing facilities were less likely to use combinations of P-binders (7% vs. 22%; p=0.002). There was no significant difference in use of other P-binders, oral vitamin D or cinacalcet use, or use of a central pharmacy between the top and bottom 5% of facilities.

Conclusions: Despite a high overall rate of P control, there appears to be a unit level treatment or demographic difference which affects the proportion of patients with serum P < 5.5 mg/dl at a facility level. Identifying practice patterns that allow a larger proportion of patients to achieve P control has the potential of further improving outcomes.

Funding: Pharmaceutical Company Support

PUB074

Oxalate Nephropathy Leading to End Stage Renal Disease after Gastric Bypass Surgery Ghaziuddin Qadri, Manish K. Saha, Tarek Hamieh, Vishal Sagar. *Regions Hospital, St. Paul, MN.*

Background: 52-year-old Caucasian female with history of DM-2, hypertension (HTN), Roux-en-Y gastric bypass (RYGB) 2 years ago, CKD stage 1-2, attributed to HTN and DM, was admitted with worsening renal function. She had no symptoms of uremia. Vital signs were normal. Trace pedal edema was noted. Renal chemistry showed BUN 56 mg/dl, creatinine(Cr) of 4.5 mg/dl, eGFR 10.9 ml/min/1.73m2, K+ 3.6 mmol/L and bicarbonate of 18 mmol/L. Urine analysis showed 27 RBCs/hpf and TP/Cr ratio was 0.7. Serological workup and renal ultrasound was normal. Hemodialysis(HD) was initiated for chemical exchange and improvement of platelet function prior to kidney biopsy. Biopsy showed calcium oxalate deposits within the tubules, early diabetic nephropathy and mild arterial thickening. Immunoflouresence was negative. Plasma oxalate level was 17 uM/L (ref <1.8 mmol/L), urine oxalate of 44.6 mg/specimen (ref 9.7-40.5 mg/spec), oxalic acid/cr ratio of 53.2 mg/g (ref 1.6-37). 72 hr fecal fat was 8g (Ref 2-7 g). She was diagnosed with oxalate nephropathy secondary to RYGB. Patient was continued on HD and started on low oxalate and fat diet and calcium carbonate, as an oxalate binder. She was later switched to home HD six times a week to maintain a plasma oxalate level less than 30 uM/L and referred for a kidney transplant.

Conclusions: Hyperoxaluria and oxalate nephropathy are an uncommon complication after RYGB. RYGB results in fat malabsorption thereby leading to more calcium binding to free fatty acids. This results in an increased quantity of oxalate, not bound to calcium, but free and readily absorbed causing increased calcium oxalate in the serum and hyperoxaluria. Patients may benefit from low oxalate diet, low fat diet and calcium supplement, but oftentimes, patients may progress to becoming HD dependent. Patients, who have undergone RYGB, especially in the setting of CKD, as in our patient, may be at increased risk of worsening renal function due to oxalate deposition. They should be closely monitored for oxalate nephropathy and counseled to follow strict dietary regimen. While those contemplating RYGB procedures may need to be screened and informed of this unfortunate complication.

PUB075

Rationale and Design of an Open-Label, Randomised, Active-Controlled Phase 3 Study Evaluating the Management of Hyperphosphatemia with PA21 in Dialysis Patients James A. Tumlin,[1] Edward M.F. Chong,[2] Sylvain Gaillard,[3] Jurgen Floege.[4] *[1]Southeast Renal Research Institute University of Tennessee, USA; [2]Vifor Pharma, Canada; [3]Vifor Pharma – Vifor (International) Inc, Switzerland; [4]Medizinische Klinik II, Aachen, Germany.*

Background: Hyperphosphatemia is a common and serious complication in patients with chronic kidney disease (CKD), and can usually not be satisfactorily controlled by dietary restriction and hemodialysis, thus necessitating pharmacologic phosphate binders. Currently available phosphate binders have limitations in regard to efficacy, safety, tolerability, pill burden and/or patient adherence that impact their effectiveness. Vifor Pharma is developing a new calcium-free, iron-based phosphate binder, PA21, for therapeutic use in the management of hyperphosphatemia. A Phase 2 study demonstrated that PA21 is effective and safe in lowering serum phosphorus (P) with a lower pill burden than most currently available phosphate binders.

Methods: The PA21 study is a randomized, active-controlled, two-stage, re-randomization, Phase 3 study designed to investigate the efficacy and safety of PA21 in lowering and maintaining serum P levels in patients with CKD on dialysis. The study population will consist of 640 subjects with hyperphosphatemia (serum P ≥6.0 mg/dL after washout). Other study endpoints include the assessment of biochemical markers of bone resorption and formation, FGF-23, and patient preference and satisfaction assessments. Subjects will be randomized 2:1 to PA21 or sevelamer carbonate (SevCa) for 24 weeks, followed by a 3-week superiority assessment of PA21 (maintenance dose) versus low-dose PA21 in a pool of 100 subjects from the PA21 treatment arm. Doses of PA21 or SevCa will be adjusted based on serum P levels and per-protocol specified guidelines to reach and maintain serum P levels between 2.5 to 5.5 mg/dl.

Results: Recruitment is currently taking place at 200 sites worldwide.

Conclusions: The study will provide robust evidence regarding the efficacy and safety of PA21 with 1 pill per meal (2-3 pills daily) in treating hyperphosphatemia in adult patients with CKD on dialysis.

Funding: Pharmaceutical Company Support

PUB076

Bone Tissue Evaluation by Bone Morphology Measurements in Type 1 Diabetes Patients with a History of Dialysis for 10 Years or Longer Rikako Hiramatsu, Keiichi Sumida, Masayuki Yamanouchi, Yoshifumi Ubara. *Kidney Center, Toranomon Hospital, Tokyo, Minato-kku, Japan.*

Background: Since the prognosis of diabetes patients undergoing dialysis is poor, little is known of bone disease in diabetes patients undergoing long-term dialysis. We therefore investigated the influence of long-term dialysis on bone disease in diabetic patients.

Methods: Six patients with type 1 diabetes mellitus who had been on hemodialysis for over 10 years and had undergone bone biopsy at our hospital between 2000 and 2011 were enrolled. There were 3 men and 3 women aged 48.7 ± 8.4 (42-58) years who had been on dialysis for 13.1 ± 4.9 (10 to 22) years. The duration of diabetes was 28.7 ± 10.8 (12 to 42) years. Right iliac bone biopsy was performed after tetracycline labeling.

Results: Laboratory tests revealed: serum intact PTH, 816.0 ± 160.4 pg/ml (247.0 to 1268.0); serum ALP, 413 ± 150 IU/l; adjusted serum Ca, 10.1 ± 2.2 mg/dl; and serum P, 6.2 ± 2.1 mg/dl. Bone histomorphometric measurements were: OB/BV (osteoid volume), 9.96 ± 5.92%; ES/BS (eroded surface), 23.50 ± 9.09%, Fb.V/TV (fibrosis volume), 1.39 ± 1.92%; mineral apposition rate (MAR), 0.82 ± 0.28 μm/day; and BFR/BS (bone surface-referent bone formation rate), 0.024 ± 0.02 (mm^3/mm^2/year). Three patients had osteitis fibrosa, two showed mixed bone disease (osteitis fibrosa plus osteomalacia), and one only had mild changes.

Conclusions: Since the report by Pei et al. in 1995, diabetes mellitus has been considered to show a close relation with low turnover bone disease, including adynamic bone. They studied diabetic patients with a dialysis period of less than 5 years. However, in Japan where renal transplantation from cadaver donors is uncommon, some diabetic patients remain on hemodialysis for longer than 10 years due to progress in dialysis technology. High turnover bone disease such as osteitis fibrosa will develop in diabetic patients receiving hemodialysis for over 10 years, as well as in non-diabetic patients.

Funding: Private Foundation Support

PUB077

Uremic Toxins Exacerbate Bone Mechanical Property in Chronic Kidney Disease Yoshiko Iwasaki,[1] Junichiro J. Kazama,[2] Hideyuki Yamato,[3] Masafumi Fukagawa.[4] *[1]Health Sciences, Oita University of Nursing and Health Sciences, Oita, Japan; [2]Division of Blood Purification Therapy, Niigata University Medical and Dental Hospital, Niigata, Japan; [3]Biomedical Research Laboratory, Kureha Corporation, Shinjuku, Tokyo, Japan; [4]Division of Nephrology, Endocrinology, and Metabolism, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

Background: Chronic kidney disease (CKD) patients are associated with greater fracture risk than general population, while the reason remains obscure. Bone quality is a prescribe factor of bone strength. Bone quality is consisted of several components including bone chemical composition. We previously revealed that cortical bones from CKD rats showed decreased mechanical property and altered chemical composition, and moreover changes of these parameters were dependent on kidney function. Thus we conducted an in vivo study to elucidate whether uremic toxins affect bone fragility.

Methods: CKD rat were divided into two groups; those administered oral charcoal absorbent which decreases the circulating levels of uremic toxins (CKD-AST), and those received vehicle (CKD-V). The Control group underwent sham operation.

Results: Storage modules in the CKD-V group were significantly less than those in the Control group, indicating decreased mechanical strength in the uremic cortical bone. However, the levels were maintained in the CKD-AST group. The raman spectroscopic analyses revealed that mineral matrix ratio, physiological collagen crosslinks and the ratio of carboxymethyl lysine (CML) were increased while crystallinity was decreased in the CKD-V group. These parameters were comparable between the CKD-AST and the Control groups. Serum parameters were comparable between the CKD-AST and the CKD-V groups except the indoxyl sulfate level, which is surrogate marker of uremic toxins, was significantly higher in the CKD-V group. Multiple regression analysis revealed that physiological collagen crosslink and the ratio of CML were independently associated with storage modulus.

Conclusions: In conclusion, accumulated uremic toxins are quite likely candidate for those deteriorate bone mechanical property through changing the chemical composition in CKD.

PUB078

Bone Turnover Markers in Haemodialysis Patients with Chronic Liver Disease Guillaume Jean, Charles Chazot. *Hemodialyse, NEPHROCARE, Tassin, France.*

Background: In chronic kidney disease (CKD) patients, bone turnover (BT) diagnosis remains challenging for nephrologists. Because it is an invasive procedure, bone biopsy cannot be performed as a routine examination. Furthermore, it is well recognized that the serum level of parathyroid hormone (PTH) is not a reliable bone turnover marker (BTM). The Kidney Disease: Improving Global Outcomes (KDIGO) foundation recommends regular sampling of BTMs such as total alkaline phosphatases (t-ALP) and bone-specific alkaline phosphatase (b-ALP) in the case of patients with liver diseases (LDs). Bone-collagen peptides such as beta-Cross-Laps (CTX) are not recommended by the KDIGO.

Methods: Between March 2004 and March 2011, all HD prevalent patients in our centre were examined for chronic LD and included in the study after at least 3 months of dialysis. Serum iPTH levels and t-ALP levels were assessed every month. B-ALP and CTX levels were assessed at least every 6 months. Linear regression between t-ALP, b-ALP, PTH, and CTX values and between CTX and Kt/V was performed.

Results: 76 prevalent HD patients (335 blood samples) with different LDs were examined: 42% were women, with a mean age of 68 ± 12 years and on dialysis for 61 ± 86 months, and 55% were diabetic, with dialysis schedule varying from 3 × 4 to 3 × 8 hours.

Linear regression showed that b-ALP (mean 28.1 ± 23 μg/l) and t-ALP (431 ± 300 U/l) levels were closely related (r^2: 0.6; $p < 0.0001$), even when the serum PTH level was <250 pg/ml (r^2: 0.56; $p < 0.001$). The b-ALP/t-ALP ratio was 0.07 ± 0.12 (range: 0.02–0.2) and correlated poorly with PTH levels (r^2: 0.03; $p = 0.01$). Both b-ALP and t-ALP levels correlated with PTH levels (233 ± 183 pg/ml); CTX levels (2.2 ± 1.2 μg/l) correlated better with PTH levels (r^2: 0.3; $p < 0.001$) and were not influenced by Kt/V or dialysis session time, even though CTXs are eliminated by dialysis. Owing to accumulation of CTXs between dialysis sessions, normal CTX values in HD patients were 4 to 5 times higher than in patients with normal renal function.

Conclusions: The bone specificity of b-ALP appears insufficient for the diagnosis of BT in the case of patients with LDs. In such cases, CTX appears to be more reliable and is independent of liver function.

PUB079

The Mechanism of Bone and Joint Pain in Chronic Hemodialysis Patients Ikuto Masakane. *Dialaysis Center, Yabuki Shima Clinic, Yamagata, Japan.*

Background: The recent concern for evaluating dialysis quality is how to relieve dialysis patients of their dialysis related symptoms, reflecting the increase of elder patients and long-dialyzed patients. The most frequent complaint of chronic hemodialysis patients is bone and joint pain, and it consists of many causes not only in dialysis patients but also in general population. In the current study, we evaluated the pathogenesis of bone and joint pain in dialysis patients.

Methods: We have performed a comprehensive survey for dialysis related symptoms twice a year, and bone and joint pain is the most frequent one prior to other typical uremic symptoms. In Jan. 2011, a further survey for bone and joint pain was performed in 301 chronic dialysis patients. The demographic data and the exact data about their pains, such as the region, degree and existence of bone deformity were collected. The demographic data and blood chemistry data were compared using t-test and Chi-square test for the pain on lumber supine, knee joint and shoulder joint.

Results: Ninety one patients (31%) had more than moderate bone and joint pain. The most frequently injured joints were lumber supine (84%), knee joint (66%) and shoulder joint (48%). The significant factors for lumber supine pain were the past history of lumbago and high level of serum parathyroid hormone. The significant factors for knee joint pain were sex, age and high level of serum parathyroid hormone. The significant factors for shoulder pain were dialysis vintage, history of the operation of carpal tunnel syndrome.

Conclusions: Based on the results of the current study, we hypothesized of two main mechanisms in the pathogenesis of bone and joint pain in chronic dialysis patients. The first mechanism is the deterioration in bone strength against the gravity and it appears as the pain on lumber supine and knee joint. This kind of pain could be worsened by secondary hyperparathyroidism. The second is the concomitant of dialysis related amyloidosis which damages the smooth movement of tendons in many joints, and it clearly appears as shoulder pain. In conclusion the protection of secondary hyperparathyroidism and dialysis related amyloidosis is essential to relieve dialysis patients of bone and joint pains.

PUB080

Oral Calcitriol Versus Intravenous Vitamin D Analogues in the Treatment of Secondary Hyperparathyroidism Sandeep Aggarwal, Ellie Kelepouris, Irfan Ahmed, Ami Patel. *Nephrology, Drexel University College of Medicine, Philadelphia, PA.*

Background: With the move toward bundled payment for dialysis to include injectable medications, alternative agents are being considered to save cost without impairing outcome in the treatment of secondary hyperparathyroidism.

Methods: We conducted a retrospective study of a single inner city dialysis unit where a cohort of dialysis patients with Medicare insurance were converted from intravenous vitamin D analogues (paricalcitol or doxercalciferol) to oral calcitriol. We investigated the differences in calcium, phosphorus, and intact parathyroid hormone (iPTH) levels before and after implementation of calcitriol therapy. A total of 52 dialysis patients were analyzed for serum calcium, phosphorus, and iPTH levels prior to calcitriol initiation and 8-12 weeks post calcitriol initiation. Equivalent doses of oral calcitriol were calculated using KDOQI guidelines.

Results: There was no significant difference in calcium and phosphorus levels detected after switching to calcitriol. The oral calcitriol dosage was significantly lower compared to the calculated equivalent doses of doxecalciferol or paricalcitol. The intact PTH levels were higher after introduction of calcitriol but did not reach statistical significance. Preliminary data suggested significant per patient cost reduction with conversion to calcitriol. The incidence of hypercalcemia(>10.2 mg/dl) was 7% in intravenous group and 9% in oral group.

Results

	Ave.	STDEV	95% CI	p
Pre PTH pg/ml	348.19	236.15	348.19±78.05	0.568
Post PTH	379.02	275.10	379.02±73.12	
Pre Phos mg/dl	5.53	1.76	5.53±0.51	0.981
Post Phos	5.52	1.68	5.52±0.47	
Pre Calcium mg/dl	9.24	0.75	9.24±0.23	0.723
Post Calcium	9.29	0.77	9.29±0.21	
Calcitriol Dose mg	4.09	3.11	4.09±1.05	0.0098
Pre Calcitriol (Dose equiv)	6.20	4.59	6.20±1.19	

Conclusions: Oral calcitriol is equivalent to injectable vitamin D analogues in the treatment of secondary hyperparathyroidism.

PUB081

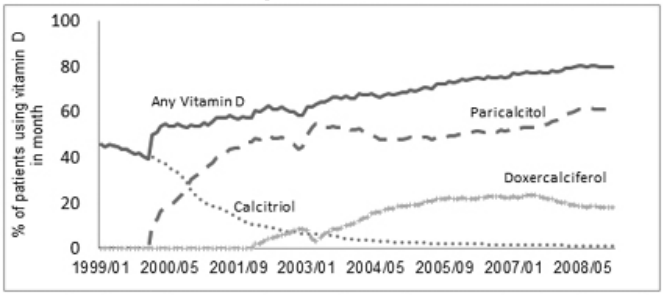
Trends in Intravenous Vitamin D Use among Dialysis Patients in the United States (1999-2008) Anne C. Beaubrun, Abhijit V. Kshirsagar, Lily Wang, M. Alan Brookhart. *University of North Carolina, Chapel Hill, NC.*

Background: Injectable vitamin D agents are commonly used to manage renal osteodystrophy in the dialysis population. Yet, there is little data documenting the temporal variability and patterns of use of these agents. We sought to to describe patient- and facility-level utilization patterns of vitamin D formulations (calcitriol, paricalcitol, doxercalciferol) in the US hemodialysis population.

Methods: We studied adult patients in the United States Renal Data System (USRDS) between January1999 and December 2008 with Medicare as a primary payer and who initiated dialysis at least 90 days prior to the month being investigated. Monthly percentages of patients treated with any vitamin D and each type of formulation were tabulated.

Results: We identified 140,790 dialysis patients in January 1999 and the number of eligible patients steadily increased to 214,113 in December 2008. Between 1999 and 2008, vitamin D use steadily increased with the exception of a slight decrease in use in the latter half of 1999. The use of calcitriol has declined since 1999, going from being administered in 44% of patients in January 1999 to 1.1% in December 2008. Paricalcitol is now the overwhelmingly preferred formulation. Doxercalciferol use in the dialysis cohort began in 2002, steadily increased and has begun to slightly decline since 2007. As of 2008, approximately 80% of the USRDS population used any vitamin D formulation.

Conclusions: Vitamin D use has increased and parallels the rise in use of paricalcitol and doxercalciferol. Furthermore, there is variation in formulation choice. Given the known pharmacologic differences in the vitamin D formulations, future research should focus on identifying the reasons for differences in vitamin D use, and, whether the variation in vitamin D use differentially affects patient outcomes.



PUB082

Switching Haemodialysis Patients from Oral to Intravenous Vitamin D May Not Improve Adherence to Prescribed Medication Alison Brown,[1] Claire Jackson,[2] James Shawcross,[1] George Hartley.[1] *[1]Renal Unit, Freeman Hospital, Newcastle upon Tyne, Tyne and Wear, United Kingdom; [2]Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*

Background: Achievement of recommended calcium, phosphate and parathyroid hormone (PTH) levels in haemodialysis (HD) patients remains a significant challenge. Our 260 HD patients are reviewed by renal dieticians who prescribe and adjust phosphate binders, and clinicians who adjust Vitamin D (Vit D) dose; medication is then dispensed in primary care. Our unit results in the UK Renal Registry compare well with the rest of the UK. However, compliance with prescribed medication remains variable. We change patients from oral self-administered Vit D to intravenous (IV) given on HD when control or compliance is poor. We describe the results of audit of bone chemistry and compliance with oral and IV Vit D.

Methods: Our local protocol based on KDOQI and UK Renal Association recommendations targets these predialysis levels :

PTH 120-540 ng/l, Adjusted calcium 2.20-2.50 mmol/l, Phosphate 1.10-1.70 mmol/l

We reviewed predialysis serum PTH, adjusted calcium and phosphate levels, and Vitamin D analogue prescription in all established HD patients during December 2010.

Results: Complete data was available for 222 of 260 patients. Only 10.8% had results within target for all parameters

Of 138 patients receiving Vit D, 55% had levels within target range for PTH, 48% for calcium and 44% for phosphate

Of 84 patients not prescribed Vit D, 55% had levels within target for PTH, 45% for calcium and 43% for phosphate

36% of all patients were taking oral Vit D doses different to that recorded in hospital notes

Only 70% of IV Vit D doses were adminstered as prescribed

Conclusions: Although achieved bone chemistry compares well with other UK renal units, 36% of oral and 30% of IV doses of Vit D differed from the recommended dose in our HD patients ; giving poorly controlled or compliant patients IV Vit D did not significantly improve compliance with medication.

Our study highlights the importance of documenting Vit D medication accurately to ensure alterations are appropriate. Future introduction of electronic and patient-held records may help to achieve this.

PUB083

Long-Term Skeletal Health and Integrity in Renal Transplant Patients – Are we Measuring the Right Parameters? Nihil Chitalia,[1] Sharon Frame,[1] Ana Sofia Rocha,[2] David Goldsmith.[1] *[1]Nephrology and Transplantation, Guy's and St. Thomas' Hospital NHS trust, London, United Kingdom; [2]Renal Medicine, St. George's Hospital NHS trust, London, United Kingdom.*

Background: As the success of kidney engraftment improves, there is a progressively larger cohort of renal transplant patients surviving a long time. We established a specialized multidisciplinary clinic to focus on best management of these patients' multiple medical issues. It is well-known that bone and mineral metabolism (CKD-MBD) parameters are abnormal after renal transplantation, but long-term data are scarcer, particularly with reference to bone mineral density as measured by DEXA scans

Methods: We took 151 well, ambulant renal transplant patients who were transplanted for more than 10 years and examined their CKD-MBD parameters and DEXA scans in detail

Results: The findings are summarized in Table 1.

Table 1: Demographic, biochemical and bone mineral density categories on DEXA scan in ambulant transplant recipients on long term follow-up (n=151)

Variable	Mean±SD or percentages
Age *(yrs)*	54±12
Gender (% Males)	62
Months post transplant (median±IQR)	207±126
eGFR *(ml/min/1.73m2)*	49±20
Haemoglobin (gm%)	12.8±1.5
Serum Calcium *(mmol/L)*	2.4±0.15
Serum Phosphate *(mmol/L)*	1.04±0.25
Serum Alkaline Phosphatase *(U/L)*	77±30
Serum PTH pmol/L	94±67
Serum 25 Hydroxy Vitamin D (nmol/L)	48±31
Vitamin D Deficient <37.5 nmo/L Vitamin D insufficient 37.5-75 nmol/L	59(39.1%) 58(38.4%)
Vitamin D sufficient >75 nmol/L	34(22.5%)
% on Steroids	77(51%)
% on Bisphosphonates	17(11%)
% on Vitamin D or Calcium supplements	26(17%)
BMD Category	
Normal	65(43%)
Osteopenia	65(43%)
Osteoporosis	20(14%)

Diagnosis of osteoporosis based on the National Osteoporosis foundation guidelines 2008.

On bivariate correlations between bone biochemistry and T-scores on DEXA scanning, serum calcium showed a negative correlation with T-score at neck of femur (NOF)(r=-0.296, p=0.002), whereas serum PTH showed a positive correlation with t-score at NOF (r=0.324, p=0.01). Serum 25 hydroxy vitamin D did not show a significant correlation with T-scores at any sites on DEXA scanning.

Conclusions: In a selected ambulant population of long-term renal transplant survivors, there were only 8% of patients with normal serum calcium, phosphate, PTH and 25(OH) ≥75nmol/L. Vitamin D deficiency and abnormal BMD indicative of osteopenia or osteoporosis is common in transplant recipients but did not seem to correlate with the usual biochemical parameters typically measured in patients on clinic visits. There is a need for further research into novel biomarkers and longitudinal study data.

PUB084

Effectiveness of a MG-Based Phosphate (P) Binder on the Development of Vascular Calcifications (VC) in Uremic Rats Tineke De Schutter,[1] Ellen Neven,[1] Geert J. Behets,[1] Mirjam Peter,[2] Sonja Steppan,[2] Jutta Passlick-Deetjen,[3] Patrick C. D'Haese.[1] *[1]Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium; [2]Fresenius Medical Care, Bad Homburg, Germany; [3]Nephrology, University of Dusseldorf, Germany.*

Background: Ca-based P-binders are widely used to control hyperphosphatemia, however go along with hypercalcemia and accelerated progression of VC. We compared the effect of 2 doses of the Mg-based P-binder CaMg (2/3 Ca-acetate and 1/3 Mg-carbonate, Osvaren®) to that of sevelamer carbonate (sev) on the development of VC in rats with CRF.

Methods: 56 male rats were divided in 4 groups: Vehicle (Veh), 375mg/kg CaMg, 750mg/kg CaMg, 750mg/kg sev. CRF was induced by feeding 0.75% adenine (AD)-2.5% protein diet for 4 wks. After 1 wk of CRF, rats were gavaged with P-binders or Veh (7d/wk) until sacrifice at wk 6.

Results: Renal function was sign. impaired after 4 wks of AD-treatment and comparable in all groups. Veh-treated CRF rats developed severe hyperphosphatemia which was well controlled in the groups receiving P-binders particularly those receiving CaMg even at the lowest dose (Cmax Serum P: Veh: 20.3 - CaMg375: 11.5 - CaMg750: 9.9 - sev: 16.3mg/dl). AUC0-6wks of serum Ca did not differ sign. between groups. Serum Mg AUC0-6wks dose-dependently increased in CaMg treated groups (Veh: 15.0 - sevelamer: 16.0 - CaMg375: 20.8 - CaMg750: 27.16 mg.wk/L). Induction of CRF went along with a sign. increase in serum PTH. Treatment with CaMg dose-dependently prevented this increase whilst sev did not. Based on tissue Ca measurements, aortas of CaMg treated rats were sign. less calcified compared to Veh. Sev had no sign. effect on aortic calcifications although a clear reducing trend was seen. In contrast, calcifications in A. femoralis and A. carotis were sign. reduced by sev and showed a clear trend for CaMg, indicating a possibly different mechanism of calcification in these arteries. Results were confirmed on Von Kossa stained sections, showing that both CaMg and sev sign. reduced the area% calcification in the aorta.

Conclusions: Treatment with the Mg-based P-binder CaMg effectively controlled serum P and PTH levels resulting in reduced aortic calcifications in uremic rats.

Funding: Pharmaceutical Company Support

PUB085

Chronic Kidney Disease-Mineral and Bone Disorder in a Cohort of Internal Inpatients: A Prospective Analysis

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Background: Patients with chronic kidney disease (CKD) are at excess risk of cardiovascular morbidity and mortality. Abnormalities of parameters related to bone and mineral metabolism are common in CKD patients. Aim of the present study was the determination of prevalence of CKD-MBD in inpatients at a general internal ward.

Methods: In a prospective analysis (November 2008 - February 2009) eGFR and serum levels of phosphate (P), calcium (Ca), parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) were measured from 238 patients who were admitted to a general internal medical ward. Patients with acute renal failure (n=3) and those who were on dialysis (n=7) were excluded.

Results: The patient population consisted of 228 patients (110 women) with a median age of 76 years (20-99); women were significant older than men ($p<0.001$). The median eGFR of the patients was 60.7 ml/min/1.73m² (10.4-171.9), no gender-specific difference was detectable. The overall prevalence of CKD (eGFR<60) was 49.1%. There was a significant correlation between eGFR and P ($r=-0.307$; $p<0.001$) and PTH ($r=-0.395$; $p<0.001$). eGFR was not associated with Ca and 25(OH)D. Compared to individuals with eGFR≥60, CKD patients were significant older [80 (37-99) vs. 67 (20-97)], showed higher P [3.24 mg/dl (1.36-6.56) vs. 2.97 (1.40-3.46)] and higher PTH levels [64.2 pg/ml (13.9-260.8) vs. 38.2 (15.8-264.0)]; ($p<0.001$). A significantly higher proportion of subjects with CKD had PTH levels >65 (23% vs. 12%; $p<0.001$). The overall prevalence of hypovitaminosis D (25(OH)D <30 ng/ml) was high (83.8%), but there was no significant difference of 25(OH)D levels between the CKD and non CKD group.

Conclusions: 50% of the acutely admitted patients had an eGFR<60 ml. Patients with CKD had higher P and PTH levels compared with those with an eGFR ≥ 60. There was a high overall prevalence of low 25(OH)D levels, however no association was found between renal function and hypovitaminosis D. The data indicate that CKD per se may not be a risk factor for developing vitamin D deficiency.

PUB086

Single-Dose Pharmacokinetics and Tolerability of Paricalcitol Capsules in Pediatric Subjects with Moderate to Severe Chronic Kidney Disease

Tushar Garimella, Samina Khan, Cheri Enders Klein. *Abbott Laboratories, Abbott Park, IL.*

Background: Paricalcitol (Zemplar) is a selective vitamin D receptor activator approved in adults for the prevention and treatment of secondary hyperparathyroidism associated with chronic kidney disease (CKD). The pharmacokinetics, safety and tolerability of single doses of paricalcitol capsules were evaluated in pediatric subjects with CKD stage 3 and 4 to support dose selection for a clinical study in similar population to study the effect of paraicalcitol on treatment of secondary hyperparathyroidism (SHPT).

Methods: This was an open-label, single oral dose study in pediatric subjects aged 10 to 16 years. Following a 3 mcg dose of paricalcitol, sequential blood samples were collected for 48 hours and pharmacokinetic parameters were determined using noncompartmental methods. Safety was assessed by adverse event monitoring, vital signs, physical examinations, ECGs and laboratory assessments.

Results: Six subjects with CKD Stage 3 (age range 10-16 years and body weight range 36-108 kg) and five subjects with CKD Stage 4 (age range 11-16 years and body weight range 37-62 kg) received study drug. Mean paricalcitol C_{max} was 0.12 ng/mL and 0.14 ng/mL and mean AUC was 2.6 ng*hr/mL and 3.2 ng*hr/mL in the Stage 3 and 4 subjects, respectively. After normalizing for dose and body weight the mean C_{max} and AUC in pediatric Stage 3-4 subjects were 18% and 13-15% lower, respectively, than adults with CKD The harmonic mean half-life of paricalcitol was 13.4 and 16.1 hours after single doses in subjects with CKD Stages 3 and 4, respectively. Overall no adverse events were reported to be related to study drug and no safety concerns were encountered in the single dose study.

Conclusions: The pharmacokinetics of paricalcitol were similar in pediatric subjects with CKD Stages 3 and 4 after single oral doses and appear to be similar to those in adults with CKD Stages 3 and 4 when corrected for dose and body weight. The pharmacokinetic data from these subjects was used to select a 1 mcg TIW dose for a clinical study in CKD stage 3 and 4 pediatric subjects with SHPT.

Funding: Pharmaceutical Company Support

PUB087

Prevalence and Factors Associated with Vitamin D Insufficiency/Deficiency in Dialysis Patients: Results of the French National Observatory for Mineral and Bone Metabolism

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Background: Vitamin D insufficiency/deficiency is very common in dialysis patients and has been associated with a less favourable survival rate.

The aim of this study was to analyse the prevalence and the factors associated with vitamin D deficiency in patients on maintenance dialysis.

Methods: Using October 2010 baseline data from a national, prospective observational study evaluating the management of mineral metabolism abnormalities in patients who were on haemodialysis for less than 12 months and not taking nutritional vitamin D, we have compared those with a serum 25-hydroxy vitamin D (25-D) < 75 nmol/l with those ≥75 nmol/l.

Results: Of 3,808 patients, 2,380 (62.5%) were tested for serum 25-D, mean age was 68.3 ± 15.5 years and 37.6% were female. Average serum 25-D was 64.3 ± 42.4 nmol/l. Among the subset of patients who were not treated with nutritional vitamin D (n=928), serum 25-D was associated with the following factors.

Table 1

Patients not treated with nutritional vitamin D	25-D < 75 nmol/l n= 672	25-D ≥ 75 nmol/l n= 256
Age (years)	68.6 ± 15.5	67.6 ± 16.3
BMI kg/m²	25.7 ± 6.0	24.6 ± 5.0 *
Kt/V	1.26 ± 0.30	1.33 ± 0.30 **
Ca mmol/l	2.20 ± 0.19	2.22 ± 0.18
P mmol/l	1.60 ± 0.58	1.56 ± 0.51
iPTH pg/ml°	236 [120-416]	193 [93-337] **
Albumin g/l	34.7 ± 5.1	36.3 ± 5.4 ***
Hemoglobin g/l	111 ± 15	113 ± 15 *
Diabetes	37.4%	31.3%
Vasc. Calcifications	40.0%	34.8%

° Median [Q1-Q3], *P < 0.05, **P < 0.01, ***P < 0.001, Chi² or t-test.

No other clinical, biochemical or therapeutic factor was significantly associated with vitamin D deficiency.

Conclusions: Vitamin D insufficiency/deficiency frequently occurs in patients on maintenance dialysis. Factors associated with this insufficiency include larger BMI, low Kt/V, albumin, haemoglobin and high iPTH.

Project Photo-Graphe 3 (SVCARB09910)

Funding: Pharmaceutical Company Support

PUB088

Prevalence and Factors Associated with Vitamin D Insuffciency/Deficiency in Non Dialysed Chronic Kidney Disease Patients: Results of the French National Observatory for Mineral and Bone Metalolism

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Background: Vitamin D insufficiency/deficiency is frequently observed in CKD patients and has been associated with a more rapid CKD progression and reduced survival rate. We report the prevalence and the factors associated with vitamin D deficiency in CKD stage 4-5.

Methods: Using October 2010 baseline data from a national, prospective observational study evaluating the management of mineral metabolism abnormalities in CKD patients with eGFR < 60 ml/min/1,73m² and not taking nutritional vitamin D, we have compared those with serum 25-hydroxy vitamin D (25-D) < 75 nmol/l with those ≥75 nmol/l.

Results: Among a total of 876 patients, 585 (67%) were tested for serum 25-D, mean eGFR 21 ±7 ml/min/1,73m² and 41% were female. Average serum 25-D was 62.9 ±40.4 nmol/l. 67.2% had a serum 25-D < 75 nmol/l. Among the subset of patients who were not treated with nutritional vitamin D (n=200), serum 25-D was associated with the following factors.

Table 1

Patients not treated with nutritional vitamin D	25-D < 75 nmol/l n= 142	25-D ≥ 75 nmol/l n= 58
Diabetes	43.7%	25.9% *
Previous CV history	64.1%	46.6% *
Age (years)	72.5 ± 12.5	68.6 ± 15.3
BMI kg/m²	27.9 ± 6.3	25.4 ± 4.5 *
iPTH pg/ml °	156 [82-246]	120 [65-210]
Albumin g/l	38.9 ± 4.5	39.3 ± 4.5
CRP mg/l °	5 [2-15]	2 [1-5] **

° Median [Q1-Q3], *P < 0.05, **P < 0.01, chi² or t-test.

Discussion: A multivariate analysis is pending to further identify independent risk factors.

Conclusions: Vitamin D insufficiency/deficiency is widely prevalent in the majority of patients with stage 4-5 CKD. Factors associated with vitamin D deficiency include diabetes, previous cardiovascular history, larger BMI and inflammation. The potential benefits of nutritional vitamin D supplementation need to be examined prospectively.

Project Photo-Graphe 3 (SVCARB09910)

Funding: Pharmaceutical Company Support

PUB089

Low 25-hydroxyvitamin D Levels Are Associated with an Increased Risk of Sepsis in a Large Managed Care Organization

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Background: Vitamin D is an important mediator of immune function and vitamin D deficiency may play an important role in the development of infections, which is the second cause of death in ESRD patients. We hypothesized that patients with vitamin D deficiency would have an increased risk of sepsis admissions.

Methods: We conducted a population-based cohort study of adult patients requiring hospitalization for sepsis who had vitamin D levels measured 3 to 15 months prior to sepsis admission. Vitamin D was evaluated as a continuous variable and a categorical predictor (< 15 ng/mL vs. higher). The primary outcome of interest was sepsis admissions identified through ICD9 codes. Patients admitted with sepsis were matched 1:1 with controls on age, sex, race and season. Conditional multivariate logistic regression was used to evaluate if low vitamin D levels were associated with an increased risk of sepsis admission.

Results: We identified 211 individuals with sepsis and 211 matched controls. The mean (SD) age of the participants was 65±14 years, 59% were female and 91% were Caucasian. Compared to controls, patients with a sepsis admission had a significantly higher percentage of diabetes (46.0% vs. 30.3 %; p=0.0009) and chronic kidney disease (36.0% vs. 21.8%; p=0.001). Mean (SD) levels of 25(OH)D were lower in patients with sepsis than controls (28.8 ±16 vs. 32.1 ± 17 ng/mL p=0.046). After adjustment for diabetes, renal disease and peripheral vascular disease higher 25(OH)D levels were protective for sepsis (OR 0.99, 95% CI 0.98-0.995; p=0.0006). Similarly, when evaluated as a categorical predictor, vitamin D levels < 15 ng/mL vs. higher (adjusted OR 2.20, 95% CI 1.41-3.50; p=0.0006) were strongly associated with a two-fold increased odds of sepsis admissions.

Conclusions: Vitamin D deficiency is associated with an increased risk of sepsis hospitalizations. Clinical trials are needed to evaluate whether vitamin D_3 supplementation can reduce the risk of sepsis in patients with and without CKD.

PUB090

Trends in Demographic and Abnormalities in Bone Mineral Metabolism Parameters in Incidents Dialysis Patients between 2009-2011. Evidence for Vitamin D Deficiency

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Background: The prevalence of elderly treated patients (pts) on hemodialysis (HD) has been increasing steadily..The.Phosphocalcic disorders(PCD) have not been studied in these pts.

Methods: A single cohort retrospective database analysis of 24 incidents (HD pts) in 2009 and 2011 was performed.Information on demographics, laboratory data and treatments were abstracted from medical records.

Results: Mean age: 77.7 year, mean plasma albumin value was 34.8 gr/L . CRP mean value was 6.8 mg/L. The mean P value was 1.5 mmol/L .The mean value of Ca was 2.15 mmol/L. Mean iPTH was 337.3pg/mL with 68.2% of pts having iPTH between 134 pg/mLand 603pg/L . iPTH was in 13 % > 603 pg/mL.

The 25(OH)D level (ng/mL) was < 30 ng/mL in 70 % of pts with a mean value of 24.1 ng/mL.

Treatments :36.4 % of pts were having Calcium with a mean dose of 1.16 gr of calcium acetate , 22.7 % of pts were receiving sevelamer with an average dose of 1.60 gr /day , 18.2 % were treated by Cinacalcet with an average daily dose of 30 mg.90.9 % of pts were supplemented with oral vitamin D 3 (cholecalciferol) with mainly an average dose of 75 µg once a week or for a few 2.5 mg (100 000 UI) once a month .18.2 % of pts were treated by an active vitamin D (IV or per os)with an average weekly dose of 1.56 µg.

Discussion: vitamin D deficiency increases in the elderly HD pts with a low 25(OH) D. Treatment strategies have been focused, until recently , on suppression of the parathyroid ‘s glands activity. But an associated age-related decline in iPTH can lead to adynamic bone disease ,these pts being already in a state of low bone remodeling.

Conclusions: Our study highlights the changing presentation of PCD and the adjustements of the treatment practices with a necessary use of vitamin D analogs which are low costs with a lack of side effects.Further studies are mandatory to evaluate the benefits on relevant patient outcomes.

PUB091

Cholecalciferol Is More Efficient Than Ergocalciferol in Normalizing 25OH Levels in Prevalent Hemodialysis Patients

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Background: Hemodialysis patients(hd) have low levels of 25(OH)vitamin D and supplementation with ergocalciferol(ergo)D2 or cholecalciferol(chole)D3 is recommended. We have previously shown that a 3 months supplementation with a 600000 IU cumulative dose of ergo failed to normalize 25(OH)D plasma levels in 40% of hd. However chole might be more efficient than ergo.

Methods: Six months, prospective, non randomized open-study was conducted in a cohort of 97 unselected prevalent hd from 2 large in-center french facilities with different modalities of supplementation. Patients were divided in 2 monthly oral treatment groups: in group1 hd received 200000IU of ergo, in group2 hd received 200000IU of chole. Plasma levels of 25(OH)D(normal 30 µg/l)and parathormone(PTH) were measured at baseline, month 3 and month 6. Total calcemia(Ca) and phosphatemia were measured monthly from baseline to month 6. Pre defined exclusion criteria at entry and per study were: native vitamin D supplementation less than 6 months before inclusion, Ca>2,65 mmol/l on 2 consecutive dosages and 25(OH)D plasma level>100 µg/l.

Results:

Baseline characteristics and evolution of biologic parameters

	Group 1 (ergo)(n=69)	Group 2 (chole)(n=29)
Age (years)	69,1	74,7
HD duration(months)	55,6 (53,6)	70,2 (66,7)
Baseline25(OH) D(mcg/l)	19,9(7,6)	18,0(6,5)
Baseline Ca(mmol/l)	2,16(0,1)	2,2(0,15)
Baseline Phosphatemia(mmol/l)	1,66(0,51)	1,36(0,55)
Baseline PTH(pg/ml)	437(351)	405(346)
25(OH)D M3** (mcg/l)	29,5(10,2)	41,6(17,3)
Ca M3**(mmol/l)	2,18(0,20)	2,30(0,13)
Phosphatemia M3(mmol/l)	1,57(0,50)	1,43(0,57)
PTH M3(pg/ml)	376(322)	428(357)
25(OH) D M6**(mcg/l)	32,5(11,7)	44,3(13,6)
Ca M6*(mmol/l)	2,18(0,19)	2,28(0,15)
Phosphatemia M6(mmol/l)	1,61(0,58)	1,49(0,53)
PTH M6(pg/ml)	386(374)	418(418)

results are expressed by means (SD) * p<0,05 ** p<0,001

At month 6, 25(OH)D was>30µg/l in 82,8% of group2 hd and in 56,5% of group1 hd (p=0,01). No significant episod of hypercalcemia nor toxic 25(OH)D levels were observed.

Conclusions: Chole is more effective than ergo in normalizing 25(OH)D in hd. Modalities of long term supplementation remain to be established.

PUB092

Efficacy and Safety of Paricalcitol Dosing Regimens for Subjects on Hemodialysis in China

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Background: In China there is a growing prevalence of hemodialysis (HD) subjects with secondary hyperparathyroidism (SHPT). The study aim was to evaluate the safety and efficacy of paricalcitol a selective vitamin D receptor (VDR) activator for reduction of intact parathyroid (iPTH) levels in HD subjects.

Methods: In this randomized, multi-center, single-blind study 216 subjects received dosing regimens based on EU (iPTH/80) or US (0.04µg/kg) package inserts (PI) of paricalcitol over 12 weeks. Primary efficacy analysis evaluated non-inferiority of EU compared to US PI in proportion of subjects achieving at least 2 consecutive ≥30% decreases from baseline iPTH. Secondary analyses evaluated the proportion achieving KDOQI (150-300pg/mL) and KDIGO (130-585 pg/mL) recommended iPTH ranges incidence of hypercalcemia (at least 2 consecutive Ca>11.0mg/dL) and changes from baseline iPTH to each post-baseline visit.

Results: A higher proportion (88.6%) of subjects initiated at iPTH/80 achieved at least 2 consecutive ≥30% decreases in baseline iPTH than subjects receiving 0.04µg/kg (55.9%). A comparable proportion treated with both regimens achieved KDOQI (17.6% EU and 19.4% US) and KDIGO (59.3% EU and 54.6% US) recommended iPTH ranges. Repeated measures analysis showed significantly greater mean reduction from baseline iPTH (p<0.001) in subjects receiving iPTH/80 compared to subjects receiving 0.04µg/kg. Overall, treatment with iPTH/80 and 0.04µg/kg dosing presented a low incidence of hypercalcemia (1 of 216).

Conclusions: Paricalcitol dosing of iPTH/80 (EU) demonstrated superiority over 0.04µg/kg (US) in achieving ≥30% reductions from baseline iPTH levels. However, both regimens were comparable in achieving KDOQI and KDIGO recommended iPTH ranges with low risk of hypercalcemia. Paricalcitol represents a potentially beneficial treatment option for subjects with SHPT on HD in China.

Funding: Pharmaceutical Company Support

PUB093

Ergocalciferol (ergo) Therapy in Calcidiol Deficient Hemodialysis (HD) Patients on Therapeutic Doses of Paricalcitol Does Not Decrease Cytokine Release in Peripheral Blood Mononuclear Cells

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Background: Vitamin D possesses immuno-regulatory activities such as inhibiting nuclear factor-κB activity, increasing IL-10 production and decreasing IL-6 and TNF-α production. It is unclear whether additional therapy with ergo in those with calcidiol deficiency would inhibit cytokine release by peripheral blood mononuclear cells (PBMC) and thus have beneficial anti-inflammatory effects in this population.

Methods: We conducted a randomized double blind cross-over trial of ergo vs placebo in 24 HD patients treated with therapeutic doses of paricalcitol with iPTH range between 150-600 pg/ml but with plasma 25(OH) vitamin D levels <30 ng/ml and high sensitivity C-reactive protein (hs-CRP) >3mg/L. 24 HD participants were randomly assigned to ergo 50,000 U/week vs. placebo for 12 wks, followed by 4 wk washout and cross-over for 12 wks. Blood was collected at baseline and wks 12, 16 and 28. PBMC were obtained using standard Ficoll-Paque isolation then incubated for 24 h plus or minus 10 ng/ml LPS, in media with 10% patient serum. Cytokine release to media was tested (ELISA).

Results: The test population demographics were 59 ± 13 yrs, 42% men, 80% Caucasian, 67% diabetic and the average duration of ESRD was 3.7±4.6 years. Ergo treatment significantly increased plasma 25(OH) vitamin D levels (p<0.001). Table 1 shows the difference between pre and post ergo vs placebo treatment in LPS-induced release of IL-6 or TNF from PBMC.

Difference in pre and post ergo vs the pre and post placebo treatment

	Mean(95%CI)	p value
Plasma 25(OH) vitamin D ng/ml	17.5(9.3-25.7)	<0.001
IL6* pg/ml	1.00(0.53-1.90)	1.00
TNF-α * pg/ml	0.93(0.61-1.42)	0.74

*LPS stimulated release from PBMCs; IL6 and TNFα are log transformed

Conclusions: Ergo treatment of HD patients increases 25(OH) vitamin D levels but had no significant effect on PBMC response to LPS treatment in vitro. These results suggest that ergo treatment will not decrease inflammation in this patient population.

PUB094

The Effect of Cholecalciferol Supplementation on Physical Function and Health Related Quality of Life in 25 Hydroxy Vitamin D Insufficient Hemodialysis Population

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Background: To determine the effects of Vitamin D supplementation on physical performance and health-related quality of life in a hemodialysis population

Methods: Subjects were followed for 4 weeks without treatment (as a control period), then were supplemented with 3000 IU cholecalciferol (vitamin D3) for 8 weeks. The following tests were performed at -4 weeks, baseline, 4 and 8 weeks post-supplementation: 25-hydroxyvitamin D (25OHD); parathyroid hormone (PTH); physical assessment including 6 minute walk,handgrip strength and Short Physical Performance Battery (SPPB); health questionnaires including cognitive screen, activities and instrumental activities of daily living, SF-36 and Geriatric Depression Scale.

Results: 30 subjects (mean age 64 + 16 years; 18 men/ 12women; 57% Caucasian) had 25 OHD levels of 19.9 + 7.7ng/dl. Twenty-four subjects (80%) had 25 OHD less than 30 ng/dl. Sixty-five percent of participants reported general health to be fair or poor. While 25 OHD increased significantly to 26.5+8.9 after supplementation, there were no changes in physical performance or health related quality of life.

Conclusions: Our data confirms that 25 OHD insufficiency is widely prevalent in the ESRD population. Further, health-related quality of life is rated below good in a majority of the patients. Modest cholecalciferol supplementation was able to increase 25OHD levels, but a significant portion of patients remained below 35 ng/dl, a level required to benefit many health outcomes including physical performance, suggesting a need for higher doses of cholecalciferol. We found no significant change in physical performance or quality of life. Further work will be required to address the appropriate dose of cholecalciferol to obtain sufficient 25OHD levels and whether physical performance or quality of life improve with supplementation in ESRD patients.

Funding: Private Foundation Support

PUB095

Cinacalcet Hydrochloride Prohibits Bone Mineral Density Loss in Dailysis Patients

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Background: In dialysis patients, bone mineral density(BMD) loosing is closely related not only to the progress of chronic kidney disease-mineral bone disorder(CKD-MBD) but also to the patient mortality.In this study we evaluated whether Cinacalcet hydrochloride (CH) is effective in keeping BMD or not.

Methods: We selected 106 patients from the consecutive 152 chronic hemodialysis patients for the current study.The inclusion criterion of the patients was that their %BMD was more than 80%.106 patients were divided into the following 3 groups by the medication for secondary hyperparathyroidism (2HPT).

Group A: intra-venous vitamin D administration (i.v. VD)+CH

Group B: i.v. VD

Group C: oral VD administration

We compared the changes in %BMD, intact-PTH (iPTH) and bone alkaline phosphate (BAP) among 3 groups 6 months and 18 months after the administration of CH.

Results: i-PTH when CH before started in group A was 450.3±266.1 pg/mL, then it was significantly reduced after CH started.%BMD was significantly reduced Group B and C but did not change in Group A.The mean values in iPTH and BAP 6 months and 18 months of each group were not significantly different (Table 1).

Parameters of each group

	%BMD		i-PTH			BAP	
Group	6 months	18 months	pre-CH	6 months	18 months	6 months	18 months
A	97.7±9.0	96.7±7.9	450.3±266.1	170.0±104.5	138.4±34.5	20.6±3.0	22.5±10.7
B	97.9±11.5*	96.3±12.3*	blank	130.2±57.4	141.3±69.5	24.8±12.9	25.1±11.3
C	102.8±11.9**	101.2±12.7**	blank	85.1±61.6	105.3±65.6	25.1±10.7	27.2±11.0

*,**p<0.01

Conclusions: In Group A, using CH together with i.v.VD has the possibility of prohibiting the decrease of the bone mineral density .And it seemed that it was preferable to use CH with i.v. VD as much as possible before the %BMD falls below 80%.

PUB096

Hipovitaminosis D in Macroalbuminuric Diabetic Nephropathy

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Background: The frequency of hypovitaminosis D in CKD patients is very high, although causal mechanisms are still unclear. In this study, we analyzed the frequency and predictors of hypovitaminosis D in diabetic nephropathy (DN) patients.

Methods: Data on 56 DN patients with proteinuria and class III CKD was collected from 2005-07. Mann-Whitney and chi-square tests were used; linear and logistic regression models were built for 25OH-vitamin D (25vitD).

Results: Mean 25vitD was 21,9 ng/mL(±10,1), with 84% of patients presenting a 25vitD <30 ng/mL. When patients were classified according to median 25vitD, those with <21 ng/mL presented lower creatinine clearance, hemoglobin, serum albumin and calcium, and higher proteinuria, serum phosphorus, urinary MCP-1, urinary VEGF and serum MCP-1. In linear regression, adjustment for creatinine clearance left only proteinuria, serum albumin and serum MCP-1 as significant predictors of 25vitD. However, further adjustment for age, sex and creatinine clearance left only proteinuria (B-1,24; 95%CI-2,26/-0,22; p=0,02) and serum MCP-1 (B-0,02; 95%CI-0,04/-0,01; p=0,002) as independent predictors. In the logistic regression model adjusted for creatinine clearance, both proteinuria and serum MCP-1 were independently related to the risk of lower 25vitD.

Logistic regression models on the risk of having 25OH-vitaminD < 21 ng/mL in DN patients.

	OR	Lower 95% CI	Upper 95% CI	p
Univariate models				
Estimated creatinine clearance (CG; ml/min/1,73m2)	0,97	0,94	1,00	0,05
24 proteinuria (g/d/1,73m2)	1,45	1,12	1,88	0,01
Serum MCP-1 (pg/mL)	1,01	1,00	1,01	0,01
Multivariate model				
Estimated creatinine clearance (CG; ml/min/1,73m2)	0,97	0,95	1,00	0,10
24 proteinuria (g/d/1,73m2)	1,31	1,01	1,70	0,04
Serum MCP-1 (pg/mL)	1,01	1,00	1,01	0,03

Conclusions: Hypovitaminosis D is extremely frequent in macroalbuminuric DN patients. While creatinine clearance may not be a major determinant of 25vitD, proteinuria and systemic inflammation were importantly associated to the risk of low 25vitD in our study.

Funding: Government Support - Non-U.S.

PUB097

Oral Cholecalciferol Therapy in Prevalent Hemodialysis Patients: A Randomized Placebo Controlled Pilot Study Karen To, Azim S. Gangji, Trevor J. Wilkieson, Cathy Z. Kotsamanes, Catherine M. Clase. *Medicine, McMaster University, Hamilton, ON, Canada.*

Background: Vitamin D is a prohormone that is activated in the liver and kidney. Besides the kidney, other tissues express 1α-hydroxylase. This local autocrine/paracrine activity is important for health maintenance and relies on adequate 25(OH)D levels. Objectives: To investigate the effects of cholecalciferol (D3) compared with placebo on i) serum 25(OH)D, calcitriol, parathyroid hormone (PTH), inflammatory and bone turnover markers; ii) blood pressure (BP); iii) the use of erythropoesis stimulating agents (ESA).

Methods: We conducted a randomized controlled pilot study of 20 prevalent adult in-center hemodialysis (HD) participants whom we assigned, using concealed randomization, to receive 50,000 IU of D3 or identical-appearing placebo weekly for 12 weeks. Health care providers, participants, and research staff were masked to allocation. The primary outcome was the change in 25(OH)D between baseline and week 12. We measured other laboratory outcomes at the same time points, and calcium and phosphorus every two weeks. We assessed BP as the mean of pre- and post-dialysis and collected information on medication use. Analysis was done according to the intention-to-treat principle. Unpaired t tests were used to assess differences between mean baseline and week 12 blood work results. Chi-squared tests were used to compare categorical variables between groups.

Results: Mean serum 25(OH)D levels of both groups were similar at baseline [14.7±6.1 (D3) versus 13.2±8.1 ng/ml (placebo);p=0.63], consistent with insufficiency.

The mean difference (week 12 minus baseline) in 25(OH)D levels were 25.1±14.3 and -1.2±3.7 ng/ml in the D3 and placebo groups, respectively (p<0.001). There were no differences in the change in calcitriol, PTH, ALP, CRP, ESR, ferritin, Alb levels between treatment groups. There were no differences between treatment groups in the mean change of BP, or in haemoglobin:ESA ratio. There were no episodes of hypercalcaemia (≥11 mg/dL). Episodes of hyperphosphatemia (≥9.3 mg/dL) were comparable between groups [n=2 (D3) versus n=3 (placebo); p>0.99].

Conclusions: Weekly D3 was effective in correcting the vitamin D status in HD participants.

Funding: Private Foundation Support

PUB098

Vitamin D Deficiency in Renal Transplantation Kristin Vibeke Veighey,[1] David C. Wheeler,[1] Mark Harber,[2] Anne B. Dawnay,[5] Francis Lam,[5] Martyn Egerton,[3] Aliyye Karasu,[4] John Cunningham.[1] *[1]UCL Centre for Nephrology; [2]Royal Free Hampstead NHS Trust; [3]Epsom & St Helier University Hospitals NHS Trust; [4]Anthony Nolan Trust; [5]UCL Hospital.*

Background: Animal studies have demonstrated a reduction in ischaemia-reperfusion (IR) injury following systemic administration of vitamin D. Vitamin D deficiency is endemic renal patients. We postulated that patients deficient in 25-hydroxyvitamin D (25OHD) and/or 1,25-dihydroxyvitamin (1,25OH2D) D might have poorer cardiac and endothelial function, greater IR injury at transplantation, and increased fibrosis on protocol biopsies.

Methods: 103 patients transplanted from 2008-2011 had protocol biopsies at 6(t6)/56 weeks(t56) and had stored serum from the time of transplantation. The population was 53.40% White, 22.33% Black, 14.56% Asian, 2.91% Mixed and 6.80% Other. None received supplemental vitamin D.

Serum 25OHD and 1,25OH2D were quantified by mass spectrometry (25OH D2/D3 by LC-MS/MS (Inter-assay CV <10%); 1,25OH2D by enzymeimmunoassay (IDS)).

Fibrosis on protocol biopsies was quantified using a validated index of chronic damage (ICD) score(%) (Mean inter-observer difference 1.0(CI 0.94-1.06)).

Results: In our cohort, 20(19.42%) were deficient(<25nmol/l), 57(55.34%) insufficient(25-75nmol/l), and 26(25.24%) replete (>75nmol/l) in vitamin D. 60% of Asian patients were deficient. In 97(94.17%), D2 was below the assay range.

62 patients (60.19%) were deficient and 41(39.81%) were replete(40-150pmol/l) in 1,25-dihydroxyvitamin D. 80% of Asian patients were deficient. 25-hydroxyvitamin D levels did not predict 1,25-dihydroxyvitamin D levels in many cases - 67.86% of those replete in the former were deficient in the latter.

Linear regression was used to assess associations between 1,25-dihydroxy-/25-hydroxy-vitamin D levels and ICD at t6 and t56. Despite adjustment for cold ischaemic time, donor age and recipient diabetes, there was no association between either measure and ICD at t6/t56.

Conclusions: Only 25% of this population was vitamin D replete at transplantation. The prevalence of deficiency amongst Asian patients was 60%. D2 status had little bearing on overall D status. There was no association between vitamin D status and chronic damage on protocol biopsies.

Funding: Pharmaceutical Company Support

PUB099

RAAS Blockade Abrogates the Effect of Parathyroidectomy on Renal Hemodynamics Liesbeth Viaene, Kathleen Claes, Bjorn K.I. Meijers, Pieter Evenepoel. *Nephrology, University Hospital, Leuven, Belgium.*

Background: Acute renal dysfunction is a common, although not universal finding after parathyroidectomy (PTX). Available experimental evidence points to a hemodynamic mechanism mediated at least partly by changes in the renin angiotensin aldosterone system (RAAS). In animal studies, parathyroid hormone (PTH) and calcium modify plasma renin activity. Supportive clinical evidence, however, is lacking.

Methods: We performed a prospective interventional study in renal transplant recipients with persistent hyperparathyroidism and hypercalcemia (NCT00452049). 16 patients (5 female, age 53±11 y) were enrolled of whom 10 were treated with RAAS blockade therapy at the time on inclusion. Mineral metabolism parameters (including PTH, FGF-23 and calcitriol) and renal hemodynamics (inulin and para-aminohippurate (PAH) clearances) were assessed before and after PTX.

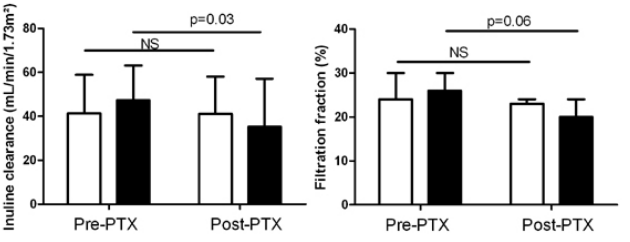
Results: PTH and calcium levels significantly decreased after PTX.

Evolution of parameters of mineral metabolism after PTX

	Pre-PTX	Post-PTX	p
Calcium (mg/dL)	10.9	8.9	0.0005
Phoshorus (mg/dL)	2.1	3.1	0.0005
PTH (ng/L)	98.9	4.8	0.0005
Calcitriol (ng/l)	55.1	50.5	0.6

Median values are shown

Inulin clearances (GFR) decreased whereas PAH-clearances (Renal Blood Flow) remained unchanged. Filtered fraction tended to decrease after PTX (p=0.07). Subgroup analysis in patients with and without RAAS blockade therapy revealed changes in renal hemodynamics in patients free of RAAS blockade only, despite similar PTH and calcium levels and similar calcitriol exposure pre- and post PTX.



Inuline and filtration fraction in patients free (black bars) and on (white bars) RAAS blockade before and after PTX.

Conclusions: GFR and filtered fraction (tend to) decrease after PTX. RAAS blockade abrogates the effect of PTX on renal hemodynamics. Our data support the thesis that PTH and calcium affect renal hemodynamics through modulation of the RAAS.

PUB100

Markedly Suppressed Parathyroid Levels in Bedridden Diabetic Patients Compared to Ambulatory Diabetic Patients on Dialysis Michael Yudd, Gabriela Wojnarska-Alvarez, Payam Benson, Parin M. Makadia, Albert Siu, Filberto D. Kelly, Shivangi Patel. *Renal Section, Veterans Affairs New Jersey Health Care System, East Orange, NJ.*

Background: Suppressed PTH levels in ESRD are noted in diabetics and the elderly, and are associated with Malnutrition- Inflammation Complex (MIC) surrogates.

Methods: We evaluated our clinical impression that sick bedridden patients had makedly depressed PTH levels. All patients in a single hemodialysis unit were divided into 3 groups: ambulatory(n=30), bedridden for at least 3 months (n=10), and wheel-chair bound (n=10). Demographic, clinical, and treatment parameters of renal bone disease were compared in the bedridden patients to the ambulatory ones. Since 90% of our bedridden patients were diabetic, we then compared these to the 14 diabetic ambulatory patients.

Results: Serum intact PTH was much lower in the bedridden patients compared to the ambulatory ones: 81.7 +/- 57.37 vs. 410.0 +/- 281.32 pg/ml (p = 0.008). This difference persisted when the diabetic bedridden pts were compared to the ambulatory diabetics: 68.7 +/- 42.3 vs. 394.4 +/- 348.5 pg/ml (p= 0.011).

There were no differences between the groups in age or dialysis vintage.

Serum albumin was much lower, and dropping over the past year in the bedridden group compared to the ambulatory (2.89 +/- 0.78 vs. 3.83 +/- 0.35 g/dl ,p = 0.0001). No differences in corrected serum calcium or phosphorus.Review of bone-related medications showed that this PTH suppression was not iatrogenic.

Review showed that most of the bedridden patients had higher PTH levels when they were ambulatory.

Conclusions: 1. Bedridden pts on hemodialysis, who were mainly diabetic, have markedly suppressed intact PTH levels, compared to ambulatory diabetic pts. This was associated with low albumin levels. It was not associated with higher calcium or phosphorus levels, or due to oversuppression by medications.

2. Before becoming bedridden, when the patients were ambulatory, PTH levels tended to be much higher.

3. The cause of this PTH suppression was not investigated. With the association of hypoalbuminemia, factors associated with the Malnutrition- Inflammation Complex may play a role. Immobilization may be a factor.

PUB101

Vitamin D Deficiency Is Associated with Poor Response to Active Hepatitis B Immunisation in Patients with Chronic Kidney Disease Emanuel Zitt,[1,2] Hannelore Sprenger-Maehr,[1,2] Florian Knoll,[1] Ulrich Neyer,[1,2] Karl Lhotta.[1,2] *[1]Department of Nephrology and Dialysis, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; [2]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Academic Teaching Hospital Feldkirch, Feldkirch, Austria.*

Background: Vitamin D deficiency is highly prevalent in patients suffering from chronic kidney disease. At present it is not known whether this condition is a cause of the poor response to hepatitis B vaccination in these patients.

Methods: We performed a retrospective analysis of 200 patients with chronic kidney disease, who had undergone hepatitis B vaccination with three to four 40 µg recombinant hepatitis B vaccine doses. Anti-HBs antibody titers and vitamin D (25(OH)D) levels were measured by chemiluminescence immunoassays.

Results: Vitamin D deficiency with serum levels <10 ng/mL was found in 35.5% of patients. These patients had a lower seroconversion rate than did patients with levels ≥10 ng/mL (45 *vs* 64%; P=0.011) and their mean antibody titres were lower (215±706 vs 476±1583 IU/L). Non-responders had lower 25(OH)D concentrations than did responders (12.9±6.5 vs 15.1±7.4 ng/mL; P=0.034). In a multiple logistic regression analysis vitamin D deficiency (OR 0.532; P=0.043) and diabetes (OR 0.493; P=0.035) remained independent and significant negative predictors of seroconversion.

Conclusions: In patients with chronic kidney disease severe deficiency of vitamin D is associated with a poor antibody formation upon hepatitis B vaccination. Vitamin D supplementation might be useful in improving response to immunisation.

Funding: Private Foundation Support

PUB102

Platelet Reactivity in Erythropoietin (EPO) Treated Long-Term Hemodialysis Patients Pravin Bhat, Angella Brown, Reisha Twanna Browne, Melissa Rampal, Anna Babinska, Moro O. Salifu. *Medicine, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Recombinant human erythropoietin (EPO) is widely used for the therapy of anemia of chronic kidney disease (CKD) however its use has been associated with increased cardiovascular mortality. The mechanisms underlying excess cardiovascular mortality with use of EPO in CKD are poorly understood. We hypothesize that given the 70% homology between erythropoietin and thrombopoietin, the administration of exogenous EPO could induce increased platelet reactivity via direct platelet stimulation by EPO.

Methods: Using washed isolated platelets obtained predialysis from 20 hemodialysis (HD) patients with no history of antiplatelet or antihistamine ingestion, we evaluated platelet aggregation using a Chronolog-Lumi impedance aggregometer model 560-CA, after exposure of platelets to 0.5 mcg/ml and 1mcg/ml final concentrations of collagen. All patients were on dialysis for at least 3 months and received EPO three times a week on dialysis. Ten healthy donors served as controls. Platelet aggregation was measured as amplitude (Amp, Ohms) and Slope (Ohms/s). Data are presented as mean±SEM. Mann-Whitney U Test was used to compare differences in aggregation parameters between patients and controls.

Results: At 0.5 mcg/ml of collagen exposure, the Amp (53.93±3.9 vs. 34.4±10.1, p=0.059) and Slope (35.5±2.6 vs. 22.7±6.0, p=0.049) of aggregation was significantly higher in HD patients compared with Controls. At 1 mcg/ml of collagen exposure, the Amp (55.6±4.0 vs.25.0±12.6, p=0.099) and Slope (34.9±9.2 vs. 17.0±8.6, p=0.029) of aggregation was significantly higher in HD patients compared with Controls. There was no significant dose response relationship.

Conclusions: EPO-treated HD patients had increased platelets reactivity ex vivo compared to control healthy subjects. Direct stimulation of platelets by EPO independent of dose may generate a chronic low grade platelet activation state and may explain the excess cardiovascular mortality associated with ESA therapy in CKD. Further studies are needed to elucidate the effect of EPO on platelet function.

PUB103

Effect of Recombinant Human Erythropoiesis-Stimulating Agent on In Vitro Platelet Reactivity in Healthy Subjects Pravin Bhat, Reisha Twanna Browne, Melissa Rampal, Angella Brown, Thin Maw, Anna Babinska, Moro O. Salifu. *Medicine, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Erythropoietin (EPO) use has been associated with increased cardiovascular mortality via unknown mechanisms. We hypothesize that given the 70% homology between erythropoietin and thrombopoietin, exogenous erythropoietin could induce increased platelet reactivity via direct platelet stimulation.

Methods: Isolated platelet aggregation, measured as amplitude (Amp, Ohms) and Slope (Ohms/s) was tested at time zero and at 4 hours of incubation in 23 healthy donors using three final concentrations of darbepoetin; low (81ng/ml), intermediate (108.8 ng/ml) and high (249.6 ng/ml). Paired sample t-test was used to compare the means ±SEM between darbepoetin-treated and untreated platelets.

Results: At time Zero, the Amp of aggregation was significantly higher at low (42.3±4.0, p=0.003), intermediate (46.8±3.7, p=0.001) and high (46.2±4.1, p=0.002) darbepoetin-treated compared to control (untreated) platelets (29.9±4.2). The Slope, representative of the speed of the aggregation curve, was significantly higher at low (24.6±2.3, p=0.001) and intermediate (25.2±1.7, p=0.002) darbepoetin-treated compared to control (untreated) platelets (17.8±2.2). At 4 hours incubation, the Amp of aggregation was significantly higher at low (41.3±3.0, p=0.001), intermediate (34.5±4.0, p=0.001) and high (30.6±4.2, p=0.013) darbepoetin-treated compared to control (untreated) platelets (21.2±3.2). The Slope was significantly higher at low (28.4±2.5, p=0.001) and intermediate years (20.2±2.3, p=0.029) darbepoetin-treated compared to control (untreated) platelets (16.36±2.3). There was no significant difference in Amp or Slope between low, intermediate and high darbepoetin treatments at zero or 4 hours.

Conclusions: Darbepoetin-treated platelets had increased platelet reactivity in vitro compared to untreated platelets but a dose response relationship was not observed. Direct stimulation of platelets by EPO independent of dose may explain the excess cardiovascular mortality associated with EPO therapy in CKD. Ex vivo platelet function studies are needed to validate these findings.

PUB104

Microarray Analysis of Genes Expressed by Glomerular Parietal Epithelial Cells Takamoto Ohse,[1] Jeffrey W. Pippin,[2] Ron D. Krofft,[2] Alice M. Chang,[2] Stuart J. Shankland.[2] *[1]Division of Nephrology and Endocrinology, University of Tokyo Graduate Medical School, Japan; [2]Division of Nephrology, University of Washington, Seattle, WA.*

Background: Glomerular parietal epithelial cells (PECs) are squamous epithelial cells which form a monolayer on the urinary side of Bowman's capsule of the glomerulus. Unlike other glomerular cells, very little is known about the normal protein expression profile of PECs. In this study differential microarray analysis was performed on RNA from capsulated (PECs present) and de-capsulated (PECs absent) rat glomeruli in order to determine which genes are constitutively expressed by PECs.

Methods: Two types of rat glomeruli, capsulated and de-capsulated ones, were isolated with the newly developed modified sieving technique and mRNAs extracted from these two fractions were compared with microarray. The levels of highly expressed genes were then verified by PCR and immunohistochemistry.

Results: Two types of analysis were pursued. First, we identified 20 genes considered highly expressed (defined as > 2 fold increase in capsulated glomeruli compared to de-capsulated glomeruli). The mRNA expression for 15 of these was verified in a cultured mouse PEC cell line. Based on the difference in expression level between PEC cells and mouse cortex, the highest expressing 5 genes in PECs were PKHD1, Aldh1a1, CDH6, FRAS1 and PAX8. Their expression was confirmed at the protein level. In the second analysis, mRNA expression was verified with Human Protein Atlas. Among 148 genes which is expressed in capsulated glomeruli with more than 2 fold difference with de-capsulated glomeruli, we found the 26 genes were included in Human Protein Atlas. Finally, we had 14 genes which were expressed in PECs in glomeruli and not expressed in glomerular tuft.

Conclusions: By microarray analysis, 29 genes were detected to be expressed in PECs, and 5 of them were verified their expression in PECs with immunohistochemistry. While none of these proteins were exclusively expressed by PECs within the kidney, these genes should serve to expand our understanding of the role and function(s) of PECs during health and disease.

Funding: NIDDK Support

PUB105

Disrupted Autophagosomal Traffic Enhances Tubular Cell HIV Replication: Role of Nef Divya Salhan, Shabina Rehman, Ashwani Malhotra, Pravin C. Singhal, Mohammad Husain. *Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Tubular cells have been considered to serve as reservoir for HIV in kidney. HIV-Nef has been demonstrated to modulate endosomal traffic in macrophages. Recently, the role of endosomes has been highlighted in HIV replication. We asked whether Nef has the potential to enhance HIV replication in human tubular cells.

Methods: To develop productive HIV infection in tubular cells, HK2 (human tubular cells) were co-cultivated with infected lymphocytes for 72 hours. Subsequently, tubular cells were extensively, washed and evaluated for Gag expression by real time PCR. To determine the role of Nef in HIV replication, HK2 cells were transfected with Nef +ve (Nef+/HK2) or Nef-ve (Nef-/HK2) constructs, followed by co-cultivation with HIV infected lymphocytes for 24 hours. Subsequently, Nef(+)/HK2 and Nef(-)/HK2 were evaluated for the quantification of Gag expression. To determine the role of autophagy in Nef induced HIV replication, cells were prepared under similar conditions and then treated with or without rapamycin (100 nM) during co-cultivation studies. To determine the mechanism involved, HK2s, Nef(+)/HK2s, Nef(-)/HK2s, and Bafilomycin/NH4Cl-treated HK2s (positive control) were immunolabled for LC3-2 (as a marker of autophagosome). To quantify the number of autolysosomes (autophagosome fused with lysososomes), double labeling for LC3-2 and lysosome tracker was carried out.

Results: Nef(+)/HK2 cells displayed enhanced HIV replication when compared to Nef(-)/HK2s. However, rapamycin decreased HIV reoplication in Nef(+)/HK2. Interestingly, Nef(+)/HK2s also showed enhanced number of autophagosomes and diminished number of autolysosomes when compared with Nef(-)/HK2. On the other hand, rapamycin treated cells displayed enhanced numbers of autolysosomes.

Conclusions: These findings indicate that Nef enhances tubular cell HIV replication by accumulation of autophagosomes through inhibiton of autophagy by disruption of fusion of autophagosomes to lysosomes.

Funding: NIDDK Support

PUB106

Tubular Expression of the Facilitative Glucose Transporter GLUT1 in Normal Human Kidney Pamela J. Fall,[2] Rokshana R. Thanadar,[2] Saadia A. Khan,[2] Sitharam C. Nandigam,[2] Rajendra Chalasani,[2] John White,[2] Muralidharan Jagadeesan,[1,2] Charles W. Heilig,[3] N. Stanley Nahman.[1,2] *[1]Medicine, Charlie Norwood VAMC, Augusta, GA; [2]Medicine, Georgia Health Sciences University, Augusta, GA; [3]Medicine, University of Florida COM Jacksonville, FL.*

Background: We showed that tubular GLUT1 expression was increased in chronic allograft nephropathy (CAN) (*J Invst Med* 59:532). In that report, ~ 41% of CAN kidney tubules expressed GLUT-1, whereas ~ 24% of tubules from normals (NL) were GLUT1(+). Due to the potential pro-sclerotic effects of increased GLUT1 we reasoned that a more qualitative assessment of GLUT1 expression from normal kidney was warranted.

Methods: We scored tubular GLUT1 staining from NL from the previous report. 10 random fields were examined by 3 readers. Each GLUT1(+) tubule was scored from 1 to 4, with 4 exhibiting the strongest staining. A GLUT1 expression score was calculated (100 to 400). NL were from the normal portion of renal cell carcinoma nephrectomy specimens.

Results: Four kidneys were reviewed. 177 fields with 7196 tubules were scored. The mean percent of GLUT1(+) tubules was 0.03±0.02, 73.5±1.5, 19.7±1.3, and 6.7±0.8 % for scores of 1, 2, 3, and 4, respectively (mean±SEM, $p < 0.05$, ANOVA), implicating that the majority of tubular GLUT1 was minimal (2+). To gauge these findings against CAN kidneys, we scored 22 fields (787 tubules) from JPEGs of CAN sections. When compared to NL, CAN had a lower percent of grade 2 tubules (61.3±4.4 vs 73.5±1.5 % for CAN vs NL, respectively, $p < 0.05$) and a higher percent of grade 4 tubules (14.8±2.2 vs 6.7±0.8 for CAN vs NL, respectively, $p< 0.05$). When assessing GLUT1 expression score, CAN showed greater GLUT1 staining (254±5.7 vs 233±2.0 for CAN vs NL, $p<0.05$).

Conclusions: There are low levels of constitutive GLUT1 staining in normal human kidney, which would be expected for ongoing metabolism of glucose in the resting state. CAN may increase tubular GLUT1 expression, however it is unclear if the increase is pathologic or adaptive.

Funding: Veterans Administration Support, Clinical Revenue Support

PUB107

5-HT1F Receptor Agonist-Mediated Mitochondrial Biogenesis *In Vitro* and *In Vivo* Sara M. Garrett. *Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: Recovery from renal cell injury requires the biogenesis of mitochondria. Studies by Xu *et al.* (2007) showed that the kidney expresses several 5-hydroxytryptamine (5-HT) receptors and we have shown that the 5-HT2 receptor agonist DOI stimulates mitochondrial biogenesis in renal proximal tubule cells (RPTC). The goal of these studies was to further explore 5-HT receptors in RPTC mitochondrial biogenesis.

Methods: Immunoblot and qPCR analyses revealed 5-HT1F receptor expression in rabbit RPTC.

Results: A mitochondrial biogenesis assay that incorporates FCCP-induced uncoupled oxygen consumption rate (OCR) and a Seahorse Biosciences analyzer demonstrated that two 5HT1F receptor agonists, LY334370 and LY344864, increased uncoupled OCR at 1 nM & 10 nM, and at 10 nM & 100 nM respectively. The increase in uncoupled OCR was not sensitive to pertussis toxin, suggesting that the signaling of 5HTR1F-agonist-mediated mitochondrial biogenesis is not through Gi/o-coupling and inhibition of adenylyl cyclase. Both agonists increased the mitochondrial proteins ATP synthase β, Cox1, and NDUFB8 at 24 h. In murine kidney tissue, mitochondrial copy number and PGC1α and Cox1 transcript levels increased in response to LY334370.

Conclusions: In summary, the 5-HT1F receptor agonists LY334370 and LY344864 induce mitochondrial biogenesis.

Funding: Veterans Administration Support

PUB108

The Expression of Barx2 and K-Cadherin in Human Proximal Tubule Cells Nileshkumar Shah,[1] Iain Macphee,[2] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]St George's University of London, London, United Kingdom.*

Background: K-cadherin (Cdh6) is the predominant cadherin expressed in the human proximal tubule and we have previously demonstrated its expression is lost in diabetic nephropathy (ASN 2010), however nothing is known about the cellular mechanisms regulating Cdh6 expression in the kidney. In a series of ovarian cancer cell lines, Cdh6 expression showed a significant direct correlation with Barx2 expression. Barx2 is a homeobox protein transcription factor that can mediate a number of signaling cascades including Ras/Raf dependent transcription. Hence we have investigated whether Barx2 might regulate Cdh6 expression in human proximal tubule epithelial cells (PTEC).

Methods: Cdh6 and Barx2 expression was investigated in 3 cell types, primary human PTEC, and two transformed human PTEC cell lines, HK-2 and HKC8, by Western blot and qPCR. Barx2 expression was suppressed using RNAi and over expression achieved using Barx2-Cherry Red expression vector.

Results: Barx2 mRNA was detectable in all 3 cell lines and its expression (by qPCR) correlated with the Cdh6; Primary cells > HK2>HKC8.

Following treatment with TGFβ1 mRNA levels of Barx2 dropped rapidly, again in a pattern similar to that seen for Cdh6.

However, treatment of primary PTEC with siRNA targeting Barx2 did not reduce Cdh6 expression and over expression of Barx2 did not increase Cdh6 mRNA expression in HKC8 cells.

Conclusions: In conclusion, we have demonstrated for the first time that human PTEC express the transcription factor Barx2 and that its expression is regulated by TGFβ1, but we do not have any evidence to support the regulation of Cdh6 (K-cadherin) expression by Barx2 in these cells. We have investigated alternative regulatory pathways in a separate abstract as we believe that understanding the regulation of Cdh6 in the human proximal tubule has genuine relevance to the development of human renal disease. The role of Barx2 remains undefined

Funding: Private Foundation Support

PUB109

Connective Tissue Growth Factor Activation of Intracellular Signaling in Human Podocytes in Culture Simon K. Winn,[1] Felicia Heidebrecht,[1] Irbaz Isaac Badshah,[1] Jochen Reiser,[2] Mysore Keshavmurthy Phanish,[1] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]Nephrology and Hypertension, University of Miami, FL.*

Background: CTGF is expressed in mesangial cells and podocytes in diabetic nephropathy. In animal models an early increase in expression in podocytes is followed by an increase in parietal epithelial and mesangial cells. Altering CTGF expression in podocytes results in a change in podocyte number and increased albuminuria; whether this is through autocrine or paracrine effects has not been defined. Consequently, we investigated the effect of CTGF on human podocytes in the absence of other cell types.

Methods: Expression of CTGF responsive receptor TrkA was demonstrated by immunofluorescence. Cells also demonstrated punctate staining for CTGF consistent with localization in intracellular vesicles. Activation of TrkA causes phosphorylation of Erk5, hence expression of Erk5 in these cells was confirmed. As the C-terminal module of CTGF (cCTGF) is believed to be the region that binds to TrkA activating Erk5 we incubated cells in the presence of full length recombinant human CTGF (flCTGF) from human renal cells and cCTGF, PeproTech Ltd.

Results: Challenge with cCTGF or flCTGF (60 min, 37°) did not increase phospho-Erk5.

flCTGF but not cCTGF resulted in increased phospho-p38 and phospho-Smad2&3. (No phospho-Smad2/3 was detectable in control cells).

The fact that flCTGF but not cCTGF caused Smad activation suggested potentiating of sub-threshold levels of TGFβ1, as TGFβ is believed to bind to the N-terminal of CTGF. We tested this by blocking the activity of the TGFβ type I receptor Alk5 which prevented flCTGF-induced signaling as did a TGFβ neutralising antibody

We confirmed flCTGF binding to TGFβ1 by plasmon surface resonance. Interestingly, we were also able to demonstrate binding of cCTGF with TGFβ1; in the light of the result above this would not appear to cause an increase in TGFβ1-mediated effects.

Conclusions: We conclude that human podocytes in culture produce sub-threshold concentrations of TGFβ1 and the presence of exogenous CTGF results in TGFβ1 signaling; although we can not exclude the possibility that CTGF activates latent TGFβ.

PUB110

Ouabain-Activated c-Src as a Potential Biomarker for Salt-Sensitivity Yanling Yan,[1] Vinai Kumar Katragadda,[1] Chiamaka Mbaso,[1] Deepak K. Malhotra,[1] Zi-Jian Xie,[2,1] Basil Akpunonu,[1] Joseph I. Shapiro,[1,2] Jiang Liu.[1] *[1]Medicine, University of Toledo College of Medicine, OH; [2]Pharmacology and Physiology, University of Toledo College of Medicine, OH.*

Background: The relationship between dietary sodium, salt sensitivity and blood pressure (BP) is well established. Excessive dietary sodium intake significantly contributes to the development of resistant hypertension and tends to be more pronounced in typical salt-sensitive subgroup (the 2008 AHA Scientific Statement). The current methods for assessment of salt sensitivity are largely depended on patients' compliance. A simple rapid in vitro test without salt intervention would be desired.

Circulating ouabain is significantly increased under conditions such as high salt diet and renal insufficiency, as well as in a large portion (about 70-80%) of essential hypertensive patients. Accumulated data suggest that Ouabain is involved in regulation of BP and renal sodium handling.

Through ligand-modulated Na/K-ATPase/c-Src signaling, we recently demonstrated that impaired or attenuated renal proximal tubular ouabain-Na/K-ATPase/c-Src signaling contributes to salt-sensitivity and salt-sensitive hypertension, by affecting renal sodium excretion. Furthermore, ouabain-activated Na/K-ATPase signaling is not tissue-specific which broadenes the choice of sampling. We found that ouabain can activate c-Src in renal proximal tubules, cardiac and skin fibroblasts, and mononuclear white blood cells (mWBCs, lymphocytes and monocytes) in Dahl salt-resistant, but not in salt-sensitive rats ($n=3$-8, $p<0.05$ or $p<0.01$). In isolated mWBCs from Dahl rats fed with low salt diet, ouabain stimulated c-Src activation in the salt-resistant rats, which was not seen or in a much less degree in the salt-sensitive rats (with ouabain as low as 1nM for 15min, $n=8$ per group per strain, $p<0.01$). In healthy normotensive volunteers, ouabain significantly activated c-Src in a dose-dependent manner (with ouabain as low as 1nM for 15min, $n=5$, $p<0.01$). c-Src activation is the proximal step in ouabain-induced Na/K-ATPase signaling and endocytosis, and may serve as a biomarker for salt sensitivity.

PUB111

Tubulointerstitial Nephritis and Uveitis in an Elderly Female Krishna M. Baradhi, Jeffrey D. Clement. *Nephrology, Brown University, Providence, RI.*

Background: Tubulointerstitial nephritis and uveitis (TINU) is an unusual oculorenal syndrome that is often under recognized in clinical practice. Dobrin et al. first described TINU syndrome in 1975 and since then, more than 150 cases have been reported.Most patients with TINU are adolescents and young women, with a median age of 15 years. It has also been reported in adults, albeit rarely in the elderly.

Methods: We briefly report a 71 year old female with Tubulointerstitial nephritis and uveitis.

Results: A 71year old female presented with eight weeks history of intermittent nausea, vomiting, fatigue, fever and anorexia. Work up revealed a creatinine of 11 mg/dl and further evaluation revealed sterile pyuria and tubular proteinuria. Serological studies in the form of ANA, ANCA, complement and protein electrophoresis were negative. Renal biopsy showed acute interstitial nephritis (AIN) with no evidence of glomerular disease. Initially etiology of AIN was unclear and patient responded to steroids with improvement in renal function. Four months later, patient presented with bilateral anterior uveitis, thus leading to a diagnosis of TINU. Uveitis responded to topical steroids.

Conclusions: TINU is a rare clinical entity characterized by interstitial nephritis and uveitis usually observed in children and young adults. The present case demonstrates that this entity may also occur in the elderly,in fact our case represents one of the oldest reported in the literature. TINU remains a diagnosis of exclusion and high degree of suspicion is essential as uveitis may precede, be concurrent or present after nephritis. The pathogenesis remains elusive though delayed-type hyper- sensitivity and suppressed cell-mediated immunity with a pre- dominance of lymphocytes have been advocated. Renal disease in patients with TINU is usually self-limited with steroids reserved for patients with progressive renal insufficiency. The optimal management of the patient with uveitis requires early referral to ophthalmologist.

PUB112

Natural History of Iron Chelation Toxicity in Patients with Thalassaemia in Oman Sunil Bhandari,[1] Shahina F. Daar,[2] Vinodh Kumar Panjwani.[2] *[1]Renal Medicine, Hull & East Yorkshire Hospitals NHS Trust & Hull York Medical School, Hull, East Yorkshire, United Kingdom; [2]College of Medicine and Health Sciences, Sultan Qaboos University Hospital, Muscat, Oman.*

Background: Chelation therapy is essential in Thalassaemia to avoid iron overload related toxicity. Chelation related toxicity/adverse effects including agranulocytosis, liver and renal dysfunction may occur. This study assessed the prevalence of iron chelator related side affects in thalassaemics exposed to Deferasirox (DFX).

Methods: A retrospective assessment of the prevalence of chelator related side affects of DFX was performed. Case notes and databases were interrogated.

Results: 70 patients, mean age 20±1y, were studied. At DFX initiation, 65 had been on deferiprone (DFP) + deferoxamine (DFO); 2 DFO only, 3 DFP only. 35 had compliance issues with DFO. 4 had agranulocytosis with DFP necessitating drug discontinuation. No patient had pre-existing renal disease or hypertension. 6 had NIDDM and 2 IDDM.

During mean follow-up of 16months, 6 patients had rashes and 6 had gastrointestinal upset. DFX was discontinued in 15 patients: 2 generally unwell, 1 severe diarrhoea, 2 high transaminases. 3 converted to DFO+DFP as DFX was not biochemically effective. DFX therapy was stopped in 7 patients because of persistently raised serum creatinine. 8 further patients had a successful dose reduction of DFX. Creatinine at baseline was 39±1.2µmol/L, rising to a peak of 61.2±5.6 ($p<0.001$). Diabetic patients had a 54% mean peak rise in creatinine. 5 of 7 others failed re-challenge with a reduced DFX dose.

Changes in serum ferritin did not correlate with a rise in creatinine. No correlation of renal function with cardiac T2* or liverT2* was observed. Cardiac MRI T2* remained stable ($p=0.4$), while liver MRI T2* ($p=0.013$) improved with DFX therapy.

Conclusions: Transient and potentially progressive renal dysfunction occur in patients exposed to DFX, especially diabetic thalassaemic patients. Haemodynamic, pre-renal hypovolaemia from diarrhoea and direct tubular toxicity related to DFX or its effects may be responsible. Further studies are required. Monitoring of renal function and flexibility of use of chelators may avoid short and long term renal toxicity.

PUB113

Contrast-Enhanced Computed Tomography and Acute Kidney Injury in Patients with Severe Acute Pancreatitis Yuan Han Chen, Xinling Liang, Fen Jiang, Wei Shi. *Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: To study the relation between contrast-enhanced computed tomography (CECT) and acute kidney injury (AKI) in patients with severe acute pancreatitis (SAP).

Methods: Eighty-four SAP cases without surgery from January 2005 to January 2011 were retrospectively reviewed. SAP was diagnosed by an Acute Physiology and Chronic Health Evaluation (APACHE) II score ≥ 8 and AKI was diagnosed by RIFLE criteria for acute renal failure.

Results: AKI incidence in the patients with CECT and without CECT was 30.6% versus 15.6%, respectively ($P=0.090$). In the APACHE II score ≥ 15 subgroup analysis, the patients with CECT had a higher AKI incidence than those without CECT (71.4% versus 35.7%, respectively, $P=0.026$). After being adjusted by the APACHE II score, CECT was a risk factor for AKI (odds ratio 1.247, 95% confidence interval 1.195-1.387). In the patients with APACHE II score ≥ 15, CECT increased the risk for AKI (odds ratio 4.292, 95% confidence interval 3.101-5.087).

Conclusions: CECT is a potential risk factor for AKI in SAP patients, especially in more severe cases.

PUB114

Etiologies, Clinical Characteristics and Outcomes of Anuric Acute Kidney Injury Hye Min Choi, Eunjung Cho, Jae-Won Lee, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Nephrology, Korea University Hospital, Seoul, Republic of Korea.*

Background: Acute kidney injury (AKI) could be clinically described as oliguric (<400ml urine volume/24hr), nonoliguric (≥400ml), or anuric (<100ml). Anuric AKI is uncommon and has been known to occur from either complete ureteral obstruction or a major vascular event such as bilateral renal infarction or cortical necrosis. However most anuric AKI were known through sporadic case reports and there are limited data regarding to the epidemiology or outcomes. We purposed to examine the causes, clinical characteristics and outcomes of recent anuric AKI cases and compare those with non-anuric AKI.

Methods: This was a prospective cohort study. We enrolled anuric AKI patients who were admitted or referred to the Nephrology division between 2009 and 2010 if they showed anuria in the initial period of AKI despite maximum dose of diuretic challenge. Age- and gender-matched non-anuric AKI patients who required RRT were also enrolled.

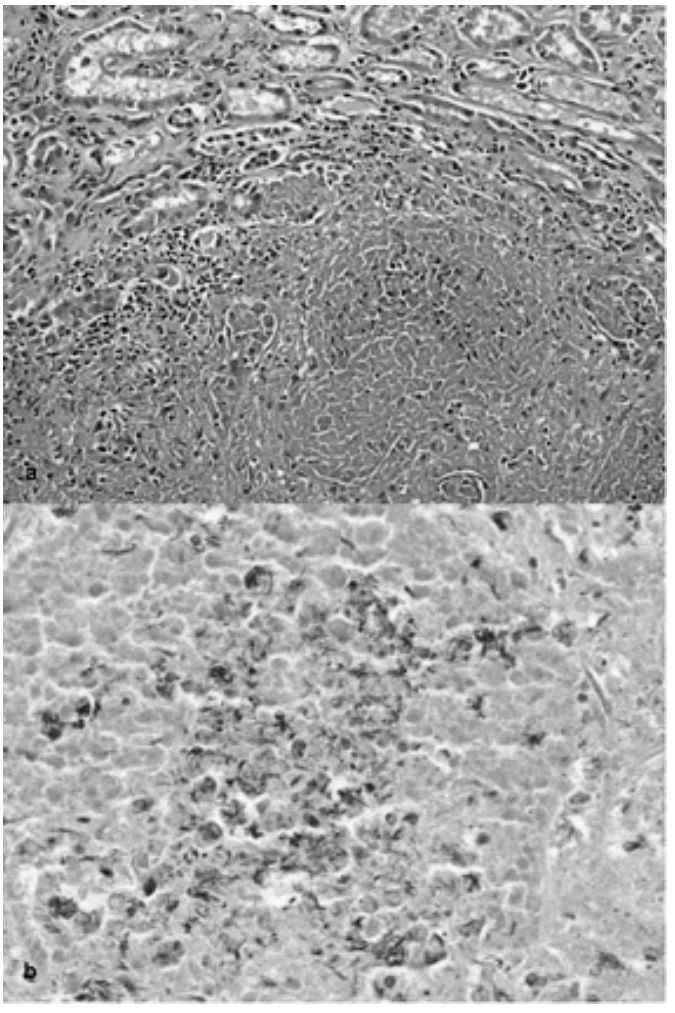
Results: Total 21 patients presented anuric AKI and the etiologies were postoperative (38.1%), sepsis (33.3%), rhabdomyolysis, ATIN, severe vasculitis and unknown. The most common cause of non-anuric AKI (n=46) was sepsis (52.1%). CKD prevalence was significantly higher in anuric compared to non-anuric group and interestingly, prevalence of malignancy was significantly much higher in anuric patients. Although CRRT requirement, duration of ICU stay, vasopressor requirement and in- hospital mortality were not different between the two groups, chronic dialysis dependence was more frequently observed in anuric group. In multivariate logistic regression analysis, the presence of anuria as well as older age, non-septic as the cause of AKI, were independent predictors of RRT dependence among AKI patients who received RRT.

Conclusions: Etiologies of anuric AKI are thought to have been changed from the past. Postoperative and sepsis were the most common causes of anuric AKI in our study. Co-morbidities with CKD and malignancy were more common in anuric patients, and anuric AKI also seems to be more commonly associated with non-recovery of renal function after episode of AKI.

PUB115

Renal Tuberculosis without Pyuria – A Fatal Case Report Paul J. Der Mesropian,[1] Snezana H. Mijovic-Das,[2] Jabulani Sidile.[1] *[1]Internal Medicine, Albany Medical Center, Albany, NY; [2]Nephrology, Albany Medical Center, Albany, NY.*

Background: Genitourinary tuberculosis is a considerably rare occurrence in developed countries, comprising only 6% of extrapulmonary cases of tuberculosis (TB) in the U.S. We report the case of fulminant miliary TB with extensive renal involvement that was diagnosed post-mortem in a 42-year-old male who presented with immunocompromise of unclear etiology, dysuria, microscopic hematuria, and sterile urine without pyuria. The kidneys were involved by a necrotizing granulomatous process (figure 1a) with AFB stain of the renal parenchyma showing numerous mycobacteria (figure 1b). The absence of pyuria, which is seen in almost all patients, makes this an atypical case of a rare disease. In fact, up to 20% of patients do not have any leukocytes in the urine. In spite of aggressive treatment with antituberculous agents, the patient succumbed to this rapidly progressing infection.



PUB116

Low 25-hydroxyvitamin D Levels Are Not Associated with an Increased Risk of Acute Kidney Injury Anna Jeanette Jovanovich,[1] Michel B. Chonchol,[1] Shailendra Sharma,[1] Kim McFann,[1] Sidney N. Thornton,[2] Jessica B. Kendrick,[1] John R. Holmen. *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Intermountain Health Care, Salt Lake City, UT.*

Background: Low 25-hydroxyvitamin D (25(OH)D) levels are associated with chronic kidney disease (CKD) but it is unknown if low 25(OH)D levels are a risk factor for acute kidney injury (AKI).

Methods: We conducted a population-based cohort study of adult patients with no history of CKD who developed AKI requiring hospitalization and had serum 25(OH)D measured 3 to 15 months prior to sepsis admission. Vitamin D was evaluated as a continuous variable and a categorical predictor (< 15 ng/mL vs. higher). AKI was defined by the RIFLE classification, which requires at least a 50% increase in serum creatinine. Cases of AKI were matched 1:1 with controls on age, sex, race and season. Conditional multivariate logistic regression analysis was used to evaluate if 25(OH)D levels were associated with an increased odds of AKI.

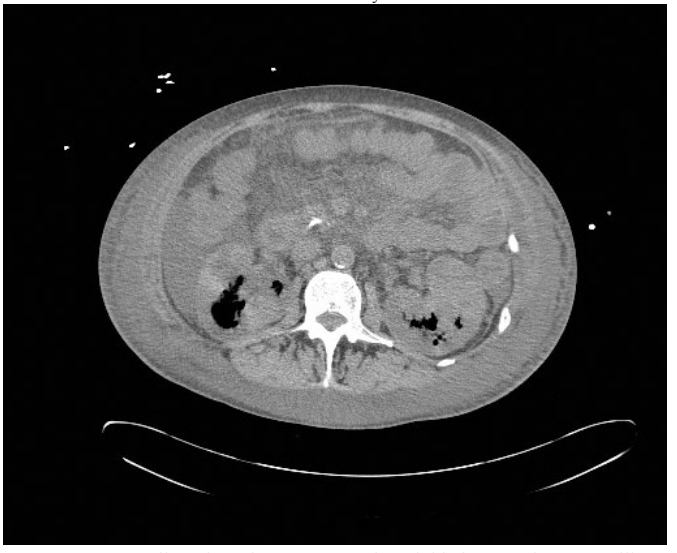
Results: We identified 51 patients with AKI and 51 matched controls. The mean (SD) age of the participants was 61 ± 18 years, 59% were female and 98% were Caucasian. Compared to patients without AKI, patients with AKI had a higher prevalence of diabetes (46.0% vs. 30.3%; p=0.0009) and chronic kidney disease (36.0% vs. 21.8%; p=0.001). There was no statistical difference between patients with and without AKI on mean (SD) 25(OH)D levels (28.8 ± 17 vs. 32.4 ± 13 ng/mL; p=0.099). After adjustment for diabetes, renal disease and peripheral vascular disease, 25(OH)D levels were not associated with AKI (OR 0.996, 95% CI 0.98-1.01; p=0.57). Similarly, when evaluated as a categorical predictor, vitamin D levels < 15 ng/mL vs. higher (OR 1.91, 95% CI 0.76-4.79; p=0.16), there was no association between vitamin D deficiency and AKI cases.

Conclusions: In this population-based cohort, low 25(OH)D levels are not associated with AKI. Larger studies are needed to evaluate whether vitamin D deficiency is a risk factor for AKI.

PUB117

Bilateral Emphysematous Pyelonephritis- A Rare But Serious Disease Praveen Kandula, Sanjiv Anand. *Nephrology, Indiana University School of Medicine, Indianapolis, IN.*

Background: A 24 year old male with history of diabetes and poly substance abuse was admitted to the hospital with diabetic ketoacidosis and acute renal failure. He had no known history of renal insufficiency and was found to have a creatinine of 3.2 on admission. His urinalysis revealed 115 WBC, bacteria, positive leukocyte esterase and nitrites. He was treated aggressively with volume repletion, insulin and broad spectrum antibiotics. A CT scan of abdomen was done on day#2 and revealed bilateral emphysematous pyelonephritis [Figure 1 and 2). Patient was also found to have severe thrombocytopenia, elevated INR, GI bleed and E.Coli bacteremia. He was deemed not to be a surgical candidate for bilateral nephrostomies as treatment of his pyelonephrtis due to his coagulopathy and comorbid illnesses. Patient's renal failure worsened and was put on continuous veno-venous hemofiltration. He succumbed to his illness 21 days later.



Conclusions: Bilateral emphysematous pyelonephritis is a rare but severe illness associated with >40% mortality in the absence of surgical intervention. Early and aggressive intervention is needed. In significantly ill patients physicians should be more aware of this entity.

PUB118

Hyperhomocysteinemia Is Independently Associated with the Risk of Contrast-Induced Nephropathy in Patients Undergoing Percutaneous Coronary Intervention Seung Jun Kim, Mi Jung Lee, Dong Ho Shin, Dong Eun Yoo, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. *Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea.*

Background: Endothelial dysfunction and oxidative stress have been considered to be responsible for the vascular damage in hyperhomocysteinemia as well as the development of contrast-induced nephropathy (CIN). To date, however, little is known on the relationship between hyperhomocysteinemia and CIN. This observational cohort study was conducted to investigate whether hyperhomocysteinemia was independently associated with CIN in patients undergoing percutaneous coronary intervention (PCI).

Methods: A total of 572 patients, who underwent PCI at Severance Cardiovascular Hospital, Seoul, Korea, were included. CIN was defined as absolute ≥ 0.5 mg/dl or relative ≥ 25% increase in serum creatinine levels at 48 hour after the procedure. Patients were divided into 2 groups; patients with and without CIN, and the clinical and laboratory data, and echocardiographic findings were compared between the two groups.

Results: Sixty-nine patients (12.1%) developed CIN after PCI. Patients with CIN were significantly older and had a higher prevalence of diabetes. Moreover, acute myocardial infarction, three vessel coronary artery disease, and the use of intra aortic balloon pump were significantly more prevalent in patients with CIN. Plasma glucose and homocysteine levels (16.9±4.9 vs. 13.5±4.2 μmol/L, p<0.001) were also significantly higher in patients with CIN compared to those without CIN. In multiple logistic regression analysis, hyperhomocysteinemia was an independent risk factor for CIN [per 1 SD change in plasma homocysteine levels (4.44 μmol/L); OR, 1.70; 95% CI, 1.07-2.71, p=0.025] even after adjusting for other major risk factors.

Conclusions: Hyperhomocysteinemia is independently associated with the risk for CIN in patients undergoing PCI, suggesting that measurement of plasma homocysteine levels may aid in the risk stratification for the development of CIN in patients undergoing PCI.

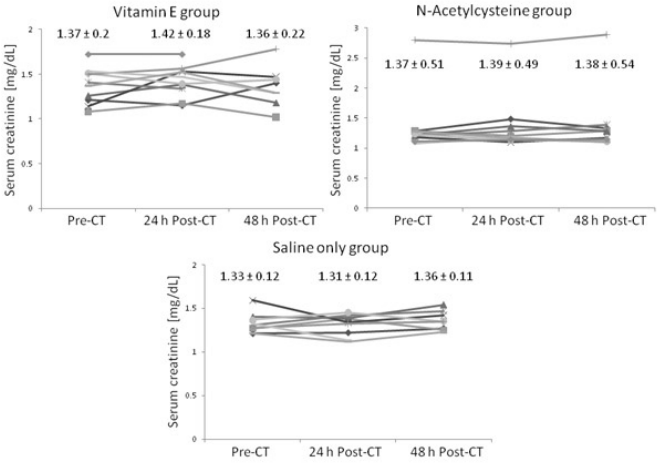
PUB119

Efficacy of Vitamin E and N-Acetylcysteine To Prevent Radiocontrast Induced Acute Kidney Injury in Patients with Chronic Kidney Disease: A Randomized Controlled Trial Thomas M. Kitzler,[1] Aala Jaberi,[1] Gerald Sendlhofer,[2] Peter H. Rehak,[2] Christian Binder,[4] Rudolf Stacher,[4] Peter Kotanko.[5] *[1]Department of Medicine, McGill University, Montreal, QC, Canada; [2]Medical University of Graz, Graz, Styria, Austria; [3]Krankenhaus der Barmherzigen Brueder Graz, Graz, Styria, Austria; [4]Renal Research Institute, New York, NY.*

Background: Contrast induced acute kidney injury (CIAKI) is a leading cause of hospital-acquired acute kidney injury, with patients suffering from chronic kidney disease (CKD) at particularly high risk. This trial tested the hypothesis that vitamin E or N-acetylcysteine improved renal outcomes following radiocontrast administration in CKD patients.

Methods: This single-center, prospective, double-blind, double dummy, placebo-controlled, randomized, parallel clinical trial enrolled patients with CKD who underwent elective computer tomography (CT) with administration of radiocontrast agents. Patients were randomized to receive either vitamin E (2160 mg i.v.) or N-acetylcysteine (4800 mg p.o) in addition to saline (1 mL/kg/h over 24 h), or saline alone. CIAKI was defined as a rise in serum creatinine > 25% within 96 h after CT. Primary and secondary outcomes were 24 h change of serum creatinine and measured creatinine clearance, respectively.

Results: Thirty patients (CKD stages 1 to 4; mean age 74.6 years; 17 F, 30% diabetics; all Caucasians) were enrolled; 1 patient was excluded after randomization because of protocol violation. No patient developed CIAKI. There was no significant difference in serum creatinine change between the three study arms.



In secondary analysis, creatinine clearance did not differ between the groups.

Conclusions: Vitamin E and NAC in addition to saline did not demonstrate a beneficial preventive effect on kidney function when compared to saline administration only.

Funding: Pharmaceutical Company Support

PUB120

Role of Novel Biomarkers for Early Detection of Acute Kidney Injury Following Gadolinium-Based Contrast Agents Administration Louis-Philippe Laurin, Jean-François Naud, François Leblond, Vincent Pichette, Martine Leblanc. *Nephrology, Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Background: Gadolinium-based contrast agents have been originally used in patients with severe renal dysfunction instead of iodinated contrast media due to their presumed non-nephrotoxicity. However, several cases of systemic nephrogenic fibrosis have been reported afterwards in patients with renal insufficiency. Cases of acute kidney injury associated with gadolinium-based media in patients with normal renal function have also been published recently, rising concerns about their potential nephrotoxicity.

Methods: We studied four novel biomarkers of acute kidney injury: interleukin 18 (IL-18), N-acetyl-{beta}-d-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL) and Cystatin C in 12 volunteers with normal renal function after the intravenous administration of a gadolinium-based contrast agent. Urinary and serum levels of biomarkers were analyzed at 0, 3 h and 24 h following injection.

Results: All urinary biomarkers were normalized for creatinine concentration. Baseline levels of urinary IL-18 were highly variable between patients (0 to 42.5 pg per mmol of creatinine). IL-18 urinary concentration was significantly increased at 3 h (0.5 to 59.2 pg/mmol; $p<0.01$) and 24 h (0.4 to 51.3 pg/mmol; $p<0.05$) in most studied patients. Similarly, urinary NAG levels were higher at 3 h compared to baseline levels (0.0 to 18.7 IU/mmol before gadolinium injection and 0.9 to 24.0 IU/mmol at 3 h; $p<0.001$). However, impact of gadolinium injection on NAG levels was no longer measurable at 24 h. Some patients also showed a significant increase of urinary NGAL (baseline at 0.9 to 7.8 ng/mmol) at 3 h (1.4 to 19.1 ng/mmol; $p<0.05$) and 24 h (1.3 to 28.7 ng/mmol; $p<0.05$). No difference in serum levels of Cystatin C and NGAL was observed following gadolinium administration compared to baseline levels.

Conclusions: Gadolinium-based contrast agents may induce a transient kidney injury shortly after their administration. These results provide new insights about the potential nephrotoxicity of gadolinium contrast media.

PUB121

Statin Initiation for the Prevention of Post-Surgical Acute Kidney Injury J. Bradley Layton, Abhijit V. Kshirsagar, M. Alan Brookhart. *UNC, Chapel Hill, NC.*

Background: Previous studies suggest preoperative statins reduce the occurrence of acute kidney injury (AKI) following coronary artery bypass graft (CABG). Yet it is unclear whether the findings result from a direct, protective effect of statins or are a result of immeasurable differences in patient characteristics among long-term users of statins.

Methods: Hospitalized CABG patients' statin use was assessed in Thomson Reuters' MarketScan Commercial Claims and Encounters database based on pharmacy dispensing prior to CABG. To reduce confounding by adherence and behavioral factors, only new statin users (initiating a statin ≤20 days before CABG) and never statin users and were included. Prevalent statin users were excluded, as were those with ESRD or AKI at baseline. AKI was identified from diagnosis codes in the 15 days post-CABG. Indicators of cardiovascular disease (CVD) management, disease severity, and acute CVD events were assessed in the 6 months prior to CABG. Logistic regression estimated the effect of statins on post-CABG AKI. An additional analysis restricted to CABG patients without recent myocardial infarction or unstable angina to better reflect planned surgeries where physicians are able to make medication prophylaxis decisions.

Results: When examining the new users of the medication in the immediate perioperative period, our results are consistent with a protective effect of statin initiation on post-CABG AKI, particularly in non-emergency CABG. This finding should be interpreted cautiously, as statin initiation may serve as a proxy for unmeasured health-seeking behaviors, disease characteristics, adherence, and other factors which cannot be fully controlled in observational analyses.

Conclusions: When examining the new users of the medication in the immediate perioperative period, our results are consistent with a protective effect of statin initiation on post-CABG AKI, particularly in non-emergency CABG. This finding should be interpreted cautiously, as statin initiation may be a proxy for unmeasured health-seeking behaviors, disease characteristics, adherence, and other factors which cannot be fully controlled in observational analyses.

PUB122

Incidence, Prognosis and Risk Factors in Hospitalized Acute Kidney Injury Defined by AKIN Renhua Lu, Yucheng Yan, Jiayuan Gao, Liu Shang, Zhaohui Ni, Jia Qi Qian. *Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: AKIN has been proposed as a consensus definition of Acute Kidney Injury(AKI) . The aim of this study is to investigate the incidence, prognosis and related risk factors in hospitalized AKI defined by AKIN to help clinicians better understand and prevent AKI.

Methods: All hospitalized patients during Jan. to Dec. 2009 were screened by Lab Administration Network. Study Cohort by AKIN. Patients' clinical data including etiology, distribution characteristics and prognosis were retrospectively collected. Logistic regression analysis was used to investigate the risk factors of prognosis.

Results: As a result, 934 patients were enrolled. The incidence of AKI in hospitalized patients was 2.14%. The comprised rate of AKI was 65.8%, 25.1% and 9.1% for Stage I, II and III respectively. The ratio of male to female was 1.88:1. The average age was (60.82±16.94) years old. 331(35.4%) patients with AKI were distributed in Intern Medicine Department while 63.4% patients in Surgical Department and 1.2% in Obstetric and Gynecologic Department. Analyzing the cause of AKI, ATN accounted for 52.0%, followed by 44.7% of renal parenchyma AKI and 3.3% of Post-AKI The mortality of AKI in hospitalized patients was 23.6%, and the rate of renal lose was 65.7%. Multivariate logistic regression analysis showed that age(OR=1.327,95%CI:1.067-1.649), renal injury drugs (OR=3.050, 95%CI: 2.023 -4.598), hypotension(OR=5.584,95%CI:2.713-11.496), oliguria (OR=4.083,95%CI: 2.586-6.447), rising amplitude of serum creatinine (OR=1.712,95%CI:1.371-2.138) and RRT(OR=3.208, 95%CI:1.365-7.537) were independent risk factors for patients death.

Conclusions: In conclusion, AKI is one of the most common clinical syndrome in hospitalized patients. The most common reasons for hospitalized AKI are pre-AKI and ATN. The mortality of AKI in hospitalized patients was high. Age, drugs, hypotension, oliguria, rising amplitude of serum creatinine and RRT were independent risk factors of patient death.

Funding: Government Support - Non-U.S.

PUB123

A Case of Auto-PEEP Induced Acute Kidney Injury Dwight M. Matthew, Karthik M. Ranganna. *Nephrology, Drexel University College of Medicine, Philadelphia, PA.*

Background: Acute kidney injury (AKI)is common among critically ill patients and results in increased mortality in this population. However, PEEP (positive end-expiratory pressure) as a cause of AKI has never been reported. We present a rare case of auto-PEEP induced AKI.

Results: A 57 year old African American male with a past medical history of hypertension and α1-anti-trypsin deficiency was admitted for a 2 day history of progressive shortness of breath. He was initially treated with steroids and bronchodilators for a COPD flare. Day 2, his respiratory status declined necessitating intubation and mechanical ventilation. A CT angiogram performed was unremarkable for pulmonary embolism.

His urine output was 50-75cc/hr on day 2 and declined to 5-10cc/hr on day 3. His serum bun/creatinine were as follows: day 1: 7/0.73, day 2: 9/0.83 and day 3: 37/2.22. His physical examination on day 3 was pertinent for bilateral wheezes and a distended, firm, tender abdomen. A non-contrast CT abdomen showed no acute pathology. Renal ultrasound revealed kidneys normal in size and echogenicity with a collapsed bladder. His urine analysis and sediment were unremarkable.

Day 4, his urine output was 0-5cc/hr, his bun/creatinine peaked at 67/3.37 and respiratory status further declined. He was noted to have significant auto-PEEP and increasing difficulty to oxygenate. His bladder pressure was measured at 30 mmHg. He then received the neuromuscular blocking agent cisatracurium (Nimbex) after other methods to remedy his auto-PEEP proved futile.

Within the first three hours of paralysis, his urine output was 300cc then continued at 100-200 cc/hr for the next 24hrs. His abdomen was no longer firm or distended on examination. A repeat bladder pressure was 5 mmHg. Day 5 his serum bun/creatinine were 64/2.87 and over the next 5 days trended to his baseline.

Conclusions: Literature addressing PEEP as a cause for IAH is very sparse. However, Verzilli and colleagues demonstrated that PEEP can have a significant impact on IAH. Acute kidney injury in the ICU can often be multifactorial. However, the rapid return of renal function after effective treatment of auto-PEEP indicates the crucial role that IAH, as a result of auto-PEEP, played in this case.

PUB124

Reno-Prevention: A New Concept for Re-Engineering of Nephrology Practice: An Economic Impact and Patient Outcome Analysis of Two Hypothetical Patient Scenarios in the CCU

Macaulay A. Onuigbo,[1,2] Nnonyelum T. Onuigbo.[3] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI; [3]Information Technology, NTEC Solutions LLC, Eau Claire, WI.*

Background: The impact of AKI on CKD progression remains uncertain. Common but untested opinion assumes that AKI in CKD is short-lived with little sequele. Ishani et al. demonstrated that 25.2% of Medicare ESRD patients all had prior AKI. Menon et al. showed that ESRD in the elderly was often precipitated by AKI. We described the new syndrome of rapid onset end-stage renal disease (SORO-ESRD) in 2010 where ESRD was a continuum of the AKI. We continue to call for more preventative measures to limit AKI (Reno-prevention, RP). We compare healthcare value outcomes with/without RP strategies (RPS).

Methods: Two similar hypothetical patients with symptomatic CAD get elective cardiac catheterization (CC)and CABG procedures, one with, and one without RPS.

Results: A more aggressive fluid repletion and one week withholding of ACEI in patient A allowed for discharge after 6 days. Patient B was continued on lisinopril 40 mg QD through CC and CABG, experienced prolonged peri-operative hypotension (POH), suffered severe oliguric AKI and needed daily RRT x 6. Kidney function improved; discharged on day 20.

Total hospital charges - \$68,580 (A) versus \$154,650 (B). 20 liters of colloid for prevention of POH in patient A, an additional hospital charge of \$2,000, was a huge return on investment (ROI).

PATIENT	ADMISSION SERUM CREATININE (mg/dL)	NUMBER OF RRT SESSIONS	LOS (DAYS)	WBC GIVEN	DISCHARGE SERUM CREATININE (mg/dL)	NUMBER OF DAYS ON THE VENT	COST OF RRT (CVVHD) ($)	COST OF VENT DAYS ($)	TOTAL CHARGES ($)
A	1.6	0	6	1	1.1	3	0	35,000	68,580
B	1.6	6	20	3	2.2	8	11,050	100,000	154,650

Conclusions: We posit that RPS in cardiac surgery patients (and other critically ill patients) - withholding nephrotoxics including RAAS blockers and avoiding POH, improves patient/renal outcomes, lessens AKI, lessens SORO-ESRD, and produces massive dollar savings. Our call for more RPS would constitute major rethinking and practice re-engineering in current nephrology practice.

PUB125

Influence of Diastolic Blood Pressure and Fluid Removal during Intermittent Hemodialysis on Incomplete Acute Kidney Injury Recovery

Milan M. Radovic,[1,2] Jelena Pavlovic.[1] *[1]Clinic of Nephrology, Clinical Center of Serbia, Belgrade, Serbia; [2]University of Belgrade, School of Medicine, Belgrade, Serbia.*

Background: Fluid overload and its removal during intemittent hemodialsyis (IHD) are independent risk factors for mortality in patients with acute kidney injury (AKI). Impact of such an event on incomplete AKI recovery has not been elucidated.

Methods: Retrospective, observational, single centre study (2000–2008) was undertaken in 386 patients (age 52.1±16.4 years, 279 male), treated by hemodialysis because AKI stage 3. Patients were assigned to one out of 4 groups according to in-hospital outcome: 1=no AKI recovery and death (N=152, 39.4%), 2=AKI recovery but death (N=5, 1.3%), 3=incomplete AKI recovery and survival (N=26, 6.7%), 4=complete recovery and survival (N=203, 52.6%, p <0.001). Individual severity score (ISS), age (ANOVA and post test), frequencies of comorbidities, systolic (Δsbp) and diastolic blood pressure (Δdbp) changes during IHD and ultrafiltration rate were compared between groups. Cox proportion hazard model was calculated.

Results: Patients in group 1 were older than groups 3 (p=0.023) and 4 (p=0.002), and had greater ISS than group 3 (p<0.001). Group 1 had significantly greater fall in Δsbp (6.323, 95% CI 0.745–4.856) fall during IHD than groups 3 (Δsbp 2.088, CI 0.8216–0.489) and 4 (Δsbp 2.745,CI 0.469–1.823; both p<0.001). Group 1 had greater Δdbp (3.462, CI 0.582 – 2.323) fall during IHD than group 3 (Δdbp 1.661, CI 0.517–0.644; both p=0.114) and group 4 (Δdbp 1.391, CI 0.310-0.783, p<0.001). Average ultrafiltration rates were similar (p=0.074). Cox regression model (p<0.001) showed significant influence of hypertension (OR 1.88, CI 1.087–3.251, p=0.024), neoplasia (OR 1.698, CI 1.136–2.537, p=0.01), cardiovascular disease (OR 6.617, CI 4.129–10.603), liver disease (OR 12.238, CI 6.284 – 21.948) and preexisting renal disease (OR 0.245, CI 0.155-0.386, all p<0.001) on incomplete ARF recovery.

Conclusions: Preventing significant dbp fall during IHD in patients with hypertension, cardiovascular disease, neoplasia and preexisting renal disease may influence risk for incomplete recovery of patients with AKI stage 3.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

PUB126

Correlation of Neutrophil Gelatinase-Associated Lipocalin with Renal Function and Proteinuria in Patients with Chronic Kidney Diseases

Xuan Shi, Xiaoliang Zhang, Bi-Cheng Liu. *Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.*

Background: Neutrophil gelatinase-associated lipocalin (NGAL) represents a novel biomarker for early detection of acute kidney injury. Recent studies have noticed the change of NGAL in some kind of chronic kidney disease (CKD) whereas the exact role of NGAL in evaluation of CKD is unknown. The aim of this study was to investigate plasma and urine NGAL (pNGAL and uNGAL) in a cohort of CKD patients in order to verify its relationship with the severity of renal impairment and proteinuria.

Methods: 100 CKD outpatients from Zhongda Hospital, who were verified at stable disease status, were recruited into this study. The random plasma and urine specimens were obtained from the eligible subjects for NGAL determination. 24-hour urine samples were collected for proteinuria measurement. Clinical data including serum creatinine (CR), eGFR and cause of CKD etc. were collected for analyzing correlation of NGAL with renal function and proteinuria.

Results: Both pNGAL and uNGAL were significantly higher than those in healthy control. Inverse correlation of eGFR with pNGAL and uNGAL were observed in all CKD patients. This relationship was also observed in subgroups with different range of proteinuria or causes. Using eGFR as a dependent variable in a multivariate model, the relationship of CR with pNGAL and uNGAL remained significant. In proteinuric patients, positive correlation of proteinuria with pNGAL and uNGAL was observed, and this relationship still existed in subgroups with different range of renal function or causes. Using pNGAL or uNGAL as dependent variables in a multivariate model, the relationship of pNGAL with eGFR remained significant in subgroups with different causes. In patients with diabetic nephropathy and chronic glomerulonephritis, uNGAL positively correlated with protienuria and eGFR, whereas only the relationship of uNGAL with protienuria remained significant in patients with hypertensive nephropathy.

Conclusions: Both pNGAL and uNGAL are significantly correlated with eGFR and proteinuria in patients with CKD. This suggests NGAL may be used as a biomarker to predict the development of CKD.

Funding: Government Support - Non-U.S.

PUB127

Coexistence of ANCA-Associated Glomerulonephritis and Anti-Phospholipase A_2 Receptor Antibody Positive Membranous Nephropathy

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Background: The etiologies of idiopathic membranous nephropathy (IMN) and anti neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN) have recently been identified. Antibodies to myeloperoxidase (MPO) and PR3 have been demonstrated to mediate ANCA-associated disease. For membranous nephropathy, antibodies to the podocyte-expressed phospholipase A_2 receptor (anti-PLA_2R) are highly associated with disease activity and have been reported in at least 70% of patients with IMN.

Methods: We now present a case of a 56 year old male with one year history of hypertension, leg edema, and proteinuria, who presented with advanced renal failure and was found to have both ANCA-associated GN and IMN on kidney biopsy.

Results: Consistent with the idea that this is due to the chance occurrence of two independent diseases, we found both anti-MPO and anti-PLA_2R antibodies in the patient's sera. Treatment with methylprednisolone, plasmapheresis, and cyclophosphamide resulted in the improvement in kidney function and proteinuria, together with the simultaneous decrease in both autoantibodies.

Conclusions: This is the first demonstration of two pathogenic antibodies giving rise to ANCA-associated GN and IMN in the same patient. It confirms the importance of classifying disease based upon the underlying mechanism, in addition to renal histopathology, to both optimize therapy and predict prognosis. Serial monitoring of changes in titers of these disease-associated antibodies, together with clinical response, should provide valuable insight into assessing response to treatment and predicting recurrent disease.

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PUB128

Therapeutic Hypothermia and Prevention of Acute Kidney Injury: A Meta-Analysis of Randomized Controlled Trials Paweena Susantitaphong,[1] Mansour Alfayez,[1] Abraham Cohen-Bucay,[1] Ethan M. Balk,[2] Bertrand L. Jaber.[1] *[1]Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Center for Clinical Evidence Synthesis, Tufts Clinical and Translational Science Institute, Tufts University, Boston, MA.*

Background: Therapeutic hypothermia has been shown to reduce neurological morbidity and mortality in the setting of out-of-hospital cardiac arrest and may be beneficial following brain injury and cardiopulmonary bypass. We conducted a systematic review to ascertain the effect of therapeutic hypothermia on development of acute kidney injury (AKI) and mortality.

Methods: We searched for randomized controlled trials in MEDLINE through February 2011. We included trials comparing hypothermia to normothermia that reported on the incidence of AKI, dialysis requirement, changes in serum creatinine and mortality. We performed Peto fixed-effect and random-effects model meta-analyses, and meta-regressions.

Results: Nineteen trials (2,218 patients) were included; in the normothermia group, the weighted incidence of AKI was 4.2%, dialysis requirement 3.7%, and mortality 10.8%. By meta-analysis, hypothermia was not associated with a significant decrease in the incidence of AKI (OR 1.01, 95% CI 0.68, 1.51; P = 0.95) or dialysis requirement (OR 0.81; 95% CI 0.30, 2.19; P = 0.68); however, by meta-regression, a lower target cooling temperature was associated with a lower incidence of AKI (P = 0.01). Hypothermia was associated with a 31% lower odds of mortality (OR 0.69; 95% CI 0.51, 0.92; P = 0.01).

Conclusions: Therapeutic hypothermia has no impact on the incidence of AKI or dialysis requirement, but is associated with lower mortality in the included trials. Different definitions of AKI and different rates of AKI and mortality in trials together with concerns about the optimal target cooling temperature preclude definitive conclusions.

PUB129

Epidemiology and Outcome of Acute Kidney Injury after Cardiac Surgery in China Jie Teng,[1] Yi Fang,[1] Jianzhou Zou,[1] Chunsheng Wang,[2] Xiaoqiang Ding.[1] *[1]Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China; [2]Cardiac Surgery, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: The aim of this study was to analyze the incidence of AKI and its risk factors after open heart surgery.

Methods: 4007 patients who underwent cardiac surgery from April 2009 to April 2011 were involved. Demographic characteristics, laboratory exam, types of surgeries and clinical outcomes were recorded.

Results: Of the 4007cases, valve surgery occupies the largest proportion (n=2090, 52.16%), followed by coronary artery bypass graft (CABG) surgery (n=695, 17.34%), congenital heart surgery (n=461,11.5%), combined surgery (n=352, 8.7%, aorta surgery (n=238, 5.9%), others (n=134, 3.3%), and orthotopic cardiac transplantation (n=37, 0.9%). AKI was defined and classified by RIFLE criteria. The overall incidence of AKI was 31.2% (n=1250), RIFLE-R 78.4% (n=837), RIFLE-I 8.4% (n=90), and RIFLE-F 13.2% (n=141). The incidence of AKI requiring replacement treatment (AKI-RT) was 2.6% (n=104). The overall hospital mortality was 1.9%. In-hospital mortality of AKI-RT group was up to 38.5%. Length of stay, ICU time, age, body mass index (BMI), basic Scr and NYHA degree were all significantly higher in AKI group than in non-AKI group. CPB time and aortic cross-clamp time of AKI group were longer than non-AKI group, and AKI group use more vasoactive drugs postoperative. The incidence of AKI was 73% after cardiac transplantation, 58% after CABG and valve combined surgery, 52% after aorta surgery, 14% after congenital heart surgery. Hospital mortality after cardiac transplantation was the highest one as 18.9%.

Conclusions: The pattern of AKI after cardiac surgery in out population was different from that in developed countries, reflected in the larger proportion of valve and aorta surgery.

PUB130

Manifestation of IgA Nephropathy by Acute Kidney Injury in Warfarin Overdose Patient Anton Y. Verbine.[1] *[1]Nephrology, St Michael's Hospital, Toronto, ON, Canada; [2]Dept of Pathology, St Michael's Hospital, Toronto, ON, Canada.*

Background: We report a biopsy-proven case of glomerular hematuria leading to acute kidney injury (AKI) with newly diagnosed IgA nephropathy in patient supra-therapeutic on warfarin. There is increased body of evidence that tubular obstruction with RBC casts can be rare but serious complication of warfarin therapy, and can lead to acute tubular necrosis (ATN).

Methods: Clinical and pathological findings are presented, available evidence on combination of warfarin anticoagulation, glomerular hematuria and AKI are discussed.

Results: 62 y.o. F was admitted to hospital with clinically worse congestive heart failure and diagnosed with pulmonary edema and new AKI. She had gross hematuria without dysuria for 3 days prior to admission. There was no previous history of hematuria, renal colic or known kidney disease. Patient was on warfarin for atrial fibrillation and previous deep venous thrombosis.

On physical exam patient was anuric and appeared hypervolemic. Admission Cr was 8.4 mg/dL (compared to 1.14 mg/dL 2 weeks prior) K 7.3 mEq/L ,Hb 98, INR on presentation 6.91. Urine dipstick grossly positive for blood and protein, microscopy showed abundant erythrocytes with rare heme-granular casts. After correction of INR with prothrombin complex concentrate temporary hemodialysis line was inserted and acute hemodialysis initiated.

Kidney biopsy demonstrated mesangial hypercellularity with early crescents, mild interstitial fibrosis. Some tubules contain red blood cell casts with evidence of ATN. Immunofluorescence shows mesangial deposits of IgA and C3.

Patient was treated with pulse steroids and cyclophosphamide and became dialysis -independent in 3 month with CKD 4.

Conclusions: AKI can be an uncommon complication combination of two common conditions combined- IgA nephropathy and anticoagulation with warfarin. Macrohematuria in patient with INR prolongation deserve serious clinical attention, and IgA nephropathy with AKI should be in differential diagnosis. In general, clinical outcomes are unfavorable. Unusual feature of our case is no previous history of hematuria and new diagnosis of IgA nephropathy.

PUB131

Early Identification of Leptospirosis as a Important Cause of Acute Kidney Injury in Taiwan: A Case Control Analysis Huang-Yu Yang,[1, 2] Chan-Yu Lin.[1] *[1]Nephrology, Chang-Gung Memorial Hospital, Taipei, Taiwan; [2]Johns Hopkins, Baltimore.*

Background: Leptospirosis, caused by the pathogenic spirochete leptospira, is the most wide spread zoonosis throughout the world.

Methods: From September 2000 to March 2006, surveillance of 455 multiple organ dysfunction syndrome patients with unclear cause or clinical suspicion of leptospirosis was performed. Diagnosis was confirmed by microscopic agglutination test or isolation. Cases were classified as excluded based on confirmed etiology other than leptospirosis or negative paired serologic test. Rapid serology tests have been applied for screening. The difference between the confirmed and excluded cases was compared by the Student t test. Statistically significant presentations of leptospirosis were tested using logistic-regression models. The relationship between delayed antibiotics treatment and days in hospital were examined by regression analysis.

Results: Forty-two patients were confirmed as leptospirosis which accounted for 9.2% of total patients with multiple organ dysfunction syndrome. Forty-nine excluded cases were identified for a case-control analysis for clinical distinction. The most common presentations of leptospirosis are fever (97.6%), acute kidney injury (85.7%), and jaundice (61.9%). The leptospirosis group showed lower urine specific gravity (cutoff value: 1.0145) and enlarged kidney size (cutoff value: 11.05 cm) as compared with the excluded cases by multivariate logistics regression. Delayed antibiotics administration prolongs the duration of hospitalization (R Square: .486, P< .01). No mortality has been found in the leptospirosis group after initiation in 2003 of rapid IgM serology assay which showed considerably high sensitivity and specificity.

Conclusions: Leptospirosis accounts for a salient cause of multiple organ dysfunctions in Taiwan. Early awareness of leptospirosis by distinct presentations, followed by prompt antibiotics therapy can dramatically save the patients. The easy-performed rapid IgM serology assay is suitable as a rapid screening test for the diagnosis of leptospirosis.

Funding: Government Support - Non-U.S.

PUB132

Monocyte ADAM17 Content Correlates with the Neovascularization of the Carotid Adventitia and with Intima Media Thickness in Normal Individuals with No Risk Factor for Atheromatous Disease M. Vittoria Arcidiacono,[1] Teresa Vidal,[2] Didac Mauricio,[3] Elvira Fernandez,[4] Adriana S. Dusso.[1] *[1]Experimental Nephrology, IRBLLEIDA, Lleida, Spain; [2]UDETMA, Hospital Arnau de Vilanova, Lleida, Spain; [3]Endocrinology Dept., Hospital Arnau de Vilanova, Lleida, Spain; [4]Nephrology Dept., Hospital Arnau de Vilanova, Lleida, Spain.*

Background: Cardiovascular (CV) disease is the leading cause of mortality in chronic kidney disease (CKD) patients. A challenge for nephrologists is to develop accurate non invasive tools for the early identification of subclinical CV disease. We focussed on ADAM17 as an early marker because mild elevations in the activity of this enzyme in peripheral blood monocytes-macrophages (PBMM) associates with a high risk for CV mortality in normal individuals. Furthermore, ADAM17 plays a key role in the development of neovascularization, inflammatory lesions in the arterial wall, and foam cell formation.

Methods: We studied the association between ADAM17 content in PBMM and both the degree of neovascularization [vasa vasorum density (VV)] of the adventicia and carotid intima media thickness (CIMT) because neovascularization is shown to precede the arterial inflammatory/atheromatous damage that leads to increases in CIMT. We examined 43 normal individuals, 51% men, average age 46 years old (Age range 30-70) with none of the classical risk factors for atheromatous or CV disease.

Results: PBMM ADAM 17 correlated positively with VV content in the carotid adventicia ($r=0.43$ $p<0.005$; $n=42$) at all ages for both genders. Importantly, PBMM ADAM17 correlated with the CIMT only in the age range 41 to 50 ($r=0.71$, $p<0.005$, $n=15$), which precedes the highest increases not only in CIMT, but also in serum cholesterol and in blood pressure (two well known risk factors for the progression of vascular lesions) that occur in the age range 50-60.

Conclusions: PBMM ADAM17 content appears to contribute to the initiation of both neovascularization and the subclinical inflammatory/atheromatous lesion leading to increases in CIMT thereby providing a novel and highly sensitive diagnostic tool for subclinical atheromatous disease.

Funding: Government Support - Non-U.S.

PUB133

Abstract Withdrawn

PUB134

Prevalence of Aspirin Resistance in Chronic Kidney Disease Patients Isabelle Ayoub, Abdallah Sassine Geara, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: Aspirin resistance is a new concern with a prevalence of 28% in the population with cardiovascular diseases without a history of CKD. In the hemodialysis population this prevalence was observed to be 34.7%. Aspirin is prescribed frequently in Chronic Kidney Disease (CKD) patients for primary and secondary prevention of cardiovascular diseases. The aim of this study was to determine the prevalence of aspirin resistance in patients with CKD.

Methods: In a cross-sectional study, we measured platelets reactivity in CKD patients on aspirin therapy. A total of 19 patients with CKD stage III and IV were evaluated in this study. The resistance to aspirin was assessed by evaluating the Aspirin Reaction Units (ARU) using platelet function analyzer-100 (VerifyNow Aspirin®). Aspirin resistance is defined as ARU > 550. The control group was a cohort of patients with cardiovascular diseases on aspirin but without CKD.

Results: In the nineteen CKD patients on aspirin the mean ARU was 425.2 compared to 468.03 in the hundred patients in control group ($p<0.003$). The control group was found to have 10% aspirin resistance with ARU >550.

Conclusions: We were able to conclude that our patient with CKD stage III and IV do not have characteristic findings of aspirin resistance by testing ARU. Although our sample size was small this information is helpful as these patients carry a high degree of cardiovascular risk.

PUB135

Presence of Anemia and Its Therapeutic Management in Patients with Chronic Kidney Disease Stages 3, 4 and 5 Not on Dialysis at Nephrology Units in Catalonia (The MICENAS I Study) Aleix Cases,[1] Alberto M. Martinez-Castelao,[2] Joan Fort,[3] Jordi Bonal,[4] J.M. Galceran,[5] J. V. Torregrossa.[1] *[1]Nephrology and Transplantation Unit, Hospital Clínic, Barcelona, Spain; [2]Nephrology Unit, Hospital de Bellvitge, Barcelona, Spain; [3]Nephrology Unit, Hospital Vall d'Hebron, Barcelona, Spain; [4]Nephrology Unit, Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain; [5]Nephrology Unit, Fundación Althaia, Manresa, Barcelona, Spain.*

Background: Anemia prevalence and its therapeutic approach in patients with CKD stage 3-5 not on dialysis in Catalonia in usual clinical practice conditions are unknown.

Methods: Cross-sectional study conducted in nephrology units in Catalonia, study approved by an Ethical Committee. Patients aged ≥18 years, diagnosed with CKD stages 3, 4 and 5 not on dialysis, with Hb measurement in the previous 2 months and give their informed consent were consecutively recruited. Anemia defined as Hb<13.5 g/dL in men, Hb<12.0 g/dL in women or receiving ESA.

Results: A total of 22 investigators recruited 531 patients, of which 504 were valid for analysis. 56.4% of patients were men; mean age: 67.8±15.5 years. Regarding CKD Stage, 61.5% of patients were in stage 3 (3a: 23.0%, 3b: 38.5%), 30.2% stage 4 and 8.3% stage 5. 58.5% of patients had anemia (stage 3a:35.3% and 3b:52.1%; stage 4:73,7%; stage 5:97,6%) showing a higher prevalence in more advanced stages. 40.5% received treatment for anemia at the time of the visit, the percentage of treated patients were 16.7%, 31.3%, 58.2% and 85.7% in Stage 3a, 3b, 4 and 5 respectively ($p<0.05$). 66.0% received iron; 14.5% folic acid; 10.0% vitamin B12 and 54.5% were treated with ESA. ESA therapy was more frequent in stage 5 (Stage 3a: 26.3%, stage 3b: 36.7%, stage 4: 61.2%, stage 5:83.3%; $p<0.001$). Darbepoetin alfa was the ESA most frequently prescribed (62.5%), mainly administered every 2 weeks; followed by methoxy polyethylene glycol-epoetin beta (30.4%) administered on a monthly basis, with a median monthly dose of 80 and 75 µg, respectively, both subcutaneously.

Conclusions: More than half of patients in stages 3, 4 and 5 not on dialysis attended in nephrology units of Catalonia had anemia and stage 5 patients were the ones who received more anemia therapy.

PUB136

Prospective Observational Multicenter Study To Evaluate the Effectiveness and Safety of MIRCERA® in Patients with Anemia Secondary to Chronic Kidney Disease. MINERVA Study Aleix Cases,[1] Jose Portoles,[2] J. Calls,[3] Alberto M. Martinez-Castelao,[4] Domingo Sanchez-Guisande,[5] Alfonso Segarra.[6] *[1]H. Clínic, Spain; [2]H. Puerta de Hierro, Spain; [3]H. Manacor, Spain; [4]H. Bellvitge, Spain; [5]H. Barbanza, Spain; [6]H. Vall d'Hebron, Spain.*

Background: Once-monthly dosing of MIRCERA® provides stable and sustained hemoglobin (Hb) levels in CKD patients. This study aimed to evaluate the effectiveness and safety of once-monthly MIRCERA® in patients with CKD stages 3-5 on pre-dialysis or receiving hemodialysis (HD) in routine clinical practice

Methods: Multicenter prospective observational study. An interim analysis at 6-months follow-up is presented. Data were collected from patients receiving MIRCERA® (at least one month of treatment at baseline visit) to correct anemia or as conversion treatment

Results: A total of 243 patients were evaluated: 55% male; age: 71±15years. Diabetic nephropathy was the most frequent etiology of CKD (27%). Naïve and converted patients: 51% and 49% respectively. 64% were on predialysis and 36% on HD. Out of converted patients 73% had previously received epoetin beta and 27% darbepoetin alfa; mean weekly doses 6,762±6,259.7IU and 38.8±32.4µg respectively. Mean Hb levels at the initiation of MIRCERA® and after 6 months of study were 10±1.1g/dL and 11.6±1.1g/dL in naïve patients. Mean Hb levels at baseline and after 6 months were 11.5±1.1g/dL and 11.4±1.2g/dL in converted patients. There were not significant differences in Hb levels between groups. Mean doses of MIRCERA® at the beginning of treatment and 6 months after study initiation were 117±104µg/month and 122±90µg/month in naïve patients and 118±72.9µg/month and 141±103.9µg/month in converted patients with no significant differences between both groups. 94% and 75% of naïve and converted patients did not require any dosage adjustment. None of the patients experienced MIRCERA®-related adverse events

Conclusions: The preliminary results of this interim analysis suggest that MIRCERA® is effective and safe for anemia correction and Hb level maintenance in CDK patients not on dialysis and on HD in routine clinical practice. These results support the data obtained in previous phase III/IV clinical trials.

Funding: Pharmaceutical Company Support

PUB137

Obesity Is Associated with Proximal Tubular Hypertrophy Avry Chagnac,[1,2] Ana Tovar,[4] Yaacov Ori,[1,2] Michal Herman-Edelstein,[1] Boris Zingerman,[1] Sydney Benshetrit,[3] Uzi Gafter.[1,2] *[1]Nephrology, Rabin Medical Center, Petah Tikva, Israel; [2]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; [3]Nephrology, Meir Hospital, Kfar Saba, Israel; [4]Pathology, Rabin Medical Center, Petah Tikva, Israel.*

Background: Obesity and Diabetes mellitus are associated with glomerular hyperfiltration and glomerular hypertrophy. Data in diabetic rats suggest that tubular hypertrophy and increased proximal sodium reabsorption may play a role in the pathogenesis of glomerular hyperfiltration. Proximal tubular sodium reabsorption is increased in obesity. No studies in obese animals and humans have yet shown that the volume of tubules is increased in obesity.

The aim of the present study is to estimate the volume of the proximal tubules in obese subjects and to determine whether tubules undergo cellular hyperplasia.

Methods: Seven obese (BMI>30) and 6 lean subjects (BMI<25) participated in the study. Subjects had undergone a kidney biopsy for renal abnormalities. Inclusion criteria were normal GFR or mild chronic renal insufficiency and no or mild chronic interstitial fibrosis. Stained sections were retrospectively examined on light microscope. All available glomerular profiles and 15 randomly chosen proximal tubular profiles were photographed at x200 and x400 magnification respectively. The cross sectional area of these structures was estimated using a grid. The number of proximal tubular cells was estimated by counting the number of nuclei per tubular section.

Results:

	Obese	Lean	P
Age (yrs)	56±11	25±6	<0.0005
BMI	40±7	21±3	significant per definition
S creat (mg/dl)	1.3±0.5	0.8±0.2	<0.05
Proteinuria (g/d)	3.2 (0.2-7.6)	1.1 (0-3.8)	NS
Fasting blood glucose (mg/dl)	104±9	87±4	<0.005
Hba1c (%)	5.95±0.3	NA	

Cross sectional area of the proximal tubules was 35% higher in the obese as compared to the lean group ($P<0.002$). Glomerular cross sectional area was 106% higher in the obese as compared to the lean subjects ($P<0.004$). The number of nuclei profiles/proximal tubule profile was similar.

Conclusions: Obesity is associated with increased proximal tubular volume, without tubular cell proliferation. These data suggest that the increased tubular volume is due to cellular hypertrophy and not hyperplasia.

PUB138

Plasma Fluorescent Oxidation Products and Risk of Chronic Kidney Disease Jing Chen,[1] Tianying Wu,[2] L. Lee Hamm,[1] Islam Enver Khan,[1] Arnold B. Alper,[1] Grace Browne,[1] Chung-Shiuan Chen,[1] Yanxi Liu,[1] Katherine T. Mills,[1] Chuan He,[1] Md Mahfuzur Rahman,[1] Stephanie E. Rogers,[3] Joseph Tarsia,[1] Kamal Ramesh Shah,[1] Eric E. Simon,[1] Myra A. Kleinpeter,[1] Jiang He.[1] *[1]Tulane University; [2]University of Cincinnati Medical Center; [3]University of California, San Francisco.*

Background: A few clinical studies have reported increased oxidative stress in patients with end-stage kidney disease. Plasma fluorescent oxidation product (FLOP) is a stable and easily measured biomarker of oxidative stress. However, its association with risk of chronic kidney disease (CKD) has not been studied.

Methods: We examined the association of FLOP and risk of CKD in 201 CKD patients and 201 controls without CKD from the community. CKD was defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m² or presence of albuminuria.

Results: Compared to controls, patients with CKD were older (56 vs. 53 yrs), more likely to be male (55% vs. 45%), less likely to have graduated from high school (59% vs. 82%), or consume alcohol (28% vs. 59%). Race and cigarette smoking were comparable between CKD patients and controls. Mean systolic blood pressure (132 vs. 122 mmHg), body mass index (32 vs. 29 kg/m²), fasting glucose (120 vs. 103 mg/dL), history of hypertension (88% vs. 24%), history of diabetes (49% vs. 6%), and cardiovascular disease (44% vs. 7%) were higher while LDL cholesterol (102 vs. 118 mg/dL) was lower

in CKD patients than in controls. After adjustment for the above risk factors, the median inter-quartile range (IQR) of FLOP was significantly higher in patients with CKD than in controls [46.19 fluorescent intensity (FI)/mL (IQR, 31.35-61.04) vs. 15.49 FI/mL (IRQ, 1.01-29.97), p=0.013]. Compared with those with a FLOP level below the 75th percentile, participants with a FLOP level above the 75th percentile had a 15.4-fold increased odds (95% CI, 6.2-38.2) of CKD after adjustment for co-variables.

Conclusions: These data indicate that an elevated FLOP level is associated with risk of CKD. Furthermore, our study findings support the notion that oxidative stress is involved in the pathogenesis of CKD.

Funding: Other NIH Support - the National Center for Research Resources

PUB139

Abstract Withdrawn

PUB140

Efficacy and Safety of Valsartan in Hypertensive Patients with Albuminuria in China Xiaoqiang Ding, Xiaoyan Zhang. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: There is limited study to demonstrate efficacy of ARB in albuminuria reduction in Chinese hypertensive patients with albuminuria.The objective of this observational study was to evaluate the safety and efficacy of valsartan in Chinese hypertensive patients with albuminuria whose blood pressure was not adequately controlled in a real-world setting.

Methods: This is an observational, open-label study. Chinese hypertensive patients with albuminuria(range for 30~1000mg/24h or UACR 30-1000mg/g Cr) whose blood pressure was not controlled received valsartan 80-160mg and were observed for 12 weeks.

Results: Significant reduction of mean sitting systolic blood pressure (MSSBP) and mean sitting diastolic blood pressure (MSDBP) from baseline at each visit were observed in the intent-to-treat (ITT) population(n=1180). General BP controlled rate in ITT population at end of week 12 was 73.2%. Besides, at end of week 12, mean albuminuria reduction rate of those patients who repeated urine albumin level test(n=904) was 33.7%. 18.9% (171 of 904) of these patients have returned to normal albuminuria and the rate of normalization was higher in diabetes patients than in non DM patients. 37.6% (340 of 904) of patients have gained over 50% reduction of albuminuria, and the rate of >50% reduction of albuminuria is also higher in diabetes patients than in non DM patients (41.7% vs 33.8%, p=0.016). The total incidence of adverse events was 4.7% in safety population. There were no investigator drug related serious adverse events reported in the study.

Conclusions: This is the first large sample size observational study to demonstrate ARB's effect in albuminuria reduction in Chinese hypertensive patients with albuminuria. Valsartan is efficacious, well tolerated and devoid of any serious adverse events both in BP reduction and in albuminuria reduction in Chinese real-world setting. Besides, valsartan may reduce more albuminria in diabetes patients than non diabetes patients.

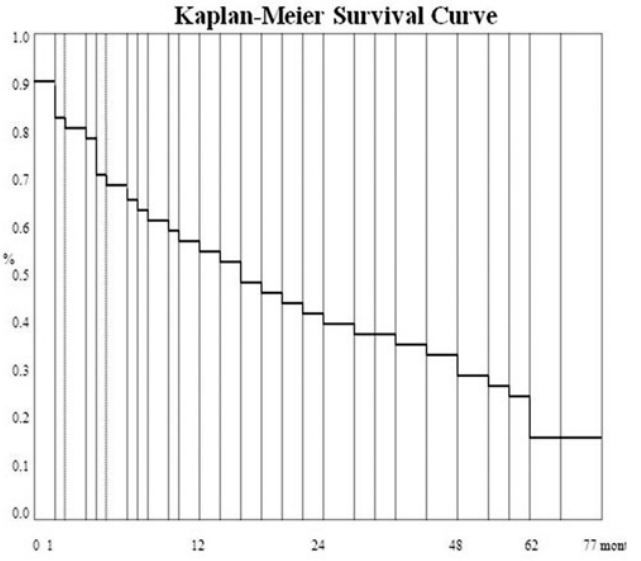
PUB141

Cardiorenal Syndrome Type 4 (crs 4) Associated with Anemia: Long Term Survival Analysis Matteo Floris,[1] Anna Clementi,[2] Dinna N. Cruz,[2] Antonello Pani.[1] *[1]G Brotzu Hosp, Cagliari, Italy; [2]S. Bortolo Hosp, Vicenza, Italy.*

Background: In Cardiorenal Syndrome type 4(CRS4)CKD is responsible for heart failure.Anemia is a complication of both conditions and is associated with an increased risk of mortality,but the possible correlation between the clinical outcome and the hemoglobin(Hb)levels still remains unclear.We analyzed the prevalence of the association between CRS4 and anemia in our hospital and the effect of the severity of anemia on long-term survival

Methods: Among all the patients admitted to our department from Jan 2001 to Jan 2008(n = 7768)we selected those who met the following criteria upon admission:signs and symptoms of systolic heart failure,left ventricular ejection fraction<40%,GFR MDRD<60 ml/min,Hb <13.5in males and <12.0in females.76 patients(0.97%)met the inclusion criteria.The primary outcome was all cause mortality and all patients were followed up for at least 18 months after discharge.Patients were stratified according to Hb levels into three groups(1,2,3)homogeneous for sex, age and comorbidities (1=Hb≥10.5, 2=Hb<9 and>10.5, 3=Hb≤9 g/dl).Univariate survival analysis were performed using Kaplan-Meier estimation and long-rank tests

Results: The overall survival is shown in the figure



60 months survival was 15%, 26%, and 18% in groups 1, 2, and 3, respectively.The difference between the survival curves in the 3 groups was not statistically significant(Log Rank Test=0.13).

Conclusions: Considering that limited data are available in this field we found that the association between CRS4 and anemia was characterized by severe prognosis.We have not observed a significant correlation between the severity of anemia and mortality risk, probably because of the elderly age of patients and the presence of comorbidities. Further studies are necessary to better understand the overall burden of disease, for a risk stratification and design of potential targets for intervention.

PUB142

Umbilical Hernia in Patients with Autosomal Dominant Polycystic Kidney Disease Yoshinari Hattori, Tatsuya Suwabe, Keiichi Sumida, Rikako Hiramatsu, Masayuki Yamanouchi, Noriko Hayami, Junichi Hoshino, Yoshifumi Ubara. *Toranomon Hospital, Nephrology Center, Kajigaya, Kanagawa, Japan.*

Background: Patients with autosomal dominant polycystic kidney disease (ADPKD) are known to have a high prevalence of abdominal wall complications such as umbilical hernia due to increased intra-abdominal pressure because of severe nephromegaly and/or hepatomegaly. However, the actual frequency has not yet been reported.

Methods: We performed transcatheter arterial embolization (TAE) for ADPKD patients who chose this option to alleviate compression symptoms related to kidney and/or liver enlargement. The frequency of umbilical hernia (apparent in the standing position) was evaluated retrospectively.

Results: Out of 497 patients receiving TAE, 159 patients were diagnosed as having umbilical hernia (31.9%) (64 men and 95 women). Their mean age was 55.3+/-8.5 years. Out of these 159 patients, hepatomegaly was dominant in 38% (n=60), nephromegaly was dominant in 54% (n=86), and both were similar in 8% (n=13). After abdominal distension was improved by TAE, umbilical hernia improved partially. Surgery for the hernia was performed successfully in five patients.

Conclusions: Although umbilical hernia is a potentially serious complication in patients with ADPKD, TAE may provide therapeutic improvement by reducing the intra-abdominal pressure.

PUB143

Neurocognitive and Social-Behavioral Functioning of Preschool Children with Mild to Moderate CKD Stephen R. Hooper,[1] Arlene C. Gerson,[2] Robert W. Butler,[3] Susan R. Mendley,[4] S. Shinnar,[5] Marc Lande,[6] Debbie S. Gipson,[7] Matthew Matheson,[8] Marjolaine M. Limbos,[9] Bradley A. Warady,[10] Susan L. Furth.[11] *[1]UNC, Chapel Hill, NC; [2]JHMI, Baltimore, MD; [3]OHSU, Portland, OR; [4]U Maryland, Baltimore, MD; [5]Albert Einstein, New York, NY; [6]U Rochester, Rochester, NY; [7]U Michigan, Ann Arbor, MI; [8]JHSPH, Baltimore, MD; [9]BC Children's Hospital, Vancouver, BC, Canada; [10]Mercy Children's Hospital, Kansas City, KS; [11]CHOP, Philadelphia, PA.*

Background: Few studies have examined the neurocognitive and social-behavioral functioning of preschool children with CKD. We used baseline data to describe the CKiD preschool sample, and to identify disease-specific factors for impaired function in this sample.

Methods: Subjects included 95 children, 12 mos. to 5.9 yrs. (median = 3.9 yrs), with a median iohexol or estimated GFR of 44.7 ml/min/per 1.73m^2. In addition to level of function and percent of subjects 1 SD or more below the test mean, multiple regression examined the associations between biomarkers of CKD (i/eGFR, anemia, hypertension, seizures, low birthweight), and IQ, attention, and parent ratings of adaptive behavior, social-emotional, and executive functions.

Results: Scores for all measures fell in the average range; however, 28% had low IQ, 30% executive dysfunction, 21% attention variability, and 36% adaptive behavior problems. None of the biomarkers were significantly associated with measures of attention, executive function, or social-behavioral functioning, but presence of seizures ($p<.03$), low birth weight ($p<.02$), and maternal eduation ($p<.02$) were related to low IQ, and presence of seizures ($p<.008$) was related to low adaptive behavior ratings.

Conclusions: Although when compared to normative expectations an increased number of preschoolers with CKD had low IQ, executive dysfunction, inattention, and low adaptive behavior, only the presence of seizures and low birthweight were related to IQ and adaptive behavior. These findings support ongoing neurodevelopmental surveillance of young children with CKD, particularly with respect to disease progression.

Funding: NIDDK Support

PUB144

Daptomycin Plus Ampicillin for Enterococcal Endocarditis in a Patient at Higher Risk for Gentamicin Nephrotoxicity Oscar A. Iznaola,[1] Matthew M. Goodwin,[2] Miguel A. Sierra-Hoffmann,[1] John Mohr.[2] *[1]Nephrology, Citizens Medical Center, Victoria, TX; [2]Cubist Pharmaceuticals; [3]Infectious Disease, Citizens Medical Center, Victoria, Tx.*

Background: Nephrotoxicity is one of the most important adverse events and therapeutic limitations of gentamicin. We present a case of a patient with CKD stage 4 , and enterococcus faecalis bioprosthetic valve endocarditis, treated with daptomycin plus amplicillin, instead of ampicillin plus gentamicin, the accepted standard of care.

Case report: An 83 year-old female, with history of CKD Stage 4, DM2, HTN, aortic bioprosthetic valve replacement, and permanent pacemaker was admitted with low grade fever and positive blood cultures for E. faecalis susceptible to ampicillin. The admitting physician thought the source of the infection was from an infected toe. The patient received levofloxacin IV for a total of 15 days and discharged. After the completion of this treatment, repeated blood cultures showed persistent E.faecalis. The patient was readmitted to the hospital and a transesophageal echocardiogram (TEE) showed small vegetations in the aortic valve. Ampicillin plus gentamicin were discussed, with consideration of gentamicin nephrotoxicity given the patient's baseline eGFR of 25 ml/min. Alternatively, a non-standard treatment for enterococcal endocarditis was proposed to the patient with daptomycin 400 mg (6 mg/kg) IV q 48 hrs plus ampicillin 2 gr bid. The patient decided to opt for daptomycin + ampicillin therapy for 6 weeks. Two weeks after completion of treatment, a set of blood cultures came back negative without any changes in her baseline renal function.

Conclusions: Gentamicin nephrotoxicity can reach 10-25% of treated patients. In enterococcal endocarditis, ampicillin plus gentamycin is the standard of care for 6 weeks. Here we show that the alternative daptomycin plus ampicillin, a non-standard option negativized the blood cultures 2 weeks after the completion of treatment without worsening the patient's eGFR. To our knowledge, this is the first publication showing the possible benefits of daptomycin plus ampicillin for enterococcal endocarditis in patients at high risk for gentamicin nephrotoxicity.

PUB145

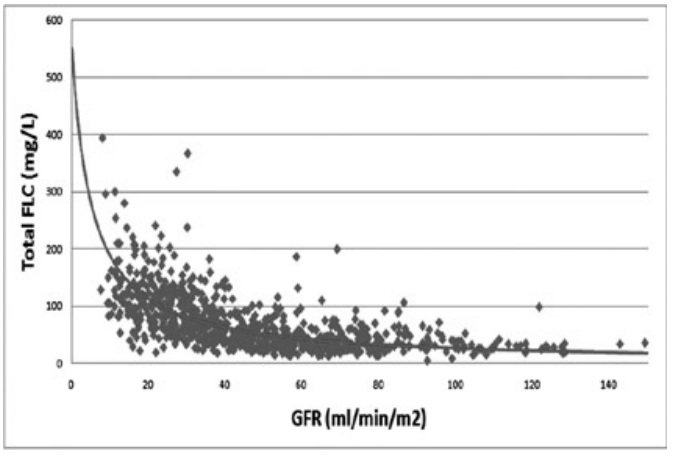
Determining Serum Polyclonal Free Light Production Rates: A Mathematical Model Richard T. Keir,[1] Neil D. Evans,[1] Anne Bevins,[2] Maarten W. Taal,[4] Colin A. Hutchison.[3] *[1]University of Warwick, United Kingdom; [2]The Binding Site Group Ltd, Birmingham, United Kingdom; [3]Renal Unit, University Hospital Birmingham, United Kingdom; [4]Nephrology, Derby Royal Infirmary, Derby, United Kingdom.*

Background: Serum concentrations of polyclonal free light chains (FLC) are determined by the activity of the adaptive immune system and when raised they independently predict adverse clinical outcomes in renal and general populations. However their absolute serum levels are also influenced by renal function. A method for determining the underlying FLC production rate independently of renal function is required.

Methods: Steady state analysis was performed on a 2 compartment mathematical model to generate a relationship between FLC production rates, total serum FLC levels and estimated GFR, namely:

$eProduction = a\text{Serum}\ (b + c\text{GFR})$

where a,b and c are constants derived from the model parameters, which include reticuloendothelial clearance, body volumes and the inter-compartmental transfer rates.



The derived production rates for kappa and lambda FLCs were 0.330 g/day and 0.178 g/day respectively.

Results: This equation was applied to data sets from two CKD cohorts. The first cohort was recruited from tertiary care nephrology clinics with moderate to advanced CKD (median GFR 29mls/min/1.73m$^{2)}$. The second cohort was recruited from a primary care with CKD stage 3 (GFR 53mL/min/1.73m^2). The work determined that FLC production rates were significantly raised in both cohorts. In each group 55% and 43% of patients, respectively, had FLC production rates more than twice normal.

Conclusions: In conclusion, we have developed a mathematical method whereby the production rate of FLCs can be determined from the absolute serum FLC level and estimated GFRs. The intention is that this new tool can be assessed in clinical studies as the role of serum FLCs is further evaluated as a prognostic marker in CKD.

PUB146

Is the Uremic Foot a Burning Emergency? Antonia Lopez,[100191] Marcora Mandreoli,[100191] Tommaso Bianchi,[100191] Maria Letizia Soverini,[100191] Juri Piattoni,[100191] Annalisa Zuccejlli,[100191] Antonio Santoro.[100191] *[1]Unit of Nephrology Dialysis Hypertension, Policlinico S Orsola Malpighi; [2]Dermatology, Local Health Unit.*

Background: Chronic Kidney Disease (CKD) is an independent risk factor for developing/worsening of wounds and the increasing risk correlates with decline of renal function. With the advent of hemodialysis, the life expectancy of these patients (pts) has improved, giving time for suffering all the consequences too.

Aim: We want to evaluate if an organized foot care team in an Hemodialysis Unit, transforming an "hard to heal" in an healing wound, can save limbs, reduce mortality, improve quality of life. We report our experience of a Dialysis Unit.

Methods: In the last year, over 83 dialysis pts, 25.3% was treated for 'hard-to-heal' wounds. The mean age was 75, the mean dialytic age 3.8 years; 41% were diabetic. The team was composed from a nephrologist, a dermatologist specialized in wound-care and three dedicated nurses of our Dialysis Unit, with an expertise in this field. The pathway required a dedicated nurse, personalized medication outline, all during the dialysis session without other hospital access of the patient.

Results: After a mean of 17 topical medications/pts and 1.5 months for each case, 13 cycles of systemic antibiotics, 2 cycles of VAC-therapy, we achieve wound healing in 77,5% of treated cases. **Comments**: Lower limb lesions and diabetic foot are well known complications in diabetic pts. However, in hemodialysis population the prevalence of uremic foot is scarcely recognized, despite, nearly 25% of dialysis pts exhibited peripheric arteriopathy (PAD). Moreover, current guidelines on foot care should recognize CKD-5d as an indipendent risk factor for foot disease.

Conclusions: Our initial experience demonstrated that a "task force" expressly dedicated to the care of wounds in the setting of Hemodialysis Unit, may contribute to change the overwhelming course of foot lesions in CKD-5d pts and underlie the clinical weight of uremic foot on the quality of life of uremic pts. Our experience is in accord to some studies reporting that structured clinical pathways and a dedicated team are associated with a decreased lenght of stay and in-hospital complications, thus reducing in-hospital costs.

Funding: Private Foundation Support

PUB147

Association between Chronic Kidney Disease and Abnormal Ankle Brachial Index Fukun Niu,[1,2] Luxia Zhang,[1] Xingyu Wang,[3] Lisheng Liu,[3] Haiyan Wang.[1] *[1]Institute of Nephrology and Division of Nephrology, Peking University First Hospital, Beijing, China; [2]Nephrology and Rheumatology, First Affiliated Hospital of Zhengzhou University, Zheng Zhou, Henan, China; [3]Beijing Hypertension League, Beijing, China.*

Background: Both low and high ankle-brachial index (ABI) has been reported to be independently associated with increased risk of cardiovascular disease and mortality. However, it is not clear whether chronic kidney disease (CKD) is associated with abnormal ABI.

Methods: This prospective cohort study included 1189 community-based participants from Beijing, China. Among them, 928 participants (78.0%) had complete data of kidney damage and ABI, and were therefore included in the present study. ABI was categorized as low ABI (< 1.00), normal ABI (1.00 – 1.30), and high ABI (>1.30) . Urinary albumin-to-creatinine ratio(ACR) and eGFR were assessed at baseline, and ABI was measured after a median of 6 years of follow-up. All participants had estimated glomerular filtration rate (eGFR) above 30mL/min/1.73m2 . Multivariable logistic regression was used to evaluate the association between CKD and abnormal ABI.

Results: The average age was 59.1±9.1 years and 45.6% were males. Among 63 participants with CKD defined by the presence of eGFR<60mL/min/1.73m2 or albuminuria, the prevalence of low ABI was significantly higher than those among participants without CKD (30.2% vs15.2%, P<0.01). After adjusting for potential confounders including eGFR, ACR was independently associated with increased risk of low ABI. For every 10 mg/g increase of ACR, the odds ratio(OR) for low ABI was 1.06 [95%CI, 1.02–1.11]. However, baseline eGFR was not significantly associated with low ABI. Among 151 participants with low ABI, 122 of them (80.8%) did not have self-reported history of cardiovascular disease. Indicators of kidney damage were not associated with high ABI in both univariate and multivariate analysis.

Conclusions: Albuminuria is independently associated with low ABI among a Chinese population with normal or mildly impaired renal function.

PUB148

Abstract Withdrawn

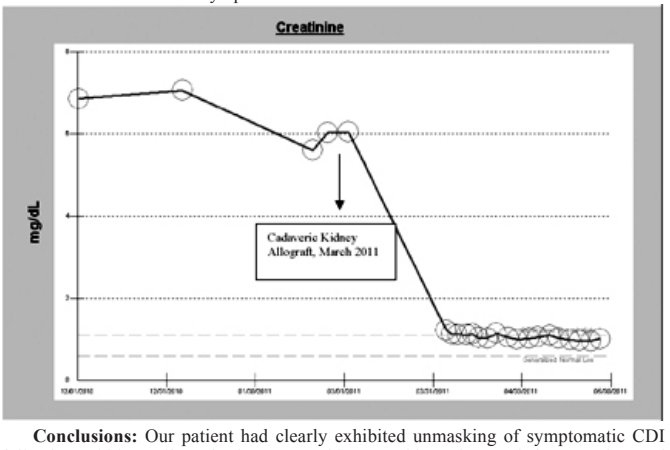
PUB149

Relapsing Central Diabetes Insipidus Following Renal Transplantation in an ADPKD ESRD Patient Macaulay A. Onuigbo.[1,2] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI.*

Background: A 59 year old Caucasian female with ESRD from ADPKD had in 2008 developed symptoms of central diabetes insipidus (CDI) following intracranial hemorrhage which were subsequently controlled with oral desmopressin. The behavior of her CDI after a successful kidney transplant had long been a subject of much speculation.

Methods: Case Report

Results: The patient had remained on HD between 2008 and 2011, serum creatinine about 7 mg/dL. In September 2009, 24-hour urine volume was 1,430 ml. She had a left elective nephrectomy in August 2010, as part of pre-transplant work up; subsequently she made less urine. She received a cadaveric renal allograft transplant in March 2011. Desmopressin was withheld post-operatively. Graft function was excellent; serum creatinine 1.2 mg/dL; on cellcept and prograf. She presented to our Transplant Office in April 2011, concerned about increasing urinary frequency, polyuria, nocturia 6x/night, and disabling thirst. Post-renal transplantation CDI exacerbation was diagnosed. Blood was sent out for a vasopressin (AVP) level. She was immediately restarted on PO desmopressin, 0.1 mg QHS. 24-hour urine volume was 3,700 ml; measured creatinine clearance 65 ml/min (Figure). Plasma AVP was not measurable. After six days, she reported no improvement in symptoms and the dose was raised to 0.2 mg QHS. She improved to 2x/night nocturia and resolution of the other symptoms.



Conclusions: Our patient had clearly exhibited unmasking of symptomatic CDI following a kidney allograft placement. This unmasking of pre-existing CDI in renal allograft recipients following kidney transplantation has been the subject of a few published case reports. Our patient, we submit, should have been maintained on pre-transplant oral desmopressin regimen post-transplantation, with the dose titrated as indicated, and not simply withheld.

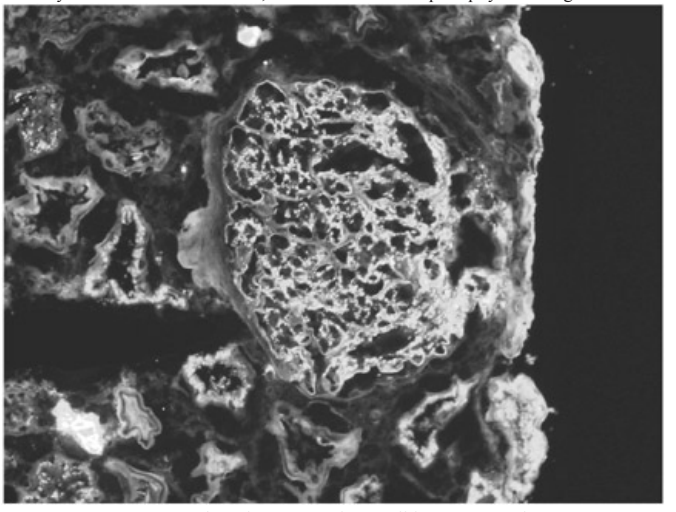
PUB150

IgA-Dominant Post-Infectious Glomerulonephritis – An Unrecognized Cause of Reversible Acute Kidney Injury in a Diabetic CKD Patient Macaulay A. Onuigbo,[1,2] Samih H. Nasr.[3] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI; [3]Pathology, Mayo Clinic, Rochester, MN.*

Background: According to Nasr et al, postinfectious glomerulonephritis (PIGN) is primarily a childhood disease, and follows URI or impetigo; its occurrence in adults is not well characterized. Immunocompromise is common; commonly diabetes or malignancy. Common infection site was skin; common causative agent staphylococcus. IgA-dominant PIGN (IgA-PIGN), a distinct subgroup, constituted 17% of cases. We report an unusual case of adult IgA-PIGN.

Methods: Case Report

Results: A 55-year old type 2 diabetic male patient presented in January 2011 with glomerulonephritis, vomiting and increased serum creatinine of 2.5 mg/dL up from 1.4. Immunologic work up for secondary GN was negative. IF staining for IgA in kidney biopsy showed coarsely granular mesangial and GBM positivity, the typical "starry sky" pattern of acute PIGN. This diagnosis triggered a search for recent infection(s). It turned out that in mid-November 2010, the patient broke his left humerus after a fall and had developed intergluteal pressure ulcers, treated with topical clotrimazole and Bactrim. Examination confirmed healed inter-gluteal ulcers. Dicloxacillin, 250 mg 4x/day for 10 days led to falling serum creatinine from a peak of 4.36 mg/dL. Hypotension from carbegoline interrupted AKI recovery. After it was discontinued, serum creatinine fell promptly to 1.5 mg/dL.



Conclusions: Our patient demonstrated reversible AKI secondary to IgA-PIGN. Lessons learnt include that kidney biopsy should be considered in undiagnosed diabetic AKI, that long after the triggering infection(s), AKI from IgA-PIGN remains potentially reversible with anti-staph therapy, and we submit that in diabetics with unexplained AKI, a trial course of anti-staph therapy may be warranted.

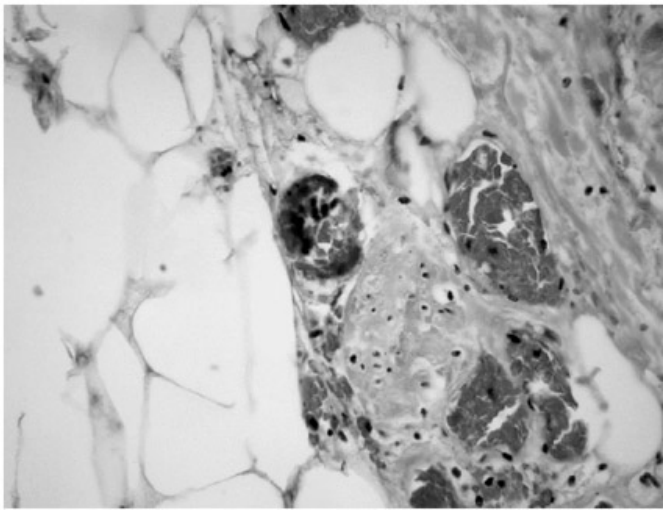
PUB151

Cutaneous Capillary Calciphylaxis – A New Variant of Calciphylaxis in End Stage Renal Disease Macaulay A. Onuigbo,[1,2] Scott A. Martin.[3] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI; [3]Pathology, Mayo Clinic Health System, Eau Claire, WI.*

Background: Calciphylaxis is a rare, usually fatal vasculopathic disorder of cutaneous iscemia/necrosis with calcification in small- and medium-sized venules/arterioles, mostly in ESRD. It has been reported infrequently among patients on warfarin. Warfarin-induced skin necrosis (WISN) and calciphylaxis can mimic each other. Typically, WISN occurs soon after drug initiation. We encountered an unusual spreading cutaneous condition in an ESRD patient also on warfarin.

Methods: Case Report

Results: A 61-year old diabetic male patient on HD for ESRD presented in March 2011 with a painless abdominal "mass". Initial diagnosis was lipoma. A surgical evaluation with ultrasound examination and biopsy suggested panniculitis; he did not improve after oral antibiotics. The lesions spread locally with increasing pain and blue-violet discoloration of the overlying skin. Calciphylaxis versus hematoma were considered. By early April, he reported "spreading" to the thighs; was admitted, started on IV antibiotics, and atypical WISN was now entertained. Warfarin was discontinued; IV heparin and IV vitamin K started. Review of ultrasound images revealed no dystrophic calcifications. He never recovered and expired. Autopsy revealed cutaneous ulceration and necrosis, mural calcification of subcutaneous capillaries, without intravascular thrombin formation, consistent with calciphylaxis.



Conclusions: Our patient demonstrated the well acknowledged diagnostic dilemma of differentiating between calciphylaxis and WISN in an ESRD patient on warfarin. Regardless of morphology, absent radiologic findings, normal PTH and concurrent warfarin, calciphylaxis must be considered in the differential diagnosis of painful, necrotic lesions occurring in the setting of ESRD.

PUB152

The Factors Associated with the Beneficial Effect of Angiotensin Receptor Blocker on Glomerular Filtration Rate and Albuminuria among Hypertensive Patients Gen Ouchi, Kentaro Kohagura, Atsushi Sakima, Kunitoshi Iseki, Yusuke Ohya. *Department of Cardiovascular Medicine, Nephrology and Neurology, Faculty of Medicine, and Dialysis Unit, University of the Ryukyus, Nishiharacho, Okinawa, Japan.*

Background: Benefit of angiotensin II receptor blocker (ARB) on estimated glomerular filtration rate (eGFR) has been reported, but not all hypertensive patients. However, the factors related to improving eGFR and decreasing albuminuria is not clear in these patients.

Methods: We conducted retrospective cohort study in hypertensive patients (N=90, 37% male) who had more than two data for eGFR and albuminuria from 2008 to 2010. We compared these data between baseline and the last visit data (mean observation period 21 months)

Results: At baseline, the mean age were 66 years, blood pressure (BP) 130/75 mmHg, eGFR 65 ml/min/1.73 m^2, albuminuria (uAlb) 124 mg/gCr, and uric acid 5.9 mg/dl. BP had not changed between the study periods. There were elevated eGFR in 44 patients (49%) and decreased uAlb in 47 patients (52%). Changes in eGFR was negatively correlated with the changes in serum uric acid (r=-0.31, p=0.003) and was not correlated with the changes in uAlb. Changes in uAlb were positively correlated with the changes in BP, serum glucose, and body mass index (BMI). Logistic analysis showed that decrease in serum uric acid was significantly associated with elevation of eGFR; age-adjusted odds ratios (95% CI) was 3.80 (1.78-8.13). While, the changes in BMI and serum glucose were significantly associated with the decreased in uAlb; age-adjusted odds ratios (95% CI) were 1.55 (1.02-2.37) for BMI and 1.02 (1.00-1.04) for serum glucose, respectively.

Conclusions: In conclusion, decrease in uric acid was associated with elevation of eGFR, while decreases in BMI and serum glucose were associated with the decrease in uAlb. Results suggest that the factors associated with the beneficial effect of ARB might be different between eGFR and uAlb.

PUB153

Low Protein Supplemented Diet in Chronic Kidney Diseases: Are They Feasible? Giorgina B. Piccoli, Federica N. Vigotti, Martina Ferraresi, Valentina Consiglio, Stefania Scognamiglio. *Nephrology and Dialysis, San Luigi Hospital - University of Turin, Orbassano, Torino, Italy.*

Background: The criticisms on the early start of dialysis, the increase in old patients with poor dialysis survival and the budget constrains have renovated interest in low protein diets, not only for slowing the progression of CKD, but also as "metabolic stabilisers", prolonging the pre dialysis care.

Low protein diets are often considered as hardly feasible in the clinical practice.

Aim of the present study was to identify the clinical profile of patients successfully treated by a vegetarian low protein diet, defining success as >6 months of diet.

Methods: Prospective analysis, December 2007-April 2011, since the start of a new Nephrology Unit. A schema of vegetarian low protein diet was proposed for progressive CKD stages 3-5 and/or refractory proteinuria: Proteins 0.6 g/Kg/day; ketoacids and aminoacid supplements 1 pill every 8- 10 Kg, 1-3 free meals/week, according to clinical situation and personal needs, monthly follow-up, the simplified "non weighted" schema was based upon allowed and forbidden foods.

To identify patients with high probability pf success (>6 months diet) the following covariates were considered: age, sex, creatinine, GFR, proteinuria, educational level.

Results: Out of over 2000 patients evaluated, 129 started the diet. The population was heterogeneous; at start: median age 67 years (20-89); creatinine 3.6 mg/dL (0.9-16); proteinuria 1.5 g/day (0.1-18), GFR 20 mL/min (3-92). Educational level was the Italian standard(University in 12%). Diabetes and/or hypertension accounted for half of the nephropathies; 22/129 displayed no comorbidity.

At April 2011, 53 patients were on the diet, 35 discontiued it, 8 died, 33 started dialysis. The main side effect was poor enteric tolerance.

No correlation with functional data at start of treatment, educational level, age and compliance was found. In the subset with >6 months of follow-up, no patient developed clinical malnutrition, weight loss or hypercalcemia.

Conclusions: Vegetarian, supplemented low protein diets are feasible in a heterogeneous, non selected CKD population. A one-month trial may help identifying patients who may benefit from the diet.

Funding: Government Support - Non-U.S.

PUB154

Depressive Symptoms Impact on Quality of Life and Cognitive Function of Elderly Patients Undergoing Chronic Hemodialysis Carmen B. Tzanno-Martins,[1] Geison Stein Meirelles Ramos,[2] Fernanda Ribeiro Nisihara,[3] Elzo R. Junior,[3] João Paulo L.B. Martins.[1] *[1]CINE - Integrate Center of Dialysis, Guarulhos, São Paulo, Brazil; [2]Home Dialysis Center, São Paulo, Brazil; [3]Renal Class, São Paulo, Brazil.*

Background: To assess the impact of depressive symptoms on quality of life and cognitive function in elderly patients undergoing chronic hemodialysis (HD) through the application of specific test for depression: Beck Depression Inventory (BECK), a test for cognitive assessment: Modified Mini-Mental State Examination (3MS) and Kidney Disease Quality of Life-short form (KDQOL-sf).

Methods: We selected 159 patients on hemodialysis and apply the BECK, 3MS and KDQOL. We evaluated demographic data, presence of depressive symptoms, quality of life and cognitive function

Results: Patients were divided into two groups: Group I: <60 years (n=85) and group II: >60 years (n=74). In group I, median time was 12.4 years, 43/85 (50,5%) were females, 50/85 (58,8%) were married, 9/85 (10,5%) were divorced or widowed and 26/85 (30,5%) were single. In group II, median time of HD was 8 years, 37/74 (47,3%) were females, 50/74 (67,5%) were married, 17/74 (22,9%) were divorced or widowed, and 7/74 (9.4%) were single. Comparing Beck Inventory with 3MS, we obtained the following results: group I, 27/85 (31,7%) had depressive symptoms, of these 7/27 (25,9%) had cognitive impairment. In Group II, 29/74 (39,1%) had depressive symptoms, of these 16/29 (55,2%) had cognitive impairment. BECK compared with who had a median scale score below 50 or above in KDQOL-sf, we obtained the following results: group I, 27/85 (31,7%) had depressive symptoms; of these 15/27 (55,5%) had scores <50. In group II, 29/74, depressive symptoms, these 17/29 (58.6%) had <50.

Conclusions: We found that depressive symptoms correlate with cognitive impairment only in group II. However the quality of life is impaired in both groups with depressive symptoms, regardless of age.

We can conclude that the presence of depressive symptoms alters the quality of life in patients with chronic kidney disease in HD, and that the cognitive impairment in this population relates more with age than with presence of depressive symptoms.

PUB155

Greater Incidence of Dental Disease and Oral Health Hygiene after Survey in Single Center of Chronic Kidney Disease Clinic Program in Thailand Manoch Rattanasompattikul.[1] *[1]Medicine Division, Medical Department, Golden Jubilee Medical Center, Mahidol University, Nakhon Pathom, Thailand; [2]Oral Medicine and Periodontics, Faculty of Dentistry, Mahidol University, Bangkok, Thailand.*

Background: Limited data exist about the effect of chronic kidney disease (CKD) on dental health problem, especially in Asian population. Thus, oral infection and inflammation should be early investigated in all uremic patients.

Methods: This cross-sectional study was conducted in CKD clinic program after National Security Policy for improvement CKD care. We divided one hundred and twenty eight patients into different stages: late-stage (GFR <30ml/min/1.73m2), moderate-stage (GFR 30-59ml/min/1.73m2) and early-stage (GFR 60-90 ml/min/1.73m2) (N = 34 vs. 44 vs. 50). Data from medical records, clinical oral examination, radiologic studies for detection of dental caries and carotid calcification, saliva parameter, and yeast culture were collected and statistically analyzed among these CKD groups.

Results: Of 128 CKD patients, 94 (73.4%) were men. Age range was between 30-86 years, with an average of 61.0 ± 10.9 years. Common etiologies of CKD in this study were hypertension (26.6%) and DM type 2 (16.4%). %HbA1c was not different among the groups (p = 0.39). The most prevalent oral health problem in CKD patients was periodontitis (65.3%) followed by gingivitis (29.4%) but no difference among groups was observed in periodontal health. In late-stage CKD had less dental caries index when compared with early-stage CKD (5±4.8 vs. 8±5.5; -5.6 to -0.9, $p < 0.05$) and was correlation with increasing growth of saliva culture for S. mutans culture in early-stage CKD ($p < 0.05$). Although DMFT index was not significantly different, it showed tendency greater in early CKD group (22±6.3 vs. 19± 9.3, p=0.19). Candida significantly grew with deterioration of GFR when adjusted with HbA1C ($p < 0.05$).

Conclusions: Our data indicate that a single center experienced. Early stage had more dental health problems than late-stage CKD. High prevalent of periodontitis was observed in CKD patients. Routine dental examination and proper preventive dental care were suggested in CKD patients, especially in early stage of CKD.

Funding: Government Support - Non-U.S.

PUB156

The Role of CXCL12 (SDF-1α) in the Uremic Endothelial Dysfunction Vanessa Ribeiro,[1] Liandra Kondrat,[1] Geison Tiberá,[1] Simone Cristina Mikosz Goncalves,[2] Sérgio Elias Gardano Bucharles,[2] Roberto Pecoits-Filho,[2] Andréa Marques Stinghen.[1] *[1]Basic Pathology Department, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; [2]Center for Health and Biological Sciences, Pontificia Universidade Catolica do Paraná, Curitiba, Paraná, Brazil.*

Background: Endothelial dysfunction play a key role in the pathogenesis of cardiovascular disease (CVD) related to chronic kidney disease (CKD). The exact mechanism by which uremic toxicity leads to endothelial dysfunction is still poorly understood. CXCL12 (SDF-1α) is a pleitropic chemokine over expressed in inflamed and injured organs, capable of mobilizing cells to the site of injury where it can support tissue repair and regeneration. In this study we investigate the plasmatic associations between systemic inflammation, endothelial dysfunction and CXCL12, in hemodialysis (HD) patients.

Methods: Plasma samples were collected from HD patients. The systemic inflammation was assessed by high-sensitivity C-reactive protein (hsCRP) and interleukine-6 (IL-6) using an automated immunoturbidimetric and ELISA assay respectively. Endothelial dysfunction (IL-8) and CXCL12 levels were investigated by ELISA.

Results: Twenty-six patients (17±3 months on HD), 52±2 years old, 38% males, 11% diabetics) were included. The mean plasma concentrations of hsCRP, IL-6, IL-8, and CXCL12 were respectively 4,9±4,8 mg/mL, 6,76±8,1 pg/mL, 128,2±206,2 pg/mL, and 2313,01±1458,1 pg/mL. There was a positive correlation between hsCRP and IL-6 (ρ=0,57, P<0,005) and CXCL12 and IL-8 (ρ=0,4294, P<0,05).

Conclusions: Our data demonstrate that chemotaxis-related factors such as CXCL12 and IL-8 are increased and correlated in HD patients. These occurs in parallel with systemic inflammation. It is also well known that these chemokines are responsible to mobilized undifferentiated cells to the injured organs. We suggest that increased levels of CXCL12 and IL-8 found in these patients could reflect an activated repair system which hypothetically would be a way of measuring the extent of cardiovascular damage caused by uremic toxicity.

Funding: Government Support - Non-U.S.

PUB157

Evaluation of Efficacy of Lanthanum Carbonate (Fosrenol) in Patients with Calciphylaxis: A Novel Pilot Study Olatokunbo O. Shobande, Micah R. Chan. *Nephrology, University of Wisconsin, Madison, WI.*

Background: Calciphylaxis is a rare and debilitating vasculopathy seen primarily in patients with end stage renal disease (ESRD). The proposed mechanism of injury of vascular calcification involves hyperphosphatemia, elevated serum PTH, and hypercalcemia. Lanthanum carbonate is a potent non-aluminum, non-calcium phosphate binder that was approved for the treatment of hyperphosphatemia in patients with ESRD.

Methods: We have designed a multi-center open-label uncontrolled pilot study in collaboration with Wisconsin Network for Health Research (WinHR) to determine the efficacy of Lanthanum carbonate in the treatment of calciphylaxis. Twelve ESRD patients will be recruited from multiple dialysis centers and clinics around Wisconsin over a period of 21 months. Patients enrolled will have a baseline physical exam, recent dermatology consult with biopsy-proven calciphylaxis skin lesions, and photograph of the skin lesions. Laboratory parameters measured will include intact PTH, phosphorus, calcium, and albumin. Lanthanum will be administered orally in a dose of 1500-3750mg daily in divided doses with meals over a 12-week period. Dose escalation will be utilized to a target dose of 3750mg daily over a 4-week period. Patients will be evaluated monthly to determine clinical response and followed for a period of 3 months for the remainder of the 21-month recruitment and treatment period. Primary endpoints will be resolution of skin lesions, while secondary endpoints will be serum levels of calcium, phosphorus, and intact PTH.

Results: Three patients are currently enrolled in our study. After 8 weeks of treatment, there are noticeable improvements of their skin lesions.

Conclusions: Lanthanum carbonate is a novel, potential treatment modality for patients with calciphylaxis.

Funding: Pharmaceutical Company Support

PUB158

Self Reported Quality of Life by EQ5D in Chronic Kidney Disease Stephanie J. Stringer,[1,2] Mary Dutton,[1] Paul Cockwell.[1,2] *[1]Renal Unit, University Hospital Birmingham, United Kingdom; [2]University of Birmingham, United Kingdom.*

Background: Patients with CKD have reduced quality of life (QoL), however many instruments used to measure QoL are too detailed for use in routine clinical practice. The EQ5D (EuroQoL) health status assessment is a simple and robust questionnaire that has been validated in non-CKD populations and can be used in a routine clinical setting. In this study we utilised EQ5D to assess QoL in patients with progressive CKD

Methods: The Renal Insufficiency In Secondary Care (RIISC) study is a prospective cohort study of patients with progressive CKD. Patients undergo a detailed social and bio-clinical assessment including self assessment of QoL by EQ5D and questions relating to educational attainment and employment.

Results: To date 100 patients have been recruited: 61% were men; 70% were white and 15% Black and South-Asian respectively; mean age was 61.3 years (SD 18.1). 44% of the cohort had no qualifications and 15% were educated to university level. 35% were unemployed, 35% were retired and 30% were in employment. 20% of those employed were in unskilled occupations, 23% were in managerial or professional occupations. 51 % reported mobility problems, 9% could not self care and 52% could not carry out usual activities of daily living. 57% reported experiencing at least moderate pain and 30% reported anxiety and/or depression. The mean self rated health score was 63.5/100 (SD 20.9) and was associated with mobility ($p = 0.012$), inability to carry out usual activities ($p = 0.016$) and the presence of anxiety or depression ($p = 0.02$).There was no significant association with co-morbid load, number of medications taken, educational attainment and employment status.

Conclusions: In people with progressive CKD, EQ5D represents a simple scoring system for self reported QoL. Self rated health is a major determinant of mobility, ability to carry out usual activities and anxiety and/or depression. These findings indicate that self related health assessment by EQ5D in progressive CKD can identify key QoL indicators. This has implication for utilising social and cognitive interventions in people with CKD.

Funding: Private Foundation Support

PUB159

Multiple Measures of Neuronal Membrane Excitability from Chronic Kidney Disease to Renal Transplantation Matthew R. Todd,[1] Juan Mason,[1] Christopher E.G. Moore.[2] *[1]Wessex Renal & Transplant Service, Queen Alexandra Hospital, Portsmouth, Hampshire, United Kingdom; [2]Department of Clinical Neurophysiology, Queen Alexandra Hospital, Portsmouth, Hampshire, United Kingdom.*

Background: Uremic neuropathy is common in Chronic Kidney Disease (CKD). Traditional nerve conduction studies (NCS) correlate poorly with clinical neuropathy and only slowly and partially resolve after the initiation of Renal Replacement Therapy (RRT). These changes are associated with histological features suggesting structural and cytoskeletal changes. Novel electrophysiological tests can show changes in neuronal membrane function after a single dialysis session (Krishnan et al., 2005), but have not been reported in pre-RRT CKD or after transplantation.

Methods: We used multiple measures of excitability as described by Kiernan et al. (2000) to demonstrate peripheral nerve function in a patient being worked up for a living-related pre-emptive renal transplant. These techniques have been validated for longitudinal measurement by University College Dublin (data presented to the British Society of Clinical Neurophysiology, March 2011). Measures were taken at 8 months and 1 day pre-transplant, and 22 hours and 4 months post-transplant. NCS were also performed at each visit.

Results: NCS showed a mild symmetrical sensorimotor neuropathy which did not change significantly over the 12 months. Membrane function, as measured by threshold electrotonus and recovery cycle, was significantly impaired at 8 months pre-transplant and progressed by the day of transplant. Within 22 hours of transplantation membrane function had normalized, and continued improvement was shown at 4 months post-transplant.

Conclusions: Rapid changes in neuronal membrane function imply a uremic toxin or toxins are responsible for nerve dysfunction in CKD, and that these toxins are readily removed by RRT. The changes seen are reminiscent of chronically depolarized neurons, e.g. in hyperkalemia. These techniques could be used in a hypothesis-generation study to investigate factors associated with progression of neuronal dysfunction, and lead to better understanding and prevention of the neurological complications of CKD.

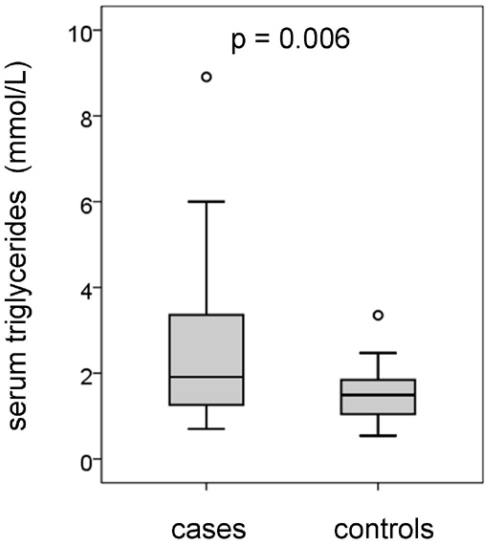
PUB160

High Triglyceride Levels Are Associated with Pancreatitis in Patients with End Stage Renal Disease Fiona S. Turkes,[1] Nadey S. Hakim,[2] Damien Ashby.[2] *[1]Imperial College School of Medicine, London, United Kingdom; [2]Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom.*

Background: Acute pancreatitis has been found to occur more frequently in renal patients than in the general population however the cause remains unclear.

Methods: This was a retrospective case control study. Data was collected from patients on renal replacement therapy (RRT) with a previous admission for acute pancreatitis without known cause such as stones or alcohol. For each case, 2 controls were selected matched for age, sex and modality.

Results: We identified 16 patients (aged 38-85, 8 male) who were admitted with acute pancreatitis of unknown cause. Compared to controls, cases tended towards a longer period on RRT (9.6+/-10.7 vs 5.2+/-4.8 years, p=0.051) and in the 6 months prior to admission had higher average levels of triglycerides (2.7+/-2.3 vs 1.5+/-0.6mmol/L, p=0.006) and CRP (47+/-88 vs 11+/-18mg/L, p=0.033). Cases did not have grossly lipaemic serum, and triglyceride levels were all below that generally thought to cause pancreatitis (10mmol/L).



Defining a poor outcome group as those patients who died or required ITU support during the same admission (n=7), the only clinical parameter associated with poor outcome was CT severity criteria (Balthazar score 3.1+/-2.4 vs 1.0+/-1.2, p=0.039). Co-morbidity and medication use were not risk factors for acute pancreatitis or related to prognosis. Of the 12 survivors, during an average follow up of 26 months, 3 died, and 4 of the remaining 9 had recurrent episodes of pancreatitis.

Conclusions: Duration of RRT and a high serum triglyceride level are risk factors for acute pancreatitis in renal patients. This illness has a high mortality and many of the survivors have recurrent episodes.

Funding: Clinical Revenue Support

PUB161

Age Interference on Quality of Life of Patients Undergoing Chronic Hemodialysis Carmen B. Tzanno-Martins,[3] Fernanda Ribeiro Nisihara,[1] Geison Stein Meirelles Ramos,[2] Elzo R. Junior,[1] João Paulo L.B. Martins,[2] Paul Clesca Troconis.[1] *[1]Renal Class, São Paulo, Brazil; [2]Home Dyalisis, São Paulo, Brazil; [3]Centro Integrado de Nefrologia, Brazil.*

Background: To assess quality of life through self-administered KDQOL-sf (Kidney Disease Quality of Life – short form) by patients on hemodialysis (HD) classified according to age.

Methods: We randomly selected 159 patients with chronic kidney disease (CKD) on hemodialysis and applied the KDQOL-sf between February 2010 and March 2011. We evaluated the quality of life based on scores below or above 50, on all scales, according to age < 60 years (n = 85) and > 60 years (n = 74).

Results: We analyzed the KDQOL-sf according to age. In patients aged 60 years or more, the scales in which they had poorer quality of life were: physical function 47/85 (55.3%), CKD burden 46/85 (54.1%), professional role 57/85 (67.1%) and sexual function 47/85 (55.3%). In patients younger than 60 years, the scales with poorer quality of life were: physical functioning 47/74 (62.2%), physical function 51/74 (68.9%), general health 38/74 (51.3 %), energy / fatigue 45/74 (60.8%), burden of CKD 56/74 (75.7%), professional role 49/74 (66.2%) and sexual function 60/74 (81.1 %).

Conclusions: We found that the quality of life in patients aged 60 years or more is below the 50 score on the following scales: physical function, CKD burden, professional role and sexual function. In patients younger than 60 years, the scales showed lower scores were: physical functioning, physical role, general health, energy / fatigue, burden of CKD, professional role and sexual function. While patients younger than 60 years have a quality of life decrease in 4 scales, patients with more than 60 years have worsened in 7 scales, with significant worsening of sexual function and energy / fatigue. We conclude that the quality of life is more impaired in the elderly undergoing HD.

Funding: NIDDK Support

PUB162

Role of Gender and Disabilities in the Development of Depressive Symptoms in Elderly Patients under Hemodialysis Carmen B. Tzanno-Martins,[1] Geison Stein Meirelles Ramos,[2] Fernanda Ribeiro Nisihara,[3] João Paulo L.B. Martins,[1] Paul Clesca Troconis,[3] Elzo R. Junior.[3] *[1]CINE - Integrate Center of Dialysis, Guarulhos, São Paulo, Brazil; [2]Home Dialysis Center, São Paulo, Brazil; [3]Renal Class, São Paulo, Brazil.*

Background: This study aims to measure the quality of life and depressive symptoms of hemodialysis patients over 60 years old, using KDQOL-sf and Beck Depression Inventory

Methods: We randomly selected 142 patients, 71 (50%) of them were males

We also analyzed separately patients with physical disabilities

We applied the KDQOL-sf test, and a screening test for depression (BDI), only in the group over 60 years. Aiming more specific ratings, for interpreting the results, we select only the item "emotional function" in KDQOL-sf

Results: We obtained 47.9% (68/142) patients above 60 years. Among them, 31 were female (45.6%).

We found a frequency of depressive symptoms in the group of female patients 13/31 (41.9%). In male, there was 14/37 (37,8%).

Concerning "emotional function", we found higher frequency of sub-standard results in the female group, 16/31 (51.6%), while the male population had rates below the standard in 14/37 (37.8%).

The presence of physical disability in females was 29% (9/31).

When the female population was evaluated separately (9/15), 4 of them (66.6%) had scores at BDI that suggested depression. Same number and same percentage rated lower than expected at KDQOL, concerning "emotional function".

When the male population (6/15) was isolated under the same conditions, 5 of them (83.34%) showed signs of depression as 6 (100%) rated lower than expected at "emotional function".

Among the female population with depressive symptoms 30.7% (4/13) showed physical disability. In male population we found 35.7%.

Associating variables disability and below expected results using KDQOL, we observed a statistically significant association in men (p=0.04), but not in women.

Conclusions: Age increases prevalence of depressive symptoms. The presence of physical disability was higher in patients with depressive symptoms, regardless of gender. However, the presence of physical disability seems to be a determining factor for the onset of depressive symptoms, especially in males

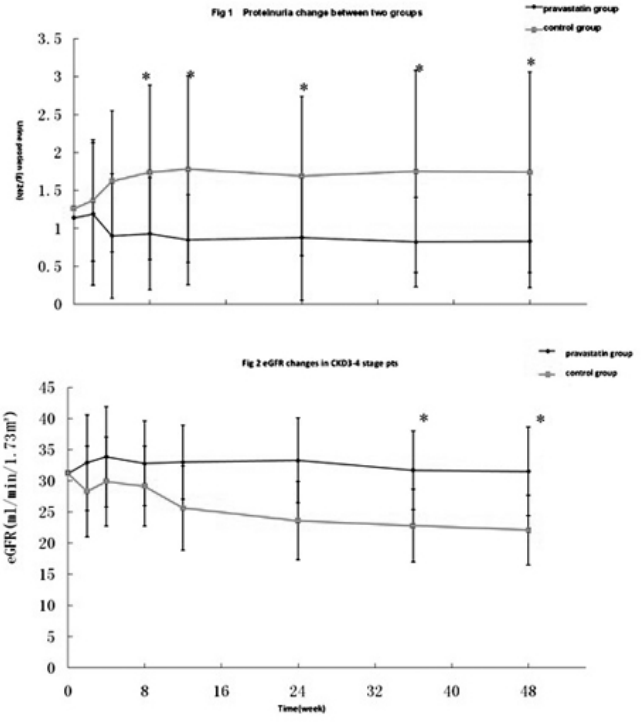
PUB163

Pravastatin Inhibition in Progression of CKD Qin Wang, Liang Ma, Shan Mou, Beili Shi, Minxia Zhu, Liou Cao, Leyi Gu, Zhaohui Ni. *Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: Lowering low-density lipoprotein (LDL) cholesterol with statin has been shown to reduce the incidence of atherosclerotic events in many diseases, but it remains uncertain whether it is of benefit among people with chronic kidney disease (CKD).

Methods: Patients with CKD who were classified based on an estimated glomerular filtration rate (eGFR) ≥15 mL/min/1.73m² and urine protein between 1 to 3.5g/day were randomized in a ratio of 1:1 to pravastatin 20mg daily versus matching placebo. The key outcome was double of serum creatinine, end-stage renal disease and atherosclerotic events, defined as the combination of myocardial infarction, coronary death, ischemic stroke, or any revascularization procedure in one-year follow-up.

Results: Totally 43 CKD pts were randomized (mean age 58 years, 46.5% male), among which 15% had diabetes mellitus, one fifth had hypertension, and 2% had cadiovascular disease. Compared with placebo group, allocation to pravastatin was not associated with any excess of myopathy, hepatic toxicity, or biliary complications. Serum cholesterol(4.25±0.23mmol/L vs 5.33±0.41mmol/L,P<0.01), triglyceride(1.33±0.29mmol/L vs 1.84±0.44mmol/L,P<0.05) and LDL(2.61±0.70mmol/L vs 3.57±0.76mmol/L,P<0.05) was lower in pravastatin group. Proteinuria start to decrease in pravastatin group at 8 week, and continue to the end of the year.



There are no significant differences in alteration of eGFR. but it was likely to slow down in pravastatin group[figure2]. 2 pts occurred CVD events in pravastatin group and 5 pts in control group. Cards-Mai's survival curve showed significant differences between two groups.

Conclusions: Pravastatin can safely adjust the lipid metabolism, reduce proteinuria and CVD risk in CKD patients. It was likely to slow down kidney disease in patients whose eGFR is between 15 to 60 mL/min/1.73m^2.

PUB164

Increasing the Dose of Lanthanum Carbonate Results in Better Serum Phosphorus Control Rosamund Wilson,[1] Lynne Poole.[2] *[1]Spica Consultants, Marlborough, United Kingdom; [2]Shire Pharmaceuticals, Basingstoke, United Kingdom.*

Background: Controlling serum phosphorus to recommended target levels is challenging. Patients should be treated with the appropriate dose of phosphate binder titrated to achieve these target levels.

Methods: To investigate whether patients had better control of serum phosphorus on a dose of 3000 mg/day of lanthanum carbonate compared with lower doses, data were analysed from a randomized controlled study conducted in Europe. After randomization, patients entered a titration phase of 5 weeks during which doses were titrated to achieve phosphorus control (≤ 5.6 mg/dL). After the titration period, patients who had achieved control continued into a 20-week maintenance phase. Data from patients randomized to the lanthanum carbonate arm, who started the maintenance period on a dose < 3000 mg/day and during the maintenance period increased their dose to 3000 mg/day, were analysed to evaluate whether increasing the dose to 3000 mg/day had a positive effect on serum phosphorus levels.

Results: Thirty-five patients started the maintenance period on 1500 or 2250 mg/day of lanthanum carbonate and had their dose increased to 3000 mg/day during this period. On average these patients had serum phosphorus levels approximately 0.6 mg/dL lower on 3000 mg/day of lanthanum carbonate compared with doses ≤ 2250 mg/day. Sixty-six percent of patients had better phosphorus control on 3000 mg than on lower doses.

Conclusions: Increasing the dose of phosphate binders may improve phosphorus control. This *post hoc* exploratory analysis suggests that increasing the dose of lanthanum carbonate to 3000 mg/day results in positive effects on serum phosphorus levels and may improve the percentage of patients achieving target levels. Lanthanum carbonate has been shown to be well tolerated when given at doses up to 4500 mg/day in patients with CKD receiving hemodialysis.

Funding: Pharmaceutical Company Support

PUB165

Glomerular Filtration Rate Using 2 Different Methods and Albumin Creatinine Ratio in the Irish Population Gemma M. Browne,[1] Joseph A. Eustace,[2] Ivan J. Perry.[1] *[1]Department of Epidemiology and Public Health, University College Cork, Cork, Ireland; [2]Department of Nephrology, Cork University Hospital, Cork, Ireland.*

Background: This study describes stages of estimated glomerular filtration rate (eGFR) and albumin creatinine ratio (ACR) in Ireland. At present the MDRD equation is used in clinical practice to estimate renal function. This is the first population based estimate in Ireland.

Methods: A population-based cross-sectional study of adults was conducted using data from the 2007 Survey of Lifestyle, Attitudes and Nutrition (SLAN). A representative sample of 1,207 adults aged 45 and over, underwent a comprehensive physical examination including serum and urinalysis. Demographically this sample was similar to 2006 national census data. Using regression equations recalibrated MDRD and CKD-EPI, eGFR was calculated from a single serum creatinine. Spot Urine Albumin Creatinine ratio (ACR mg/g) was measured

Results: eGFR <60 ml/min/1.73 m^2 based on the CKD EPI regression equation occurred in 12.1% (95%CI 10.3-14%). ACR >30mg/g occurred in 13.2% (95%CI 11.2-15.2%). ACR >30mg/g varied from 11.1% in GFR Stage 1 to 100% in GFR stage 4-5. Using the MDRD regression equation, more subjects were categorised as eGFR<60, and similarly more subjects were described as GFR Stage 2 compared to GFR Stage 1 (Table 1). Younger and female subjects were more likely to be categorised as a lower GFR using the MDRD equation.

Prevalence of GFR Stages (G1-5) by CKD EPI & MDRD

	ALL N=1160* (95%CI)	
GFR STAGES	CKD EPI	MDRD
G1	33.2% (30.9, 36.3%)	19.2% (17, 21.5%)
G2	54.22% (51.4, 57.1%)	65.5% (62.8, 68.3%)
G3A	9.22% (7.6, 10.9%)	11.98% (10.1, 13.9%)
G3B	2.5% (1.6, 3.4%)	2.84% (1.9, 3.8%)
G4-5	0.43% (0.05, 0.8%)	0.43% (0.05, 0.8%)

*Subjects with an available serum creatinine

Conclusions: Lower estimates of GFR, which may be dependent on the method of estimation, can impact on the individual. In laboratories in Ireland and in the UK, the MDRD regression equation has widespread use. The CKD-EPI formula results in higher estimated GFR especially in younger and female subjects. Albuminuria, as an additional measure of cardiovascular risk, is prevalent in this population.

Funding: Government Support - Non-U.S.

PUB166

Prevalence of Albuminuria in Polish Elderly Population (The Polsenior Study) Jerzy Chudek,[1,2] Katarzyna Wieczorowska-Tobis,[3] Andrzej Wiecek.[1] *[1]Department of Nephrology Endocrinology and Metabolic Diseases, Medical University of Silesia; [2]Department of Pathophysiology, Medical University of Silesia; [3]Department of Geriatric Medicine and Gerontology, Poznan University of Medical Sciences.*

Background: Albuminuria is used as a screening tool for detection of chronic kidney disease (CKD) in epidemiological studies. Therefore, the aim of this study was to assess albumin excretion, in addition to serum creatinine concentration, in a representative group of Polish elderly population.

Methods: The study was carried out as a part of the nationwide PolSenior project in the population of randomly selected 4,979 people aged 65 and older (2,567 males and 2,412 females) using the national PESEL database (the National Electronic System of Population Records). Data concerning the prevalence of arterial hypertension, diabetes, kidney stones, refractory urinary tract infections, prostate diseases were collected. Urinary albumin and creatinine concentrations were assessed in 3792 of 3915 obtained urine samples. Microalbuminuria was scored as an albumin to creatinine ratio of 17-250 mg/g in men and 25-355 mg/g in women. Higher albumin excretion was scored as a macroalbuminuria. eGFR was estimated according to CKD-EPI formula, based on serum creatinine concentration.

Results: Micro- and macroalbuminuria were found in 2150 (56.7%) and 652 (17.2%) urine samples, respectively. The prevalence of participants with microalbuminuria was similar in all examined age categories (varied from 55.3% to 60.1%), while the prevalence of macroalbuminuria was significantly increasing with age from 10.14% in subjects aged 65-69 to 29.04% in those aged 90 and older. In consequence, the percentage of subjects with albumin excretion within the normal range was diminishing from 29.8% in the age group 65-69 yrs. to 14.42% in the age group 90 and older. Surprisingly enough , a substantial percentage of subjects with normal range of albumin excretion showed eGFR below 60 ml/min/1.73m^2 (from 9.5% aged 65-69 to 48.0% aged 90 and older.

Conclusions: In Polish elderly population the prevalence of normal range albumin excretion is declining with aging. Albuminuria should not be used as a screening tool of CKDin elderly subjects.

Funding: Government Support - Non-U.S.

PUB167

Performance of Serum Cystatin C Versus Serum Creatinine as a Marker of Glomerular Filtration Rate Referred for Inulin Renal Clearance Masaru Horio,[1] Enyu Imai,[2] Yoshinari Yasuda,[2] Tsuyoshi Watanabe,[3] Seiichi Matsuo.[1] *[1]Functional Diagnostic Science, Osaka University Graduate School of Medicine, Osaka, Japan; [2]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Third Department of Medicine, Fukushima Medical University, Fukushima, Japan.*

Background: Serum cystatin c was recently proposed as an alternative marker of GFR. Better performance of cystatin C compared with creatinine has been suggested. However, detailed studies are limited. We evaluated the performance of cystatin C as a GFR marker.

Methods: GFR was measured by inulin clearance in 763 Japanese subjects. Factors other than GFR influencing serum cystatin C or serum creatinine were analyzed by multivariate analyses. Correlations between GFR and 1/cystatin C or 1/creatinine were evaluated in subjects stratified by gender and age groups with 18-39, 40-59, and 60-79 y.o.

Results: After adjustment for GFR, value of serum creatinine was 25.2% lower in female than male, and was decreased 5.2% every 20 years of age. Serum cystatin C was 8.2% lowerin female than in male, and was not significantly changed with aging. Creatinine but not cystatin c was significantly affected by body weight, height and body mass index after adjustment for GFR, gender and age. Correlation coefficient between GFR and 1/cystatin C was significantly higher than that of 1/creatinine in total subjects (0.866 and 0.810, respectively, $p<0.001$) and consistently higher in subjects stratified by gender and all age groups although not reaching statistical significance. Unlike serum creatinine, serum cystatin C did not increase in association with reduction of GFR in subjects with very low GFR. Regression line of 1/cystatin C against GFR showed a significantly negative intercept of about -8ml/min/1.73m^2

Conclusions: Age, gender, body weight, height and BMI had smaller effect on serum level of cystatin C than that of creatinine. Reciprocal cystatin C had a better correlation with GFR than reciprocal creatinine, suggesting a superior GFR marker. On the other hand, cystatin C appeared to have an apparent non-renal elimination that could affect the performance as the marker of GFR.

Funding: Government Support - Non-U.S.

PUB168

Performance of GFR Estimation Equations in the Elderly Harbir Singh Kohli,[1] Ashesh Dhungana,[1] B.R. Mittal.[2] *[1]Nephrology, Post Graduate Institute of Medical Education and Research; [2]Nuclear Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India.*

Background: It is important to know the glomerular filtration rate (GFR) in elderly population for staging of chronic kidney disease (CKD) and also for drug dosing. GFR can be estimated from e GFR equations and from creatinine clearance (Cr Cl) by estimating 24 hour urinary creatinine. Which one is an accurate reflection of true GFR is controversial.

Methods: A total of 51 elderly patients with serum creatinine up to 3.5 mg/dl were included. GFR was measured in all with Tc-99m DTPA plasma sample method which was considered the standard GFR (DTPA GFR) and with 4 GFR estimating equations: Cockroft-Gault (CG), Cockroft-Gault adjusted for body surface area (CG-BSA), 4 variable MDRD (4MDRD) and 6 variable MDRD (6MDRD) equations. Cr Cl was also calculated. Pearson's correlation coefficient was used to compare the correlation of equations with DTPA GFR. Bland and Altman plots were derived for each method of GFR measurement to estimate agreement with DTPA GFR results. Limits of agreement were calculated as mean ± 2SE for each pair. The accuracy was calculated as percentage of estimated GFR lying within 10%, 30% and 50% of measured GFR.

Results: The mean age was 70.14 ± 4.6 years (65-80), mean DTPA GFR was 65.42 ± 26.28 ml/min/1.73m^2 (23-147). Of 51, 23 were in CKD 3, 17 in CKD 2 stages. Overall correlation of 6MDRD was the highest (r- 0.714) followed by 4MDRD (r- 0.706), CG-BSA (r- 0.635) Cr Cl (r- 0.608), and CG (r- 0.542). On Bland and Altman plots, mean bias was least and limits of agreement narrowest with 6MDRD (-2.8 ± 2.7) followed by 4MDRD (-3.7±2.8), Cr Cl (-9.6 ± 3.2), CG- BSA (-10.6± 2.9), and CG (-11.3 ± 3.3). The accuracy of 6MDRD (33%, 77% and 94% accuracy at 10, 30 and 50 percent respectively) was the best followed by 4 MDRD (28%, 71% and 92% accuracy at 10, 30 and 50 percent respectively) and CG- BSA (22%, 67% and 90% accuracy at 10, 30 and 50%). The accuracy of CG and Cr Cl was low.

Conclusions: 6MDRD equation was found to be superior in terms of correlation, bias, and accuracy, and can be used as a good surrogate for actual GFR in the elderly. This requires validation in a larger population.

Funding: Government Support - Non-U.S.

PUB169

Improved GFR Estimation by BP Neural Network Xun Liu,[1,2] Xiaoming Wu,[2] Ningshan Li,[2] Tan-Qi Lou.[1] *[1]Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China; [2]College of Biology Engineering, South China University of Technology, Guangzhou, Guangdong, China.*

Background: Chronic kidney disease (CKD) is a growing health problem that needs to be addressed. This paper attempts to develop and validate a GFR-estimating model using BP neural network.

Methods: 831 Chinese patients with CKD enrolled, of whom 562 were randomly selected as the training data set; the remaining 269 patients constituted the internal validation data set. Additional 349 patients were included in the external validation data set. Serum creatinine (SC) was determined enzymatically. The ^{99m}Tc-DTPA-GFR was used as the reference GFR (sGFR). The input layer of the BP network consisted of seven units, the patients' records of serum SC, albumin, urea, age, sex, height and weight. The output layer consisted of only one unit representing sGFR. Average sGFR was 46.1±27.0 (3.3-130.1) ml/min/1.73 m^2 in the training data set, 44.2±28.0 (4.4-137.6) ml/min/1.73 m^2 in the internal validation data set and 49.1±26.6 (2.8-122.9) ml/min/1.73 m^2 in the external validation data set. The Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation, reexpressed 4-variable MDRD equation and BP network were compared in both two validation data sets.

Results: In both two validation data sets, bland-Altman analysis demonstrated that BP network was better than the other equations. Only the precision of the BP network exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m^2. The slope of the regression line estimated by the BP network was smaller than those of the other equations**.** Differences as well as the accuracy with a deviation less than 30% from the sGFR of the BP network were significantly better than those of the other equations. When compared the internal validation data set with external validation data set, the bias as well as accuracy of eGFR estimated by BP network were not statistically significant.

Conclusions: Our data indicated this BP network model is suitable for the specific Chinese population tested. Relevant procedures are being developed to facilitate the validation of the model.

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PUB170

Is the GFR Estimation Equation in Japan and China Useful in Elderly Chinese Patients with Chronic Kidney Disease? Xun Liu, Cailian Cheng, Tan-Qi Lou. *Division of Nephrology, Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.*

Background: China faces an aging crisis. Recently, some modified glomerular filtration rate (GFR) estimating equations had been established in Japan and China. In this study, we sought to evaluate the applicability of these formulas in elderly Chinese patients with CKD.

Methods: 332 patients enrolled. Serum creatinine (SC) was determined enzymatically. The ^{99m}Tc-DTPA-GFR was used as the standard GFR (sGFR). The mean age was 69.8±6.7 years (range, 60-93 years). The mean sGFR was 39.7±21.6 ml/min/1.73 m^2 (range, 23.7-116.6 ml/min/1.73 m^2). The reexpressed 4-variable MDRD equation, Chinese-equation and new Japanese equation were tested. The performance of estimated GFR (eGFR) was compared with sGFR in various stages of CKD.

Results: Median of difference ranged from -9.79 ml/min/1.73 m^2 to 6.81 ml/min/1.73 m^2. Median percents of the absolute difference ranged from 25.38% to 38.04%. Accuracy with a deviation less than 15% ranged from 20.5% to 28.9%. Accuracy with a deviation less than 30% ranged from 38.9% to 55.4%. Accuracy with a deviation less than 50% ranged from 64.5% to 77.7%. CKD stage misclassification ranged from 42.2% to 57.5%. However accuracies with a deviation less than 30% of all the equations were less than 70%. When the overall performance as well as bias and accuracy were compared in different stages of CKD, GFR estimated by reexpressed 4-variable MDRD equation showed promising results.

Conclusions: When SC was measured by the enzymatic method, GFR estimation equation in Japan and China showed great bias in elderly Chinese patients with CKD. Further improved equations are needed. If conditions are not available, reexpressed 4-variable MDRD equation may be more accurate to assess GFR in elderly Chinese CKD patients.

Correspondence to: Prof. Lou Tan-qi

PUB171

Chronic Kidney Disease Prevalance in Primary Care – Missing Cases Donal O'Donoghue,[1] Beverley Matthews,[2] Stephen Green.[2] *[1]Salford Royal Foundation NHS Trust; [2]NHS Kidney Care.*

Background: The aims of this study were:

to compare QOF reported CKD prevalence and Public Health (PH) estimated prevalence,

to identify the extent of under-diagnosis; and

to aid commissioners and primary care in improving ascertainment.

Methods: The prevalence of Chronic Kidney Disease in people aged 18 years plus in Primary Care is reported annually through the Quality and Outcomes Framework (QOF). The East Midlands Regional Public Health Observatory has used the NEOERICA study to model estimated CKD Prevalence, nationally, at SHA and PCT levels. The gap in % prevalence and the estimated "missing people" have been calculated by subtracting the QOF results from the PH estimates.

Results:

CKD Prevalence (%)

People with CKD	QOF Reported	PH Estimated	% Identified	QOF Reported	PH Estimated	Missing People
England	4.3%	8.8%	48%	1,817,871	3,770,267	1,952,396
East Midlands	5.3%	9.0%	59%	189,602	322,613	133,011
East Of England	4.3%	9.3%	46%	199,106	430,491	231,385
London	2.7%	6.8%	40%	181,575	456,593	275,018
North East	5.0%	9.1%	55%	105,248	191,117	85,869
North West	4.5%	9.0%	51%	258,350	510,800	252,450
South Central	3.9%	8.6%	46%	131,577	287,963	156,386
South East Coast	4.4%	9.8%	45%	158,367	351,237	192,870
South West	4.7%	10.1%	47%	202,428	433,033	230,605
West Midlands	4.5%	9.1%	49%	200,056	406,096	206,040
Yorkshire & Humber	4.6%	8.8%	52%	191,562	371,729	180,167
PCT - High	7.4%	8.2%	90%	15,939	17,634	1,695
PCT - Low	1.4%	5.9%	23%	2,769	12,096	9,327

Conclusions: The key findings from this assessment show that:

CKD Prevalence as reported through QOF has increased from 3% in 2006/7, 3.7% in 2007/8, 4.1% in 2008/9 to 4.3% in 2009/10.

However, compared with Public Health estimated prevalence of 8.8% for England, national ascertainment is still only 48% of expected prevalence.

At SHA level in 2009/10, ascertainment ranges from 40% of estimated prevalence in London to 59% of estimated prevalence in East Midlands.

At PCT level, the range is even wider, with the PCT with the lowest estimated ascertainment reporting 27% of expected prevalence and the PCT with the highest reporting 90% of estimated.

Overall, there is an estimated 1.95 million people with undiagnosed CKD and not being treated and at risk of faster disease progression, emergency admission and poor outcomes.

PUB172

Pregnancy in Chronic Kidney Diseases and Chronic Kidney Diseases in Pregnancy: Outcome in 188 Singleton Pregnancies with Renal Involvement

Giorgina B. Piccoli,[1] Rossella Attini,[2] Silvia Parisi,[2] Valentina Consiglio,[1] Stefania Scognamiglio,[1] Federica N. Vigotti,[1] Martina Ferraresi,[1] Piero Gaglioti.[2] *[1]Nephrology and Dialysis, San Luigi Hospital - University of Turin, Orbassano (TO), Italy; [2]Materno-Foetal Unit, S. Anna Hospital - University of Turin, Torino, Italy.*

Background: The relationship between pregnancy and CKD is complex, entangled and difficult to explore.

The present study was aimed at assessing pregnancy outcomes in a large cohort of CKD women followed in a tertiary care Center where a conjunct Outpatient Unit is run by Nephrologists and Obstetricians.

Methods: Prospective, Singel Center, observational study. In 2000-2011 262 pregnancies in 235 women were referred. The results were compared with a cohort of "low-risk" pregnancies, followed in the same setting. PE patients were not referred unless there was the need for a differential diagnosis with CKD. The following data were gathered: age, parity, educational level, CKD (cause and stage); acute kidney disease, creatinine, GFR, proteinuria, arterial pressure, delivery (week, caesarian, need for intensive care, weight and entile of the newborn, major clinical problems).

Results: The prevalence of early CKD stages is high (192 in stage 1; 44 in stage 2; 20 in stage 3; 4 in stages 4-5, 2 not yet classified), underlining the importance in the of the wider population of early CKD stages; the prevalence of 24 proteinuria <0.3 g was 66%; 0.3-1 g 18%; 1-3 g 9% and over 3 grams per day 6% (2/262 not yet classified: prevalence 1%).

Diagnoses are related with referral patterns. Glomerular diseases are the most frequent diagnoses in patients who are first diagnosed CKD in pregnancy, urological disorders are the frequently known but overlooked.

188 singleton deliveries were observed, 167 in stages 1-2; the prevalence of preterm delivery (<37th week) was 21% and of "early" preterm (<34th week) was 15%, significantly higher then prevalence recorded in control group (>37 weeks:5%). Caesarean section: 91/188 singleton deliveries (48.4%)versus 25%.

Conclusions: CKD is a risk factor for pregnancy related morbidity, since the early stages; pregnancy is a valuable occasion for early CKD diagnosis.

Funding: Government Support - Non-U.S.

PUB173

MDRD or EPI Equations for Estimating Glomerular Filtration Rate (GFR) in a Stage 1-3 CKD Population: Any Relevant Differences?

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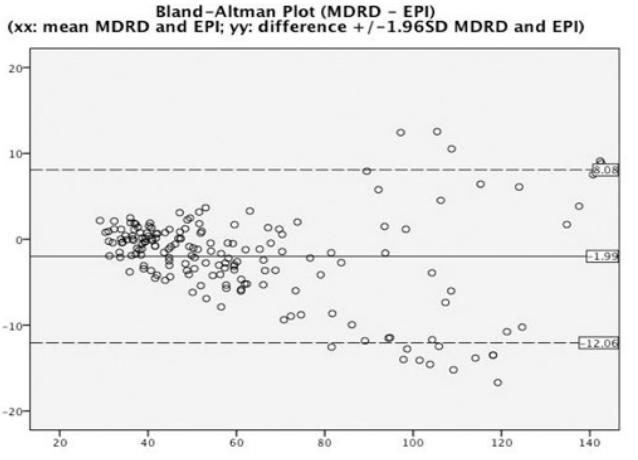
Background: MDRD equation is considered accurate for estimated GFR <60 ml/min. The new equation EPI was developed to enable a better performance for GFR ≥60 ml/min. Our aims were to understand the difference of estimated GFR using MDRD or EPI equations for stage 3 CKD.

Methods: We randomly selected 175 patients from our clinic with stage 1-3 CKD and stable kidney function. Serum creatinine (SCR) was measured by an IDMS traceable method. We compared eGFR by each formula using Wilcoxon paired samples test. Prevalence of stage 3 CKD was compared by McNemar's χ^2 test. Agreement between equations in categorizing patients in stage 3 versus stage 1-2 CKD was evaluated by Kappa statistics. Pearson correlation was determined between the difference between formulas (calculated as MDRD_GFR minus EPI_GFR for each paired estimates) and age, SCR and MDRD_GFR.

Results: Patients enrolled were mainly old (median age, 66 years), with a high prevalence of stage 3 CKD (by MDRD and EPI formulas 65.7% and 59.4% respectively, *P*=0.003), median MDRD_GFR of 52.3 ml/min and EPI_GFR of 52.2 ml/min. Estimated GFR by each equation were significantly different (P<0.001) but Kappa statistics showed a good agreement (K=0.84). The string of the differences between formulas correlated significantly with age (r=-0.30, *P*=0.02), SCR (r=-0.48, *P*<0.001) and MDRD_GFR (r=0.60, *P*<0.001). Bland-Altman analysis between formulas is plotted in Figure 1.



Conclusions: Estimated GFR varied significantly between equations, with noticeable lower EPI_GFR in younger and with lower SCR patients. However, a significantly lower prevalence of stage 3 CKD by the EPI equation was shown. In patients with mild kidney dysfunction, EPI_GFR estimates seemed more accurate, permitting a more adequate CKD staging.

PUB174

Profile of Chronic Kidney Disease Practice in Queensland, Australia – CKD. QLD Phase 2 Survey

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Background: Chronic kidney disease (CKD) management is evolving from doctor and hospital centric models towards nurse practitioner (NP) led multidisciplinary community models of care. We assessed CKD practice management in Queensland, Australia, which has a multiethnic population of 4.47 million.

Methods: Profile of CKD management was undertaken using a web-based questionnaire (Survey-monkey) completed by senior medical and nursing staff across all public renal units in Queensland, Australia.

Results: The response rate was 100% with participation of all clinics in the survey. The majority of CKD practice was hospital based (85%) with community CKD clinics available in major cities only. Total number of patients followed in all public CKD clinics was 11668, exceeding the number (10,469) estimated during the first CKD site profiling 12-months previously.

The majority of patients (90%) were seen by a nephrologist, as most clinics were hospital based, while 5 NPs using the NP model seeing the rest. Numbers of indigenous CKD patients varied according to location, ranging from 0 to 40%. Indigenous staff were available in 50% of units surveyed and there was a high level (88%) of cultural awareness and training.

Patient follow up was based on CKD stage and co-morbidities, and ranged between intervals of 6-weeks to 1-year. Allied health professionals were available in all clinics, with 80% of patients seen by a dietician and follow-up was according to need. The multi-disciplinary care model including pharmacist, psychologist, social worker and podiatrist was being implemented in all units, but variably limited by resources.

Conclusions: CKD management has become a major focus of renal services in Queensland Health with almost 12,000 patients to care for, with increasing numbers as new referrals are seen regularly. Community based services and NP models are increasingly explored for CKD service delivery.

Funding: Pharmaceutical Company Support

PUB175

Evaluation of Polyclonal Free Light Chains in Chronic Kidney Disease Stage 3 Lakhvir Assi,[1] Natasha J. McIntyre,[2] Colin A. Hutchison,[3] Philip Young,[1] Richard Hughes,[1] Stephanie J. Stringer,[3] Richard J. Fluck,[2] Chris W. McIntyre,[2] Paul Cockwell,[3] Maarten W. Taal.[2] *[1]The Binding Site Group Ltd, United Kingdom; [2]Royal Derby Hospital, United Kingdom; [3]Renal Institute of Birmingham, United Kingdom.*

Background: Polyclonal free light chains (FLCs) are elevated in patients with kidney impairment and serve as an independent risk factor for renal progression in patients with advanced chronic kidney disease (CKD). The aim of this study was to assess serum (S) and urine (U) FLCs in CKD stage 3 patients, in relation to their disease status.

Methods: 1741 patients were recruited from primary care practices. Subjects were predominantly white (98%) and female (60%) with an average age of 73 years. 17% had diabetes. The median eGFR was 53ml/min/1.73m2. FLCs were measured in the S and U using the Freelite™ assay. SFLCs (Sκ, Sλ) were compared to established normal ranges (Sκ: 3.3-19.4mg/L, Sλ.: 5.71-26.3mg/L,). UFLCs (Uκ and Uλ) and Ucreatinine values were obtained from healthy volunteers (Table).

Results: Median Sκ 19mg/L (4-181mg/L) and Sλ 17mg/L (2-74mg/L) concentrations were elevated, in the CKD population. Multivariable linear regression identified several factors as independent determinants of SFLCs including eGFR, UACR, albumin and cholesterol. UFLCs corrected using Ucreatinine (κCR and λCR, mg/mmol) were also elevated (Table). UFLCs and FLCs/CR were further increased in patients with albuminuria (Table). κCR and λCR correlated significantly with ACR (r=0.38 and r=0.47 respectively). Of the CKD patients with an abnormal ACR, 59% had abnormal UFLCs. 43% patients with a normal ACR had abnormal UFLCs, indicating UFLCs are an earlier indicator of renal damage.

Conclusions: The clinical relevance of these observations will be determined as the population is prospectively followed up.

	Control (N=106) Median (range)	CKD (N=865*) Median (range)
	Uκ	Uκ
Normal ACR	5.72 (0.05-29.90)	17.89 (3.48-455.25)
CKD N=650	κCR	κCR
	0.92 (0.02-4.62)	3.30 (0.36-53.36)
	Uλ	Uλ
	0.51 (0.02-9.76)	1.84 (0.78-66.12)
	λCR	λCR
	0.07 (0.01-0.34)	0.38 (0.05-8.65)
Abnormal ACR		Uκ
CKD N=215		37.09 (3.3-1416)
		κCR
		7.49 (0.86-142.46)
		Uλ
		5.23 (0.78-116.49)
		λCR
		1.08 (0.10-18.21)

*N=847 < detection limit of the assay, removed from analysis

Funding: Pharmaceutical Company Support

PUB176

Is Glycosylated Hemoglobin a Predictor for Onset of Diabetes Mellitus in Chronic Kidney Disease? Eleni Chelioti, Sotiris Mikros, Evagelia Chrisanthopoulou, Thnasis Georgiou, Maria Sotiraki, Theodora Fragou, Maria Tsilivigou, Gabriel Papadakis. *Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, Athens, Greece.*

Background: Glycosylated hemoglobin (HbA1c) is a powerful predictor of future cardiovascular events in diabetic patients with chronic kidney disease (CKD). The optimal target for glycemic control has not been established in these patients. However, few studies have found that HbA1c maybe related to manifestation of diabetes mellitus (DM) in patients with CKD. Aim of this study was to evaluate the association between HbA1c and onset of DM in non-diabetic patients (DP) on stage 3 and 4 of CKD.

Methods: A retrospective study was performed in Outpatient Clinic. Participants were 121 non-DP on stage 3 and 4 of CKD (51,2% men, age range 29 to 97 years, mean duration 12 months). Fasting glucose levels (FGL), HbA1c and serum creatinine (SCr) were evaluated at time 0 and after 12 months (time 1) in patients with stable renal function. They were separated in 2 groups according to the initial HbA1c and FGL: normal (HbA1c < 5,7%, FGL<100 mg/dl) and prediabetics (HbA1c > 5,7%, FGL > 100 mg/dl).

Results: Of the 121 patients, mean HbA1c was 5,99% at time 0 and 5,78% at time 1. Mean FGL were 105,6 mg/dl at time 0 and 104,7 mg/dl at time 1. Mean SCr did not change significantly at time 0 and 1. Normal patients (HbA1c < 5,7%)presented a statistically significant increase in HbA1c levels during the study year (p=0,04). The same applies to patients with normal FGL (<100 mg/dl). Their FGL were significantly higher after one year (p=0,013). However, mean FGL and HbA1c levels remained below 100 mg/dl and 5,7% respectively. For the group of patients with baseline HbA1c and FGL suggestive of prediabetes there was no significant difference after a year of observation.

Conclusions: In non-DP with stage 3 and 4 of CKD, HbA1c and FGL cannot be used as predictors for the onset of DM while their renal function remains stable. Only in normal patients did we notice a significant deterioration of HbA1c and FGL, but always within normal levels. Finally, the stability of renal function in these patients contributes to their stability in terms of glycemic control.

Funding: Clinical Revenue Support

PUB177

The Chronic Kidney Disease Prognosis Consortium (CKD-PC): A Global, Collaborative, Individual Participant Data Meta-Analysis (for CKD-PC Collaborators) Josef Coresh, Kunihiro Matsushita, Shoshana Ballew, Brad C. Astor, Mark Woodward, Brenda Hemmelgarn, Adeera Levin, Chi Pang Wen, Paul E. de Jong, Ron T. Gansevoort, Andrew S. Levey. *Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Background: The Chronic Kidney Disease Prognosis Consortium (CKD-PC) was established in 2009 and currently consists of 46 cohorts from 17 countries of North America, Europe, Asia, and Oceania. The consortium conducts complex individual participant data meta-analyses with the goal of providing comprehensive evidence regarding CKD prognosis.

Methods: The consortium governance includes a steering committee, operations committee, and data coordinating center (DCC). Cohorts can join the consortium at any time following operating principles posted at www.jhsph.edu/ckdpc. Each cohort opts in or out for each proposed manuscript. Statistical code for each manuscript is written by the DCC, distributed to participating cohorts, and shared publicly on the website for each manuscript. Piece-wise linear spline models allow a detailed examination of the dose-response association between eGFR and albuminuria with outcomes (mortality, cardiovascular disease, ESRD, AKI, and progression of CKD) and can be pooled across cohorts using the variance-covariance matrix of the regression coefficients.

Results: The consortium includes cohorts representing general (24 cohorts), high risk (10 cohorts), and CKD (12 cohorts) populations including over 1.5 million participants. CKD-PC published four meta-analysis manuscripts in 2010 (phase 1), with seven meta-analyses to be completed in 2011 (phase 2) and meta-analyses of individual risk and definitions of CKD-progression in 2012 (phase 3). Forty-three cohorts from phase 1 opted in for phase 2 analyses and three new cohorts joined. Authorship includes ~15 authors and ~100-200 collaborators per paper.

Conclusions: CKD-PC has established a productive model allowing flexible collaborative meta-analyses. Distribution of statistical code allows inclusion of cohorts which cannot share the raw data due to legal/administrative constraints.

Funding: Private Foundation Support

PUB178

Single Center Experience with Rituximab: Indications, Use, and Response in Pediatric Patients Roshan P. George, Leonard C. Hymes, Rochelle Schmidt, Sandra Amaral. *Pediatric Nephrology, Emory University and Children's Healthcare of Atlanta, Atlanta, GA.*

Background: Rituximab is an anti-CD20 monoclonal antibody, initially approved for use in Non-Hodgkin's Lymphoma. It is frequently used off-label for various other conditions, including renal diseases. There is scant knowledge about response to rituximab use in pediatric patients for these renal conditions.

Methods: We performed a retrospective cohort study of the indications, use and response to rituximab within our nephrology center in the Southeastern US. We included all patients who received rituximab from January 2003 to May 2011 for any indication.

Results: 39 patients received rituximab. 9 patients had renal transplants with rejection unresponsive to other therapies. 9 patients had EBV infection and post-transplant lymphoproliferative disease (PTLD). 15 patients had lupus nephritis; 14 (93%) had either class IV, V or a combination of these. 6 patients had other conditions, including 4 with nephrotic syndrome, and 1 each with Wegener's Granulomatosis and Thrombotic Thrombocytopenic Purpura (TTP). Among transplant patients, rejection improved significantly in 4 of 9 cases (44%) with resolution in 11%. All 9 PTLD patients responded with complete resolution of disease. Among the 15 lupus patients, 4 had clinical and serologic response, 4 had partial response with improved hypocomplementemia but residual significant proteinuria. These 8 patients received 2 or more doses. None of the patients with other conditions showed a response. 18 (46%) experienced adverse effects including 4 with anaphylaxis requiring aborting the dose. 3 had minor infusion reactions. 3 (33%) of the transplant patients with PTLD had persistent hypogammaglobulinemia requiring monthly IVIG. 3 patients had altered mental status, 3-30 days following rituximab dose.

Conclusions: In our single center, rituximab was most effective for PTLD with heterogeneous responses in patients with renal transplant rejection, lupus nephritis and other conditions. There was a high incidence of adverse events (46%). These results emphasize that rituximab should be used with caution and more research is needed to discover optimal therapies for these challenging renal conditions.

PUB179

Combined Impact of Anemia, Hepcidin-25 and Proteinuria on Early Mortality in Cancer Patients Masaki Hara,[1,2] Minoru Ando,[1,2] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Bunkyo-ku, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Tokyo, Shinjuku-ku, Japan.*

Background: Anemia of chronic disease (ACD) occurs in patients with either cancer or kidney disease or both. Hepcidin-25 is a key player in the pathologic state of ACD. We examined associations among hepcidin-25, proteinuria and mortality in cancer patients.

Methods: Two-year prospective cohort study was conducted in a total of 55 cancer patients receiving chemotherapy. Underlying malignancies included malignant lymphoma (47%), gastric cancer (25%), and other cancers (28%). Serum hepcidin-25 level was measured by liquid chromatography mass spectrometry. Proteinuria was defined as a dipstick

test ≥±. Anemia was defined as Hb <10 g/dl. Cumulative survival rate was analyzed using Kaplan-Meier curves with stratification by median value of serum hepcidin-25 (25.1 ng/ml), proteinuria, or anemia. Multivariate Cox proportional hazards analysis, adjusted for age, gender, performance status and estimated GFR, was used to calculate mortality HR for the combined effect of such 3 markers on mortality. 'Score 1' was assigned to each marker.

Results: Mean hepcidin-25 level was 46.7±48.8 ng/ml, which was nearly 2-fold greater than the reference value(22.2±12.3 ng/ml). Cumulative survival rate was significantly lower in the high hepcidin-25, proteinuria (+) or anemia (+) group than in each corresponding opposite. Multivariate analysis showed that the HR (95% CI) was 27.8 (6.27-155.2) for patients with 3 points; 4.0 (1.26-19.4) for those with 2 points; and 1.6 (0.53-7.19) for those with 1 point, as compared to the reference patients with neither of them.

Figure1-a; 2-year Survival curves stratified by each factors

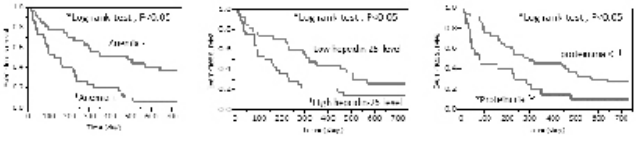
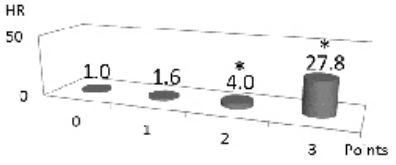


Figure1-b; The combined impact of anemia, hepcidin-25 and proteinuria for mortality



Conclusions: High hepcidin-25 may be a novel predictor for early mortality in cancer patients receiving chemotherapy. Adding either anemia or proteinuria or both to hepcidin-25 increases its predictive power.

PUB180

Serum Uric Acid as a Marker of Mortality in an Elderly Patients Cohort

Manuel M. Heras, María José Fernández-Reyes, Rosa Sanchez, Alvaro Molina, Astrid Rodriguez, Fernando Alvarez-Ude. *Nephrology, General Hospital, Segovia, Spain.*

Background: There is growing evidence of the role of serum uric acid (SUA) as a risk factor for cardiovascular and renal disease. We analyze the association between baseline SUA and overall mortality in elderly patients followed prospectively for 5 years

Methods: 80 clinically stable patients, median age 83 years (range 69-97), 31.3% men, 35% diabetic, 83% hypertensives, recruited in Geriatrics and Nephrology consultations between January-April 2006, were followed for 5 years. Predictive variables were: baseline SAU and plasma creatinine; estimated glomerular filtration rate (GFR) (abbreviated MDRD formula); recorded age, gender, baseline comorbidity (Charlson index), cardiovascular individualized treatment and mortality. Statistical analysis: SPSS15.0.

Results: Baseline SUA was normally distributed and its median was 5.85 mg/dl. We found not significant differences in levels of SUA by gender, diabetes mellitus, hypertension, diuretic use, heart failure (HF), peripheral arterial disease or stroke. Patients with an history of HF had significantly higher SUA (7.00 ± 1.74 vs. 5.90 ± 1.71, P = 0.031). 41 deaths occured during follow-up (15 men and 26 women): 15 general deterioration, 8 infections, 4 stroke, 4 tumors, 3 cardiovascular disease, 2 fractures and 5 unknown. The table shows how patients with SUA higher than the median had significantly lower GFR and higher mortality at 5 years.

Comparison of variables between groups according to SUA median

	Group 1: SUA < 5,85;N=40	Group 2: SUA>5,85;N=40	P
Baseline SUA (mg/dl)	4,77 ± 0,86	7,45 ± 1,27	0,000
Creatinine (mg/dl)	1,15 ± 0,45	1,46 ± 0,51	0,005
MDRD (ml/min/1.73m2)	56,94±15	45,60± 16	0,001
Age, years	81,55±6	83,42±6	N. S.
Sex (male/female)	10/30	15/25	N. S.
Charlson	1,62±1	1,97±1,29	N. S.
Mortality at 5 years	32,5%	70%	0,001

In logistic regression analysis for overall mortality (independent variables: age, gender, Charlson Index, history of HF, SUA, creatinine and GFR), only age (HR:1.14, 1.04-1.025, P=0.004) and SUA levels (HR:1.92, 1.28-2.88, P=0.002) were independently associated with mortality.

Conclusions: In our study, levels of SUA are shown as independent risk factor for mortality in elderly patients.

PUB181

Better Outcomes of Chronic Kidney Disease (CKD) Patients in Okinawa, Japan: Single Renal Clinic Report Okinawa Tokuyama Clinic Epidemiology and Nephrology Study (OCEANS)

Kunitoshi Iseki.[2] *[1]Internal Medicine, Tokuyama Clinic, Urasoe, Okinawa, Japan; [2]Dialysis Unit, University Hospital of the Ryukyus, Nishihara, Okinawa, Japan.*

Background: The purpose of the study is to examine the outcomes of the registered patients in single renal clinic in Okinawa, Japan. Tokuyama clinic is one of the referral centers taking care of CKD patients in Urasoe City which the population is about 110,000. All the members of general practitioners in the city are cooperative with the support of the Urasoe Medical Association. Medical records are filed to the registry database of Okinawa Tokuyama Clinic Epidemiology and Nephrology Study (OCEANS).

Methods: The registration period was from April 2004 to March 2008, 4 years, and followed up until March 2011. Serum creatinine was measured in 3,141 patients during the study period. Among them, a total of 1,962 (62.4%) patients were followed until events such as death and dialysis program or followed regularly, at least more than 3 months. Others (N=1,179) were not followed regularly as 1) visited simple medical reasons such as health-check, Cold, gastro-intestinal problems and miscellaneous reasons (N=661), moved outside to other area (36), transferred to other hospitals and hospitalization (307), and unknown (175). Serum creatinine was measured using the enzymatic method and the GFR was estimated by the formula of the Japanese Society of Nephrology.

Results: Baseline characteristics of the study cohort (N=1,962) were men (58.2%), mean (SD) age of 55.0 (15.4) years, systolic blood pressure 131.3 (23.1) mmHg, diastolic blood pressure 78.3 (13.6) mmHg, proteinuria 20.1%, serum creatinine 0.95 (0.89) mg/dl, and eGFR 71.4 (25.0) ml/min/1.73m^2. The distribution of eGFR was <15 in 2.9%, 15 to 29 in 3.5%, 30 to 59 in 21.6%, 60 and over in 72.0%. During the follow-up period, 69 patients entered dialysis program (3.5% of the total) and 33 patients (1.7%) died. None were entered dialysis program among those referred at eGFR>;;;45 ml/min/1.73m^2.

Conclusions: Based on our observation, early referral from general practitioners, eGFR>45 ml/min/1.73m^2 is warranted for the reduction of ESRD.

Funding: Private Foundation Support

PUB182

Determinants of Vitamin D Deficiency in Korean Adult: Kidney Function and Other Factors

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Background: Vitamin D deficiency has been associated with increased cardiovascular risk, mortality and progression of various chronic diseases including chronic kidney disease (CKD). But, there is limited information about the other factors interacting with the kidney function regarding vitamin D deficiency.

Methods: We examined the association between vitamin D deficiency and CKD by analyzing the data from the Fourth Korea National Health and Nutrition Examination Surveys 2008, consisted of 6529 adults aged 20 years or older. Vitamin D deficiency was defined as a serum 25-hydroxyvitamin D (25(OH)D)≤20 ng/ml, and the kidney function was estimated by the equation of Modification of Diet in Renal Disease equation.

Results: The overall prevalence rate of vitamin D deficiency was 54.3%, and the mean value of vitamin D showed a tendency of increase in proportion to age from twenties to sixties, pointing out that 25(OH)D level was lowest in the most young-aged group. Though there was a significant positive correlation between 25(OH)D level and GFR in multiple linear regression models, there was no significant difference in prevalence of vitamin D deficiency between two groups divided by GFR level of less or greater than 60 ml/min/1.73m^2. However, the prevalence of vitamin D deficiency at GFR level of less than 45 ml/min/1.73m^2 was higher than the other, even after adjustment for age, sex, smoking, residential region (urban/rural area), physical activity (regular exercise and walking), other medical co-morbidities; odds ratio: 2.040; 95% confidential interval: 1.096-3.799; p=0.025. In general adults population, vitamin D deficiency was significantly more common among those who were female, young aged, urban resident, seldom exercise, anemic, having proteinuria, and also with renal impairment.

Conclusions: Decreased renal function and young aged urban female resident are the prominent predictors of vitamin D deficiency. Based on the above data, the longitudinal observation for the association between vitamin D deficiency and clinical outcome should be studied.

PUB183

Evidence of the Syndrome of Rapid Onset End-Stage Renal Disease (SORO-ESRD) in the Acute Kidney Injury (AKI) Literature – Preventable Causes of AKI and SORO-ESRD – A Call for Re-Engineering of Nephrology Practice Paradigms

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Background: We published the first report of the previously unrecognized syndrome of rapid-onset end-stage renal disease (SORO-ESRD) in 2010. We investigated the AKI literature to ascertain to what extent this syndrome may have been reported in the past without acknowledging its uniqueness.

Methods: We reviewed the AKI literature for SORO-ESRD as defined by sudden unanticipated AKI requiring RRT and quickly terminating in irreversible ESRD.

Results: The AKI reports revealing SORO-ESRD appear below. The 15 reports spanning the world, 20-1095 patients each, age 39-65 years, published from 1975-2010, demonstrated SORO-ESRD rates from 1%-85%. AKI was commonly caused by hypovolemia/hypotension, infections/sepsis, and nephrotoxics - radiocontrast, NSAIDs, aminoglycosides and ACEIs/ARBs.

Results of Literature Review Revealing SORO-ESRD from AKI Studies From Around the World

(Authors Publication, Year)	Country	Number of AKI patients	Mean age (Years)	Number of AKI patients that received RRT (%)	Number with SORO-ESRD	SORO-ESRD rate (%)	RAAS Blockade Implicated in AKI	Common Causes of AKI
Merino et al Ann Surg, 1975	USA	187		187 (100)	2	1		Perioperative Hypotension, Sepsis, Nephrotoxics
Kjellstrand et al Trans ASAIO, 1981	USA	432	Half older Than 60	432 (100)	4	1		Not Available
Bonomini et al Nephron, 1984	Italy	227			10	1		Acute Tubular Necrosis, Acute Glomerulonephritis
Lameire et al NDT, 1987	Belgium	287	56	201 (70)	6	2		Acute Tubular Necrosis From Hypovolemia, Septic Shock and Nephrotoxics
Bhandari/Turney QJM, 1996	UK	1095	64		107	10		Not Available
Robertson et al QJM, 2002	UK	193	64	193 (100)	108	56		Not Available
Al-Homrany East MHJ, 2003	Saudi Arabia	150			1	0.7		Acute Tubular Necrosis Sepsis commonest cause
Yallop et al Nephrology, 2005	New Zealand	43	69	10 (23)	2	5		Post-CABG AKI Study
Bahar et al Perfusion, 2005	Turkey	168		168 (100)	8	5		All Cases Followed Cardiac Surgery Old Age Increased AKI
Bagshaw et al Crit Care, 2005	Canada	240	65	240 (100)	19	8		Acute Tubular Necrosis, Nephrotoxics Especially Radiocontrast Media
Jayakumar et al Ren Fail, 2006	India	1112	37	768 (69)	91	8		Volume Depletion From Acute Diarrhea, Nephrotoxics, Glomerulonephritis, Sepsis
Kaballo et al Saudi JKDT, 2007	Sudan	89	39	89 (100)	6	7		Volume Depletion, Infections (Malaria, Typhoid Fever), Nephrotoxics
Ozturk et al Ren Fail, 2007	Turkey	192	56		18	9	+	Acute Tubular Necrosis, Acute Glomerulonephritis, Nephrotoxics including ACEI/ARB in 10 patients
Al-Azzam et al Ren Fail, 2008	Jordan	111		20 (18)	4	4	+	Dehydration, Sepsis, Nephrotoxics as NSAIDs, Contrast, ACE Inhibitors
Kelly/Dominguez AJN, 2010	USA	20			17	85		Not Available

Conclusions: Not surprisingly, at least to us, several published AKI reports dating back to 1975 had demonstrated SORO-ESRD. This has substantiated our hypothesis that SORO-ESRD is prevalent worldwide. The human body is a complex adaptive system (CAS), so small changes can lead to huge effects; thus AKI can lead to irreversible ESRD, fairly quickly. The studies incriminated the same preventable causes of AKI. Reno-prevention, a new concept that we reported in the QJM in 2009, measures to prevent AKI, would benefit CKD patients everywhere. Note worthily, Merino et al in 1975 who described irreversible post-operative ESRD in older CKD patients following AKI from hypotension, with or without septicemia, had asked the rhetorical question whether indeed this should not represent a new syndrome. Now we know - the rest of the story. It is SORO-ESRD.

PUB184

Outcomes of Valvular Heart Surgery in Patients with Chronic Kidney Disease Almothana Shanaah,[1] Eleanor D. Lederer,[1, 2] Ihab Hamzeh,[1, 2] Michael E. Brier.[1, 2] *[1]Medicine, University of Louisville, KY; [2]Medicine, Robley Rex Veterans Affairs Medical Center, Louisville, KY.*

Background: Few studies have focused on the surgical outcome of patients with chronic kidney disease (CKD) undergoing valvular heart surgery. Furthermore, the optimal choice of valve type in this population remains unknown (ACC/AHA guidelines). A previous report showed that mortality was lower in patients undergoing aortic valve replacement (AVR) with mechanical vs. tissue valve in general VA population. The purpose of this study is to examine short-term and long-term outcomes in CKD patients undergoing cardiac valve surgery at our VA.

Methods: A retrospective review of patients with chronic kidney disease undergoing valve replacement from January 2000 through December 2010 at Robley Rex Veterans Affairs Hospital. ICD-9 and CPT codes were used to identify patients from electronic medical records. Outcomes were compared using Pearson chi-square.

Results: A total of 16 patients met the selection criteria for inclusion in this study. The average age at time of surgery was 68 years. The majority of patients were white (75%). The average body mass index was 28.6 kg/m^2. The prevalence of hypertension, diabetes mellitus and cerebrovascular accidents was 88%, 75% and 31%, respectively. Most patients (81%) had CKD-III, while 19% had end stage renal disease requiring hemodialysis. 63% underwent concomitant coronary artery bypass surgery. 9 underwent AVR, 5 underwent MVR and 2 underwent combined valve replacement. 19% or 3/16 received mechanical heart valves while 81% received tissue heart valves. Overall mortality was 5/16, 2 with mechanical valves and 3 with tissue valves. Six month mortality was 19%, one of three with mechanical valve and two of thirteen with tissue valve (p=0.49). Two mechanical valves, but no tissue valves, developed perivalvular regurgitation.

Conclusions: The mortality rate after valvular heart replacement surgery is similar to the previously reported mortality rate in general VA patients. While the small sample size precludes a definitive conclusion, these results suggest that bioprosthetic valves may provide a survival advantage in CKD population.

Funding: Veterans Administration Support

PUB185

Epidemiology of Chronic Kidney Disease: Results from a Polycentric Community Based Population of Middle-Older Aged Adults in Shanghai, China Yi Wang,[1] Shougang Zhuang,[1,2] Haidong Yan.[1] *[1]Department of Nephrology, Tongji University Affilated Shanghai East Hospital, Shanghai, China; [2]Department of Medicine, Brown University School of Medicine, Providence, RI.*

Background: The purpose of this study is to investigate the prevalence, awareness and the risk factors of chronic kidney disease (CKD) among community adult population in Pudong New Area, Shanghai, China.

Methods: 2000 adult residents (≥45 years old) from Pudong New Area were randomly selected to answer a questionnaire and to receive health examinations from July 2006 to October 2009. The morning spot urine dipstick test was used to evaluate proteinuria, creatinine and hematuria. Urine protein to creatinine ratio ≥ 30 mg/g and urine red blood cells>3 /Hp were considered abnormal. The association of kidney damage indicators including age, gender, hypertension, diabetes mellitus, smoking, income, education, cholesterol, triglyceride, body mass index and waist-to-hip ratio were assessed as well. The Chinese improved abbreviated MDRD equation was applied to estimate the glomerular filtration rate (eGFR, abnormal: <60ml/min^{-1}(1.73m^2)). A SPSS13.5 statistical software was used for statistical analysis.

Results: 1905 residents with complete data were enrolled in the study, with mean age 59.02±9.42 years old. After the adjustment of age and gender components, the prevalence of albuminuria, hematuria and the reduced renal function was 12.5% (95%CI:10.5%–13.5%), 7.90% (95%CI:6.7%-9.1%), and 1.9% (95%CI:1.7%-2.1%), respectively. Approximately 13.2% subjects had at least one indicator of kidney damage. Age, hypertension, diabetes mellitus and hyperuricemia were independently associated with CKD.

Conclusions: The prevalence of CKD in adult residents (≥45 years old) from Pudong New Area in Shanghai is 13.2%, and the awareness is 20.9%. The independent risk factors associated with CKD include age, triglyceride, hypertension, diabetes mellitus and hyperuricemia.

Funding: Government Support - Non-U.S.

PUB186

Regional CKD Medical Network System, OCKD-NET: Design and Method of Prospective Cohort Study of Japanese CKD Patients Hiroko Yamasaki,[1] Yohei Maeshima,[1] Norikazu Hinamoto,[1] Daisuke Saito,[1] Hiroyuki Watatani,[1] Haruyo Ujike,[1] Shinji Kitamura,[1] Hitoshi Sugiyama,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ. Graduate School of Medicine, Okayama, Japan; [2]Center for Chronic Kidney Disease and Peritoneal Dialysis, Okayama Univ. Graduate School of Medicine, Okayama, Japan.*

Background: The continuous increase of patients requiring dialysis therapy is a major clinical and socioeconomical issue. Effective management of CKD patients requires association between nephrologists and primary care physicians (PCPs). Okayama city CKD Network (OCKD-NET) was established in 2007. Six referral hospitals with nephrologists and 115 PCPs in Okayama city have registered. Prospective cohort study within OCKD-NET was designed to identify risk factors for progression of CKD and cardiovascular disease (CVD).

Methods: CKD patients, aged ≥ 20 yrs, attending to the PCPs are enrolled. Clinical information on age, gender, blood pressure (BP), proteinuria and eGFR will be collected annually, up to 5 yrs. Progression of CKD stage, annual decline of eGFR, CVD event and initiation of dialysis therapy will be assessed. The outcome between groups of CKD patients attending to 1) PCPs and 2) both PCPs and nephrologists will be compared. Study protocol was approved by the ethical review committee of our university.

Results: A total of 111 Japanese CKD patients were enrolled until May, 2011. In Group 1 (n=17), mean age was 79 yr, 33% were male and mean eGFR was 32 ml/min per 1.73m2 at enrollment. In Group 2 (n=94), mean age was 67, 64% were male and mean eGFR was 41. Overall, 48% had hypertension, 22% had diabetes, 43% were at CKD stage 3, 32% were at CKD stage 4, and 48% had proteinuria. Lower eGFR was associated with older age (P<0.001), proteinuria (P<0.01) and complication of Diabetes (P=0.03). Proteinuria was significantly greater in diabetic group compared with non-diabetic group (P<0.01).

Conclusions: OCKD-NET was established to facilitate regional CKD medical association. Long-term follow-up will provide critical insights into potential risk factors of CKD and CVD progression, leading to the establishment of an effective CKD care system.

Funding: Government Support - Non-U.S.

PUB187

Cost of Treatment Schemes of Intra-Venous Iron in CKD Patients with Iron Deficiency Anaemia: A Patient Record Study Patricia R. Blank,[1] Thomas D. Szucs.[2] *[1]Institute of Social and Preventive Medicine, University of Zurich, Switzerland; [2]Institute of Pharmaceutical Medicine, University of Basel, Basel, Switzerland.*

Background: Iron deficiency (ID) and anaemia in chronic kidney deficient (CKD) patients can be treated by erythropoiesis-stimulating agents (ESAs) or intravenous (i.v.) iron. This study assessed costs of treating CKD patients with i.v. iron based on record data.

Methods: Nephrologists (5 EU countries) completed questionnaires on anaemic CKD patients seen within 3 months (Aug-Sept 2010). Demographics and iron parameters were recorded at initiation and last visit of ID treatment (n=1143). Administration and drug costs were identified from a Swiss payer perspective.

Results: Mean dosage was 583mg i.v. (n=428) and 11604mg oral iron (n=467). respectively. ESA dose was not measured (n=425). Treatment costs are shown in Tab.

Table 1. Treatment scheme and costs (EUR) per single dose and over survey group (rounded values)

	Ferric Gluconat (n=23)	Iron dextran (n=20)	Iron sucrose (n=244)	Ferric carboxymaltose (n=251)
Mean total dose given (mg)	305	1000	482	692
Number of session needed	3.7	1.6	2.4	2.5
Infusion administration scheme as per approved label	62.5mg per 10min	1134 mg in 7h	200 mg in 30min	500-1000 mg in up to 15min
Administration type	65% infusion, 35% injection**	Infusion	80% infusion, 20% injection**	65% infusion, 35% injection *
Costs of administration (EUR)	164	594	149	128
Costs of total i.v. iron (EUR)	35	205	110	193
Total costs (EUR)	199	799	259	321

*push injection with max. 200mg; ** slow injection with max 200mg

HB levels for iron sucrose or ferric carboxymaltose (FCM) were 9.3g/dl (standard deviation (SD) 1.1) or 9.4g/dl (SD 1.3) at initiation and 10.9g/dl (SD 1.2) or 11.5g/dl (SD: 1.4) at last visit, respectively. ESA patients had levels of 9.3g/dl (SD: 1.5; initiation) and 11.0g/dl (SD: 1.6; last visit).

Conclusions: This survey identified practice related treatment schedules in CKD patients with ID. The i.v. iron group achieved similar HB levels compared to ESA. Due to methodological limitations, it was not possible to assess data on treatment duration or the superiority of a therapy. I.v. iron might be a clinically and economically viable alternative to expensive ESAs in CKD patients.

Funding: Pharmaceutical Company Support

PUB188

Automated Reporting of Estimated Glomerular Filtration Rate Varies among Veterans Affairs Laboratories Rasheeda K. Hall,[2] Bradley G. Hammill,[2] George L. Jackson,[1,2] Virginia Wang,[1,2] Matthew L. Maciejewski,[1,2] Uptal D. Patel.[2] *[1]VA Health Services Research & Development; [2]Duke University, Durham, NC.*

Background: The prevalence of CKD among veterans is increasing, and to facilitate earlier detection of CKD, clinical practice guidelines have recommended automated reporting of eGFR by clinical laboratories. We sought to better understand the diffusion of this innovation within the Veterans Health Administration (VHA) by evaluating time to adoption of eGFR reporting at individual VHA facilities and associations between adoption and site-specific organizational characteristics.

Methods: Using VHA laboratory data, we estimated time to adoption from the date VHA delivered a mandate for eGFR reporting (July 2004). For each medical facility with a laboratory (n= 135), adoption was defined by the presence of an associated eGFR for at least 1% of creatinine values. VHA facilities were classified by adoption status, and adopters were further divided into 3 categories (early-, mid-, and late-adopters) based on tertiles of time to adoption. Then, we compared organizational characteristics between facilities with bivariate analyses.

Results: By September 2009, 109 (81%) facilities adopted eGFR reporting while 26 (19%) facilities had not. Time to adoption varied widely with range, 0.2 to 4.3 years, and median of 1.8 (IQR 2.2) years. Comparing facilities by adoption status, dialysis units were only present among adopters (59%)($p<0.05$). Among adopters, there were no significant differences in organizational characteristics.

Conclusions: Despite a uniform mandate to adopt this laboratory reporting innovation, 1 in 5 facilities did not adopt eGFR reporting and among those who did, time to adoption varied widely. These findings suggest that adoption may be due to availability of local resources (e.g., dialysis units). Still, reasons for the wide range in adoption remain unclear, but may be the result of local decision-making between clinical and information technology administrators. With the emergence of newer eGFR equations to improve CKD detection, our findings should be considered prior to future rollouts of other laboratory reporting innovations to promote more uniform and timely adoption.

Funding: NIDDK Support, Other U.S. Government Support

PUB189

Trial Announcement: Vitamin K2 To Slow Vascular Calcification in Hemodialysis Patients, "VitaVasK" Thilo Krueger,[1] Georg Schlieper,[1] Mario Cozzolino,[2] Johannes Jacobi,[3] Ralf-Dieter Hilgers,[11] Michel Y. Jadoul,[4] Markus Ketteler,[5] Tom Cornelis,[6] Lars C. Rump,[7] Peter Stenvinkel,[8] Andrzej Wiecek,[9] Ralf Westenfeld,[10] Leon J. Schurgers,[12] Jurgen Floege.[1] *[1]Nephrology, University Clinic Aachen, Aachen, Germany; [2]Renal Division, University of Milan, Milan, Italy; [3]Nephrology, University of Erlangen Nuremberg, Erlangen, Germany; [4]Nephrology, University of Louvain, Brussels, Belgium; [5]Nephrology, Coburg Hospital, Coburg, Germany; [6]Nephrology, University Hospital Maastricht, Maastricht, Netherlands; [7]Nephrology, University Duesseldorf, Duesseldorf, Germany; [8]Renal Medicine, Karolinska Institute Stockholm, Stockholm, Sweden; [9]Nephrology, University of Katowice, Poland; [10]Cardiology, University Duesseldorf, Duesseldorf, Germany; [11]Medical Statistics, University Clinic Aachen, Aachen, Germany; [12]CARIM, University Maastricht, Maastricht, Netherlands.*

Background: Patients on hemodialysis (HD) exhibit an increased cardiovascular mortality associated with vascular calcification (VC). Matrix Gla protein (MGP) is a powerful vascular wall-based inhibitor of VC. MGP needs activation by vitamin K-dependent carboxylation.

The ERA/EDTA sponsored VitaVasK study will be the first clinical trial in HD patients to target the progression of VC using dietary vitamin K-containing supplements. VitaVasK is a randomized, double-blind, placebo-controlled trial with parallel groups. Participants are recruited from nine European nephrology centers. Multislice spiral computed tomography (MSCT) will be used to screen for coronary artery calcification (CAC). Stable HD patients with CAC scores >100 will be randomized to a daily oral supplementation of vitamin K2 (n=178) or placebo (n=178) for 18 months. Primary outcomes will be attenuation of progression of coronary and aortal calcification at 12 and 18 months using volume scores determined by MSCT. Secondary outcomes will be regression of VC, attenuation of progression of aortic and mitral valve calcification, major cardiac events, mortality, and change in serum levels of undercarboxylated and carboxylated MGP. Patients will be followed beyond the primary study duration to determine the mortality at three and five years.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

PUB190

CKD.QLD Phase 2 Survey: Risk Factor Modification for Prevention of Chronic Kidney Disease Progression Sree Krishna Venuthurupalli,[1,4] Anne Salisbury,[3] Wendy E. Hoy,[3] Helen G. Healy,[2] Robert G. Fassett.[2,4] *[1]Renal Medicine, Toowoomba Hospital, Toowoomba, Queensland, Australia; [2]Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia; [3]Centre for Chronic Disease, University of Queensland, Brisbane, Queensland, Australia; [4]School of Medicine, University of Queensland, Brisbane, Queensland, Australia.*

Background: Management of traditional risk factors for chronic kidney disease (CKD) progression include antiproteinuria therapy, hypertension and diabetes control, diet, exercise and lipid lowering therapy. We investigated the current clinical practice for prevention of CKD progression in Queensland, Australia.

Methods: Using a web based questionnaire, nephrology medical and nursing staff from each CKD clinic in Queensland were surveyed to assess risk factor modification in CKD management practices.

Results: The participation rate was 100%. Restriction of salt and fats formed the corner stone (80%) of diet modification. Routine protein restriction was not advised. Dietary potassium and phosphate limitations were used as case by case.

Anthropometric measurements included weight (100%), height (81%), body mass index (61%) and waist circumference (10%). An exercise physiologist was not available in all clinics, though physical activity and exercise was routinely recommended.

Angiotensin converting enzyme inhibitors and angiotensin receptor blockers were routinely prescribed for blood pressure reduction and to slow CKD progression. However, combination therapy was only used in 50% of cases, based on level of proteinuria. Lipid lowering therapy was a frequent practice (90%), with use of statins being the main strategy. Interestingly, statins were used with the aim of reducing cardiovascular mortality and morbidity but not to slow CKD progression.

Diabetes control was routine with HbA1C levels used as the main tool for follow up (85%). Patients are screened for vascular complications related to diabetes with annual follow up.

Conclusions: Traditional risk factors management was incorporated in CKD practice across renal units in Queensland, Australia. A longitudinal study in this population will determine the impact of these on CKD progression and cardiovascular events and mortality.

Funding: Pharmaceutical Company Support

PUB191

Relationship between Clinicopathological Findings and Renal Outcomes of Diabetic Nephropathy in Type 2 Diabetes Tomoaki Funamoto,[1] Miho Shimizu,[1] Tazuko Ohama,[1] Chikako Nose,[1] Yasuyuki Shinozaki,[1] Shinji Kitajima,[1] Tadashi Toyama,[1] Akinori Hara,[1] Kiyoki Kitagawa,[1] Kengo Furuichi,[1] Shuichi Kaneko,[2] Takashi Wada.[1] *[1]Division of Nephrology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; [2]Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Ishikawa, Japan.*

Background: The purpose of the present study was to evaluate the relationship between clinicopathological findings and renal outcomes of diabetic nephropathy in Japanese patients with type 2 diabetes.

Methods: Two hundred and sixty, biopsy-proven type 2 diabetic patients with diabetic nephropathy (164 males and 96 females) were examined. Observation period was 7.9±6.5 years. Renal outcomes were assessed by requirement for dialysis or 50% reduction of estimated glomerular filtlation rate (eGFR). For presumption of clinicopathological factors that affected renal outcomes, multivariate analysis by the Cox proportional hazards model was employed.

Results: The number of cases of normal albuminuria or urinary protein (-) or (±), microalbuminuria or urinary protein (+), and overt nephropathy were 47, 50 and 163 respectively. 2) Seventeen patients (36.2%) with normal albuminuria or urinary protein (-) or (±), 21 patients (42%) with microalbuminuria or urinary protein (+), and 116 patients (71%) with overt nephropathy were under 60ml/min/1.73m^2 of eGFR. 3) In patients with microalbuminuria and overt nephropathy, renal outcomes were good in patients with over eGFR values of 45 ml/min/1.73m^2. 4) Higher albuminuria was the most important clinical factor for poor renal outcomes. Lower eGFR was an independent risk factor for renal outcomes in patients with overt nephropathy. Pathological factors for poor renal outcomes were nodular lesions and interstitial fibrosis in microalbuminuria patients, while those were atherosclerosis, exudative lesions, and nodular lesions in overt nephropathy patients.

Conclusions: Our observations suggest that development of albuminuria play an important role for renal outcomes of diabetic nephropathy in type 2 diabetes. Lower eGFR was an independent risk factor in renal outcomes among patients with overt nephropathy.

PUB192

Low 25-hydroxyvitamin D Levels Are Associated with an Increased Risk of Non-Alcoholic Fatty Liver Disease (NAFLD) in a Large Managed Care Organization Anna Jeanette Jovanovich,[1] Giovanni Targher,[2] Jessica B. Kendrick,[1] Shailendra Sharma,[1] Kim McFann,[1] Sidney N. Thornton,[3] Michel B. Chonchol,[1] John R. Holmen. *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Section of Endocrinology, Department of Medicine, University Hospital, Verona, Italy; [3]Intermountain Health Care, Salt Lake City, UT.*

Background: To explore associations between serum 25-hydroxyvitamin D levels and NAFLD.

Methods: We conducted a population-based cohort study of adult patients with NAFLD who had vitamin D levels measured 3 to 15 months prior to diagnosis of NAFLD. Vitamin D was evaluated as a continuous variable and a categorical predictor (< 15 ng/mL vs. higher). The primary outcome of interest was diagnosis of NAFLD identified through ICD9 codes and sex-specific elevation in liver function tests. Patients with a history of alcoholic liver disease were excluded from the analyses. Patients with NAFLD were matched 1:1 with controls on age, sex, race and season. Conditional multivariate logistic regression was used to evaluate if low vitamin D levels were associated with an increased risk of NAFLD.

Results: We identified 607 individuals with NAFLD and 607 matched controls. The mean (SD) age of the participants was 56±14 years, 74% were female and 90% were Caucasian. Compared to controls, patients with NAFLD had a significantly higher body mass index (31±17 vs. 28 ±14 Kg/m2; p<0.0001), and higher percentage of diabetes (36.0% vs. 30.0 %; p<0.0001). Mean (SD) levels of 25(OH)D were lower in patients with NAFLD than controls (30 ± 7 vs. 34 ± 8 ng/mL p=0.0003). After adjustment for BMI, diabetes, chronic kidney disease and peripheral vascular disease higher 25(OH)D levels were protective for NAFLD (OR 0.97, 95% CI 0.97-0.98; p<0.0001). Similarly, when evaluated as a categorical predictor, vitamin D levels < 15 ng/mL vs. higher (adjusted OR 1.98, 95% CI 1.33-2.97; p=0.0008) were strongly associated with almost a two-fold increased odds of NAFLD.

Conclusions: Vitamin D deficiency is associated with an increased risk of NAFLD. Clinical trials are needed to evaluate whether vitamin D_3 supplementation can reduce the risk of NAFLD in patients with and without CKD.

PUB193

Effects of Dual Blockade of the Renin Angiotensin System in Diabetic Kidney Disease: A Systematic Review and Meta-Analysis Jacqueline Pham,[1] Brian P. Schmitt,[2] David J. Leehey.[1] *[1]Renal and Hypertension, Edward Hines Jr. VA Medical Center, Hines, IL; [2]Edward Hines Jr. VA Medical Center, Hines, IL.*

Background: There is much evidence to support a renoprotective effect of inhibitors of the renin- angiotensin system in diabetic kidney disease. However, it remains unclear whether dual renin- angiotensin system blockade has additional benefits in this population and whether any benefits outweigh the risks.

Methods: *Study Design:* Systematic review and meta-analysis

Setting and Population: Diabetic patients with overt proteinuria

Selection Criteria for Studies: Randomized, controlled, parallel or crossover design studies

Intervention: Combination renin-angiotensin system blockade vs. monotherapy

Outcomes: The primary outcome measure was the post-treatment difference in proteinuria with combination therapy versus monotherapy. Secondary outcomes included percent change in proteinuria, changes in systolic blood pressure, glomerular filtration rate, and serum potassium, and incidence of hyperkalemia. Sensitivity analyses that evaluated differences in outcome based on study quality (assessed by Jadad scores), baseline systolic blood pressure, and drug types and doses were conducted.

Results: There was significantly less proteinuria (by 334 mg/24 hr) after treatment with combination therapy vs. monotherapy. Systolic blood pressure (BP) after treatment with combination therapy vs. monotherapy was significantly lower (by 4.1 mmHg). However, clinically significant hyperkalemia was 3.5-fold more common with dual blockade. Sensitivity analyses did not identify subgroup differences that altered these findings.

Conclusions: Dual renin-angiotensin system blockade in patients with diabetic kidney disease reduces proteinuria and BP but is associated with a higher incidence of clinically significant hyperkalemia. Further studies assessing long-term outcomes are needed to weigh the benefits versus risks of combination renin-angiotensin system inhibitor therapy.

PUB194

Effect of Blockers of the Renin-Angiotensin System on Renal Tissue Oxygenation in Type 2 Diabetics as Measured by BOLD-MRI Menno Pruijm, Lucie Hofmann, Anne Zanchi, Marc P. Maillard, Valentina Forni, Meryll Cassat, Grégoire Wuerzner, Bruno Vogt, Michel Burnier. *Nephrology, Centre Hospitalier Universitaire Vaudois, Lausanne, VD, Switzerland.*

Background: Previous studies have shown that acute intake of certain drugs alters renal tissue oxygenation, and animal studies suggest that blockers of the renin-angiotensin system might exert their renoprotective effect by correcting diabetes-induced renal hypoxia.

The objective of this study was to investigate the chronic effect of the ATII- type 1 receptor blocker (ARB) candesartan, compared to the ACE-inhibitor (ACEI) enalapril on renal tissue oxygenation in type 2 diabetics with microalbuminuria and/or hypertension, using blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI).

Methods: After a wash out period, ten patients (aged 63.8±8.4 y, BMI 35.0±3.0, 30% women) underwent BOLD-MRI at baseline, after one month of enalapril (20mg/day), and after one month of candesartan (16 mg/day). BOLD-MRI was performed before and after intravenous administration of furosemide.

Four coronal slices were selected, and a modified MEDIC sequence was used to acquire T2* weighted images. The mean R2* values (=1/T2*) were calculated, a low R2* indicating a high tissue oxygenation.

Results: Baseline characteristics and their changes are shown in table 1. The mean cortical and medullary R2* did not differ significantly between groups (ANOVA, p=0.88 and 0.24). Furosemide did not change cortical R2*, and decreased medullary R2* to a similar extent in all groups (mean decrease 7.51±2.17 s^{-1}).

	Baseline	Candesartan	Enalapril
SBP (mmHg)	135.4 (10.2)	132.4 (12.2)	132.4 (10.5)
DBP (mmHg)	74.4 (14.6)	72.4 (9.2)	73.0 (10.3)
Creatinine (µmol/l)	86 (67-186)	89 (58-210)	89 (64-202)
Clearance (ml/min)	105.4 (48.7)	99.8 (30.8)	99.6 (36.2)
Urinary sodium (mmol/24h)	198.6 (23.6)	185.0 (51.7)	174.0 (46.9)
HbA1c (%)	7.60 (0.9)	7.47 (1.07)	7.45 (0.8)
Medullary R2* (1/s)	28.5 (1.3)	27.9 (1.6)	29.1 (1.6)
Cortical R2* (1/s)	17.8 (1.5)	17.8 (1.4)	18.1 (1.7)

Values are shown as mean±SD, or median (min-max), as appropriate

Conclusions: At the current stage, chronic intake of ACEI or ARB does not seem to alter renal tissue oxygenation measured by BOLD-MRI in type 2 diabetics.

PUB195

Design and Rationale for a Randomized, Double-Blind, Placebo-Controlled Phase 2 Study To Evaluate the Safety and Efficacy of CTP-499 in Patients with Diabetic Nephropathy James Shipley, LuAnn A. Sabounjian, Virginia Braman, Dolly A. Parasrampuria, Lijun Wu, David J. Turnquist, Philip B. Graham. *Concert Pharmaceuticals, Lexington, MA.*

Background: CTP-499 is a deuterium-containing analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine (HDX), an active metabolite of pentoxifylline (PTX). Small published clinical trials with PTX have shown evidence of beneficial effects in CKD patients. CTP-499 is being investigated as a novel treatment for diabetic nephropathy and

is expected to be additive to RAS blockade. Deuterium is a safe, naturally-occurring, non-radioactive isotope of hydrogen. Selective incorporation of deuterium does not appear to alter the binding, potency or selectivity, but in select cases may provide beneficial effects on compound metabolism. In vitro and ex vivo studies have shown CTP-499 possesses anti-inflammatory, anti-fibrotic and anti-oxidative properties, making it potentially useful in slowing the progression of DN. In rats with streptozotocin-induced diabetes, CTP-499 reduced urinary protein excretion and improved other renal function markers.

Methods: Design: Approximately 170 patients with type 2 diabetic nephropathy, macroalbuminuria (UACR ≥400 mg/g) and an eGFR ≥25 mL/min/1.73m2 (MDRD), who are receiving concomitant ACEi and/or ARB therapy, will be enrolled at approx. 30 US centers. After a 4-8 week stabilization period, during which BP will be managed to a target of <145/90 mmHg, patients will be randomized (1:1) to receive 24 weeks of treatment with CTP-499 600 mg bid (600 mg qd for the first 2 weeks), or placebo. UACR will be assessed monthly using the geometric mean of 3 consecutive first morning voids. eGFR will be determined at each visit along with a biomarker panel incl. cytokines and markers of fibrosis and kidney function. Plasma conc. of CTP-499/metabolites will be assessed monthly. In a subset of patients, 24h plasma and urine conc-time profiles will be obtained after 1st and last doses.

Results: Primary Endpoint: UACR change from pre-treatment baseline to post-treatment using a longitudinal model with data from weeks 16, 20, and 24.

Conclusions: Study enrollment is expected to begin in late 2011; top-line data are expected in 2013.

Funding: Pharmaceutical Company Support

PUB196

A Systematic Review and Meta-Analysis: Impacts of Albuminuria and Low GFR on Renal Failure, Cardiovascular Death, or Mortality in Patients with Diabetic Nephropathy Tadashi Toyama,[1] Kengo Furuichi,[1] Toshiharu Ninomiya,[2] Shuichi Kaneko,[3] Takashi Wada.[1] *[1]Division of Nephrology, Kanazawa University Hospital, Kanazawa, Japan; [2]Department of Nephrology, Hypertension,and Strokology, Kyushu University Hospital, Fukuoka, Japan; [3]Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa, Japan.*

Background: The relationships between albuminuria and complications of diabetes mellitus have been reported. However consistency and strength of these evidences have not been cleared so far. In this study, we explored the impacts of albuminuria on renal failure, cardiovascular death, and all-cause mortality in diabetes mellitus.

Methods: Relevant studies were identified from MEDLINE, EMBASE, and CINAHL by using relevant text words and medical subject headings that includes all keywords related to "diabetes mellitus," "renal failure," "cardiovascular diseases," and "mortality." The literature search and data extraction was conducted for three endpoints (renal failure, cardiovascular death, all-cause mortality) by two independent researchers. The extracted estimates were combined using a random-effects model.

Results: The literature search yielded 6546 articles, of which 328 papers were reviewed in full. In diabetic patients, the presence of microalbuminuria is associated with an approximate 3.5-fold increase in risk of renal failure (relative risk 3.52, 95% CI 2.04-6.05). And there are the same trends in cardiovascular death (relative risk 1.90, 95% CI 1.56-2.32), and all-cause mortality (relative risk 1.62, 95% CI 1.45-1.82). In addition, there are significant trends towards greater effects with macroalbuminuria in each outcome.

Conclusions: These findings suggest that microalbuminuria and macroalbuminuria are risk factors for renal failure, cardiovascular mortality, and all-cause mortality in diabetic patients. Individual patient data meta-analysis is needed in the future.

PUB197

Clinical and Histological Features of Patients with IgA Nephropathy Who Have Attained Stable Clinical Remission Lasting Longer Than 5 Years Alessandro Amore,[1] Roberta Camilla,[1] Laura Morando,[1] Rachele Gallo,[1] Licia Peruzzi,[1] Cristiana Rollino,[2] Raffaella Cravero,[4] Marco Quaglia,[5] Loredana Colla,[6] Gianna Mazzucco,[6] Rosanna Coppo.[1] *[1]Nephrology, Dialysis, Transplant, R. Margherita H., Turin, Italy; [2]Nephrology and Dialysis, G. Bosco H., Turin, Italy; [3]Nephrology, University of Turin, Italy; [4]Nephrology, Infermi H, Biella, Italy; [5]Nephrology, East Piedmont University, Novara, Italy; [6]Biomedical Science and Human Oncology, University of Turin, Italy.*

Background: IgA nephropathy (IgAN) progresses to ESRD in 30% of cases within 20 years, but some patients have a long-lasting clinical remission. Little attention has been devoted to them.

Methods: This retrospective study enrolled IgAN patients who showed for more than 5 years a stable remission defined as absence/trace of hematuria (Hb-/+ or < 5 RBC/hpmf),absence/trace of proteinuria (< 250 mg/day or uP/uCr < 0.2),stable renal function (loss of eGFR <1 ml/min/1.73m2/year)and normal BP with/without RAAS inhibition.

Results: Entering criteria were fulfilled by 51/320 patients.They were followed for 15±7.2 years from onset to December 2010. Median age at onset was 25 y(7-65); 61% had history of gross hematuria; e-GFR was 106±25(60-159) ml/min/1.73m2 and uP/uCr 1.1±.5 (0.01-14).

Renal biopsies were reviewed according to Oxford classification for IgAN. Treatments included steroids in 14 cases(3-9 i.v. MP pulses or oral for 6-8 months), oral cyclophosphamide for 8 weeks in 2 cases, ACE-inhibitors/ARB in 24.The median time from renal biopsy to remission was 24 months (IQ range 6-48). In subgroups of patients presenting with clinical or histological risk factors for progression, time to remission resulted longer in proteinuric cases.

Factors affecting time to remission

	N of cases	Time to remission, months (median - IQ range)
proteinuria > 1 (uP/uCr)	18	30 (6-57)
hematuria Hb > ++	43	24 (6-48)
eGFR < 90 ml/min/1.73m2	14	24 (12-57)
MEST > M0E0S0T0	22	24 (6-60)

Conclusions: A subset of patients with IgAN can enter a long-lasting clinical remission even when presenting clinical or histological risk factors at renal biopsy. These patients are good candidate for genetic and biomarker investigations to be compared with progressive cases.

PUB198

Dense Deposit Disease Associated with a CFH Gene Mutation, CFH Autoantibodies and C3 Nephritic Factor: What Is Responsible? Gianluigi Ardissino,[1] Francesca Tel,[1] Nicolò Borsa,[2] Richard J. Smith,[3] Yuzhou Zhang,[3] Silvana Tedeschi,[2] Stefania Salardi,[2] Sara Testa,[1] Alberto Edefonti.[1] *[1]Ped Nephrol Unit, Fondazione IRCCS Osp. Maggiore Policlinico, Milano, Italy; [2]Lab. Medical Genetics, Fondazione IRCCS Osp. Maggiore Policlinico, Milano, Italy; [3]Molecular Otolaryngology and Renal Research Laboratories, Departments of Pediatrics and Medicine, Divisions of Nephrology, University of Iowa, Iowa City, IA.*

Background: DDD is a rare disease leading to ESRD ~50% of pts < than 10 yrs of age at diagnosis. The pathophysiology of DDD is the uncontrolled activation of the alternative pathway (AP) of complement cascade. AP dysregulation can be caused by mutations in *CFH* and antibodies to complement proteins such as factor H (FHAA) and C3 convertase (C3 nephritic factor, C3Nef).

Methods: We report a 9-yr-old boy who developed DDD and was found to have a CFH mutation, FHAA and C3Nefs.

Results: This child presented with occasional macrohematuria, persistent microhematuria, proteinuria and hypocomplementemia. A renal biopsy showed DDD with endo-extracapillary proliferation (10%). Over a 2 yrs period, he was treated with 4 cycles (x 3) of methylprednisolone followed by oral prednisone. uPr/uCr temporarily normalized and sC3 levels increased. Additional studies identified a novel heterozygous *CFH* variant as well as FHAA and C3Nefs. Steroid treatment was stopped and plasma exchange (PEX) with fresh frozen plasma (FFP) was initiated, followed by weekly FFP infusions over a period of 3 months. This treatment led to a stable reduction in FHAA with normalization of sC3 and uPr/uCr ratio for as long as 5 mos, after which FHAA increased, proteinuria recurred and sC3 levels dropped. A single PEX followed by weekly infusions of FFP was temporary effective in lowering FHAA but uPr/uCr did not improve. The addition of mycofenolate mofetil (MMF) was ineffective.

Time (d)		FHAA (IU)	C3 (mg/dl)	uPr/uCr
0	Baseline pre-PEX	>100	42	1.75
0	Post-PEX	6	80	0.36
10	FFP	13	75	0.38
45	FFP	8.7	97	0.3
162	NO TX	100	9	0.9
204	FFP	3.4	19	1.9
232	MMF	3.5	18	2
262	MMF	11	18	3.1

Conclusions: Herein we share this unique case of DDD where several possible causes of the disease coexist and the response to different treatment modalities.

PUB199

Necrotizing Crescentic Glomerulonephritis Complicating Membranous Nephropathy: A Rare Coexistence Syed N. Babar, Richard A. Sherman. *Nephrology, Robert Wood Johnson University Hospital, New Brunswick, NJ.*

Background: Membranous glomerulonephritis (MG) is among the most common causes of nephrotic syndrome in adults. The majority of MG cases represent primary or idiopathic disease, whereas the remaining cases represent secondary forms related to infection, drugs and malignancy. Necrotizing crescentic glomerulonephritis (NCGN) is characterized by glomerular necrosis and crescent formation. One half of NCGN is associated with SLE, Goodpasture's or Wegener's while the other half is idiopathic. We present a case demonstrating the rare coexistence of MG and NCGN.

Methods: A 38 year old male with biopsy proven MG maintained initially on prednisone, and then mycophenolate mofetil and later cyclosporine was noted to have worsening lower extremity swelling and fatigue two years after his diagnosis. Labs revealed worsening nephrotic range proteinuria and azotemia along with positive p-ANCA and MPO Abs. Serologies for lupus and anti-GBM disease were negative. Repeat renal biopsy revealed NCGN superimposed on MG with segmental and global sclerosis. Aggressive salvage therapy with pulse methylprednisolone and IV cyclophosphamide failed to improve renal function. Four months after starting hemodialysis he received a kidney transplant. A year and a half post-transplant, he remains stable with a creatinine of 1.5 mg/dl.

Results: The concurrence of MG and NCGN is a rare phenomenon with only a handful of reported cases. The presence of fibrinoid necrosis and crescent formation in the setting of membranous nephropathy may be seen in lupus nephritis, anti-GBM disease, or ANCA-associated RPGN. Renal biopsy is required to make the diagnosis and treatment typically involves pulse steroids with cyclophosphamide. The outcomes vary and generally correlate with the percentage of globally sclerotic glomeruli seen on the biopsy. The manifestation of both membranous nephropathy and necrotizing crescentic glomerulonephritis likely represents a coincidental happening given their distinct pathogenesis.

Conclusions: Unexpected or sudden worsening of proteinuria and renal function in patients with established glomerular disease should prompt consideration of a superimposed renal lesion.

PUB200

Validation of Histology Classification of ANCA Associated Vasculitis and Patient Outcomes Sophie Louise Bennett,[1] Ajay Prabhakar Dhaygude.[1] [1]*Renal Medicine, Royal Preston Hospital, Preston, United Kingdom;* [2]*Histopathology, Royal Preston Hospital, Preston, United Kingdom.*

Background: Recently published meta-analysis of outcome 535 ANCA associated systemic vasculitis (AASV) patients from four different clinical trials suggested that 48% of the mortality was due to infections in the first year and 19% mortality due to active vasculitis. Validation of Berden histological classification of AASV in 100 patients demonstrated good correlation with histology class of AASV and renal outcomes at 1 and five years and is likely to be a valuable tool to guide therapy of AASV. In this study we validated this classification in 35 patients with renal vasculitis.

Methods: We retrospectively classified all kidney biopsies of newly diagnosed patients with small vessel vasculitis who presented between 1/1/2003 and 31/12/2005. Data was collected regarding their ANCA serology, overall survival and renal survival.

Results: Thirty-five patients were analysed. Fifteen patients were cANCA positive, 13 patients were pANCA positive and seven patients were ANCA negative. Average creatinine at the time of diagnosis was 535.97 mmol/lr and average creatinine at the time of last follow up was 253.08 mmol/lr. Outcomes are presented in table-1

Table-1: Outcome of patients with renal vasculitis.

Histology Class	Average Diagnosis Creatinine (mmol/lr)	Average Follow up Creatinine (mmol/lr)	Patient survival	Renal survival	Reduction in creatinine
Crescentic (11)	543	208	10 (90.9%)	8 (72.7%)	61.7%
Focal (14)	378	239	12 (85.7)	13 (92.8%)	36.8%
Mixed (8)	648	285	3 (37.5)	8 (100%)	56%
Sclerotic (2)	1151	800	1 (50%)	1 (50%)	30.5%

Conclusions: These results suggests that patients with

1] Patients with crescentic class of renal vasculitis have most improvement in their renal function.

2] Patients with mixed lesions had poor patient survival

3] Patients with sclerotic lesions have poor patient and renal survival

Limitations: Small sample size and retrospective study.

PUB201

Membranoproliferative Glomerulonephritis with IgMk Deposits in an Anti-HCV Positive Patient with Concurrent Small B-Cell Lymphoproliferative Disease Eleni Chelioti, Sotiris Mikros, Evagelia Chrisanthopoulou, Maria Tsilivigou, Gabriel Papadakis. *Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, Athens, Greece.*

Background: Hepatitis C(HCV) infection has been associated with Membranoproliferative Glomerulonephritis(MPGN),usually in the context of type II cryoglobulinemia with polyclonal IgG and monoclonal IgM precipitates. On the other hand, MPGN has been reported as the most frequent histological diagnosis of renal involvement in the context of B-cell lymphoproliferative diseases. Renal complications of malignant IgM-secreting proliferations are rare, the most common cause being Waldenstrom's Macroglobulinemia,but small B-cell non-Hodgkin and Marginal Zone Lymphoma are also reported occasionally.

Methods: We report the case of a 50-year-old male with history of alcohol and IV drug abuse,chronic HCV infection and renal failure of undetermined origin who presented in the emergency department with uremic symptomatology. The patient was dyspnoeic, hypertensive, without peripheral oedemes, with metabolic acidosis, hyperkalemia, hyperphosphatemia, hypoalbuminemia, anemia, thrombocytopenia and severe renal failure with signs of glomerular involvement(urine red blood cells=40–60/HPF,24-hour urine proteins=3.06g).

Results: The clinical and laboratory findings on admission necessitated the immediate start of dialysis.The imaging exploration revealed hepatosplenomegaly and normal sized kidneys with high echogenicity. There was no clinical or radiological sign of lymph node involvement.Laboratory analyses suggested monoclonal IgM+κ gammopathy,present in serum and urine immunofixation,type I cryoglobulinemia and low C4 complement levels. After clinical stabilization and restoration of anemia and thrombocytopenia,a bone marrow biopsy was performed,suggestive of a small B-cell lymphoproliferative disorder. A renal biopsy revealed a MPGN with IgMκ deposition and lymphocyte infiltration of the interstitium.

Conclusions: The rapidly deteriorating renal function in the context of nephrotic syndrome and emergence of MPGN secondary to IgMκ deposits and monoclonal gammopathy by B-cell lymphoproliferative disorder is a rare diagnosis.In similar cases, renal biopsy is a powerful diagnostic tool.

Funding: Clinical Revenue Support

PUB202

Study of Malignancy and Chronic Infections in Patients with Biopsy Proven Membranous Nephropathy Marie B. Condon, Tom Cairns, Megan Griffith. *Imperial College NHS Trust, United Kingdom.*

Background: Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults. Most cases of MN are idiopathic, however historical studies have shown MN can also occur in association with malignancy or chronic infections. This study investigated the prevalence of these secondary causes of MN in all patients presenting to this institution with nephrotic syndrome (NS) as a consequence of MN from March 2008.

Methods: Forty-one pts presented with biopsy proven MN, 24 male, 17 female. 37 de novo presentations and 4 relapses of NS, at median time of 6 years post 1st presentation (3-18). The median age was 54 yrs (25-80), 10/41 were aged >60. 18 Caucasian, 18 Asian and 5 African/Caribbean. Blood was tested for hepatitis B, C and HIV. Pts were screened for malignancy with CT chest/abdomen/pelvis, prostate specific antigen in males and underwent endoscopic investigations if any gastro-intestinal symptoms were present.

Results: Three pts had asymptomatic chronic infections; 1 Hep B, 1 HIV and 1 pulmonary TB detected on CT scan. 2/41(5%) had solid tumours. Pt 1 (male, 74 yrs) had an asymptomatic lung malignancy, detected on CT scan. Pt 2 (male, 72 yrs) a colonic adenocarcinoma on colonoscopy performed for bowel symptoms. Both pts were presenting with a relapse of nephrotic syndrome due to MN; pt 1, 18 years after the initial diagnosis (treated with IV cyclophosphamide); pt 2, 3 years after initial diagnosis (treated with Rituximab, Mycophenolate Mofetil and Tacrolimus).

Conclusions: This prevalence of malignancy in this study is lower (5%) than some previous reports (7-16% in all patients with MN and >50% in pts >60 yrs.) Interestingly the 2 cases of malignancy in our study were in pts presenting with a relapse of their MN. Others have reported an increase in malignancy in pts with MN in the years post presentation. The contribution of malignancy to the pathogenesis of MN and the role of immunosuppressive treatment for MN in the development of malignancy is not yet clearly defined. Surveillance is required not only at diagnosis but also during follow up. The association of MN with infection and malignancy needs to be taken into account when considering treatment with immunosuppressive therapy.

PUB203

A European Network for IgA Nephropathy Focused on the Validation of the Oxford Clinico-Pathology Classification Rosanna Coppo, Laura Morando, John Feehally, H. Terence Cook, Stephan Troyanov, Daniel C. Cattran, Ian Roberts. *for the VALIGA European Immunonephrology working group, R. Margherita H.*

Background: A recent publication from an International Consensus - based on a retrospective analysis of 265 patients with IgA Nephropathy (IgAN) from 4 continents - focused on prognostic information provided by renal biopsy. According to this Oxford Classification of IgAN, mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity and tubular atrophy/interstitial fibrosis predict renal outcome independently from all clinical indicators at the time of biopsy and during follow up (KI 2009;76:534-45; and 2009;76:546-56). The number of patients and their heterogeneous origin indicated a need for validation studies involving large cohorts of patients. Some validation studies have now been published, but these are of limited size (273 cases in a Korean study, 187 in a study from USA).

Methods: To validate the Oxford classification in Europe,the ERA-EDTA scientific community was invited to participate in this study, as a new model for researches needing large integration and collaboration, aiming to enrol at least 500 European patients with IgAN.

Results: We have presently enrolled 674 IgAN patients with long follow-up or rapidly progressive course from 29 nephrology and renal pathology centers in 10 European Countries: Italy (14 centers); Spain (3 centers); Turkey, Greece, Sweden Netherlands(2 centers each); Germany, Croatia, Czech Republic, Poland (1 Center each). Renal biopsies have been scored by the local pathologist and are under central review in Oxford, UK. Clinical data at renal biopsy and during follow-up were provided by local nephrologists to the Coordinating Center, 481 further cases are being checked for completeness of clinical data and their biopsies are under review.

Conclusions: Networking is the only possible way to obtain sufficient case enrolment in studies of rare rare conditions, such as glomerular disease. This European Network, now established, is open to further research opportunities, for example genetic studies, and the development of clinical trials. This presentation at the ASN is aimed at encouraging future scientific networks.

PUB204

Epidemiology of Patients with Pulmonary Haemorrhage from Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis Admitted to the Intensive Care Unit Partha Das, Scott R. Henderson, Maria Ostermann. *Department of Nephrology and Transplantation, Guy's & St Thomas' Hospitals NHS Foundation Trust, London, United Kingdom.*

Background: Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides are associated with significant morbidity and mortality despite advances in diagnosis and immunosuppressive treatment. Pulmonary haemorrhage is a much feared and life-threatening manifestation of the disease and may lead to admission to the Intensive Care Unit (ICU). Our aim was to describe the epidemiology of patients with pulmonary haemorrhage admitted to the ICU in a tertiary care teaching hospital.

Methods: A retrospective case note review was performed on all patients with pulmonary haemorrhage due to new or previously diagnosed ANCA associated vasculitis admitted to ICU between 2008 - 2011 for pulmonary haemorrhage. Demographics, disease duration and previous treatments were recorded alongside reason for ICU admission, APACHE II and SOFA scores, level of organ support, immunosuppression and patient mortality.

Results: 11 patients (9 men, mean age 56.2 years ± 16.1, 10 Caucasian, 1 mixed race) were admitted to the ICU with pulmonary haemorrhage. This was thefirst presentation for 8 patients. Proteinase 3 (PR3) ANCA was positive in 8 patients, myeloperoxidase (MPO) ANCA in 1 patient, and 2 patients had no detectable ANCA. 45% of patients had received immunosuppressive treatment prior to admission in the preceding 3 months. Patients were managed by a multidisciplinary team. 63% of patients had invasive ventilation, 54% had renal replacement therapy, and 54% were treated for sepsis. 8 patients (72.7%) received steroids and cyclophosphamide treatment and 7 patients (63%) had plasmapheresis.

Mean length of stay in ICU was 13 days ±10.5 and in hospital 29 days ±20 days. 2 patients (18%) died in ICU due to bowel ischaemia. 1-year mortality of ICU survivors was 100%.

Conclusions: Outcome of patients with vasculitis associated pulmonary haemorrhage in ICU is good, even when significant organ support, immunosuppressive therapy and plasma exchange are required.

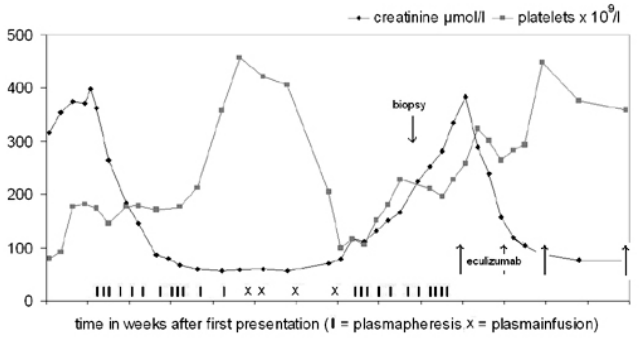
PUB205

Child with Plasma Resistant Atypical HUS Escaped End Stage Renal Failure by Eculizumab Therapy Eiske Dorresteijn,[1] Nicole Van De Kar,[2] Karlien Cransberg.[1] [1]*Pediatric Nephrology, Erasmusmc-Sophia, Rotterdam;* [2]*Pediatric Nephrology, UMC-St.Radboud, Nijmegen.*

Background: Recently, eculizumab, a humanized monoclonal antibody against C5 that blocks the terminal complement pathway, is reported to be effective in the treatment of atypical haemolytic uremic syndrome (aHUS).

Methods: We report the successful treatment of plasma resistant aHUS with eculizumab.

Results: A 6-year-old girl developed aHUS with severe renal insufficiency and hypertension. Plasmapheresis was immediately started, after which serum creatinine levels returned to almost normal after 3 weeks. Then, however, aHUS recurred shortly after an upper respiratory tract infection with influenza B virus. Her platelet count normalised after immediate intensive plasmapheresis, but the renal failure progressed, necessitating dialysis. C3 and C4 serum complement levels at the first presentation were normal, but C3d was elevated by 4.4%. Complement factors H, I, B, C3 or MCP showed no mutations. No other possible cause of aHUS was found. As end stage renal failure rapidly developed despite aggressive plasma therapy, plasmapheresis was stopped and treatment with eculizumab was initiated. Thereupon the girl's renal function promptly improved; after 8 weeks' therapy the estimated creatinine clearance had increased from 12 to 70 ml/min.1.73m^2. The multiple anti hypertensive treatment could be tapered to monotherapy with ACE inhibition. Four months after initiating eculizumab treatment, her renal function was stable at an estimated creatinine clearance of 85 ml/min.1.73m^2 with therapy every other week.



Conclusions: This case of aHUS shows that timely started, intensified plasmapheresis restored the haematological parameters, but could not restore kidney function. However, the change to eculizumab improved kidney function without the need of dialysis. Treatment with complement inhibitors might well be promising for patients with aHUS.

Funding: Pharmaceutical Company Support

PUB206

Case Report: Thrombotic Thrombocytopenic Purpura (TTP) like Syndrome in the Setting of Systemic Lupus Erythematosus (SLE) Vince Faridani, Adil Akthar, Ahmed M. Awad. *Nephrology, University of Missouri - Kansas City, Kansas City, MO.*

Background: We report a unique case of a patient with SLE , manifesting as acute renal failure, thrombocytopenia, and altered mental status. TTP-Like Syndrome may present with a myriad of symptoms and laboratory abnormalities. This case highlights the diagnostic challenges raised by the occurrence of microangiopathic hemolysis in the setting of multi-organ involvement in a patient with underlying SLE.

Case: A 42-year-old African American female with a history of hypertension and systemic lupus erythematous presented with altered mental status, renal failure, and thrombocytopenia. Vitals signs revealed blood pressure of 161/113, pulse rate 153, respiratory rate 36, saturation 92% on 4 liters of nasal cannula. On examination, crackles present at both lung bases bilaterally, 3+ pitting edema in lower extremities. Notable laboratory studies were hemoglobin 9.2, wbc 11.2, platelets 138, BUN 37, creatinine 2.6. CT of the head revealed no acute processes. Echocardiography revealed an EF 10-15% with global LV-hypokinesis. Lab studies showed low complement levels, a positive dsDNA antibody screen. Dialysis was initiated in addition to 5 rounds of plasmapharesis, 1gm of methylprednisone for 3 days, and mycophenolate mofetil. Renal biopsy showed thrombotic microangiopathy with secondary segmental sclerosis and mild interstitial fibrosis and no evidence of lupus nephritis. On day 8, repeat echo showed ejection fraction of 35%, a lower serum creatinine, urine output improved and dialysis was ceased.

Discussion:

This case highlights the diagnostic challenge raised by the occurrence of microangiopathic changes in the setting of multiorgan involvement in patients with underlying SLE. Microangiopathic changes can be caused by active SLE, malignant hypertension, antiphopholipid syndrome, TTP; differentiation between these syndromes is difficult given their numerous overlapping clinical and laboratory features. Prompt institution of aggressive blood pressure control, plasma exchange, corticosteroids should be instituted within 24hrs of presentation as delay in treatment initiation may increase treatment failure.

PUB207

Microscopic Polyangiitis: Case Report in Coexistence with Systemic Lupus Erythematosus Nephritis Vince Faridani, Ahmed M. Awad, Adil Akthar. *Nephrology, University of Missouri - Kansas City, Kansas City, MO.*

Background: We report a unique case of microscopic polyangiitis (MPA) manifesting as a renal-pulmonary syndrome in the setting of systemic lupus erythematosus (SLE) nephritis.

Case:

A 66-year-old female with a history of biopsy confirmed Class III SLE nephritis, presented with shortness of breath. On examination, right lower extremity revealed a 5x8 cm erythematous, painful, palpable purpura. Notable serum studies were creatinine 1.84 mg/dL, Hb 12.8 g/dl. Urinalysis was positive for a large amount of blood. CT chest demonstrated patchy bilateral infiltrates. Bronchoscopy showed diffuse alveolar hemorrhage and endoscopy revealed diffuse erosive gastritis. Immune serology was negative for anti-GBM, anti-dsDNA, but positive for ANA, with a pANCA, and myeloperoxidase 100 units (0-9 units). She underwent 11 consecutive treatments of plasma exchange along with 1 gram IV cyclophosphamide and 1 gram daily methylprednisolone and made a full recovery.

Discussion:

This is the first reported case of MPA in the setting of established SLE nephritis. Our case exemplifies the importance of a methodical approach to an extensive list of differential diagnoses. The main clinic clues to the alternative diagnosis of renal-pulmonary syndrome were diffuse alveolar hemorrhage and erosive gastritis. A positive pANCA and high anti-MPO titers without upper respiratory involvement strongly supports our diagnosis.

PUB208

Systemic Lupus Erythematous Masking Pre-Eclampsia Vince Faridani, Ahmed M. Awad, Adil Akthar. *Nephrology, University of Missouri - Kansas City, Kansas City, MO.*

Background: Systemic lupus erythematous (SLE) is one of the most common autoimmune disorders that affect women during their childbearing years. We report a unique case of pregnancy induced SLE nephritis presenting as pre-eclampsia.

Case:

A 27-year-old African American woman, with no previous medical illnesses presents seven days status post cesarian section at 29 weeks of gestation due to worsening pre-eclampsia. She was admitted with shortness of breath and uncontrolled hypertension. Vitals, blood pressure 173/123, pulse rate 106, oxygenation 92% on 2 liters nasal cannula. Physical exam revealed, diffuse scattered rhonchi bilaterally, 2+ lower extremity edema bilaterally. Labs revealed, hemoglobin 7.2, hematocrit 22, WBC 15, Platelets 619, creatinine 1.4, albumin 1.4, positive ANA, normal C3 and C4 level, all other serologies were negative. Urine analysis was positive for RBC 12, moderate hemoglobin, protein > 300. CT chest done was suggestive of pulmonary hemorrhage which was confirmed by bronchoscopy. Percutaneous renal biopsy showed type V lupus nephritis, diffuse proliferative and membranous lupus nephritis. She was treated with methylpredisolone 1 gram for 3 days, followed by oral prednisone and mycophenolate mofetil. The patient was followed up at 2 months and 4 months at which time her creatinine returned to her baseline and she was asymptomatic.

Discussion:

Pregnancies for patients with lupus have a greater risk of fetal loss, pre-eclampsia, intrauterine growth retardation, and neonatal lupus syndrome. Thus, it is important to do a thorough assessment in patients with new onset of gestational proteinuria not to delay diagnosis. The patients course was consistent with preeclampsia and the renal biopsy confirmed the diagnosis of lupus which explained her multisystemic involvement. Clinicians should be cautious and consider SLE nephritis when treating pre-eclampsia whom present with new onset proteinuira and hypertension.

PUB209

Procalcitonin – A Good Predictor for Persisting Kidney Damage in Patients with Goodpasture's Syndrome? Susanne V. Fleig, Jan T. Kielstein. *Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: Autoantibodies to the NC-1 domain of the type IV collagen's α3 chain are the pathogenetic basis for anti glomerular basement membrane (GBM) disease, otherwise known as Goodpasture's syndrome. It involves both the kidneys and lungs to varying degrees. Prediction of renal outcome in the phase of acute presentation of disease is difficult. Procalcitonin (PCT) has been proposed as a parameter of potential prognostic value. Based on a small group of patients (n=7), high procalcitonin (PCT) had been proposed as a predictor of poor renal outcome.

Methods: We report on a case demonstrating a severe manifestation of Goodpasture's syndrome, in which a high PCT on admission was fortunately not associated with detrimental renal outcome.

Results: Apart from dialysis, high intensity immunosuppressive therapy was begun immediately after admission in spite of high PCT. Our patient recovered to full renal function and remains free of symptoms after two years of follow-up. In contrast, a further three cases observed during 2010 presenting with acute renal failure and low procalcitonin remained dialysis dependent.

Conclusions: Our data do not confirm the notion that high procalcitonin is a good prognostic factor for renal outcome in Goodpasture's syndrome. Causal immunosuppressive therapy should not be delayed because of high PCT.

PUB210

Neurohumoral Control of Sodium Excretion in Nephrotic Syndrome (NS): A Case Report Musab S. Hommos, William G. Haynes, Bradley S. Dixon. *Int Med, U Iowa & VAMC, Iowa City, IA.*

Background: The importance of aldosterone (ALDO) and the sympathetic nervous system (SNS) in mediating sodium retention in NS has been questioned.

Methods: A 63 y/o woman, healthy except for hypertension treated with metoprolol and lisinopril, presented with renal salt wasting despite nephrotic range proteinuria (NP) from membranous nephropathy. Serial clinical exams and lab tests were performed. Autonomic function was assessed by respiratory variation in heart rate (RVHR) and by changes in BP and pulse with the valsalva maneuver and orthostasis.

Results: Two months earlier she had acute bronchitis requiring ibuprofen for chest pain. She subsequently developed incapacitating orthostatic hypotension, requiring recurrent admissions for intravenous saline during which NP was discovered. On transfer to us she had no edema and jugular venous pressure was undetectable. She was too dizzy to sit or stand. Her urine protein/creatinine ratio=8.3 gr/gr. Plasma creatinine=1.0 mg/dl and BUN=22 mg/dl. Serum electrolytes were normal. After 2 liters of intravenous saline she felt better. No further saline was administered and a day later she had recurrent orthostatic hypotension and tachycardia (supine BP 134/85, pulse 80/min; standing BP 117/77, pulse 125/min) with a urine sodium of 91 mEq/L. Plasma ALDO was undetectable and renin=0.6 ng/ml/hr. Treatment with florinef and oral salt led to edema, supine BP=160/110 and urine protein/creatinine ratio=25.3 gr/gr and was stopped. Cyclosporine with a burst and taper of prednisone led to complete remission of her proteinuria, which has not recurred. Following remission, she continued to have profound orthostatic hypotension but without tachycardia and persistent hyporeninemic hypoaldosteronism (24 hour urine ALDO=undetectable). Autonomic function testing including RVHR and valsalva confirmed primary autonomic failure (PAF) with severe sympathetic dysfunction and partially preserved parasympathetic function.

Conclusions: This patient developed PAF (possibly from a viral infection) with associated hyporeninemic hypoaldosteronism and renal salt wasting despite the presence of NP from membranous GN. Hence, ALDO and the SNS are required for the renal sodium retention of NS.

Funding: Other NIH Support - National Center for Research Resources, Grant Number UL1RR024979, Veterans Administration Support

PUB211

Complete Remission Is Severe Lupus Nephritis: Assessing the Rate of Loss in Proteinuria Stephen M. Korbet, Edmund J. Lewis, The Collaborative Study Group. *Internal Medicine, Rush University Medical Center, Chicago, IL.*

Background: The prognosis of severe lupus nephritis (SLN) is improved in pts attaining a complete remission (CR). The time to remission ranges from 10-16 mos with many pts not attaining a CR until after 12 mos. We assess whether the rate of loss of proteinuria (UPro) at 3 and 6 mos is predictive of a CR in SLN pts.

Methods: We studied the 86 adult pts in the previously reported trial of plasmapheresis in SLN (NEJM 1992). All pts had ISN/RPS class IV ± class V lesions. All pts received prednisone and oral cyclophosphamide and 40 pts received plasmapheresis. A CR was defined by a serum creatinine (SCr) of ≤ 1.4 mg/dL and UPro (g/d) of ≤0.33. The change in UPro from baseline in g/d per week was determined at 3 and 6 months.

Results: CR was attained in 37 pts (44%) by 16±14 mos. The level of UPro at baseline was similar in CR and No Remission (NR) pts (5.5 vs. 6.4 g/d). CR pts had a lower SCr (1.2 vs. 2.4, P <0.0001). At 3 mos UPro decreased in CR pts by 2.3 g/d but increased by 0.19 g/d in NR pts (P-0.01) and at 6 mos UPro decreased by 4.1 vs. 1.8 g/d (P=0.006). The rate of change of UPro in CR vs. NR pts at 3 mos was (-)0.259 vs. (+)0.033 g/d/wk (P=0.01) and at 6 mos was (-)0.224 vs. (-)0.107 g/d/wk (P=0.01). The time to a CR was ≤12 mos in 19 pts and >12 mos in 18 pts. The baseline SCr was similar but the UPro was lower in pts with CR in ≤12 mos (3.9±2.7 vs. 7.2±3.0 g/d, P =0.001). The proportion of pts with MGN was similar (16% vs. 22%). At 3 mos the UPro decreased by 2.0 vs. 2.6 g/d in CR ≤12 vs. >12 mos pts (P=NS), and at 6 mos the UPro had decreased by 3.4 vs. 4.8 g/d (P=NS). The rate of change in UPro in CR ≤12 vs. >12 mos pts at 3 mos ((-)0.236 vs. (-)0.214 g/d/wk) and 6 mos ((-)0.214 vs. (-)0.235 g/d/wk) was similar (P=NS). Additionally, the rate of change in UPro at 3 and 6 mos was similar within each group.

Conclusions: The rate of change in proteinuria is significantly greater at 3 and 6 months in pts attaining a CR relative to NR pts. In pts attaining a CR at >12 mos the rate of change in UPro was similar to that in pts with CR in ≤12 mos. Thus, the rate of loss of UPro at 3 and 6 mos may help in predicting which pts will attain a CR.

PUB212

Renal Biopsy Patterns in an Urban Immigrant Population Kayode C. Lawrence, Salwa Rhazouani, Saad A. Bhatti, Sudhanshu Jain, George N. Coritsidis. *Nephrology, Elmhurst Hospital Center, Elmhurst, NY.*

Background: The types and incidence of glomerular diseases vary worldwide. Elmhurst and Queens Hospital Centers (QHN) are public hospitals located in Queens, New York, the most ethnically diverse county in the United States. We were interested in reviewing the pattern of glomerular pathology presenting to our centers from various immigrant population..

Methods: 222 patients had kidney biopsies between 2001 and 2010. 74 patients were removed from the final analysis due to incomplete data, 14 patients due to non glomerular pathology.

Data reviewed included sex, age, proteinuria, estimation of glomerular filtration rate (eGFR) at time of biopsy, this was further divided by areas of origin into Caribbean, Subcontinent Asia (India, Pakistan, Bangladesh, Nepal), Far Asia (China, Tibet, Hong Kong, Philippines, Korea, Indonesia) South and Central America.

Statistical analysis was done using analysis of variance

Results: Baseline characteristics was statistically similar in all groups.

Origin	South America	Central America	Caribbean	Subcontinent Asia	Far Asia	Total
Number	45	30	22	22	15	134
*Age	46±2.1	37±2.4	43±2.9	39±2.4	43±4.1	42±1.0
Sex (Male)	58%	37%	41%	55%	63%	51%
*Proteinuria (g/day)	7.9±2.5	6±0.8	8.1±1.7	5.7±0.8	5.4±1.5	6.6±1.0
*eGFR (mdrd ml/min)	66±9.2	66±5.5	64±8.5	67±8.9	43±8.0	61±3.1
* Serum albumin (g/dl)	2.5±0.1	2.8±0.3	2.3±0.2	2.7±0.3	2.4±0.3	2.5±0.1
African ancestry **	8	3	40	0	0	10
Lupus**	33	10	27	14	33	23
IgA**	9	20	9	18	27	16
Membranous**	13	14	27	14	13	16
Minimal change**	2	10	9	9	7	7
FSGS**	13	10	5	23	7	12

*= Mean ± SEM,** percent

Within the immigrant groups lupus continued to be the most common diagnosis, IgA was highest in far asians, while focal segmental glomerulosclerosis (FSGS) was increased in subcontinental asians. Despite 40% African ancestry within the caribbean populace FSGS was only seen in 5%.

Conclusions: Over all expected global trends were found in our population. Increased FSGS in subcontinent Asians and not caribbeans is surprising and suggests possible geographical and environmental influences in glomerular pathology that go beyond genetics.

FSGS incidence was surprisingly high among patients from subcontinent Asia.

PUB213

A Fascinating Case of Vasculitis with Mixed ANA and P-ANCA Seropositivity Sunggeun Lee, Marie-Alex Michel. *Medicine, Woodhull Medical Center, Brooklyn, NY.*

Background: To present a case of RPGN type 3 with SLE features

Methods: The patient's medical chart was reviewed to obtain the clinical course/relevant laboratory/ancillary work-up/pathology/interventions.

Results: A 60 y/o female with no past medical history who initially presented to clinic complaining of multiple joint pain. She has been using NSAIDs. The out-patient work up demonstrated Cr 1.6 ANA (+) 502, anti-SSA (+). ESR 77. normal.complements

UA: 3+ protein, 3+ heme

1 month later, the patient presented to the ER with painless hematuria and was found to have H/H 6.2/ 19.7 BUN 82 Cr 8.9

Renal U/S demonstrated large kidneys,14cm with increased cortical echogenicity

Prednisone 100mg/d and HD were started

Kidney biopsy: 25 out of 45 glomeruli showed complete obliteration with sclerosis and fibrous crescents. No immune deposits. The finding was compatible with pauci-immune GN.

Subsequently P-ANCA was (+) and C3 low

During the hospital course, patient was found to have aortic aneurysm (5.1 cm), NSTEMI , DVT , Pericardial effusion,VRE bacteremia,clostridium difficile, sepsis.

On day 31, she received pulse cytoxan and methylprednisolone.

On day 45, she was off the dialysis for 2 weeks maintaining her Cr around 3.5. HD was restarted after she developed generalized rash, edema and sepsis. Therefore, subsequent pulse cytoxan was held.

Patient had RPGN III with P-ANCA vasculitis featuring medium and large vessel involvement with NSTEMI and thoracic aortic aneurysm. In addition, she had strong clinical features of SLE: polyarthritis, pericarditis, pleuritis, anemia, ANA(+). The kidney biopsy revealed pauci-immune GN with crescents without IC deposits. She originally responded to pulse therapy. Subsequently septic course prevented further immunosupression, she became HD dependent.

Conclusions: P-ANCA RPGN can be present with SLE concurrently. This mixed seropositivity should be further investigated. Multiple cases have been reported in the literatures. Since RPGN progresses rapidly, it is important to be aggressive in diagnosing and treating. Sometimes initial symptom can be non-specific like arthritis or painless hematuria, which is why it is paramount for clinicians to always suspect RPGN in a setting as such.

Funding: Clinical Revenue Support

PUB214

Clinicopathological Findings, Long-Term Mortality and Renal Outcome in Japanese Patients with Idiopathic Focal Segmental Glomerulosclerosis

Taito Miyake,[1] Shinji Kitajima,[1] Yasuyuki Shinozaki,[1] Tadashi Toyama,[1] Akinori Hara,[1] Kiyoki Kitagawa,[1] Kengo Furuichi,[1] Hitoshi Yokoyama,[2] Shuichi Kaneko,[3] Takashi Wada.[1] *[1]Division of Nephrology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; [2]Division of Nephrology, Kanazawa Medical University Hospital, Uchinada, Ishikawa, Japan; [3]Department of Disease Control and Homeostasis, Kanazawa University, Kanazawa, Ishikawa, Japan.*

Background: The 20-years renal survival of idiopathic focal segmental glomerulosclerosis (iFSGS) was reported around 30% in Japan. In this study, we examined clinicopathological findings, long-term mortality and renal outcome of iFSGS.

Methods: Forty seven Japanese patients (34 males and 13 females; mean age 39.0 years, mean time of follow-up was 96.0 months) with biopsy proven iFSGS from 1961 to 2010 in Kanazawa University Hospital were examined in this study. The patients were followed for more than one year, or until renal or patient death. Clinicopathological findings were evaluated for renal and patient death.

Results: Twelve renal death (25.5%, 9 males and 3 females) and 12 patient death (25.5%, 9 males and 3 females) were observed. Age of onset was higher in the group of patient death. The percentage of nephrotic syndrome out of all patients was 95.7%. Thirty out of 45 nephrotic syndrome patients (66.7%) achieved complete remission (CR) or incomplete remission I (ICRI) (proteinurea < 1 g/day) after initial treatment within 6 months, and only one renal death was observed. We treated 46 patients with corticosteroids, and 28 patients with additional immunosuppressive drugs (cyclophosphamide, 36%; cyclosporine, 64%). Sixteen out of 18 patients with additional cyclosporine treatment were achieved CR or ICRI, and only one renal death was observed. Regarding pathological findings, percentage of glomerulosclerosis was higher in group of renal death. The pathologic variants were as follows; Perihilar, 13%; Cellular, 13%; Tip, 36%; and not other specified (NOS), 38%. Five renal deaths were observed in 6 patients with perihilar variant.

Conclusions: The good response of initial treatment within 6 months, and cyclosporine may be favorable prognostic factors.

PUB215

Clinicopathological Study of the Possible Relation between Hepatitis C Virus Infection and Nephrotic Syndrome in Children

Arwa Nada,[1,2] Morris J. Schoeneman,[1] Mona Salem,[3] Dalal Elkaffash,[4] Hanan K. Tawadrous,[1] Mahmoud El-Kersh.[2] *[1]Pediatrics, Division of Nephrology, SUNY Downstate Medical Center, NY; [2]Pediatrics, Division of Nephrology, Alexandria University Children's Hospital, Alexandria, Egypt; [3]Pathology, Alexandria University, Alexandria, Egypt; [4]Clinical Pathology, Alexandria University, Alexandria, Egypt.*

Background: Glomerulonephritis (GN) is one of the major consequences of HCV infection in adults. Neither the course of nephrotic syndrome (NS) in children with HCV infection nor the effect of interferon therapy were studied thoroughly in children.

Objective: To study the possible clinicopathological relation between HCV infection and cases of NS in children who are steroid resistant or dependent

Methods: Sixty children were found to be HCV positive out of 378 who presented with NS between 2004 and 2009 at Alexandria University Children's Hospital, Egypt, 20children were steroid resistant or dependent on high dose corticosteroids. Those twenty patients were subjected to comprehensive biochemical, histological, virological and immunological investigations. RT-PCR to detect HCV RNA was performed in the kidney biopsy. The pattern of steroid responsiveness was studied before and after HCV infection. Interferon and ribavirin therapy was given to 9 children with assessment of proteinuria before and during therapy.

Results: FSGS accounted for 50% of the patients, MPGN accounted for 20%, 5% endocapillary proliferation, 5% minimal change NS, 5% fibrillary glomerulonephritis, 5% mesangioproliferative GN, and 10% inadequate renal biopsies. None of the patients showed clinical or laboratory manifestations of cryoglobulinemia. RT-PCR for HCV RNA was positive in 80% of children. Significant worsening was observed in the pattern of steroid responsiveness after HCV infection (p= 0.001). Significant reduction of proteinuria was observed in the 9 children who received therapy with interferon and ribavirin (p= 0.004).

Conclusions: HCV infection worsens the course of NS in children based on detecting the virus RNA in the renal biopsy, increased steroid requirements after HCV infection and the significant reduction of proteinuria after treatment with interferon and ribavirin.

Funding: Government Support - Non-U.S.

PUB216

Retrospective Analysis of Enteric Coated Mycophenolate Sodium Therapy in Patients with Adult Primary Nephrotic Syndrome Who Relapsed or Were Resistant to Standard Treatment

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Background: Mycophenolic acid is an optional treatment of Adult Primary Nephrotic Syndrome relapsant/resistant to standard therapy with Corticosteroids (Cs) and/or alkylating agents (Al). Enteric coated mycophenolate sodium (ecMPS) is a formulation designed to improve gastrointestinal tolerability.

Methods: A retrospective analysis of nephrotic syndrome patients resistant to standard (st) therapy and treated with ecMPS was performed in Argentina. They were 53 patients, 60% males, aged 44 ± 17 years old. Baseline biopsies: Idiopathic membranous nephropathy IMN I: 2 and IMN II-III: 18, membrano proliferative glomerulo nephritis MPGN: 5, focal segmental glomerulosclerosis FSGS: 12, mesangial glomerular disease 1, and Minimal Changes MCD: 9, IgMN: 4, C1qN: 2. Entry criteria: diagnosis of Nephrotic Syndrome (urinary protein ≥ 3g/24hs and serum albumin ≤ 3g/dl) with BP ≤ 140/90 mmHg (under treatment) after relapse or resistance to st therapy (Cs and/or Al). Patients received ecMPS 1440 mg bid during 12 months. Primary endpoint: percentage of patients achieving complete (urinary protein <0.5 g/day and albumin > 3 g/dl) or partial (urinary protein 0.5-3 g/24hs and albumin > 3g/dl) response. Analysis applied consisted of descriptive statistics

Results: Patient effectiveness results are summarized by response to therapy in the table.

Response N (%)	Albumin g/dl		Creatinine mg/dl		Urinary protein (g/24hs)	
	Baseline	Month 12	Baseline	Month 12	Baseline	Month 12
CR: 22 (42)	2.6±2.2	3.8±0.6	1.1±0.4	1.0±0.0	9.5±7.9	0.1±0.0
PR: 11 (20)	2.6±0.8	3.9±0.3	0.9±0.1	1.3±0.9	8.6±5.1	1.5±0.6
NR: 20 (38)	2.4±1.1	3.3±1.2	1.3±0.7	3.3±2.6	7.4±2.3	7.6±3.0

Responders (complete or partial) included: IMN I: 1/2, IMN II-III: 11/18, MPGN: 5/5, FSGS: 7/12, MCD: 4/9, IgMN: 3/4, C1qN: 2/2. Safety findings included five infections: 4 herpes virus and 1 cellulitis; 2 anemia and 5 mild diarrhea episodes.

Conclusions: One year treatment with ecMPS provided a complete or partial response of 33/53 (62%) in patients relapsant/resistant to prior standard therapy including Cs and or AI.

Funding: Pharmaceutical Company Support

PUB217

Clinicopathological Analysis of Primary Focal Segmental Glomerulosclerosis in 560 Cases

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Background: To investigate the relationship between clinical and pathological features of primary focal segmental glomerulosclerosis (FSGS) .

Methods: The data of 560 patients diagnosed as primary FSGS from January 2002 to December 2010, excluding secondary FSGS by renal biopsies in Shanghai Ruijin Hospital, were reviewed and analyzed retrospectively.

Results: Of the 560 cases of primary FSGS, 331 were males (55.9%) and 247 females (44.1%). The median age of onset was 41 yrs (ranging 16-79 yrs). The median case history was 6 months (range 2 days to 40 years). Proteinuria (97.7%) was the most common clinical manifestations. However, its severity varies from minor or moderate, that is, 1 g/day to 3.5 g/day the most common (41.2%), followed by nephrotic levels, >3.5g/day (27.7%). Primary FSGS is always accompanied by microscopic haematuria (61.4%) and hypertension (31.4%). The percentage of patients in CKD stages 1~3 was approximately 81.9%. Pathologically, the NOS (not other specified) variant was the most frequent type (91%) .The percentage of patients with glomerular sclerosis grade 1~2 and TIL stage 0~2 was 75.7% and 92.1%, respectively. Patients with renal vascular lesions accounted for 58.2%. The results of immunofluorescent study were negative in 68% of the cases while deposits of IgM (19.1%) and C3 (21.3%) component of complement were seen within the segmental scars. The degree of glomerulosclerosis and tubulointerstitial lesion was positively with serum creatinine (P<0.01) . A positive corelation was also found between glomerulosclerosis and tubulointerstitial lesion (P<0.01). Patients with vascular disease suffered much more severe clinical manifestations and renal pathological changes.

Conclusions: We retrospectively analysed the largest data of clinical and pathological features in 560 primary FSGS. The main clinical manifestation of primary FSGS is proteinuria. In addition, haematuria, hypertension and renal dysfunction are common presenting features. Glomerulosclerosis, tubulointerstitial and renal vascular lesions, which contribute to renal insufficiency, are common when the patients are diagnosed as primary FSGS.

Funding: Government Support - Non-U.S.

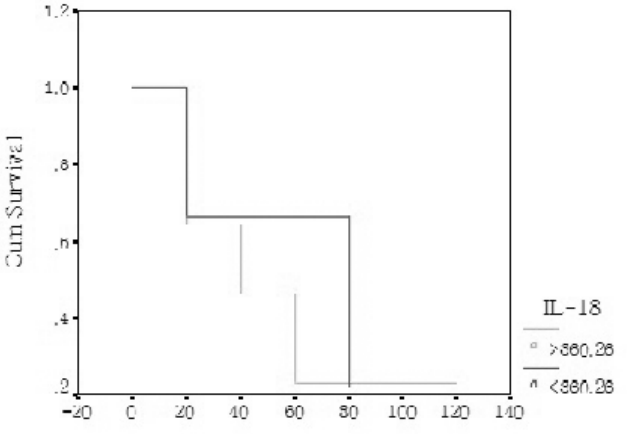
PUB218

Serum Interleukin 18 Levels Are Closely Associated with the Progression of IgA Nephropathy Beili Shi, Minjie Zhou, Liou Cao, Shan Mou, Qin Wang, Ling Wang, Wei Fang, Yucheng Yan, Aiwu Lin, Minfang Zhang, Renhua Lu, Mingli Zhu, Jia Qi Qian. *Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: IgA nephropathy (IgAN) was once thought to be benign but recently found to be slowly progressive. The aim of this research is to investigate the value of serum IL-18, a biomarker for tubule injury, for assessing the disease progression in IgAN patients.

Methods: Serum IL-18 in patients with IgAN and healthy controls were measured by ELISA. Percentage of global glomerular sclerosis (GGS) and extent of tubulointerstitial damage (TID) were semiquantitatively estimated. Deterioration in renal function was defined as a 50% increase of serum creatinine than baseline.

Results: 36 patients (19 females, 37.9±9.7 years) were enrolled and presented with 2.6 (1.7-3.1)g/day proteinuria . Compared with controls, serum IL-18 levels were significantly elevated in IgAN (360.3±25.2 vs 51.2±8.9 pg/ml, P<0.01). Univariate analysis showed that albumin(r=-0.395, P=0.001), proteinuria (r=0.494, P=0.002), Serum creatinie (r=0.61, P<0.001), and eGFR (r=-0.598, P<0.001) were significantly correlated with IL-18 levels. TID scores showed a borderline significance with serum IL-18 levels (r=0.355, P=0.057), whereas GGS did not. During 38 (12-60) months of follow-up, 14 patients (38.89%) had a deteriorated renal function. Spearman correlation analysis showed that baseline IL-18 were correlated with decline of eGFR (r=-0.127, P=0.045). Kaplan-Meier analysis found those who with elevated IL-18 had a poorer renal outcome (P=0.008) and Cox analysis further confirmed that serum IL-18 levels were independent predictor of renal outcome (β=0.984, 95%CI 0.926-1.,042, P<0.001).



Figue 2. IgAN patients with elevated serum IL-18 levels at baseline (>360.26pg/ml) predict renal function deterioration in the follow-up period.

Conclusions: Elevation of serum IL-18 levels may be served as an early biomarker of predicting renal disease progression in patients with IgAN.

PUB219

Intravenous Corticosteroid Pulse Therapy Predicts the Remission of Proteinuria in Patients with Minimal Change Nephrotic Syndrome, along with Serum Creatinine and Age Maki Shinzawa,[1] Ryohei Yamamoto,[1] Yasuyuki Nagasawa,[1] Susumu Oseto,[2] Kakuya Niihata,[3] Yoshitsugu Obi,[1] Megumu Fukunaga,[2] Yoshiharu Tsubakihara,[3] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Internal Medicine, Toyonaka Municipal Hospital, Toyonaka, Osaka, Japan; [3]Nephrology and Hypertension, Osaka General Medical Center, Osaka, Japan.*

Background: Little information is available about predictors of effectiveness of corticosteroid in adult patients with MCNS.

Methods: In the present multicenter retrospective cohort study in 3 major nephrology centers, eligible patients were 75 patients aged ≥15 yr who were diagnosed as new-onset primary MCNS by kidney biopsy between 2000 and 2009. The outcome was time from initiation of corticosteroid to complete remission of proteinuria (CR), defined as urinary protein <0.3g/day, urinary protein/creatinine ratio (UPCR) <0.3, or negative/trace for UP by dipstick. Predictors of CR were identified using log-rank test and multivariate Cox proportional hazard (CPH) models.

Results: Baseline characteristics; age 38 (20-61) yr median (interquartile range), male 45(60%), serum creatinine (Cr) 0.9 (0.7-1.3) mg/dL, UPCR 10.8 (5.8-19.9). No significant difference was observed in baseline characteristics of 26 patients with intravenous methylprednisolone pulse (mPSL) and 49 patients with prednisolone (PSL), except serum albumin (1.7±0.6 vs. 2.0±0.5 g/dL, P=0.015) and UPCR (6.2 (3.5-15.8) vs. 12.9 (8.4-19.9)). During 5.5 (3.0-9.5) yr of observational period, mPSL patients had significantly higher cumulative probability of CR, compared with PSL patients (time to CR, 10 (7-22) vs. 22 (9-44) days, P=0.023 for log-rank test). Use of mPSL (HR 1.93 [95%CI 1.11-3.34], P=0.020) was identified as predictor in multivariate CPH models adjusting for clinically relevant factors, besides age (per 10 yr, 0.84 [0.73-0.96], P=0.012) and log(Cr) (0.22 [0.06-0.73], P=0.013).

Conclusions: The present study, based on one of the largest MCNS cohort in the world, identified intravenous corticosteroid pulse therapy as a predictor of CR in patients with MCNS, along with serum creatinine and age.

PUB220

Treatment of Hepatitis B Associated IgA Nephropathy In O Sun, Yu Ah Hong, Hyun Gyung Kim, Sun Ryoung Choi, Byung Ha Chung, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim, Bumsoon Choi. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: A strong association between IgA nephropathy (IgAN) and hepatitis B surface (HBs) antigenemia has been reported in endemic regions. However, there are few data about treatment of HBV-associated IgAN. We describe our experiences about treatment of IgAN with HBsAg.

Methods: From 1996 to 2010, biopsy-proven IgAN was diagnosed in 498 patients and 22 (4.4%) had HBsAg. We evaluated the clinical feature and therapeutic responses of IgAN with HBsAg.

Results: The patients included 16 men and 6 women, with a mean age of 37 years (range, 18-56). All were seropositive for HBsAg, and hepatitis e antigen (HBeAg) or circulating HBV DNA was detected in nine patients (41%). The mean follow-up duration was 77 months (range, 14-160). Out of 22, two patients (9%) developed end-stage renal disease requiring hemodialysis during follow-up. Comparing the patients (N=9) with HBeAg or circulating HBV DNA and those (N=13) without, there were no significant differences in the changes of estimated GFR, urinary protein excretion and renal survival between two groups during follow-up. Six out of nine patients with HBeAg or HBV DNA were treated with anti-viral agents, but the treatment didn't show any benefits on the clinical outcomes in comparison with patients not treated with. Five patients were treated with steroid. Out of 5 with steroid therapy, 2 had HBeAg or HBV DNA, whereas 3 didn't have. During steroid treatment, no hepatic flare occurred in three patients who didn't have HBeAg or HBV DNA despite no anti-viral therapy. But one patient out of 2 with HBV DNA experienced active viral replication when the patient stopped receiving lamivudine during follow-up. On the other hand, one patient who had maintained entecavir consistently had no hepatic flare.

Conclusions: There were no differences of clinical outcomes between the patients with HBeAg or HBV DNA and those without. However, when the patient with HBeAg or HBV DNA should receive immunosuppressant including steroid, it is necessary to combine anti-viral therapy to prevent viral replication.

PUB221

A Case Report of Chronic Osteomyelitis Causing Secondary Renal Amyloidosis Dhwanil Vyas, George George Sunny, Anuja Vyas. *University of Connecticut.*

Background: Secondary Amyloidosis is usually seen in diseases of chronic inflammation such as Rheumatoid Arthritis, Inflammatory Bowel Disease, Bronchiectasis and Familial Mediterranean Fever. It is rarely reported in patients with chronic osteomyelitis.

Results: A 50 year old Hispanic female was admitted for right lower extremity swelling secondary to cellulitis. Her past medical history includes spinal cord tumor resection at the age of 27 years with residual paraplegia. Over past few years, patient developed a sacral decubitus ulcer and chronic osteomyelitis after unsuccessful surgical interventions. The patient also has Type 2 Diabetes Mellitus without known complications.

Her physical examination was significant for right lower extremity cellulitis and paraplegia.

A urinalysis, obtained as a part of routine studies, showed proteinuria. A 24 hour urine sample had 19 grams of protein. Of note, her serum creatinine was within normal range. Work up including Hepatitis panel, ANA, Anti- dsDNA Ab, complement levels, SPEP and UPEP were negative/within normal level. Fundoscopy did not show diabetic retinopathy.

Renal biopsy was obtained. Light microscopy of glomeruli, tubulo-interstitium and the walls of the blood vessels showed signs of Amyloid deposition with positivity to Congo Red and Thioflavine T stains. Immunohistochemical stain for Amyloid AA was positive. Electron microscopy showed matted deposits of randomly oriented, non-branching fibrils consistent with Amyloid material in the mesangium. Diagnosis of Renal Amyloidosis (AA) was made. In this patient, the triggering factor was chronic osteomyelitis.

Patient's renal function kept worsening as we could not treat her underlying osteomyelitis. Patient was placed on hemodialysis within 2 months after the diagnosis of Secondary Renal Amyloidosis was made.

Conclusions: Outcomes in Secondary Amyloidosis usually depend on the control of the underlying disease. There have been case reports of Secondary Amyloidosis caused by osteomyelitis disappearing clinically and pathologically after successful treatment of osteomyelitis. However, if the underlying disease is not treated, the outcome can be detrimental.

PUB222

Mycophenolate Mofetil Rescue Therapy in Primary Glomerulonephritis with Chronic Renal Injury Suya Wang, Xianping Yu, Jianghua Chen. *Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

Background: The role of mycophenolate mofetil (MMF) in chronic kidney disease (CKD) is not conclusion. MMF may be an effectively therapy for chronic renal injury with active lesions.

Methods: During 2005.2 to 2011.1, 5 patients with CKD-III-IV stage were treated by MMF. Renal biopsy proved the diagnosis were IgA nephropathy (lee IV-Vstage) in 4 cases, and proliferative sclerosis glomerulonephritis in 1 case. The kidney pathological changes in the 5 patients were characterized by glomerular sclerosis by 10.34% to 56.25%, respectively; adhesion of glomeruli and Bowman's capsule in 4 cases; proximal tubular atrophy by 10% to 70%, respectively; 4 patients shown cellular and/or segmental crescent by 6.25%, 6.9%, 17.65%, 25%, respectively, and diffuse mesangial proliferation. The inflammatory cell infiltration in vascular loop cavity were seen in 2 cases; 4 patients presented with tubulitis; multiple foci or diffuse interstitial infiltration of inflammatory cells in all 5 patients, and inflammatory cells in peritubular capillaries cavity were visible in 2 patients. Immunofluorescence IgA and C3 deposited 3-4+ as mass shape in mesangial region in 4 and 5 cases, respectively. According to the renal pathological activity targets, 5 patients were treated with oral MMF (0.75-1.0g/d).

Results: All patients were followed up by 22.7±28.31 (range 4-72) months.The duration of MMF therapy was 6.2± 2.59 (range 3-10) months. The average serum creatinine (Scr) level at renal biopsy was 247.4±62.19 (range 178-322) μmol/L, compared with the last Scr level after treatment (147.6±39.07, range 63-191 μmol/L), P=0.016. The mean eGFR before treatment was 30.17±8.55 (range 23.5-40.3) mL/min/1.73m^2, related to the last eGFR after treatment (53.87±19.36, range 38.98-86.31 mL/min/1.73m^2), P=0.035. The Scr level in 3 patients decreased to normal at 1-3 months after treatment. Scr levels decreased to less than 200 umol /L at 1.5 and 6 months after treatment in the other 2 cases.

Conclusions: This small sample study showed MMF therapy can effectively save kidney function and delay renal failure in primary glomerulonephritis with chronic renal injurry and active lesions.

PUB223

Histopathologic Classification of Glomerular Lesions in ANCA-Associated Glomerulonephritis in Children: A Single Center Experience Adam Weinstein, Swarupa R. Eskapalli, Samuel K. Okoh, Martha L. Graber, Thomas M. Kaneko, Alan R. Schned. *Dartmouth Hitchcock Medical Center, Lebanon, NH.*

Background: There are few reports of outcome of antineutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis (GN) in the pediatric population, in particular regarding the classification schema by Berden et al, which found class an independent predictor of outcome in a cohort of 100 adults.

Methods: We performed a retrospective analysis of all 5 children, age 7 to 13, with ANCA-associated GN at a Northern New England center over the past 10 years. We compared clinical outcomes to Berden's schema that distinguishes between 4 classes of ANCA-associated GN based on the appearance of glomeruli: Focal (F), crescentic (C), mixed (M) and sclerotic (S).

Results: All biopsies were categorized: 3 as class C; 2 as class M; none as class F or S. Biopsies had a mean of 12.4 glomeruli per sample. Patients were followed for 34.4 (7-79) months. 3 of 5 patients were female. All patients received cyclophosphamide and corticosteroids for initial therapy; none received apheresis. Estimated GFR (eGFR) at entry for the class C patients was 21, 24, and 30 mL/min/1.73m^2. These patients responded to treatment with eGFR's of 90, 107, and 115 mL/min/1.73m^2 at most recent follow-up ($p<0.001$). The 2 class M patients presented with eGFRs of 79 and 80. These have decreased to 47 and 66 mL/min/1.73m^2 at last follow-up. None have reached ESRD. One class C patient relapsed after 2 years follow-up. She required acute dialysis, but died from complications of immunosuppression.

Conclusions: We describe a 10 year single center experience of ANCA-associated GN in 5 pediatric patients. Though this is a small sample size, our patients responded as predicted by Berden et al's study and classification scheme. Further, our patients responded similarly to other pediatric cohorts, demonstrating a better prognosis compared to published reports in adults. We conclude that the proposed schema should be studied further in the pediatric population to gauge whether it will be a reliable tool in these patients. Due to small numbers of patients, multicenter collaboration will likely be needed.

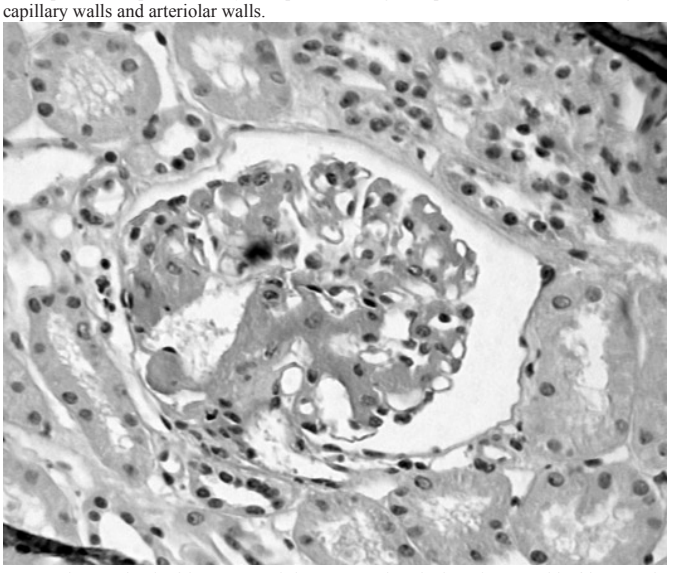
PUB224

AH-Amyloidosis Related to Franklin Disease Elena Zakharova,[1] Ekaterina Stolyarevich.[2] *[1]Nephrology, Clinical City Hospital n.a. S.P. Botkin, Moscow, Russian Federation; [2]City Nephrology Center, Moscow, Russian Federation.*

Background: Firstly described in 1990, AH-amyloidosis is exceptional condition, associated with plasma cell/B-cell lymphoproliferative disorders, with only few cases derived from heavy chains μ and γ thus far reported

Methods: 45 y.o. Caucasian female presented with weakness, night sweats, mild anemia (Hb 10.4 g/dl), proteinuria (1.2 g/day) and splenomegaly. We performed kidney biopsy, serum and urine protein electrophoresis, immunoelectrophoresis, immunofixation electrophoresis, and bone marrow biopsy with immunohistochemical examination

Results: Kidney biopsy revealed no proliferative changes, and showed deposition of eosinophilic, amorphous, weakly PAS-positive, Congo red-positive material in mesangium, capillary walls and arteriolar walls.



Sections stained with Congo red demonstrated typical apple-green birefringence in polarized light. Immunofluorescent study of infixed frozen tissue for IgA, IgM, IgG, C3, C1q, κ and λ proved to be positive only for IgG in mesangial zones, immunohistochemistry on formalin fixed/paraffin embedded tissue with immunoperoxidase staining for amyloid A-protein was negative. Immunochemical study showed presence of paraprotein heavy chain γ both in serum and urine, profound secondary immunodeficiency and glomerular proteinuria. Bone marrow smear found elevated lymphocyte count up to 32%, bone marrow biopsy revealed clusters of CD20+ B-lymphoid cells, and a great number of CD38+, CD138+ plasma cells

Conclusions: Based on clinical presentation with weakness, night sweats, anemia, splenomegaly, and workup showing lymphoplasmacytic infiltration of bone marrow, presence of paraprotein heavy chains γ, secondary immunodeficiency and kidney amyloidosis with positive immunofluorescence for IgG, the patient was diagnosed with Heavy chain disease γ (Franklin disease) with AH-amyloidosis and treated with rituxima b+bortezomib+dexametasone

PUB225

The Safety and Efficacy of Percutaneous Renal Biopsy by Physcian-in-Training in an Acadamic Teaching Setting Bereket Alemu,[1] Mehrdad Hamrahian,[1] Albert W. Dreisbach,[1] Eva Csongradi,[2] Luis A. Juncos,[1,2] Tibor Fulop.[1] *[1]Department of Medicine, University of Mississippi Medical Center, Jackson, MS; [2]Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS.*

Background: The safety and efficacy of percutaneous renal biopsy (PKB) is relatively little studied in a purely training setting. We performed a retrospective cohort review of our consecutive 3-year renal biopsy experience (01/2007 - 12/2009) at the University of Mississippi Renal Fellowship Program.

Methods: We collected data on numerous baseline and procedure related variables, as well as complication rates. All biopsies were performed exclusively by Renal Fellows under real-time ultrasound (US) visualization within a framework of structured US-PKB training course. Data was analyzed with PAWS Statistics 18 and the results expressed as either percents or means with standard deviation (±SD).

Results: 70 patients underwent PKB during the index period, 50 (71.4%) of native kidneys. Age was 40.4 (±13.7) years, blood pressures 140/84.7 (±20.8/14.6) mmHg and biopsied kidneys measured 11.7 (±1.6) cm. Serum creatinine was 3.16 (±3.09) mg/dL, random urine protein/creatinine ratio 5.22 (±7.16) and urine sediments showed 12.9 (±24.8) RBC and 10.7 (±20.7) WBC/high-power fields. Specimens appeared "Sufficient" in 64 (91.4%), "Borderline" 4 (5.7%) at bedside inspection. We recovered 18.6 (±11.4) glomeruli per procedure (range: 0-72; median 17.5); 2 biopsies (2.8%) remained unsuccessful. There was a very close correlation between preceding history and recovered diagnoses of diabetic changes (r 0.580; $p=1.74\ 10^{-7}$) and lupus nephritis (r 0.847; $p=5.04\ 10^{-20}$). Only 3 specimens returned with "No diagnostic changes". Initial Hemoglobin of 10.7 (±1.8) g/dL dropped by 0.5 (±0.73) after PKB (NS). Three (4.3%) patients needed transfusion; 1 patient experienced persistent urine leakage, but no one needed surgical or radiological intervention or died.

Conclusions: In the setting of well-structured training environment, US guided PKB performed by relatively inexperienced operators in-training is safe and an essential component of fellowship training.

Funding: Clinical Revenue Support

PUB226

Prenatal Ultrasound Prediction of Postnatal Renal Outcome in Fetuses with Renal Abnormalities Gianluigi Ardissino,[1] Simona Boito,[2] Elisa Dusi,[3] Francesca Tel,[1] Sara Testa,[1] Stefano Ghirardello,[3] Fabio Mosca.[3] *[1]Pediatric Nephrology Unit, Fondazione Ca' Granda Osp Maggiore Policlinico, Milano, Italy; [2]Department of Obstetrics and Gynecology 'L. Mangiagalli', Fondazione Ca' Granda Osp Maggiore Policlinico, Milano, Italy; [3]Neonatal Intensive Care Unit, Fondazione Ca' Granda Osp Maggiore Policlinico, Milano, Italy.*

Background: To examine the predictive role on renal function at 6 mos of age, of amniotic fluid (quantitative) and renal echogenicity in fetuses with bilateral renal abnormalities.

Methods: We searched our database and identified all cases of fetal renal abnormalities, normal karyotype and no evidence of other structural defects on ultrasound for which data on post natal renal function were available. Post-natal renal function was classified into 3 severity categories: a. CKD1 b. CKD2-4, c. CKD5.

Results: We identified 29 cases of isolated renal abnormalities in fetuses subsequently born at term of pregnancy (ge] 38 weeks), which were divided into two groups. The first group included 12 cases of obstructive uropathy with bilateral moderate to severe hydronephrosis. The second group included fetuses with PKD (N: 6), ranal dysplasia (N:11). The mean gestational age at the time of the scan was 26.5 (range 14-36) weeks. In all fetuses with obstructive uropathy both amniotic fluid and renal echogenicity were normal and had a normal renal function (CKD1) postnatally. In the second group, 8 patients (47%) were classified as CKD1: 7 (88%) had normal amniotic fluid and 6 cases were associated with hyperechogenic kidneys. Five patients (29%) were classified as CKD2-4; all were characterized by oligo (4) or anhydramnios (1) and hyperechogenic kidneys. In those pts requiring dialysis (CKD5), 3 out of 4 had anhydramnios and the remaining one oligohydramnios. In all of these cases the kidneys were hyperechogenic.

Conclusions: Amniotic fluid production is the main predictive indicator of post-natal renal outcome. The finding of hyperechogenic kidneys with normal amniotic fluid is associated with a normal postnatal renal function at 6 mos of age.

PUB227

Safety and Efficiency of Native Kidney Percutaneous Biopsy Stanislas Bataille,[1] Laurent Daniel,[2] Stephane Burtey.[1] *[1]Nephrology, Hôpital Conception, Marseille, France; [2]Anatomopathology, Hôpital Timone, Marseille, France.*

Background: Renal biopsy (RB) procedures vary among nephrology units. We compared efficacy and complications of different native kidney percutaneous RB procedures.

Methods: RB data from 5 nephrology units (C1 to C5) performed between 2006 and 2010 were recorded: age, gender, number of glomeruli on light microscopy (LM) and immunofluorescence (IF) and serious adverse event.

Results: Before RB, all centers required platelet count, coagulation test and clotting time except in C3 which required no clotting time and C4 which required no platelet count and no clotting time. C1 performed US assessment before RB, C2 and C4 real-time US guidance, C3 and C5 tomodensitometry guidance. C1, C2 and C3 used 14 gauge core cutting needles, C4 and C5 16 gauge.

Data from 943 RB were collected (C1:408; C2:254; C3:81; C4:136; C5:64). Number of failed biopsies, was 48/943(5%) on LM. It was higher in C1 and C3 than in other units ($p<0.001$). Number of failed biopsies on IF was 99/943(10%). It was higher in C3 than in other nephrology units ($p<0.001$). On LM, mean number of glomeruli was 14.2+/-8.6(mean +/-SD). It was higher in C1 and C2 than in C3, C4 and C5 ($p=0.01$). On IF, mean number of glomeruli was 4.4 +/-3.3. It was higher in C1 and C2 than in C3, C4 and C5 ($p<0.001$). In multivariate analysis, the only factor that influenced number of glomeruli was the nephrology unit ($p=0.004$). Complications occurred in 14/943(1.5%) of biopsies with no difference between nephrology units.

Conclusions: Number of failed RB was higher in C1 and C3. In these two units, failed RB are mainly due to the absence of renal core. The absence of kidney sample is certainly due to an imprecise kidney location. Thus, practices could be improved, especially in C1 where no real-time radiological guidance is performed. In C1 and C2, mean number of glomeruli were higher than in other units. These two units which have the higher number of glomeruli are the two units who perform the more RB: experience may be important for efficiency. Technical procedure does not affect the rate of serious complications.

RB is safe. Experience, radiological guidance or core-cutting needle-size could influence quality of RB.

PUB228

Free Light Chains in Urine – An Additional Diagnostic Tool To Detect Cast Nephropathy? Raoul Bergner, Nikolaos Karapanagiotidis, Jochen Breuer, Michael J. Uppenkamp, Martin Hoffmann. *Medizinische Klinik A, Klinikum Ludwigshafen, Ludwigshafen, Germany.*

Background: The examination of free light chains (FLC) in serum is a very useful diagnostic tool in patients with monoclonal light chain (MLC) disease. About 30% of patients with multiple myeloma (MM) have renal involvement at the time of diagnosis increasing to 50% during the course of disease. But different kinds of renal involvement have different prognostic values. Cast nephropathy (CN) usually progresses very rapidly to end stage renal disease. Currently the type of renal involvement can be diagnosed correctly only by kidney biopsy. We investigated if the analysis of FLC in urine may be helpful to predict the type of renal involvement.

Methods: We analysed the excretion of FLC in urine in patients with MLC associated disease who underwent kidney biopsy because of unclear proteinuria or renal insufficiency. Patients were grouped according their histological findings: 1: CN, 2: light chain deposit disease (LCDD), 3: AL amyloidosis (ALA), 4: other renal disease (ORD). Urine FLC was determined by immune electrophoresis and immunoessay.

Results: Kidney biopsies of 94 patients with MM (n=52), B-cell Non Hodgkin lymphoma (B-NHL n=5), AL-amyloidosis (ALA n=15) and monoclonal gammopathy of undeterminated significance (MGUS n=22) were available. The findings in kidney biopsy were CN n=27, LCDD n=6, ALA n=19 and ORD n=42.

If the critical FLC concentration for detecting a CN was defined with >50mg/dl, the sensitivity was 85%, the specificity 79%. With FLC concentration >75mg/dl the sensitivity was 78% the specificity was 85%, respectively. Adjusted to the renal function (FLC x 1/GFR) the sensitivity and specificity was 93% and 87% for a quotient > 2 and 81% and 94% for a quotient >5, respectively.

Conclusions: This data demonstrate that the examination of FLC in urine is helpful to find patients with CN. Patients with LCDD or ALA had significant lower FLC concentrations in urine and better renal function at the time of diagnosis. This might be important in patients who are not eligible for kidney biopsy. Because CN decreases very often rapidly with renal function the correct diagnosis of renal involvement is important for treatment decisions.

PUB229

Renal Function in Myeloma Patients Treated with Ibandronate Raoul Bergner, Jochen Breuer, Nikolaos Karapanagiotidis, Michael J. Uppenkamp, Martin Hoffmann. *Medizinische Klinik A, Klinikum Ludwigshafen, Ludwigshafen, Germany.*

Background: Treatment of myeloma bone disease (MBD) with bisphosphonates (BP) is standard of care. However, renal toxicity due to BP treatment tends to be a major problem in myeloma patients (MP), especially. At highest risk are patients with reduced renal function (RF) and patients who had switched the BP. Toxicity appears typically after few month of BP treatment.

We evaluated the renal safety of ibandronate (IBD) in MP, who were treated with IBD for MBD. The patients were stratified according to pretreatment and kidney function.

Methods: In a prospective noninterventional study (NIS) about safety and efficacy of IBD in breast cancer patients it turned out that MP were also included unintentionally. Out of 3540 documented patients 105 MP were identified. The data from these MP were evaluated separately.

The patients were subdivided in 4 groups according to their RF: GFR >90 (1), 60–90 (2), 30–59 (3) and <30 (4) ml/min. Since pretreatment with other BP could be of influence, patients were analysed according to former pretreatment: no BP, IBD, and other BP. The RF was calculated every month over a period of six month by the MDRD-formula.

Results: 105 patients were available for evaluation. In 99 patients RF was documented over a minimum of 5 months. The initial RF was (1) n=14, (2) n=36, (3) n=38 and (4) n=17, respectively. At baseline there were no differences in RF according to their pretreatment. The IBD dosage was 6 mg in 90% of all infusions, 4 mg in 3,6%, 3 mg in 2% and 2 mg in 4.4%, respectively. The GFR was stable over time in the groups 1-3 and improved significantly in group 4: GFR +15.6 ml/min [95%CI 1.0–32.9];

In 9 patients the treatment was terminated prematurely, due to disease progression (n=3), death due to myeloma during study period (n=3). Two patients were lost by follow up and one patient declined further treatment.

Conclusions: The data of this NIS demonstrate, that there is no evidence for renal toxicity of IBD in MP in all stages of RF. Quite in contrary to previous observations with other BP patients with worse kidney function (stage 4) had a significant improvement of GFR over the study period.

Funding: Pharmaceutical Company Support

PUB230

The Role of a CT Urogram in a Stone Clinic Farrukh M. Koraishy,[1] Thuy-Trang T. Ngo,[2] Gary M. Israel,[3] Neera K. Dahl.[1] *[1]Internal Medicine, Yale University School of Medicine, New Haven, CT; [2]Internal Medicine, Norwalk Hospital, Norwalk, CT; [3]Diagnostic Radiology, Yale University School of Medicine, New Haven, CT.*

Background: Medullary sponge kidney (MSK) is traditionally diagnosed by intravenous pyelography. When Computed Tomography Urography (CTU) is combined with three-dimensional post-processing using MultiDetector-Row Computed Tomography (MDCT), the diagnosis of MSK can be made with high accuracy.

Methods: Patients with multiple bilateral stones or with a finding of medullary calcinosis were referred for a CTU in addition to standard laboratory testing, and a 24 hour urine collection for evaluation of metabolic risk factors for recurrent stones. A retrospective analysis of the first 16 of these patients is presented. Patients were seen between January 2008 and March 2011 for initial evaluation of stones. Characteristic findings of MSK on CTU included dilatation of the collecting tubules manifested as a papillary "blush", "paintbrush" or "bouquet of flowers" pattern. In addition, medullary calcinosis and medullary cysts were also evaluated. This information was assessed in light of demographic and laboratory data of the patients.

Results: Four patients were diagnosed with MSK based on the characteristic radiologic features seen on the CTU. All 4 of these patients had features of medullary calcinosis, while only three had medullary cysts. Three of the four patients also had features suggestive of distal RTA. Six patients had medullary calcinosis without MSK. 3 of these 6 patients (without MSK) also had features of distal RTA. 2 of 8 patients with features of distal RTA did not have medullary calcinosis or MSK.

Conclusions: CT Urography effectively demonstrates characteristic radiologic findings of MSK and helps distinguish between medullary calcinosis and MSK. Medullary cysts were not seen in all cases of MSK. Most but not all cases of distal RTA were associated with medullary calcinosis.

PUB231

Kidney Cysts in a Patient with Systemic Toxicity from D-Penicillamine Farrukh M. Koraishy,[1] Gary M. Israel,[2] Neera K. Dahl.[1] *[1]Internal Medicine, Yale University, New Haven, CT; [2]Diagnostic Radiology, Yale University, New Haven, CT.*

Background: D- Penicillamine (PCA) has been used for treatment of cystinuria due to its cysteine binding properties. PCA has also been known to cause impaired collagen deposition and dysfunction in the elastic fibers resulting numerous systemic toxicities. Nephrotoxicity reported with PCA is typically a membranous glomerulonephritis. We describe a patient who developed large bilateral kidney cysts during long-term treatment with PCA for cystinuria.

The patient was treated effectively for cystinuria for over 20 years with a PCA dose of 2.0 grams per day His renal function remained normal and he had no proteinuria. In 1999, an Intravenous pyelogram (IVP) with tomography showed normal kidney sizes and no cysts were noted. In 2003, the patient was admitted progressive dyspnea. A CT of the chest and abdomen revealed new, multiple, bilateral cysts in the kidneys. The largest cyst on the right was 11.9 x 8.8 cm, the largest on the left was 8.3 x 5.8 cm. Both kidneys were between 9-10 cm in size. Patient also had a rise in his serum creatinine. PCA was discontinued in 2005 when the patient developed systemic toxicities of PCA including loosening of the skin, keratoconus and popliteal aneurysms. A skin biopsy diagnosed cutix laxa. Other systemic toxicities included cardiomyopathy, restrictive lung disease and thickening of the walls of the esophageous and duodenum. During these 2 years, the size of his kidney cysts had continued to progress and his renal function also worsened. He also developed proteinuria that ranged between 0.5 to 1.0 grams per day.

Since the discontinuation of PCA, the size of his kidney cysts and his renal function has been stable. Other systemic toxicities have also remained stable.

Conclusions: To our knowledge, this is the first report of kidney cysts acquired during PCA therapy. The etiology of cyst formation due to PCA is unknown. The possible mechanisms include the alteration in the connective and elastic fibers of the kidney and the malfunction of the extra-cellular matrix and epithelial cell interactions.

Cystic renal disease should be evaluated in patients treated with PCA.

PUB232

Estimating the Range of Kidney Length in Adults with Normal GFR by Automatically Extracting Data from Dictated Ultrasound Reports Brian J. Lee,[1] Ekamol Tantisattamo.[2] *[1]Nephrology, Kaiser Moanalua; [2]Medicine, University of Hawaii.*

Background: Abnormal kidney size can reflect renal disease. However, normal kidney size is not well-characterized, particularly in the mixed ethnic population in Hawaii. Maximum ultrasound kidney length is the most practical way to estimate kidney size. Ultrasound dictations are unstructured freetext having data that must be extracted manually, and this limits the number of data points available. To analyze the largest possible sample, we create a tool to automatically extract kidney lengths from the ultrasound reports.

Methods: Freetext ultrasound reports were extracted by using natural language processing principles. Programs to download and parse the text and extract data were written in SAS and Excel/VBA to extract the longest kidney length from 45,020 renal and abdominal ultrasound reports from 2002-2010. The top 1% of outliers were manually reviewed. To validate the data extracted from the program, we compared computer-extracted maximum kidney lengths from 500 reports to two independent physicians extracting the same data manually. To determine which ultrasounds were eligible to represent "normal" kidneys, we excluded patients 18 years old, diabetes, HIV, polycystic kidneys, eGFR ≥60 ml/minute/1.73 m^2, kidney or other organ transplant, and abnormal echogenic kidneys.

Results: The computer-extracted data was validated, with no significant difference in human vs computer error rate. Of the 45,020 dictated reports, 4,493 qualified for this study. The maximum kidney length was 11.62 cm (SD 1.09) and 11.18 cm (SD 1.11) in male and female adults, respectively. The kidney length peaked in the 30-39 age group and decreased with increased age. Kidney length was positively correlated with both height and weight.

Conclusions: We created a novel technique to collect and analyze a massive amount of data and characterize the normal range of kidney length within Hawaii population. The maximum kidney length depends on age, gender, height, and weight. Ultrasound kidney length can be used as a diagnostic tool for early detection of renal disease. We plan to examine the use of kidney length as a predictor of risk of progressive loss of kidney function in a future study.

PUB233

Urine Neutrophil Gelatinase-Associated Lipocalin in Early Diagnosis of Urinary Tract Infection in Children Inseok Lim, Do Soo Kim, Hakyoung Kim. *Pediatrics, Chung-Ang University Hospital, Seoul, Korea.*

Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein mainly found in activated neutrophils and renal tubular cells. The expression of NGAL can rise 1000-fold in response to renal tubular injury, so that it has been proposed as an early biomarker of acute kidney injury. But, there are a few studies for NGAL level in urinary tract infection (UTI) so far. And result of urine culture that is the most accurate method for diagnosing UTIs may not be available for 2-3days, so a new technique that is swift and as least as accurate would be valuable. The present study assessed whether urine and serum NGAL levels could be an early biomarker of UTI.

Methods: Fifty febrile UTI patients and a control group of 50 febrile non-UTI patients were enrolled. Serum and urine NGALs were quantified using the BioPorto® NGAL enzyme-linked immunosorbent assay within 48 hours after fever onset in both patient groups.

Results: Mean urine NGAL level was significantly higher in UTI patients than in control (49.06 ng/ml vs 12.30 ng/ml, p=0.001). But, there was no significant difference of mean serum NGAL level in the UTI and control groups (116.29 ng/ml vs 101.90 ng/ml, p=0.651). Using 10.4 ng/ml of urine NGAL as the cutoff -value for diagnosis of UTI, sensitivity was 95.8% and specificity was 66.7%.

Conclusions: The results indicate that urine NGAL may be useful for additive marker to urinalysis in the early diagnosis in UTI in children.

PUB234

Baclofen Toxicity in Renal Failure: An Underappreciated Occurrence Julie Ann T. Linatoc, Domenic A. Sica, Daniel E. Carl. *Nephrology, Virginia Commonwealth University, Richmond, VA.*

Background: Baclofen (BA) is used for the treatment of musculoskeletal spasticity and occasionally for hiccups. It is mainly eliminated by renal mechanisms and its half life of 4 to 7 hours in normal subjects, extends to 3 to 4 times higher in patient with advanced chronic kidney disease (CKD). This pattern of drug accumulation puts patients with impaired renal function particularly prone to BA toxicity.

Methods: We report 6 patients with impaired renal function and BA-related toxicity seen at our institution from Oct 2010 to Feb 2011.

Results: These 6 cases all had altered mental status (AMS) as the major presenting symptom following BA administration. In all cases, either conventional hemodialysis or continuous renal replacement therapy (RRT) were used to accelerate BA clearance and where obtained blood levels dropped proportionately. Central nervous system (CNS) symptoms resolved in all patients following initiation of RRT. The time to resolution of BA-related symptoms varied substantially reflective of the known CNS compartmentalization of BA.

Characteristics of 6 Patients with Baclofen Toxicity

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age (y)/Sex	56/F	49/M	62/M	66/M	52/F	66/M
Renal Function	CKD 4(eGFR 20-25)	AKI	AKI (2 days prior giving BA)	AKI, on HD	ESRD on PD	CKD 3(eGFR 30-35)
Reason for Use	low back pain	spasticity	spasticity	hiccups	spasticity	hiccups
BA dose (mg), cumulative	25	90	10	30	50	10
Duration of use (days)	1	3	1	1	1.5	< 1
Symptoms	coma, respiratory distress	coma, respiratory distress (1 day after onset of AKI)	AMS	AMS, hypotension	AMS	AMS
Intervention	4 hr HD	4 hr HD x 2 days	supportive	CRRT x 24 hr, then switched to HD	4hr HD x 3 days	supportive
Recovery Time	4 hr	48 hr	2.5 days	36 hr	3 days	2 days

Abbreviations: AKI, acute kidney injury; ESRD, end-stage renal disease; HD, hemodialysis; PD, peritoneal dialysis; eGFR, estimated glomerular filtration rate

Conclusions: These cases illustrate the poor knowledge of this drug's pharmacokinetics and its concentration-dependent toxic profile in patients with renal failure. Our institution has now inserted precautionary statements into the usage criteria for BA and a ban on its use at an eGFR below 15 mL/min is under consideration.

PUB235

Awareness of Chronic Kidney Disease and Association with Proteinuria in Japanese General Population Yohei Maeshima,[1] Norikazu Hinamoto,[1] Daisuke Saito,[1] Hiroko Yamasaki,[1] Hiroyuki Watatani,[1] Haruyo Ujike,[1] Masashi Kitagawa,[1] Hitoshi Sugiyama,[1] Yoshinari Yasuda,[2] Tadao Akizawa,[3] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ. Med. School, Okayama, Japan; [2]Medicine, Univ. of Nagoya, Nagoya, Aichi, Japan; [3]Medicine, Univ. of Showa, Tokyo, Japan.*

Background: In spite of high prevalence of CKD in Japan, majority of CKD patients may not be aware of CKD due to the lack of subjective symptoms, thus leading to the increasing number of patients on dialysis therapy. The aim of the current study is to survey the awareness of CKD in the Japanese general population and to examine association with proteinuria.

Methods: Questionnaire form and urinary dipstick check kit was distributed to participants in the annual meeting of International Rotary 2690th district held in 2010. The questionnaire form of Research program of Ministry of Health, Labour and Welfare was used. The questionnaire included questions on awareness of CKD, self-reported renal function, knowledge questions on diagnosis, risk factors, clinical symptoms and effective life-style modification of CKD, and the degree of proteinuria using urinary check kit.

Results: Of 1238 participants, 505 general population excluding medical professionals were studied. The mean age was 60 years and 95% were male. The awareness of CKD was 30.9%, and complication of hypertension, diabetes mellitus or dyslipidemia (33%) did not affect CKD awareness. In regard to CKD diagnosis, proteinuria (61%) was recognized sufficiently, but eGFR (14%) was poorly recognized. In regard to the symptoms of CKD, hypertension (73%) was recognized sufficiently, but renal anemia (49%) and mineral bone disorder (38%) were poorly recognized. Mild (34.3%) and heavy proteinuria (1.7%) was observed. The degree of actual life-style modification significantly correlated with proteinuria (r=-0.11, P=0.016) and self-reported renal function (r=0.25, P<0.01).

Conclusions: These results suggest that awareness of CKD remains low in Japan, and efforts to improve knowledge of CKD and to promote life-style modification may play important roles in delaying the progression of CKD.

Funding: Government Support - Non-U.S.

PUB236

Urine Protein-to-Creatinine Ratio Does Not Correlate with 24-H Urine Total Protein Excretion in Nephrotic Proteinuria Nuria Montero,[1] Maria Jose Soler,[1] Maria Jose Pascual,[1] Clara Barrios,[1] Eva Marquez,[1] Eva Rodriguez,[1] Maria Antonia Orfila,[1] Luis Coca,[2] Julio Pascual.[1] *[1]Nephrology, Hospital del Mar., Barcelona, Spain; [2]Laboratori de Referencia de Catalunya.*

Background: Measurement of protein content of a timed 24h urine collection is the definitive method for establishing presence of abnormal proteinuria, however, urine collection is cumbersome. Spot urine protein to creatinine ratio seems to be a reliable diagnostic tool for urine protein measurement. Our aim is to evaluate spot urine protein-to-creatinine ratio compared to 24h urine total protein excretion in different proteinuria ranges.

Methods: Observational, cross-sectional study of 159 consecutive paired determinations of 24h urine total protein excretion and spot urine protein-to-creatinine ratio in renal patients. The strength of the correlation was determined by calculating intraclass correlation coefficient(ICC) and Spearman correlation coefficient(SCC).

Results: Among all groups, ICC was 0.756(CI 95% 0.680-0.816) and SCC was r=0.91(p<0.05). There is an excellent significant correlation between the spot urine protein to creatinine ratio and 24h urine total protein excretion when proteinuria was<3500mg/24h. This correlation decreased when it was<300mg.

TABLE: Intraclass correlation coefficient between spot urine protein to creatinine ratio and 24-h urine total protein excretion

Proteinuria 24h (mg)	< 300	300-3499	≥3500
Number	60	77	22
Intraclass correlation coefficient (Confidence interval 95%)	0.456 (0.230-0.635)	0.656 (0.508-0.766)	0.340 (-0.041-0.650)
Spearman correlation coefficient (r)	0.498	0.828	0.181
p	<0.001	<0.001	0.420

When patients were stratified according to eGFR, the correlations between spot urine protein-to-creatinine ratio and 24h urine total protein excretion were similar between groups.

Conclusions: In summary, a strong correlation is observed between spot urine protein to creatinine ratio and 24h urine total protein excretion when the level of proteinuria is <3500mg/day. In our experience, there is no relevant correlation between spot urine protein to creatinine ratio and 24h urine total protein excretion in nephrotic-range proteinuria. Further studies with larger sample sizes are needed to confirm these results.

PUB237

Impact of Reduced Glomerular Filtration Rate on Japanese Acute Stroke Naoki Nakagawa. *Asahikawa Medical University, Japan.*

Background: Patients with chronic kidney disease (CKD) are at high risk of stroke and other cardiovascular diseases, and recent guidelines for the management of stroke stress the importance of managing such patients. However, the characteristics of the subtypes of stroke that occur in patients with CKD remains to be determined. The present study investigates stroke subtypes in patients with or without CKD using the stroke database at our hospital.

Methods: We analyzed data from 470 (male, 251; mean age, 70.6 y) patients admitted to our hospital with stroke between 2006 and 2010. Relationships between the type of stroke and estimated glomerular filtration rate (eGFR) for Japanese, age, sex, hemoglobin and the presence or absence of various risk factors for arteriosclerosis were investigated.

Results: The type of stroke was transient ischemic attack in 17 patients (4%), lacunar infarction in 40 (9%), atherothrombosis in 30 (6%), cerebral embolism in 129 (27%), unclassified cerebral infarction in 90 (19%), subarachnoid hemorrhage in 41 (9%), and cerebral hemorrhage in 104 (22%). Among the 470 patients, 140 (30%) had CKD with eGFR < 60 mL/min/1.73 m^2. Compared with a group without CKD (mean eGFR 85 mL/min/1.73 m^2), the rate of complications with all risk factors was higher in the CKD group (mean eGFR 46 mL/min/1.73 m^2) with more advanced age (76 vs. 68 years, P < 0.01), atrial fibrillation (31% vs. 15%, P < 0.01) and a history of cardiovascular disease (38% vs. 19%, P < 0.01) being the most prevalent. The prevalence of hypertension tended to be higher in the group with CKD, but the difference did not reach significance (58% vs. 53%). A comparison of stroke subtypes revealed a significantly higher incidence of cardiogenic cerebral embolism (34% vs. 25%, P < 0.05) and a significantly lower incidence of subarachnoid hemorrhage (3.6% vs. 11%, P < 0.01) in the group with, than without CKD.

Conclusions: About 30% of patients with stroke admitted to our hospital had CKD. Those with CKD were older, had a higher prevalence of atrial fibrillation and a significantly higher rate of cardiogenic cerebral embolism. Thus, strict control of blood pressure and prevent the onset of atrial fibrillation should be important to prevent stroke among patients with CKD.

PUB238

Evaluation of Rectus Sheath Hematoma Risk Factors Heena S. Sheth, Hoda Kaldas. *Medicine, UPMC, Pittsburgh, PA.*

Background: The following risk factors for rectus sheath hematoma (RSH) are known: systemic or prophylactic anticoagulation, trauma, abdominal surgery or injections, cough, old age, female gender and pregnancy. Platelet dysfunction associated with kidney disease, administration of antiplatelet agents, steroids, or immunosuppressants may increase the risk of bleeding complications such as RSH. RSH may increase morbidity/mortality, length of stay and readmissions.

Purpose: To evaluate the risk factors for RSH such as Chronic Kidney Disease, antiplatelet therapy, steroids and immunosuppresants.

Methods: The patients who developed RSH during 2006-6/2009 were identified from the CT reports from UPMC MARS data system. Patient charts were reviewed for demographics and risk factors. Descriptive statistics are reported for the risk factors.

Results: The MARS query identified 114RSH CT reports. 12 patients were ineligible : NO RSH (10) and inadequate documentation (2). The 102 patients evaluated, demographics were: 60.8% females, 10.8% AA, mean age 62.6 years (SD 16.9, range18.9-87.6). 6 patients (5.8%) were underweight and 35 (34.3%) obese. 23 (22.5%) were admitted for RSH management. 24 (23.5%) patients had not received any anticoagulation. 60 (58.8%) were on chronic anticoagulation or received anticoagulant treatment in the hospital, 18 (17.7 %) received prophylactic anticoagulant doses. The presenting symptoms were pain, drop in hematocrit, hypotension, or incidentally diagnosed on CT. Among the 42 patients administered prophylactic doses or no anticoagulants; 11 (26%) had CKD stage 4/ 5, 28 (66.6%) were on immunosuppressant, steroids or antiplatelet therapy and 23 (54.7%) had abdominal surgery. RSH treatment was conservative [pain management, transfusion and coagulopathy reversal] in 91 (89 %) and surgical in 11(11%) patients. Mortality was 24.5% (25) and RSH was contributing to death in one patient.

Conclusions: Our study highlights the importance of RSH as a bleeding complication in patients who are on anticoagulation and among those who are not on anticoagulation. High percentage of patients may have platelet dysfunction related to Kidney disease, steroids, immunosuppressants, antiplatelet therapy. These risk factors warrant further exploration in a large sample study.

PUB239

A Systematic Review of Maternal Deaths in Women with Systemic Lupus Erythematosus and Lupus Nephritis Andrew Smyth,[1,2] James Ritchie,[3,4] Clare Tower,[7] Michael Venning,[5] Vesna D. Garovic.[6] *[1]Medicine, National University of Ireland, Galway, Ireland; [2]Nephrology, Galway University Hospitals, Galway, Ireland; [3]Renal Medicine, Salford Royal Hospital, Salford, United Kingdom; [4]Manchester Academic Health Science Centre, University of Manchester, United Kingdom; [5]Manchester Institue of Nephrology & Transplantation, Central Manchester Trust, Manchester, United Kingdom; [6]Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [7]Fetal & Maternal MEdicine, St Mary's Hospital, Manchester, United Kingdom.*

Background: Pregnancies in women with systemic lupus erythematosus (SLE) and lupus nephritis are considered high risk due to high rates of maternal and fetal complications. However, there has not been a formal analysis addressing the issue of maternal deaths in these women. The aim of this study was to perform a systematic literature review of the maternal deaths in women with SLE and lupus nephritis to: (1) identify the main causes of death and (2) discuss possible reasons for these, and strategies that may improve patient care and outcomes.

Methods: We performed an extensive electronic literature search from 1966 to 2009 using online databases (PubMed, Embase, Lilacs, Cochrance Controlled Trials Register, Medline and Science Citation Index). Studies were included if they reported pregnancies in patients with SLE and lupus nephritis with at least one reported death.

Results: We identified eleven studies reporting sixteen deaths in the immediate post-partum period that were attributable to SLE and lupus nephritis. In all cases, death occurred in the setting of active disease, and was attributed either to infection (n=7) or disease activity (n=7). The two remaining deaths were due to pulmonary embolus (n=1) and steroid withdrawal (n=1).

Conclusions: All maternal deaths in patients with SLE and lupus nephritis occurred in those with active disease, with disease activity/complications and infections (mainly opportunistic) being two major causes. The presented evidence further supports timing of pregnancy relative to SLE activity, and judicious use of immunosuppressive agents in pregnant patients.

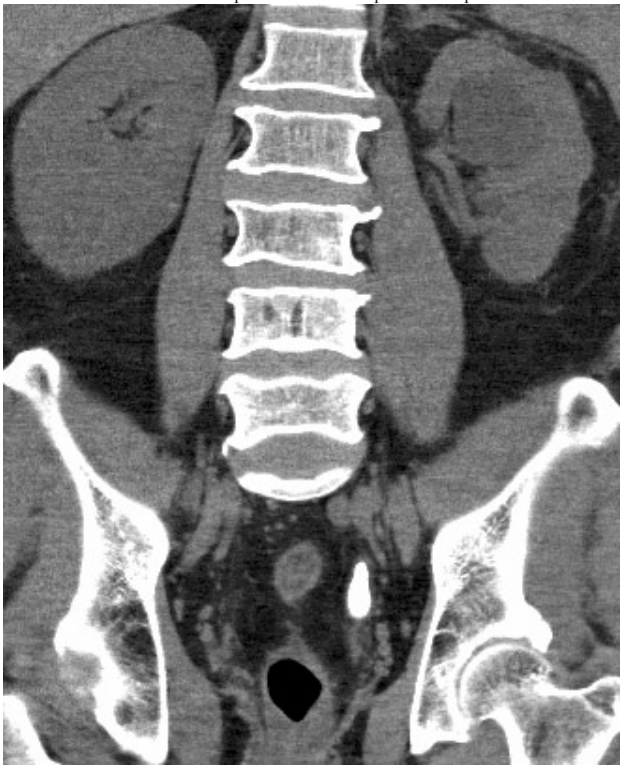
PUB240

Recurrent *Proteus mirabilis* Urinary Tract Infection Presenting with a Large Struvite Stone Ekamol Tantisattamo, Chuong Dinh. *Department of Medicine, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI.*

Background: Urinary tract infection (UTI) is uncommon in adult male, and always results from a complicated UTI. Urease forming bacteria cause the urine to become alkaline, thus leading to favorable conditions for struvite stone formation. We report a case of a man who had recurrent *Proteus mirabilis (P. mirabilis)* UTI and was found to have a large ureterovesical junction (UVJ) struvite stone complicated by severe sepsis and acute kidney injury.

Results: TThe patient is a 59-year-old man with a history of *P. mirabilis* UTI 5 years ago. Urine pH was 8. He was successfully treated with antibiotics but lost to follow up. One day prior to admission, he presented with fevers, chills, and severe sepsis. Abdominal exam was unremarkable. He had no costovertebral angle tenderness. Digital rectal exam showed 5 fingerbreadths nontender prostate gland. His scrotum was erythematous, mildly tender, but with no swelling or penile discharge. He developed acute kidney injury. His serum creatinine was 7.6 mg/dl rising from baseline serum creatinine of 1 mg/dl. Urinalysis showed an alkali urine with pH of 8. Blood and urine cultures grew *P. mirabilis*. Abdominal CT scan revealed a 1.5 * 5 cm left distal ureteric stone extending across the UVJ. He underwent left percutaneous nephrostomy tube placement, and then laser lithotripsy of the left ureteral stone. Stone analysis was consistent with struvite stone. His sepsis ultimately resolved and serum creatinine returned to his baseline.

Conclusions: *P. mirabilis* is a common cause of struvite stone which is difficult to treat medically and needs surgical treatment. Incomplete treatment of *P. mirabilis* can result in progressive struvite stone formation with complications of acute kidney injury and urosepsis. Infected alkaline urine is an important clue to the presence of persistent *P. mirabilis* UTI.



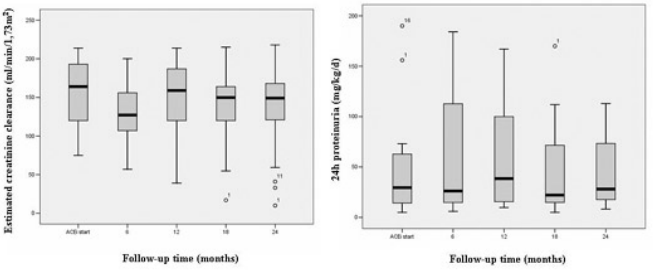
PUB241

Collagen 4-Related Nephropathies: A Series of Patients Treated with ACEi Silvia M. Titan,[1] Helen Takagi,[2] Denise Maria Avancini Costa Malheiros,[3] Benita Schvartsman,[2] Maria Helena Vaisbich.[2] *[1]Nephrology Division, Sao Paulo University Medical School, Sao Paulo, Brazil; [2]Nephrology Division, Instituto da Criança, Sao Paulo University Medical School, Sao Paulo, Brazil; [3]Pathology Division, Sao Paulo University Medical School, Sao Paulo, Brazil.*

Background: Collagen IV-related nephropathies (COLIV) are associated with a high frequency of ESRD. ACEI and ARBs are the only pharmacological measures, but the impact of this treatment remains unclear. In this study, we have analyzed the effect of ACEI in COLIV patients.

Methods: Retrospective data on 19 COLIV patients treated with ACEi was collected. Primary outcome (PO) was defined as an estimated creatinine clearance <60 ml/min/1,73m².

Results: At diagnosis, 70% of cases presented macroalbuminuria, 47% hypertension and 63% hyperfiltration. Figure 1 shows the laboratorial evolution during the first 2 years of treatment, with no significant change in proteinuria.



	ACEi initiation	6 months	12 months	18 months	24 months	p value*
Urea (mg/dL; mean / std)	32,7 (15,6)	32,6 (11,6)	30,3 (13,0)	37,3 (24,7)	43,7 (35,8)	0,1
Creatinine (mg/dL; mean / std)	0,6 (0,2)	0,7 (0,2)	0,7 (0,4)	0,9 (0,9)	1,2 (1,7)	0,004
Estimated creatinine clearance (mL/min/1,73m²; mean / std)	161,3 (52,7)	131,7 (36,8)	149,1 (47,0)	143,5 (63,7)	134,7 (60,6)	0,14
24h proteinuria (mg/kg/d; median, min - max)	25 (5 - 190)	25,3 (5 - 184)	28,9 (5 - 167)	22 (5 - 170)*	27 (4 - 113)*	0,94

* 2 missing values
# Friedman test (pairwise data, non parametric)

During follow-up, 4 patients reached the PO. Table 1 shows that renal function, proteinuria and hypertension were significantly related to the PO.

Clinico-laboratorial features according to PO.

	no-PO (n=15)	PO (n=4)	p
At baseline			
Age (months; mean/std)	117,3 (39,4)	150,3 (37,2)	0,15
Sex (men; n / %)	9 (60)	4 (100)	0,25
Creatinine (mg/dL; mean / std)	0,5 (0,2)	0,8 (0,1)	0,01
Estimated creatinine clearance (ml/min/1,73m2; mean / std)	165,5 (40,6)	118,0 (45,1)	0,06
24h proteinuria (mg/kg/d; median, min - max)	23 (6-87)	172 (40-225)	0,004
Mean ACEi dose (mg/kg/d; mean / std)	0,25 (0,08)	0,18 (0,07)	0,22
Hypertension (n / %)	5 (33,3)	4 (100)	0,03
Abnormalities at optic microscopy (n / %)	4 (30,8)	2 (66,7)	0,52
Follow-up			
Ratio of proteinuria during follow-up (median, min - max)	1,1 (0,25 - 8,3)	0,5 (0,5 - 2,0)	0,60

Conclusions: In our series, no major effect of ACEi on proteinuria could be seen. RCTs are needed to further explore the real impact of ACEi and ARBs in the natural history of COLIV.

PUB242

Is There a Relation between ADPKD and Valvular Heart Disease? Yoshiki Tsuchiya,[1] Yoshifumi Ubara,[2] Tatsuya Suwabe,[2] Keiichi Sumida,[2] Kenmei Takaichi,[2] Masayuki Yamanouchi,[2] Rikako Hiramatsu,[2] Noriko Hayami.[2] *[1]Nephrology Center, Tohkatsu Hospital, Nagareyama, Chiba, Japan; [2]Nephrology Center, Toranomon Hospital Kajigaya, Kawasaki, Kanagawa, Japan.*

Background: Hossack (NEJM 1988) reported that ADPKD has a close relation with valvular heart disease, including mitral valve incompetence. However, Ecder (Nat Rev Nephrol. 2009) argued that there was no significant difference of valvular abnormalities between ADPKD patients and healthy controls, so this issue still remains unclear and further study is needed.

Methods: A retrospective analysis of echocardiographic data was performed with comparison among 4 groups: group 1 (ADPKD patients on hemodialysis, CKD stage 5, n=421), group 2 (ADPKD patients in CKD stage 3, n=49), group 3 (chronic glomerulonephritis patients, mainly IgA nephropathy, on hemodialysis, CKD stage 5, n=60), and group 4 (IgA nephropathy patients with CKD 3, n=70).

Results: In group 1, 48% had mitral incompetence (MI), 29.2% had aortic incompetence (AI), and 41.8% had tricuspid incompetence (TI). The total valvular disease (TVD) rate (MI, A1, and TI) was 77.9%.

Comparison among five groups

	MI	AI	TI	PI	TVD	pericardiac effusion	ejection fruction
GROUP1 ADPKD CKD stage5 (N=421)	48.0%	29.2%	41.8%	20.7%	77.9%	19.2%	67.3 +- 11.0%
GROUP2 ADPKD CKD stage3 (N=49)	20.4%	14.3%	24.5%	57.1%	71.4%	4.1%	70.0 +- 7.7%
GROUP3 CGN CKD stage 5 (N=60)	43.3%	31.7%	61.7%	23.3%	80.0%	10.0%	67.7+- 11.7%
GROUP4 IgAN CKD stage3 (N=70)	22.3%	8.6%	38.6%	47.1%	72.9%	0%	71.6 +- 5.3%

However, the rates of MI, AI, and TI were significantly different (P<0.01) for group 1 vs. group 2 and group 3 vs. group 4.

Conclusions: Patients with ADPKD and CGN (including IgA nephropathy) showed no differences whether they were CKD 3 or CKD 5. However, ADPKD and non-ADPKD patients with CKD3 or 5 showed significant differences. Valvular heart disease may not be characteristic of ADPKD, but could have a close relation with some hemodynamic factors secondary to hemodialysis.

PUB243

The Impact of Gene Polymorphisms of Interleukin-18 (IL-18), Transforming Growth Factor-β (TGF-β) and Vascular Endothelial Growth Factor (VEGF) on Development of IgA Nephropathy or Thin Glomerular Basement Membrane Disease Jang-Hee Cho,[1,2] Hee-Yeon Jung,[1,2] Mi-Kyung Jin,[1,2] Owen Kwon,[1,2] Kyung-Deuk Hong,[1,2] Ji-Young Choi,[1,2] Se-Hee Yoon,[1,2] Sun-Hee Park,[1,2] Yong-Lim Kim,[1,2] Chan-Duck Kim.[1,2] *[1]Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea; [2]Clinical Research Center, End Stage Renal Disease in Korea, CRC for ESRD, Republic of Korea.*

Background: We investigated the effects of gene polymorphisms on the development of IgA nephropathy and thin glomerular basement membrane (GBM) disease by analyzing polymorphisms in the interleukin (IL)-18, transforming growth factor (TGF)-β, and vascular endothelial growth factor (VEGF) genes in Korean patients.

Methods: The study included 146 normal subjects (control group) and biopsy-proven 69 IgA nephropathy and 44 thin GBM disease patients. The gene polymorphisms A-607C and G-137C in *IL18*, C-509T and T869C in *TGFB1*, and C-2578A and C405G in *VEGF* were investigated in DNA extracted from peripheral blood.

Results: The frequencies of the *IL18* -607CC genotype (p=0.002) and the *VEGF* 405GG genotype (p=0.002) were significantly increased in the IgA nephropathy group compared with the control group. No significant differences in genotype frequency were observed between the thin GBM disease and control group. There were no significant differences in genotype and allele frequencies between IgA nephropathy and thin GBM disease groups.

Conclusions: Significant differences of genotype and allele frequencies were observed only between IgA nephropathy and control group. However, because of the small size of the IgA nephropathy and thin GBM group, additional extensive studies are required to clarify the potential role of gene polymorphism to discriminate IgA nephropathy and thin GBM disease.

Funding: Government Support - Non-U.S.

PUB244

DNA Methylation and CpG Dinucleotide Frequencies in Promoter Regions of Human Kidney Development Genes Yasunobu Kawata,[1] Walter L. Ruzzo,[2] Karol Bomsztyk.[3] *[1]Department of Medicine, Shimonoseki Municipal Saiseikai Toyoura Hospital, Shimonoseki, Yamaguchi, Japan; [2]Department of Computer Science and Engineering, University of Washington, Seattle, WA; [3]Department of Medicine, University of Washington, Seattle, WA.*

Background: DNA methylation of CpG dinucleotides in genome regulates gene expression. CpG islands are frequently found in gene promoters, but the detailed analysis of these sequences are not known.

Methods: Here, we analyzed sequences from -5kb to +5kb of transcriptional start site (TSS) of human genomic genes by computing CpG dinucleotide frequencies every 500 bps. From Gene Ontology and UCSC website, human genomic genes including kidney development, cell cycle, mitosis, chromatin, extracellular matrix and receptor genes were examined.

Results: This analysis revealed that CpG frequencies flanking TSS regions in genes encoding kidney development, cell cycle, mitosis, chromatin genes are significantly higher than average. The TATA box is a critical promoter element that regulates transcriptional initiation and is located in 10% of all genes. We found that as a whole TATA box-containing promoters have lower CpG frequencies flanking TSS than the average. In contrast, interestingly, 15% of the kidney development genes have promoter TATA boxes associated but with higher CpG frequencies flanking TSS regions. We used GSE17001 of Gene Expession Omnibus datasets in NCBI of the same set of genes of primary human fibroblasts and breast adenocaricinoma cell lines to analyze DNA methylation patterns. This analysis revealed that compared to the average, kidney development genes show higher DNA methylation level in promoter regions but lower methylation levels at TSS. This unique pattern at these genes was not found in the adenocarcinoma cell lines, which show higher methylation level at TSS and lower level at the promoter region. This analysis suggest that regulation of CpG islands methylation flanking TSS is different in kidney development genes than an average gene.

Conclusions: This suggest that renal development may involve transcriptional mechanisms that are unique.

PUB245

^{1}H NMR Spectroscopy Analysis of Metabolites in the Kidneys Provides New insight into Pathophysiological Mechanisms: Applications for Treatment with *Cordyceps sinensis* Fang Zhong, Qiao Zhou, Ying Lu, Xu Hao, Cong Li, Shanmai Guo, Weiming Wang, Nan Chen. *Department of Nephrology, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China.*

Background: The number of patients with chronic kidney disease (CKD) is continuously growing worldwide. Treatment with traditional Chinese medicine might slow the progression of CKD.

Methods: In this study, we evaluated the renal protective effects of the Chinese herb *Cordyceps sinensis* in rats with 5/6 nephrectomy. Male SD mice (weighing 150-200 g) were subjected to 5/6 nephrectomy. The rats were divided into three groups: (a) untreated nephrectomized group (OP group, n=16), (b) oral administration of *Cordyceps sinensis*-treated (4 mg/kg/d) nephrectomized group (CS group, n=16) and (c) sham-operated group (SO group, n=16). The rats were sacrificed at four and eight weeks after 5/6 nephrectomy, and the kidneys, serum and urine were collected for ^{1}H nuclear magnetic resonance (^{1}HNMR) spectral analysis. Multivariate statistical techniques and statistical metabolic correlation comparison analysis were performed to identify metabolic changes in aqueous kidney extracts between these groups.

Results: Significant differences between these groups were discovered in the metabolic profiles of the biofluids and kidney extracts. Pathways including the citrate cycle, branched-chain amino acid metabolism and the metabolites that regulate permeate pressure were disturbed in the OP group compared to the SO group; in addition, these pathways were reversed by *Cordyceps sinensis* treatment. Biochemistry and electron microscopic images verified that *Cordyceps sinensis* has curative effects on chronic renal failure. These results were confirmed by metabonomics results.

Conclusions: Our study demonstrates that *Cordyceps sinensis* has potential curative effects on CKD, and our metabonomics results provided new insight into the mechanism of treatment of this traditional Chinese medicine.

Funding: Government Support - Non-U.S.

PUB246

Changing Demographics of Autosomal Dominant Polycystic Kidney Disease over Four Decades Imed Helal, Berenice Y. Gitomer, Xiang-Dong Yan, Kim McFann, Godela M. Brosnahan, Robert W. Schrier. *Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: ADPKD is the most life threatening hereditary disease. Over the last four decades there have been considerable improvements in clinical diagnosis and management of patients with ADPKD. The impact of these changes on the overall demographics of the ADPKD patient population is however, unknown. The present study compared the demographics of ADPKD patients who were seen at our ADPKD center from 1961-1992 versus 1993-2007.

Methods: Data was collected from the ADPKD clinical database at the University of Colorado for the first subject visit with full clinical information and patient clinical and demographic information compared for both time intervals. All patients were evaluated during a 2-day in-patient visit at the Clinical Translational Research Center at University of Colorado Hospital.

Results: The results are depicted in the table.

Table 1: Demographics of ADPKD

Parameter	1993-2007	1961-1992	P-value
N	579	281	
Age (years)	41 ± 12	43 ± 13	0.003
Gender: Male/Female	219/360	133/148	0.007
Sysstolic blood pressure (mm Hg)	129 ± 16	142 ± 24	<0.0001
Diastolic blood pressure (mm Hg)	81 (144-37)	92 (160-60)	<0.0001
CrCl (ml/min/1.73m2)	75 ± 35	49 ± 33	<0.0001
Proteinuria (mg/24h)	0.24 (3.31-0.01)	0.81 (5.9-0.04)	<0.0001
ACE/ARB use	275 (34.6%)	25 (3.3%)	<0.0001

While the earlier cohort was somewhat older and had more male patients, it seems unlikely that these demographics alone explain the major differences in blood pressure (BP), kidney function and proteinuria. The improved BP control was accompanied by the increased use of the renin-angiotensin system (RAS) inhibitors.

Conclusions: Over the last 4 decades the demographics of ADPKD have changed in a more favorable manner.

Funding: NIDDK Support, Private Foundation Support

PUB247

Congenital Heart Disease in Autosomal Dominant Polycystic Kidney Disease
Maria V. Irazabal,[1] Amber Harmon,[1] Heidi M. Connolly,[2] Marie C. Hogan,[1] Jamie L. Sundsbak,[1] Sandro Rossetti,[1] Peter C. Harris,[1] Vicente E. Torres.[1] *[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

Background: *Pkd2* and *Pkd1l1*, but not *Pkd1*, are expressed in the embryonic node and required for left-right patterning. Disturbances in embryonic left-right patterning are an important cause of disrupted cardiac development and congenital heart disease (CHD). *Pkd2* and *Pkd1l1* null mice exhibit cardiac septation and outflow tract defects. Although cardiac valvular abnormalities occur with increased frequency in Autosomal Dominant Polycystic Kidney Disease (ADPKD), it is uncertain whether an association between ADPKD and CHD exists.

Methods: To examine this question we reviewed the echocardiogram reports obtained between 1999 and 2010 in ADPKD patients (554 patients).

Results: A diagnosis of CHD (including previous diagnoses, excluding patent foramen ovale and atrial septal aneurysm) was present in 25 patients (4.5 %). Atrial or ventricular septal defect (n=4 or 0.7 %), patent ductus arteriosus (n=1), isolated bicuspid aortic valve (BAV n=9 or 1.6 %), aortic coarctation (n=4 or 0.7 %, 3 with associated BAV), hypoplasia of upper descending thoracic aorta (n=1), left coronary artery to pulmonary artery fistula (n=1), congenital pulmonic stenosis (n=1), Ebstein anomaly (n=1), tetralogy of Fallot (TOF n=1), tricuspid atresia (n=1), and double inlet left ventricle with transposed great vessels and pulmonic stenosis (n=1). Four cases had been referred to our institution for evaluation of CHD; in the remaining the diagnosis of CHD was incidental to evaluation for ADPKD or other conditions. Three additional ADPKD patients with TOF, VSD and isolated left superior vena cava were seen during the same period of time but had no echocardiogram. Genetic studies (in progress) have so far revealed 7 *PKD1*, no *PKD2* mutations, and no mutation detected in one patient.

Conclusions: CHD is rarely associated with ADPKD, but the observed prevalences seem higher than those in the general population 2.9 vs 0.8 % (excluding isolated BAV). Preliminary results do not demonstrate an enrichment of *PKD2* mutations in these patients.

Funding: NIDDK Support

PUB248

Mutations in KIF7 Link Joubert Syndrome with Sonic Hedgehog Signaling and Microtubule Dynamics
Max C. Liebau,[1,2] Claudia Dafinger,[2,4] Yorck Hellenbroich,[5] Christoph Korenke,[8] Francesca Fabretti,[2,3] Inga Ebermann,[4] Gudrun Nürnberg,[3,6] Thomas Benzing,[2,3] Hanno Jörn Bolz,[4,7] Bernhard Schermer.[2,3] *[1]Department of Pediatrics, University Hospital of Cologne, Cologne, Germany; [2]Department of Medicine and Centre for Molecular Medicine, University Hospital of Cologne, Cologne, Germany; [3]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Cologne, Germany; [4]Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany; [5]Institute of Human Genetics, University Hospital of Schleswig-Holstein, Luebeck, Germany; [6]Cologne Center for Genomics and Centre for Molecular Medicine, University of Cologne, Germany; [7]Bioscientia Center for Human Genetics, Ingelheim, Germany; [8]Elisabeth Kinderkrankenhaus, Oldenburg, Germany.*

Background: Joubert syndrome (JBTS) is characterized by a specific brain malformation with various additional pathologies including a nephronophthisis-like cystic renal phenotype in around 25% of the JBTS patients. JBTS can result from mutations in at least 10 different genes and has been linked to dysfunction of primary cilia. We have identified a disease locus, JBTS12, with mutations in the KIF7 gene, a homolog of the Drosophila kinesin Costal2, in a consanguineous JBTS family and subsequently in other JBTS patients. Interestingly, KIF7 is a known regulator of Hedgehog signaling and a putative ciliary motor protein. In human epithelial cells knockdown of KIF7 expression caused defects in cilia formation and induced abnormal centrosomal duplication and fragmentation of the Golgi network. These cellular phenotypes likely result from abnormal tubulin acetylation and microtubular dynamics induced by loss of KIF7 function. Further functional studies concerning the mechanisms involved are ongoing. In summary we suggest that modified microtubule stability and growth direction caused by loss of KIF7 function may be an underlying disease mechanism contributing to JBTS.

PUB249

Mutational Analysis of the TRPC6 Gene in Czech Adult Patients with Steroid-Sensitive and Steroid-Resistant Idiopathic Nephrotic Syndrome
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Background: Focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) are frequent causes of nephrotic syndrome (NS). About 50% of patients are resistant to immunosuppresive therapy.TRPC6 mutations are the cause of idiopatic NS in about 2-7 % of steroid-resistant patients. These mutations lead a variable rate of progression to end stage renal disease. The aim of the study was the identification of mutations in adult patients with FSGS/MCD.

Methods: 40 patients (22 females, 18 males) with steroid- resistant FSGS/MCD and 44 patients (22 females, 22 males) with steroid-sensitive FSGS/MCD were studied. Renal biopsy with the histological finding of FSGS/MCD was performed in the years 2004-2008. The mean age of the onset of NS was 39± 20.7 years. Family history for proteinuria was positive in three patients. 300 healthy Czech individuals formed control group with mean age 58.4±19.5 years. High resolution melting method (HRM) was established for all 13 exons and intron-exon boundaries of the TRPC6 gene. Suspected samples were analysed by direct sequencing on ABI Prism 3130 Genetic Analyzer.

Results: No TRPC6 gene mutation was identified. Two polymorphisms were described: in exon 1 C43T (P15S) with prevalence 32.5% of heterozygotes and in exon 4 C1211T (A404V) with prevalence 20% of heterozygotes (resp. 1% of T/T homozygotes) in patients with steroid-resistant FSGS/MCD. The prevalence of heterozygotes in steroid-sensitive patients was 13.9% for C43T polymorphism and 29.5% for C1211T polymorphism. The prevalence of heterozygotes in control group was 16.5% for C43T and 20.5% for C1211T (resp. 2.5% of T/T homozygotes). T allele of C43T polymorphism in exon 1 was significantly more frequent in patients with steroid-resistant FSGS/MCD.

Conclusions: TRPC6 gene mutations are rare causes of FSGS/MCD in adult patients. The C43T polymorphism could have some influence on the therapeutic response and progression of the disease.

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PUB250

Ambiguous Genitalia in a Neonate; Cause and Consequence
Sarah Hasham,[1] Tetyana L. Vasylyeva.[1] *[1]Pediatrics, Texas Tech University Health Sciences Center, Amarillo, TX; [2].*

Background: Denys-Drash syndrome (DDS) is a rare disorder consisting of the triad of congenital nephropathy, Wilms tumor, and intersex disorders resulting from mutations in the Wilms tumor suppressor (*WT1*) gene. Worldwide, more than 200 cases of DDS have been reported since 1967 when Denys et al originally described a child with nephropathy, ambiguous genitalia, and Wilms tumor. WT1 gene is thought to have tumor suppressor activity and play an important role in nephrogenesis, genitourinary development, haematopoiesis and sex determination.

Methods: We report a case of DDS in a baby boy who was born with ambiguous genitalia and transferred to our NICU.

Results: He was found to have persistent HTN and proteinuria in the NICU. His kryotype was XY and WT1 gene showed mutation *P.D 396N*: c.1186G>;A consistent with DDS. We followed him in Nephrology clinic for 8 months and his proteinuria reached nephritic range and he became albumin transfusion dependent.

Conclusions: DDS is the result of mutations in the *WT1* gene on chromosome band 11p13. The WT1 protein is a transcription factor predominantly expressed in the embryonic kidneys and gonads. Patients with Denys-Drash syndrome develop early-onset nephrotic syndrome, have a high prevalence of severe hypertension, and experience rapid progression to end-stage renal disease (ESRD). The vast majority of patients with DDS is destined to develop Wilms tumor in the native kidneys and are at significant risk for development of gonadoblastoma in the dysgenetic gonads. DDS therapy includes management of fluid and electrolyte balance, treatment of hypertension, renal replacement therapy for ESRD or after bilateral nephrectomy and renal transplant. DDS and WT1 related syndromes are under recognized. Late diagnosis of DDS may lead to late diagnosis of Wilms' tumor, which is often associated with this syndrome. Diagnosis of WT1 related syndromes and molecular testing for WT1 mutations should be considered in the presence of ambiguous genitalia and hypertension or proteinuria.

PUB251

Genetic Factors in the Development of Primary Chronic Glomerulonephritis
Magdalena Mostowska,[1] Stanislaw Czekalski,[1] Pawel P. Jagodzinski,[2] Andrzej P. Oko.[1] *[1]Department of Nephrology, Transplantology and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland; [2]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland.*

Background: Genetic factors can play a significant role in the onset of primary chronic glomeluronephritis (GN). Genes encoding proteins of the renin - angiotensin - aldosterone system (RAAS) are candidate genes for primary chronic GN because of influence on renal function in physiological and pathological conditions. The aim of the study was to evaluate the association of 10 polymorphisms of RAAS genes (ACE rs4646994; AGT rs699; AGTR1 rs5186; ATP6AP2 rs2968917 and rs2971597; CMA1 rs1885108 and rs1956923; CYP11β2 rs1799998; REN rs11571080 and rs2368564) with the risk of primary chronic GN in the Polish population.

Methods: The analysis of selected single nucleotide polymorphisms (SNPs) was conducted in the group of 148 patients from the Polish population with biopsy proven primary chronic GN. Control group consisted of 193 healthy persons matched by sex, age, and place of birth. SNP genotyping was performed using PCR-RFLP (Restriction Fragment Length Polymorphism) or HRM (High - Resolution Melting) analyses.

Results: The significant result was found for the rs1799998 polymorphism of the CYP11β2 gene. Individuals carrying two copies of the rs1799998 C allele has nearly twofold increased risk of primary chronic GN (ORCCvsCT+TT = 1.941; 95%CI: 1.171 - 3.218; p = 0.0095). The genetic variations in ACE (rs4646994), AGT (rs699), AGTR1 (rs5186), ATP6AP2 (rs2968917, rs2971597), CMA1 (rs1885108, rs1956923), REN

(rs11571080, rs2368564) are not associated with the risk of primary chronic GN. Moreover, the gene-by-gene interaction analysis conducted using the Multifactor Dimensionality Reduction approach revealed no significant interactive genetic effect on primary chronic GN occurrence.

Conclusions: Polymorphism rs1799998 of the CYP11β2 gene is associated with primary chronic GN in the group of patients from the Polish population.

PUB252

Acute Caffeine Overdose Requiring Hemodialysis Syed N. Babar, John A. Walker. *Nephrology, Robert Wood Johnson University Hospital, New Brunswickl, NJ.*

Background: Caffeine is commonly consumed by humans in the form of beverages like coffee, tea and soft drinks. In the brain, it acts as an antagonist of adenosine receptors, and with adenosine having vasodilatory properties, it is a common composition of various headache pills. We present a rare case of caffeine intoxication requiring hemodialysis.

Methods: A 40 year old woman was found unresponsive after she had consumed approximately 100 pills of Fioricet (Composition: Caffeine 40 mg, Acetaminophen 325 mg, Butalbital 50 mg). Labs revealed hypokalemia, lactic acidosis, and an elevated serum acetaminophen level. Gastric detoxification was initiated with activated charcoal along with IV N-acetyl cysteine. The patient became agitated and developed seizures. Three hours of emergent hemodialysis was administered empirically. Immediately post dialysis, she responded to her name and the next day was fully awake and oriented. Pre and post hemodialysis serum caffeine levels were 51 and 4 mcg/ml respectively (therapeutic level 5-15 mcg/ml).

Results: In quantities found in most foods and beverages, caffeine is unlikely to cause any acute medical problems. However, its presence in higher concentrations in OTC products like energy drinks, diet aids, analgesics and in prescription medications may be a potential cause for acute toxicity. Average doses may result in feelings of alertness and decreased fatigue, whereas high doses (250-500 mg) results in restlessness, nervousness, insomnia and tremors. Even higher doses can cause a hyperadrenergic syndrome resulting in seizures, cardiovascular instability and altered mental status. Hypokalemia, lactic acidosis, and hyperglycemia are classic features of caffeine overdose.

Treatment of severe acute intoxication is generally supportive and includes providing treatment of the immediate symptoms. However, extracorporeal therapy is indicated for caffeine concentrations of >100 mcg/ml or in patients who develop seizures or arrhythmias regardless of the caffeine concentration.

Conclusions: With the widespread availability and consumption of caffeine, its toxicity, (intentional or unintentional) should be recognized by physicians and appropriate treatment provided in a timely fashion.

PUB253

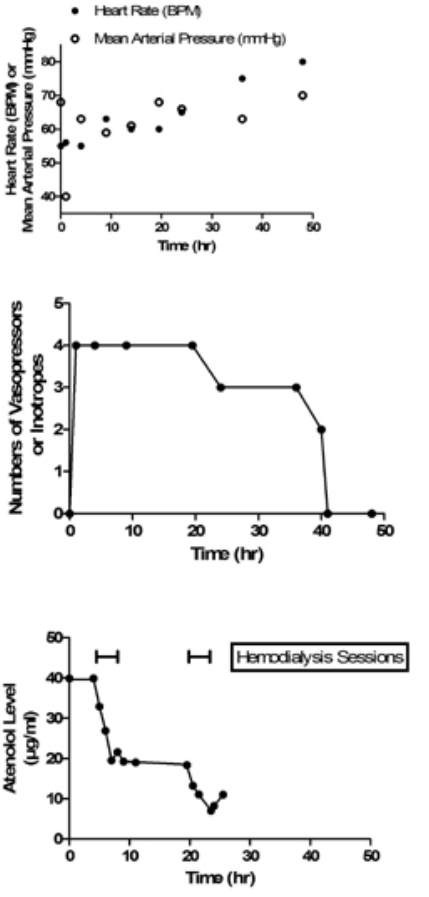
Atenolol Overdose Successfully Treated with Hemodialysis: A Case Report Shih-Han S. Huang, Rita Suri. *Medicine, Nephrology, London Health Sciences Centre, London, ON, Canada.*

Background: Atenolol overdose is common. Because of its hydrophilic characteristic, a few case reports have demonstrated the benefits of hemodialysis treatments. However, the amount of atenolol clearance throughout hemodialysis sessions has not been studied.

Methods: In this case report, a patient with impaired renal function was successful treated with two 5-hour intermittent high-flux high-efficiency hemodialysis therapies after atenolol overdose. Serial atenolol levels were measured during his hemodialysis treatments.Atenolol in plasma was determined using liquid chromatography, tandem mass spectrometry.

Results: We demonstrated an over 50% atenolol reduction after each 5 hours hemodialysis therapy. The parameters for mean arterial blood pressure (mmHg), heart rate (BPM), the numbers of inotropes and vasopressors and the plasma atenolol levels required during the first 48 hours of hospital admission, are presented in Figure 1The zero hour was set at time when the patient first present to emergency room.

Figure 1 A-C. The Recorded Heart Rate (BPM) and Mean Arterial Blood Pressure (mmHg) (Figure 1A), Numbers of Vasopressors and Inotropes Used (Figure 1B), and Atenolol Levels (μg/mL), (Figure 1C) During the First 48 Hours in Our Patient.



Conclusions: Hemodialysis therapy is an effective treatment for atenolol overdose, especially in patients with impaired renal function.

PUB254

Retrospective Comparison of Renal Replacement Therapies (RRT) in Critically Ill Patients with Acute Kidney Injury (AKI): Intermittent Hemodialysis (IHD) vs. Sustained Low Efficiency Daily Dialysis (SLEDD) vs. 24 hrs SLEDD (c-SLEDD) Sudhanshu Jain,[1,2] George N. Coritsidis,[1] Andrew Chao,[1] Yiannis Apergis,[1] Edward T. Zawada.[2] *[1]Elmhurst Hospital Center, Elmhurst, NY; [2]Avera Mckennan Hospital, Sioux Falls, SD.*

Background: Use of SLEDD is steadily increasing in critical care units due to its relative ease of usage and cost effectiveness. Recently there has been interest in its continuous use even though data is lacking

Methods: We reviewed charts for all patients with AKI requiring RRT admitted to ICU (Surgical or medical). Data was collected from 1998-2009 from Elmhurst Hospital Center and Avera Mckennan Hospital. SLEDD modalities were available after 2004. Statistical analysis was done using analysis of variance.

Results: 31 pts (IHD) vs 48 pts (SLEDD) vs 45 pts (c-SLEDD) were compared. There was no significant difference between the groups with the following: age, gender, organ failure rate, diabetes mellitus and cardiac disease history. IHD group had significantly lower rate of sepsis. Vasopressor use (38.7% vs 79.2% vs 77.7%) was significantly higher in both SLEDD modalities as compared to IHD. APACHE II score at admission was significantly lower in SLEDD group where as pre RRT APACHE II scores and organ failure index showed no significant difference. In hospital mortality was significantly higher in SLEDD modality when unadjusted for acuity.

	IHD	SLEDD	c-SLEDD	p
Patients #	31	48	45	
Age	57 ± 3.1	62.6 ± 2.4	60± 2.2	NS
Gender(M)	80.6%	75%	69%	NS
Vasopressor(VP)	38.7%(n=12)	79.2% (n=38)	77.7%(n=35)	<0.0002
Organ Failure	2.26 ± 0.15	2.35 ± 0.15	2.55 ± 0.14	NS
Cardiac Ds Hx	38.7%	27.1%	25%	NS
DM	43.3%	47.9%	40%	NS
Sepsis	25.8%	45.8%	51.1%	0.04
APACHE II(adm)	20.8 ± 1.4	16.8 ± 1.1	21.08 ± 1.4	0.02
APACHE II(preRRT)	26.1 ± 1.4	28.2 ± 0.9	25.1 ± 1.1	NS
Mortality	48.4%	78.8%	57.8%	0.04
Mortality(no VP)	42.1%	40%	20%	0.05
Mortality(w/ VP)	58.3%	79.6%	74.3%	NS

Conclusions: When comparing the three modalities in critically ill patients, mortality was higher in the SLEDD population. This difference disappeared after correcting for acuity and vasopressor (VP) use.

PUB255

Continuous Renal Replacement Therapy with Regional Citrate Anticoagulation Induces a Negative Calcium Balance Victor Sato, Renato Antunes Caires, Igor Marques, Renato Pontelli, Patricia T. Goldenstein, Emmanuel A. Burdmann, Maristela Carvalho Costa, Rosa M.A. Moyses. *Nephrology, University of Sao Paulo School of Medicine, Brazil.*

Background: Continuous Renal Replacement Therapy (CRRT) associated with regional citrate anticoagulation (RCA) is commonly employed in intensive care units, especially in patients with acute kidney injury (AKI), hemodynamic instability and high risk of bleeding. However, this therapy carries the potential risk of negative calcium (Ca) balance associated with a worsening of hemodynamic instability. The aim of the study was to better understand the safety aspects of CRRT with RCA.

Methods: In order to assess the effects of CRRT with RCA in Ca kinetics, 10 continuous venovenous haemodialysis (CVVH) sessions, performed with Ca-free dialysate, were evaluated. The delivered dialysis dose and the blood flow were fixed, and intravenous Ca reposition was titrated based on ionized Ca values.

Results: During therapy, our patients (7 males, 66±11yrs, 6 with AKI) presented a diuresis rate of 0.3L (range, 0; 2.4L). No cardiac arrhythmia neither any circuit clotting was observed during therapy. Biochemical parameters are shown below.

Comparison of biochemical parameters at the baseline and the end

	Baseline	End
Ionized calcium (mg/dL)	4.7±0.4	4.2±0.4*
Phosphate (mg/dL)	5.6±2.6	3.1±0.6*
PTH (pg/mL)	140±110	68±3*

Results are shown as mean ± SD. * p<0.05

Median Ca balance was -372mg (range, -855; 290mg), and was negative in 8 patients. Mean effective ultrafiltration (UF) was 6.3±1.9 L. Ca balance presented a trend toward a negative correlation with UF (R= -0.58: p=0.09). Curiously, despite a negative Ca balance and a decrease in serum Ca, PTH decreased after 24h of CRRT.

Conclusions: In conclusion, this study showed a negative calcium balance in patients submitted to CRRT, which was not accompanied by a compensatory increase in PTH, probably due to the critic clinical status of these patients. These results indicate that Ca should be closely monitored in critically ill patients submitted to CRRT therapy, mainly in those with a high UF rate.

PUB256

Outcomes of Patients with End Stage Renal Disease (ESRD) under Chronic Hemodialysis and Patients without ESRD in Acute Renal Failure Requiring Continuous Renal Replacement Therapy: A Single Center Study Ho Sik Shin, Yeon Soon Jung, Hark Rim, Jin Hee Park, Sung Bin Kim. *Internal Medicine, Kosin University College of Medicine, Busan, Korea.*

Background: The purposes of this study were to compare the survival of conventional hemodialysis (HD) patients with the survival of non-end stage renal disease (ESRD) patients.

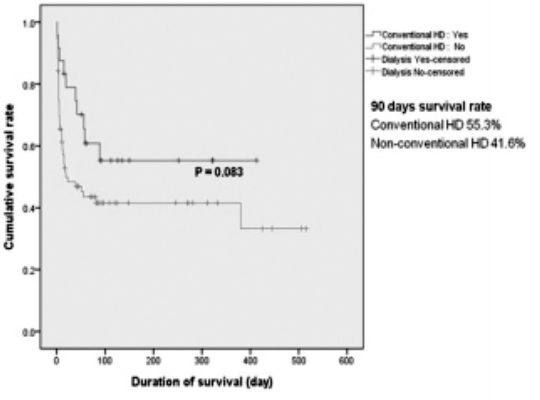
Methods: We evaluated adults (> 18 years) requiring CRRT who were treated in the ICU of Kosin University Gospel Hospital, Busan, Korea from January 1, 2008 to November 30, 2010. A total of 100 (24 ESRD, 76 non-ESRD) patients received CRRT during the study period. Predictors of all-cause death were examined using Kaplan-Meier analysis and Cox proportional hazards analyses in both treatment groups.

Results: For non-ESRD patients, the 90 day survival rate was 41.6 %. For ESRD patients, the 90 day survival rate was 55.3 %. Multivariate Cox proportional hazards analyses demonstrated that conventional HD was not a significant predictor of mortality [hazard ratio (HR) 0.334, 95% confidence interval (CI) 0.063–0.1.763, P = 0.196].

Table 1. Comparison between ESRD Group and Non-ESRD Group

	ESRD (n=24)	Non-ESRD (n=76)	P value
Age, years	53.6 ± 31.6	49.5 ± 28.2	0.324
Admission to CRRT, day	6.4 ± 8.5	8.4 ± 28.7	0.819
APACHE score	89.2 ± 34.9	89.0 ± 32.5	0.982
Medical setting (%)	20 (83.3)	54 (71.1)	0.293
No. of organ failure (range)	1.4 ± 0.8	1.8 ± 0.9	0.131
Serum BUN (mg/dL)	59.2 ± 33.9	53.2 ± 28.5	0.391
Serum creatinine (mg/dL)	6.5 ± 4.0	3.5 ± 2.3	0.002
Leukocyte (× 103/μL)	12.6 ± 5.1	14.6 ± 10.2	0.351
Hemoglobin (g/dL)	10.3 ± 1.7	10.4 ± 2.1	0.701
Platelet (× 103/μL)	126.2 ± 5.5	105.6 ± 82.2	0.534
Serum albumin (g/dL)	2.5 ± 0.6	2.7 ± 0.5	0.179
UFR (mL/kg/hr)	22.4 ± 4.6	21.7 ± 4.4	0.609
Sepsis (%)	12 (50)	48 (63.2)	0.251
Death	10 (41.7)	43 (56.6)	0.202

Fig. 2. Patient survival in conventional hemodialysis group and non-ESRD group



Conclusions: The survival rates of non-ESRD and ESRD patients requiring CRRT did not differ, and conventional HD was not a significant predictor of mortality.

PUB257

The Efficacy of Ferumoxytol in Peritoneal Dialysis Patients Manaf Alroumoh, Farhanah Yousaf, Sayed Husain, Hani Judeh, Prince Mohan, Chaim Charytan, Bruce S. Spinowitz. *Department of Nephrology, New York Hospital Queens, Flushing, NY.*

Background: Iron deficiency is one of the major elements contributing to anemia in CKD patients. Oral iron is often not tolerated and ineffectively absorbed. Intravenous infusion is time consuming and inconvenient in peritoneal dialysis (PD) patients self-treating at home. A new iron preparation , ferumoxytol, which can be administered as a bolus intravenous injection, would allow PD patients to more easily comply with current IV iron dosing regimens. Therefore, we evaluated the effect of ferumoxytol on hemoglobin, hematocrit, ferritin, and iron saturation.

Methods: We reviewed the medical records of PD patients aged ≥ 18 years, who received at least one dose of ferumoxytol between January 2010 and August 2010 at our institution.

Results: 9 males and 8 females, aged 53.5 ± 16.6 years with average weight of 83.94 ± 22.8 kg were included. 15 patients received 2 doses and 2 patients received only 1 dose of ferumoxytol.

table 1

Lab	Baseline	4 Wk Post Feraheme	8 Wk Post Feraheme	12 Wk Post Feraheme	16 Wk Post Feraheme
Hemoglobin (g/dL)	10.4±1.0	11.0±0.9*	11.3±0.9*	11.3±1.0*	10.9±1.2
Hematocrit (%)	32.3±3.9	33.8±3.5*	34.6±3.2*	34.6±3.4*	33.6±3
Ferritin (mg/dL)	279±152	625±248*	743±259*	N/A	640±367*
TSAT (%)	18.1±6.3	37.4±14.7*	36.9±23.7	N/A	32.9±12.9*
Epoetin(U)/ week	39,573	29,764	27,058	27,329*	26,585

*p<0.05 versus baseline

The average time interval between the two doses of ferumoxytol was 6.9 ± 3.6 days. The average intervals between the 2nd dose and subsequent labs were 24.6±11.7 days (Wk 4), 56.1±8.4 days (Wk 8), 87.3±13.6 days (Wk 12), and 116.7±13.8 days (Wk 16). Data presented as mean±SD. Epoetin dose presented as mean.

Conclusions: Ferumoxytol has the expected efficacy of an intravenous iron compound, with improvements in anemia and iron being evident as early as 4 weeks post ferumoxytol. Additionally, significant decrease in monthly epoetin dose was noted at 12 weeks post ferumoxytol dosing. Ferumoxytol is a desirable therapeutic option in peritoneal dialysis patients, who typically visit the clinic at monthly intervals. Alternative iron therapies would require lengthy infusions, or frequent visits to achieve comparable iron delivery.

PUB258

Gender Related Differences in Mortality in Hemodialysis Sonia Maria Holanda Almeida Araujo,[1] Constance Almeida de Alencar Araújo,[1] Nicole Araujo,[2] Andre Pantaroto,[2] Elizabeth De Francesco Daher,[1] Pedro Bruin,[1] Veralice Meireles Sales Bruin.[1] *[1]Medicina, Universidade Federal do Ceara, Fortaleza, Ceara, Brazil; [2]Medicina, Faculdade de Medicina de Jundiai, Jundiai, Sao Paulo, Brazil.*

Background: Long-term prognosis of patients undergoing chronic hemodialysis remains poor. Presently, we hypothesize that factors influencing survival/mortality in hemodialysis are different for each gender.

We aimed to evaluate factors influencing mortality in men and women on chronic hemodialysis.

Methods: All cause mortality was examined in a two-year follow-up study of 400 patients (59% men, mean age 51.6±5.5 years) from three hemodialysis centers. Analyzed factors were age, body mass index, albumin levels, hemoglobin, eKt/V<1.2, ferritin, parathormone<150pg/L, diabetes, stroke, heart failure, hypertension, excessive daytime sleepiness, depressive symptoms (Beck Depression Inventory, BDI), and comorbidity severity (Charlson Comorbidity Index, CCI). Time-dependent Cox regression analysis was performed in all cases and in the cohort grouped by gender.

Results: Two-year mortality was 13.5%. Crude analysis showed that old age, diabetes, stroke, depressive symptoms BDI and CCI scores were associated with all-cause mortality, in all cases. In men, old age, hypoalbuminemia, low hemoglobin, low parathomone, heart failure, excessive daytime sleepiness and comorbidity severity were associated with mortality; in women, old age, diabetes, depressive symptoms and comorbidity severity. In the final model and in all cases, old age and comorbidity severity were associated with increased mortality; in women, comorbidity severity was the determinant of mortality; in men, old age, hypoalbuminemia, low parathormone and excessive daytime sleepiness were determinant of mortality.

Conclusions: Determinants of mortality are specific for each gender in hemodialysis patients. In men, old age, hypoalbuminemia, low parathormone and excessive daytime sleepiness are independent risk factors. These differences must be considered when evaluating effects of therapy.

Funding: Government Support - Non-U.S.

PUB259

Statins, Vitamin D, Cholesterol in End-Stage Renal Disease Ishir Bhan, Sagar U. Nigwekar, Ravi I. Thadhani. *Department of Medicine, Division of Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Randomized trials of statins in end-stage renal disease (ESRD) have failed to convincingly demonstrate an effect on cardiovascular mortality. However, recent studies in the general population have suggested a possible effect of statins on the metabolism of 25-hydroxyvitamin D (25-OH D). We sought to examine the relationship between statin use, 25-OH D levels, and total cholesterol levels in ERSD.

Methods: We obtained statin usage status, and 25-OH D levels, total cholesterol levels in a subset of patients in the Accelerated Mortality in Renal Replacement (ArMORR, n=10044) cohort of incident hemodialysis patients. We then determined associations between these factors using univariate and multivariate modeling, controlling for potential confounding factors.

Results: 26% of individuals were on statins at baseline. 25-OH D levels were available in 12.3% of subjects. In univariate analysis, there was no association between statin use and 25-OH D levels (p=0.4). However, both statin use and higher 25-OH D levels were associated with lower total cholesterol (Figure 1). In multivariate analysis controlling for age, race, sex, albumin, and season, both statin use (p=0.001) and 25-OH D (p=0.017) continued to be independently associated with lower cholesterol levels. There was no evidence for interaction between the effects of 25-OH D and statins on cholesterol.

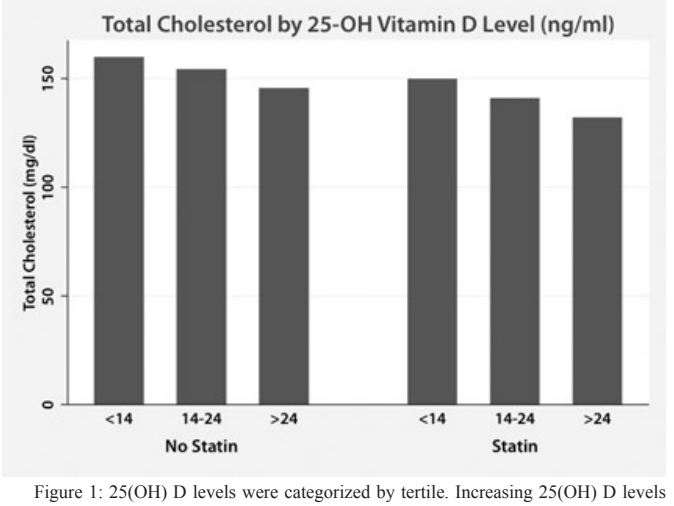


Figure 1: 25(OH) D levels were categorized by tertile. Increasing 25(OH) D levels were associated with lower cholesterol levels among statin users (p=0.003) and non-users (p=0.004). Statin use was also associated with lower total cholesterol (p<0.001).

Conclusions: In patients initiating hemodialysis, both statin use and higher 25-OH D are independently associated with lower total cholesterol levels. Future studies of statins in ESRD should examine the additional value of optimizing vitamin D status.

Funding: NIDDK Support

PUB260

Frequent Epoetin Alfa Dose Titrations Observed in End-Stage Renal Disease (ESRD) Patients T. Christopher Bond, Steven Wang, Jaime Rubin. *DaVita Clinical Research, Minneapolis, MN.*

Background: There is increasing pressure to maintain hemoglobin (Hb) levels in the range of 10-12 g/dL in U.S. dialysis patients (pts). Physicians use dose titration to attempt to keep patients in Hb range. We sought to characterize EPO dose titrations patterns in patients at a large dialysis organization.

Methods: We examined all EPO doses in patients treated at DaVita dialysis clinics between 1/2009-12/2010. Pts were age ≥18 years, prevalent (>120 days on dialysis), receiving in-center hemodialysis 3 times per week. We segmented EPO utilization into 3 categories. "Dose holds" were defined as ≥3 consecutive sessions with zero EPO dose. "Stable periods" were defined as consecutive doses within 10% of 1st dose in the period, ignoring zero EPO doses lasting <3 consecutive sessions. Non-dose hold and non-stable periods were defined as "transition periods". We defined titrations as absolute differences of ≥ 10% between mean EPO dose in consecutive periods. Dose changes on either end of a dose hold were considered 1 titration if the dose returned to within ±10% of the dose in the period immediately prior to the dose hold; they were considered 2 titrations otherwise. Titration frequency was calculated at the clinic level as the total number of titrations divided by the total number of patient-months.

Results: 84,126 pts from 1,627 clinics met the inclusion criteria; mean per clinic = 52; mean follow up = 15 months. Results show that EPO is titrated 1.1 times per month on average (median = 1.1; SD = 0.25). Only 2% of clinics averaged < 0.6 titrations per patient-month, while majority of clinics (71%) averaged ≥1 titrations per patient-month. Of the observed titrations, there were an equal number of up and down titrations not from/to a dose hold (41%). The remaining titrations were to or from a dose hold.

Conclusions: Results suggest that physicians titrate EPO in ESRD patients very frequently, with the majority of patients receiving at least 1 dose titration per month. Further research is needed to determine if these frequent dose titrations are associated with time in target range.

Funding: Pharmaceutical Company Support

PUB261

The Effect of Long Acting ESA on Maintaining Stable Hb Levels in Chronic HD Patients; 3 Year Observational Study Christopher Brown, Rupinder Rai, Jennifer Williams, Sadananda V. Aithal, Ashraf I. Mikhail. *ABM ULHB.*

Background: Increasingly, the focus of anaemia management of CKD has shifted from absolute Hb levels to achieving stable Hb with minimal variability. The risk of failing to maintain or overshoot Hb levels in chronic dialysis patients has been a concern with long acting ESA.

We assessed the efficacy of long acting ESA in maintaining Hb levels in chronic HD patients.

Methods: This study used data from 83 patients switched from shorting acting sc epoetin β to CERA. Six months treatment period with epoetin β were compared with upto 3 years treatment with CERA. Hb stability was analysed using Hb levels, number of Hb excursions and residual standard deviation of Hb regression. Two methods of analysis was used to assess Hb stability:

Method 1:- Quantifying excursions of ≥1g/dL outside individual mean Hb levels.

Method 2:- Regressional residual SD, a surrogate marker for Hb stability. RSD has been validated to be one of the best measure of Hb stability.

Treatment approach to iv Iron was not changed throughout the study.

Results: Throughout the observation, ESA dose was modified to maintain the Hb within the target range. During the observation period and due to the publication of large multi-centre studies and regulatory advice, target Hb range was lowered from (10.5 - 12.5) to (10 - 12) g/dL.

6 monthly Hb parameters before and after switch

	Pre-switch (months -6 to -1)	Months 12 to17	Months 18-23	Months 24-29	Months 30-35
Mean Hb	11.8	11.2	11.2	11.2	11.5
Epoetin (IU/wk)	8575				
CERA (mcg/Mth)		212	230	272	260
No. Hb excursions/pt	1.07	0.94	0.95	0.84	0.88
No. low excursions/pt	0.53	0.49	0.44	0.34	0.42
No. high excursion/pt	0.54	0.45	0.51	0.50	0.46
Residual SD	0.7	0.63	0.61	0.60	0.62

Aspiration Hb targets were maintained throughout the observation period. The no. of Hb excursions decreased 1 year post switch; this reduction was maintained throughout the 3 year observation period. The residual SD of Hb (a marker for Hb variability) showed a trend for reduction but did not reach statistical significance.

Conclusions: Long acting ESA therapy can effectively maintain stable Hb in chronic HD patients.

Further analysis of these data assessing the relationship of Hb stability to patient outcomes will be presented.

Funding: Government Support - Non-U.S.

PUB262

Influence of Different Erythropoietin Stimulating Agents on Stability of Hemoglobin Levels in Patients on Maintenance Hemodialysis Maciej Drozdz, Dominik Cieniawski, Karolina Dudek, Maja Koziarz, Sylwester Smialek. *Dept. of Nephrology, Jagiellonian University Collegium Medicum, Cracow, Poland.*

Background: Fluctuations of hemoglobin (Hb) level in dialysed patients leading to changes its concentration outside the target range (10 to 12g/dl) may contribute high cardiovascular mortality in this group. One of the factor affecting these fluctuations might be the choice of erthropoiesis stimulating agent (ESA).

The aim of the study was to find out if using different ESAs - methoxy polyethylene glycol-epoetin β (PEG-epoietin β) and darbepoetin α may affect the stability of Hb level in patients on maintenance hemodialysis.

Methods: The study was composed of 54 (35 M and 19 F) stable hemodialysed patients aged was form 23 to 85 years, mean 57.7 yrs. Time on dialysis was between 16 to 218 months and all of the patients were treated with ESA at least 12 months before beginning of the study. During 12 months of observation Hb level, transferrin saturation and ESA dose were controlled monthly. ESA dose was determined so that Hb level was between 10 to 12g/dl. Only patients, who finished a year of observation were covered in analysis (population per protocol). Before the study patients were randomly (2:1 ratio) assigned to group treated with methoxy PEG-epoetin β (n=36) and group treated with darbepoetin α (n=18).

Results: At the beginning of the study Hb level was: 10.65 vs 11.21g/dl and after 12 months of observation: 10.61 vs 11.6g/dl (no statistically significant difference), respectively. Mean transferrin saturation was 43.9 vs 44.1% and there was no significant difference between groups. During observation in the group treated with methoxy PEG-epoetin β 26.67% of Hb measurements were outside target level. In group treated with darbepoetin α 45.92% of them were outside target level and the difference was statistically significant (Chi^2=12.4; p<0.004). 3.58 changes of methoxy PEG-epoetin β dose and 5.18 changes of darbepoetin α dose was needed during observation, but this difference wasn't statistically significant.

Conclusions: Therapy with methoxy PEG-epoetin β provided better stability of Hb level achieved by lower number of dose changes.

PUB263

Maintenance of Hemoglobin Levels with Once-Monthly C.E.R.A. in Chronic Kidney Disease Patients – Data from the MORAL Study, a Phase IIIb, Single Arm, Open Label Study Neval Duman, Abdullah Uyanik, Abdulkadir Unsal, Siren Sezer, Taner Camsari, Mustafa Cirit, Mehmet Emin Yilmaz, Bulent Altun, Murat Duranay, Alaattin Yildiz, Idris Sahin, Ayhan Dogukan, Sedat Ustundag, Ibrahim Karayaylali, Arzu Kahveci, Sukru Sindel, Yavuz Yenicerioglu, Ahmet Alper Kiykim, Ertugrul Akbas, Fatih Ozdener. *Ankara Univ. School of Med.*

Background: MORAL study was conducted to assess the long term maintenance of hemoglobin (Hb) levels, with once-monthly IV administration of continuous erythropoietin receptor activator (C.E.R.A.) in patients with chronic kidney disease (CKD) previously treated with other erythropoiesis stimulating agents (ESAs) and to evaluate safety and tolerability.

Methods: This study consisted of a 4-week Study Verification Period (SVP), a 16-week Dose Titration Period (DTP), an 8-week Efficacy Evaluation Period (EEP) and a 4-week follow-up period.

Results: Totally 173 patients were screened in the SVP, 132 of which entered the DTP and 107 the EEP. Forty-four patients reported 73 adverse events, 15 of which were serious. Hypertension and bronchitis were the most frequent adverse events reported, occurring in 3.8% and 2.3% of patients, respectively. Two treatment-related serious adverse events were reported and both resolved.

Table 1. Study results

	ITT n=127	PP n=84
Mean (SD) BL Hb concentration g/dL	11.1 (0.56)	11.1 (0.54)
Mean (SD) EEP Hb concentration g/dL	11.3 (1.06)	11.3 (1.07)
Hb concentration ±1 g/dL of baseline & within 10-12 g/dL during EEP n (%)	44 (34.7)	39 (46.4)
Hb concentration 10-12 g/dL during EEP n (%)	65 (51.2)	54 (64.3)
Mean (SD) change in Hb concentration from baseline g/dL	0.29 (1.08)	54 (64.3)
Median time in target range days	38	38

Conclusions: The results of this study further demonstrate that once-monthly C.E.R.A. administration maintains Hb levels within the target range. The data contributes to the clinical efficacy and safety experience of once-monthly C.E.R.A. in clinical practice.

Funding: Pharmaceutical Company Support

PUB264

Effectiveness and Safety of Methoxy Polyethylene Glycol-Epoetin beta for the Treatment of Anemia in Patients with Chronic Kidney Disease in Hemodialysis (EPOSEM Study) Joan Fort,[1,3] Fernando Marco,[2] Rafael Ramos,[2] Mouhssen El Manouari,[3] Bernat De la Torre,[4] Antoni Pelegri,[3] Jorge Bartolome,[5] Irene Agraz,[1] Daniel Seron.[1] *[1]Nephrology, Vall d'Hebron Hospital, Barcelona, Spain; [2]Nephrology, CD Diaverum Nephros, Barcelona, Spain; [3]Nephrology, CD Diaverum Verge de Montserrat, Barcelona, Spain; [4]Nephrology, Diaverum Clinica E. Rotellar, Barcelona, Spain; [5]Nephrology, Clinica Secretari Coloma, Barcelona, Spain.*

Background: To evaluate the effectiveness of Mircera® in prefilled syringes in the treatment of anemia in patients with chronic kidney disease (CKD) on hemodialysis.

Methods: Multicenter, observational, retrospective study. Included patients aged ≥ 18 y.o. with anemia associated with CKD on hemodialysis (HD), who have begun treatment with Mircera® at least 6 months before and were on hemodialysis 6 months before the start of Mircera®.

Results: A total of 57 patients were included in the analysis (61.4% men and 38.6% women). Mean time on HD was 4.4 years. All patients received previous treatment with an eritrophoietic stimulating agent (ESA) before starting treatment with Mircera® (70.2% epoetin beta, 10.5%epoetin alfa, 24.6% darbepoetin). Average dose of conversion to Mircera® were: 123.7 µg/month (DA 40-80 or 8000-16000 epoetin weekly) 102.8 µg/month for lower doses and 200 µg/month for patients with higher doses being below those recommended in SPC. Median Haemoglobin (Hb) at baseline was 11.2 g/dl, 11.6 g/dl at the start of treatment Mircera® and 11.0 g/dl at follow-up.

19.3% and 15.8% of patients were within the Hb range of 10-12 g/dl (p> 0.05), without differences between the two time periods . No differences either between the percentage of patients with Hb <10 and Hb> 12 g/dl, or between the percentage of patients maintaining Hb between 10-13 g/dl or complications (p> 0.05) during the two treatment periods.

Conclusions: In HD patients the conversion to monthly Mircera from other ESAs is safe and effective, maintaining Hb levels within the desired targets.

PUB265

Hemoglobin Variability in Peritoneal Dialysis Patients Treated with Erythropoiesis Stimulating Agents Ashwani K. Gupta,[1] Sumit Narula,[1] Ramesh Saxena.[2] *[1]Nephrology, University of Florida, Jacksonville, FL; [2]Nephrology, University of Texas Southwestern, Dallas, TX.*

Background: Hemoglobin (Hb) variability and cycling have been documented both in Hemodialysis and Pre-dialysis patients receiving Erythropoiesis stimulating agents(ESA). Increased Hb variability (HbVar) has been linked to greater morbidity and mortality in these populations. Similar data for PD patients is extremely limited. A previous report of 12 PD patients from the Netherlands reported the average amplitude of HbVar to be 3.1 g/dL.

We hypothesized existence of HbVar in PD patients and conducted a pilot study to characterize it.

Methods: A random sample (10%, n=20) of prevalent PD patients at a university based PD program was chosen for analysis. Hb, ESA doses and laboratory parameters were abstracted for a period between September 2009 and February 2011(mean follow up= 14 months). HbVar was examined by calculating the standard deviation of Hb within each subject and across all subjects.

Results: 95% of the observed Hb values varied by upto 2.8g/dL(2SD) from the mean. Table 1 summarizes the study findings. 3 patients (15%) did not require therapy with EPO. HbVar was significantly lower in patients not requiring EPO in comparison to those requiring EPO(0.65 vs 1.4 g/dL, p value<0.001). The mean EPO dose was 12,000 U/wk(Range 1000-54000 U/wk). Mean number of dose chages were 10/pt(range 3-15).

Patient Characteristics and HbVar

Mean(SD)	Hb(g/dL)	Albumin	PTH	Ferretin	Saturation(%)
All Patients(n=19)	11.5(1.7)	3.6(0.6)	433(400)	800(400)	35(18)
No ESA(n=3)	12.9(0.6)				
Recieving EPO(n=14)	11.2(1.4)				

Table 1

Conclusions: This pilot study confirms the existence of HbVar in PD patients. Intercurrent events, varying iron stores and inflammatory mediators have been implicated in HbVar in pre-dialysis and hemodialysis patients. Similar factors may play a role in PD patients. However, significantly greater HbVar in patients treated with ESA suggests a causal role of ESA administration in HbVar. PD patients may benefit from an improved ESA dosing protocol to reduce HbVar. A larger analysis including more patients will be undertaken to confirm these findings. Studies relating HbVar to outcomes are needed in PD patients.

PUB266

Factors Affecting the Levels of Serum Glycated Albumin in Peritoneal Dialysis Patients Ami Hayashi, Yoshitaka Miyaoka, Toshiyuki Nakao. *Nephrology, Shinjuku-ku, Tokyo, Japan.*

Background: Glycated albumin (GA) is an alternative glycemic maker in diabetic hemodialysis patients. However, GA levels may be affected by albumin turnover, independent of glycemic status. We investigated the dialysis-related factors which affect GA levels in peritoneal dialysis (PD) patients.

Methods: The levels of GA, HbA_{1c}, serum glucose, and other clinical variables related to PD, namely, daily peritoneal protein loss (PPL), peritoneal weekly creatinine clearance (pwCcr), and dialysate-to-plasma creatinine ratio (D/Pcr) were measured in 33 stable PD patients (13 diabetic, 20 non-diabetic; age, 59.9 ± 10.5 years; men/women, 25/8; dialysis duration, 44 ± 35 months). The variables for each patient were calculated as the mean values of 3 monthly measurements. The correlations between GA and these variables were examined using the Pearson correlation coefficient and stepwise multivariable regression analysis.

Results: GA, HbA_{1c}, and serum glucose levels were significantly higher in diabetic patients than in non-diabetic patients (diabetic, 17.0% ± 2.91%, 6.4% ± 0.8%, 140 ± 49 mg/dl; non-diabetic, 13.3% ± 1.89%, 5.1% ± 0.3%, 99 ± 11 mg/dl; all, $p < 0.001$). In diabetic patients, a significant correlation was found among serum glucose, HbA_{1c} and GA levels. In non-diabetic patients, a significant correlation was found between pwCcr and GA levels. There were no significant correlations among serum albumin, PPL, D/Pcr, and GA levels in either diabetic or non-diabetic patients. Stepwise multivariable regression analysis showed that serum glucose and pwCcr were significant variables associated with GA levels in all patients (β= 0.69, -0.44, R^2 = 0.72). In the diabetic patients, serum glucose, pwCcr and age were significant variables (β= 0.62, -0.31, -0.42, R^2 = 0.87). In the non-diabetic patients, only pwCcr was a significant variable associated with GA levels (β= -0.64, R^2 =0.41).

Conclusions: GA was affected by pwCcr in both diabetic and non-diabetic PD patients.

PUB267

Injection of Darbepoetin alfa at the Start of a Hemodialysis Session Might Be More Efficient Than Injection at the End of the Session Noritomo Itami. *Kidney Center, Nikko Memorial Hospital, Muroran City, Hokkiado, Japan.*

Background: It has been noted that the injection of darbepoetin alfa(DA) at the end of hemodialysis(HD) was often neglected because of the confusion that can occur at the end of the HD session due to complications such as hypotension, vomiting and cramps. Injection of DA can be performed with more certainty at the start of HD but there is the possibility that an increased dose of DA will be required because of its adherence to the dialysis system's tubing.

Methods: Thirty-eight patients were recruited (M/F Ratio, 20:18; Age: 63.6±10.5yrs; Primary disease: Diabetes(13), Glomerulonephritis(12), Polycystic kidney(3); others(10)). Written informed consent was obtained. At the start of the study, DA treatment was changed from the end of HD (Period I) to the start of HD (Period II). Other anemia treatment was carried out as usual. DA dose was altered biweekly according to our algorithm which was presented at the 52nd Congress of the Japanese Society of Dialysis Therapy. The algorithm maintains a target hemoglobin(Hb) level of 10-12g/dL while keeping the change in Hb to within 0.5g/dL to prevent the occurrence of Hb cycling. After 24 weeks, the average DA dose and clinical parameters were examined.

Results: Hb was unchanged at the end of both periods (Period I: 11.01±0.95g/dL and Period II: 10.91±0.87g/dL, not significant(NS)). Serum ferritin increased (143.9±94ng/mL and 193.2±126.5ng/mL, $p<0.001$) although the number of patients undergoing iron treatment (Period I: 18.8±4.1 and Period II: 16.3±2.4, NS) was unchanged. Average DA dose in Period II was significantly reduced from 18.0±11.6mg/week in Period I to 16.1±10.7mg/week in Period II($p<0.05$). The average fulfillment of target Hb(%) was 69.4±8.4% in Period I and 77.6±4.3% in Period II($p<0.01$). There was no neglection of injections of DA in Period II.

Conclusions: The injection of darbepoetin alfa at the start of an HD session might be more efficient and reliable than injection at the end of the HD session. A multicenter study providing access to more patients and a longer time period is warranted.

PUB268

Poor Performance of Correction Formula for the Prediction of True Hypocalcemia in Dialysis Patients Zainab Khan, Christine A. White, Alexander R. Morton, Rachel M. Holden. *Medicine, Queen's University, Kingston, ON, Canada.*

Background: Abnormalities in serum calcium are frequently encountered in patients receiving dialysis therapy. Total calcium is most frequently measured because of sample handling and cost concerns. Given that the ionized form is biologically active a number of adjustment formulae have been derived to "correct" the total calcium for changes in serum albumin (SA) and phosphorus (P). International guidelines for nephrology recommend that calcium be kept in the normal range reference range and K/DOQI recommends using SA-corrected formulas. We determined the accuracy of noncorrected calcium and 5 published corrected calcium formulae in the identification of abnormal ionized calcium in a cohort of hemodialysis (HD)patients.

Methods: Ionized calcium (iCa), total calcium (tCa), serum albumin (SA), total protein (TP) and phosphorus (P) were measured in HD patients. The accuracy with each each SA and P corrected formulae (Table 1) identified abnormal iCa was determined.

Results: There were 73 HD patients with a mean age of 63 y. There were only 3 cases of hypercalcemia (ica > 1.31 mmol/L). There were 50 cases of hypocalcemia (iCa < 1.19 mmol/L). The mean SA was 35.1. The accuracy of each formula to predict abnormal iCa is demonstrated in the table. Non-corrected tCa identified true hypocalcemia in 38% of cases with minimal improvement achieved with mathematical correction. The single P-corrected formula was least accurate in this sample.

	low ionized Ca	high ionized Ca
	calculated low (%)	calculated high (%)
tCa+0.0176 x (34-SA)	42	33.3
tCa+0.02 x (40-SA)	22	66.7
tCa+(0.015 x (40-SA)+0.07 x (1.5 - P)	24	66.7
tCa+0.01 x (30-SA)	52	33.3
tCa+0.018 x (35-SA)	40	66.7
Uncorrected tCa	38	66.7

Conclusions: There was a high prevalence of hypocalcemia in this cohort of HD patients. In patients with difficult to control hyperparathyroidism, it is important to know that true hypocalcemia is not present particularly when vitamin D analogues and calcimimetics are being used. There is little benefit of SA or P corrected forumulae in identifying true hypocalcemia. Future studies should identify those dialysis patients best monitored by ionized calcium values.

PUB269

The Association of Nutrition with Interdialytic Weight Gain and Depressive Disorder in Hemodialysis Patients Hyun Gyung Kim, Young Ok Kim. *Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: Malnutrition was reported to occur in 23-76% and known as an important predictor of increased mortality in maintenance hemodialysis (HD) patients. This study was performed to evaluate the association of nutrition with interdialytic weight gain (IDWG) and depression in HD patients.

Methods: Sixty-five HD patients for at least 3 months were enrolled. We investigated malnutrition by OSND (Objective score of Nutrition on dialysis) score and depressive disorder by Montgomery depression rating scale and Hamilton depression rating scale. We compared the clinical and biochemical profiles according to the malnutrition.

Results: Mean age of the patients was 55.7 ± 12.6 years and patients with diabetes accounted 55.4%. Mean duration of HD was 36.2 ± 32.0 (4 - 129) months. Depressive disorder was diagnosed in 21 (32.3%), Malnutrition (OSND score < 22) in 24 (36.9%) and large IDWG (>1kg/day) in 40 (61.5%) out of the 65 HD patients. Patients with malnutrition had lower incidence of large IDWG (45.8% vs 70.7%, p=0.046) and depression (19.5% vs 54.1%, p=0.004) than those without. BMI (21.2 ± 3.0 vs 23.4 ± 3.0 kg/m^2 ,p=0.006), TSF (triceps skin fold thickness, 8.8 ± 4.2 vs 15.0 ± 6.2 mm, $p<0.001$), MAC (mid-arm circumference, 23.6 ± 2.7 vs 26.4 ± 3.0 cm, p=0.001), serum albumin (3.6 ± 0.3 vs 3.8 ± 0.2 g/dL, p=0.029) and total cholesterol (142.5 ± 29.2 vs 169.7 ± 31.2 mg/dL, p=0.001) were also lower in patients with malnutrition, compared to the patients without malnutrition. There was no difference in age, gender, diabetes and HD duration between the two groups. In multivariate analysis, Depressive disorder was an independent risk factor for malnutrition.

Conclusions: This study showed that depressive disorder was significantly related to malnutrition in maintenance HD patients. Therefore, we suggest that the depressive disorder may be considered in HD patients at the time of the assessment of nutritional status in maintenance HD patients.

PUB270

The Factors Affecting Health-Related Quality of Life in Dialysis Patients Owen Kwon,[1,3] Ji-Young Choi,[1,3] Jung-Ju Seo,[1,3] Jang-Hee Cho,[1,3] Mi-Kyung Jin,[1,3] Kyung-Deuk Hong,[1,3] Chung-Hoon Yu,[1,3] Se-Hee Yoon,[1,3] Sun-Hee Park,[1,3] Chan-Duck Kim,[1,3] Ki-Soo Park,[2,3] Yong-Lim Kim.[1,3] *[1]Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea; [2]Preventive Medicine, Gyeongsang National University School of Medicine, Jinju, Korea; [3]Clinial Research Center for End Stage Renal Disease, Korea.*

Background: To investigate the factors influencing health-related quality of life (HRQOL) in ESRD patients on dialysis, we measured the level of HRQOL.

Methods: The study subjects were 237 patients with ESRD on HD or PD over 6 months in two university hospitals in Korea. Patients completed the Korean version of KDQOL (kidney disease quality of life)-36 with five subscales. Measures of self-efficacy and treatment satisfaction in other studies were translated and modified for this study. Various sociodemographic and clinical variables were also recorded.

Results: The quality of life score were 46.3, 49.2, 67.6, 58.5, and 41.1 in the subscales of physical, mental component, symptoms and problems, effect of kidney disease on daily life, and burden of kidney disease, respectively. Variables associated with better physical component were male gender, lower age, higher educational level, having an occupation, higher self-efficacy, and higher treatment satisfaction; a variable associated with better mental component was male gender; variables associated with better symptoms and problems component were hemodialysis and self efficacy. Burden of kidney disease component was associated with educational level, primary cause of ESRD and self efficacy. Effect of kidney disease component correlated positively with self-efficacy and treatment satisfaction. Multiple linear regression analysis showed that independent variables associated with HRQOL were age, educational level, duration and adequacy of dialysis, primary cause of ESRD, self-efficacy, and treatment satisfaction.

Conclusions: Adequacy of dialysis, self-efficacy and treatment satisfaction were associated with HRQOL in ESRD patients on dialysis. Strategies to increase adequacy of dialysis, self-efficacy and treatment satisfaction may be helpful to enhance the HRQOL in dialysis patients.

Funding: Government Support - Non-U.S.

PUB271

The Newly Identified Anorexigenic Adipokine Nesfatin-1 in Hemodialysis Patients: Associations with Protein Intake and Body Composition Denise Mafra,[1] Juliana Saldanha,[1] Julie Lobo,[3] Milena Barcza Stockler-Pinto,[3] Viviane Oliveira Leal,[1] Antonio Calixto,[4] Bruno Geloneze,[4] Denis Fouque,[5] Juan J. Carrero.[2] *[1]Clinical Nutrition, Federal University Fluminense, Niteroi, Rio de Janeiro, Brazil; [2]Division of Renal Medicine, Karolinska Institutet, Sweden; [3]Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [4]Laboratory of Investigation on Metabolism and Diabetes, State University of Campinas, Campinas, Brazil; [5]Nephrology, Universite Claude Bernard I - Hôpital Edouard Herriot, Lyon, Rhone, France.*

Background: Nesfatin-1 is a anorexigenic peptide. Anorexia and malnutrition are common features of chronic kidney disease (CKD) that predispose patients to worse outcomes. The aim of this study was to evaluate the plasma nesfatin-1 levels in hemodialysis (HD) patients.

Methods: Twenty five HD patients were studied and compared to 15 healthy subjects matched for body mass index (BMI), % body fat mass (BF) and age. Nesfatin-1 levels were analyzed using ELISA and, leptin levels were measured by a multiplex assay kit manufacture by R&D Systems®. Appetite was measured using a specific questionnaire and food intake were recorded.

Results: Plasma nesfatin-1 levels did not differ between HD patients and healthy subjects.

Clinical and anthropometric characteristics of the HD patients and healthy subjects

Parameters	HD Patients	Healthy Subjects
Age (years)	53.2±11.9	47.9±14.8
BMI (kg/m2)	23.1±2.8	24.9±3.9
Triceps skinfold thickness (mm)	13.1±4.9	15.2±5.9
Body fat (%)	28.6±6.5	29.3±5.2
Leptin (ng/mL)	13.9±21.2*	5.1±4.3
Nesfatin-1 (ng/mL)	0.16±0.07	0.17±0.10

*p<0.05

Nesfatin-1 levels showed significant negative correlations with protein intake (r=-0.42; p=0.03), and positive correlations with BMI (r=0.33; p=0.03), % body fat (r=0.35; p=0.03) and the triceps skinfold thickness (r=0.36; p=0.02). Nesfatin-1 levels also correlated positively with leptin levels (r=0.45; p=0.006).

Conclusions: In conclusion, nesfatin-1 levels did not differ between HD patients and healthy subjects who were matched for BMI, % body fat and age. However, nesfatin-1 correlated with protein intake and body composition.

Funding: Government Support - Non-U.S.

PUB272

Is Body Mass Index of 23kg/m² a Reliable Marker of Protein-Energy Wasting in Hemodialysis Patients? Denise Mafra,[1] Viviane Oliveira Leal,[1] Cristiane Moraes,[1] Milena Barcza Stockler-Pinto,[2] Julie Lobo,[2] Najla Elias Farage,[3] Denis Fouque.[4] *[1]Clinical Nutrition, Federal University Fluminense (UFF), Niterói, Rio de Janeiro, Brazil; [2]Institute of Biophysic Carlos Chagas Filho, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil; [3]Nutrition, RenalCor Clinic, Rio de Janeiro, Brazil; [4]Nephrology, Université Claude Bernard I, Lyon, Rhône, France.*

Background: There are several parameters that may be indicative of protein-energy wasting (PEW) in hemodialysis (HD) patients and the International Society of Renal Nutrition and Metabolism panel reccomends that a body mass index (BMI) less than 23kg/m² is a marker of PEW. However, BMI can be heavily influenced by fat mass and the adiposity is related to inflammation. Therefore, the adequate cut-off point by BMI remains controversial and this study compared the body composition and inflammatory status of HD patients according to the cut-off of 23kg/m² for BMI.

Methods: Forty seven HD patients (30 men, 11 diabetic, 53.8 ± 12.2 yr, 58.2 ± 50.9 months on dialysis) were studied. Anthropometric data and handgrip strenght (HGS) were evaluated. Inflammatory markers (CRP, TNF-α, leptin and IL-6) were measured.

Results: Nineteen (40.4%) patients presented BMI values < 23kg/m², and leptin levels, midarm muscle circunference and free fat mass (FFM) were significantly lower in these patients when compared to patients with BMI > 23kg/m². However, the prevalence of muscle functional loss was not different between the BMI groups. The percentage of body fat (%BF), fat mass (FM), FM/FFM ratio and waist circumference (WC) were significantly lower in patients with BMI < 23kg/m² but the mean values of %BF did not indicate energy wasting. Patients with BMI > 23kg/m² presented higher prevalency of inflammation and the WC and %BF values were compatible with metabolic derangements associated with obesity. The adiposity parameters were correlated with CRP and leptin.

Conclusions: HD patients with BMI < 23kg/m² did not presented signs of energy wasting while those with BMI > 23kg/m² were more inflammed probably due higher adiposity. Thus, the BMI of 23kg/m² seems not to be a realiable marker of PEW in HD patients.

Funding: Government Support - Non-U.S.

PUB273

Efficacy of Intradialytic Parenteral Nutrition on the Aminoacid Status: Preliminary Results of a Kinetic Study Elena Mancini, Antonio Santoro. *Nephrology Dialysis Hypertension, Policlinico S.Orsola-Malpighi, Bologna, Italy.*

Background: Intradialytic parenteral nutrition (IDPN) may be useful to contrast malnutrition of hemodialysis (HD) patients. However, it is essential to verify whether IDPN is actually able to draw positively upon metabolism, enriching the aminoacid (AA) status, thereby enhancing protein synthesis.

A kinetic study was planned to evaluate whether IDPN can induce an effective AA gain in the short-run in malnourished HD patients.

Methods: Ten HD patients (3 x week; Bologna Malpighi & Trento Hospitals) with albumin <3.5 gr/dl and pre-albumin < 30 mg/dl for at least one month were studied (low-flux membrane, 240 min/session). After a dialysis study session without IDPN, patients received IDPN for one month (*all-in-one* bag NutriSpecialipid: 625ml, Proteins 35.9 gr, 10 AA: Ser, Pro, Gly, Ala, Val, Met, Leu, Phe, Lys, His). In the first session, as well as after 2 and 4 weeks, we measured the plasma (pre- and post-HD), and dialysate AA concentration (dialysate spilling technique at 120 and 240 min). Albumin, pre-albumin, glucose and lipid metabolism, inflammation indices and blood cell count were also evaluated.

Results: Preliminary results show that even with a low-flux dialyzer and without IDPN infusion an AA loss in dialysate does exist. With the IDPN infusion that loss actually increases (43±13 with IDPN *vs* 23±14mmol/L without IDPN, p<0.001). Nevertheless, after one month IDPN, the pre-dialytic plasma concentration of each infused AA was increased as compared with the basal value (minimum 5.2% for Tyr, maximum 66% for Try), with a mean increment of 36.8%. No derangement was found in glucose and/or lipid metabolism.

Conclusions: In one month of IDPN, a time range that is quite insufficient to induce a clinically relevant change in the nutritional state, a homogeneous growth in the plasma concentration of all the infused AA was evident, however, leading us to hypothesise that the use of IDPN for a longer time-span may actually translate into increased protein synthesis.

Funding: Private Foundation Support

PUB274

Stopping of Dialyzer Reuse and Switch to High-Flux Dialyzers Is Associated with Lower ESA Requirement and Better Phosphare Control Andrzej Milkowski,[1] Teresa Rydzynska,[1] Jolanta Malyszko.[2] *[1]Fresenius Nephrocare, Poland; [2]Nephrology, Medical University, Bialystok, Poland.*

Background: Dialyzer reuse is still common practice in some dialysis units. There are three major concerns with reuse: the risk of infection; biochemical and immunologic effects; and loss of performance with impairment in clearance and/or ultrafiltration.

Methods: The aim of our study was to assess the dialysis adequacy, anemia control and use of ESA as well as phosphate control in 68 patients from a single center before and 1 year after stopping of reuse of dialyzers. In addition, together with single use of dialyzers, all the patients were switched to high-flux dialyzers (Fresenius, Germany).

Results: All the parameters are given in the Table 1.

Parameters before and 1 year after stopping of dialyzer reuse

	before	after
Phosphate (mmoL/L)	1.87±0.55	1.73±0.51 *
Urea before HD (mmoL/L)	24.01±6.35	24.24±6.90
Urea after HD (mmoL/L)	7.40±2.41	6.64±2.15
Urea reduction ratio (%)	69.34±6.79	72.54±4.73 ***
Kt/V	1.46±0.54	1.54±0.17
HD time weekly (hours)	11.71±2.27	12.34±1.54 *
ESA weekly dose (IU)	3485±2179	2846±2706 **
ESA dose per kg dry body weight	55.12±38.41	45.42±45.56 **
Serum iron (nmoL/L)	12.07±4.38	13.76±5.99
TSAT (%)	32.34±19.02	34.64±17.77
Iron iv weekly (mg)	54.76±71.84	53.73±67.03
Hb (mg/dL)	10.74±1.66	11.13±1.58 p=0.06

*p<0.05, ** p<0.01. ***p<0.001

One year after switch to single use high flux dialyzers a highly significant rise in urea reduction ratio was observed, together with a tendency to rise in Kt/V. Moreover, use of ESA dropped significantly in the study group with a tendency to higher achieved hemoglobin, whereas the iron supplementation did not change. A significant fall in serum phosphate was also observed.

Conclusions: Single use high-flux dialyzers are more efficient in clearing urea and other molecules. Moreover, after switch amelioration of anemia with lower use of ESA was demonstrated together with better phosphate control. It may be associated with better survival and lower costs. However, further investigation is required to accurately assess the morbidity and mortality associated with reuse or single use as well as cost effectiveness.

PUB275

C-Peptide (CPR) Levels of Diabetic Patients (pts) on Maintenance Hemodialysis (HD) as Compared to Non-DM HD pts Ibuki Moriguchi,[1] Michihito Okubo,[1] Yoko Itoh,[1] Naoyuki Kobayashi,[1] Shokichi Naito,[2] Kei Kobayashi,[2] Yasuo Takeuchi,[2] Kouju Kamata.[2] *[1]Sohbudai Nieren Clinic, Zama, Kanagawa, Japan; [2]Dept. of Internal Med., Kitasato University, Sagamihara, Kanagawa, Japan.*

Background: CPR concentration in HD pts was known to be higher as compared to pts with normal renal function.

The purpose of this study was to investigate the plasma CPR levels in 28 type 2 DM HD pts and to compare them with levels in 57 non-DM HD pts(control). Further, DM HD pts were split into three treatment groups, i.e., 12 pts given insulin, those under diet control only(12pts) and the remaining 4 treated with other anti diabetics. Their respective CPR levels were studied.

Methods: Mean age was 64.3±9.5 (mean ±SD) years in DM pts versus 57.8± 16.0 yrs in non DM. Vintage on HD was 5.1±3.7 yrs (DM) vs. 10.3±8.0 yrs (non-DM). CPR (ng/ml), HbA1c (%) and glycated albumin (GA, %) were measured in pre-and post-HD samples of the first weekly session. Statistical difference between groups was analyzed by Mann-Whitney U test and chi-square test. Correlation coefficient was also calculated.

Results: 1. Pre-HD CPR levels in DM pts was significantly lower than those in non-DM pts (8.59±5.06 vs. 11.68±4.47 p<0.0052). Post-HD CPR levels were significantly lower than pre-HD levels both in DM and non-DM pts (2.74±1.47 vs. 5.44±4.40, p<0.001). CPR in pts on insulin were significantly lower than those in pts on dietary control (pre-HD: 6.01±3.49 vs. 10.70±5.92 p<0.0243; post-HD: 1.97±0.98 vs. 3.24±1.57, p<0.0282)

2. Pre-HD GA as well as HbA1c levels in DM pts were significantly higher than those in non DM pts. Post-HD GA levels were significantly decreased compared with pre-HD levels both in DM and non-DM pts.

3. A significant negative correlation was observed between serum levels of CPR and GA only after HD session both in DM (r=-0.373, p<0.0499) and non-DM pts (r=-0.388, p<0.0003).

These data indicate that DM HD pts may have a lower insulin secretion from their pancreas than that in non DM HD pts. Post-HD decrease in CPR levels might be induced by an effective removal through high-flux membrane used in this study.

Conclusions: Pre-HD CPR level may reflect pancreatic secretion of insulin as well as the effect of disturbed renal disposal.

PUB276

Changes in Cholesterol Related to Dialysis: Magnitude, Impact on Statin Use and Relation of Statin Use to Eythropoetin Requirements Janice G. Nigos, Jeannie P. Co, Barbara A. Clark. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: While CKD is associated with abnormailites in lipid profiles and CV risk, hemodialysis (HD) patients may have improved profiles, possibly explaining a lack of proven CV benefit of statins in this population. The purpose of the present study was to determine the magnitude of changes in lipid profiles for individual dialysis patients related to hemo or peritioneal dialysis (PD) and if these changes impacted statin use before and after regular dialysis. In addition, since statins may have pleitropic effects we wanted to see if statin use could be associated with reduced erythropoetin (Epo) requirements in HD patients.

Methods: Retrospective review of 88 charts revealed 35 HD patients and 8 PD patients with enough data to compare from 1 to 3 years before dialysis and after at least 3 months of regular dialysis. Crosssectional comparison was also made of Epo doses in stable iron replete HD pts with (n=30) or without statin use (n=34).

Results: In HD there were reductions in total cholesterol (181 ±8 vs 142±5, p<0.001), LDL (104±6 vs69±4, p<0.001), TG (163±17 vs 117±13, p<0.01), and significant improvements in HDL (44±2 vs 50±2, p<0.05) and serum albumin (3.5±0.1 vs 3.96 ±0.06, p<0.001). Statins were used in 65.7% pre versus 54.3% after. Conversely, In PD, LDL levels increased significantly (103±22 vs 131±28, P<0.05) with no significant changes in the other parameters (TC 183±22vs 208±39; TG 168±51 vs 153±57; HDL 45±19 vs 47±18; albumin 3.8±0.4 vs 3.8±0.4). Statins were used in 50% pre and 62.5% after PD. HD lowers TC, LDL, and TG by about 25%, while in PD, LDL levels rise by 25%. Epo doses were significantly lower with statin use than without (3160±353 vs 5717±664 units/ treatment, p<0.05).

Conclusions: HD is associated with favorable lipid profiles, while profiles worsen with PD. Statin use may be becoming less prevalent in HD patients. Since statins have pleitropic efffects aside from lipid lowering, including possible lower Epo requirements, additional studies of trends in statin use and the clinical impact of this in ESRD are warranted and more study is needed regarding CV outcomes with statin use in the PD population.

PUB277

Changes of Plasma Volume Play a Major Role in Intradialytic Hemoglobin Variations Esteban L. Siga,[1] Raul Coste,[2] Hugo De Palma,[3] Vito Mesina,[4] Mario Galarza,[5] Miguel Fernandez.[6] *[1]Htal Madariaga; [2]Htal Interzonal; [3]Dialysis Azul; [4]Dialysis Tandil; [5]Dialysis Olavarria; [6]CENU Trenque Lauquen, Argentina.*

Background: It is known that ultrafiltration induces cyclic Hemoglobin (Hg) variations, but others factors could also affect Hg level. Therefore, the "true" functional Hg may be different. We studied the role of changes in plasma volume and if choosing functional Hg would modify estimation of ESA needs.

Methods: Multicentric, three phases study. Post minus Pre Dialysis changes of Hg (ΔHg, gr.dl) and body weight (ΔBW, Kg) were measured. Functional Hg was calculated by the average Hg (AVGHg) and by the time-averaged Hg concentration (TACHg, Krisper P et al). Percent changes in relative plasma volume were estimated by Albumin changes (%ΔRPValb, Van Stone J et al).

Results: Phase 1: in 57 Patients (Pts), pairs of Pre and Post samples were measured separately and the repeatability coefficient was 0.29 (Bland and Altman). In the next phases, only Pts with a ΔHg 0.3 were included. Phase 2: Out of 291 Pts, a ΔHg 0.3 was observed in 241: 144 male and 97 female. ΔHg (median 1.1, Interquartile Range: 0.75-1.6) was correlated (r = 0.38, p=0.000) only to ΔBW (median 2.4, IR: 1.8-3.2). Pts of the two upper quartiles of ΔBW showed a ΔHg of 1.41 (95% CI 1.3–1.52). This change was higher than the observed in Pts of the two lower quartiles : 1.05 (95% CI 0.95–1.15, p = 0.000). Phase 3: 76 Pts with ΔBW > 2.5 were studied. %ΔRPValb was 11.4 (95% CI 10.3-12.5). Regression analysis of %ΔHg as outcome and %ΔBW or %ΔRPValb as predictor, estimates a r^2 of 0.16 and 0.41 (p= 0.041), respectively. PreHg level of phase 2 and 3 Pts (total n=312) was significantly lower than AVGHg and TACHg. Both estimations of functional Hg were similar. Of the Pts with ΔBW >2.5 and PreHg between 9-9.9, the AVGHg was ≥10 in 62%. Conversely, odds of getting out of target was high in Pts with PreHg between 11-12 and ΔBW >2.5, since 67% of them showed an AvgHg >12.

Conclusions: i) changes in RPV play a major role in Hg intradialytic variations. ii) ESA dosing in dialysis Pts with more than 2.5 Kg of weight loss should be based on the average of the Hemoglobin levels measured before AND after dialysis.

PUB278

Patterns and Predictors of Blood Transfusion among Hospitalized Hemodialysis Patients Ben C. Wong, Rick Chin, Brenda Hemmelgarn, Pietro Ravani, Robert R. Quinn. *University of Calgary, Calgary, AB, Canada.*

Background: Hospitalized hemodialysis patients may be at increased risk of blood transfusion due to acute illness and relative erythropoietin resistance. The primary objective of this study was to quantify the risk of transfusion in the three months following hospital admission and to identify important baseline predictors.

Methods: Administrative datasets were used to identify adult hemodialysis patients admitted to hospital between September 2004 and March 2008 in Calgary, Canada. Primary clinical data was abstracted from electronic medical records and linked to blood bank records and laboratory holdings. Logistic regression was used to model the risk of transfusion in the three months following admission to hospital and to identify important clinical predictors, accounting for clustering of hospitalizations within individuals.

Results: A total of 475 patients experienced 695 eligible hospital admissions. The risk of blood transfusion was 25% at 3 months. Higher baseline erythropoiesis stimulating agent (ESA) dose was an independent predictor of transfusion risk. The presence of congestive heart failure and a higher baseline hemoglobin level were protective. Of note, there was a significant interaction between baseline hemoglobin level and ESA dose such that, for a given baseline dose of ESA, a 10 unit increase in hemoglobin level was associated with a 1% reduction in transfusion risk. Age, sex, serum albumin, and a medical history of other conditions (ischemic heart disease, diabetes mellitus, peripheral vascular disease, atrial fibrillation, and stroke) were not independently associated with the risk of transfusion.

Conclusions: Among hospitalized, prevalent hemodialysis patients, there was a high risk of blood transfusion in the 3 months following admission. Future work will focus on the impact of changes in ESA dose on transfusion risk in high-risk patients admitted to hospital.

PUB279

Risk Factors of Cardiovascular Events in Peritoneal Dialysis Patients: A Single Center Experience Ana Cabrita,[1] Ana Pinho,[1] Helena Morim Carreira,[2] André Fragoso,[1] Anabela Malho,[1] Idalecio Bernardo,[1] Pedro Neves.[1] *[1]Serviço de Nefrologia, Hospital de Faro, EPE, Faro, Portugal; [2]Public Health Institute, Oporto University, Oporto, Portugal.*

Background: Cardiovascular (CV) disease is the main cause of morbidity and mortality in chronic kidney disease. Several risk factors for CV disease have been described and it is crucial to know their real predictive value. However, the number of studies regarding peritoneal dialysis (PD) patients is scarce.

The aim of our study was to evaluate the predictive factors of cardiovascular events in a sample of PD patients.

Methods: Since 2000 we prospectively followed 105 incident PD patients (mean follow up = 31 months). Data on comorbidities and laboratorial data were collected at the start of PD and the CV events were collected during the follow-up. We defined CV events as one of the following: cardiac event, cerebrovascular event, peripheral vascular event.

Results: We included in our study 105 patients, 57% males, with a mean age of 53 years at the start of PD. During the follow-up period, 36.2% patients had a CV event (1.4 episodes/patient-year). The ocurrence of CV events was significantly associated with all causes of death (p<0.001). The univariate analysis showed an association between CV events and previous hemodialysis treatment, age and C-reactive protein (CRP), albumin and parathyroid hormone levels. After adjusting for potencial confounders (gender, diabetes, hypertension, pulse pressure, peritonitis, treatment with vitamin D), the albumin (hazard ratio (HR)=7.8, 95% CI- 14.4 to 42.8), age at start of PD (HR=1.1, 95% CI- 1.03 to 1.2), male gender (HR=0.2, 95% CI- 0.03 to 0.98), pulse pressure (HR=1.1, 95% CI- 1.01 to 1.12) and CRP (HR=1.1, 95% CI- 1.05 to 1.18) remained as significant predictors of CV events in PD patients.

Conclusions: Patients with higher age at the beginning of PD, with lower levels of albumin, elevated pulse pressure and CRP levels have an increased risk of developing a CV event.

PUB280

Statin Therapy Correlates with Lower Cardiovascular Death, but Not with Lower Cardiovascular Events among Patients on Hemodialysis Edmond Deda,[1] Chrisoula Pipili,[1] Paraskevi Tseke,[1] Konstantinos Pantelias,[1] Petros Korfiatis,[1] Zoi Tegou,[2] Helen Tzanatos,[2] Eirini Grapsa.[1] *[1]Nephrology, Aretaieion University Hospital; [2]Dialysis Unit Specimed, Loutraki, Greece.*

Background: Cardiovascular events are the major cause of death among patients on maintenance hemodialysis. Regulation of cholesterol and triglyceride levels within target goals is paramount. Our purpose was to examine whether statin therapy correlated with evidenced (presence or absence) cardiovascular events among patients receiving hemodialysis.

Methods: A total of 106 patients (32 women and 74 men, aged 66 ± 14 years) on chronic hemodialysis were studied. The lipidemic profile (total cholesterol, LDL-cholesterol, triglycerides) and all the cardiovascular events (acute coronary syndrome, stroke and heart failure) as well as deaths caused from them were recorded within two - year follow up.

Results: Patients on hemodialysis with dyslipidemia showed higher rates of cardiovascular events (20% vs. 2,4%, odds ratio 3.75, p =0.005). Cardiovascular deaths were noted among 41% of patients not receiving treatment for dyslipidemia and only in 22% among them who were under treatment (statins, omega-3 or both) (p = 0.039). Logistic regression analysis showed that patients receiving treatment for dyslipidemia had 60% reduced risk for cardiovascular death compared with patients without treatment (odds ratio = 0.41, p = 0.042).

Conclusions: Patients on chronic hemodialysis present impaired lipid profile. Those received treatment for dyslipidemia presented lower risk for cardiovascular deaths, but not for cardiovascular events.

PUB281

Evolution of Diabetic Patients Older Than 65 Years Starting Hemodialysis. Comparation with Control Group Herrero Juan Carlos. *Nephrology, Hospital Severo Ochoa, Leganes, Madrid, Spain.*

Background: In the last years we have seen a significative increase of patients starting hemodialysis (HD) due to diabetic nephropathy older than 65 years. This patients has more cardiovascular risk factors and morbi-mortality.

Methods: We want study evolution, demographic and clinical characteristics of these patients (Group I) starting HD in our service, versus patients starting HD with similar age and other cause of ESRD (Group II).

Results: Between September-2006 to September-2010, 165 patients incident HD. 68 (41%) patients older than 65 years. Median follow up was 23 months (5-51). Main results shown in the table.

Characteristics	Group I (n=20)	Group II (n=48)	p
Mean age (year)	73,5±4 (65-82)	74,5±6 (65-85)	ns
Males, %	50%	56%	ns
Body Mass Index (kgr/m2) *	24±3	23±3	ns
Charlson Comorbidity Index	8,5±1,4	7,6±2	0,029
Ischemic heart disease (%)	35	15	0,06
Cerebrovascular disease (%)	25	33	ns
Peripheral vascular disease (%)	35	42	ns
Hypertension (%)	95	81	ns
Dyslipidemia (%)	55	40	ns
Hemoglobin >11 gr/dl * (%)	60	64,5	ns
Ferritin levels 100-500 mg/ml * (%)	55	64,5	ns
Serum calcium levels 8,4-9,5 mg/dl * (%)	65	60,4	0,002
Phosphate <5,5 mg/dl * (%)	95	92	ns
PTH levels 150-300 pg/ml * (%)	35	31,5	ns
Albumin >3,5 gr/dl * (%)	40	50	ns
Number Hospitalization Index (NHI)	0,35 (0-1,05)	0,19 (0-0,88)	0,053
Day's Hospitalization Index (DHI)	5,13 (0-23)	2,88 (0-29)	0,034
Death	7 (35%)	22 (46%)	ns
Survival at 12 months	75%	80%	ns
Survival at 36 months	26%	46%	ns
Renal transplant	2 (10%)	4 (8%)	ns

NS, no significant. NHI: number hospitalization / patients / month. DHI: day's hospitalization / patients / month. *: end study level

Cause of death in Group I: 4 due to infection disease, 3 to decline in general status. Main cause in Group II: 6 to decline in general status, 6 for cardiological pathology. In multivariate analysis, survival was significantly influence by DHI (p 0.001, HR 1.26, 95%CI 1.114-1.401).

Conclusions: In conclusion, patients starting HD with diabetic nephropathy older than 65 years have similar survival at 12 months, although less at 36 months, that patient in Group II. The main reason is major comorbidity, in relation with major percentage of ischemic heart disease and peripheral vascular disease, with more number and day's hospitalization index.

PUB282

Audit of the Management of Atrial Fibrillation in Haemodialysis Patients Jonathan Reaney, Cathal L. Steele, Agnes Masengu, Camille Harron, Robert Mullan, Ronan Cunningham. *Department of Nephrology, Antrim Area Hospital, Antrim, United Kingdom.*

Background: The prevalence of atrial fibrillation (AF) in haemodialysis (HD) patients ranges from 7.7% to 27%. A recent study found one-year mortality rates were twice as high in HD patients with AF than in those without. An audit of management of AF in the haemodialysis unit was carried out.

Methods: Baseline electrocardiograms (ECGs) were performed on patients attending for HD sessions and the rhythm analysed by medical staff. In addition, serum samples were analysed for high sensitivity C reactive protein (hsCRP) and high sensitivity Troponin-T (hsTropT). The notes and electronic records of patients identified with AF were reviewed to determine if AF had been previously documented. Patients identified with AF had their CHADS2 stroke risk index score calculated and management reviewed.

Results: ECGs were carried out on 57 dialysis patients. Median age was 73 years, ethnicity 100% Caucasian, female 47.4% (27/57) and diabetic 41.1% (24/57). The incidence of AF on ECG was 10.5% (6/57). No cases of undiagnosed AF were identified. No significant association between the levels of hsCRP and hsTropT and presence of AF was found. In this study, all patients with AF had a CHADS2 score ≥2. Oral anticoagulation therapy is recommended for patients with CHADS2 score ≥2. All patients with AF were on oral antithrombotic agents (Warfarin 3/6, Aspirin 2/6, Clopidogrel 1/1). The presence of AF was not clearly documented in the patient's medical records in 3/6 cases.

Conclusions: Rates of AF in our HD population were similar to rates reported from larger studies. AF in haemodialysis patients carries a significant risk of mortality. All patients identified with AF were receiving oral antithrombotic therapy however little evidence exists for the use of this for AF in this population. HD patients on warfarin have an increased risk of stroke and major haemorrhage. An individual risk stratification with respect to oral antithrombotic agents needs to be performed and documented for each haemodialysis patient with AF.

PUB283

With Increased Inflammation, Fibrinogenolysis May Counterbalance Platelet Hyperactivity in Diabetic Hemo and Peritoneal Dialysis Patients Saila V. Ventrapragada,[1] George P. Bayliss,[2] Bijan Roshan,[1] Ray E. Gleason,[1] Larry A. Weinrauch,[1] John A. D'Elia.[1] *[1]Harvard Medical School, Boston, MA; [2]Brown Medical School, Providence, RI.*

Background: Study subjects with Type1 diabetes (DM) had elevated levels of hemostasis factors [Factor VII (FVII), fibrinogen (fibr), vonWillebrand factor (vWf)], decreased plasminogen activator inhibitor (PAI) levels, accelerated platelet adhesion/aggregation. With intensive treatment over 6-12 months, FVII, fibr, PAI factor levels improved significantly. Follow-up study examined impact of hemostasis, inflammation, oxidative stress on cardiovascular events (CVE) in 124 diabetic study subjects on dialysis (103 HD and 21 PD) vs 26 nondiabetic controls (HD+PD).

Methods: DM + dialysis subjects divided into CVE + history (n= 58) vs CVE– (n=66): CVE + history associated with significantly higher levels of CRP ($p < 0.05$). DM – subjects had significantly higher levels of low MW/fibr and PAI-1 if CVE history +. Dialysis study group data analyzed using platelet activation index: fibrinogen x vWf x p- selectin. Result for DM+ group significantly greater than DM – group ($p< 0.05$). Data analyzed using an index of fibrinogenolysis: low MW/ intact fibr (% degradation) x fibrinolytic activity x 1/PAI-1. Result for DM + group significantly greater than DM – group ($p< 0.05$).

Results: Results at follow up: CVE + DM + study group had significantly higher inflammation index (fibr x interleukin-6 x C-reactive protein) vs CVE – DM + dialysis patients (p= 0.02). Accelerated platelet function and fibrinogenolysis indices didn't discriminate DM + dialysis patients + or – CVE. When CVE free interval analyzed for 150 DM + and – subjects, there was no difference between tertiles for platelet activation index. When CVE free interval analyzed for 150 subjects, tertiles for fibrinogenolysis index reflected more favorable prognosis of DM – (p = 0.04).

Conclusions: DM dialysis patients with CVE on followup had higher inflammation index. Increased fibrinogenolysis may counterbalance accelerated platelet aggregation/adhesion. Impact of fibrinogenolysis on CVE free survival reflects more favorable prognosis of non-diabetic subjects on dialysis.

Funding: Pharmaceutical Company Support, Private Foundation Support

PUB284

Hemodialysis Induced Bacteremia in Our Dialysis Unit, Where Do We Stand from International Rates? Jafar Al-Said, Aimee Pagaduan, Soni Murdeshwar. *Nephrology and Internal Medicine Department, Bahrain Specialist Hospital.*

Background:

Aim:

(1) Identify the prevalence rate of hemodialysis related bacteremia in our dialysis unit and compare it with published North America data.

(2) Measure the admission rate for hemodialysis related bacteremia and vascular access infection and compare it to USRDS 2010 data.

Methods: In this retrospective observational study we reviewed all our patients' dialysis electronic records over 87 months, from January 2004 through March 2011. Demographic information was collected from the files. The definition used for dialysis related bacteremia is: the combination of clinical symptoms and positive blood culture within one week.

Contaminated blood cultures were excluded. The prevalence rate of dialysis induced bacteremia per 100 patient months and per 1000 hemodialysis sessions were estimated. Admission rates for dialysis related bacteremia and access related infection was measured. Our data was compared to USRDS 2010 and other published North America data.

Results: Total 4,300 hemodialysis sessions were included which were done on 114 patients. Thirteen dialysis related bacteremia were encountered in 7 patients over 87 months. Six of them had cuffed tunneled catheter and one had an AV fistula. Two patients required admission during their bacteremia infection. Sphingomonas paucimobilis was the only organism causing all the infection episodes. This was significantly different from the type of bacteria causing dialysis related infection in other units. The source was found to be the main dialysis water tank.

Table(1): Prevalence of dialysis infection bacteremia in our unit as compared to International data:

Item	Our data	International data	P
Prevalence of Dialysis related bacteremia per 100 patient months	0.02	3.59	0.002
Prevalence of bacteremia per 1000 Hemodialysis procedure.	3.02	3.07	0.9
Prevalence of admission for Hemodialysis related bacteremia per 1000 patient years	0.76	180	0.006
Prevalence of admission for cuffed tunneled access infection per 1000 patient years.	Zero	456	NS

Conclusions: Dialysis related bacteremia per patient in our unit is less than North America data while the prevalence per dialysis session is the same.

We had lower admission rates compared to USRDS 2010.

PUB285

Increasing Mean Body Mass Index among Adults Initiating Treatment for End-Stage Renal Disease, by Diabetes Status, United States, 1996–2008

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Background: Diabetes is a major risk factor for end-stage renal disease (ESRD), and high body mass index (BMI) is associated with developing type 2 diabetes. We assessed trends in mean BMI among U.S. adults initiating ESRD treatment.

Methods: Using 1996–2008 data from the U.S. Renal Data System, we obtained mean BMI at initiation of ESRD treatment among people aged ≥18 with diabetes (ESRD-DM) or other conditions (ESRD-OT) listed as the primary diagnosis. Mean BMI was examined by age group. Joinpoint regression was used to analyze trends and calculate an average annual percentage change (AAPC) with 95% confidence interval (CI).

Results: From 1996 to 2008, mean BMI increased among adults initiating treatment for ESRD-DM (from 25.7 to 29.6) and ESRD-OT (from 23.6 to 26.7). Among ESRD-DM incident cases, mean BMI increased from 24.4 to 29.6 (AAPC=1.6% per year, CI=1.5%–1.8%) among those aged 18–44, from 26.8 to 30.6 (1.2%, 1.0%–1.5%) among those aged 45–64, from 25.2 to 29.8 (1.5%, 1.3%–1.7%) among those aged 65–74, and from 23.9 to 27.3 (1.2%, 1.1%–1.3%) among those aged ≥75. Among ESRD-OT incident cases, mean BMI increased from 24.6 to 27.8 (1.0%, 0.8%–1.1%) among those aged 18–44, from 24.6 to 27.9 (1.0%, 0.9%–1.1%) among those aged 45–64, from 23.2 to 26.7 (1.2%, 1.0%–1.3%) among those aged 65–74, and from 22.1 to 24.7 (0.9%, 0.8%–1.0%) among those aged ≥75. Throughout the period, among incident cases aged ≥45, mean BMI was higher among ESRD-DM than among ESRD-OT. From 2005 to 2008, mean BMI was ≥30 among incident ESRD-DM cases aged 45–64.

Conclusions: Mean BMI at initiation of ESRD treatment increased during the study period irrespective of diabetes status. Higher BMI is associated with greater likelihood of survival in the first 12 months of ESRD treatment, but also associated with comorbidities, such as cardiovascular disease (CVD), which could impact survival. Evaluation of the nutritional profile of people preparing for ESRD treatment and reduction of CVD risk factors is important to improve outcomes.

PUB286

Schizophrenia and Schizoaffective Disorder in a Dialysis Population: A Pilot Sample

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Background: Schizophrenia (Sp) affects about 1% of the world's population. It complicates the treatment of patients with ESRD, and the stresses of dialysis may exacerbate Sp. We compared the prevalence of Sp and schizoaffective disorder (Sa) in dialysis patients treated in the VA Loma Linda Healthcare System over a 9 year period with that in matched patients without kidney disease in the same system.

Methods: Medical records of all chronic hemodialysis patients in the Loma Linda VA from January 1, 2000 through December 31, 2008 were examined for a diagnosis of Sp or Sa made by a mental health professional. Each ESRD patient was matched with a control by age, race (non-African American vs. African American), sex, and the diagnosis of diabetes and hypertension. Continuous variables were compared using the Student's T-test, and dichotomous variables ware compared using the Chi-squared test.

Results: The 410 patients with ESRD were matched with 407 controls without kidney disease. Three of the ESRD patients had rare characteristics (e.g. female gender, young age) and could not be matched. Of the 817 remaining patients, 197 (24.1%) were African American, 17 (2.1%) female, 770 (94.3%) had hypertension, and 541 (66.2%) had diabetes. The prevalence of Sp/Sa in cases vs. controls was not significantly different, 10 (2.4%) vs. 6 (1.5%) (p = 0.32). Consistent with other published reports, African American race was associated with a higher prevalence of Sp/Sa, 8 of 197 (4.1%) vs. 8 of 620 (1.3%) non-African Americans in our combined population (p = 0.014).

Conclusions: Our control population had a prevalence of Sp/Sa similar to the accepted prevalence of these diseases. The ESRD population showed a trend toward a higher prevalence of Sp/Sa at 2.4%, but in this small population the difference was not significant. If this trend is correct, power analysis based on our findings, assuming an alpha of 5%, would require a sample size of 3000 ESRD patients and their matched controls to have a power of 80% to detect a difference of 1% in the prevalence of Sp/Sa between the two groups.

Funding: Veterans Administration Support

PUB287

Co-Existence of Peripheral Vascular Disease(PVD) Is Associated with High 5 Yr Mortality in Diabetics on Dialysis

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Background: Patients with diabetic nephropathy starting dialysis are at high risk for cardiovascular(CV) events. A 70% 5yr mortality has been reported in them (USRDS data). Data on the influence of glycaemic status on survival in patients on dialysis is conflicting.

We retrospectively looked at the impact of coexistent CV disease and conventional risk factors on 5yr survival in a cohort of 98pts(28 diabetic)who started dialysis in 2005. We audited our management of prevalent diabetic patients on dialysis in 2010.

Methods: Data collected and analysed from the renal data base.

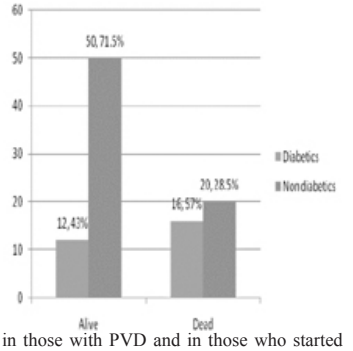
Results: 82.66% of the 98 pts started haemodialysis and 17.34% peritoneal dialysis. 28.6% were diabetic and 71.42% were non diabetics.Ischaemic heart disease IHD(42.85% vs 18.5%)and PVD(25% vs 2.85%)was significantly higher in diabetics.Mean age in both groups was 70yrs.

The 5yr mortality in diabetics was significantly higher when compared to non-diabetics(57%vs28.5%).The median survival in the mortality group was 2years.15.7%of nondiabetics and 10.7%of diabetics were transplanted.

Diabetic mortality data

	Alive	Dead
Age(yrs)	66.3	73.4
HbA1c(%)	6.8	7.5
IHD(%)	58.3	31.2
PVD(%)	8.3	37.5

Diabetic/Non diabetics



5yr mortality in diabetics was higher in those with PVD and in those who started dialysis at a later age.

Audit of 53 prevalent diabetic patients on dialysis in 2010 showed that risk factors were managed satisfactorily [Mean HbA1c(SD)7.2 (1.8),cholesterol 3.68(0.70),predialysis systolic BP(SD)144(22.9),diastolic BP 67.35(11.34) mmHG and CaxPO4 product 3.39(0.81)].75.4% were on antiplatelets,90.6%were non-smokers.

Conclusions: Diabetes is an independent predictor of mortality in patients starting renal replacement therapy. PVD in diabetics worsened survival significantly at 5years. Management of risk factors may not have an impact on survival in patients on dialysis.

PUB288

Views of Renal Healthcare Professionals About the Role of Palliative Care in Patients with End Stage Kidney Disease

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Background: Palliative care is increasingly recognized as an important part of end stage kidney disease (ESKD) care. Health professionals' own beliefs and knowledge about palliative care, death and dying impact on their decision to offer and support palliative care involvement. This study explored the perceptions of renal health professionals regarding palliative care and how this influenced how palliative care was integrated into ESKD patient management. The aim of this study was to identify barriers and facilitators experienced by renal healthcare professionals to incorporating palliative care into ESKD management.

Methods: All renal healthcare professionals in North and Northwest Tasmania were invited to complete a questionnaire exploring their views on the role of palliative care in the management of patients with CKD.

Results: Of 105 surveys distributed 41 were completed (response rate 39%). Health professionals' beliefs that emerged included; Loss of will to live was the greatest influence on dialysis withdrawal. Loss of will to live mainly followed pain and depression. Acute co-morbidity or depression delayed withdrawal of treatment. Malignancy and functional decline influence health professionals and family towards palliation. End of life care should be discussed early at pre-dialysis education, openly, honestly and sensitively with full disclosure by senior renal medical or nursing staff. Final decisions should occur depending on the patient's ongoing condition. The patient should make the decision supported by family.

Conclusions: Renal health professionals believed the patient, with involvement of the family, should make end of life decisions. Preparation should occur at the start of ESKD management with the actual decision made when the patient is ready.

Funding: Government Support - Non-U.S.

PUB289

Factors Affecting Quality of Life in Caucasian Octogenarians and Nonagenarians on Dialysis in Southern Indiana – A Cross Sectional Study

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Background: The elderly constitute the fastest-growing segment of the ESRD population. The numbers of octogenarians & nonagenarians starting dialysis have more than doubled in the last decade, corresponding to an average annual increase in dialysis initiation of 9.8%. Literature mainly focuses on elderly in general (≥ 65 years). While management is focused on cardiovascular end points, mortality & lab parameters including KT/V, albumin, & mineral bone disorder. There is paucity of literature focusing on patients ≥ 80 years & on their symptomatology. Symptoms affecting quality of life (QOL) may involve HEENT, musculoskeletal or nervous systems. Also, majority of these patients may find it hard to respond to extensive questionnaires used by most studies. Moreover, access to primary care is not readily available in this patient population.

Methods: We performed a cross sectional study to look at the factors affecting QOL in patients ≥ 80 years, in order to provide comprehensive care to this population. Two hundred thirty two (232) patients from 4 outpatient dialysis units in Southern Indiana were screened. Both hemo and peritoneal dialysis patients were included. Patients had to be on dialysis for at least 3 months. Patient with underlying dementia were excluded.

Results: Twenty five (25) (10.7%) met criteria. A simple questionnaire was devised to look at factors that could affect QOL. This included identifying problems related to hearing, vision, dizziness, headache, gait, joint pain, back pain & appetite. Patient's responses were recorded. Management of these symptoms was integrated in care plan along with lab parameters for optimal health care delivery.

Conclusions: 1.Symptoms related to gait, vision, back pain & joint pain predominated in effecting QOL irrespective of modality of dialysis.

2. Patients indicated that dialysis per se did not affect QOL.

3. Symptoms related to volume issues were present in about 10% patients only.

3. We propose, a simplified questionnaire to determine symptoms affecting QOL be integrated in care plan for comprehensive care.

4. Palliative care should be considered when attempt to relieve symptoms is unsuccessful.

Funding: Clinical Revenue Support

PUB290

Achievement of CKD-MBD (Chronic Kidney Disease-Mineral and Bone Disorder) Management with Vitamin D Analogs and Minimal Calcium Load in Patients on Maintenance Hemodialysis

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Background: CKD-MBD (chronic kidney disease-mineral and bone disorder) is an important issue associated with increased mortality in hemodialysis (HD) patients.We had a strict calcium,phosphorus management and administration in order to actively tried to use vitamin D.

Methods: In 86 patients in our hospital,serum concentrations of calcium, phosphorus, and intact parathyroid hormone (iPTH), administrated dose of calcium carbonate,sevelamer, and vitamin D analogs in 2004, 2006, and 2008 were examined. In addition, the rate of the patients with achievement of Clinical Practice Guideline for the Management of Secondary Hyperparathyroidism in Chronic Dialysis Patients by the Japanese Society for Dialysis Therapy (JSDT guideline) was investigated.

Results: Datas shown in Table1. We were maintaing the value of phosphorus, increased the dose of severamaer without increasing the level of serum calcium. Calcium carbonate was administered in 76.7% of the patients in 2004, and in 33.7% in 2008 ($P < 0.001$). Sevelamer was administered in 34.9% in 2004, and in 82.6% in 2008($P < 0.001$). Vitamin D analogs were administered in 93.0% in 2004, and 100% in 2006 and 2008.

parameters of each year

	in 2004	in 2006	in 2008
Ca(mg/dL)	9.47±0.57*	9.28±0.70*	9.10±0.37*
Ca.Carbonate (g/day)	2.1±0.8**	blank	1.5±0.7**
Severamaer (g/day)	3.4±1.4***	blank	3.5±1.6***

*,**,***:$p<0.001$

Conclusions: In 2004, mean calcium concentration in our hospital was within target range of JSDT guideline, but was mainly dependent on calcium carbonate. We consider that this was fake and fruitless achievement. Subsequent replacement of calcium carbonate with sevelamer successfully resulted in the administration of vitamin D analogs in 100% of the patients.

PUB291

Antithrombotic Therapy and Bleeding in Hospitalized Patients with End-Stage Renal Disease Receiving Hemodialysis

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Background: In end-staged renal disease (ESRD) patients, use of anticoagulation and antiplatelet agents for cardiovascular disease is common but may increase the risk of major hemorrhage because of uremic platelet dysfunction. The aims of this study were to evaluate transfusion burden and hospital length of stay (LOS) associated with antithrombotic therapy in hemodialysis (HD) patients with ESRD who developed bleeding during hospitalization.

Methods: We analyzed medical records from 151 consecutive HD patients who developed bleeding during hospitalization between January 2008 and June 2010. The patients were stratified into two groups: Group 1 (n=73) were those who received antithrombotic therapy (aspirin, clopidogrel, warfarin, or heparin but exclusive of its use during HD). The antithrombotic therapy followed the practice standard for clinical needs such as prophylaxis against cardiac or venous thrombosis, or after percutaneous coronary intervention. Group 2 (n=78) did not receive antithrombotic therapy.

Results: In the cohort, the age was 62±1 yr (mean±SE), 46% male, and 79 patients received blood transfusion. Similar clinical characteristics were noted in the two groups, including age, gender, race, urea reduction ratio, platelet count, baseline INR, and HD time. In Group 1, the nadir hemoglobin before transfusion was 7.9±0.3, similar to 8.0±0.2 g/dl in Group 2. In Group 1, 48% patients received blood transfusion, similar to 52% in Group 2. In patients who received transfusion, Group 1 received 5.5±0.6 units of blood, similar to 4.7±0.6 units in Group 2. LOS in Group 1 was 11.6±1.0 days, longer than 6.1±0.6 days in Group 2 ($P<0.001$). In a multivariate survival analysis, exposure to antithrombotic agents was associated with longer LOS ($P = 0.0018$); however, blood transfusion as clinically indicated did not affect LOS ($P = 0.38$).

Conclusions: In conclusion, in hospitalized ESRD patients receiving HD, antithrombotic therapy did not adversely affect severity of bleeding or transfusion burden and the significantly increased LOS in patients on antithrombotic therapy was linked to the clinical conditions requiring its use.

Funding: Clinical Revenue Support

PUB292

Analysis of Factors Associated with Complaints in Patients Treated with Hemodialysis or Hemodilafiltration

Atsuhiro Kanno, Ikuto Masakane, Satoko Ito, Minoru Ito, Kiyotaka Yabuki. *Yabuki Hospital.*

Background: Recently, the growing concern for dialysis is considered to be the increase in the number of aged and long-term patients in Japan. Therefore, the objective of dialysis treatment is not only the longevity of the patients but the improvement of quality of life among them. For that reason, it is essential to determine factors related to these complaints. In our cross-sectional study, we examined the relationship between the severity of the uncomfortable symptoms and various clinical parameters.

Methods: A total of 342 patients performed with chronic maintenance dialysis in our three facilities were participated. Additionally, they were evaluated by the self-rating score questionnaire based on fifth graded face scales from 0 (none) to 4 (very strong) according to the severity. The symptoms were composed of 20 common physical and psychological complaints. Odds ratios for each of moderate to severe (score = 2, 3, 4) or severe (score = 3, 4) levels were calculated using a multiple logistic regression model adjusted for confounding factors including age, gender, time of dialysis, history of diabetes mellitus, Kt/V, normalized protein catabolic rate, body mass index, predialytic values of hemoglobin, albumin, β2-microglobulin, sodium, potassium, phosphate.

Results: Among participants, 146 (42.7%) patients were treated with hemodiafiltration and the overall mean values were 66.7 years for age and the median values were 5.4 years for years of dialysis.The adjusted significant factors for the presence of reduction of blood pressure during dialysis were age (odds ratio [OR] 1.04, P =0.0002), body mass index (OR 1.13, P =0.001) and β2-microglobulin (OR 1.04, P =0.045). Potassium was significantly related to severe disturbance of sleep (OR 0.55, P =0.0014) and constipation (OR 0.65, P =0.018).

Conclusions: In our study, dialysis-related symptoms are significantly associated with various clinical parameters. This comprehensive strategy based on self-assessment of common problems could provide an opportunity for us to revisit the therapeutic modality and ameliorate these symptoms, therefore, it would lead to improve the quality of life among each of the patients.

PUB293

Patients with Type 2 Diabetes Admitted for RRT – Are There Any Differences According to Primary Renal Disease and Referral? Radka Kleckova, Jana Veresova, Petra Ronova, Katarina Nehezova, Ivan Rychlik. *Dialysis Unit, FMC, Prague 10, Czech Republic.*

Background: Late referral of type 2 diabetic(T2D) patients(pts) is well-known risk factor for morbidity/mortality in RRT,but the role of primary renal disease(PRD)is not well determined.

Methods: All T2D pts admitted for hemodialysis(HD) in 3-yrs period were divided according to PRD /1=diabetic nephropathy(DN);2=non-diabetic renal disease(NDRD)/ and referral as early(E)>3months vs.non-referral(N).Selected lab&clinical parameters were compared.

Results: 38 T2D pts entered HD(=34% of all incident pts).50% were diagnosed as DN,while atherosclerotic renal disease represented majority of NDRD.All were managed by diabetologist, but only 68% were referred early.

Starting HD,following parameters differed significantly comparing all groups: E1(n=14):highest 24h proteinuria(PU)(4.8g),GFR (0,138 ml/s),DM vintage(21yrs),insulin th (86%),RAS blockers(86%) and lowest mean age(64yrs); E2(n=12):highest s-albumin(ALB)(34.7g/l),hemoglobin(Hb)(99.9g/l),systolic BP(161mmHg) and lowest smoking(0%),coronary artery dis.(CAD)(33%),peripheral arterial dis.(PAD)(8%),congestive heart failure(CHF)(25%),stroke(CS)(8%); N1(n=5):highest HbA1c(7.2%),CHF(60%)and lowest GFR(0.10ml/s),ALB(27.6g/l); N2(n=7):highest s-creatinine(s-cr)(716 umol/l),mean age (75 yrs) and lowest PU(1,0g),DM vintage(7.3yrs),Hb(88.8g/l).

Comparing groups according to PRD(1 vs.2),significantly differed: s-cr(577/671umol/l), PU(4,5/1,8g), cholesterol(4,5/3,7mmol/l), mean age(64,6/71,3 yrs), DM vintage (19,3/10yrs),insulin(76/5%),RAS blockers(74/53%),smoking(47/18 %),CS (26,3/10,5%)but not CAD,CHF,PAD,while according to referral(E vs.N) these were: PU(3.6/2.0g),ALB(33.9/29.8g/l),s-cr(600/676umol/l),DM vintage(16.8/10.4 yrs),insulin(50/25%),RAS blockers(77/33%),statins(46/25%),CHF(31/50%),PA D(27/42%).

Conclusions: 1/early referral had positive clinical-lab consequences;2/non-referred DN pts had the worst metabolic status,the highest CHF and shortest DM vintage;3/non-referred NDRD had highest prevalence of atherosclerotic complications,while early referred NDRD pts had lowest ones;4/anemia treatment was poor in all groups,while RAS blockers and lipid lowering drugs were used highly in early referrals.

PUB294

Perceived Stress in Chronic Kidney Disease Patients Jennifer Ling, Amy L. Jones, Amir Toussi, Sean Wo, Sarah Ramer, Mark L. Unruh. *University of Pittsburgh School of Medicine, PA.*

Background: Stress has been linked to increased risk of cardiovascular events and insufficient immune response. This study examined the relationship between kidney disease and stress to see whether modifiable risk factors may attenuate it.

Methods: The sample consisted of 88 chronic kidney disease (CKD) and 95 end-stage renal disease (ESRD) patients on dialysis as well as 224 controls from the Sleep-Strategies Concentrating on Risk Evaluations study of sleep and cardiovascular risk. Participants completed the Perceived Stress Scale-4 (PSS4), a 4-item instrument that rates a respondent's stress (maximum score of 16). The PSS4 has been shown to be reliable and valid and has been widely used in studies of chronic illness. Participants' demographics, blood pressure, depression, and objective and subjective sleep quality were also assessed.

Results: The sample (median age 58 years) was 56.3% male and 62.1% white. The mean PSS4 score differed between the control (3.8±2.6), CKD (5.5±2.7) and ESRD patients (5.6±3.5), p<0.001. In a multivariable linear regression adjusted for sex, race, and employment, age (-0.06, 95% CI -0.08, -0.03), CKD (vs. control) (1.19, 95% CI 0.43, 1.95), and ESRD (vs. control) (1.29, 95% CI 0.53, 2.05) independently predicted higher stress. In a second model adjusted for the same covariates as above with depression also entered, age (-0.05, 95% CI -0.07, -0.03), CKD (vs. control) (1.17, 95% CI 0.42, 1.92), ESRD (vs. control) (1.28, 95% CI 0.54, 2.03), and depression (1.18, 95% CI 0.63, 1.72) independently predicted higher stress. In a final model adjusted for the same covariates as in the second model with the Pittsburgh Sleep Quality Index (PSQI) also entered, age (-0.06, 95% CI -0.08, -0.03), CKD (vs. control) (0.99, 95% CI 0.25, 1.75), depression (0.89, 95% CI 0.35, 1.42), and PSQI score (0.18, 95%CI 0.11, 0.25) independently predicted higher stress.

Conclusions: The CKD and ESRD patients had significantly more stress than control patients. Poor sleep quality appears to attenuate the association of ESRD and higher stress. Both depression and sleep quality can potentially be modified as part of interventions to reduce stress in this at-risk population.

Funding: NIDDK Support, Private Foundation Support

PUB295

The Troponin T Level at Initiation Time of Hemodialysis May Be Considered an Independent Prognostic Factor for All-Cause and Cardiovascular Mortality for a Short Term Shoichi Maruyama, Seiichi Matsuo. *Kidney Internal Medicine, Nagoya University, Nagoya, Aichi, Japan.*

Background: Cardiac troponin T , a useful marker for diagnosing acute myocardial infarction (AMI) in the general population, is significantly higher than the usual cut-off value in many hemodialysis patients without clinically apparent evidence of AMI. The aim of this study was to examine whether the troponin T level at initiation time of hemodialysis is related to the occurence of cardiovascular diseases (CVD) afterwards and short term mortality.

Methods: 170 patients began hemodialysis between January 2006 and November 2008 at our hospital. Among them we examined 124 patients(76 male and 48 female) without ischemic heart disease concurrence. These patients were followed until death or November 2010. We examined 1)the association of troponin T level at initiation time of hemodialysis and other clinical data and 2) occurence of CVD afterwards .

Results: Positive troponin T level was found in 39 of 124 patients. The 74% of this group were diabetes and it were significantly many compared with non DM ($P < 0.01$). On the other hand, patients with diabetes were 29% in a negative group. In addition, the laboratory data were as following: Creatinin 8.67mg/dl (positive group) vs 9.71 (negative group), serum total protein 5.95g/dl vs 6.34, serum Alb was 3.06g/dl vs 3.58 at the beginning time of hemodialysis. These datas were significantly a low tendency in comparison with negative group. ($P < 0.01$) High sensitive CRP was 0.54mg/dl vs 0.31mg/dl(positive group vs negative group). at the beginning time of hemodialysis ($P < 0.05$)

As for the 2-year occurence of CVD were significantly higher in positive group (36%)than in negative group (11%) ($P < 0.01$) and the total mortality was higher in positive group (13%), compared with that of negative group (2%).

Conclusions: The troponin T level at initiation time of hemodialysis may be considered an independent prognostic factor for all-cause and cardiovascular mortality for a short term.

PUB296

Novel Methicillin Resistant S. aureus (MRSA) Reduction Practices in Outpatient Hemodialysis Mark G. Parker,[1] Elisabeth Tillotson,[2] Dottie Lagasse,[2] Rosalie Blenkhorn,[1] Michelle Duval,[1] Nicole Boutet,[1] Ioan Cosma,[1] Bradley N. Doebbeling.[3,4] *[1]Maine Medical Center, Portland, ME; [2]Fresenius Medical Care North America, Westbrook and Biddeford, ME; [3]HSR&D Center, Indianapolis VAMC, Indianapolis, IN; [4]Regenstrief Institute, Indiana University School of Medicine, Indianapolis, IN.*

Background: The MRSA Reduction Collaborative is an AHRQ-funded seven hospital Quality Improvement consortium studying the application of systems improvement and positive deviance to reduce MRSA infection.

Methods: In April, 2010, staff from two southern Maine FMCNA dialysis units and MMC introduced lean methods and social/behavioral change dialogues from positive deviance methods in dialysis to identify effective practice changes for prevention of MRSA transmission. Process measures identified and introduced include new patient and staff infection prevention education strategies, reinforcing staff and patient hand hygiene adherence, standardized communication tools, and dissemination strategies. Patient outcome measures include MRSA infection rates, all bloodstream infection rates, and costs.

Results: In the intervention period to date, practice changes have included improved identification of patients with drug-resistant organism colonization or infections, development of hand hygiene education tools, ongoing random infection control and hand hygiene audits, and improved reporting and planning between inpatient and outpatient dialysis teams. An average 10% reduction in monthly unit supply costs and 2.6% reduction in per treatment costs have been observed.

Conclusions: Our results demonstrate successful application of combined lean manufacturing principles and positive deviance in outpatient dialysis, including quantifiable process improvements to reduce infection transmission. Challenges have included limits in time and human resources in the outpatient dialysis setting available for positive deviance dialogues, as well as coordination of inpatient/outpatient teams across institutions. Interrupted time series analysis of MRSA infection and bloodstream infection rates will be analyzed after completion of intervention and follow-up periods.

Funding: Other U.S. Government Support

PUB297

Hepatitis C Virus Infection among End Stage Renal Disease Population of Guam Saied Safabakhsh, Christina Vasques, Brittany L. Balajadia, Ali H. Ahangari. *Micronesian Institute for Disease Prevention & Research.*

Background: The purpose of this study was to establish the prevalence and incidence of Hepatitis C Virus (HCV) among End Stage Renal Disease (ESRD) population of Guam and whether there was a correlation between HCV infection and cholesterol levels.

Methods: This research was a retrospective study that was conducted at four Hemodialysis (HD) centers on Guam, a total of 341 subjects were screened using electronic medical records and written chart records. Subjects with Hepatitis C were identified and recorded.

Results: 341 ESRD patients participated in this study out of 410 patients in Guam. 23 out of 341 ESRD patients were positive for HCV. Two patients had converted to HCV positivity in 2010. The average cholesterol level was 138 for all non-HCV patients, while the average cholesterol level was 127 for all HCV patients. 197 (62%) of non-HCV patients were on cholesterol reducing agents. 263 (79%) of all patients were diabetics. 19 (7%) of diabetic patients had HCV. 4 (5%) of non-diabetic patients had HCV.

Conclusions: Hepatitis C Virus infection is considered to be one of the factors increasing morbidity and mortality among ESRD patients. Our study shows that the prevalence of HCV among ESRD patients on Guam is significantly lower than in the United States and other countries. Rate of annual conversion to HCV was less than 1%. Although there was no statistically significant difference between the average cholesterol levels of all non-HCV patients and HCV positive (+) patients, knowing that majority (197 or 62%) of non-HCV (+) patients were on cholesterol reducing medication, we can conclude that HCV infection among ESRD patients affects cholesterol synthesis; therefore, reducing their total cholesterol levels. This may play an important factor in management of ESRD patients. Diabetic patients were found to be at higher risk for HCV infection compared to non-diabetic patients.

PUB298

Increase of HCV RNA Levels during Hemodialysis Treatment in Patients with Chronic Hepatitis C Gernot Schilcher,[1] Csilla Putz-Bankuti,[2] Daniel Schneditz,[3] Harald Kessler,[4] Alexander R. Rosenkranz,[1] Rudolf E. Stauber.[2] *[1]Department of Internal Medicine, Division of Nephrology and Hemodialysis, Medical University of Graz, Graz, Austria; [2]Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria; [3]Institute of Physiology, Center for Physiological Medicine, Medical University of Graz, Graz, Austria; [4]Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria.*

Background: Previous studies suggest a reduction of hepatitis C virus (HCV) viremia by hemodialysis. The aim of this study was to quantify the effect of hemodialysis (HD) and hemodiafiltration (HDF) treatment on HCV RNA levels.

Methods: HD as well as hemodiafiltration (HDF) was delivered to chronic HD patients (n=11). Blood samples were taken before dialysis (t=0 min) and from the arterial and venous line of the extracorporeal circulation during dialysis at times t=30 min and t=180 min for quantitative detection of HCV RNA using real-time PCR.

Results: HCV RNA levels significantly increased during extracorporeal therapy (p<0.001, Tab.1). After 180 minutes and correction for hemoconcentration, HCV RNA levels increased relative (R180) to baseline by 56%. Importantly, no significant differences could be observed between serum samples collected pre and post dialyzer as well as between HD and HDF treatments.

Tab. 1 HCV viral load during extracorporeal blood treatment (N=11, mean ±SD)

	Unit	all	SD	p
HCV RNA_0	10^6 IU/mL	2.88	4.92	
HCV RNA_{30}	10^6 IU/mL	3.44	5.97	
HCV RNA_{180}	10^6 IU/mL	3.61	5.11	<0.001
R_{30}		1.13	0.26	n.s.
R_{180}		1.56	0.89	<0.0001

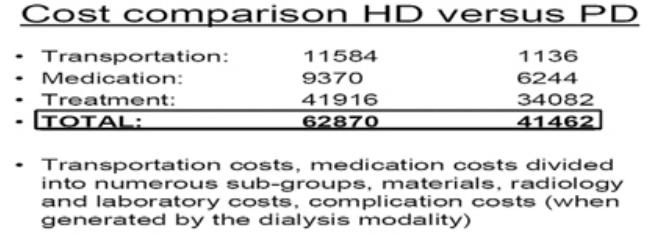
R: HCV RNA level changes relativ to baseline (t=0'), ultrafiltration corrected

Conclusions: Contrary to published data that HCV viremia would be reduced during HD/HDF, a significant increase of HCV RNA was observed after 180 min of treatment. These virological changes could not be explained by HCV RNA absorption on the dialysis membrane, by hemoconcentration or inhibition of real-time PCR due to uremic toxins. After 48 hours of dialysis free time all patients underwent a reduction in viremia by yet unknown mechanisms. Our results also document the importance of timing to collect HCV serum in HD and HDF.

PUB299

Quality of Life in Patients with Various Dialysis Modalities: A Multi-Center, Retrospective Study Klaus Schuster. *Regionalmanagement Mostviertel, Niederösterreichische Landeskliniken-Holding, Amstetten, Austria.*

Background: Public health planning often tries to balance between medical outcomes like survival, etc. and economical/operational reasons. A measurement of quality of life (QuoL)in comparable patients with ESRD seldom is done. Patients with ESRD mainly have 2 options: in-center-dialysis, mainly with hemodialysis (HD), or a home-therapy like peritoneal-dialysis (PD) or home-hemodialysis(HHD). In a recent full-cost analysis we could successfully demonstrate, that treatment at home with PD is obviously cost benficial, but with equal or even better medical outcomes using PD.



Now in a subsequent analysis we did a patient-questionnaire to evaluate the individual QuoL of those patients.

Methods: The questionnaire was completed by 258 patients (62 PD, 196 HD) in 6 dialysis-centres in Lower-Austria, a federal state in Austria. Beside some epidemiological data the questionnaire has three parts, as three different questionnaires are combined. We chose the following surveys: SF-36,an international standard in QuoL-analysis, PLC, a german assessment tool especially for patients with chronic diseases and HADS, the Hospital Anxiety and Depression Scale.

Results: After adjusting age and sex, the results proof our hypothesis, that a home-dialysis-therapy like PD serves the patients better in their subjective self-assessment of various QuoL-aspects. All three parts of the questionnaire show a tendency favouring home-dialysis-therapy, but according to the subscore HADS, the significance is striking.

subscore HADS (points, max. 10)

PD	7.9
HD	6.3

Conclusions: Home-therapy is the choice for patients with ESRD. Not only medical outcomes like better survival and economical/operational (cost saving) reasons, but also the individual self-assessments in QuoL show an advantage for home-dialysis-modalities and so has to be the first choice according to ethics and limited resources.

Funding: Government Support - Non-U.S.

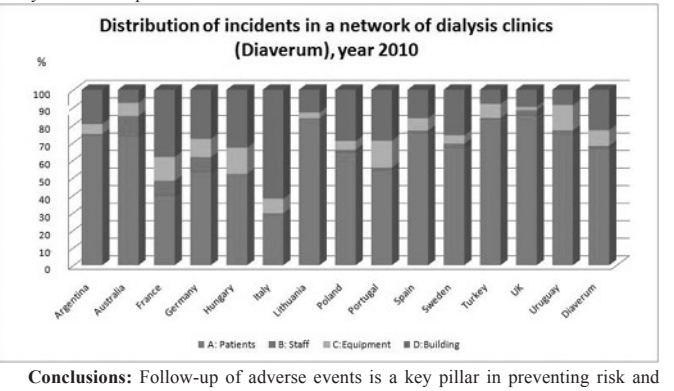
PUB300

Top 5 Incidents in a Worldwide Dialysis Network Paul Stroumza, Jorgen B.A. Hegbrant, Giovanni F.M. Strippoli. *Diaverum, Lund, Sweden.*

Background: The management of adverse events management is a key priority in the overall management policy for quality of care in international dialysis provider groups. Hemodialysis treatments are prone to risks. To our knowledge, a systematic analysis of these incidents in the dialysis world has never been presented. We presents the collection and the analysis of adverse events data for in 2010 in a network of 220 dialysis centers, treating 18,000 patients in Europe, Australia and South America.

Methods: All centers report annualy their incidents through an on-line reporting system. All staff members may report an incident. There are 4 categories of incidents (related to patients, staff, equipment, buildings (fluids, computer, water treatment, other) divided in 49 subgroups. Data on these incidents are presented by monthly analyses with standard descriptive statistics.

Results: In 2010, 25,960 incidents were reported in our organization, with large inter country variability. This is 1.56 incidents per patient per year or 10 per 1000 treatments. The five most common incidents represent 60% of the total. They are, in order: 16% sessions missed-patient did not show up, 15% interruptions of sessions per monitor malfunction, 12% dialyzer and/or blood lines changed due to clotting, 10% vascular access problems, 7% hypotension requiring a filling of more than 300 ml. Distribution of the four groups of incidents were different from country to country; incidents relating to patients were always the most represented.



Conclusions: Follow-up of adverse events is a key pillar in preventing risk and improving the quality of care for dialysis patients. We are implementing a more detailed analysis specially for the first cause: sessions missed; is it voluntary or not? If yes, what about the right for stopping treatment and the physician's responsibility?

PUB301

Characteristics of Bacteremia in Hemodialysis Patients Masashi Suzuki,[1,2] George Seki,[2] Norio Hanafusa,[3] Toshiro Fujita,[2,3] Kyoji Moriya.[1] *[1]Department of Infection Control and Prevention, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan; [2]Department of Nephrology and Endocrinology, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan; [3]Department of Hemodialysis and Apheresis, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan.*

Background: Hemodialysis patients are thought to be immunocompromised. Although infection is the second cause of death in all hemodialysis patients and the first cause of death in patients within one year after initiation of hemodialysis therapy in Japan, the underlying mechanisms are unclear.

Methods: To clarify the characteristics and prognosis of bacteremia, retrospective chart reviews were performed for 850 patients who were admitted to The University of Tokyo Hospital in 2007-2009 and received hemodialysis therapy.

Results: Within 50 cases of bacteremia, the most common types were catheter related infection (20%) and pneumonia (18%), with a substantial portion (22%) unspecified. The common causes of bacteremia were *Staphylococcus aureus* (28%),*Escherichia coli* (10%), *Staphylococcus epidermidis* (8%), *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Candida glabrata* (6%). The frequency of detection was similar to patients who did not receive hemodialysis therapy. Age, types of blood access, catheter, hypertension, cerebrovascular disease, smoking, peripheral vascular disease, serum albumin, hemoglobin, body mass index did not affect the causative organisms. In all hempdialysis patients, the mortality rates within one and three months after bacteremia were 20% and 34%, respectively. These values were even higher (28% and 52%) in a subset of patients who received hemodialysis for less than one year. In these patients, mortality after *S. aureus* bacteremia was extraordinary high (60% and 80% within one and three months, respectively). Mortality was also very high after *Candida glabrata* bacteremia (67% within one and three months).

Conclusions: There was no difference in the cause of bacteremia between hemodialysis and non-hemodialysis patients. Because the bacteremia of *S. aureus* and *Candida glabrata* in the first-year after initiating hemodialysis predicted very bad prognosis, careful observation and intensive care would be required in such cases.

Funding: Government Support - Non-U.S.

PUB302

Abstract Withdrawn

PUB303

Patient Characteristics Associated with Dialysis for Veterans with ESRD in VA and Non-VA Settings Virginia Wang,[1,2] Matthew L. Maciejewski,[1,2] Uptal D. Patel,[1,2] Morris Weinberger.[1,2] *[1]HSR&D, Durham VA Med Ctr, Durham, NC; [2]Gen Int Med, Duke Univ Med Ctr, Durham, NC.*

Background: Dialysis is a limited resource within the VA, and growing demand for ESRD care has led VA to purchase dialysis treatment from non-VA providers on a fee for service basis. Decisions about the optimal mix of "making" dialysis in-house or "buying" non-VA services requires an understanding of current users within the VA system. This study examined characteristics of veterans with ESRD who utilized outpatient dialysis in VA and non-VA settings.

Methods: We constructed a cohort of veterans with ESRD in 2 regional networks who received VA-financed outpatient dialysis treatment in 2007-8. Using VA administrative data, we identified veterans who received dialysis in VA; non-VA fee basis; or both settings ("dual users"). We performed bivariate and multinomial probit analysis to identify patient characteristics associated with dialysis setting.

Results: 1,388 veterans received chronic hemodialysis financed by VA: 25% received VA dialysis, 36% used non-VA fee basis dialysis, and 39% were dual users. VA dialysis users were more likely to be non-White, unmarried, sicker, and living closer to VA dialysis units than veterans in non-VA and dual settings (p<.05-.01). After covariate adjustment, non-VA dialysis was positively associated with marital status (p<.05) and negatively associated with comorbidity burden (p<.001). Greater geographical distance to VA dialysis was associated with non-VA and dual dialysis (p<.001). There was regional variation in veterans' use of dual dialysis (p< .001).

Conclusions: A significant proportion of VA-funded dialysis is delivered via fee basis. Non-VA providers benefit from favorable selection of patients, as veterans with lower comorbidity burden are more likely to obtain dialysis outside of VA. With limited service capacity and increasing ESRD prevalence in VA, veterans are likely to continue receiving non-VA dialysis. VA may need to account for selection bias in determining the optimal 1) mix of making vs. buying dialysis services and 2) payment to non-VA dialysis providers. More research is needed to understand the implications on the quality and cost of VA's make-buy decisions.

Funding: Other U.S. Government Support, Veterans Administration Support

PUB304

Prosthetic Status and Treatment Needs for Lost Masticatory Function in Hemodialysis Patients Magdalena Wilczynska-Borawska,[2] Jolanta Malyszko,[1] Michal Mysliwiec.[1] *[1]Nephrology, Medical University, Bialystok, Poland; [2]Dentistry, Medical Universiy, Bialystok, Poland.*

Background: Premature loss of permanent teeth leads to stomatognathic system disability, loss of masticatory functions, speech and alterations in face aesthetics. Loss of masticatory function may result in severe malocclusion which, if not treated, may lead to irreversible dysfunction of the whole masticatory system. Premature loss of permanent teeth is a very serious but underrated problem for patients with chronic renal failure.

Methods: The analysis of the degree of loss of masticatory function and number of teeth present for hemodialysis patients, and defining, based on the results, patients' needs for prosthetic treatment, which could restore correct occlusal condition. We studied the total number of teeth and number of teeth separately for upper and lower jaws, 2) the existing prosthetic restorations and 3) the preserved masticatory function in 68 HD patients.

Results: Nearly 70% of tested hemodialysis patients did not have a reconstructed masticatory function. Female patients had less remaining natural teeth in both jaws compared to males and constituted a higher percentage of edentulous patients in the test group. All patients with at least 28 natural teeth with retained occlusal contacts whilst chewing were males (4; 10% males; 5,7% of the whole group). There were 15 edentulous patients: 7 males (10%,) and 8 females (11,5%). More of them had their masticatory functions restored, implementation of prosthetic treatment was needed to a lesser degree. In this group, more extensive restorations like partial, skeletal and complete dentures were observed.

Conclusions: The population of hemodialysis patients from the North East part of Poland are patients with severe stomatognathic system dysfunctions which developed as a result of the loss of physiological function of the masticatory system (more than 20% of patients were edentulous). It is of importance for dentists, as well as nephrologists, to understand the essence of the problem, as the general health of a patient cannot be improved without ensuring functional comfort of such as important system as the masticatory one.

Funding: Government Support - Non-U.S.

PUB305

A Prospective, Randomized, Multicenter Study Comparing Survival in Subjects Receiving Peritoneal Dialysis or Hemodialysis (SURinD) Xueqing Yu,[1] Anders P. Tranaeus,[2] The SURinD Study Group.[1] *[1]Renal Department, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; [2]Baxter Asia Pacific, Shanghai, China.*

Background: There is currently no consensus on whether peritoneal dialysis (PD) or hemodialysis (HD) is associated with better survival outcomes in patients with end-stage renal disease (ESRD). Results from a pilot study, where 61% of eligible subjects were willing to be randomized to either dialysis modality, suggest that it is feasible to conduct an adequately powered, randomized controlled study to investigate survival outcomes in patients with ESRD receiving PD or HD.

Methods: SURinD is a prospective, randomized, multicenter, open-label study being conducted in China (registered at www.clinicaltrials.gov; sponsor: Baxter Healthcare Corporation). The primary objective is to compare survival in subjects receiving PD or HD; the primary endpoint is the PD/HD mortality hazard ratio (HR). Secondary objectives include a comparison between the two modalities of technique failure, cause of death, residual renal function, erythropoietin-stimulating agent dose, systemic inflammation, quality of life, and safety. Eligible subjects are ≥ 18 years, diagnosed with ESRD (glomerular filtration rate ≤ 15 mL/min/1.73 m² body surface area), and predicted to need dialysis within 10 weeks after the pre-screening period. Approximately 1,370 subjects will be randomized (1:1) to PD or HD (centralized randomization, stratified by investigator site) and followed for up to 5 years. The sample size is based on an overall type I error of 5% and overall power of 80%, and assumes that a non-inferiority margin of 1.25 for the PD/HD mortality HR is clinically relevant. Assessments will be conducted to ensure that internationally accepted standards of care (including adequacy, anemia, calcium, phosphate, and blood pressure) and clinical practice guidelines for dialysis are met for both modalities.

Results: Recruitment will start soon and the final study results are expected in 2017.

Conclusions: This large randomized study will assess whether survival outcomes differ between PD and HD in patients with ESRD, and will provide information on factors that influence modality choice, including quality of life.

Funding: Pharmaceutical Company Support

PUB306

Female Chinese Hemodialysis Patients with a Better Prognosis Than Man When Treated by the Same Dialysis Parameters Jianzhou Zou, Yi Fang, Jie Teng, Wenlv Lv, Xiaoqiang Ding. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: Dialysis dose would be higher in female hemodialysis patients than that in males when treated by the same dialysis parameters. Literatures suggest that greater dialysis dose may significantly benefit women, but not men. So we speculate that prognosis of female hemodialysis patients will be better than that of males.

Methods: All 151 patients were treated by the same dialysis parameters and would be followed up for 30 months or ceased by death, transforming to the other renal replacement therapy, or transferring to other dialysis centers. The demographic datas, laboratory test results and dialysis dose would be collected when the patients enrolled in the cohort study.

Results: During the follow-up, five patients changed to kidney transplant and one to peritoneal dialysis, one patient transferred to the other dialysis center. Among the analyzed 144 patients, almost 45% were females. Thirty one patients died from all causes and the all-cause mortality was 21.53%, Adjusted death rate per 100 patient years was 9.83 by the end of follow-up. The all-cause mortality for male (29.11%) was significantly higher than that for females(12.30%, p<0.05). Adjusted death rate per 100 patient years was 13.82 in males which was higher than that in females (5.27, p<0.05). The level of hemoglobin, white blood cell count and predialysis serum creatinine were significantly higher in males than that in females, but URR and spKt/V were significantly lower in males. When analyzed by Cox proportional hazards regression modeling, we found that for male patients, C-reactive protein, preablumin and iPTH were independent predictors of death; however for the females, the independent predictors of death were C-reactive protein and iPTH.

Conclusions: When treated by the same HD parameters in Chinese patients, the prognosis of females was better than that of males. Meantime, we found that iPTH was associated with mortality in males or females, but the impact was different by gender.

PUB307

Hemodialysis Catheter Adherent to Jugular Vein Solange Bourque. *Nephrology, CHUS, Sherbrooke, QC, Canada.*

Background: Hemodialysis catheters are used in patients without any other possible vascular access. However, they have multiple complications. We present an unusual complication, namely a catheter adherent to the jugular vein.

Methods: A 50 year old man was dialysed with a permanent right jugular tunneled catheter since 1999. He presented his first tunnel infection on July 7th 2010. Infective organism was staphylococcus aureus. At that time, there was a first, unsuccessful attempt to remove the catheter at the dialysis clinic. The patient was sent to radiology for further attempt. Ultrasound showed that the dialysis catheter seemed to be attached to the jugular vein. The surgeon was called in for help but was unable to remove the catheter even with further incision and dissection. It was then decided to proceed to surgical dissection under general anaesthesia. A partial sternotomy had to be done to reach the jugular vein. An incision was made along the jugular vein to retrieve the catheter. This caused a massive

2L bleed with severe hypotension. After hemostasis was completed, the procedure was finished. The patient fully recovered and is now dialysed with a permanent left jugular tunneled catheter.

Results: Ten cases of hemodialysis catheters embedded or adherent to central vein are described in the literature. Clinically, there was either resistance or chest pain while trying to remove the catheter. Every patient needed surgery to try to remove the catheter. Some could not be removed and needed to be ligatured, cut and buried under the fascias. Some catheters were fragmented during the attempt to remove them. The most important risk factor was the length of time since the installation of the catheter, which varied from one and a half to seven years. Other risk factors are the presence of thrombus around the catheter and stenosis of the vessel.

Conclusions: Resistance or chest pain during attempt to remove a hemodialysis catheter should make us think of possible adherence to the blood vessel. There currently aren't any recommendations available about optimal or maximal time for which a catheter can be left in place or if routine investigation such as fluoroscopy or cardiac ultrasound should be done.

PUB308

When To Refer for Vascular Access Fistulogram in Hemodialysis Patients with Decreasing Intra-Access Flow? Sun Ryoung Choi, Hoon Suk Park, In O Sun, Hyun Gyung Kim, Yu Ah Hong, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Chul Woo Yang, Young-Soo Song. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: Intra-access flow measurement is preferred to surveil vascular access in hemodialysis patients. K/DOQI guidelines emphasized prospective trend analysis of access flow on a monthly basis. They recommend refer for fistulogram if access flow 1,000 ml that has decreased by more than 25% over 4 months in graft, and do not mention about fistula.

Methods: This study was a prospective observational trial. The surveillance used was intra-access flow measured by ultrasound dilution (Transonic HD03 hemodialysis monitor; Transonic Systems, Inc., Ithaca, NY) performed monthly. We defined 25% decrease in Qac compared to that of the last measurement as significant finding and thus performed intervention without follow-up over four months.

Results: Out of total 52 cases, 38 patients were male, mean age 63 years old, 22 (42.3%) diabetics, 38 (73.1%) fistulae, and 37 (71.2%) accesses were on the forearm. At the time of referring for fistulogram, the access flow has been decreased from 816±367 ml/min to 614±312 ml/min in fistulae, and from 1140±448 ml/min to 849±393 ml/min in grafts. The mean decrease in access flow was 30±7% in fistulae and 34±11% in grafts. Significant stenosis was found in 48 cases (94%) on the fistulogram. The result of fistulogram showed outflow stenosis (55%), inflow stenosis including juxta-anastomotic stenosis (36%), accessory vein (3%), and no stenosis (6%). Twenty two percent of patients had increasing venous pressure or negative arterial pressure during dialysis. However, none of the patients showed abnormal physical finding

Conclusions: Our study suggests that the patient should be immediately referred to fistulogram if access flow has decreased more than 25% regardless of access type, absolute value of access flow, or duration of decreased access flow

PUB309

Impact on Autologous Arteriovenous Fistula Aneurysm by Improved Puncture Technique: Centripetal and Exodic Alternate Xia Fu, Wei Shi, Xinling Liang. *Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Background: To study the appropriate puncture technique of autologous arteriovenous fistula for reducing the occurrence of aneurysm or avoiding aneurysm increasing.

Methods: 298 maintenance hemodialysis patients with autologous arteriovenous fistula were randomized into 3 groups, respectively punctured by centripetal method, exodic method and alternate ways. Incidence of aneurysm, aneurysm increasing, blood flow and Kt/v were compared among the three groups.

Results: Aneurysm incidence and aneurysm increasing in the centripetal group and the alternate group had no significant difference ($p>0.05$). The incidence and increasing of exodic group was significantly higher than those of centripetal group and alternate group ($p < 0.05$). The values of blood flow and Kt/v in the centripetal group were significantly lower than those in the exodic group and alternate group ($p < 0.05$).

Conclusions: Alternating centripetal and exodic puncture not only guaranteed blood flow but also effectively avoided occurrence or increase of aneurysms of autologous arteriovenous fistula.

Funding: Government Support - Non-U.S.

PUB310

Evaluation of Intravascular Ultrasound (IVUS) Observations with AVF in Which Intravascular Stents Were Inserted into Their Central Veins Tomonari Ogawa, Tota Kiba, Yuta Kogure, Shimpei Okazaki, Mizuki Iwanaga, Chie Noiri, Akihiko Matsuda, Hajime Hasegawa, Tetsuya Mitarai. *Nephrology and Blood Purification, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan.*

Background: In hopes for long-term patency, insertion of intravascular stents into sites of stenosis in the central vein is not a rare case in recent years. Intravascular assessments were conducted before and after treatment on patients with stents placed inside the lesion of the central vein.

Methods: This study is based on 8 cases where intravascular ultrasound (IVUS) analysis was conducted on dialysis patients with AVF and have a stent inserted into sites of venous stenosis in or near their trunk.IVUS was used to measure the vascular diameter and to assess the lumen of the stegnotic site for cases in which central vein stenosis was confirmed through angiography. The vascular diameter was measured again after stent placement. In addition, blood flow was measured before and after treatment using ultrasonography in order to make functional assessments of the stent.

Results: A clear blood clot was found in 2 out of the 8 cases, but not in the other remaining 6 cases. A low echoic area was identified around the blood vessel, and thus a form of exclusion was confirmed in all 8 cases. A Wallstent was placed in patients whose improvement in vascular diameter could not be confirmed using only balloon dilation. An improvement in vascular diameter after Wallstent placement, relative to that before treatment, was observed in all cases. In terms of functional assessment, there was a significant increase in blood flow in the brachial artery before and after treatment.

Conclusions: Stenosis was confirmed through angiography in the patients who received treatment in this study, and exclusion of the stenosis was confirmed through IVUS examinations. A clear morphological improvement was observed as a result of stent insertion in these cases, and an improvement in blood flow was also confirmed through functional assessments. Although there are negative opinions against stent insertion, it could be concluded that this treatment could be effective depending on the morphology of the stenosis.

PUB311

Identifying Factors Associated with Type of Hemodialysis Access: Efforts To Reduce Catheter Rates Maria Aurora C. Posadas, Aleksandra Gmurczyk, Robert M. Rosa, Daniel Batlle, James J. Paparello, Jennifer A. Tuazon, Shubhada N. Ahya. *Department of Medicine - Division of Nephrology and Hypertension, Northwestern Memorial Hospital, Chicago, IL.*

Background: HD catheters are suboptimal due to associated increased infection, morbidity, and mortality. Identifying factors that affect the patients' choice of HD access is valuable to reduce the catheter rate.

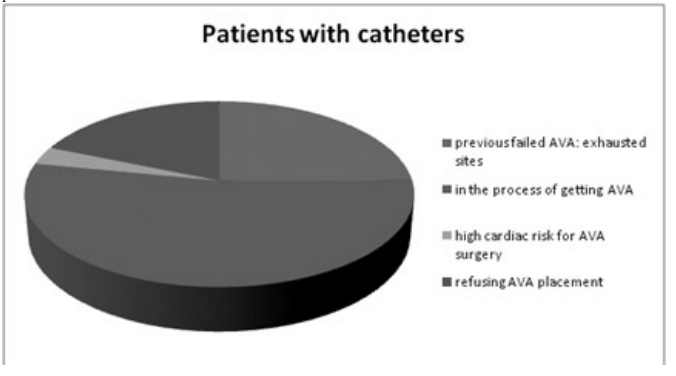
Methods: This is a cross-sectional study of 83 ESRD patients on HD at one outpatient dialysis unit. The subjects were grouped based on HD access type: catheter or arteriovenous access (AVA). T-test was used to look at differences in demographics, presence of pre-dialysis care, health literacy, HD vintage, and labs.

Results: Subjects from both groups were similar. Patients with catheters had shorter HD vintage (P=0.02) and were more likely to have received pre-dialysis care from a nephrologist (P=0.049) compared to patients with AVA.

Demographic Data

Parameters	Catheter	AVA	P-value
no. of subjects	42	41	
gender			0.063
male	14	22	
female	28	19	
received pre-HD care	31	26	0.049s
HD vintage (months)	29	57	0.020s
STOFHLA numeracy	3.3	3.5	0.230
STOFHLA reading comprehension	32.6	33.6	0.328

Nineteen percent of patients with catheters had previous AVA that either had primary non-function or ceased to function and 14% of the patients with catheters refuse AVA placement.



Conclusions: Patients with catheters had shorter mean HD vintage compared to patients with AVA. While patients with catheters were more likely to have received pre-dialysis care compared to patients with AVA, these patients had previous failed AVA, or were undergoing evaluation for AVA placement at the time of the study, were either awaiting kidney transplantation or switching to peritoneal dialysis. More studies need to be done to explore qualitative factors for high catheter rates including patients' refusal for AVA placement.

PUB312

Malfunction of Tunneled Cuffed Venous Catheter with Small Gap between Cuff and Catheter: A Report of Four Cases Nami Shibahara,[1] Hiroshi Shibahara,[2] Susumu Takahashi. *[1]Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; [2]Blood Purification Center, Sagamihara Kyodo Hospital, Sagmihara, Kanagawa, Japan.*

Background: The cuff of a tunneled cuffed venous catheter (TCC) provides a barrier against infection, and firmly anchors the catheter by fixing it to the subcutaneous tissue. However, we experienced four cases of malfunction of a Soft Cell (Bard Access Systems, Salt Lake City, UT, USA), a type of TCC, with a small gap between the cuff and the catheter.

Methods: In case 1 (74-year-old woman), case 3 (95-year-old woman), and case 4 (74-year-old woman), a Soft Cell (12.5 Fr diameter, 19 cm long) was used as a permanent access, while in case 2 (86-year-old woman) a Soft Cell was used as a bridge access for arteriovenous graft failure. The Soft Cell was removed because of tunnel infection at 290 catheter-days in case 1 and at 29 catheter-days in case 2, suspected catheter-related blood stream infection at 513 catheter-days in case 3, and catheter dysfunction at 734 catheter days in case 4.

Results: In all of the removed Soft Cells, the presence of a gap was confirmed by passing a probe through this gap. Sections of formalin-fixed cuffs of these Soft Cells showed that the cuff had detached from the catheter at the part where it was possible to pass a probe, despite the cuff being tightly attached to the adjacent dermal connective tissue with marked foreign body reaction and fibrosis histologically.

Conclusions: In the four cases presented, the cuff was tightly attached to the dermal connective tissue histologically. Even if the cuff were firmly fixed to the subcutaneous tissue, bacteria and foreign substances would have been able to get into the tunnel site through the gap. The gap between the cuff and the catheter, representing a break in the barrier against infection, might have been the cause of the two cases of tunnel infection. Fortunately these patients improved immediately without hospitalization, and all Soft Cells were hardly anchored to the skin. The cause of this malfunction of the Soft Cell is currently being investigated by the manufacturer.

PUB313

An Assessment of International Frequent Hemodialysis Utilization and Practice Patterns: A Collaboration between the Canadian Home Hemodialysis Study Group and Nephrology Now Nathan D. Allen,[1] Daniel Schwartz,[2] Paul Komenda,[1] Deborah Lynn Zimmerman,[3] Robert P. Pauly,[4] Gemini Tanna,[5] Jeffrey Schiff,[5] Manish M. Sood.[1] *[1]Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]Medicine, University of British Columbia, Abbotsford, BC, Canada; [3]Medicine, University of Ottawa, ON, Canada; [4]Medicine, Univeristy of Alberta, Edmonton, AB, Canada; [5]Medicine, University of Toronto, ON, Canada.*

Background: The purpose of our study was to determine influences for the utilization of frequent HD and attitudes regarding initiation of and the evidence for frequent HD.

Methods: An international cohort of subscribers of a nephrology education website (www.nephrologynow.com) was invited to participate in an online survey. Survey questions assessed indications, reasons for withdrawal support of evidence and attitudes towards frequent HD. Frequent dialysis was defined as nocturnal, short daily or long conventional hemodialysis, either in-centre or at home. Variables associated with frequent HD use were determined using multivariate logistic regression analysis.

Results: Our survey had a 40.9% response rate. The final cohort was limited to 311 physicians.125 (40.2%) physicians had patients treated with frequent HD. In the multivariate model, adequate training (OR 2.47 CI 1.25-4.16), government physician reimbursement (OR 2.66, CI 1.11-6.40), higher national health care expenditure and number of ESRD patients per centre were independently associated with frequent HD utilization. Hemodialysis providers with patients on frequent HD were significantly more likely to support the evidence base and less likely to agree with the statements that frequent HD increases fistula/graft thrombosis and is too costly. The most common reasons to initiate frequent HD were driven by patient preference and the desire to improve volume control and global health outcomes

Conclusions: Frequent HD use and support of the evidence vary according to identifiable factors. Interventions and health policy targeting these areas and increased physician education and training in frequent HD modalities may be effective in increasing frequent HD utilization.

PUB314

Recurrent Pancreatitis Associated with Icodextrin Mehrdad Hamrahian, Tibor Fulop, Luis A. Juncos. *Division of Nephrology, University of Mississippi, Jackson, MS.*

Background: Acute pancreatitis is an infrequent condition in peritoneal dialysis (PD) patients; determining its cause can be challenging. We present a patient with recurrent bouts of chemical pancreatitis that appear to be due to icodextrin.

Methods: A 42 year-old diabetic and hypertensive female on PD presented with recurrent pancreatitis. Laboratory studies consistently showed high serum lipase levels, normal peritoneal fluid white blood cell counts of macrophage predominance, and negative cultures (Table 1). Imaging studies were consistent with pancreatitis. The results raised the possibility of chemical pancreatitis, but no clear culprit was identified. Her cycler automated PD dialysate consisted of 2.5% and 4.25% dextrose plus a daytime dwell of icodextrin. A chart review revealed that the patient rapidly improved with admission, despite the lack of specific therapy. The only intervention identified that was consistently instituted during each admission was a change from icodextrin to dextrose in the daytime dwell (due to formulary restrictions). We therefore entertained the possibility that icodextrin was inducing chemical pancreatitis and thus eliminated the icodextrin dwell from her outpatient PD prescription. Removal of icodextrin not only led to a complete resolution of her symptoms, but she did not have any further recurrences.

Admission	1st	2nd	3rd	4th
Lipase/Amylase	2669/-	683→534/17	1266→173/23	931→245/-
WBC/Culture	15-131/∅	22/∅	17/∅	29/∅

Results: Discussion: Icodextrin is an osmotic glucose polymer which is especially useful in patients with high peritoneal membrane transport, because of its limited diffusion across this membrane. While it is usually well-tolerated, it can cause cutaneous allergic reactions, abdominal pain, sterile peritonitis, hyponatremia and decreased plasma amylase activity (with unaltered lipase levels). Our report suggests that it may also cause chemical pancreatitis. This is suggested by the clinical presentation, chronologic relationship and the resolution of symptoms with no further recurrence of episodes upon icodextrin withdrawal.

Conclusions: Chemical pancreatitis should be considered in recurrent unexplained pancreatitis in PD patients when icodextrin is used.

PUB315

Changing Picture in the Microbiology of Peritoneal Dialysis Associated Peritonitis – A Single Center Experience over Almost Three Decades Martin Kimmel, Niko Braun, Mark Dominik Alscher. *Department of Internal Medicine, Division of General Internal Medicine and Nephrology, Robert-Bosch Hospital, Stuttgart, Germany.*

Background: Peritonitis is the most important complications of peritoneal dialysis (PD) and is still a major cause of morbidity and technique failure. In previous reports concerning the microbiology of peritonitis and its characteristics the longest observation period was one decade.

Aim of this retrospective study is to analyze the microbiological spectrum of PD-associated peritonitis from July 1983 over an almost three decade-long period up to December 2010 in a local peritoneal dialysis reference center.

Methods: Retrospective study analyzing >300 peritonitis episodes at a peritoneal dialysis reference center in south Germany. All peritonitis episodes in three timeframes (a-c) were analyzed: a: 1983-1989, b: 1993-2006 and c: 2007-2010. The spectrum of organisms causing PD-associated peritonitis and the antibiotic resistance profiles were assessed for each pathogen.

Results: Organism spectrum: the ratio gram-positive to gram-negative organism is changing: gram-positive organisms are decreasing (a: 74%, b: 66%, c: 66%). Staphylococcus aureus and coagulase-negative Staphylococcus (CoNS) is decreasing, but Methicillin-resistant CoNS is increasing and formerly rare organisms appeared more frequently (e.g. Serratia marcesens or Stenotrophomonas maltophilia). Antibiotic susceptibility: there is an increasing rate of resistant organisms with the need to adapt the initial treatment protocol.

Conclusions: There is a changing picture in the microbiology of peritonitis episodes over almost three decades with an ongoing need to adapt continuously the initial treatment protocol

PUB316

How Home Hemodialysis Programs Deal with the Non-Adherent Patient: A Survey of 17 HHD Programs in Canada Paul Komenda,[1] Deborah Lynn Zimmerman,[2] Robert P. Pauly.[3] *[1]Medicine, Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Medicine, Nephrology, University of Ottawa, ON, Canada; [3]Nephrology, Univeristy of Alberta, Edmonton, AB, Canada.*

Background: Despite growing interest in intensive hemodialysis (HD) prescriptions (more frequent and or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns. This abstract pertains to how programs address non-adherence in patients performing independent home hemodialysis.

Methods: A survey was developed and then sent to 19 physician experts to ensure content and face validity. Physicians were encouraged to seek input from allied health team members. The survey was modified based on responses received and then circulated prior to a face-to-face meeting with the same physician experts before final changes to the instrument were made. The survey was completed on line.

Results: Seventeen of 19 (89%) physicians representing individual programs in Canada participated in survey development and provided program information. 11/17 (65%) HHD Medical Directors felt that patients were entitled to make decisions clinicians may deem "unsafe". 12/17 (71%) programs have discontinued HHD therapy to patients against their wishes. Reasons for removing patients from HHD therapy could include missing routine bloodwork (15/17), missing clinic visits (14/17), or not performing routine machine maintenance (12/17).

Conclusions: The majority of HHD programs in Canada communicate that informed patients may be permitted to make their own decisions regarding safety in performing HHD. The majority of these programs however have removed a patient from therapy against the patient's wishes. Consensus guidelines may help guide HHD teams in setting thresholds for involuntarily removing patients from this modality.

PUB317

Training Variability in Home Hemodialysis in Seventeen Canadian HHD Programs: The Case in Favor of Standardization Paul Komenda,[1] Deborah Lynn Zimmerman,[2] Robert P. Pauly.[3] *[1]Medicine, Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Medicine, Nephrology, University of Ottawa, ON, Canada; [3]Nephrology, University of Alberta, Edmonton, AB, Canada.*

Background: Despite growing interest in intensive hemodialysis (HD) prescriptions (more frequent and or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns. This abstract addresses practice patterns pertaining to training schedule.

Methods: A survey was developed and then sent to 19 physician experts to ensure content and face validity. Physicians were encouraged to seek input from allied health team members. The survey was modified based on responses received and then circulated prior to a face-to-face meeting with the same physician experts before final changes to the instrument were made. The survey was completed on line.

Results: Seventeen of 19 (89%) physicians representing individual programs in Canada participated in survey development and provided program information. Programs vary in training schedules with 7/17 (41%) training three days per week, 4/17 (23%) four days/week and 6/17 (35%) five days/week. 9/17 programs report a median training time of 5-6 weeks, with 5/17 training for 7-8 weeks. Training more days per week did not always correlate with shorter training duration. 14/17 programs maintain a 1:1 nurse to patient ratio throughout training. Most programs (16/17) generate their own training manual.

Conclusions: Significant heterogeneity exists between Canadian HHD programs in terms of the intensity and duration of training provided to new patients. As up front training represents a significant fixed cost in providing access to this modality, standardized training manuals across centres may augment efficiency in helping new programs to grow quickly and safely.

PUB318

A Flexible Approach to Extended Hours Hemodialysis at Home Rathika Krishnasamy, Carmel M. Hawley, David W. Johnson, David Mudge, Nicole M. Isbel, Scott B. Campbell, Carolyn L. Van Eps. *Department of Nephrology, University of Queensland at Princess Alexandra Hospital, Brisbane, Queensland, Australia.*

Background: Home hemodialysis (HHD) allows greater tailoring of prescription to individual needs both medically and socially. The aim of this survey is to review current dialysis prescription practices of HHD patients and to explore factors involved in their decision making.

Methods: 60 HHD patients from PAH were invited to complete a written survey containing 24 questions in April 2011.The survey covered duration and timing of HHD, patient's health perception, support system and perceived barriers.

Results: 60% of patients responded to the survey. 82% of patients had been on HHD for more than a year. At least 35% of these patients stayed on HHD for more than 5 years.

Duration and Timing of HHD

Variable	HHD(n=35)	Nocturnal HHD (n=21)
Frequency(sessions/week)		
daily	6%	5%
alternate days	57%	67%
< 4 days	37%	28%
Duration(hours/session)		
> 8	20%	28%
6-8	49%	62%
4-6	26%	10%
< 4	5%	0%
Start time		
morning(0400 -1100)	22%	0%
midday and afternoon(1100-1700)	20%	3%
evening(1700-2000)	18%	40%
night(2000-0400)	40%	67%

Nearly 80% of them rated their overall health, mental health and their current dialysis as being good, very good or excellent. 75% of patients felt that doing dialysis at home was both beneficial to their health and their family.70% of patients had a support person during dialysis and mostly were satisfied with the care from the dialysis training centre.

The duration of dialysis was mainly influenced by lifestyle preferences (42%) and advice from the dialysis training centre (39%) rather than work choices (3%) or dietary allowances (10%). The concerns regarding their current dialysis included medical problems (28%), expenses for dialysis (17%), storage space (14%) and maintenance of equipment (14%).20% of patients dropped out from previous nocturnal HHD and reported sleep disturbance as a primary problem. Lifestyle limitation and inability to contact dialysis staff were the concerns raised by patients who declined nocturnal HHD.

Conclusions: This survey suggests that the flexibility of HHD is perceived as highly beneficial for patients and their families. We also identified patients' concerns and barriers towards HHD.

PUB319

The Effect of Newly Developed Semi-Long Peritoneal Dialysis Catheter on the Inflow- and Outflow Time of Peritoneal Dialysis Fluid Akihiro Kuma,[1] Narutoshi Kabashima,[2] Tetsu Miyamoto,[1] Nana Ishimatsu,[1] Yumi Furuno,[1] Kaori Kanegae,[2] Ryota Serino,[1] Masahito Tamura,[2] Yutaka Otsuji.[1] *[1]Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitkakyushu, Japan; [2]Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Japan.*

Background: To reduce the incidence of omental wrapping, we developed a newly designed peritoneal dialysis catheter (PDC) for the upper abdominal exit. This catheter (JBS-2) called "CAVA CATHETER" has smaller end-holes (2.0 mm) and smaller side-holes (0.5 mm) with 4-line slits on the surface to prevent omental entanglement. While JBS-2 could prevent a suction of omental tissue, this catheter could cause an inflow- or outflow-delay of peritoneal dialysis fluid (PDF).

Methods: We provided five different types of catheters according to the existence of slits and size/number of end-hole or side-hole. The effect of JBS-2 (n=5), the five different types of catheters (n=3-6) and conventional Swan Neck catheter (JB5A, n=3) on inflow- and outflow- times were assessed with artificial abdominal cavities.

Results: The mean PDF outflow of the PDC with 2.0 mm of end-hole (JBS-2) was delayed compared to the PDC with 3.0 mm of end-hole (15min 2 sec vs 9 min 33 sec). We observed trivial difference of inflow time between the two types of catheters (8 min 3 sec vs 6 min 9 sec). Meanwhile, the existence of slit, side-hole size/number had no impact on the inflow or outflow time of PDF. It took 8 minutes of mean inflow time with JBS2.

Conclusions: The bag exchange procedure with JBS-2 will finish within 30 min, which is considered to be permissible in clinical settings. The smaller side-holes and slit structure of the PDC may contribute to the lower incidence of catheter-related problems including omental wrapping without a delay of PDF inflow- or outflow- time.

PUB320

First Year Outcomes of Incident Peritoneal Dialysis Patients Eduardo K. Lacson, Nien-Chen Li, Raymond M. Hakim, J. Michael Lazarus, Franklin W. Maddux, Joseph P. Pulliam. *Fresenius Medical Care, North America, Waltham, MA.*

Background: There have been few large multi-center studies on peritoneal dialysis (PD) outcomes in incident dialysis patients. We describe a contemporary cohort of patients starting PD and their outcomes in the first year of therapy.

Methods: All adult (age ≥18 years) patients admitted to Fresenius Medical Care North America facilities between January 1 to December 31, 2009 who initiated PD within their first 90 days were included. Patient follow-up is for one year from their first home PD treatment. We describe the cohort and their outcomes including deaths (with withdrawals), hospitalization, transplants, hospitalization, peritonitis and technique failure rates (a switch from PD to hemodialysis for ≥30 days).

Results: Patients (N=1,960) had mean age of 57.0±14.8 years, BMI was 32.2±12.0 kg/m^2, 56.2% male, 74.7% white, 54.1% diabetic, 3.0% with CHF, 7.1% with PVD, and 3.6% with limb amputation. During follow-up, 136 died (6.9%), 47 withdrew (2.4%), 128 were transplanted (6.5%) and 34 recovered kidney function (1.7%). No patient was lost to follow-up - leaving 1,615 (82.4%) active patients on PD by year-end. The median time to to death/withdrawal was 167 days. Patients stayed on PD until being censored 78.6% of the time while 21.4% switched to HD. The median time to technique failure was 158 days. More than half (57.9%) were hospitalized and 28.2% had at least one episode of peritonitis. Median time to 1st hospitalization was 111 days and to 1st peritonitis episode was 123 days. Overall peritonitis rate was 0.68/24 patient-months.

Conclusions: Four out of 5 patients initiating PD within their first 90 days continue with PD for the first year. 1 of 5 patients who died or were discharged switched to HD beforehand. Causes of high hospitalization and peritonitis rates will need to be explored.

PUB321

Psychological Problems and Nursing Intervention of Patients Undergoing Continuous Ambulatory Peritoneal Dialysis Rong Li. *Deparment of Nephrology, Xijing Hospital, FMMU, Xi'an, China.*

Background: To promote psychological care for patients undergoing the continuing ambulatory peritoneal dialysis(PD), and help patients can cooperate with the medical staff in order to improve dialysis results and quality of patients' life.

Methods: Professional staff provide self-management education, self-care guide and targeted communication for PD patients with different psychological problems, and provide psychological intervention by such means as the combination of individual guidance and group education. Firstly, investigations on PD patients' age, occupation, personality and family background may be carried out and the psychological problems of patients should be confirmed. The specialist, professional nurses and dietitians will provide self-management education and self-care guide for patients and their families by means of lectures, videos, pictures and brochures. Patients club will be established and patients with successful experience in the treatment will be invited to give speeches on successful treatments, Individual guidance and group education will be integrated to achieve better effect.

The nurse staff should encourage patients to face the reality with positive attitude and receive dialysis treatment and therapy. The nurse staff should narrow the mental differences among the patients, establish harmonious relationship among doctors, nurses and patients by individualized services. The nurse staff should detect problems and help resolve the problems timely thereby to effectively improve the PD quality and patients' life quality.

Results: Psychological problems of PD patients are one of the growing concerns of the medical personnel. Active intervention can improve the life of patients and reduce the burden brought to individuals, the families of the patients and the society. So patients are willing to receive psychological care, and the dialysis will have a significant effect, which will improve patents' life significantly.

Conclusions: Psychological care for patients undergoing the long-term dialysis has significant meaning.

PUB322

Percutaneous Versus Surgical Insertion of Peritoneal Dialysis Catheters: Experience of an Irish Center Samar A. Medani, Mohamed Shantier, Wael F. Hussein, Catherine A. Wall, George Mellotte. *Nephrology, Adelaide & Meath Hospital Dublin, Dublin, Co Dublin, Ireland.*

Background: Peritoneal dialysis (PD) is the preferred available option of renal replacement therapy for a growing number of end stage kidney disease patients. A major limiting factor to the successful continuation of PD is long-term viability of the PD catheter (PDC). Percutaneous placement of PDCs is not commonly practiced despite recently published data encouraging use of this technique. Its advantages include faster recovery and avoidance of general anaesthesia.

Methods: We carried out a retrospective analysis of the outcomes of 313 PDC insertions in our centre comparing all percutaneous PDC insertions between July 1998 and April 2010 (group P) with all surgical PDC insertions from January 2003 to April 2010 (group S).

Results: 151 group P and 162 group S catheter insertions were analysed. Significantly more patients in group S had previously undergone abdominal surgery or PDC insertion compared with group P (41.8 % vs 9.3 % and 33.3 % vs 3.3% respectively; P=0.00). There were more exit site leaks in group P than in group S (22.1 % vs 7.4 %; P= 0.00) but no significant differences in peritonitis rates (1 episode per 16.5 catheter months vs 1 episode per 12.5 catheter months; P= 0.36), poor initial drainage (9.9 % vs 11.7 %; P= 0.1) or secondary drainage failure (8.7% vs 13.7 %; P= 0.183). Technical survival at 3 months was significantly better for group P than for group S (86.6 % vs 77 %; P= 0.037) and at 12 months was 77.7 % vs 68.7 % respectively (P= 0.126). No life threatening complications attributable to the insertion of the PDC occurred in either group.

Conclusions: We have demonstrated further encouraging outcomes of percutaneous PDC placement in comparison with the open surgical technique. The percutaneous insertion group were primarily a selected subset of patients without prior abdominal surgery or PDC insertion, therefore limiting this comparability. Studies eliminating these confounding factors are required although local expertise may affect generalisability of results. We recommend formal training of junior nephrologists on this bedside technique, particularly in healthcare systems with limited resources.

PUB323

Medical (Trocar & Cannula Method) Versus Open Surgical Insertion of Peritoneal Dialysis Catheters: A Retrospective Cohort Study Girish S. Namagondlu,[1] Vivian W. Yiu,[2] Thomas F. Hiemstra,[2] Paul F. Williams.[1,2] *[1]Nephrology, Ipswich Hospital, Ipswich, United Kingdom; [2]Nephrology, Addenbrookes Hospital, Cambridge, United Kingdom.*

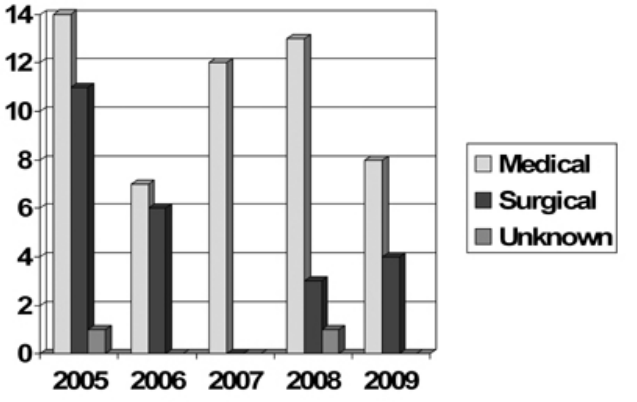
Background: There is little evidence in literature about direct comparison of trocar & cannula method vs open surgical technique for PD catheter insertions. A 5year(Jan 2005 - Dec 2009) retrospective cohort study of medical insertion(MIs) versus surgical insertion(SIs) of PD catheters, was performed at our centre (Ipswich Hospital). Primary end point was catheter patency at 1 year (Censored for death & elective modality switch).

Methods: Data were extracted from an electronic database & patient case records.

Results: We identified 84 catheter insertions in 80 patients during the study period,54/84 (64.2%) were MIs.Age did not differ significantly between groups.More MIs were male (44/54) compared to SIs (10/24, P=0.013).7/24 (29.2%) SIs and 5/54 MIs (9.2% overall) were no longer patent after 12 months(P=0.052).Combined catheter patency at 12 months (censored for death and transplant) was 79.2% (ISPD recommendations suggests >80%).

	MIs	SIs	Unknown	Total
Mean age in years, at the time of catheter insertion	56.5	52.7		58.1
No. of catheter insertions	54(64.2%)	24(28.5%)	6	84
Catheter patency at 1 year(Censored for death and Transplantation)	48(88.8%)	15(62.5%)	2(33.3%)	65(79.2%)
Catheter failure within 1 year	5(9.2%)	7(29.2%)	1	13(16.6%)
Immediate mechanical Complications (< 4 weeks post insertion)	4(7%)	2(8%)		6(7.1%)
Catheter related infections (< 4 weeks post insertion)	0	0		0

Rates of immediate complications (<4 weeks) were similar in both the groups and comparable to ISPD recommendations.



Conclusions: Our data indicates that MIs of PD catheters by trocar & cannula method, are comparable to open SIs in terms of safety and better in efficacy. Strict selection criteria for MIs is required to acheive these results.Our data is comparable to ISPD recommendations 2010.

PUB324

Equipment and Home Requirements for Home Hemodialysis (HHD) among Canadian Renal Programs Robert P. Pauly,[1] Paul Komenda,[2] Deborah Lynn Zimmerman.[3] *[1]University of Alberta, Canada; [2]University of Manitoba, Canada; [3]University of Ottawa, Canada.*

Background: There is growing interest in HHD though little published information on establishing and maintaining an HHD program. The purpose of this study was to survey Canadian HHD programs to describe practice patterns in a variety of domains. The current abstract focuses on equipment and home requirements for HHD.

Methods: A questionnaire was developed by 19 physician experts with input from allied health team members. The instrument underwent multiple modifications to ensure content and face validity, and was completed online between Jul and Dec 2010; data reflect practices during this period.

Results: Seventeen of 19 (89%) programs responded. All programs use conventional HD machines in the home setting. Maintenance of the equipment is provided by the vendor in 6/17 (35%) or by the renal program in 11/17 (65%). Wetness detectors are routinely used around cannulation sites and on the floor beside the HD machine in 14/17 (82%) while 3/17 (18%) use detectors at one site or the other. Centrifuges (for home blood sample preparation) and weigh scales are provided by 14/17 (82%) and 10/17 (59%) of programs respectively. Real-time remote monitoring is performed in a minority of programs (3/17 – 18%). Reasons for denying access to HHD include substandard water (11/17 – 65%), and substandard/ nonmodifiable plumbing (13/17 – 76%) or electrical (11/17 – 65%). Only 2 programs (12%) do not reimburse patients for some or all plumbing/electrical renovations, with 11/15 (73%) covering in excess of $1,500 (CDN) in expenses. Five of 17 (29%) reimburse patients for additional monthly utility costs incurred by dialyzing at home.

Conclusions: Significant heterogeneity exists among Canadian renal programs in terms of delivery of and equipment requirements for HHD. It is unknown whether such differences change patient outcomes or pose potential safety concerns; this will require prospective study.

PUB325

Teaching Home Haemodialysis: An e-Learning Platform Defined by a Multidisciplinary Group in a Series of Brainstorm Sessions Giorgina B. Piccoli,[1] Martina Ferraresi,[1] Federica N. Vigotti,[1] Valentina Consiglio,[1] Stefania Scognamiglio,[1] Franca Giacchino.[2] *[1]Nephrology and Dialysis, San Luigi Hospital - University of Turin, Orbassano, Torino, Italy; [2]Nephrology and Dialysis, Ivrea Hospital, Ivrea, Torino, Italy.*

Background: Home hemodialysis is a hot topic; it conveys self management and empowerment of the patients; lower costs and availability of non conventional, highly efficient techniques. Lack of knowledge and confidence are the main barriers for its wider use.

Aim of this study was to design an e-learning platform, for home dialysis patients and their caregivers, designed by a multdisciplinary group.

Methods: The elements needed were identified in 4 brainstorm sessions, with the participation of a senior engineer, a senior nephrologist, a nephrologist in training, 1-3 dialysis nurses (skilled on home-self care), plus either 1-2 patients/caregivers on home dialysis (5-34 years of experience) or 2 experts in computer programming/communication. At the end of each session, the suggestions were gathered by the two nephrologists, and presented, for approval, at the start of the next session.

Results: The following elements were identified as crucial for a e-learning home support:

1. tutorial (video recording in real time) on the preparation dialysis machine and water treatment, alarms, end of the session, disinfection.
2. lessons: generalities on kidney diseases, indications for dialysis start; other dialysis modalities, transplantation; details on hemodialysis membranes, efficiency and water and biochemical tests, generalities on imaging tests.
3. Kt/V calculation and other formulae (equivalent clearances, BMI, Calcium phosphate balance).
4. videos on different aspects of dialysis and transplantation, including patients' histories
5. laws and legal support.
6. video-calls and video-conferences with the center and the caregivers.

The main patients' request was a high degree of detail, and simple, not oversimplified explanation, and the possibility to directly interact with the Center.

Conclusions: A flexible approach to e-learning and home hemodialysis support, including technical experts, patients and caregivers may help tailoring the programme to the specific needs of each clinical and cultural setting.

PUB326

Non Planned Dialysis and Peritoneal Dialysis Choice Maite Rivera, Antonio Gomis Couto, Nuria Rodriguez Mendiola, Franz Fernandez Rodriguez, Francisco Díaz Crespo, Sara Jiménez álvaro, Robert Marcen-Letosa, Jose L. Teruel, Milagros Fernandez Lucas, Carlos Quereda. *Nephrology, Hospital Universitario Ramón y Cajal, Madrid, Spain.*

Background: The underutilization of Peritoneal Dialysis (PD) as a renal replacement therapy is well known . It has been published that predialysis information about the different modalities of dialysis would increase the patients who chose PD as dialysis treatment. Furthermore, some authors affirm that an unplanned starting of dialysis influences the election of dialysis modality towards Hemodialysis (HD).

Aim: To analyze the patients who started dialysis treatment in a non-scheduled manner in our renal unit during 2010 and what was the treatment modality chosen.

Methods: From January to December 2010, 64 patients started dialysis in our Hospital. 44 patients (69%) started dialysis treatment on a programmed basis and dialysis was unplanned in 20 patients (31%). In those patients acute HD was required through a central venous access for several weeks.

Results: 16 non-programmed patients started HD (80%) while 4 chose PD (20%) despite. They were 18 male and 2 female with a mean age of 61.3±13.2 years (range 37-85 years). Causes of unplanned dialysis were: acute renal failure (n=2), unexpectedly rapid deterioration of end stage renal disease (n=7), late start of dialysis (n=8) and other (n=3).

1 out of 20 non-programmed dialysis patients was prescribed HD because having an impracticable abdomen. Finally, 19 patients chose dialysis modality after being informed of the different dialysis modalities. The reasons why 15 patients chose maintenance HD were: personal preference (n=8), social reasons (n=6), expected short survival (n=1). All 4 patients who chose PD to maintain their independence (n=2) and to continue working (n=2). HD patients were older than patients who chose PD (64.3 vs 49.5 years).

Conclusions: When informed, 20% of patients who started on an unscheduled basis were finally included in PD. Younger patients choose PD over HD in order to maintain their independence. In our experience, unplanned dialysis does not always mean HD.

Funding: Government Support - Non-U.S.

PUB327

Factors That Influence the Dialysis Modality Choice Maite Rivera, Antonio Gomis Couto, Nuria Rodriguez Mendiola, Francisco Díaz Crespo, Sara Jiménez álvaro, Robert Marcen-Letosa, Jose L. Teruel, Carlos Quereda. *Nephrology Service, Hospital Ramon y Cajal, Madrid, Spain.*

Background: Peritoneal dialysis is an underutilized dialysis technique. The reasons why this occurs are not well known. In this work we analyze the reasons that condition the choice of hemodialysis (HD) or peritoneal dialysis (PD) in incident patients of our hospital during 2010.

Methods: 64 patients started dialysis from January to December 2010 (49 male and 15 female),with a mean age of 58 years (range17-85 years). The most frequent cause of chronic renal failure was diabetic nephropathy (23%). 13 patients had a dysfunctioning graft and were transferred to dialysis (20%). 39 patients (61%) started HD and 25 PD (39%). 44 patients (69%) started dialysis treatment on a programmed basis and dialysis was unplanned in 20 patients (31%). At the start of dialysis 18 (28%) were occupationally active and expressed their desire to keep on working. All patient received information about dialysis modalities.

Results: Treatment modality was a personal choice in 55 patients (86%). The remaining patients could not choose to have a contraindication to any form of dialysis.Of the 55 patients who could choose, 31 chose HD and 24 PD. Patients who chose HD were older (62±16 vs 52±14 years, $p< 0.05$) and had a higher comorbidity Charlson index (6.6±2.6 vs 4.7±2.2, $p<0.01$). The choice of technique was not influenced by patient sex, cause of chronic renal failure, previous failed graft, planned dialysis or time of follow-up in predialysis consulting. PD was chosen by 54% of patients who wished to continue working while only 10% of patients that chose HD wanted to work ($p<0.001$).

Conclusions: In our unit, the dialysis options information program provides 39% of patients began treatment with PD. In our experience, the main factor that influences the choice of PD , mainly in young patients, was the decision to continue working.

PUB328

Effect of Interventions in Peritonitis Rate and Technique Failure in Renal Therapy Service Colombia Network Angela S. Rivera. *Medical, Baxter, Bogota, Colombia.*

Background: Peritonitis and technique failure are the most frequent complications in peritoneal dialysis (PD). Interventions in education and training impact clinical outcomes

Renal Therapy Service (RTS) is a network of renal units in Colombia, with a large PD program (proportion of PD patients 47% and an average of 3515 patients), in 49 clinics with a Clinical Coordinated Care Model focused on quality assurance process (standardized clinical protocols, ongoing education and training), internal national and regional clinical audits (continuous quality improvement) for protocol compliance, disease management (anemia, hypertension, diabetes, nutrition, mineral & bone disorder, hepatitis B vaccination and Dialysis management) and quality standards adherence monitored by nephrologists and nurses.

Methods: A retrospective analysis of peritonitis rate and technique failure trend after care model implementation was performed from 2004 to 2010.

The interventions were:

Monthly comprehensive evaluation by nephrologist and nurse

Attention by nutrition, psychology and social work according to risk

Programmed home visits and prioritized visits according to risk

PD Training for clinical team

Standardized nurse/ patient ratio: 1 / 45

Protocol Standardization for peritonitis prevention: Prophylaxis for exit site infection with antibiotic ointment

Change in antiseptic for cleaning hands and surfaces: Not use of iodinated solutions and introduction of chlorhexadine and glycerinated alcohol

Results: Mean age was 58.4 years, 53.7% were female, 38% were diabetic and 63% has a low socioeconomic level. There was a significant decrease in peritonitis rate from 1 episode each 25.2 to 1 episode each 44.1 patient months in risk (RR 0.55 [0.52 – 0.59], $p< 0.05$) and decrease in technique failure defined as transfer to hemodialysis due to clinical complications from 16.20% to 8.11%(RR 0.52 [0.45- 0.59] $p< 0.05$)

Conclusions: here is an improvement of peritonitis rate and a decrease in PD technique failure after a systematic compliance of protocols and the development of continuous education programs.

Funding: Pharmaceutical Company Support

PUB329

Pasteurella Multocida Peritonitis in a Peritoneal Dialysis Patient Manish K. Saha,[1] Tarek Hamieh,[1] Vishal Sagar.[2] *[1]Internal Medicine, Regions Hospital, Saint Paul, MN; [2]Nephrology, Regions Hospital, Saint Paul, MN.*

Background: 64 year old male with end stage renal disease on automated peritoneal dialysis(PD) with a cycler at home presented with abdominal pain and vomiting for 1-2 days. He noted that his PD fluid was cloudy a day before presentation. On exam, there was diffuse abdominal tenderness but no evidence of erythema or tenderness over the PD catheter site. Hemogram was unremarkable . Blood cultures were negative. CT scan of abdomen did not show any evidence of bowel perforation or abscess. Initial PD fluid was cloudy and showed 1195 nucleated cell/ul with 91 % of PMNs. Patient was started on intraperitoneal vancomycin and tobramycin after PD fluid was sent for culture for presumed peritonitis. Initial gram stain showed gram negative bacillus and cultures grew Pasteurella multocida. On further review of history, patient disclosed that he had 2 pet cats that had been licking/ biting on his PD tubing while he was on the cycler at night. A diagnosis of pasteurella multocida peritonitis was made and his antibiotic regimen was changed to intraperitoneal ampicilin-sulbactum for 2 weeks with improvement of symptoms. Patient was counseled and educated about pasteurella multocida and routes of transmission prior to discharge. He was also advised to keep cats, cats oral secretions at bay while performing dialysis.

Discussion: Pasteurella multocida is a gram-negative bacilli and is found the oropharyngeal flora of cats and felines. Patients on peritoneal dialysis are at increased risk for peritonitis if PD catheter gets exposed to cats or felines' oral flora. Peritonitis is major cause of mortality and morbidity in PD patients. The usual symptoms are fever, abdominal pain, and cloudy dialysate as in our patient. Since our patient had significant history of

cat's oral secretion exposure to PD catheter, this is a rare form of transmission without PD catheter break or scratches. Increasing awareness through patient education is of utmost importance given the prevalence of patients having animal pets at home.

PUB330

Prevalence and Clinical Relevance of Sick Euthyroid Syndrome in a Peritoneal Dialysis Population Hugo Mário Silva, Joana Tomás, Anabela S. Rodrigues, Maria João Carvalho, António Manuel Nunes Cabrita. *Nephrology, HSA, ICBAS, Portugal.*

Background: Sick euthyroid syndrome (SES) has been linked with severity and outcomes in acute illness, however scarce data is available in dialysis patients.

We aimed to evaluate the prevalence of low T3 syndrome in our peritoneal dialysis population and determine its relation with peritoneal transport rate, peritoneal protein loss, inflammation, nutrition and volume status.

Methods: Cross sectionall design: 56 prevalent patients on PD, aged 57.3 (45.4-68.0) years, 46.4% male, 16.1% diabetics, median Charlson comorbidity score of 5; 28.6% anuric, residual GFR 5.0 (2.7-6.8) mL/min; time on PD 28.6 (11.5-55.4) months.

Prevalence of SES and comparisons between subgroups according to the median T3 value were evaluated. Nutritional and volume parameters were obtained by multifrequency bioimpedance. Serum protein C reactive (PCR), D/P creatinine, protein losses, normalized protein catabolic rate, anemia, albumin and dose of dialysis were also explored. Multinominal linear regression model was applied to determine relevant correlations.

Results: SES was diagnosed in 5.3% of patients. Median plasma free T3 level (FT3) was 2.9mg/dL (2.5-3.1). Lower FT3 group was older (58.7 vs. 48.3 years, P=0.003), had lower albumin (3.6 vs. 3.9 mg/dL, P=0.005) and haemoglobin levels (11.6 vs. 12.5 mg/dL, P=0.028); higher ferritin (540.0 vs. 356.2 mg/dL, P=0.021) and PCR (7.9 vs. 5.0 mg/dL P=0.03); a trend for higher extracellular/intracellular water (ECW/ICW) ratio (0.98 vs. 0.91, P=0.052) was found. No differences were documented in peritoneal transport rate, protein losses or dialysis dose. In a multivariate linear model, entering with age, haemoglobin, PCR, ferritin and ECW/ICW ratio as independent variables only haemoglobin and PCR were able to predict FT3 (Beta + 0.33, P=0.004 ; and Beta - 0.27 P=0.042 respectively).

Conclusions: There was a very low prevalence of SES in our PD population. Thyroid dysfunction was associated with markers of systemic inflammation, volume status and malnutrition, however only hemoglobin and PCR were good predictors of FT3. Longitudinal evaluation of this overlooked parameter might elucidate more its clinical impact on morbidity and mortality.

PUB331

Peritoneal Dialysis Technique Outcome and Associated Risk Factors: A 9-Year Single-Center Study Namita Singh,[1] Ramesh Saxena.[2] *[1]Internal Medicine, JHU/ Sinai Hospital, Baltimore, MD; [2]Internal Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: To review the peritoneal dialysis (PD) technique outcomes at our center and assess factors affecting the technique survival (TS).

Methods: This is a retrospective study on 315 patients who initiated PD between January 2001 and September 2009 at UT Southwestern/ DaVita PD-Clinic, Dallas. Medical records were reviewed for demographic and clinical information. The primary end point was PD technique failure (TF), defined as discontinuation of PD due to catheter-related, technique-related or other medical/surgical/social complications. TS was analyzed by Kaplan Meier method. Cox proportional hazard regression model was used to identify factors independently associated with TS.

Results: There were 54.6% females, and 42.5% African Americans and 43.2% diabetics in our study population. More than 90% of patients had co-morbidities; and 57.5% had previous abdominal surgery. The mean BMI was 28.6 +/- 13.8 kg/m^2.

Infectious complications included 39.7% peritonitis and 22.9% catheter-related infections. Non-infectious/ mechanical catheter problems were observed in 24.1%. There were total of 203 PD failures, of which 12.8% were due to catheter-related problems and 20.2% due to peritonitis. Poor performance on PD, and medical/ surgical events contributed to 7.4% and 6.9% PD discontinuations, respectively.

Overall PD TS rates at 1, 2 and 3 years were 82.12%, 69.42% and 58.38%, respectively. Two variables significantly affecting TS rates were PD catheter-related non-infectious problem (Hazard ratio 1.812; 95% CI 1.193-2.750), and Diabetes as the etiology of ESRD (Hazard ratio 1.712; 95% CI 1.146-2.556). No significant association was observed between TS and other risk factors including age, BMI, previous abdominal surgeries or infections.

Conclusions: Our study shows 58.4% 3-year PD TS. Diabetes and PD catheter-related non-infectious problems are significantly associated with PD TF. Other factors such as age, gender, race, BMI, previous abdominal surgeries, peritoneal infections or exit-site/ tunnel infections do not affect the PD technique survival and should not be considered barriers to PD initiation.

Funding: Clinical Revenue Support

PUB332

Comparison of Change in the Adequacy of Peritoneal Dialysis over Time in Diabetic and Non-Diabetic Patients – A Single Centre Observational Study Subash Somalanka, Manivarma Kamalanathan, Bhrigu Raj Sood. *South West Thames Renal Unit, Epsom and St Helier NHS Trust, Carshalton, Surrey, United Kingdom.*

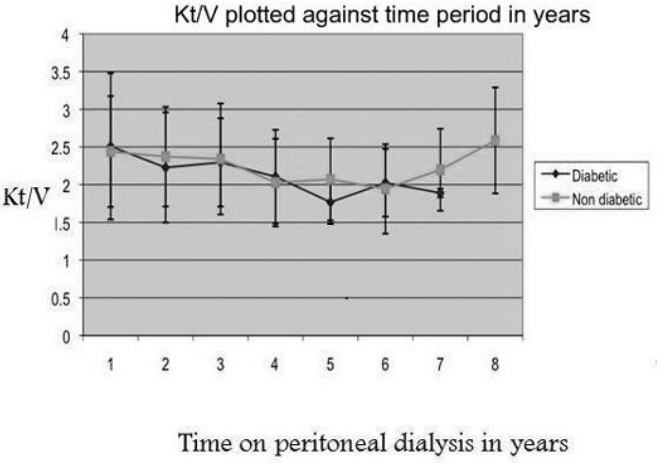
Background: The diabetic patient with end stage renal failure presents many therapeutic challenges, some of which are particularly difficult when peritoneal dialysis is selected as modality for renal replacement. Given the advantages of home based therapy, more patients with diabetes are taking up peritoneal dialysis. We wanted to compare the difference in change of adequacy of peritoneal dialysis over time in patients with and without diabetes undergoing peritoneal dialysis.

Methods: We studied a cohort of peritoneal dialysis patients in our unit. The data of all the patients who are currently on the peritoneal dialysis programme in 2011 was obtained from our renal unit database. We compared the dialysis adequacy between diabetic and non-diabetic patients over the last 8 years. We studied variable including age, sex, diabetes status, modality of PD, and annual Kt/V. The follow up period varied between 2 to 8 years.

Results: A total of 64 patients were identified from the renal unit database who had annual adequacy data. 19 patients had a diagnosis of diabetes and 45 were non-diabetic.

Patient characteristics

	Diabetic	Non-diabetic
N	19	45
Male	8 (42%)	21 (47%)
Female	11 (58%)	24 (53%)
APD	14 (74%)	34 (75%)
CCPD	5 (26%)	11 (25%)



Conclusions: No significant difference was observed in serial change in adequacy of dialysis when compared over time in diabetic and non-diabetic population. This observational study shows that peritoneal dialysis can be used effectively as a form of RRT in diabetic patients.

PUB333

Early Start of Combination Therapy with Hemodialysis and Peritoneal Dialysis Prolongs Survival Term and Reduces Cardiovascular Events in Male Patients Hiromichi Suzuki, Tsuneo Takenaka, Hirokazu Okada, Tsutomu Inoue. *Department of Nephrology, Saitama Medical University, Iruma gun, Saitama, Japan.*

Background: Although peritoneal dialysis (PD) has been proposed as the initial type of dialysis therapy, a majority of patients with end-stage renal disease choose hemodialysis (HD) therapy. Several previous studies comparing survival rates and cardiovascular events between these two therapies have not clearly demonstrated the superiority of one over the other. Recently our data have indicated that renal replacement therapy with a combination of HD and PD prolonged survival rates and reduced cardiovascular events compared with either HD or PD alone. However, combination dialysis therapy is currently not a widely adopted procedure.

Aim: To analyze the efficacy of combination dialysis therapy of PD and HD in patients who were started with PD as the initial dialysis therapy.

Methods: A single center, retrospective cohort study was performed that included 401 patients (female/male: 165/236; age: 61± 12/62 ± 9 years) who started PD as the initial dialysis treatment from 1995 to 2005. Chart and electronic databases were used to obtain information on the course of dialysis therapy including mortality and cardiovascular events.

Results: A total of 103 patients were treated with PD and HD in combination. In 5 years of follow-up after the start of PD, 80 patients died. There were no significant differences in cumulative mortalities between males (49; 20%) and females (31; 18%), and in the cumulative incidence of catheter removals (35 vs. 31%) for various reasons. A significant

difference ($p<0.05$) was seen in the start of hemodialysis between males and females. In male patients on PD, HD therapy was started 24± 16 months after the start of PD. In contrast, in female patients, HD was started 55± 16 months after the start of PD.

Conclusions: Although females have a survival advantage in the general population as well as among dialysis patients, females undergoing PD have a similar mortality to men. The reasons for these findings remain to be explained. However, this analysis suggests that early start of hemodialysis therapy will prolong survival rate in patients on PD, and especially in male patients.

PUB334

Developing Bioimpedance (BIA) as a Tool for Fluid Management in Peritoneal Dialysis Patients: A Validation Study Boon Kay Tan,[2] Zanzhe Yu,[1] Frauke Wenzelburger,[1] Martin E. Wilkie,[3] Sarah Jenkins,[3] Jia Qi Qian,[4] Zhaohui Ni,[4] Simon J. Davies.[1] *[1]Institute for Science and Technology in Medicine, Keele University, Stoke on Trent, Staffordshire, United Kingdom; [2]Nephrology Department, University Hospital of North Staffordshire, Stoke on Trent, Staffordshire, United Kingdom; [3]Sheffield Kidney Institute, Sheffield, South Yorkshire, United Kingdom; [4]Renal Department, Renji Hospital, Shanghai, China.*

Background: The assessment of fluid status in PD patients using simple clinical parameters is insensitive due to spontaneous body composition changes with time on therapy. We hypothesize regular monitoring of body composition using BIA adds value to the fluid management in PD patients.

Methods: This is an ongoing multi-centre, prospective, randomized study of incident and prevalent PD patients in the UK and China stratified by country and residual renal function >200ml vs <200ml at entry. To detect a between group difference in extracellular fluid volume (ECFv) of 0.8 Kg requires 38 patients per group with 80% power. Following a 2-3 months run in period where fluid status is deemed optimal baseline BIA measurement is obtained in all patients. Measurements are taken in both groups 3 monthly and in addition at any time of clinical need in the active limb where BIA data is available. Interventions can be a combination of advice on dietary salt and fluid intake, increased use of diuretics, hypertonic solutions and icodextrin. Any interventions based on BIA data and their intended effects will be recorded prospectively. This study makes no assumptions as to the ideal desirable fluid status in PD patients at any given time.

Results: The primary outcome is the maintenance of ECFv determined from BIA in the active limb. Other outcome measures are BP, residual urine volume, membrane function, biomarkers and cardiac functions by echocardiography.

Conclusions: Measurement of fluid status in PD is challenging and BIA can be a powerful clinical tool in the routine management of fluid status. This study will give insight and valuable information as to the best application of this technique.

Funding: Pharmaceutical Company Support

PUB335

Variability in Water Source and Dialysate Composition for Canadian Intensive Home Hemodialysis Programs Deborah Lynn Zimmerman,[1] Robert P. Pauly,[2] Paul Komenda.[3] *[1]Medicine, University of Ottawa, ON, Canada; [2]Medicine, University of Alberta, Edmonton, AB, Canada; [3]Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: Despite growing interest in intensive hemodialysis (HD) prescriptions (more frequent and/or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns.

Methods: A survey was developed with the assistance of 17 physician experts with input from their allied health teams. The survey was modified based on reviews received. The instrument was finalized after a face to face meeting with the expert physician panel. The survey was completed on line. The current abstract addresses water quality and dialysis prescription.

Results: Seventeen of 19 programs contactied participated in survey development and provided information. For water sources, 16 and 6 programs have used well or surface water respectively. Testing for microbial contamination, endotoxin units (EU), organics/inorganics is done by 15, 13, and 13 programs respectively. All programs set a limit of 50 or 100 CFU for the product water. There was more variability in EU limits of < 0.25/ml (3), <1.0/ml (9), <2.0/ml (1) and not measured or not answered (4). Water sampling is variable and done by patients, technicians and/or the equipment manufacturer. For nocturnal HD, starting dialysate prescription was Na (136-140mmol/L), K (2-3mmol/L), HC03 (28-37mmol/L), Ca (1.5-1.75mmol/L), Mg (0.5-0.75mmol/L) and glucose (5.55-11.1mmol/L). Dialysate flow was 150-500mls/min and most programs are using high-flux dialyzers. For short daily HD, variability was also seen in dialysate concentrations except the lowest HC03 was 30mmol/L and Ca was usually 1.25mmol/L. Dialysate flow was 500-800mls/min and all programs used high flux dialyzers.

Conclusions: Despite a long history of intensive HD utilization in Canada, there is tremendous practice variability. Deciphering the results of observational data is made more complicated by these differences in prescription. A lack of published patient outcomes or guidelines for patient management may contribute to variability.

Funding: Clinical Revenue Support

PUB336

Malnutrition Inflammation Complex Syndrome in Haemodialysis Patients: Assessing the Prevalence and Severity Using Malnutrition Inflammation Score Christopher T. Agbo, Sumith C. Abeygunasekara. *Renal Medicine, Broomfield Hospital NHS, Chelmsford, United Kingdom.*

Background: The combination of inflammation and protein energy malnutrition (PEM) is very common condition among maintenance haemodialysis patients. The term malnutrition-inflammation complex syndrome (MICS) has been widely used to describe this condition and is associated with increased morbidity and mortality in maintenance haemodialysis(MHD) patients. Malnutrition-inflammation score (MIS) is a comprehensive tool for evaluating MICS and there has been recorded correlation between MIS and mortality among haemodialysis patients. In this study, we used MIS to assess the prevalence and severity of MICS in MHD patients.

Methods: Observational cross-sectional study of 139 maintenance haemodialysis patients in our renal unit. The MIS tools was used to evaluate all the patients within the study period, other laboratory parameters such as C-reactive protein (CRP), Haemoglobin(Hb), protein catabolic rate (nPCR), urea reduction ratio were measured.

Analysis of the data was done using SPSS statistical software. Pearson's correlation coefficient was used for selected continuous variables, Spearman's rank correlation used for non-parametric variable.

Results: The mean age was 68.4 +/- 15.1, 68.9% were men and mean duration on dialysis in months was 42.92 +/-51.45 ; mean MIS was 6.17 +/- 3.03; MIS < 3 (3.8%), 3 – 5 (40.6%), 6 – 8 (36.8%), > 8 (18.8%). MIS showed a strong correlation with serum CRP level ($p = 0.014$), Haemoglobin level ($p =0.004$), Age ($p=0.034$) and nPCR ($p=0.039$). This result showed high CRP,low Hb,low nPCR and age were associated with increase morbidity and mortality.

Conclusions: Malnutrition- inflammation score is a powerful tool in assessing the prevalence and severity of MICS. It is useful also in predicting the factors that affect morbidity and mortality in haemodialysis patients such as CRP,Hb,nPCR and age. MIS showed a strong correlation with CRP, haemoglobin, nPCR and age. The entire patients studied had some degree of MICS, the greater the MIS the increased risk of morbidity and mortality. Apart from using the MIS to risk stratify haemodialysis patients, there is need to establish a universal cut off point in order to define MICS.

Funding: NHS

PUB337

N-acetylcysteine (NAC) Supplemented in the Reinfusate during Acetate-Free Biofiltration (AFB) to Blunt Oxidative Stress Generated during Dialysis (HD) Alessandro Amore, Roberta Camilla, Licia Peruzzi, Roberto Bonaudo, Rosanna Coppo. *Nephrology, Dialysis, Transplant, R. Margherita H., Turin, Italy.*

Background: The oxidative stress (OXS) plays a key role in triggering the dialytic vasculopathy. Bioincompatible reactions during HD amplify the phenomenon. In an in vivo study we showed that NF-kB is activated during HD and it is blunted by i.v. infusion of NAC at the end of HD. We realized that both NF-kB activation during HD and NAC blunting effects were too sharp to be satisfactory, apart from prolonging the time of each HD, not liked by the patients.

We aimed this study to improving a highly biocompatible HD, acetate free biofiltration (AFB), adding NAC to the infusion fluid in order to blunt the OXS generated during HD.

Methods: We performed 8 AFB ex vivo sessions by circulating healthy blood donors 250 ml/min, in AN60-ST dialyzers, using a buffer-free dialysate (Safebag,Hospal) 500 ml/min flow and NaHCO3 reinfusate (Hospasol 145, Hospal) 30 ml/min. Reinfusate was additioned by NAC, 10 mM. NAC SH residues were measured in blood, reinfusate and dialysate at 0, 1, 5, 15 , 30, 60 min by cation exchange chromatography, Biochrom, specific for -SH and S-S residues, after derivatization with Ninhydrin (440 and 570 nm) correcting values for hematocrit.

Results: A SH peak in blood as well as in dialysate was detected. At 5 min after start of dialysis, we added to the recirculating blood Nitroprusside (SNP) 5 µM which, reacting with NAC SH groups,leads to the production of S-S compounds.

The addition of SNP significantly reduced the SH peak, while a peak of S-S appeared indicating a buffering effect on OXS during in vitro HD.

Data, expressed as mM of SH and S-S compounds, are reported in the table.

Table1

Time minutes	1	5	15	30	60
Dialysate effluent SH compounds	5.4±0.68	5.7±0.63	5.6±0.66	5.3±0.07	5.9±0.42
Blood +NAC in reinfusate SH compunds	0.92±0.16	1.13±0.09	1.15±0.05*	1.72±0.01*	0.73±0.11*
Blood+NAC 10mM+ Sodium Nitroprusside 5µM SH compounds		0.77±0.03*	0.78±0.09*	0.71±0.03*	0.73±0.11*
Reinfusate+ SNP 5µM S-S compounds		0.32±0.12	0.45±0.21	0.47±0.18	0.38±0.09

*$p<0.01$ vs blood +NAC in reinfusate

Conclusions: In conclusion, we proved that the addition of NAC to the reinfusate fluid of AFB, can blunt the OXS generated during HD.

PUB338

The Relationship between TRAIL Concentration and Inflammatory Markers Marek Kuzniewski,[1] Danuta Fedak,[2] Dariusz Giza,[1] Pawlica Dorota,[3] Beata Kusnierz-Cabala,[2] Paulina Dumnicka,[3] Bogdan Solnica,[2] Wladyslaw Sulowicz.[1] *[1]Department of Nephrology, Jagiellonian University, Collegium Medicum, Cracow, Poland; [2]Department of Clinical Biochemistry, Jagiellonian University, Collegium Medicum, Cracow, Poland; [3]Department of Medical Diagnostics, Jagiellonian University, Collegium Medicum, Cracow, Poland.*

Background: TRAIL (TNF-related apoptosis-inducing ligand) is a member of TNF ligand superfamily. Five different receptors of TRAIL has been identified. Two receptors containing a death domain (TRAIL-R1 and TRAIL-R2) are capable of rapidly inducing apoptosis. Two decoy receptors (TRAIL-R3 and TRAIL-R4) unable to transducer apoptosis signals but may activate nuclear factor kappa-light-chain enhancer of activated B cells and block apoptosis and osteoprotegerin (OPG)-soluble decoy receptor (TRAIL-R5). TRAIL can activate both apoptotic and anti-apoptotic signals. TRAIL, TRAIL-R2 and OPG are present in human atherosclerotic lesions and their expression levels are higher in vulnerable plaques than in stable ones. Chronic kidney disease is associated with accelerated atherosclerosis and exacerbated but ineffective inflammatory response.

Aim of the study was to investigate the relationship between soluble TRAIL (sTRAIL) and selected markers of inflammation in patients on maintenance hemodialysis.

Methods: Studied group: 76 patients (36 female and 40 male) of average age 60 12 years on maintenance hemodialysis (25 5 months). sTRAIL, IL-6 and IL-8 were determined by ELISA and hsCRP using immuno-nephelometry.

Results: The mean values of TRAIL was 959.6 ± 204.0 pg/ml, hsCRP 11.5 ± 18.8 mg/l, IL-6 6.4 ± 8.7 pg/ml and IL-8 20.0 ± 15.7 pg/ml. The obtained correlations between TRAIL and tested parameters were given in table 1.

Correlations between TRAIL and selected inflammatory parameters

TRAIL			
Parameter	r (Pearson's)	r^2	p
hs-CRP	-0.143050	0.020463	<0.217663
IL-6	-0.275220	0.075746	<0.048301
IL-8	-0.304642	0.092807	<0.015199

Conclusions: Nevertheless we found no correlation between TRAIL and CRP the interrelations between TRAIL and IL-6 and IL-8 indicate that sTRAIL may be considered as is a negative inflammation marker.

PUB339

Clinical Characteristics and Cytokine Expression Profile in Hemodialysis Patients Following Hepatitis B Vaccination Roy Mathew,[1] Darius Mason,[2] Jeffrey S. Kennedy.[3] *[1]Internal Medicine, Stratton VA Medical Center, Albany, NY; [2]Nephrology, Albany College of Pharmacy and Health Sciences, Albany, NY; [3]Department of Health of the State of New York, Wadsworth Laboratories, Albany, NY.*

Background: Hepatitis B vaccine (Hbvax) efficacy is suboptimal in Hemodialysis (HD) patients. We proposed to study the role of inflammation using cytokine profile surrounding vaccination to understand the impact on adaptive immune responses to Hbvax in HD patients.

Methods: Eligibility - HD patients at the Stratton VA Medical Center receiving the EngerixB® (GlaxoSmithKline) Hbvax as a primary or repeat vaccination. Exclusion - Coinfection with HIVor Hepatitis C, acute infection, severe anemia precluding blood draws, <30days vaccination with any agent, inability to give consent. Vaccination series: Day 0, Day 30, Day 60 and day 180. Optimal anti-HepB titer is defined >100 pg/mL. Following informed consent, 50ml of blood was collected at Day 0, 3, 7, 10, 14, 30, 180 following the last vaccination in the series. All bloods were drawn pre-dialysis, mid-week. Cytokine analysis: IL-2, -6, -12, -18, TNF-alpha, IFN-gamma. Descriptive statistics were used to evaluate baseline variables. Repeated measures ANOVA was used to compare change in cytokine following vaccination.

Results: N=13. Mean age 73.9 years, mean duration of dialysis 23 months. 4 of 13 received Hbvax previously. 8 developed optimal response (mean 504 pg/ml), and 5 suboptimal (mean 51 pg/ml). No significant differences were noted between dialysis adequacy, paricalcitol or erythropoietin dose, and 25-OH Vitamin D levels (31.4 ng/ml v 31.8 ng/ml, p>0.05; responder v non-responder). No significant cytokine modulation was noted following vaccination from pre-vaccine levels. Antibody titers dropped an average of 30% from 30 days to 180 days post final booster.

Conclusions: Dialysis patients demonstrate a poor response to Hbvax despite higher dosing, as well as an impaired ability to maintain adequate titers. Vitamin D use or adequate serum levels do not improve ability to respond to Hbvax. Cytokine analysis suggests an inability to modulate inflammatory response to stimulate cellular response to vaccine.

PUB340

Usefulness of Biomarkers of Inflammation in Predicting Subsequent Cardiovascular Events in Diabetic Patients on Renal Replacement Therapy Bijan Roshan, George P. Bayliss, Saila V. Ventrapragada, Ray E. Gleason, Larry A. Weinrauch, John A. D'Elia. *Renal Unit, Joslin Diabetes Center/Harvard Medical School, Boston, MA.*

Background: Biomarkers of inflammation, oxidative stress and hemostasis (BIOMARK) were abnormal in hemodialysis pts, but only levels above vs. below the median of IL-6 and CRP were associated with an increased prevalence of prior CVE. Nevertheless, we hypothesized that BIOMARK levels would predict event-free follow up in stable RRT patients.

Methods: In a prospective study, 177 stable renal failure patients (pts) were followed for 0.04-13.69 years for CE (myocardial infarction, coronary arterial intervention, peripheral arterial bypass or amputation, cerebrovascular accident, or carotid artery intervention). Treatment groups were based on modality at entry into the study. 128 hemodialysis pts (64 male, age 58.1±1, 63 [49%] with prior CE); 22 peritoneal dialysis pts, (12 male, age 49 ± 3, 10 [45%], with prior CE); 27 renal transplant pts (10 male, age 44±1, 13 [48%] with prior CE) were included. Tests of inflammation included: Il-6, CRP, fibrinogen; of hemostasis included: fibrinogen, PAI-1, fibrinolytic activity, vWF, factor VII, p-selectin, viscosity; oxidative stress: Advanced glycated end products and antibody to oxidized LDL. For each, upper vs. lower median and tertiles were compared for evaluation of event free follow up.

Results: Median levels of all BIOMARK were in an abnormal range. 14 tests assessing thrombosis, inflammation and oxidative stress were evaluated for their relationship with subsequent event free survival. When upper vs. lower tertiles and above/below medians were compared for event free survival no BOMARK was related to duration of event free survival.

Conclusions: In clinically stable patients undergoing renal replacement therapy, levels of most biomarkers were not associated with differences in event free survival. The use of BIOMARK for prediction of CVE in stable RRT pts is not supported. Further studies of metabolism of fibrinogen and its relationship to event free survival may be worthwhile.

Funding: Pharmaceutical Company Support

PUB341

Pre and Post Dialysis Echocardiography Performed by a Nephrologist. A Useful and Accessible Tool for Hemodynamic Evaluation of Chronic Hemodialysis Patients Adriano Luiz Ammirati,[1] Maria C.C. Andreoli,[1] Fellype Barreto,[1] Wercules Antonio De Oliveira,[2] Marcelo Luiz Campos Vieira,[2] Claudio Henrique Fischer,[2] Bento Santos.[1] *[1]Dialysis Unit, Albert Einsten Hospital, Sao Paulo, Brazil; [2]Cardiology, Albert Einsten Hospital, Sao Paulo, Brazil.*

Background: Echocardiographic (ECHO) parameters are useful to evaluate the volume status in hemodialysis (HD) patients. In this work, we studied the feasibility of a handheld ECHO analysis by a nephrologist, and the correlation of different ECHO parameters and hemodynamic and volume status in HD patients

Methods: Subjects were recruited from a single HD clinic and underwent, at the beginning and at the end of a HD section, a focused handheld transthoracic ECHO by a trained nephrologist. The variables measured were fractional shortening; inferior vena cava diameter and inspiratory collapse index (VCCI), mitral inflow early diastolic velocity (E), late diastolic velocity (A), mitral annular early (E') diastolic velocities and TVI (time–velocity integral obtained across the aortic valve). These parameters were compared with pre and post HD arterial blood pressure, blood volume monitoring data (Fresenius 4008®, Germany) and HD ultrafiltration volume (UF).

Results: Eleven patients were enrolled (age=70.5 ±13 yo). The mean UF was 1.43 ± 0.9 kg. Pre and post HD ECHO parameters values were: TVI (15,6 ± 4; 13.7± 4 cm); VCCI (28.9 ±14%; 29.4± 14%), Fractional shortening (33.6 ± 10.5%; 32.4 ± 6%), E/A ratio (0.62±0.2; 0.61 ± 0.2). Of note, VCCI variations were related with UF (r=0.70; p=0.03) and changes in A wave (r=0.73; p=0.04). Additionally, there was a correlation between changes in VCCI and relative plasma volume slope (r=-0.54; p= 0.10). Furthermore, VCCI changes were related with the variation in systolic blood pressure (r=0.78; p=0.02)

Conclusions: ECHO parameters, especially VCCI, were associated with fluid removal and the variation of blood pressure during a HD session. In addition, ECHO method performed by nephrologist is a helpful and feasible tool in evaluation of hemodynamics status and dry weight in HD patients.

PUB342

Combined Ultrafiltration and Plasmaexchange for Patients with Steroid Resistant Nephrotic Syndrome Gianluigi Ardissino,[1] Fabio Paglialonga,[1] Rosalia Viviana Scarfia,[2] Sara Testa,[1] Antonietta Biasuzzi,[1] Maria Elena Albion,[1] Giovanna Bagnaschi,[1] Cristina Felice Civitillo,[1] Francesca Tel.[1] *[1]Pediatric Nephrology Unit, Fondazione Ca' Granda Osp. Maggiore Policlinico, Milano, Italy; [2]Nephrology and Dialysis Unit, AOU Policlinico V. Emanuele, Catania, Italy.*

Background: Plasmaexchange (PEX) is one of the possible treatment strategies for steroid resistant nephrotic syndrome (SRNS), in particular when associated to focal segmental glomerulosclerosis. Most of the patients with SRNS becomes resistant to diuretics and the management of their fluid overload sometimes becomes a major clinical challenge.

Methods: Here we describe our experience with combining ultrafiltration (UF) together with plasmaexchange aimed at removing part of the fluid overload in patients with SRNS undergoing PEX for other indications.

Results: The described procedure was performed in 2 patients, a boy and a girl, 11 and 19 years old, with a baseline body weight of 39 and 80 Kg and an estimated overhydration of 3 and 16 Kg, respectively. The total number of PEX sessions was 7 for the boy and 5 for the girl.

PEX treatment (BM25 device) was performed with a substitution of 150% of plasma volume (10 with albumine in Ringer Lactate, 2 with fresh frozen plasma) in 3-6 hrs. Heparinization schedule was an initial bolus of 40-50U/kg followed by 20U/Kg/hr. On the arterial line, just before the plasmafilter (Gambro PF2000N), a hemofilter (Edwards Lifesciences HF 0.3) was placed, with a single line out for collecting and measuring

ultrafiltrate. The UF volume obtained ranged between 320 and 2800 mL/session (equal to 2 to 7 mL/kg/hr), the Qb was 80-120 ml/min. The UF was well tolerated, hematocrit (monitored by CritLine2000) remained stable during the sessions.

No significant adverse events were observed, except urticarial reactions. Only one session was complicated by increased transmembrane pressure requiring treatment discontinuation.

Conclusions: The combination of UF and PEX is feasible, safe and efficacious and it represents an additional tool whenever fluid removal is necessary in patients unresponsive to diuretics and requiring PEX.

PUB343

The Impact of a Prior History of Cardiovascular Events on Outcomes in Patients on Renal Replacement Therapy George P. Bayliss,[1] Bijan Roshan,[2] Saila V. Ventrapragada,[2] Larry A. Weinrauch,[2] Ray E. Gleason,[2] John A. D'Elia.[2] *[1]Division of Kidney Diseases and Hypertension, Department of Medicine, Alpert Medical School, Brown University, Providence, RI; [2]Joslin Diabetes Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

Background: The majority of morbidity and mortality on renal replacement therapy (RRT) is cardiovascular. Prior cardiovascular events (CVE) have an unknown impact in the presence of uremia, however they are considered when important therapeutic decisions are made.

Methods: In a prospective study, 177 stable renal failure patients (pts) were followed for 0.04-13.69 years for CVE (myocardial infarction, coronary arterial intervention, peripheral arterial bypass or amputation, cerebrovascular accident, or carotid artery intervention). Pts group was based on treatment at entry into the study. 128 hemodialysis pts (64 male, age 58.1±1, 63 [49%] with prior CVE); 22 peritoneal dialysis pts, (12 male, age 49 ± 3, 10 [45%], with prior CVE); 27 renal transplant pts (10 male, age 44±1, 13 [48%] with prior CVE).

Results: Clinical endpoints included CVE (total of 61; 29 heart, 27 peripheral vascular, 5 cerebrovascular), and 27 deaths (6 cardiovascular). 38 patients underwent renal transplant. Of 150 pts with diabetes mellitus, 24 of 76 (31.6%) type 1 and 34 of 74 (45.9%) type 2 study subjects experienced a CVE, as opposed to 27 pts without diabetes, 3 had CVE (11.1%), p= 0.043, 0.001. There were no significant differences for median event free follow up between groups defined by the presence vs. absence of prior CVE for either dialytic (2.0 vs. 2.3 yrs) or transplant therapy (2.0 vs. 2.9 yrs) (all p>0.20).

Conclusions: In clinically stable patients undergoing renal replacement therapy, diabetes mellitus is associated with a higher CVE rate such that the presence of prior CVE may not predict future CVE. Clinical decision-making for such pts should not overemphasize the importance of prior cardiovascular morbidity.

Funding: Pharmaceutical Company Support, Private Foundation Support

PUB344

Abstract Withdrawn

PUB345

Responses to Furosemide in Hemodialysis Patients with Residual Renal Function Louis A. Carrera, Maria V. DeVita, Michael F. Michelis. *Department of Nephrology, Lenox Hill Hospital, New York, NY.*

Background: It has been generally accepted that residual renal function provides a contribution to the well-being of end stage renal failure patients. Studies regarding the use of furosemide in peritoneal dialysis patients have shown some benefit, however its role in hemodialysis patients is less well known. We report our experience with the use of furosemide at our outpatient hemodialysis center.

Methods: All patients were screened for residual renal function, which was defined by urine output of > 400 ml over a 24 hour period. From a total of 167 patients, 28 patients met our criteria and 14 patients agreed to participate. For 3 weeks, patients were given either furosemide 100 mg daily if their weight was <80 kg, or 160 mg daily for weight > 80 kg. Baseline and post furosemide measurements were obtained for urine volume, blood pressure (BP), potassium, phosphorous and interdialytic weight gain (IDWG). Twenty-four hour urine collections were obtained commencing on the day preceding the first dialysis session of the week. BP was quantified by using the average of three readings obtained predialysis on the week preceding the use of furosemide and during the last week of the study. The average of three IDWG on the week preceding administration of furosemide and the last week of the study period were used for comparison. The effect of furosemide on these parameters was analyzed using a paired t-test**.** During the study, 3 of the 14 patients were non-responders and were excluded from further analysis.

Results: The mean increase in urine volume was statistically significant at 575 ml (p<0.001). The mean systolic BP decreased 2.72 mmHg (p=0.58) but diastolic BP decreased by 5.45 mmHg (p=0.06), which approached statistical significance. Changes in serum potassium and phosphorus were not statistically significant. Interestingly, IDWG was not significant suggesting that patients were liberalizing fluid intake.

Conclusions: Our study suggests that furosemide is effective in increasing urine output in hemodialysis patients. Furthermore, it may improve diastolic pressure and lessen fluid restriction.

PUB346

Comparison of In-Vitro Metabolism of Medications in Uremic Serum Using Human and Recombinant Hepatic Microsomes Brian S. Decker, Nitesh Thakker, James Slaven, Zhangsheng Yu, Sharon M. Moe, David Jones. *Medicine, Indiana University School of Medicine, Indianapolis, IN.*

Background: Studies have demonstrated that uremia can diminish the hepatic metabolism of medications. Human and recombinant hepatic microsomes are used to evaluate the in-vitro hepatic metabolism of medications. The purpose of this study was to compare the magnitude and variability of the metabolism of medication substrates in uremic serum using human and recombinant hepatic microsomes.

Methods: The medication substrates evaluated for this study were midazolam and dextromethorphan. Uremic serum was obtained at the midpoint of a four hour hemodialysis session from six anuric subjects with end-stage renal disease (ESRD). The control used in each experiment was pooled normal serum obtained from subjects with normal renal function. Midazolam and dextromethorphan were incubated with human liver and recombinant microsomes in normal and uremic serum. Hepatic metabolism by the microsomes was tested according to validated protocols. The analysis of the 1-OH and 4-OH midazolam and dextrorphan metabolites was performed by mass spectrometry. Statistical analyses using Students t-test were then performed to compare the magnitude of metabolite formed from the human and recombinant microsomes. Coefficients of variation were also analyzed to look at variability between the human and recombinant microsomes. All data were normalized with the normal pooled serum values, to control for day-to-day run variation.

Results: There was no statistical difference in the magnitude of the metabolites formed from either human or recombinant liver microsomes. Analysis of metabolism using normalized data found no significant difference between human and recombinant live microsomes in all three outcomes. Without normalization, recombinant microsomes demonstrate significant higher level of activities than human microsomes. There were no significant differences in coefficients of variation within each metabolite.

Conclusions: Human and recombinant microsomes do not differ in the magnitude or variability of metabolites formed when incubated in uremic serum, after normalizing to control for day-to-day run variation.

Funding: NIDDK Support

PUB347

Urea, Creatinine and β2-Microglobulin Concentrations in Spent Dialysate Measured by Spectrophotometric and Spectrofluorimetric Measurements Carlo Donadio,[1] Dario Calia,[2] Fabio Di Francesco,[2] Silvia Ghimenti,[3] Angeliki Kanaki,[1] Massimo Onor,[3] Danika Tognotti,[1] Roger Fuoco.[2] *[1]Internal Medicine, Nephrology, University of Pisa, Italy; [2]Dept. Chemistry & Industrial Chemistry, University of Pisa, Italy; [3]ICCOM, CNR, Pisa, Italy.*

Background: The aim of this study was to evaluate the possibility to determine the concentration of urea (URE), creatinine (CRE) and ß2-microglobulin (ß2M) by spectrophotometric and spectrofluorimetric measurements in spent dialysate samples during hemodialysis (HD) sessions.

Methods: Eight maintenance HD males patients, 61±17 years, treated with a low-flux polysulfone membrane (4 pts) or a high-flux triacetate membrane (4 pts).

Spent dialysate samples were collected 5, 15, 30, 60, 120, 180, and 240 min after the beginning of HD. URE, CRE and ß2M concentration were determined by standard laboratory methods. The absorbance and the fluorescence spectra of standard solutions of URE, CRE and ß2M were determined to select the best analytical conditions, then samples were analyzed by flow injection in a high pressure liquid chromatograph mounting a spectrophotometric and a spectrofluorimetric detector.

Results: The concentrations of URE, CRE and ß2M in spent dialysate decreased, according to a single exponential function. The spectrometric analyses of the pure compounds showed 1) a peak of maximum absorbance at 235 nm and no fluorescence for CRE; 2) a peak of absorbance at 280 nm and a peak of fluorescence at 340 nm (excitation wavelength 220 nm) for ß2M; 3) neither absorbance nor fluorescence for URE. High correlations were found between concentration and absorbance values: 0.918 for URE (at 280 nm), 0.855 for CRE (at 235 nm), and 0.979 for ß2M (at 235 nm). The correlations between fluorescence and concentration values were definitely lower: 0.675 (URE), 0.621 (CRE), and 0.765 (ß2M).

Conclusions: URE, CRE and ß2M concentrations into spent dialysate strictly correlate with absorbance values. A predominant contribution to the spectrometric signals is linked to undetermined substances, which should be identified, before validating spectrometric analyses in spent dialysate to monitor the removal of toxins and to predict dialytic efficiency.

Funding: Government Support - Non-U.S.

PUB348

The Results of Multicenter Study Comparing Effects of Different Renal Replacement Therapy Modalities (Low-Flux Hemodialysis vs. Online Hemodiafiltration) Maciej Drozdz, Wojciech Marcinkowski, Andrzej Milkowski, Teresa Rydzynska, Tomasz Prystacki, Ryszard August, Ewa Benedyk-Lorens, Katarzyna Bladek, Jan Cina, Grazyna Janiszewska, Andrzej Kaczmarek, Teresa Lewinska, Malgorzata Mendel, Mariusz Paszkot, Ewa Trafidlo, Malgorzata Trzciniecka-Kloczkowska. *Fresenius Nephrocare Polska, Poznan, Poland.*

Background: Online hemodiafiltration (online HDF), as compared to low-flux hemodialysis (low-flux HD), is postulated as a superior method of renal replacement therapy (RRT), but the effect of both methods on clinical effects of treatment still remains unclear.

The aim of this multicenter (11 dialysis units) study was to compare the dialysis adequacy, anemia and calcium-phosphate disturbances correction as well as the frequency of intradialytic hypotension (IH) in patient treated with different RRT modalities – HDF vs. low-flux HD.

Methods: The study was composed of 423 patients (171F and 252 M) aged 21 to 87 yrs. (mean 61.6), on RRT for 0.9 do 275 mths. (mean 44.2). 192 (5 dialysis units) patients was treated using online HDF and 231(6 dialysis units) using low-flux HD.

Patients were observed for 24 mths. 274 of them (65%) finished the observation period. Other patients died, were transplanted or transferred to other units. The assessment of biochemical and clinical parameters was performed at the start of the trial and next every year.

Results: All results are shown in table.

The results of tested parameters

Parameter	start	after 12 mths.	after 24 mths.
spKt/V	1.51 vs. 1.16 p<0.0001	1.52 vs. 1.2 p<0.0001	1.54 vs. 1.45 p<0.05
Hb (g/dl)	11.2 vs. 11 p=NS	11.5 vs. 11.1 p<0.05	11.3 vs. 11.2 p=NS
P (mmol/l)	1.57 vs. 1.77 p<0.05	1.69 vs. 1.88, p<0.005	1.5 vs. 1.85 p<0.001
CaxP (mmol2/l2)	3.5 vs. 3.8, p<0.05	3.7 vs. 4.1, p<0.05	3.3 vs. 3.8, p<0.001
SBP (mmHg)	124 vs. 128, p<0.005	123 vs. 130, p<0.005	119 vs. 128, p<0,005
DBP (mmHg)	69 vs. 77, p<0.0001	69 vs. 77, p<0.0001	66 vs. 77, p<0.001
IH episodes (per week)	0.2 vs. 0.5, p<0.01	0.08 vs. 0.4, p<0.005	0.2 vs. 0.4, p<0.05

The erythropoiesis stimulating agents dose did not differ between the groups.

Conclusions: Treatment with online HDF brings important biochemical and hemodynamic benefits for RRT patients.

PUB349

Comparison of Online Clearance Monitor with Other Blood-Sampling Methods in Hemodialysis Adequacy Evaluating Jiayuan Gao, Renhua Lu, Zhaohui Ni, Jia Qi Qian, Yucheng Yan. *Nephrology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: To compare the accuracy of hemodialysis adequacy evaluated by online clearance monitor (OCM) with other blood-sampling methods, using direct dialysate quantification method (DDQ) as a gold standard.

Methods: We included 19 anuric maintenance hemodialysis patients. Drained dialysate samples were collected continuously during the entire therapy by partial dialysate collection method (PDC). Urea nitrogen levels were assessed to calculate Kt/V by DDQ. Blood urea nitrogen (BUN) at the beginning of dialysis, at the end of dialysis and as well as 30 minutes after therapy were measured. Single pool Kt/V (Sp Kt/V) by Daugirdas II formula was calculated. Equilibrated Kt/V (Eq Kt/V) and rate adjusted Kt/V (Ra Kt/V), which seem close to double pool Kt/V, were also employed. OCM Kt/V was provided by OCM module from Fersenius. The value and correlation of Kt/V by different methods were analyzed. Urea distribution volume (Vurea) used in the three formulas internally installed in OCM were compared with the standard Vurea level from DDQ.

Results: As a result, the value of OCM Kt/V, Ra Kt/V and Eq Kt/V were very similar with DDQ Kt/V (1.24±0.24, 1.39±0.24, 1.41±0.27 vs. 1.34±0.26, all P >0.05), while Sp Kt/V was significantly higher than DDQ Kt/V (1.58±0.27 vs. 1.34±0.26, P<0.05). OCM Kt/V, Sp Kt/V, Ra Kt/V or Eq Kt/V was all well correlated with DDQ Kt/V. The relationship between OCM Kt/V and DDQ Kt/V (r= 0.706, P<0.05) was lower than that of Sp Kt/V, Ra Kt/V or Eq Kt/V (R values were 0.901, 0.891, 0.963, separately. all P<0.05). Vurea levels calculated from all three formulas internally installed in OCM were significantly higher than DDQ (P<0.05).

Conclusions: In conclusion, the correlation between OCM Kt/V and DDQ Kt/V was ideal with almost similar value. It can ideally evaluate the actual delivered dialysis dose of hemodialysis patients. The advantage of online quick monitoring implys its further value in application, yet still need more validation.

Funding: Government Support - Non-U.S.

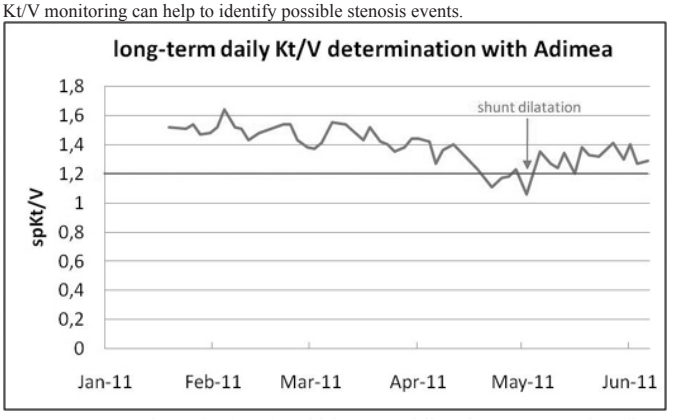
PUB350

Daily Long-Term Kt/V Determination by Ultraviolet Absorbance (Adimea) in Hemodialysis Helps To Detect Clinical Complications Marten Kelm,[1] Roman Guenthner,[2] Martin Kuhl,[1] Juergen Wagner.[1] *[1]B. Braun Avitum AG, Melsungen, Germany; [2]PHV Dialysezentrum, Melsungen, Germany.*

Background: Online Kt/V-determination with Adimea has become an established measuring principle in regular hemodialysis and has proven its measurement adequacy in various clinical studies (Castellarnau et al., 2010). The aim of this work was to show that daily and long-term Kt/V-measurement with Adimea can lead to the detection of possible events during hemodialysis such as shunt stenosis.

Methods: In this case study, patient data from the PHV Dialysezentrum Melsungen, Germany was analysed.

Results: A case of a 54 year old woman is reported who had chronic bicarbonate hemodialysis 3x per week for nearly 1 year. The patient underwent the third vascular access dilatation due to a shunt stenosis in 05/2011. Before and after this intervention, the patient underwent regular hemodialysis with a Dialog+ machine and daily Kt/V measurements with Adimea. Kt/V data was analysed retrospectively over a period of 5 months to identify a possible change in administered dialysis dose. It can be seen that the Kt/V dose gradually decreases over a period of 7 weeks before the patient was sent to shunt dilatation. Prior to shunt revision, the administered blood flow had to be decreased. After the patient returned from shunt dilatation, the blood flow and Kt/V dose increased and the patient was able to reach the Kt/V target again. This data shows that in patients prone to shunt stenosis, daily Kt/V monitoring can help to identify possible stenosis events.



Conclusions: The results show that with long-term daily Kt/V measurement by Adimea it is possible to detect changes in dialysis efficacy over time and clinical complications such as shunt stenosis.

PUB351

Utility of Multifrequency Bioimpedance Analysis in HD Patients Vijay K. Kher, Salil Jain, Shyam Bihari Bansal, Saurabh Pokhariyal. *Nephrology and Transplant Medicine, Medanta - The Medicity, Gurgaon, Haryana, India.*

Background: Clinically estimating the dry weight (DW) in dialysis pts is subjective & often inaccurate. Several methods used include natriuretic peptides, measurement of IVC diameter and collapsibility on inspiration by ultrasound, intradialytic relative blood volume change and single frequency bioimpedance. These methods suffer from poor specificity . Multifrequency Bioimpedance Spectroscopy (MBIS)to determine TBW & ECV has been validated by applying dilution methods as gold standard.

Aim

To study the utility of MBIS in HD pts in addition to clinical method of assessment of DW.

Methods

A prospective study in prevalent HD pts at a single dialysis centre.

Inclusion Criteria

HD pts on 3/week treatment for 4 hours for more than 3 months, clinically euvolemic.

Exclusion Criteria

Active infection, CHF, Liver disease with ascitis ,Pacemaker/ ICD,Metallic Implants,URR< 60%,Hb <9.0 gm/dl, Albumin < 3.0 gms/dl

20 pts were monitored over 2 week for BP, no.& dosage of antihypertensive drugs, weight & no. of hypotensive episodes during dialysis.

MBIS(Body Composition Monitor BCM-Fresenius) machine was used to assess TBW and ECV. The new calculated DW was obtained from BCM . We tried to achieve this new DW over next 2 weeks and the parameters were reassessed.

Results

20 pts with mean age of 60.25 years ,mean weight of 73.4kgs , median SBP of 145.5 mmHg, median DBP of 75mmHg & average number of anti hypertensive drugs was 1.7. Average Hb, Albumin and URR were 10.82 mg%,3.6 mg/dl and 69.105. All pts were hypertensive , 20% had coronary artery disease and 65%were diabetics . All pts finished the study.

SBP significantly decreased from 145.5 to 131.5 mmHg. DBP decreased from 78 to 75 mmHg (NS). The mean weight decreased significantly from 73.4 to 71.09 kg. Hypotension episodes increased in 35% decreased in 25% and remained constant in 40% cases.

The numbers of antihypertensive drugs reduced in 20% & remained constant in 80% (NS)

Conclusion

This study shows that MBIS is helpful in achieving DW in addition to clinical examination & a longer follow up will demonstrate if it translates into decreasing cardiovascular morbidity & mortality.

PUB352

Dialyzer Reuse in Short Daily Online Hemodiafiltration: Impact on Solutes Extraction Natalia C.V. Melo,[1, 2] Rosa M. Moyses,[1] Manuel C. Castro.[1] *[1]Nephrology Department, University of Sao Paulo School of Medicine, Brazil; [2]CDRB/HRT, Brasilia, Brazil.*

Background: In daily online hemodiafiltration(D-OL-HDF), there are no studies evaluating the impact of dialyzer reuse on solutes extraction.

Methods: 14 patients(47.9±13.5 years) in daily hemodialysis(D-HD) program were included. The impact of high flux dialyzer reuse on solutes extraction in D-OL-HDF sessions was evaluated and compared to that in D-HD.

Results: Directly quantified small solutes total mass removal(MTdq) and clearance(Kdq) were similar when 1st, 7th and 13th dialyzer D-HD uses were compared to D-OL-HDF respective uses. Small solutes MTdq and Kdq were similar among dialyzer uses in D-HD and in D-OL-HDF sessions. β2-microglobulin(β2-m) MTdq and Kdq were greater in D-OL-HDF dialyzer 1st, 7th and 13th uses than in the respective D-HD uses. β2-m MTdq and Kdq were similar among dialyzer uses in D-OL-HDF; but were inferior in 13th D-HD dialyzer use than in 1st and 7th D-HD uses.

Solutes removal in D-HD and D-OL-HDF

	1st D-HD dialyzer use	7th D-HD dialyzer use	13th D-HD dialyzer use	1st D-OL-HDF dialyzer use	7th D-OL-HDF dialyzer use	13th D-OL-HDF dialyzer use
MTdq Urea(g)	25.2±6.1	23.9±6.5	25.1±4.1	25.7±6.1	24.9±4.9	24.7±5.2
Kdq Urea(mL/min)	262.8±25.4	265.3±31.0	268.1±27.6	275.3±35.9	264.3±30.0	271.5±25.9
MTdq Phosphorus(mg)	810.4±164	755.3±213	754.5±126	790.7±199	747.9±136	789.9±135
Kdq Phosphorus(mL/min)	149.1±24.0	144.4±23.5	143.9±21.7	142.0±23.9	143.4±21.7	150.8±21.8
MTdq Creatinine(mg)	1523±397	1441±470	1448±413	1512±416	1489±385	1511±448
Kdq Creatinine(mL/min)	171.9±12.6	168.8±22.5	164.1±25.9	177.0±19.4	176.5±21.5	174.0±22.9
MTdq Uric Acid(mg)	821.7±158	685.3±163	671.1±182	748.6±195	678.1±118	699.0±107
Kdq Uric Acid(mL/min)	213.0±26.6	181.7±74.0	184.0±41.6	205.8±41.6	196.9±25.1	201.2±26.9
MTdq β2-m(mg)	* ♣123.1±49.5	♦ ♣119.6±34.3	† ♣109.5±42.5	*148.8±43.4	♦141.4±43.8	†144.8±42.5
Kdq β2-m(mL/min)	° ‡52.2±9.9	◊ ‡49.9±9.4	♣ ‡44.2±13.9	°77.8±11.7	◊77.8±15.8	♠73.7±13.1

*p=0.0009, ♦p=0.0295, †p=0.0067,♣p=0.0065, °p=0.0002, ◊p=0.0002, ♠p=0.0001, ‡p=0.0128

Conclusions: Dialyzer reuse did not impact on solute extraction in D-OL-HDF sessions. β2-m extraction was greater in D-OL-HDF sessions than in D-HD, without differences in other solutes extraction.

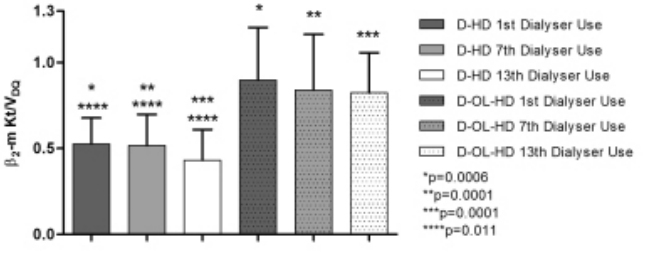
PUB353

High-Flux Polysulfone Dialyzers Reprocessment in Short Daily Online Hemodiafiltration: Impact on Dialysis Dose and β2-Microglobulin Kinetics Natalia C.V. Melo,[1, 2] Rosa M. Moyses,[1] Manuel C. Castro.[1] *[1]Nephrology Department, University of Sao Paulo School of Medicine, Brazil; [2]CDRB/HRT, Brasilia, Brazil.*

Background: In daily online hemodiafiltration(D-OL-HDF), there is a lack of studies evaluating the impact of dialyzer reuse on dialysis dose and β2-microglobulin(β2-m) kinetics.

Methods: 14 patients(47.9±13.5 years) in daily hemodialysis(D-HD) program were included. Impact of high flux polysulfone dialyzer reuse on dialysis dose and β2-m kinetics, in D-OL-HDF sessions, was evaluated and compared to that in D-HD.

Results: Dialysis dose -measured by single-pool Kt/V, equilibrated Kt/V, standard Kt/V and direct dialysis quantification Kt/V (DDQ Kt/V)- was similar when 1st, 7th and 13th dialyzer D-HD uses were compared to 1st, 7th and 13th D-OL-HDF uses, respectively. Additionally, dialysis dose was similar among 1st, 7th and 13th dialyzer uses in D-HD sessions and also in D-OL-HDF sessions. β2-m directly measured Kt/V (Kt/Vdq) was statistically higher in D-OL-HDF dialyzer 1st use(0.90±0.30) than in D-HD dialyzer 1st use (0.53±0.15)(p=0.0006). β2-m Kt/Vdq in D-OL-HDF dialyzer 7th use (0.84±0.32) was superior to that in D-HD dialyzer 7th use (0.52±0.18)(p=0.0001). β2-mKt/Vdq in D-OL-HDF dialyzer 13th use(0.82±0.23) was also superior to that in D-HD 13th dialyzer use (0.43±0.18)(p=0.0001). β2-m Kt/Vdq was decreased in 13th D-HD dialyzer use (0.43±0.18) when compared to 1st (0.53±0.15) and 7th (0.52±0.18) D-HD dialyzer uses (p=0.011). β2-m Kt/Vdq was similar among the 1st, 7th and 13th dialyzer uses in D-OL-HDF sessions.



Conclusions: Polysulfone high flux dialyzer reprocessment did not result in reduction in dialysis offered dose or in β2-m kinetics in D-OL-HDF sessions. There was an increase on the β2-m Kt/Vdq in D-OL-HDF sessions when compared to D-HD sessions, without significant variations in dialysis dose.

PUB354

Differences and Similarities among Daily High Flux Hemodialysis, Online Hemofiltration and Online Hemodiafiltration: A Kinetical Approach Natalia C.V. Melo,[1,2] Rosa M. Moyses,[1] Manuel C. Castro.[1] *[1]Nephrology Department, University of Sao Paulo School of Medicine, Brazil; [2]CDRB/HRT, Brasilia, Brazil.*

Background: There is a lack of studies comparing dialysis dose and solutes removal among short daily high flux hemodialysis(D-HD), short daily online hemofiltration(D-OL-HF) and short daily online hemodiafiltration(D-OL-HDF).

Methods: 14 patients(47.9±13.5 years) in daily hemodialysis(D-HD) program were included. There were collected blood pre, post and in the middle of the sessions and dialisate (partially and homogeneously collected) in two-hour D-HD, pre-dilution D-OL-HF and post-dilution D-OL-HDF sessions.

Results: Dialysis dose measured by direct dialysis quantification Kt/V (DDQ Kt/V) was significantly smaller in D-OL-HF sessions (0.43±0.12) than in D-HD (0.92±0.26) and D-OL-HDF sessions (0.96±0.28)(p=0.0002). Urea directly measured total extracted mass (MTdq) and clearance (Kdq) were smaller in D-OL-HF sessions (14.28±7.73g; 136.4±54.5mL/min) than in D-HD (25.22±6.1g; 262.8±25.4mL/min) and D-OL-HDF(25.68±6.1g; 275.3±35.9mL/min) sessions (p<0.0001). Phosphorus MTdq and Kdq were smaller in D-OL-HF sessions (475.8±169mg; 84.03±28.0mL/min) than in D-HD (810.4±165mg; 149.1±24.0mL/min) and D-OL-HDF(790.7±199mg; 142.0±23.9mL/min) sessions (p<0.0001).). Creatinine MTdq and Kdq were smaller in D-OL-HF sessions (915.0±352mg; 97.84±30.7mL/min) than in D-HD (1523±397mg; 171.9±12.56mL/min) and D-OL-HDF(1512±416mg; 177.0±19.4mL/min) sessions (p<0.0001). Uric Acid MTdq and Kdq were smaller in D-OL-HF sessions (417.5±140mg; 110.4±34.8mL/min) than in D-HD (821.7±158mg; 213.0±26.6mL/min) and D-OL-HDF(748.6±195mg; 205.8±41.6mL/min) sessions (p<0.0001). β2-microglobulin MTdq and Kdq were greater in D-OL-HDF sessions (148.4±43.4mg; 108.0±25.8mL/min) than in D-OL-HF(94.05±45.9mg; 67.62±14.2mL/min) and D-HD(123.1±49.5mg; 62.95±12.1mL/min) sessions (p<0.0001).

Conclusions: Removal of small solutes (urea, phosphorus, creatinine and uric acid) was significantly smaller in pré-dilution D-OL-HF than in D-HD and in post dilution D-OL-HDF sessions, undergone with the same dialyzer type. The removal of β2-microglobulin was greater in D-OL-HDF sessions than in the other studied methods.

PUB355

Viral Serology in Dialyzed Patients Dilip Kumar Pahari. *Nephrology, Medica Super Speciality Hospital, Kolkata, West Bengal, India.*

Background: Hepatitis B and Hepatitis C outbreaks in dialysis units are well known. Contamination from a reprocessed dialyzer often blamed for the increased transmission. We started a new dialysis unit and wanted to study prospectively the incidence and nature of transmission.

Methods: We have 280 patients on maintenance dialysis. We check viral serology(Hepatitis B, Hepatitis C and HIV) for all patients at entry and every 3 months interval (January, May, September,December). 15 Hepatitis B positive patients dialyzed in a separate room with isolated washing area. 13 hepatitis C positive patients dialyzed on machines in a separate room with isolated washing area. Hepatitis B and hepatitis C negative patients are dialyzed in two different rooms with two different washing areas. We have no patients with HIV positive status. We reprocess dialyzers for 240 patients who are Hepatitis B and C negative, and 12 patients use single dialyzers. of the 240 patients, 102 patients who regularly use Erythropoietin, and some of them also have single use dialyzer are dialyzed in a separate room with separate washing area. Patients hepatitis B and hepatitisC negative but depend on transfusion and do not take take Erythropoeitin are dialyzed in forth area. 2 patients of hepatitis B and 3 patients of hepatitis C use single dialyzers. 102 patients (36%) receive EPO during dialysis, rest depend on blood transfusion whenever necessary.

Results: In one year April 2010 to March 2011, 3 (0.01%) patients became Hepatitis C and 2 (0.007%) patients got Hepatitis B positive. No patient acquired HIV infection at any point of time. 2 patients became Hepatitis B positive from non EPO group and 1 from EPO group, and all 2 patients became Hepatitis C positive from Non EPO group. The single patient who became Hepatitis B positive also received multiple transfusions earlier during bladder resection due to transitional cell cancer.

Conclusions: Hepatitis B and Hepatitis C transmission during dialysis is only 0.01% and 0.007% for Hepatitis B and C respectively. The seroconversion is from blood transfusion rather than from dialysis procedure or dialyzer reprocessing if safe practice is followed.

PUB356

"This Is Not Your Father's Dialyzer" – Leukocyte and Platelet Stability Indicate That Newer Dialyzers Are Significantly More Biocompatible Than Previous Dialyzers Norbert Shtaynberg, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: One factor associated with the poor outcomes with HD patients is the exposure to a foreign membrane. Older membranes were very bioincompatible and increased complement activation, caused leukocytosis by activating circulating factors which caused sequestration of leukocytes (WBC) in the lungs, and activated platelets. Recently, newer membranes have been developed which were designed to be more biocompatible. We investigated what effect these membranes had on platelet levels and white blood cell count.

Methods: 99 maintenance hemodialysis patients with no known systemic or hematologic diseases affecting their platelets or WBC had blood drawn immediately prior to, ninety minutes into, and immediately following their first hemodialysis session of the week. All patients were dialyzed using a Fresenius Medical Care Optiflux polysulfone membrane F160, F180 or F200 (polysulfone synthetic dialyzer membranes, 1.6 m2, 1.8 m2, and 2.0 m2 surface area respectively, electron beam sterilized). WBC and platelet counts were measured from each sample by analysis on a CBC analyzer (Sysmex XT-4000i)

Results: The average age of the patients was 62.7 years; 36 were females and 63 were males. The mean platelet count pre, mid and post dialysis was 193 (SD 74.86), 191 (SD 74.67), and 197 (SD 79.34) TH/mm3 (Fig. 1), showing no statistical difference. The average WBC count pre, mid and post dialysis was 6.84, 6.44, and 6.16 TH/mm3, with a p-value of 0.05, showing a statistically significant, but clinically insignificant, decrease in WBC.

Conclusions: Newer membranes have no significant effect on platelet count, and have a statistically significant, but very minimal effect on leukocytes. This suggests that they are, in fact, more biocompatible than their predecessors and may explain their association with increased survival.

PUB357

Dialysis Patients' Fluid Overload, Antihypertensive Medications and Obesity Mihaly Tapolyai,[1,2] Maria Faludi,[1,2] Virag Reti,[1,2] Zsolt Lengvarszky,[3] Tibor Szarvas,[3] Klara Berta.[1,2] *[1]Dialysis, Semmelweis University, Budapest, Hungary; [2]Dialysis, Fresenius Medical Care, Budapest, Hungary; [3]Mathematics, Louisiana State University Shreveport, Shreveport, LA.*

Background: Overhydration (OH) is both a major etiology of hypertension in hemodialysis patients and a serious risk factor for mortality. We investigated the association of multiple variables and OH.

Methods: This is a cross sectional study of prevalent hemodialysis patients examining the hydrational status with a portable bioimpedance apparatus to measure the degree of hydrational status and comparing these with demographic, dialysis and laboratory data.

Results: We completed our study in 79 patients mean age was 60.7 ±16.9 years, 49.3% men, 30.7% diabetic, vintage: 66.5 ±57.1 months, residual urine output of 442 ±521 mL/day. Patients were overhydrated by 2.6 ±2.4 L and had a percent body fat of 36.4 ±11.6 kg. The average medication count was 2.4 ±1.5 and 45% had diuretics. We found a significant correlation between OH and systolic BP (r^2: 0.152 p: 0.0006), each liter of OH generating 3.6 mmHg. We also found a positive correlation between the use of diuretics and OH (p: 0.003 two tailed Student t test) but no correlation between OH and body weight (r^2: <0.00001 p: 0.99), body mass index (r^2: 0.03), age (r^2: 0.008) and vintage (r^2: 0.002). Every 10% increase in body fat OH decreased by 1.2 L; residual urine output gave no protection from OH (r^2: 0.006) and did not correlate with BP (r^2: 0.0001).

Conclusions: OH is strongly associated with the use of antihypertensive medications and the use of diuretics in this dialysis population. Obesity seems to afford some protection from OH.

PUB358

Rapid Correction of Chronic Metabolic Acidosis in Hemodialysis Olga R. Carmona. *Nephrology Center, University of Uruguay, Montevideo, Uruguay.*

Background: Metabolic Acidosis is associated with chronic renal failure for the incapacity of H+ excretion and the decrease of NH4+ production, besides the increase of protein catabolism and the decrease of protein syntesis.

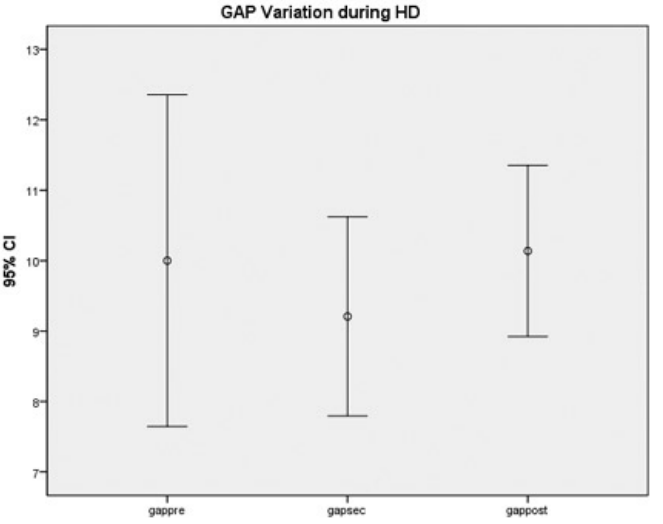
Methods: 27 Chronic HD patients(mean age 54,55 ± 16,44 years , 44,7%F) were study. Samples for acid base and blood gases analysis were drawn from the arterial side of the a-v fistula at pre, second hour and post HD using a Radiometer Copenhagen 700 and $NaHCO_3$ (39mmol/L) in the dialysate. Measured parameters were: pH,pCO_2, pO_2, SO_2. Calculated parameters were: HCO^-_3,CO_2t, SBE, GAP, p50 (pO2at 50 % Sat.) Student t test was used to evaluate differences between means (p<0,05).

Results: A significant increase of HCO_3-, pH, pCO_2, CO_2t, SBE were observed fundamentally at the second hour. The decrease of GAP in the first two hours was not significant but at the end of HD, increase significatly respect to the second hour. Besides a significant decrease of pO_2 and p50 were produced.

Acid Base and Blood gases variation during HD

Parameters	Pre HD(mean [plusmn st]	Second hour (mean [plusmn st]	Post HD (mean [plusmn st]	Second-Pre (p value)	Post-Pre (p value)	Post -Second (p value)
[HCO3-] mmol/L	21,06±2,57	27,05±2,22	29,04±2,15	0,00 ↑S	0,00↑S	0,00 ↑S
pH	7,36±4,56	7,43±4,08	7,48±4,2	0,00 ↑S	0,00↑S	0,00 ↑S
[CO2]t mmol/L	22,15±2,68	28,22±2,38	30,58±2,80	0,00 ↑S	0,00↑S	0,00 ↑S
SBE mmol/L	-4,08±3,29	2,94±2,45	5,11±2,39	0,00 ↑S	0,00↑S	0,00 ↑S
GAP mmol/L	10,00±5,95	9,20±3,57	10,14±3,07	0,19↓NS	0,88↑NS	0,04 ↑S
pO2 mmHg	97,23±9,55	92,68±10,52	90,12±13,12	0,04 ↓S	0,02↓S	0,34↓NS
p50 mmHg	26,94±2,14	25,86±2,75	24,61±2,78	0,02 ↓S	0,00↓S	0,00 ↓S

p<0,05



Conclusions: The rapid correction of metabolic acidosis occurred in the first two hours of HD may contribute to the significant decrease of pO2 and the Oxygen delivery to the tissue (↓p50) that may cause increase in intermediates of Krebs acid cycle, with the consequences of a significant increase of anion gap at the end of HD.

Funding: Government Support - Non-U.S.

PUB359

An Unusual Case of Refractory Hypokalemia Nwamaka Mukoso Osakwe,[1] Rajnish Dhingra,[2] Mohammad G. Saklayen.[3] *[1]Department of Internal Medicine, WSU Boonshoft School of Medicine, Dayton, OH; [2]Department of Internal Medicine, WSU Boonshoft School of Medicine, Dayton, OH; [3]Department of Internal Medicine, WSU Boonshoft School of Medicine, Dayton, OH.*

Background: Extrapituitary causes are identified in 10-15% of patients with Cushing's syndrome. When in excess, cortisol may act as a mineralocorticoid resulting in hypokalemic alkalosis and hypertension. Hypokalemic alkalosis is seen in 74 to 95% of patients with ectopic ACTH secreting syndrome but in fewer than 10% of all patients with Cushing's disease.

Methods: Single Case Report

Results: A 37 year old ten-week pregnant woman was admitted for vomiting, generalized fatigue and weakness since onset of pregnancy. Physical examination revealed BP 117/70mmHg [Normal 70s-80s/50 mmHg] and no evidence of striae or cervico dorsal fat pad. Laboratory data showed severe hypokalemia of 2.0 meq/L, Mg 2.2mg/dl, HCO_3 32meq/L and WBC 16,000. She was commenced on aggressive intravenous and oral repletion of potassium. However, patients' potassium levels remained very low. Three days after admission patient had a missed abortion and her BP was in the 130s/70s.

Laboratory Findings

Lab	Results	Normal Range
Plasma renin assay [ng/ml/hr]	3.6	1.9-3.7
Aldosterone [ng/dL]	4	2-16
Urinary free Cortisol [mcg/24hours]	37,627.2	10-100
Morning cortisol [mcg/dL]	194	6-23
ACTH [pg/ml]	901	9-52
CT abdomen/pelvis	bilateral hypertrophic changes of the adrenal glands without a definite adrenal mass	
PET/CT	mild to moderate uptake in a 2.1cm left mediastinal lesion	

She was transferred to a regional referral hospital for surgical removal of the mediastinal mass and she died from complications of surgery. Final pathology report revealed she had an atypical carcinoid tumor.

Conclusions: This case illustrates the challenges clinicians face in diagnosing ectopic ACTH-secreting tumor and also the pitfalls of fragmented medicine practice. Ectopic ACTH-secreting syndrome can present in several ways ranging from the traditional Cushingoid features (refractory hypertension, diabetes etc) to hypokalemic alkalosis as seen in this patient. In many cases, optimal management is dependent on early localization and surgical resection of the ACTH secreting tumor.

PUB360

Serum Acetone: A Cause of Elevated Serum Osmolal Gap in Wide Anion Gap Metabolic Acidosis Ekamol Tantisattamo, Alexander L. Pan. *Department of Medicine, University of Hawaii, Honolulu, HI.*

Background: Serum osmolal gap is useful to screen for toxic alcohol ingestion in wide anion gap metabolic acidosis (WAGMA). However, often times there is limited history and delay before blood toxic alcohol levels are available. Serum acetone is one of the overlooked

causes of a serum osmolar gap. While awaiting for serum alcohol levels and considering syndromes of toxic alcohol ingestion, checking a serum acetone level may avoid costly and invasive treatments such as fomepizole and hemodialysis.

Results: A 45-year-old male with a history of alcohol abuse and poor oral intake presented with chest pain and nausea. He drank 4 glasses of wine the previous night. There were no visual symptoms. His laboratory data, below, showed a WAGMA with an elevated anion gap of 22 mmol/l and lactic acid of 8.8 mEq/l. Measured and calculated serum osmolality were 301 and 278 mOsm/kg, respectively; resulting in an osmolal gap of 23 mOsm/kg. His alcohol level was negative. Urine sediment showed no crystals. Initially, toxic alcohol ingestion was considered, so was treatment with fomepizole and hemodialysis. However, serum acetone returned elevated at 100 mg/dl, likely explaining of his osmolal gap. Patient responded well to supportive treatment, both anion and osmolal gaps closed with the normalization of his acetone level; and, one week later, both serum methanol and ethylene glycol levels returned negative.

Laboratory data

pH 7.33	Na 134	Blood glucose 150 mg/dl
PaCO2 22	K 4.3	Albumin 4.6 g/dl
PaO2 100	Cl 99	BUN 5
BE -14	HCO3 13	Creatinine 0.8
HCO3 12		
SaO2 97%		

Conclusions: WAGMA and serum osmolal gap in our patient could be the result of high serum acetone levels (likely from starvation ketoacidosis). Alcohol was not a contributor for his anion gap, nor was lactic acid - as it is dissociated at physioligic pH. The below formula calculates acetone's contribution towards the patient's osmolality:

Serum osmolality = 2xNa + (1.15 glucose/18) + BUN/2.8 + ETOH/3.7 + Acetone/5.8

His recalculated osmolality is 297mOsm/kg, resulting in no significant osmolal gap. Therefore, serum acetone should be included in the calculation of serum osmolal gap if the clinical presentation is inconsistent with toxic alcohol ingestion.

PUB361

Differences in Serum Sodium Related to Sex While Patients Grow Older

Noemí Esparza,[1] Cesar Garcia-Canton,[1] Marta Riaño-Ruiz,[2] Santiago Suria,[1] Ana Sánchez Santana,[1] Batista Fatima Garcia,[1] Pablo Marcos Braillard Poccard,[1] Eduardo Baamonde,[3] Maria Dolores Checa.[1] *[1]Nephrology, Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Las Palmas, Spain; [2]Biochemistry, Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Las Palmas, Spain; [3]Centro de Hemodiális Dialsu, Avericum, Santa Brígida, Las Palmas, Spain.*

Background: Elderly patients become more susceptible to clinical complications involving salt and water abnormalities. Both hypernatremia and hyponatremia were reported more frequently in geriatric population and both confer a high risk of death.

Methods: Thanks to the cooperation of the biochemistry service of our hospital, we could use a file with all measurements (n = 27973) including uric acid from June 1 to December 31, 2010. The following determinations were removed from this file: 1182 from measurements without number of clinical history, 1822 from dialysis patients, 300 from the occupational health service, 151 patients under 14 years old and 260 from patients with a transplanted kidney. The determination with a higher uric acid was always chosen, out of the determinations from patients with more than a determination during the semester. In total 15413 patients (7320 women and 8093 men) were left to study.

Results: In 15413 patients studied, 14563 had normal serum sodium (serum sodium > to 135 mmol / l and < to 145 mmol / l), 675 had hyponatremia and 175 had hypernatremia. Women were older than men (56,17 + 17,58 vs 55,25 + 17,10 years, p = 0.01). There were not differences related to age between hyponatremic and hypernatremic patients (63,07 + 17,39 vs 65,54 + 16,63 years) but both were older than normonatremic patients (55,58 + 17,30) (p = 0.000). When performing the Pearson´s correlation for age and serum sodium variables we obtained the results shown in Table 1.

Pearson´s correlations between serum sodium and age in women and in men

	Age (years) (n= 15413 patients)	Age (years) (n = 8093 men)	Age (years) (n = 7320 women)
Serum sodium (mmol/l)	r = 0.26 p = 0.03	r =- 0.36 p = 0.04	r = 0.76 p = 0.000

Conclusions: Serum sodium increases while patients grow older only in women. In men, the serum sodium decreases as age increases.

Funding: Other NIH Support - Avericum

PUB362

Is Hyponatremia Managed Appropriately in an Academic Tertiary Care Center?

Chelsea Estrada, Navdeep Kaur, Nand K. Wadhwa. *Nephrology, Stony Brook University, Stony Brook, NY.*

Background: Hyponatremia is a common electrolyte abnormality in hospitalized patients and has been shown to be associated with increased morbidity, mortality, length of stay and health care costs. We examined if hyponatremia is appropriately diagnosed and/or treated in an academic tertiary care center.

Methods: We reviewed 415 serial electronic medical records retrospectively on 3 different medical services over a two month period. We collected demographic, diagnostic and therapeutic data on each patient who had hyponatremia (hyponatremia was defined as having 2 or more values of serum sodium (Na)<135 mEq/L). The study was approved by the Stony Brook University IRB.

Results: 48(11.5%) patients (18 females and 30 males with a mean age of 65.8, range 24-97 years) were found to be hyponatremic during their hospitalization. Mean duration of hospital stay was 9.7 days. 8(16.6%) patients had serum Na levels <126 mEq/L, 20(41.6%) with 126-130mEq/L, and 20 (41.6%) with Na levels of 131-134mEq/L. The mean serum Na(133.0±4.0 mEq/L) on discharge was unchanged to admission mean serum Na(133.9±3.9 mEq/L). 17 patients had normal mean serum Na (138.3±2.3 mEq/L) on admission and became hyponatremic (serum Na 128.5±3.8 mEq/L) during their hospital stay. Their mean discharge serum Na (133.0±3.7 mEq/L) was significantly (p= 0.002) lower than admission serum Na. 31 patients were hyponatremic on admission (mean Na 131.5±2.1 mEq/L)and were discharged with mean Na of 133.1±4.3 mEq/L(p=0.02). The mean serum glucose, potassium, bicarbonate and chloride were similar on admission and discharge. Of 48 hyponatremic patients, only 6(12.5%) had serum osmolarity measured,11(22.9%) had urine osmolarity done and 17(35%) had urine electrolytes checked during their hospital stay. Regarding therapy 11(22.9%) were given IV 0.9% NaCl infusion with diuretics simultaneously, and 26(54.1%) were on Na and fluid restricted diet while receiving IV 0.9% NaCl infusion.

Conclusions: The data suggest that hyponatremia was not managed appropriately in majority of patients at the tertiary care institution. Further studies are needed to evaluate the reasons and to assess it's impact on patient care and length of hospital stay.

PUB363

Electrolyte Disorders Following Major Cardiac Surgery in Patients in the ICU

Vijay Lapsia,[1] A. Ahsan Ejaz.[2] *[1]Nephrology, Mount Sinai School of Medicine, New York; [2]Nephrology, University of Florida, Gainesville.*

Background: Electrolyte disorders are an important cause of various complications in the ICU. We studied the incidence of electrolyte abnormalities following major cardiacthoracic surgery (CTS) in patients with renal dysfunction (RD).

Methods: A retrospective review of patients post CTS from 2001 to 2006 with ICU stay of atleast 5 days was performed. Serum levels of creatinine (SCR, mg/dl), sodium, potassium, chloride, calcium, magnesium and phosphate were collected pre-op and 5 consecutive postop days. For each electrolyte, patients with abnormal preop levels were excluded from analysis. RD was defined as SCR>1.4, patients with SCR≤1.4 served as controls. The chi square test was used for statistical analysis.

Results: We included 836 patients; mean age 60 ± 16.7 years, mostly men (64%). Comorbidities included coronary artery disease (55.4%), hypertension (60%), diabetes mellitus (21.8%), CKD (20.1%), COPD (19.3%) and peripheral vascular disease (10.8%). Most surgeries were elective (65.2%). CABG (40.8%) and aortic surgeries (20.9%) were the commonest procedures. The mean length of stay in the ICU was 12.7 ± 16.1 days. Hyperkalemia (44.4% Vs 24.9%), hypercalcemia (14% Vs 6.8%), hypermagnesemia (26.9% Vs 14.8%) and hyperphosphatemia (52.5% Vs 29.1%) were significantly more common in RD (p<0.05). Hypophosphatemia (73.7% Vs 85.5%) and hypocalcemia (69.1% Vs 89.1%) were significantly more common in controls (p<0.05).

Electrolyte abnormalities

	RD	Control
Hyponatremia	190 (34.9%)	34 (27.4%)
Hypernatremia	193 (35.4%)	54 (43.5%)
Hypokalemia	34 (5.6%)	11 (7.2%)
Hyperkalemia*	152 (24.9%)	68 (44.4%)
Hypochloremia	33 (6.4%)	4 (3.3%)
Hyperchloremia*	451 (87.7%)	90 (75.0%)
Hypocalcemia*	447 (81.9%)	94 (69.1%)
Hypercalcemia*	37 (6.8%)	19 (14.0%)
Hypomagnesemia	13 (2.1%)	1 (0.6%)
Hypermagnesemia*	93 (14.9%)	45 (26.9%)
Hypophosphatemia*	288 (85.5%)	87 (73.7%)
Hyperphosphatemia*	98 (29.1%)	62 (52.5%)

*p<0.05

Conclusions: Electrolyte abnormalities are common in the first 5 days following CTS. Patients with RD prior to surgery are at a higher risk for post-op hyperkalemia, hypercalcemia, hypermagnesemia and hyperphosphatemia. Patients with SCR<1.4 were more likely to develop hypophosphatemia and hypocalcemia.

PUB364

An Uncommon Case of Hyponatremia – A Case of Cerebral Salt Wasting

Reginald Ifeanyi Obi, Melanie I. Hames. *Nephrology and Hypertension, East Carolina University, Greenville, NC.*

Background: A 71 year- old female with a history of Stage IIIB Non-small cell adenocarcinoma of the lungs admitted with mental status change and found to have a large solitary left parietooccipital mass consistent with brain metastasis . At presentation, laboratory studies included a serum sodium of 137mEq/L and a serum creatinine of 0.8 mg/dl.

Methods: She subsequently underwent a left parietooccipital craniotomy with total excision of the brain tumor. Five days post-surgery her sodium had decreased to 131mEq/L, and serum creatinine was 0.67 mg/dl. By postoperative day 8, her sodium had decreased further to 129 mEq/L with urine osmolality of 629 mOsmol/kg, serum osmolality of 274 mOsmol/kg, and urine sodium of 88 mEq/L. Nephrology was consulted. The syndrome of inappropriate antidiuretic hormone secretion (SIADH) was suspected and a trial of fluid restriction was implemented. The patient did not respond and had a further drop in serum sodium to 126mEq/L.

Results: Further evaluation revealed evidence of orthostatic hypotension. Cerebral salt wasting was then suspected and she was started on intravenous normal saline and oral salt tablets. Her urine osmolality improved from a high of 707 mOsmol/kg down to 323 mOsmol/kg and her serum sodium trended back up nicely to 135mEq/L upon hospital discharge.

Conclusions: Cerebral salt wasting(CSW) is defined as the renal loss of sodium during intracranial disease leading to hyponatremia and a decrease in extracellular fluid volume. It was first introduced as a concept in 1950 but lost ground to SIADH and only in recent years has it come back in favor as a distinct entity. This case illustrates the challenges of differentiating CSW from SIADH as a cause of hyponatremia. The major difference between them which was exemplified in this case is the presence of a volume depleted state in CSW as opposed to euvolemia or hypervolemia in SIADH. Treatment involves salt and volume repletion using isotonic or hypertonic saline, salt tablets and fludrocortisone. Further research is needed to better define incidence of CSW, identify other disorders that can lead to this and elucidate the mechanism underlying this syndrome.

PUB365

Does Bio-Impedance Analysis Predict Volume Overload States and Clinically Relevant Outcomes in Septic Intensive Care Unit Patients with Acute Kidney Injury? A Prospective Observational Study Bram Rochwerg,[2] Jason H. Cheung,[2] Catherine M. Clase,[1, 2] Scott K. Brimble,[1, 2] Deborah Cook,[1, 2] Peter Margetts,[1, 2] Azim S. Gangji.[1, 2] *[1]Division of Nephrology, St. Joseph's Healthcare, Hamilton, ON, Canada; [2]McMaster University, Hamilton, ON, Canada.*

Background: The initial management of sepsis has been well established through early-goal directed therapy. Beyond the acute setting, persistent hypervolemia results in prolonged ventilation, the need for renal replacement therapy, and increased mortality. Clinical assessment of volume status is limited, however, bio-impedance analysis (BIA) may be a more accurate measure. The use of BIA to assess volume status and prognosis in septic ICU patients is unknown. We hypothesized that the change in BIA vector length (VL) is predictive of ventilator-free days in those have systemic inflammatory response syndrome (SIRS) due to infection.

Methods: A prospective observational study targeting 100 ICU patients. BIA will be measured on ICU admission days 2, 5, 10 and 15. Patients will be followed up for 60 days. Secondary outcomes include mortality, acute kidney injury requiring dialysis, and length of stay in the ICU. A correlation between BIA with known measure of volume status including physical exam, central venous pressure, brain natriuretic peptide and chest radiographs will be completed.

Results: To date, 16 patients have been recruited & enrolment is ongoing. Initial data suggest patients are persistently volume overloaded at both days 2 and 5 (mean VL 188 +/- 26 at day 2 and 174 +/- 20 at day 5). A high correlation existed between edema scores and VL (r = -0.645, p = 0.007); CVP also correlated with VL (r = -0.237, p = 0.376) although this was not significant. Finally, fluid balance between days 2 & 5 correlated inversely with change in VL (r = -0.648, p=0.009).

Conclusions: Septic patients in the ICU are persistently volume overloaded at days 2 and 5 based on BIA measures. VL correlates with known measures of volume status adding to BIA's construct validity. This pilot study has proved to be feasible and may identify BIA as an easy bedside measure to assess ICU patients' volume status. At the current pace, by the fall we will have recruited 50 patients.

Funding: Private Foundation Support

PUB366

Wine Potomania Takashi Shinha. *Internal Medicine, Long Island College Hospital, New York, NY.*

Background: Beer potamania caused by excessive alcohol consumption other than beer has rarely been reported. Recongition is particularly important because the patients usually respond quickly to normal saline and therefore they are put at high risk of complications from rapid correction.

Methods: We describe a case of beer potomania caused by wine abuse and analyze the pathophysiology and literature review.

Results: A 47-year-old female with no significant past medical history was admitted to ICU for tonic-clonic seizures. She had a history of wine abuse which constituted her major dietary intake. Her labs revealed serum sodium 120 mEq/L, plasma osmolality 265 mOsm/L, urine osmolality 65 mOsm/L and urine sodium 12 mEq/L. She was started on intravenous normal saline. In a 24-hour period, her sodium went up to 135 mEq/L. The pivotal pathophysiologic mechanism in beer potomania is the minimal intake of solute and the hypoosmolality of beer. This will lead to the inability to excrete sufficient amounts of free water. Wine is as hypotonic as beer. Sodium content of beer is 18 mg per 12 fluid ounce (fl oz) while wine contains 5 mg of sodium per 3.5 fl oz. From our literature review, only 2 cases of beer potomania caused by other alcohol beverages have been reported. One was cider and the other was Japanese rice wine. The consumption of large amounts of hypotonic solution, whether beer or wine, can lead to the dilution of serum sodium. Differentiating beer potomania from other causes of hyponatremia is paramount because the patients usually respond quickly to normal saline and therefore they are put at high risk of complications from rapid correction. In fact, central pontine myelinolysis associated with beer potomania has often been reported. In this case, sodium level was corrected by 15 mEq/L in a 24-hour period only with normal saline infusion. This case re-illustrates the value of controlled correction of hyponatremia in the case of beer potomania because of its rapidity and unpredictability.

Conclusions: Beer potomania should be considered as a cause of hyponatremia in alcohol misusers regardless of the type of alcohol consumed. Extra caution should be exercised because of its rapidity and unpredictability when correcting sodium level.

PUB367

Unsung Hero or Unrecognized Villain: Correction of Hyponatremia with Potassium Lonika Sood,[1] John Kevin Hix,[1,2] Jonathan W. Bress,[1] Richard H. Sterns.[1,2] *[1]Dept. of Medicine, Rochester General Hospital, Rochester, NY; [2]Nephrology Division, University of Rochester School of Medicine and Dentistry, NY.*

Background: An 80 year old female on chlorthalidone and a selective serotonin reuptake inhibitor presented with one week of poor dietary intake and ten pound weight loss. On admission, blood pressure 143/76 mm Hg, SaO2 99% on room air, weight 45.8 kg. Examination was unremarkable except for lethargy. Her serum sodium (sNa) was 110 mEq/L, serum potassium 2.2 mEq/L. She was treated initially with 50 ml of 3% NaCl (25 mEq). Because of a 1.1 L water diuresis, sNa rose by 5 mEq/L, five times the increase predicted by formulas that ignore urine output. A tonicity balance analysis was performed to explain the sNa increase after 3% NaCl during the first 2.5 hours of therapy, using the following equation:

Predicted sNa=
sNa*Total Body Water + (Infused Na + Infused K)=
Total Body Water + Net fluid balance
110 mEq/L*(0.45 L/kg*45.8 kg) + (50 ml*0.513 mEq/mL + 0)
(0.45 L/kg*45.8 kg) – 1.05 L
Predicted sNa = 117 mEq/L (actual = 115 mEq/L)

After the 3% NaCl she was given subcutaneous DDAVP every 8 hours to prevent unintended over-correction of sNa (Am J Kidney Dis. 2010; 56:774-9). In addition she received 60 mEq KCl orally and 60 mEq KCl intravenously as a 400 millimolar solution, and 180 ml 0.9% NaCl (27 mEq). Twenty-four hours after admission her sNa was 121 mEq/L, an 11 mEq/L increase.

Methods: A tonicity balance analysis was performed to explain the additional 6 mEq/L increase in sNa that occurred in response to KCl and a small amount of 0.9% NaCl despite the prevention of excess water losses with DDAVP:

Results: Predicted sNa =
115 mEq/L *(0.45 L/kg*44.8 kg) + (27 mEq + 120 mEq)
(0.45 L/kg*44.8 kg) + 0.172 L
Predicted sNa = 121 mEq/L (actual = 121 mEq/L)

Conclusions: Administration of hypertonic potassium chloride can be substituted for hypertonic saline in the treatment of hyponatremic patients who are also hypokalemic. Because a reversible impairment in water excretion is common in such patients, concurrent administration of DDAVP with KCl is an attractive strategy.

PUB368

Hyponatremia, but Not Hypernatremia, Is Significantly Associated with Rhabdomyolysis in a Burn Population Ian J. Stewart,[1] Molly A. Tilley,[1] Chris A. Gisler,[2] James K. Aden,[3] Evan Renz,[3] Kevin Chung.[3] *[1]Medicine, San Antonio Military Medical Center, San Antonio, TX; [2]Medicine, University of Texas Health Science Center at San Antonio, TX; [3]Burn Center, U. S. Army Institute of Surgical Research, San Antonio, TX.*

Background: Both hypernatremia and hyponatremia have been associated with rhabdomyolysis, correlations based largely on case reports and small series. We sought to examine this relationship in a large population of burn patients, where it has never been reported.

Methods: All admissions to the burn center at our institution from January 2003 to December 2008 were examined. Patents less than 18 years old, those with end stage renal disease, without a measured creatine kinase (CK) or serum sodium (SNa), or who died within 24 hours of admission were excluded from review. Independent variables included age, inhalation injury, percentage total body surface area burned (%TBSA), percentage of full thickness burns, Injury Severity Score (ISS), presence of electrical injury, hyponatremia (SNa less than 130) and hypernatremia (SNa more than 150). These variables were examined via a multiple logistic regression analysis against those with rhabdomyolysis, as defined by a maximum CK level > 5000 Units/L.

Results: In 530 subjects with a mean age of 44±19, average %TBSA of 28±24, and average ISS of 18±15, hypernatremia occurred in 24.0% (n=137) while hyponatremia occurred in 14.2% (n=75) during their admission. The prevalence on rhabdomyolysis was 17.2% (n=91). On multiple logistic regression, presence of electrical injury (OR 17.5, 95% CI 8.8-34.5, p <0.0001) and hyponatremia (OR 3.1, 95% CI 1.7-5.7, p=0.0003) had the greatest correlation with rhabdomyolysis. Hypernatremia (OR 1.1, CI 0.6-2.1, p=0.69) had no effect.

Conclusions: In the burn population, presence of electrical injury as well as hyponatermia, but not hypernatremia, is significantly associated with rhabdomyolysis. The results from this large retrospective review call into question prior assumptions of a relationship between hypernatremia and rhabdomyolysis, specifically in burn patients.

Funding: Other U.S. Government Support

PUB369

Severe Hypokalemia and Hypomagnesemia in a Patient Presenting with a History of Muscular Dystrophy Heino R. Anto, Rong Rong, Gurjeet Singh Sandhu. *Nephrology, St. John's Episcopal Hospital, New York, NY.*

Background: A 52 year old female from Trinadad presented with acute cholecystitis requiring a cholecystectomy. In Trinadad the patient was diagnosed to have muscular dystrophy since her 30's. Another sibling was also diagnosed with the same muscular condition. On this admission serum potassium was 2.9 mEq/L, serum magnesium 0.8-1

mg/dL. Bun 12 mg/dL , serum creatinine 0.2 mg/dL and CPK of 85 U/L.Two 24 hour urines for calcium ranged from 35 to 100 mg.It was felt that the diagnosis of muscular dystrophy was incorrect and that the patient had Gitleman's syndrome. The patient was treated with spironolactone , KCL, and magnesium oxide . Serum magnesium corrected to 1.8-2 mg/dL and serum potassium is 4-4.8 mEq/L. Gitleman's syndrome must be considered in a patient presenting with generalized muscle weakness associated with severe hypokalemia and hypomagnesemia.

PUB370

High Glucose Modifies NHE Activity in hSGLT1 Transfected Cells Olivia Beloto-Silva, Maria Oliveira-Souza. *Physiology and Biophysics, University of Sao Paulo, SP, Brazil.*

Background: Na^+/glucose co-transporter 1 (SGLT1) is one of the proteins responsible for the glucose transport into the cells. Protein kinase A (PKA) can phosphorylate the *Serine* 418 of the SGLT1 changing its cellular distribution. In addition, high glucose can modulates the activity of the Na^+/H^+ exchange 1 and 3 (NHE1 and 3, respectively) which are important to regulation of intracellular pH (pHi). In this study was investigated if the chronic treatment with high glucose can modulate the intracellular pH recovery rate (dpHi/dt) in Human Embryonic Kidney (HEK-293) cells overexpressed with wild-type human SGLT1 (WT) or transfected with human SGLT1 mutant of S418 to Histidine (S418H).

Methods: The cDNA of WT and S418H were cloned into the expression vector pEGFP-N1. All the recombinants were transfected into HEK-293 cells and the stable transfection was confirmed by Confocal microscopy. The presence of mRNA for a glucose sensor, G-Protein Receptor 1 (GPR1) was investigated by RT-PCR. The HEK-293 cells not-transfected (NT), transfected with WT or S418H grown to confluence in DMEM medium containing glucose 25 mM for 20 days. The dpHi/dt was analyzed by fluorescence microscopy using BCECF-AM fluorescent probe.

Results: The dpHi/dt decreased in WT and S418H when compared with NT cells [0.148±0.02 (n=5); 0.208±0.02 (n=6) and 0.4321±0.02 (n=7), respectively, $p<0.05$]. The addition of H-89 (PKA inhibitor; 10^{-6}M) did not prevent this effect but reduced it partially only in WT [WT: 0.282±0.02 (n=6) and S418H: 0.242±0.02 (n=7), $p<0.05$]. Western blot analysis showed that the chronic treatment increased the NHE1 expression only in WT cells [NT: 0.327±0.046 (n=3); WT: 0.807±0.050 (n=3); S418H: 0.2976±0.030 (n=3), $p<0.05$].

Conclusions: Our results indicate that despite having increased NHE1 expression, the dpHi/dt decreased in WT and S418H cells indicating that in NT cells another NHE isoform could be involved. GPR1 could activate PKA which phosphorylates SGLT1 and NHE1 and modulates differently the activity of these proteins, justifying the reduction on the inhibitory effect on dpHi/dt, observed only in WT.

Financial Support: FAPESP, CNPq and CAPES
Funding: Government Support - Non-U.S.

PUB371

Dysnatremias in Polytrauma Patients Shilpa Reddy Carlson,[2] George M. Feldman.[1] [1]*Hunter Holmes Mcguire Veterns Affairs Medical Center, Richmond, VA;* [2]*Virginia Commonwealth University.*

Background: Dysnatremias are associated with increased morbidity and mortality. (Waikar, 2009, O'Donoghue, 2009). Mild hyponatremia can cause deficiencies in posture, gait and can cause diminished cognition. (Decaux, 2006). There is a high incidence of hyponatremia in spinal cord patients (Furlan, 2009). Recently, patients with polytrauma, i.e., patients sustaining injuries to multiple body parts, are being treated in VA hospitals and may be at risk of dysnatremia. The aim of this study was to evaluate the incidence of abnormalities in serum sodium concentration (sNa) in a population of polytrauma patients.

Methods: Charts of patients discharged from the Polytrauma Unit at the Richmond VAMC between 12/2007 and 12/2009 were reviewed (n=64). Laboratory records were reviewed retrospectively from the time of injury until discharge. A sNa of less than 135 meq/L was defined as hyponatremia and a sNa above 145 meq/L was defined as hypernatremia

Results: The average age was 30 ± 11.6 years old; 98.4% were male; 10.9% were African American; 1.5% were Hispanic; and 87.5% were caucasian. Intracranial hemorhage occurred in 32% and fracture of cranial/peripheral bones occurred in 41%. Two (2) had been diagnosed with diabetes mellitus, 2 with diabetes insipidus, and 1 with SIADH. None had been diagnosed with adrenal insuffiency, hypothyroidism, congestive heart failure, or cirrhosis. Upon review of the laboratory records 29 patients (45.3%) had had abnormalities of sNa. Of these patients, 10 (34.5%) had hypernatremia only, 11 (37.9%) had hyponatremia only, and 8 (27.6%) had both hypo and hypernatremia during their hospital course. 44.4% of the hypernatremic patients had hypernatremia on their first available sNa determination, and 36.8% of the hyponatremic patients had hyponatremia on their first available sNa determination. At discharge, 94% of the patients with dysnatremia had documented normal sNa.

Conclusions: Polytrauma patients appear to be at high risk of experiencing dysnatremia. It is uncertain whether dysnatremia contribute to the mortality or morbidity of patients with acute polytrauma or whether dysnatremia and associated neurological sequelae delay recovery or prolong hospitalization and rehabilitation.

PUB372

Novel Reconfiguration of the Fractional Excretion of Sodium (FENa) Equation Vernon S. Chiu, Eric J. Bloom, Rasib Raja, Daranee Chewaproug, Saurabh A. Pande, Elio A. Torres, Pankaj Jawa, Sayed A. Kazi, Kawin Tangdhanakanond. *Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

Background: FENa is calculated when the true volume status of a patient is in question. Creatinine clearance (CrCl) is widely used as an estimate for glomerular filtration rate (GFR) and is part of the FENa formula. CrCl overestimates renal function as creatinine is freely filtered by glomeruli and secreted at the level of the proximal tubules. Urea clearance underestimates GFR since filtered urea is not secreted but reabsorbed in the proximal and distal nephrons. Our aim is to test a potentially more accurate equation for determining FENa, by implementing both creatinine and urea in an attempt to offset the inherent errors of creatinine and urea clearance alone. This should translate to improved volume assessments and medical management.

Methods: All patients seen in consult between 7/1/10 to 12/15/10 with AKI or any stage of CKD. Subjects must have had metabolic panels drawn simultaneously with urinary electrolytes, urea, and creatinine. FENa was calculated using both conventional and modified equations.

Conventional FEna

$$FEna = \frac{(Una) \bullet V}{(Pna) \bullet GFR}$$

Modified FEna

$$FEna = \frac{(Una) \bullet V}{(Pna) \bullet \left[\frac{\left(\frac{(Ucr) \bullet V}{(Pcr)} \right) + \left(\frac{(Uurea) \bullet V}{BUN} \right)}{2} \right]}$$

Results: When using the modified FENa equation, 23 of 26 (88%) subjects had good correlation between conventional and modified FENa.

Conventional vs Modified FENa

Subject	Conventional FEna	Modified FEna
1	0.15	0.22
2	0.22	0.35
3	3.02	2.32
4	0.33	1.6
5	0.24	0.35
6	0.36	0.52
7	1.93	2.86
8	2.17	3.56
9	5.65	6.55
10	4.6	6.16
11	1.79	2.16
12	0.82	1.26
13	1.74	2.72
14	2.8	3.57
15	0.25	0.4
16	0.55	0.79
17	0.33	0.46
18	0.16	0.22
19	0.47	0.74
20	3.11	2.68
21	14.8	16.68
22	3.18	2.11
23	0.97	1.39
24	0.27	0.48
25	0.69	0.93
26	3.3	4.21

Conclusions: The conventional FENa equation may be sufficient to determine an accurate volume status in most clinical scenarios.

PUB373

Normalization of Fractional Excretion of Urate (FEurate) after Correction of Hyponatremia Differentiates SIADH from Cerebral/Renal Salt Wasting (RSW) James Drakakis, Louis J. Imbriano, Nobuyuki (Bill) Miyawaki, Shayan Shirazian, John K. Maesaka. *Division of Nephrology & Hypertension, Winthrop University Hospital, Mineola, NY.*

Background: Differentiating SIADH from RSW has been an unresolved diagnostic conundrum that has important therapeutic consequences, to water-restrict those with SIADH and administer salt and water in RSW.The normalization of a previously elevated FEurate of > 12% to < 10% in SIADH can be contrasted to a persistent elevation in RSW after correction of hyponatremia.We utilize this normalization of FEurate after timely correction of hyponatremia to confirm the diagnosis of SIADH in 2 patients

Results: A 93 year old female presented with a pneumonia and serum sodium (SNa) 115 mmol/L, urine osmolality (Uosm) 336 mosm/kg, urine sodium (UNa) 82 mmol/L and FEurate 18.1%,; her plasma renin was increased and aldosterone decreased on ACE inhibitor. She was treated with intravenous antibiotics and fluid restriction.By the fifth hospital day, SNa increased to 132 mmol/L, FEurate 11.3% and on the sixth day, SNa increased to 136 mmol/L and FEurate now normal at 6.5%.

An 87 year old male with bronchogenic carcinoma was admitted after a fall due to a SNa of 112 mmol/L/L.The Uosm was 547 mosm/kg, UNa 90 mmol/L and FEurate 27.5% with low plasma renin and aldosterone.The initial treatment of isotonic saline, fluid restriction and salt supplementation for probable RSW failed to correct SNa, briefly exhibiting desalination.Isotonic saline was switched to 1.5% hypertonic saline and SNa gradually increased from 131 to 138 mmol/L over two days as FEurate normalized from 27.5 to 8.5%.The normalization of FEurate after correction of hyponatremia in both cases is consistent with SIADH.

Conclusions: A normal FEurate after correction of hyponatremia is consistent with SIADH.This contrasts to a persistently increased FEurate in RSW

Correction of hyponatremia can be achieved gradually with hypertonic saline over several days. Determination of FEurate after correction of hyponatremia can be used to differentiate SIADH from RSW.Normalization of FEurate after correction of hyponatremia by hypertonic saline confirms our notion that saline has a meager effect on FEurate

PUB374

Effect of Angiotensin Converting Enzyme Inhibitors on Serum Potassium Concentrations in Hemodialysis Patients. An Observational Study Ezio Movilli, Corrado Camerini, Paola Gaggia, Roberto Zubani, Giovanni Cancarini. *Division of Nephrology, Spedali Civili and Section of Nephrology, University of Brescia, Brescia, Italy.*

Background: Angiotensin converting enzyme inhibitors (ACEi) are drugs increasingly used in uremic patients (pts). However, their effect on serum potassium concentrations (sK) in pts on chronic hemodialysis (HD) is controversial. Aim of the study: To evaluate sK before and after ACEi therapy.

Methods: From 1-1-2009 to 31-12-2010, 87/206 prevalent HD pts started ACEi therapy. Mean age was 67±14 years, 57/87 were men, dialytic vintage was 6-204months. In the 2 months before (24 HD sessions) and after (24 HD sessions) the start of ACEi, were evaluated in pre dialysis after the long interdialysis interval: sK (mean of 8 determinations) (mmol/L), maximum sK (maximum K value observed during observations) (sKmax; mmol/L), serum sodium (Na; mmol/L), hemoglobin (Hb; g/dL), EPO dose (U/Kg/sett), pre dialysis systolic (SBP: mmHg) and diastolic (DBP: mmHg) blood pressure, body weight (BW; Kg), interdialytic weight gain (IWG; Kg), Kt/V. SBP, DBP, IWG are the mean values of the 24 HD sessions. Duration of HD, blood and dialysate flow rate were kept constant. Data are expressed as mean±SD, t test for paired data was employed. Significant differences were defined as $p<0.05$.

Results: sK increased from 5.0±0.4 mmol/L to 5.7±0.5 mmol/L ($p< 0.0001$). sKmax increased from 5.4±0.5 mmol/L to 6.2±0.6 mmol/L ($p<0.0001$). 7/87 pts reduced the K dialysate concentration. 15/87 pts (18%) stopped ACEi therapy. sK in these pts varied from 5.2±0.3 mmol/L to 6.5±0.2 mmol/L at the moment of suspension ($p<0.0001$). sKmax varied from 5.5±mmol/L to 6.9±0.3 mmol/L at the moment of suspension ($p< 0.0001$). After the suspension of ACEi, sK and sKmax decreased to basal levels within 1 month. There were no significant changes of BW, IWG, SBP, DBP, Na, Hb, EPO dose, Kt/V in both periods.

Conclusions: Treatment with ACEi causes a significant 13% increase of the sK concentrations in chronic HD pts. This fact can lead, in 18% of cases, to the need to stop the drug for sK values greater than 6.5 mmol/L. This suggests caution in the increasingly wider utilization of this class of drugs in HD patients.

PUB375

Hypokalemia in Hospitalizaed Patients Fahad Saeed, Muhammad Omer Toor, Noaman Siddiqi, Digant V. Bhatt, Jae Hyung Cho, Nadia Kousar, Jean L. Holley. *Department of Medicine, University of Illinois at Urbana Champaign, IL.*

Background: Hypokalemia is common in hospitalized patients and can lead to prolonged length of stay and increased mortality. The aim of this study was to assess causes of hypokalemia and the average time taken for repeat potassium (K) orders to be entered in electronic medical records.

Methods: Data of all adult patients (> 18 years) with any low K level (K<3.5 mEq/L) admitted between January 2009 to March 2009 for any reason were reviewed. Average time taken by the treating physicians to order IV or oral K replacement was noted as were any events ascribable to hypokalemia and possible causes of low K.

Results: A total of 375 hypokalemic lab values in 202 patients were analyzed. Severe hypokalemia (K<2.5meq/L) occurred 10 times in six patients (2.7% of all hypokalemic episodes). Average patient age was 60.24±16.81 years and 68.8% of hypokalemic episodes were in women (P=<0.05). Use of loop diuretics (19.2%) was the most common cause of hypokalemia, followed by beta 2 agonists (15.2%), hypomagnesemia (12.0%), steroids (10.7%), insulin (10.1%), diarrhea (9.1%), thiazide diuretics (5.9%), and amphotericin B (5.3%). Use of corticosteroids (40 %) and amphotericin B (40%) were the most common causes of severe hypokalemia. Steroid (13.5 % vs 4.4 %) and amphotericin b (7.3 % vs 0.9 %) usage was more common in women than men who received more loop diuretics than women (23.9% vs 17.3 %). Mean time between low K lab value and orders for repeat K value was 15.41 ±11.4 hours, the longest being for patients on surgical services (21.5± 8.5 hrs vs 15.7±10.2 hrs for patients on medical services, p = <0. 01).

Conclusions: Loop diuretics are the most common cause of hypokalemia in hospitalized patients. Steroids and amphotericin B are associated with severe hypokalemia. Women are more prone to hypokalemia than men despite less frequent diuretic usage. The electronic medical record can be a useful tool in the analysis of causes and management of hypokalemia.

PUB376

Pseudohyponatremia: Not a Diagnosis of the Past Dana F. Work, Ayesa N. Mian, Marc Lande. *Pediatric Nephrology, University of Rochester, NY.*

Background: Pseudohyponatremia, the finding of hyponatremia associated with normal serum osmolality, occurs in disorders with marked elevation of serum lipids or protein. The fraction of water in the serum is thereby reduced, causing an artificially low serum sodium concentration. Low measured sodium values observed in pseudohyponatremia are not clinically relevant. The use of direct potentiometry via ion-selective electrode (ISE) rather than flame photometry for measurement of the serum sodium can avoid artificially low sodium readings.

Methods: Case Report

Results: A 12-mo-old male with Alagille Syndrome was found to have a low serum sodium concentration of 127 mmol/L, but a normal serum osmolality of 284 mOsm/Kg H2O. Total cholesterol was markedly elevated at 1,455 mg/dl, a finding thought to be related to the patient's hepatic disease. The diagnosis of pseudohyponatremia was originally considered, but thought unlikely given the use of ISE measurements in our laboratory. Oral sodium supplementation was initiated. However, analyses of sodium on subsequent blood samples by blood gas analyzer were normal, whereas simultaneous measurements by ISE continued to be low. On further investigation, it was discovered that the ISE analyzer in our laboratory automatically dilutes the serum sample 1:31 prior to direct potentiometry sodium measurement. The dilution step had introduced the potential for pseudohyponatremia, despite the use of ISE technology. By contrast, the blood gas analyzer measures sodium directly, without a dilution step. The diagnosis of pseudohyponatremia was made, and oral sodium supplementation was discontinued.

Conclusions: The diagnosis of pseudohyponatremia was delayed due to the incorrect assumption that the use of ISE measurement of serum sodium precluded that possibility. Commercially available ISE analyzers differ in whether potentiometry is performed on diluted or undiluted serum. This case serves to underscore that clinicians need to know the method used in their laboratory when considering a diagnosis of pseudohyponatremia.

PUB377

Central Diabetes Insipidus and Acute Myeloid Leukemia Solange Bourque. *CHUS.*

Background: Central diabetes insipidus (CDI) can occur in acute myeloid leukemia (AML) by infiltration of the central nervous system (CNS). We present a case of CDI in AML without evidence of CNS involvement.

Methods: A 48 years old woman was treated since 2004 for essential thrombocytosis. She was hospitalised in November 2010 with a diagnosis of AML. At that time her platelet count was 1950 x 10^9/L. Her karyotype showed an inversion of the chromosome 3 (q21q26) without any abnormality of the chromosome 7. In January 2011, while she was receiving her third induction chemotherapy for her AML, she started to urinate more than 20 litres per day. Her natremia was 151 mmol/L, plasmatic osmolarity 311 mosm/L, urinary osmolarity 203 mosm/L, TSH and cortisone levels were normal. DDAVP 4mg IV reduced her diuresis to 5 liters per day and normalised her sodium to 140 mmol/L. Brain MRI was normal; lumbar puncture didn't show any cells.

Results: CDI without CNS involvement is a rare but recognised complication of myelodysplastic syndrome, AML and chronic myeloid leukemia. Most cases are associated with a monosomy of the 7th chromosome. There are also some cases of the 3q21q26 syndrome with or without 7th monosomy. 3q21q26 syndrome implies an inversion, an insertion or a translocation of the chromosome 3. MRI and cerebrospinal fluid are usually normal. Etiology of CDI is unknown. Hypothesis includes infiltration, thrombosis, bleeding or infection too small to be detected without an autopsy. A paraneoplasic factor could also inhibit, degrade or interfere with the formation of arginine vasopressin. Thrombocytosis in 3q21q26 syndrome can also interfere with the level and the function of arginine vasopressin as more than 90% of this hormone circulates with platelets. Nine cases of CDI with 3q21q26 syndrome are described in the litterature. They are usually but not always associated with monosomy 7.

Conclusions: CDI in AML without CNS involvement is rare. It is usually associated with chromosomal abnormalities such as monosomy 7 or 3q21q26 syndrome. It is unknown whether the chromosomal abnormalities cause the CDI or if it is just a marker.

PUB378

Pioglitazone Suppresses Renal Tubular Senescence in Dahl Salt-Sensitive Hypertensive Rats Yuki Kusano, Yoshimitsu Hayashi, Shigeatsu Hashimoto, Kimio Watanabe, Yoshihiro Tani, Kaoru Sakurai, Koichi Asahi, Masaaki Nakayama, Tsuyoshi Watanabe. *Nephrology and Hypertension, Fukushima Medical University, Fukushima, Japan.*

Background: The PPAR-g agonist pioglitazone has several functions including anti-inflammation, anti-oxidative stress and anti-senescence. Increased oxidative stress plays a pivotal role in premature senescence. Dahl salt-sensitive (DSS) rats are characterized by increased oxidative stress in the renal outer medulla caused by salt loading, which results

in chronic tubulointerstitial damage to the kidney. Because pioglitazone protects against aging-related tubular damage in SD rats, we investigated whether it also affects tubular senescence in DSS rats.

Methods: Six-week-old male DSS rats were assigned to either a group fed with 4% salt (high salt [HS] group; n = 10) or a group fed with 4% salt containing 0.005% pioglitazone (HSP group; n = 10). The rats were weighed and blood pressure was regularly measured during the study period. The rats were sacrificed after 12 weeks to examine kidney function, metabolic markers and kidney parameters such as histological changes, positive areas of SA-b-gal (cellular senescence marker) activity and Sirt1 mRNA expression.

Results: Body weight, blood pressure, blood glucose, insulin levels and proteinuria did not significantly differ between the two groups. However, creatinine clearance and serum adiponectin levels significantly differed (HS vs. HSP: 2.34 ± 0.37 vs. 3.60 ± 0.52 mL/min, $p < 0.001$ and 2.32 ± 0.39 vs. 3.78 ± 0.32 mg/mL, $p < 0.001$, respectively). The tubular injury index and positive area of SA-b-gal activity in tubular cells were significantly lower in the HSP group (8.53 ± 3.4 vs. 4.49 ± 3.3, p=0.016 and 11.0 ± 4.1 vs. 4.48 ± 2.0%, $p < 0.001$), whereas Sirt1 mRNA expression in the kidney was significantly higher (1.0 ± 0.4 vs. 1.6 ± 0.4, p=0.012).

Conclusions: Pioglitazone suppressed tubulointerstitial damage and tubular senescence in DSS rats, although it did not confer a benefit in terms of reducing blood pressure, urinary protein and blood glucose levels. Increased levels of serum adiponectin and of Sirt1 mRNA expression in the kidney might be involved in the anti-senescence mechanism of pioglitazone.

PUB379

Serum Protein and Podocin Expression Changes in Living Mouse Glomeruli under the Acute Hypertensive Condition Zilong Li, Juan Wang, Fengxia Yu, Xiaohui Yin, Jun Wang, Hua Zhou, Lining Wang. *Department of Nephrology, First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China.*

Background: The purpose of this study is to visualize topographical serum protein and podocin changes in living mouse glomerular capillary loops (GCL) under various hemodynamic conditions by novel "in vivo cryotechnique"[1, 2].

Methods: "In vivo cryotechnique" group: The "in vivo cryotechnique" was performed on left kidneys of anesthetized C57BL/6 mice, as reported before[3]. **Control group:** the kidney tissues were fixed with the conventional immersion and perfusion preparation methods. Their serial sections were stained and observed by light, confocal laser scanning microscopy[4] and immunoelectron microscopy. The animals were also evaluated for renal podocin mRNA, and protein expression.

Results: By the "in vivo cryotechnique", the distribution of serum proteins: albumin and immunoglobulinG (IgG; Ig kappa light chain and IgG1 heavy chain) were disorder and the immunoreactivity of albumin or Ig kappa light chain was markedly increased and immunolocalized in apical areas of the foot processes and urinary space, not slit-diaphragm, but the podocin expression was seriously decreased under the acute hypertensive condition. The abnormal distribution of serum proteins and podocin was also found in the control group under the normotensive condition was similar to that under the acute hypertensive condition with "in vivo cryotechnique".

Conclusions: These results suggest that the redistribution of serum proteins and podocin was the important factor about proteinuria under the acute hypertensive condition. The "in vivo cryotechnique" followed by freeze-substitution should be a reliable tool to observed the serum or podocyte foot process proteins in situ and capture transient images of functioning glomeruli in living mice. The artifacts seemed to be due to immersion and perfusion fixation procedure.

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PUB380

Characterization of Blood Pressure and Urine Sodium Retention in Mouse Models of Insulin Resistance Robert B. McClellan,[1] Wuxing Dong,[2] Michael Pellizzon,[3] Vivek Bhalla.[2] *[1]Pediatrics, Stanford Univ., Stanford, CA; [2]Medicine, Stanford Univ., Stanford, CA; [3]Research Diets, Inc., New Brunswick, NJ.*

Background: The optimal mouse model for elucidating the role of the kidney in mechanisms of hypertension in diet-induced insulin resistance, obesity, and the metabolic syndrome is unknown.

Methods: At 4 weeks of age, C57BL/6J male mice were fed 10% fat, 45% fat, 60% fat, 30% fructose H2O, or 60% fat plus 30% fructose H2O (n=4/group). At 10 months of age, the diets were supplemented with 4% NaCl. Fasting serum insulin (ELISA), body weight, and systolic BP (tail-cuff) were measured at 1, 2, 6, and 12 months after initiation. Urine sodium excretion rates were measured via calibrated flame photometry. Results are expressed as mean±SEM and p-values< 0.05 were determined by t-tests or ANOVA.

Results: Compared with control mice, fasting serum insulin increased significantly at 2 months in the 45% fat diet (0.57±0.11 ng/mL vs. 1.40±0.29, $p = 0.04$) and at 6 months in the 45%, 60%, and 60% fat/30% fructose diets [1.0 ng/mL vs. 2.9 (p=0.001), 3.0 (p=0.01), and 3.6 (p=0.0006)], respectively. Body weight was also significantly increased in the 45%, 60%, and 60% fat/30% fructose mice compared with control or 30% fructose-fed mice. The mice in all groups maintained similar BP(mean systolic 108.6mmHg±11.9, 106.8mmHg±12.4, 106.7±11.2, 108.3±13.4, and 107.4±11.9). No significant differences in BP were noted after 3 weeks of high-salt food. Urine sodium excretion after initiation of a high-salt diet was significantly higher in control mice vs. other groups (19.26 µmol/12-hrs±2.75 vs.1.76±1.10, 4.22±1.56, 7.32±4.02, and 1.63±1.62, $p<0.007$).

Conclusions: High-fat but not high-fructose med mice develop sustained hyperinsulinemia and weight gain compared with normal fat-fed control mice. Despite defects in urine sodium excretion rates compared with controls, the mice did not develop systolic hypertension with or without high-salt chow. Ongoing radiotelemetry-based measurements may discriminate amongst these mouse models further. High-fat (45% or 60%) fed mice are useful models for insulin resistance, obesity, and decreased urine sodium excretion in male mice.

Funding: NIDDK Support, Private Foundation Support

PUB381

Effect of Tamsulosin on Bladder Microcirculation in a Rat Bladder Outlet Obstruction Model, Evaluated by a Pencil Lens Charge-Coupled Device Microscopy System Hideki Mizuno,[1,2] Tokunori Yamamoto,[1] Yasushi Yoshino,[1] Momokazu Gotoh.[1] *[1]Urology, Graduate School of Medical Sciences, Nagoya University, Nagoya, Aichi, Japan; [2]Urology, National Hospital Organization Nagoya Medical Center, Nagoya, Aichi, Japan.*

Background: To investigate the effect of tamsulosin hydrochloride on blood flow in a submucosal capillary of the bladder (SCB) in a rat bladder outlet obstruction (BOO) model, using a pencil lens charge-coupled device microscopy system (PLCMS).

Methods: BOO was produced in rats by partial ligature of the proximal urethra, which was maintained for 2 weeks. Tamsulosin or saline (control) was subcutaneously administered via an osmotic pump for 2 weeks immediately after the BOO surgery. Using the PLCMS, bladder microcirculation was visualized and quantitatively assessed by measuring the velocity of blood flow in a SCB of the bladder at the two site the base and dome of the bladder. The blood flow in the SCB of the sham-operated rats, the control BOO rats, and the tamsulosin-treated BOO rats were compared.

Results: The blood flow in the SCB was significantly greater at the base than at the dome. The blood flow in the SCB both at the base and the dome was significantly reduced in BOO rats as compared with sham-operated rats, but pretreatment of tamsulosin significantly increased the blood flow in the SCB at both sites in the BOO rats than in the control . The PLCMS imaging morphologically revealed that BOO rats showed chronic ischemic capillary injury and tamsulosin ameliorated it.

Conclusions: The present study suggests that tamsulosin hydrochloride exerts a protective effect on the blood flow in the SCB from ischemic injury following bladder outlet obstruction.

PUB382

The Disturbed Circadian Rhythm and Salt Sensitivity of Blood Pressure in Adriamycin Nephropathy Rats Lijun Mou, Yan Qin, Xuewang Li, Xuemei Li. *Kidney Department, Peking Union Medical College Hospital, Beijing, China.*

Background: Adriamycin Nephropathy rats developed significant proteinuria as an useful mouse model for nephrotic syndrome. In the present study we determined the blood pressure levels, their circadian rhythm, and their regulation by changes in salt intake.

Methods: Circadian characteristics of DBP, HR, PP and locomotor activity were measured in conscious and unrestrained ADR nephropathy rats and age-matched SD control rats by the radiotelemetry system. After baseline studies were obtained, the rats were were provided a high salt diet (8.0%) for a 1-wk period prior to the 3 day telemetry study.

Results: Adriamycin Nephropathy rats demonstrated reversed diurnal rhythms of DBP, and PP compared with SD control rats. However, there is no significant difference in 24-h mean value of MAP or SBP. In ADR nephropathy rats, the circadian rhythm of the urine sodium excretion was also reversed. The RUNa in Dark period was significantly lower than that in the Light period [(14.69 ±3.65)µmol/h v.s (27.66± 5.84)µmol/h, P=0.001] . In the ADR nephropathy rats, the FENa in Dark period was significantly lower than that in the Light period (0.15±0.06 v.s 0.29±0.06,P=0.008) and also significantly lower than that in the Dark period of the control group(0.15±0.06 v.s 0.31±0.19,P=0.05). Both the ADR nephropathy rats and control rats adapted to the high NaCl diet with significant blood pressure elevated($P<0.001$). The control rats maintained the normal BP rhythm and ADR nephropathy rats still presented disturbed BP circadian rhythm after 1week high salt diet.

Conclusions: We concluded that circadian patterns of blood pressure were dramatically altered in ADR nephropathy rats with the disturbed circadian rhythm of the urine sodium excretion and FENa. The ADR nephropathy rats also showed a striking salt sensitivity of blood pressure. So the ADR nephropathy rat was a suitable CKD animal model with disturbed circadian BP rhythm and sodium sensitivity.

PUB383

Indoxyl Sulfate Accelerates Vascular Smooth Muscle Cell Senescence through Oxidative Stress Gulinuer Muteliefu,[1,2] Hidehisa Shimizu,[1] Masahide Takahashi,[2] Toshimitsu Niwa.[1] *[1]Department of Advanced Medicine for Uremia, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Pathology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: We previously demonstrated that indoxyl sulfate (IS), a uremic toxin, induced oxidative stress in human aortic smooth muscle cells (HASMCs). This study aimed to clarify whether IS contributes to cellular senescence in cultured HASMCs and the aorta of hypertensive rats.

Methods: The mRNA expression of cyclin-dependent kinase inhibitors, p16 and p21, and tumor suppressor proteins, p53 and pRb, in HASMCs was analyzed by RT-PCR. The protein expression of p53, p21, prelamin A, a senescence marker of VSMCs, and FACE1

(also named Zempste 24) was detected by western blotting. The activity of senescence-associated β-galactosidase (SA β-gal) was evaluated by SA β-gal staining in HASMCs. The expression of prelamin A in the arcuate aorta in rats was examined by immunohistochemical analysis using (1) Dahl salt-resistant normotensive rats (DN), (2) Dahl salt-resistant normotensive IS-administered rats (DN+IS), (3) Dahl salt-resistant hypertensive rats (DH), (4) Dahl salt-resistant hypertensive IS-administered rats (DH+IS).

Results: IS treatment enhanced the mRNA expression of p53 and p21 in a time- and dose-dependent manner, whereas the mRNA expression of p16 and pRb showed no significant change. The IS-induced mRNA expressions of p53 and p21 in HASMCs were suppressed by the addition of N-acetylcysteine (NAC), an antioxidant. IS significantly promoted the protein expression of p53, p21 and SA β-gal activity, and NAC and pifithrin-α, p-nitro (PFTα), a p53 inhibitor, blocked these effects in HASMCs. IS upregulated prelamin A and downregulated FACE1 protein expression, and NAC suppressed these effects in HASMCs. In aorta, DH+IS rats showed significantly increased expression of prelamin A in the cells embedded in the calcification area as compared with DH and DN rats.

Conclusions: IS accelerates cellular senescence through oxidative stress in HASMCs and the aorta of hypertensive rats. Thus, accumulation of IS in blood due to renal dysfunction may be one of the risk factors for vascular senescence in CKD patients.

Funding: Government Support - Non-U.S.

PUB384

Effects of Angiotensin II on Vasomotor Function of Pregnant Rats Rosemary E. Nwoko,[1] Iasmina Craici,[1] Steven Wagner,[1] Livius V. d'Uscio,[2] Joseph P. Grande,[1] Zvonimir S. Katusic,[2] Vesna D. Garovic.[1] *[1]Department of Nephrology, Mayo Clinic, Rochester, MN; [2]Department of Anesthesiology, Mayo Clinic, Rochester, MN.*

Background: The mechanism of resistance to the pressor effect of Angiotensin II (Ang II) in pregnancy is not well understood.

Methods: Pregnant and non-pregnant Sprague Dawley rats underwent subcutaneous implantation of Ang II (0.96mg/kg/day) or saline (sham) via osmotic pumps on day 5 of gestation. Animals were then sacrificed on day 19, and vasomotor function was studied in the isolated thoracic aortic rings suspended in organ chambers (37°C; 95% O_2, 5% CO_2).

Results: The contractile effect of KCL (20 mmol) was significantly potentiated in both non-pregnant and pregnant rats treated with Ang II. However, this Ang II-induced potentiation of contractions to KCL was significantly attenuated by pregnancy (p=0.01). Contractions to an alpha1-adrenergic agonist, phenylephrine (10^{-9}-10^{-5} M), were significantly increased in both Ang II treated pregnant and non-pregnant rats (maximal contraction: 78±7% and 71±4%, respectively; P<0.05 vs. sham: 52±5%; n=7). However, the sensitivity to phenylephrine was significantly reduced in Ang II treated pregnant rats (pD_2: 7.03±0.03, P<0.05 vs. Ang II treated non-pregnant rats: 7.44±0.10). We also examined the effect of pregnancy on endothelium-dependent and endothelium-independent relaxations. In the presence of acetylcholine (10^{-9}-10^{-5} M), endothelium-dependent relaxation did not differ between saline and Ang II treated non-pregnant rats (n=7). In contrast, in pregnant animals treated with Ang II, relaxations to acetylcholine were significantly enhanced as compared to sham animals: maximal relaxation: 99±1% vs. 92±3% for saline treated pregnant rats, respectively, P=0.02; (n=7). Endothelium-independent relaxations to nitric oxide-donor, DEA-NONOate (10^{-10}-10^{-5} M) were not significantly different among groups.

Conclusions: Pregnancy may modulate pressor effect of Ang II by: a) attenuating vasoconstrictor effects dependent on depolarization of smooth muscle cells and activation of alpha1-adrenergic receptors, b) by enhancing endothelium-dependent vasodilatation mediated by production and release of nitric oxide.

PUB385

Identifying Adolescents at Risk for High Blood Pressure Eleni Chelioti,[1] Dimitrios Athanasopoulos,[1] Ekaterini Garopoulou,[2] Maria Sotiraki,[1] Theodora Fragou,[1] Thnasis Georgiou,[1] Maria Tsilivigou,[1] Gabriel Papadakis.[1] *[1]Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, Athens, Greece; [2]Dept. of Pediatrics, General Hospital of Kalymnos, Kalymnos, Greece.*

Background: The increased prevalence of childhood obesity and the strong relationship of blood pressure (BP) with the body weight indicate that high blood pressure (HBP) prevalence in adolescents could increase as well. Therefore accurate diagnosis in young people is problematic.Primary objective of the study is to estimate the prevalence of HBP in adolescents of a remote Greek island. Secondary objective is to reveal which factors are associated with HBP.

Methods: A cross sectional study was carried out.Eligible subjects were adolescents aged between 13 to 15 years from a high school of the island of Kalymnos. Somatometrics and BP were measured at the school environment.HBP was defined according to simplified abnormal BP screening table based on the 4th report of National HBP Education Program Working Group in Children and Adolescents. Nutritional status was defined according to International Obesity Task Force.

Results: Participants were 215 adolescents (106 boys and 109 girls). 60 subjects were classified with HBP (27.9%, 95%CI 22.1-34.5%). Significant differences between the two genders were the outschool sport activities (p=0.03), the mean weight (p<0.01), height (p<0.001), systolic and diastolic BP (p<0.001and p=0.01). Statistically significant factors associated positively with HBP were the male gender and the overweight. The adjusted odds ratios of the best fitted model showed that the independent factors associated with HBP were still the male gender and the overweight. Female subjects had 83% lower odd in comparison with male subjects, taking into account other model terms. The odds of subjects with increased body weight were approximately 3 times higher compared to subjects with normal body status, adjusting for other model terms.

Conclusions: A great number of adolescents from the island of Kalymnos are in danger to develop hypertension in adulthood. Both screening of adolescents for HBP and recognition of risk factors for HBP could give us the opportunity for early prevention and intervention of hypertension.

Funding: Clinical Revenue Support

PUB386

Genetic Variants of the Renin Angiotensin System (RAS) and Blood Pressure Response to the RAS Blocking Drugs -A Systematic Review for Pharmacogenomics in the Renin Angiotensin System- Tadashi Konoshita. *Third Department of Internal Medicine, Fukui University School of Medicine, Eiheiji, Fukui, Japan.*

Background: The concept of "pharmacogenomics" promises to offer the ultimate in personalized medicine and the renin-angiotensin system (RAS) is one of the most plausible candidates for this approach in the area of hypertension. For the past two decades, genetic variants of the RAS have been tested for association with blood pressure response, but the results have been inconsistent. The most fundamental concern is thought to be the statistical power. Therefore, we have tried to put together a new systematic review using a database search including only reports with adequate subjects numbers.

Methods: Studies were identified by a PubMed search with condition 1 (("pharmacogenetics"[All Fields] OR "pharmacogenomics"[All Fields]) AND ("renin"[All Fields] OR "ACE"[All Fields] OR "angiotensinogen"[All Fields])) or condition 2 (("genetic variant"[All Fields] OR "polymorphism"[All Fields]) AND ("blood pressure response"[All Fields] OR ("blood pressure responder"[All Fields]) AND "angiotensin"[All Fields]).

Results: Condition 1 identified 136 studies, and condition 2 found 52. Practical clinical implications require detection of a difference in diastolic blood pressure (DBP) of almost 5 mm Hg. Thus, we calculated the necessary sample size, assuming a standard deviation for the DBP of 10 mm Hg with protection against type I error of 5% and 80% of power, and determined that a study required 200 subjects. We therefore adopted studies with about 200 or more subjects. Evaluated genes were ACE, AGT, AT1, AT2, renin (REN) and ACE2. The evaluated drugs were ARB and ACEI. Finally 11 studies were recruited and put together to a new systematic review.

Conclusions: From the results, we were able to draw conclusions with nearly consistent findings that the conventional variants (ACE I/D, AGT M235T, AT1 A1166C and AT2 variant) are not associated with antihypertensive effects by RAS blockade, at least by one individual SNP. By contrast, significant associations have been reported (by one report each) for AGT rs7079, AT1 haplotype, REN, and ACE2. For these variants, further evaluations and confirmation are anticipated.

PUB387

The Impact of Endothelin Receptor Antagonists on Predictors of Mortality in Patients with Heart Failure Natallia Maroz, Amir Kazory. *Nephrology, Hypertension, and Renal Transplantation, University of Florida, Gainesville, FL.*

Background: Elevated blood urea nitrogen (BUN) and decreased level of serum sodium have being consistently shown to predict adverse outcomes in patients with heart failure (HF) both at short and long-term. Endothelin receptor antagonists (ERA) represent an emerging therapeutic option for HF but their impact on mortality is not yet well-known. This study was designed to explore the currently available data on the impact of these agents on predictors of mortality in HF.

Methods: Articles cited in Pub Med database from 1980 to 2011 using key words: "endothelin receptor antagonist" and "heart failure" were searched. Those clinical randomized controlled trials that exclusively included HF population were identified, and relevant articles were selected. The results of these studies were then reviewed and compared with regards to impact on levels of BUN and serum sodium.

Results: A total of 40 relevant articles were identified that used four different ERA agents (tezosentan, erasentan, bosentan, and darusentan,). Twelve randomized, placebo-controlled trials were selected to be included in this study. While 10 studies could not find any significant change in the levels of BUN and serum sodium prior to and after therapy, 2 studies did not report them.

Conclusions: Although there is a promising theoretical basis for the use of ERA in patients with HF, currently available data does not support any beneficial impact on predictors of mortality. Future large-sized trials are needed to further evaluate long-term effects of ERA and their potential impact on patient's outcome.

PUB388

Resistant Hypertension and Vitamin D Deficiency in a Tertiary Care Clinic Alexis Payette, Jean-Philippe Lafrance, Michel Vallee. *Department of Nephrology, HMR, University of Montreal, Montreal, QC, Canada.*

Background: Vitamin D deficiency is thought to play a significant role in cardiovascular disease and hypertension. Suppression of the renin gene could be involved, although the precise mechanisms underlying this association are incompletely understood.

The goal of this study is to evaluate the possible association between vitamin D deficiency and resistant or difficult-to-control hypertension.

Methods: We conducted a retrospective study among patients referred for resistant or difficult-to-control hypertension in a tertiary center in Montreal. We collected the demographic, clinical and laboratory data of 49 patients via review of their medical records.

Blood pressure levels were measured using 24-hour ambulatory blood pressure monitoring (ABPM) which correlates more closely with cardiovascular complications.

A possible association between circulating 25(OH)D concentration and ABPM levels was analyzed in 28 patients.

Results: Up to 65.3% of all patients received three or more antihypertensive drugs and 36.7% also had a 24-hour ABPM average above 130/80 mmHg.

24-hour Ambulatory BP monitoring (mmHg)

Visit Number	Number of measures	Systolic BP	Diastolic BP
1	41	134.6	77.0
2	16	128.3	75.6
3	10	135.9	81.5
Follow-up		Systolic BP variation	Diastolic BP variation
Visit 2 vs1	11	-6.6	-3.5
Visit 3 vs 2	5	-8.6	-4.0
Visit 3 vs 1	7	0.0	+3.7

Among these patients with resistant or difficult-to-control hypertension the mean circulating level of 25(OH)D was 29.7 ng/mL, and was low (<20 ng/mL) in 28.6% of patients. This mean value is similar to that of the general Canadian population which is 27.1 ng/mL.

We did not observe a significant relationship between 25(OH)D circulating level and systolic or diastolic 24-hour average blood pressure in this population.

The majority of patients (73.5%) involved in this study received a final diagnosis of essential hypertension.

Conclusions: We did not observe a significant relationship between circulating levels of 25(OH) vitamin D and blood pressure levels in our population of patients with resistant or difficult-to-control hypertension.

These results suggest that vitamin D deficiency is not a significant factor in the severity of resistant or difficult-to-control hypertension.

PUB389

Blood Pressure Control in Chronic Kidney Disease Hypertensive Patients Using Home Blood Pressure Monitoring Deepali Prasad,[1] Thilagavathi Venkatachalam,[1] Akash Ajmera,[1] Krystal Hunter,[2] Barry Milcarek,[2] Christopher B. McFadden.[1] *[1]Medicine, Cooper University Hospital, Camden, NJ; [2]Cooper Research Institute, Cooper University Hospital, Camden, NJ.*

Background: Home Blood Pressure Monitoring (HBPM) is frequently used by subjects with hypertension. Literature supports its role in predicting cardiovascular risks better than office blood pressure (OBP). Limited information is available about the relationship between use of HBPM and blood pressure control in a population with Chronic Kidney Disease (CKD). We hypothesized that HBPM use in a CKD population would be associated with better blood pressure control as measured by OBP.

Methods: We estimated a sample size of 112 subjects would be needed to show a higher rate of BP control in the HBPM group (one-sided difference) with a power of 80% and a p value of <.05. A significant difference was defined as 20% or more subjects at goal BP (<130 mm SBP and 80 mm DBP). Subjects filled out a survey about their use of HBPM (yes or no) as well as other demographic and medical questions. BP control was determined by clinic BP at time of enrollment.

Results: Preliminary results follow enrollement of 70 subjects (44% male and 56% female). This included 26 HBPM and 44 non HBPM users from 2 Nephrology clinics at Cooper University Hospital. No significant difference in BP control was noted. 42% of subjects with HBPM had controlled BP compared to 45% of non HBPM subjects. No significant difference in mean age (65 years in both groups) or CKD stage between HBPM and non HBPM groups existed. Men were more likely to use HBPM (54%) then women (23%). 50% of Caucasians used HBMP compared to 37% of enrolled African Americans and 27% Hispanics. No difference in education existed between the groups.

Conclusions: A preliminary analysis of our study shows no clear difference between HBPM use or non-use in BP control in this population with CKD. The study has not reached target enrollment. Preliminary enrollment and more severe HTN in the HBPM group are potential explanations for a possible type 2 error. The study is ongoing. A difference in frequency of HBPM use between Caucasians, African-Americans, and Hispanics may exist.

PUB390

Role of Ambulatory Blood Pressure Monitoring as a Predictor of Renal and Cardio-Vascular Endpoints Mihail Ion Soare, Dianne T. Sandy, Mauro Braun, Rute C. Paixao, Umabala Pasupala. *Nephrology and Hypertension, Cleveland Clinic Florida, Weston, FL.*

Background: Ambulatory blood pressure monitoring has traditionally been used to diagnose white coat hypertension and only recently has had a more widespread use. The loss of circadian blood pressure rhythm on ABPM correlates with the presence of obstructive sleep apnea and renal insufficiency and may be a prognostic marker. Our study aimed to determine the prevalence of the different types of hypertension in our clinic population, and whether ABPM was useful as a predictor of renal and cardiovascular outcomes.

Methods: Retrospective chart review done from July 2005 to June 2009. 175 charts were reviewed. 34 patients excluded due to incomplete monitoring (<90 % successful readings). 141 patients included in the final statistical analysis.

BP was classified according to JNC-7 guidelines.Nocturnal dipping status: nocturnal drop in SBP, DBP and heart rate of > 10%. Statistical analysis was performed using the SPSS Statistical Analysis Software.

Results: Mean age: 58 years, 44% male, 70.9% Caucasian. Mean serum creatinine 0.87 mg/dl (range 0.5 -1.7 mg/dl), 15% diabetic. 10% GFR< 60 ml/min, 11% OSA

Primary outcome: the composite outcome of renal (doubling in serum creatinine / reaching ESRD) and cardio-vascular (acute myocardial infarction, acute CHF) endpoints. Mean study follow-up period: 3 years.

Six patients (4.3%) reached the composite outcome. There was a trend towards the composite outcome in patients with isolated systolic HTN and Stage 2 HTN. OSA was associated with the loss of circadian rhythm.(67% vs 31%, p =0.01). The composite outcome was associated with the presence of diabetes, CHF and microalbuminuria

Conclusions: The study shows a trend towards renal and cardiovascular outcomes in patients with isolated systolic hypertension, stage 2 hypertension and loss of circadian rhythm as measured with ABPM. These trends could be confirmed with a larger study population and longer follow up period. Our study showed a strong association of these endpoints in patients with diabetes, CHF and albuminuria. ABPM is useful not only in the diagnosis of hypertension, but can be a valuable prognostic tool.

PUB391

Blood Pressure Measurement in Diabetes – New Techniques and Validity of Current Modalities in Hypertension Diagnostics Simone Theilade, Maria Lajer, Christel Joergensen, Frederik I. Persson, Peter Rossing. *Steno Diabetes Center, Gentofte, Denmark.*

Background: Tonometry is a novel technique for measuring BP. A watch-like device captures radial pulsewave reflection and calculates ambulatory brachial blood pressure (BP). Hypertension (HTN) is often diagnosed by office blood pressure (OBP) rather than ambulatory BP (AMBP). In this study we investigate if tonometry is applicable and reliable, and if OBP is reliable in HTN diagnostics in patients with diabetes.

Methods: In 24 Caucasian diabetic patients we compared tonometric (BPro) to cuff-based (Takeda TM2421) BPs. Patients were seen twice<2 weeks. At visit 1, 15 minutes rest was followed by 3 Takeda BPs and 2 minutes continuous BPro BPs. At both visits AMBP was recorded with BPro.

Another 450 Caucasian type 1 diabetic patients, 262 men(58%), (mean±SD) 55±13years, 261(58%) with albuminuria (>30mg/24h), regularly attending clinical controls were randomly selected for AMBP with BPro. OBP was calculated as the mean of cuff-based BPs at 3 separate office visits<1 year prior to AMBP. AMBP and OBP≥130/80mmHg were classified as HTN. Masked HTN was present with normal OBP and elevated AMBP, and white coat HTN was present with normal AMBP and elevated OBP.

Results: Validation: At visit 1, Takeda BP (mean±SD) was 136±19/72±8mmHg vs. BPro 138±19/78±8mmHg. Visit 1 AMBP was 132±20/76±9mmHg vs. 131±13/75±9mmHg at visit 2. Correlations between Takeda and BPro systolic and diastolic BP were r=0.86 and 0.65, (p<0.001). Mean differences (±SD) between devices were 1.9±10 and 5.5±6.6mmHg for systolic and diastolic BP.

Mean AMBP, day BP, night BP and dipping at the 2 visits were similar (p>0.40).

Evaluation: OBP was 137±14/76±8mmHg vs. AMBP 129±15/75±10mmHg. HTN with both AMBP and OBP was present in 211(47%), 79(18%) had normal BP, 117(26%) had white coat HTN and 43(10%) had masked HTN.

In 65% OBP and AMBP were in concordance. HTN (incl masked, and excl white coat HTN) was seen in 254(57%). In 26% BP was ≥140/90.

Conclusions: In patients with diabetes tonometric and cuff-based BPs are comparable and tonometric AMBPs are reproducible.

In 35% there is discrepancy in HTN diagnosis between AMBP and OBP. In a majority of patients BP is not below target <130/80mmHg.

PUB392

The Association of Renal Arteriolopathy and Proteinuria in Chronic Kidney Disease Kentaro Kohagura,[#1] Tsuyoshi Miyagi,[#1] Yusuke Ohya,[#1] Kunitoshi Iseki.[#2] *[1]Department of Cardiovascular Medicine, Nephrology and Neurology, Faculty of Medicine, University of the Ryukyus, Nishihara-cho, Okinawa, Japan; [2]Dialysis Unit, University of the Ryukyus, Nishihara-cho, Okinawa, Japan; [3]Japan; [4]Japan.*

Background: Preglomerular arteriole has a pivotal role in the regulation of glomerular pressure. Therefore, its damage might be associated with proteinuria by inducing glomerular hypertension. However, an association of renal arteriolopathy with urine protein in patients with chronic kidney disease (CKD) has not been investigated.

Methods: In the present study, we examined the cross-sectional association between the urine protein and renal arteriolar hyalinosis using renal biopsy specimen. Arteriolar hyalinosis was assessed by semi quantitative grading for arterioles among 162 patients with non-nephrotic (serum albumin ≥ 3 g/dl) CKD (mean age, 42 yrs; 52% male).

Results: Hyalinosis was seen in 96 patients. The patients with hyalinosis were older and higher prevalence of hypertension and metabolic disorder compared with the patients without hyalinosis. Among the patients with hyalinosis, max grade of hyalinosis (r=0.37, p=0.0002) in addition to systolic blood pressure (SBP) and metabolic risk factors such as body mass index, triglyceride, and uric acid were significantly correlated with urine protein. On the other hand, SBP was only factor, which correlated with UP among the patients without hyalinosis. Multivariate regression analysis showed that triglyceride (β= 0.31, p= 0.002) and max grade of hyalinosis (β= 0.25, p= 0.01) were independent predictor of the urine protβein among the patients with hyalinosis (R^2=0.30, ANOVA P<0.0001).

Conclusions: In conclusion, significant association between the severity of renal arteriolar hyalinosis and the amount of urine protein was observed among the patients with hyalinosis. Besides primary disease, renal arteriolopathy might be associated with the amount of proteinuria and subsequent progression of CKD.

PUB393

Plasma Desmosine and MMP-2 Levels as Potential Predictors of Arteriovenous Fistula (AVF) Maturation in a Porcine Model Yan-Ting E. Shiu,[1] Huan Li,[1] Sun Hyung Kwon,[2] Alfred K. Cheung.[1,3] *[1]Medicine, Univ. of Utah, SLC, UT; [2]Pharmacology & Toxicology, Univ. of Utah, SLC, UT; [3]Medical Service, VASLCHCS, SLC, UT.*

Background: AVFs for chronic hemodialysis often fail to mature and become usable. The underlying factors that cause maturation failure are not well understood. Elastin and collagen are the major extracellular matrix (ECM) proteins in large vessel walls, whose mechanical properties are dependent on these two proteins, which in turn are regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). We postulate that aberrant ECM turnover plays a role in the pathogenesis of AVF maturation failure, and that the plasma levels of desmosine and ICTP (degradation products of elastin and type I collagen, respectively), MMPs and TIMPs reflect ECM turnover of the AVF wall and hence can be used as predictors of AVF maturation.

Methods: We examined plasma MMP-2 and desmosine levels and the AVF maturation outcomes in a porcine AVF model. An AVF was created between the common carotid artery and external jugular vein on one side on each pig; the un-operated contralateral vessels served as controls. Serial plasma MMP-2 and desmosine levels were measured using respective ELISA. The patency, blood flow rate (BFR) and vein diameter (VD) of the AVF were assessed using serial duplex ultrasound.

Results: Compared to the animals with AVF patency at 10 weeks, the animal with occluded AVF before 10 weeks had higher plasma MMP-2 and desmosine concentrations and lower BFR and VD prior to that time point.

Conclusions: These preliminary data support the notion that increased proteolysis activity and elastin degradation in the AVF wall, as reflected by increased plasma MMP-2 and desmosine levels, respectively, are predictive of AVF maturation failure. Work is ongoing to further examine the relationships among vascular wall characteristics, plasma levels of ECM and their regulatory proteins, and AVF maturation outcomes in both experimental animal and clinical settings.

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PUB394

The Role of Vitamin D Supplementation on Aortic Augmentation Index and Pulse Wave Velocity in Healthy Humans Ahmed Abdi Ali, David Donald McTavish Nicholl, Brenda Hemmelgarn, Jennifer M. MacRae, Darlene Y. Sola, Sofia B. Ahmed. *University of Calgary.*

Background: Vitamin D (VD) deficiency is a risk factor for kidney and cardiovascular (CV) disease. Animal studies suggest a role for VD in modulation of the renin-angiotensin system (RAS), though the mechanism in humans is unclear. Arterial stiffness is an important predictor of CV risk in both healthy and chronic kidney disease (CKD) populations. We sought to determine the effect of VD supplementation on arterial stiffness and the RAS, both at baseline and in response to angiotensin II (AngII) challenge, a well-accepted measure of RAS activity, in VD-deficient healthy humans.

Methods: Ten VD-deficient (25(OH)-VD<50nmol/L), normotensive, non-obese, healthy subjects (7 females and 3 males) were studied before and after ingestion of 5000U of VD daily for 28 days. All subjects were studied in a high salt balance state, a state of maximal RAS suppression, and female subjects were studied in the same phase of their menstrual cycle. Arterial stiffness (aortic augmentation index (AIx) and pulse wave velocity (PWV) (brachial, n=9 and femoral, n=1) was measured by tonometry at baseline and in response to AngII infusion (3ng/kg/min x 30 minutes followed by 6ng/kg/min x 30 minutes).

Results: Baseline mean serum 25(OH)-VD levels increased from 51.2±3.8nmol/L to 101.8±9.5nmol/L after VD supplementation (p=0.001). VD supplementation resulted in a non-significant improvement in baseline arterial stiffness (baseline AIx pre- vs. post-VD supplementation, 12.3±3.3% vs. 10.8±4.3%, p=0.6), while the AIx response to AngII challenge increased non-significantly (AIx pre- vs. post-VD supplementation, 11.1±2.2% vs. 12.6±3.8%, p=0.8), consistent with downregulation of RAS activity. In contrast, VD supplementation did not alter the PWV at baseline (p=0.8) or in response to AngII challenge, p =0.058).

Conclusions: VD supplementation may improve arterial stiffness and down-regulate intrinsic vascular RAS activity. Further studies are needed to determine if VD supplementation ultimately decreases CV disease in patients with CKD.

PUB395

The Equilibrium and Dynamic Interactions of Albumin with Anionic Polyions Demonstrate That Charge Selectivity and Electrical Effects of the Glomerular Filter on Albumin Filtration Are Small Wayne Comper. *Exosome Diagnostics Inc, New York, NY.*

Background: Recent studies have suggested that the restricted transport of albumin across the glomerular filter may be due charge repulsion offered by the endothelial cell glycocalyx (charge selectivity) or repulsion offered by an electric field induced through convection across the filter (electrical effects).

Methods: The equilibrium charge and size interaction of albumin with polyanions (charge selectivity) has been accurately studied by a number of techniques including exclusion chromatography and light scattering under physiological conditions. The dynamic interaction of albumin in polyion systems where there is a convection-induced charge separation (electrical) has been studied by sedimentation analysis in the ultracentrifuge.

Results: The equilibrium interaction studies all demonstrated that the interaction of albumin with the polyanion was dominated by size exclusion and that charge interactions were small and would not have a major influence on transglomerular transport. Sedimentation analysis of albumin transport in polyion solutions with induced-charge separation at hydraulic conductivity levels equivalent to physiological GFRs again demonstrates that albumin transport is governed by size exclusion effects and that the electrical effects of induced charge separation were small if not negligible.

Conclusions: The lack of significant charge selectivity and electrical effects clearly reflects the influence of the relatively high ionic strength and screening of charge interactions that occur under physiological conditions. These results also support recent direct measurements of the glomerular sieving coefficient of albumin by 2-photon microscopy to be in the range of 0.01-0.03 as predicted by purely size selectivity transport studies.

Funding: Government Support - Non-U.S.

PUB396

Mechanisms for the Enhanced Plasma Retention of Albumin and IgG Are Coupled and Renal Centric: Uncoupling and Loss of Enhancement Occurs in Nephrotic States Wayne Comper,[1] Leileata M. Russo,[1] Maria Koltun.[2] *[1]Exosome Diagnostics Inc, New York, NY; [2]Biochemistry, Monash University, Clayton, Victoria, Australia.*

Background: The hypthothesis to be tested here is whether hypoalbuminemia and hypoimmunoglobulinemia are related and whether this relationship is centered at the kidney. The study employs plasma elimination measurements as they offer certain advantages over renal clearance studies particularly in terms of examining the behaviour of high molecular weight proteins

Methods: Plasma elimination rates measured over 24h were determined for tritium-labeled tracers of Ficolls (radii range 3.5-8.5nm), dextrans (5.0-10.5nm), albumin and IgG in healthy control and nephrotic (induced by puromycin aminonucleoside (PA)) Sprague Dawley rats. Nephrotic state resulted in a 1000-fold increase in the urinary excretion of intact albumin and 500- fold increase in intact IgG. Tissue uptake was also measured.

Results: Plasma elimination rate of albumin (n=18) (3.6nm radius) and IgG (n=6) (5.5nm radius) in control rats were coupled as their elimination rates were identical at 0.019±0.003 h^{-1} independent of their size. Their elimination rate was far enhanced as compared to the elimination rates of inert transport markers of equivalent hydrodynamic radius; their elimination rate corresponded to the elimination of a 7.5nm radius Ficoll (n=5) and >10.5nm radius dextran (n=5). The renal centric mechanism of the enhanced plasma elimination was demonstrated in nephrotic states where the increase in the plasma elimination rate for albumin and IgG was equal to the increased quantities of material excreted in the urine. In nephrotic states plasma retention was uncoupled and enhancement destroyed as the plasma elimination of both albumin and IgG was identical to Ficolls with radii of 3.6nm and 5.5nm respectively.

Conclusions: Given recent studies that the glomerular sieving coefficient of albumin is in the range of 0.01-0.03, the present studies indicate that the renal centric mechanism is the albumin retrieval pathway in proximal tubular cells and the retrieval pathway participates in retrieving filtered IgG.

Funding: Government Support - Non-U.S.

PUB397

Idiopathic Edema (IE) in Patients with Polycystic Ovary Syndrome (PCOS): Importance of Nocturia Sudharani Dikkala,[1] Jonah Feldman,[1] Siham Accacha,[2] Louis J. Imbriano,[1] Nobuyuki (Bill) Miyawaki,[1] Shayan Shirazian,[1] John K. Maesaka.[1] *[1]Department of Medicine, Winthrop University Hospital, Mineola, NY; [2]Department of Pediatrics, Winthrop University Hospital, Mineola, NY.*

Background: IE is characterized by weight gain of more than 2 lbs between 8 AM and 8 PM unrelated to menses in obese women.Nocturia is not included as a feature of IE but is a needed compensation to maintain fluid balance and avert progressive fluid retention.We present 3 cases of IE in patients with PCOS in whom nocturia was prominent

Three female patients, ages 42, 47 and 50 yrs, with PCOS, obesity, type II diabetes mellitus, hypertension, hypercholesterolemia met criteria for IE by daily orthostatic weight gains of 5-8 lbs from 8 AM to 8 PM with bloating, increased abdominal girth, tightening of clothes, 4X nocturia with full bladders and loss of weight gained during the day.Two patients noted edema of hands and face in the morning.Two have glomerular hyperfiltration, 1 obesity-related FSGS by biopsy, 1 gout, sleep apnea and antiphospholipid syndrome with 7 miscarriages, 2 emotional instability.None were on diuretics before development of edema.In 1 patient, while on hydrochlorothiazide, her orthostatic daily weight gains decreased to 3-5 lbs on a 3gm salt diet, 1-2 lbs on a 2gm salt intake and no weight gain and elimination of nocturia on 1gm salt intake.Weight gains were aborted by furosemide on the morning after salt intake exceeded 1gm/day.Successful treatment of her edema led to greater emotional stability

Conclusions: IE may be common in PCOS.Increased vascular permeability appears to be abnormal in IE (edema of hands and face) and accounts for the orthostatic weight gain, nocturia must be an essential feature of IE to prevent continuing fluid retention and weight gain.Salt restriction with judicious use of diuretics can reduce or eliminate fluid retention.Control of fluid retention and understanding the pathophysiology of their disorder in 2 patients led to greater emotional stability.Additional studies must be performed to determine the prevalence of IE in PCOS and effectiveness of judicious use of diuretics and salt restriction in controlling orthostatic fluid retention

Funding: Clinical Revenue Support

PUB398

Hemodynamic and Renal Function in Rats with AngiotensinII-Induced Hypertension Claudia Ferreira-Figueiredo, Maria Oliveira-Souza. *Physiology and Biophysic, University of Sao Paulo, Brazil.*

Background: The angiotensin II (Ang II) has been shown to control glomerular hemodynamic and renal function, including the sodium homeostasis. Thus, this study investigated the renal function and modulation of epithelial transporters of rats with angiotensin-induced hypertension.

Methods: 4-weeks-old male Wistar rats were divided in two groups: Sham or treated with Ang II (200 ng/kg/min) by 15 days, using osmotic minipumps (Alzet). Blood pressure (BP) was monitored weekly by tail cuff plethysmography. Renal plasmatic flow (RPF) and glomerular filtration rate (GFR) were determined by para-aminohippurate sodium (PAH) and inulin clearance. Plasmatic levels of renin were analyzed by Enzime Immunoassay Kit (EIA), Urinary excretion of ions were also analyzed. The expression of renal transporters, Ang II and aldosterone receptors will be analyzed by Western Blot.

Results: Our preliminary results showed that the dose of Ang II used was enought to induce hypertension [114 ± 2,2 (Sham) versus 142 ± 2,66 (Ang II)*p<0,0001]. RPF decreased [12,65 ± 0,55 (Sham) versus 9,80 ±0,44 (Ang II) ml/kg/min p<0,0001]. The GFR decreased [8,8 ± 0,33 (Sham) versus 4,75 ± 0,52 (Ang II) ml/kg/min, p < 0,0001]. The plasma renin level increased [4,54 ± 3,2 (Sham) versus 9,29 ± 1,8 (Ang II) ng/ml]. Urinary Na+ and K+ also decreased in Ang II treated rats. The expression of Na+/H+ exchanger (NHE1) decreased [0,973 ± 0,009 (Sham) versus 0,788 ± 0,04 (Ang II), p<0,001]. The expression of Na+/H+ exchanger (NHE3) did not change [0,911 ± 0,052 (Sham) versus 0,93 ± 0,071 (Ang II)], nephrin did not change [1,19 ± 0,057 (Sham) versus 1,22 ± 0,032 (Ang II)].

Conclusions: Our results suggest that the dose of Ang II (200 ngr / kg/ min) was enough to affect hemodynamic parameters, tubular function and renin plasmatic level. The decrease of RPF and GFR may be involved with the action Ang II intraglomerular The handling ionic by tubule should be evaluate by expression of other transporters.

Financial Support: CNPq and FAPESP

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PUB399

Arterial Stiffness Is an Independent Determinant of Compensatory Hyperfiltration after Kidney Donation Pierre Fesler, Jean Ribstein, Guilhem Du Cailar, Georges J. Mourad, Albert Mimran. *Department of Internal Medicine and Nephrology, Lapeyronie Hospital, Montpellier, France.*

Background: After kidney donation, the remaining kidney tends to hyperfiltrate thus limiting the initial loss of renal function. However, the potential determinants of this compensative hyperfiltration (CHF) and the possible influence of arterial function are not known.

Methods: In 26 normotensive healthy kidney donors (51 +/- 9 yrs [mean+/-SD], 22 females), glomerular filtration rate (GFR) was measured by the clearance of continuously infused Tc99m-DTPA and timed urine collections at baseline -i.e. before donation- and 1 year after donation. CHF was computed as post-donation GFR minus half of baseline GFR. Arterial function was assessed at baseline through carotido-femoral pulse wave velocity (PWV) and carotid augmentation index (AIx).

Results: After kidney donation, there were no significant changes in blood pressure (BP), but 2 subjects became hypertensive. GFR decreased from 104+/-17 to 71+/-11 mL/min/1.73m² and mean CHF was 19+/-9 mL/min/1.73m². In univariate analysis, CHF was inversely correlated to baseline age (r^2=0.15, p=0.049) and PWV (r^2=0.23, p=0.012), but not mean BP or AIx. In multivariate analysis, CHF remained inversely correlated to PWV (p=0.020), independently of baseline age and mean BP (model r^2=0.54, p=0.002).

Conclusions: In healthy subjects, increased arterial stiffness seems to be associated with a limited magnitude of post-donation hyperfiltration. This could reflect an influence of arterial function on renal reserve, providing further insights into the relationship between macrocirculation and renal microcirculation.

PUB400

Evaluation of Vascular Function in Hemodialysis Patients: Small Artery Elasticity Index (SAE) Correlates with Pulse Wave Velocity (PWV) and Flow Mediated Dilatation (FMD) William D. Paulson,[1,2] John White,[2] David M. Pollock,[2] Jennifer S. Pollock,[2] Allison Dubner,[2] Gaston K. Kapuku.[2] *[1]Charlie Norwood VA Medical Center, Augusta, GA; [2]Georgia Health Sciences University, Augusta, GA.*

Background: Cardiovascular disease is the most important cause of mortality in dialysis patients, and contributes to vascular access failure. Tests of vascular function are important in evaluating and treating these problems, but standard methods such as PWV and FMD may not be fast and easy enough for widespread use. Measurement of arterial elasticity may meet this need since it is fast and easy to apply and has correlated with maturation failure of arteriovenous fistulas (Kheda NDT 2010). We report herein early results from an ongoing study of the correlation of SAE with PWV and FMD.

Methods: Sixteen hemodialysis patients underwent measurement of arterial blood pressure, SAE (HDI/PulseWave System), carotid-femoral PWV (SphygmoCor System), and FMD by standard protocol. SAE is measured by a tonometer that is applied noninvasively to the skin over the radial artery. The HDI device performs pulse contour analysis of the radial artery waveform. We plan to study a total of 60 dialysis patients.

Results: Mean values were generally consistent with arterial dysfunction (high systolic and pulse pressures, low SAE, high PWV, low FMD):

Systolic BP	Pulse Pressure	SAE	PWV	FMD
138.7 mmHg	60.8 mmHg	6.03 ml/mmHg x100	9.48 m/sec	5.31 %

In simple regression analysis, SAE negatively correlated with systolic blood pressure (R^2 = 0.27, P = 0.038) and PWV (R^2 = 0.35, P = 0.027), but did not reach significance with FMD (R^2 = 0.10) in this small initial sample. In multiple regression analysis, SAE correlated with PWV, FMD, and mean arterial pressure (R^2 = 0.80, all P <0.014), indicating that 80% of SAE variation was due to its relation with these 3 variables.

Conclusions: SAE closely correlated with standard measures of vascular function and is fast and easy to apply. These results support the concept that elasticity may be a feasible alternative measure of vascular function in dialysis patients. Further study with a larger number of patients is needed to confirm these promising results.

Funding: Clinical Revenue Support

PUB401

Increased Baroreceptor Sensitivity Predicts Intradialytic Hypertension Dan Sapoznikov, Rebecca Backenroth, Dvora Rubinger. *Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.*

Background: Autonomic hyperactivity may be involved in the pathogenesis of intradialytic hypertension (HD-H).

Methods: To assess intradialytic changes in autonomic activity in patients without (H (-)) and with (H (+)) intradialytic hypertensive episodes and to identify HD-H predictors, continuous beat to-beat blood pressure and heart rate recordings were performed in 108 patients during 113 hemodialysis sessions without (n=51) and with (n=62) HD-H episodes. HD-H was defined as a period of at least 10 mmHg increase in the systolic blood pressure (SBP) between the start (*Begin*) and the end (*End*) of dialysis, or hypertension resistant to ultrafiltration occurring during or immediately after dialysis. SBP, diastolic blood pressure (DBP), SBP and interbeat intervals (IBI) variability and baroreceptor sensitivity (BRS) in the low frequency (LF) range (LF SBP, LF IBI and LF BRS) were assessed using the complex demodulation method (CDM). LF oscillations are believed to be representative of sympathetic activation.

Results: SBP, IBI, LF SBP, LF IBI and LF BRS at *Begin* and at *End* of HD (median and interquartile range) were:

Table 1.

	SBP (mmHg)	DBP (mmHg)	IBI (msec)	LF SBP (mmHg)	LF IBI (msec)	LF BRS (msec/mmHg)
H(-) *Begin*	139 (42)	69 (19)	802 (198)	1.98 (0.93)	5.39 (4.49)	3.03 (1.95)
H(-) *End*	125 (34)	71(18)	813 (274)	1.95(1.00)	6.01 (6.80)	3.21 (3.20)
p	0.023	0.056	NS	0.051	0.046	0.001
H(+) *Begin*	132 (30)	67 (17)	850 (162)	1.92 (0.64)	6.11 (4.92)	3.62 (2.79)*
H(+) *End*	143 (36)*	76(21)*	878 (175)	1.88 (0.86)	7.16 (5.49)	3.67 (2.67)
p	0.001	0.001	NS	NS	NS	NS

* p <0.05 vs. H (-)

In multivariate analysis, including clinical and CDM data, pre-dialysis LF IBI , LF SBP, LF BRS, age and serum albumin were the main predictors of HD-H.

Conclusions: These results show: 1. H (-) sessions are associated with blood pressure normalization and enhanced IBI variability and BRS. 2. H (+) sessions are associated with increased post dialysis blood pressure. 3. pre-dialysis LF SBP , LF IBI, and LF BRS predict HD-H. Our data suggest that sympathetic activation with enhanced BRS, in concert with clinical factors contribute to HD-H. Intradialytic hypertension has to be further evaluated as a potential cause of interdialytic hypertension.

PUB402

IgA Nephropathy and Alcoholic Cirrhosis Display Common IgA Abnormalities Laureline Berthelot,[1] Emilie Tissandie,[1] Willy Morelle,[2] Eric Daugas,[3] Ivan Cruz Moura,[1] Francois Vrtovsnik,[3] Richard Moreau,[4] Renato C. Monteiro.[1] *[1]INSERM U699, Faculté Xavier Bichat, Paris, France; [2]Unité Mixte de Recherche CNRS/USTL 8576, Université des Sciences et Technologies de Lille, Villeneuve d'Ascq, France; [3]Service de Néphrologie, Hôpital Bichat-Claude Bernard, Paris, France; [4]INSERM U773, Centre de Recherche Biomédicale Bichat-Beaujon CRB3, Paris, France.*

Background: Alcoholic cirrhosis (AC) patients present IgA abnormalities including mesangial IgA deposits. Contrary to primary IgA nephropathy (IgAN), very little is known about IgA1 glycosylation and IgA-circulating immune complexes containing soluble CD89-IgA or IgG-IgA complexes in secondary IgAN which occurs in AC.

Methods: Here, purified IgA1 from serum of 32 patients with compensated or advanced AC were analyzed for O- and N-glycosylation. IgA-IgG and soluble CD89-IgA complexes presence in the sera of AC patients was assessed by ELISA.

Results: Similar to IgAN, galactose deficiency and decreased sialylation of IgA1 were observed in advanced AC. Increased amounts of abnormally O-glycosylated polymeric IgA1 were detected in serum from these patients. Moreover, aberrant IgA1 formed complexes with IgG and soluble CD89 in serum of patients with advanced AC as observed in primary IgAN. However, IgA1 from AC patients exhibited also several modifications of N-glycosylation, not observed in IgA1 from primary IgAN patients. In AC patients with IgAN, IgA deposits were associated with CD71 overexpression in mesangial areas suggesting that CD71 might be involved in deposit formation. Although AC purified IgA1 bound more extensively to human mesangial cells than control IgA1, they differ from primary IgAN by not inducing mesangial cell proliferation.

Conclusions: To conclude, abnormally O-glycosylated IgA1, soluble CD89-IgA and IgA-IgG complexes, features of primary IgAN, are also present in AC and could indicate common environmental factors influencing the development of IgAN.

Funding: Government Support - Non-U.S.

PUB403

Transgenic Rat with GEC Targeted HO-1 Overexpression Ling-Mei Chiang,[2] Pu Duann,[1] Elias A. Lianos.[1] *[1]Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Pediatrics, Chang-Gung Memorial Hospital, Keelung, Taiwan.*

Background: Induction of heme oxygenase (HO)-1 is a key defense mechanism against oxidative stress. Compared with tubules, glomeruli are refractory to HO-1 upregulation in response to injury. This can be a disadvantage as it may be associated with insufficient production of cytoprotective heme-degradation metabolites. We, therefore, explored whether 1) targeted HO-1 expression can be achieved in glomeruli without altering their physiological integrity and 2) this expression reduces proteinuria in immune injury induced by an anti-glomerular basement membrane (GBM) antibody (Ab). We previously reported generation a transgenic mice mice, pNeph-hHO1, in FVB/N strain with confirmed GEC-targeted HO-1 expression and had shown a reduced proteinuria in both glomerular immune injury and adriamycin glomerulopathy.

Methods: Using Sleeping Beauty(SB) transposon system we subcloned pNeph--FLAG-hHO1 expression cassette between inverted repeats of the transposon vector SB10. We then injected pNeph-FLAG-hHO1 transgene with an mRNA source of SB transposase into newly fertilized Sprague Dawley eggs. This resulted the transposition of the transgene, pNeph-FLAG-hHO-1, and its integration as a single copy unit.

Results: We thus obtained transgenic rats with GEC targeted HO-1 overepression. These rats show no apparent changes in weight, eating habits, fur color or motility. In addition, there is no infertility or deviated Mendelian ratio in offspring. Assessed by electron microscopy revealed no glomeruli microstructure deformity such processes effacement, flattening or microvillus transformation of GEC.

Conclusions: Transgenic rats with GEC targeted HO-1 overexpression could help us study the role of renoprotective HO-1 in the GEC pathobiology.

Funding: Other NIH Support - AHA-SDG

PUB404

Caspofungin Efficacy in Re-Establishing Chronic Haemodialyzed and Renal Transplant Patient Phagocyte Primary Functions Against Candida albicans and C. glabrata Infections Franca Giacchino,[1] Giuliana Banche,[2] Valeria Allizond,[2] Narcisa Mandras,[2] Giuseppe Garneri,[1] Daniela Scalas,[2] Rosaria Patti,[1] Janira Roana,[2] Chiara Merlino,[2] Vivian Tullio,[2] Anna Maria Cuffini.[2] *[1]Nephrology and Dialysis Unit, Civil Hospital, Ivrea, Turin, Italy; [2]Public Health and Microbiology, University of Turin, Italy.*

Background: Although invasive fungal infections attributed to *Candida spp.*, particularly to *C. albicans,* are prevalent, a tendency of an increasing proportion of *C.* non-*albicans* species has also been reported: the most important species is *C. glabrata.* Due to the impaired phagocyte-dependent host defences, patients with chronic renal failure are more frequently at risk of candidiasis than healthy subjects (HSs). Thus, for candidiasis resolution immunomodulating antifungal drugs may be decisive. This study aimed to evaluate the potential immunomodulating activity exerted by caspofungin on polymorphonuclear cells (PMNs) from haemodialysed patients (HDs) and renal transplant recipients (RTRs), compared with HSs, towards both *C. albicans* and multidrug-resistant *C. glabrata*.

Methods: PMNs were separated from venous blood samples of 66 HDs, 54 RTRs and 30 HSs. The effects of caspofungin on both phagocytosis and intracellular killing by PMNs towards *Candida* spp. were investigated by incubating yeasts and PMNs with caspofungin at MIC values. Drug-free controls were included.

Results: A reduced fungicidal activity towards intracellular *C. albicans* and *C. glabrata*, was detected in HD-PMNs and RTR-PMNs, in comparison with HS-PMNs. This decreased PMN antibacterial activity was re-estabilished by the addition of caspofungin that significantly improved the intracellular killing of HD-PMNs and RTR-PMNs against both *C.albicans* and *C.glabrata*, without affecting phagocytosis.

Conclusions: These data provide confirmation that caspofungin in addition to its antifungal activity possesses immunomodulating properties that make it highly suitable for the treatment of infections caused by both *C. albicans* and multidrug-resistant *C. glabrata* in patients with altered phagocyte-dependent innate immunity which represent a high risk population.

PUB405

Involvement of Anaphylatoxins C3a, C5a in Tubulointerstitial Fibrosis in Rats with Unilateral Ureteral Obstruction Fang Liu, Ping Fu. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.*

Background: To investigate the role of anaphylatoxins C3a, C5a in tubulointerstitial fibrosis in rats with unilateral ureteral obstruction (UUO) and to clarify the possible mechanism.

Methods: In vivo study, the expressions of C3a, C5a and their receptors C3aR, C5aR (CD88) were detected by immunohistochemistry staining in UUO rats. In vitro study, HK-2 cells were divided into C3a and C5a group which were divided into four subgroups: control group, 10 ng/ml TGF-β1, 50 nmol/L C3a, 50 nmol/L C3a plus 1 μmol/L C3aRA; control group, 10 ng/ml TGF-β1, 50 nmol/L C5a, 50 nmol/L C5a plus 2.5 μmol/L C5aRA. 10 μg/ml TGF-β1 receptor antagonist (TGF-β1RA) was used to investigate the mechanism of C3a- and C5a-induced tubular epithelial-myofibroblast transdifferentiation (TEMT). Electron microscopy was used to observe the morphological changes. Immunocytochemistry staining, real time PCR and Western blot were used to detect the expressions of α-SMA, E-cad, Col-I, C3aR, CD88, CTGF and TGF-β1.

Results: In vivo study, the expressions of C3a/C3aR and C5a/CD88 were detected in tubular epithelial cells 7 days after UUO established, especially in dilated tubules. In vitro study, after HK-2 cells were cultured with C3a and C5a for 72 hr, many cells had strong staining for α-SMA, lost the positive staining of E-cad, and showed a slightly spindle-like shape and loss of microvilli on the cell surface. The expressions of α-SMA, E-cad, Col-I, C3aR, CD88, TGF-β1 and CTGF in C3a- and C5a-treated groups were higher than normal control group ($P < 0.05$). C3aRA and C5aRA inhibited the expressions of α-SMA, Col-I, C3aR, CD88, and up-regulated the expression of E-cad ($P < 0.05$). TGF-β1 and CTGF mRNA expressions induced by C3a and C5a were blocked by TGF-β1RA ($P < 0.05$).

Conclusions: Anaphylatoxins C3a, C5a and their receptors were involved in the early pathophysiologic process of tubulointerstitial fibrosis in rats with UUO by inducing TEMT via the up-regulations of C3aR and CD88 and the activation of TGF-β/CTGF signaling pathway.

PUB406

The Pivotal Role of Midkine in the Pristane-Induced Lupus Nephritis Mice Kayaho Maeda, Tomoki Kosugi, Waichi Sato, Hiroshi Kojima, Yuka Sato, Hiroshi Nagaya, Mayuko Hori, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: The growth factor midkine (MK) exerts pleiotrophic effects such as cell proliferation and migration in various organs. We have previously demonstrated that the major biological activity of MK in the kidneys affects in the progress of ischemic renal injury, diabetes and hypertension. A recent study proposed that inhibition of MK alleviates experimental autoimmune encephalomyelitis through the expansion of $CD4^+CD25^+$ regulatory T cell (Treg) population. However, the precise mechanism remains unclear yet. In order to clarify the molecular mechanism of autoimmune disease, we examined the role of MK in pristane-induced lupus nephritis model.

Methods: Lupus nephritis was induced in MK deficient mice ($Mdk^{-/-}$) or wild-type mice ($Mdk^{+/+}$) with an intraperitoneal injection of pristane (0.5ml/each mice). Mice were measeured on albuminuria and anti-nuclear antibody once a month and sacrificed at 6 months later. Kidney, spleen and lipogranulomas were analyzed.

Results: There was no difference between $Mdk^{+/+}$ and $Mdk^{-/-}$ in serum anti-nuclear antibodies during the experimental period. Albuminuria in $Mdk^{+/+}$ was more than $Mdk^{-/-}$. Histological analysis showed that there were no differences in IgG and C3 depositions of glomeruli, whereas mesangial and endocapillary proliferations were more severe in $Mdk^{+/+}$ mice. Macrophage infiltration in glomeruli was prominent in $Mdk^{+/+}$. By electron microscopy, electron dense deposits were more found in the mesangial area in glomeruli of $Mdk^{+/+}$. Macrophage-related cytokines such as monocyte chemotactic protein-1 were suppressed in the $Mdk^{-/-}$. Serum IL-12, IL-6 and IL-1β levels were higher in $Mdk^{+/+}$, but not IL-10. By flow cytometry analysis, IFN-γ and IL-17A in $Mdk^{+/+}$ spleen are higher than $Mdk^{-/-}$ mice, but not $CD4^+CD25^+$ Treg population and $CD4^+/CD8^+$ T cell ratio in spleen.

Conclusions: MK is involved in the pathogenesis of lupus nephritis through Th1 and Th17 cell activations. This finding opens up new avenues that may facilitate research on the development of therapeutics for SLE.

PUB407

Comparison of Everolimus and Sirolimus on Cyclosporine A-Induced Pancreatic and Renal Injury in Rats ShangGuo Piao, Sunwoo Lim, Byung Ha Chung, In O Sun, Sun Ryoung Choi, Chul Woo Yang. *Internal Medicine, Seoul St. Mary's hospital, The Catholic University of Korea, Seoul, Republic of Korea.*

Background: It is well known that sirolimus (SRL) aggravates cyclosporine A (CsA)-induced renal and pancreatic injury. But**,** the effect of everolimus (EVL) on CsA-induced organ injury is undetermined.This study was conducted to investigate the pharmacologic interaction between EVL and CsA at the blood and tissue level.

Methods: Male Sprague-Dawley (SD) was subcutaneously administrated with CsA (15 mg/kg/day) and EVL or SRL (0.3 mg/kg/day) for 4 weeks. The effect of EVL on CsA-induced nephrotoxicity and pancreatic islet dysfunction was compared with that of co-administered CsA and SRL. To confirm the drug interaction between CsA and EVL or SRL, concentration of each drug was evaluated in the whole blood and tissue samples using liquid chromatography-mass spectrometry.

Results: The EVL or SRL treatment did not cause severe renal injury and pancreatic dysfunction, but combination treatment with CsA aggravated CsA-induced toxicity. Macrophage infiltration, TIF, 8-OHdG excretion were significantly increased in the CsA group compared to the VH group. Addition of EVL or SRL on CsA showed much more aggravation of pancreatic islet dysfunction than CsA group. In whole blood, CsA concentration did not differ among CsA, CsA + EVL, and CsA + SRL groups. In kidney and pancreas tissue, CsA concentration was also not significantly different between the CsA + EVL and the CsA group. However, a surprising higher CsA concentration was detected in the CsA + SRL group in the kidney (2-fold) and pancreas (2.1-fold) compare to the CsA group. There was no difference of concentration of EVL in kidney and pancreas from the CsA + EVL group compared with EVL group . However, concentration of SRL in kidney and pancreas tissues from CsA + SRL was higher than that of SRL group.

Conclusions: EVL aggravates CsA-induced organ injury but pharmacologic interaction between EVL and CsA is less than that of SRL at tissue level. This finding provides a better understanding of difference of EVL and SRL in combined treatment with CsA.

PUB408

Novel Transcription Factors in Regulation of M2 Macrophage Phenotype
Changqi Wang,[1] Qi Cao,[1] Di Yu,[2] Guoping Zheng,[1] Ya Wang,[1] Junyu Lu,[1] Yiping Wang,[1] David C. Harris.[1] *[1]Centre for Transplantation and Renal Research, University of Sydney at Westmead Millennium Institute, Sydney, New South Wales, Australia; [2]Department of Immunology (Clayton), School of Biomedical Sciences, Monash University, Melbourne, Victoria, Australia.*

Background: Macrophages can be classified phenotypically into two main groups, namely classically activated macrophages (M1) and alternatively activated macrophages (M2). M2 macrophages can be polarized further with cytokines into M2a (after exposure to IL4 or IL-13) and M2c (IL-10 or TGFbeta). Previous studies showed that M2a and M2c macrophages have a protective effect in reducing renal injury, and that endogenous macrophages can undergo phenotypic switch in response to microenvironmental stimuli. However, the contribution of transcription factors to M2 macrophage differentiation and phenotypic switching is poorly understood. The aim of this study was to identify key transcription factors involved in M2 macrophage differentiation.

Methods: Peritoneal and bone marrow-derived macrophages were isolated and polarized into M0 (non-activated macrophages), M1, M2a and M2c respectively. Whole genome array analysis was employed to identify transcription factors. Changes in all selected novel genes were validated by real time PCR.

Results: By comparison of differential gene expression, our data showed that several transcription factors were highly upregulated in M2a and M2c. Some of these transcription factors have been associated previously with M2 (Irf4 and Tcfec for M2a; Cebpb for M2c), but some uncharacterized transcription factors (4 with M2a, 3 with M2c) were identified for the first time in this study. In addition, several novel specific surface markers for M2 (3 with M2a; 3 with M2c) were identified.

Conclusions: Several transcription factors involved in M2 macrophage differentiation and M2 specific surface markers have been identified. Gene transfer and gene silencing techniques are currently being employed to demonstrate the possible relevance to M2 differentiation of these newly identified genes. This finding could provide clues for application of M2 macrophages as a therapy for chronic inflammatory diseases, by controlling M2 phenotype.

Funding: Government Support - Non-U.S.

PUB409

Influence of the Tariable Domain Glycosylation on Anti-Neutrophil Cytoplasmic Autoantibodies and Anti-Glomerular Basement Membrane Autoantibodies
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Background: Pathophysiological significance of variable region of IgG is still unclear by now. The influences of the variable region N-linked oligosaccharides on the antigen binding ability of three kinds of autoantibodies were investigated with Sambucus nigra agglutinin (SNA), which mainly binds to oligosaccharides with terminal α2, 6-linked N-acetylneuraminic acid on the variable region of IgG.

Methods: Twenty-seven patients with serum autoantibodies against myeploperoxidase (MPO, proteinase 3 (PR3), or noncollagenous domain of α3 chain of type IV collagen (α3(IV) NC1) of glomerular basement membrane (GBM) were enrolled. IgG was divided into non-SNA-binding and SNA-binding fractions with SNA affinity chromatography. Antigen-specific IgG were purified with immunoaffinity chromatography.

Results: Variable glycosylation level of total IgG was significantly lower than that of purified anti-MPO antibodies IgG (1.021±0.201 *vs.* 1.434±0.134, P=0.004, expressed by absorbance value 405nm). Variable glycosylation level of total IgG was significantly higher than that of purified anti-GBM antibodies IgG (1.034±0.340 *vs.* 0.734±0.333, P=0.007). The antigen binding level of non-SNA-binding IgG was significantly lower than that of SNA-binding IgG for anti-MPO antibodies (0.572±0.590 *vs.* 0.962±0.670, P<0.001) and anti-PR3 antibodies (0.362±0.530 *vs.* 0.560±0.531, P=0.003), while significantly higher for anti-GBM antibodies (1.301±0.594 *vs.* 1.172±0.583, P=0.044). SNA-binding fraction of anti-MPO and anti-PR3 antibodies-containing IgG could induce higher level of neutrophils respiratory burst than non-SNA-binding fraction.

Conclusions: Variable region glycosylation levels were different among different antigen-specific IgG, and could influence the antigen-binding ability of antigen-specific IgG, which might be dependent on the type of target antigens.

Funding: Government Support - Non-U.S.

PUB410

Plasma Induces Non-Muscle Myosin Type IIA-Mediated Changes in Neutrophil Migration and Morphology during Phagocytosis
Chen Yu. *Department of Nephrology, East Hospital, Shanghai, China.*

Background: Neutrophils are the primary effector cells in the pathogenesis of transfusion-related organ injury or multiple organ failure (MOF) after blood transfusion. However, the mechanisms underlying the effect of plasma transfusion on neutrophil migration and morphological changes during phagocytosis remain largely unknown.

Methods: We investigated the effect of fresh (one day preparation) and aged (42 day preparation) plasma on neutrophil morphology, migration and phagocytosis. We evaluated the production of reactive oxygen species (ROS) and the expression of non-muscle myosin heavy chain IIA (MYH9) in plasma-treated neutrophils. We used western blots and antibody arrays to evaluate changes in signal transduction pathways in plasma-treated neutrophils.

Results: Aged plasma elicited a stronger oxidative burst in neutrophils when compared with fresh plasma. Antibody arrays showed increased phosphorylation of NF-κB proteins (p105, p50 and Ikk) in plasma-treated neutrophils. The expression of non-muscle myosin IIA (MYH9), a cytoskeleton protein involved in immune cell migration and morphological change, was also significantly upregulated in neutrophils treated with aged plasma. Pretreatment of neutrophils with blebbistatin (a specific type II myosin inhibitor), ascorbic acid (an antioxidant), or staurosporine (a protein tyrosine kinase inhibitor), effectively abrogated the morphological changes, neutrophil migration, and phagocytosis in response to plasma.

Conclusions: Our data provide an insight into the cellular and molecular mechanisms of transfusion-related injury

Key Words: neutrophils, migration, MYH9, NFκ

PUB411

Activation Markers of Thrombogenesis and Endothelial Dysfunction in End Stage Renal Disease
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Background: The pathogenesis of ESRD is complex involving inflammatory, thrombotic, and calcification processes. Current studies suggest that chronic inflammation is a major risk factor in ESRD. This study profiles markers of inflammation, thrombogenesis, and vascular dysfunction in the ESRD population.

Methods: Samples from 117 ESRD patients over the age of 18 on maintenance hemodialysis for at least 3 months and 50 aged matched control samples obtained from healthy volunteers with no known kidney disease were included in the study. The Randox Evidence Investigator (Antrim, United Kingdom) was used to measure IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, Vascular Endothelial Growth Factor (VEGF), Interferon-γ (INF-γ), Tumor Necrosis Factor-α (TNF-α), Monocyte Chemotactic Protein-1 (MCP-1), Epidermal Growth Factor (EGF), C-Reactive Protein (CRP), D-Dimer, and Thrombomodulin (TM). Plasminogen Activator Inhibitor -1 (PAI-1) and Asymmetric Dimethylarginine (ADMA) were measured using ELISA kits by Diagnostica Stago (Paris, France) and DLD Diagnostika GmbH (Hamburg, Germany), respectively.

Results: IL-6 levels in ESRD were 4.3 times the controls. VEGF and INF-γ were both elevated at 2.6 times the controls. MCP-1, IL-8, IL-10, EGF, TNF-α, and IL-1α were elevated at 2.0, 1.6, 1.4, 1.4, 1.3, 1.2 times the controls, respectively. IL-4, IL-2, and IL-1 β levels were reduced at 0.9, 0.9, and 0.5 times the controls. TM, CRP, DDMER, PAI-1, and ADMA were increased at 5.9, 4.4, 3.0, 1.4, and 1.6 times the controls, respectively, each demonstrating statistically significant p-values.

Conclusions: The elevation of the interleukins, INF-γ, TNF-α and MCP-1 revealed intense inflammatory activation of leukocytes. The increase in these cytokines also demonstrates the vascular and tissue damage occurs in ESRD. VEGF and EGF are both elevated in the ESRD samples indicating a state of cellular proliferation, possibly in an attempt to replace damaged tissue caused by the heightened inflammatory state. These markers should be evaluated for their role in kidney disease risk stratification and prognosis.

Funding: Private Foundation Support

PUB412

A Mouse Glomerular Epithelial Cell Line with HO-1 Overexpression
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Background: The study of cultured glomerular cells has greatly extended our knowledge of the individual cells in the normal and changed glomeruli. Podocytes have primarily been cultured from rat or human glomeruli. But GEC from glomeruli of normal mice, although available, still limited. GEC are terminal differntiated cells thus left only limited replication and proliferation potency. GEC Only limited exception, ie. derived from an immortalized transgenic mice (TsA58), conditional immortal GEC cells are available. Previously we reported a transgenic mice with GEC targeted HO-1 overexpression which show attenuation of proteinuria in GEC injury (Am J Physiol. 297[5]:F1476, 2009). In order to explore the emchanistic role of HO-1 in renoprotection, we generated a GEC cell line from the pNeph-hHO1 tranbsgenic mice.

Methods: Glomeruli are isolated from pNeph-hHO1 transgenic mice by standard differential sieving (80-, 107- and 75- μm) several cell populations were subcloned by the differential speed of growth and subcloning enhanced medium according to previously report (J Cell Physiology 105, 369-378, 1980). Cultured glomeruli was trypsionizd on day 7 and outgrowth cells were subcloned. Several single cell clones were identified and isolated. From these single cells we were able to clone a specific cell types.

Results: The cell with GEC specific surface cilia were further identified for GEC marker C3 (55%) and nephrin (64%) immunohistochemically. The pNeph-hHO1 transgenic mice, as we previously reported, with GEC targeted HO-1 overexpression. The FLAG-Tagged HO-1 was exclusively expressed in GEC with no leakage. This provided us a solid basis to assess FLAG-tagged HO-1 in the newly cloned GEC candidate line(s) and identified

87% FLAG(+) in FACS assay. The homogenous GEC cells are capable to replicated in limited passage, thereby, this newly clone homogenous GEC will preserve its physiological characteristics, with transprotein, FLAG-tagged hHO1, constantly expressed.

Conclusions: This novel GEC cells, with hHO1 over expression will assist future mechanistic studies, supplemental to animal model, of the renoprotective role of HO-1 in GEC injury.

Funding: Other NIH Support - AHA-SDG

PUB413

Imaging Activation of Complement Sarah De Freitas, Adam Badar, Richard Smith, James Clark, Greg Mullen, Steven H. Sacks. *MRC Centres for Transplantation and Imaging, King's College London, London, United Kingdom.*

Background: Ischemia reperfusion injury (IRI) is an important result of cardiovascular disease as well as impacting on organ transplantation. Complement activation contributes to inflammation and the degree of injury in IRI.

We characterised the first in vitro radiopharmaceutical targeting of activated complement. The SPECT tracer, based on the N-terminal binding domain of endogenous complement receptor 2 (CR2) binds C3d a product of complement activation that remains bound to IRI tissue.

Preliminary imaging studies evaluating the radiotracer in the setting of IRI were done using a mouse myocardial IRI model.

Methods: rCR2 was radiolabelled with [99mTc(CO)3]+ via the engineered C-terminus hexahistidine tag. Serum stability was assessed at 37°C in human and mouse serum and analysed using instant thin layer chromatography and gamma detection via radio TLC scanner. Samples were further analysed using SDS-PAGE followed by autoradiography and densitometry of the protein bands. Induction of IRI was achieved in mice by occluding the left anterior descending coronary artery for 30 min prior to reperfusion. After 24 hr, 100 µL of 1mg/mL rCR2-[99mTc(CO)3]+ at a specific activity of 7 MBq/µg was injected via tail vein and mice imaged 1 hr later using a preclinical SPECT/CT system. Controls included sham operated mice, and injection of a radiolabelled inactive mutant of rCR2 (K41E CR2). Myocardium slices were sectioned for autoradiography and histology (H&E and C3d staining).

Results: After 8hrs serum stability was over 94%. Emission tomography revealed significant uptake in the hearts of mice (n=3) induced with MIRI compared with controls. Autoradiography of heart sections suggested that this was localised to the left ventricle. No activity was detected in control hearts. Histological staining confirmed that C3d was present in areas of tissue damage on myocardial sections, and absent in hearts of control mice.

Conclusions: Initial in vivo evaluation of the rCR2-[99mTc(CO)3]+ SPECT tracer indicates that this has potential as an imaging ligand for delineating the footprint of complement activation in organs affected by IRI.

Adam Badar is joint first author.

PUB414

Repression of Heme Oxygenase(HO)-1 Expression in Glomerular Epithelial Cells (GEC) Maria Detsika,[2] Pu Duann,[1] Elias A. Lianos.[1] *[1]Medicine, RWJ Medical School, New Brunswick, NJ; [2]Thorax Foundation, University of Athens, Medical School, Athens, Greece.*

Background: Of the glomerular cells, the least capable of upregulating HO-1 expression in response to the natural HO-1 substrate/inducer, heme, or to various forms of injury are the GEC (J Lab Clin Med. 147, 150, 2006). While this may be a disadvantage, given the well-established cytoprotective effects of heme degradation products, it may also be justified because production of catalytically active Ferrous iron also occurs following HO-1 induction thus promoting Fenton-type oxidant reactions, i.e. hydroxyl radical formation, in these terminally differentiated cells. Indeed, HO-1 repression does occur in organ injury and was shown to serve as a defense mechanism (Exp Biol Med. 228, 472, 2003). To assess whether GEC do repress HO-1 expression, cultured rat glomeruli were used as a means to obtain a short-term (18h) co-culture of endothelial, mesangial and epithelial cells. In addition, GEC were cultured alone. Both preparations were incubated with heme at concentrations (0, 2.5, 5.0 and 10µM) likely to be encountered in the glomerular milieu following intravascular hemolysis or hematuria. There was a concentration-dependent increase in HO-1 expression (Western blot analysis) in cultured glomeruli. An opposite effect (a concentration-dependent HO-1 decrease) was observed in cultured GEC, indicating a suppressor effect of heme on HO-1 that is apparent in GEC only. We next employed a pNeph-hHO1 transgenic mice in which GEC-targeted hHO-1 expression was achieved as previously described (Am J Physiol. 297[5], F1476, 2009). To upregulate the hHO-1 in GEC, we induced glomerular inflammation by administration of an anti-GBM Antibody to transgenic (Tg) and wild-type (Wt) control mice. While proteinuria was attenuated in Tg mice compared to Wt, this effect was dissipated on day 9 following anti-GBM Ab, and this was associated with a decrease in glomerular hHO-1 levels.

Conclusions: These observations point to the presence of HO-1 repressor mechanisms in GEC that can be activated both by its natural substrate and by proinflammmatory mediators.

Funding: Other NIH Support - AHA-SDG

PUB415

TNF-α and Tunicamycin Interfered with Glycosylation of Nephrin Protein, Then Resulted in Endoplasmic Reticulum (ER) Stress Shokichi Naito, Tomoko Okamoto, Togo Aoyama, Mariko Kamata, Chikako Okina, Yasuo Takeuchi, Kouju Kamata. *Department of Internal Medicine, Kitasato University School of Medicine, sagamihara, Kanagawa, Japan.*

Background: Accumulation of deglycosylated proteins in ER induces unfolded protein response. However, it is difficult to directly identify these deglycosylated proteins. We have successfully generated antibody against deglycosylated nephrin protein. (Naito et al; Clin Exp Nephrol, 2011, in press) In this study, we investigate ER stress by deglycosylated nephrin protein.

Methods: HEK293-NW cells producing nephrin protein were cultured with 0-10 µg/ml of tunicamycin, an N-linked glycosylation inhibitor, or 0-30 ng/ml of TNF-α for 20 hrs. Cell survivals were evaluated by using Trypan blue dye exclusion test. Then, production of deglycosylated nephrin proteins and expression of GRP78 and calreticulin were analyzed using immunoprecipitation method and western blotting.

Results: Cell death in HEK293-NW cells increased in more than 1 µg/ml of tunicamycin, while did not increase in any dose of TNF-α. Tunicamycin with more than 0.05 µg/ml and TNF-α with more than 0.1 ng/ml induced deglycosylated nephrin proteins in HEK293-NW cells. Tunicamycin with more than 0.5 µg/ml incresed GRP78 and calreticulin in the cells. TNF-α with more than 10 ng/ml increased GRP78 in the cells, and did not increase calreticulin in any dose.

Conclusions: Deglycosylated nephrin proteins induced unfolded protein response in the cells. TNF-α showed a different response compared with it in tunicamycin.

PUB416

Novel Method for Simultaneous Determination of P-cresylsulphate and P-cresylglucuronide: Clinical Data and Pathophysiological Implications Eva Schepers, Natalie Meert, Griet L.R.L. Glorieux, Nathalie Neirynck, Annemieke Dhondt, Raymond C. Vanholder. *Internal Medicine, Nephrology, University Hospital Gent, Gent, Belgium.*

Background: The uremic retention solutes p-cresylsulphate and p-cresylglucuronide, two conjugates of p-cresol, were never determined simultaneously. In the present paper an HPLC method was developed and used to quantify both compounds in parallel in an *in vivo* observational study and their *in vitro* effect was evaluated by flow cytometry.

Methods: P-cresylsulphate and p-cresylglucuronide were determined in serum. For the validation specificity, linearity, recovery, precision and the quantification limit were evaluated. In vivo, concentrations of both compounds were determined in 15 controls and 77 hemodialysis patients, as well as protein binding in the dialyzed group and the reduction ratios during hemodiafiltration. In addition, the i*n vitro* effect of the solutes on leukocyte free radical production at measured concentrations was assessed.

Results: A fast and accurate HPLC method was developed to simultaneously quantify p-cresylsulphate and p-cresylglucuronide. Both conjugates are retained in uremia with a substantially higher total serum p-cresylsulphate in comparison to p-cresylglucuronide (31.4 ± 15.8 vs 7.3 ± 6.5 mg/L) but also a substantial difference in protein binding (92.4 ± 3.0 vs 8.3 ± 4.4 %) and in reduction ratio during post-dilution hemodiafiltration (37.4 ± 7.1 vs 78.6 ± 6.4 %). P-cresylglucuronide *per se* has no effect on leukocyte oxidative burst activity whereas in combination with p-cresylsulphate a synergistic activating effect was observed.

Conclusions: Serum concentrations of p-cresylsulphate and p-cresylglucuronide are elevated in uremia. Both conjugates show a different protein binding, resulting in a different dialytic behavior. Biologically, both conjugates are synergistic in activating leukocytes.

PUB417

Preeclampsia, Hemopexin and Extracellular ATP. Pro-Inflammatory Activation by Hemopexin and Hemopexin-ATP Complexes Floor Spaans, Marijke M. Faas, Chiwan Chiang, Theo Borghuis, Harry Van Goor, Winston W. Bakker. *Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands.*

Background: Preeclampsia (PE) is clinically characterized by hypertension and proteinuria, whereas also activated inflammatory cells in the circulation and altered endothelium is observed. We previously showed that hemopexin (Hx), which is able to down regulate vascular responsiveness to angiotensin II, rises in healthy pregnancy , leading to an expandend vascular bed. In PE this does not occur due to enhanced plasma ATP, which inhibits Hx activity, leading to persisting vascular responsiveness upon angiotensin II and hypertension.

As activated endothelium and monocytes occur in PE and to a lesser extent in normal pregnancy, the question emerged whether Hx, ATP, or Hx inactivated by ATP is associated with the pro-inflammatory status of PE and (to a lesser extent) of normal pregnancy.

Methods: Human monocytes (monomac-6 cell line; $6x10^6$ cells/ml) or confluent human endothelial cells (HUVEC) were stimulated with either LPS (2 µg/ml; positive control), ATP (150 µM), plasma Hx (100µg/ml), Hx-ATP complexes (100µg/ml), or saline (negative control) for 4 hours under standard conditions. Cells were subsequently washed, stained for ICAM-1 and processed for flow cytometry.

Results: In monocytes enhanced ICAM-1 expression was shown after stimulation with Hx-ATP complexes ($p<0.01$) and to a lesser extent after Hx alone ($P<0.05$) as compared with saline stimulation. ATP alone was negative in this respect. In endothelial

cells, ICAM-1 expression was upregulated after stimulation with either Hx or with Hx-ATP complexes as compared with saline stimulation ($p<0.05$). Again ATP alone did not affect ICAM-1 expression.

Conclusions: Endothelial cells are activated by both Hx or HX-ATP complexes equally, whereas monocytes are activated by Hx-ATP complexes and to a lesser extent by Hx. This may reflect the in vivo situation, i.e. enhanced pro-inflammatory stimulation in PE (due to inactivated Hx and enhanced plasma ATP), and moderate stimulation in healthy pregnancy associated with increased plasma Hx activity.

PUB418

Renal Lymphangiogenesis in Experimental Proteinuric Nephropathy Parallels Fibrosis-Development over Time Saleh Yazdani,[1] Menno Hovingh,[1] Maartje C.J. Slagman,[1] Andrea B. Kramer,[1] Klaas A. Sjollema,[3] Gerjan Navis,[1] Harry Van Goor,[2] Jacob Van den Born.[1] *[1]Nephrology, University Medical Center, Groningen, Netherlands; [2]Pathology, University Medical Center, Groningen, Netherlands; [3]Microscopy Center, University Medical Center, Groningen, Netherlands.*

Background: Renal lymphangiogenesis was reported in transplantation and in nephropathies with interstitial fibrosis, mostly cross-sectionally. Proteinuria (UP) is an important cause of progressive renal fibrosis. Whether UP in itself could trigger a renal lymphangiogenic response has not been established, moreover, the temporal relationship between development of fibrosis and lymphangiogenesis is unknown.

Methods: To evaluate the time course of lymph vessel (LV) formation related to UP and morphological damage we studied unilateral adriamycin nephropathy (1.5 mg/kg; left kidney clipped for 12 minutes), UP was measured and rats sacrificed at 6, 12, 18, 24, and 30 weeks after nephrosis induction, and kidneys were harvested (n=6 /time point). LVs were quantified by tissue FAXS/Image J using podoplanin/VEGFR3 IF double staining. Myofibroblasts (α-SMA), collagen III, macrophages (ED1), lymphangiogenic factors VEGF-C and -D and focal glomerulosclerosis were also quantified.

Results: After 6 weeks UP (100-200 mg/24h) was established however without influx of interstitial macrophages, myofibroblasts, collagen III deposition and LV formation. LV density gradually increased over time (18.6 ± 3.4 LV/HPF at 30 wks vs. 4.6 ± 1.5 at 6 wks; $p<0.0001$), especially in fibrotic regions. Increase in LV density was associated with macrophage and myofibroblast numbers, focal glomerulosclerosis and proteinuria (all $p<0.05$). Besides, increase in LV size was observed over time. VEGF-C was expressed by interstitial cells, VEGF-D by tubular epithelium. All parameters remained low in non-adriamycin exposed kidneys.

Conclusions: UP up to week 6 is not associated with renal lymphangiogenesis. However, subsequent chronic proteinuria induces lymphangiogenesis in temporal conjunction with influx of macrophages and myofibroblasts and development of interstitial fibrosis.Whether modulation of lymphangiogenesis may modulate fibrosis development should be subject of future studies.

PUB419

The Prognostic Significance of Crescentic Lesions in IgA Nephropathy Abdulkareem Alsuwaida,[1] Mohammed A. Al-Ghonaim,[1] Hala M. KFoury,[2] Sufia Husain.[2] *[1]Medicine, King Saud University, Riyadh, Saudi Arabia; [2]Pathology, King Saud University, Riyadh, Saudi Arabia.*

Background: Oxford classification of IgA Nephropathy has been developed to predict renal outcome. However, this classification system does not include crescentic lesions. The objectives is of this study is to assess the prognostic significance of crescentic lesion in predicting renal outcome.

Methods: A retrospective review of all biopsied cases of IgA nephropathy from May 1998 to May 2011 was undertaken done at the King Khalid University Hospital, Saudi Arabia. The renal biopsy routine slides were reexamined and clinical and laboratory parameters were also collected. Predictors of worsening of renal function (WRF), which was defined as 50% increment in baseline serum creatinine, were examined.

Results: Among 59 patients included in the study 17 (28.3%) had histological evidence of crescentic lesion. Baseline levels of serum creatinine, but not proteinuria, were significantly higher ($p = 0.01$) in the patients with crescentic, versus patients without crescent (310 umol/l vs. 137 umol/l, respectively). Worsening of renal function was observed in 11 patients (18.3%). The odd ratio of WRF among those with any crescent was 1.37 (CI: 1.02-1.82).

Conclusions: The existence of crescentic lesion is an independent risk factor for WRF among patients with IgA nephropathy. Our data raises the question of whether attempts should be made to incorporate the crescentic lesion into other parameters of Oxford Classifications. This is would allow more precise prediction of renal outcome.

Funding: Government Support - Non-U.S.

PUB420

Tamm Horsfall Protein Promotes Adherence of Uropathogenic Bacteria to Urinary Catheters James M. Bates, Hajamohideen S. Raffi, Satish Kumar. *Medicine/Nephrology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Catheter associated urinary tract infection (CAUTI) is a common problem. Tamm-Horsfall Protein (THP) is urine's most abundant protein and has been shown to bind to uropathogenic bacteria. We hypothesized that Tamm-Horsfall Protein (THP) may adsorb to the surface of urinary catheters and induce bacterial binding. In this study, we determined the effect of THP on adherence of *Escherichia coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*) to silicone and latex urinary catheters.

Methods: *E. coli* strain UTI89 and *P. aeruginosa* (ATCC 27314) were grown in tryptose broth and brain heart infusion broth, respectively for 24 hours at 37°C with 5 uCi/ml of methyl-^{3}H Thymidine (64 Ci/mmol specific activity). The radiolabelled bacteria were washed in Dulbecco's phosphate buffered saline (DPBS) and resuspended in artificial urine with or without THP. The bacterial solutions were incubated with 1-cm sections of the two types of catheter at 37°C. Three sections were removed from each tube at 1, 6 and 24 hrs, and washed with DPBS. Bacterial binding to the catheter segments was measured by scintillation counting. On days 2, 4 and 7, three sections were removed from each tube, and sonicated in DPBS to dislodge the bacteria from the catheters. The bacteria were quantitated by serial dilution and culture on agar. The data were expressed as the mean ± SE and analyzed with Student's t-test.

Results: *E. coli* adherence (expressed as Log CFU/cm catheter), in the artificial urine with THP, was greater to the silicone catheter at 1 hr (6.25 ± 0.17 vs 5.73 ± 0.01, $P = 0.02$), 6 hr (6.08 ± 0.04 vs 5.67 ± 0.13, $P = 0.02$) and 1 day (6.1 ± 0.02 vs 5.57 ± 0.02, $P = 0.00004$) and to the latex catheter at 6 hr (5.95 ± 0.06 vs 5.68 ± 0.05, $P = 0.01$) than in artificial urine alone. *P. aeruginosa* adherence was more to the latex catheter at 2 days (6.7 ± 0.03 vs 5.57 ± 0.01, $P = 0.0003$) in the artificial urine with THP versus the artificial urine alone.

Conclusions: THP facilitates the binding of *E. coli* to both silicone and latex catheters, and the binding of *P. aeruginosa* to latex catheters. THP may play a role in the pathogenesis of CAUTI.

Funding: NIDDK Support, Veterans Administration Support

PUB421

Effect of Pioglitazone in Controlling Proteinuria and Renal Failure in Focal Segmental Glomeruloscelosis Induced by Adriamycin in Male Wistar Rats Pratik Das. *Nephrology, Majula Ben Kidney Hospital, Kolkata, India.*

Background: Focal Segmental Glomerulosclerosis (FSGS) results in impaired renal function and proteinuria. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) modulate angiogenesis and PPAR- γ agonists have been shown to be protective in nondiabetic glomerulosclerosis. This study compares the effects of Pioglitazone and Losartan in controlling progressive renal damage in Doxorubicin- induced FSGS.

Methods: Progressive renal damage was induced with I.V. Doxorubicin (5mg/kg) and baseline serum creatinine and 24-hour urinary protein were estimated. The animals were randomized into 4 treatment groups, each comprising of six animals after proteinuria developed at Day-7.Oral treatment was distilled water (control-C),Losartan 9mg/kg/day(L), Pioglitazone 4mg/kg/day (P) or a combination of L and P(L+P), for 8 weeks.24 hour urinary protein was estimated at 14-day intervals, serum creatinine was estimated at Day-63 and excisional renal biopsy obtained. The pathological changes were estimated using a semi-quantitative histological score.

Results: Proteinuria continued to progress in all groups but the degree of proteinuria varied. At Day-21, 35, 49 and 63, L, P and L+P treated rats had significant reduction in proteinuria.

Proteinuria in 24 hours in different groups after adriamycin treatment

Group No.	Treatment	D-0	D-7	D-21	D-35	D-49	D-63
1	Saline treated control (C)	1.72±0.27	40.58±4.47	78.83±10.81	138±7.75	217.43±33.01	302.17±41.36
2	Losartan (L)	1.69±0.26	25.46±1.02	43.67±2.34*	119.55±4.87	134.5±3.58	150.72±3.25***
3	Pioglitazone (P)	1.76±0.23	37.08±1.56	65.33±2.80	120.7±3.05	147.45±5.46	181.22±7.26**
4	Losartan+ Pioglitazone (L+P)	1.83±0.22	32.03±2.83	39.15±3.25**	91.85±3.58**	135.27±2.87*	140.4±3.31***

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Doxorubicin caused significant rise in serum creatinine in C rats from D-0 to D-63 . P and L+P treatment caused significant reduction in serum Creatinine. Histological changes reflected the protective nature of both drugs.

Conclusions: Pioglitazone controls the rise in serum creatinine in Doxorubicin -induced glomerulosclerosis. Combining Losartan with Pioglitazone produced greater reduction of proteinuria, serum creatinine and sclerosis.

PUB422

Calcium Oxalate Crystal Deposition Is Associated with Production of More Urinary Osteopontin, Monocyte Chemoattractant Protein-1 and Kidney Injury Molecule Than Hyperoxaluria Alone in an Experimental Rat Model Saeed R. Khan, Aslam Khan. *Pathology, University of Florida, Gainesville, FL.*

Background: Calcium oxalate (CaOx) crystal deposition in the kidneys is associated with renal injury. The cause of injury, oxalate (Ox) or CaOx crystals, has been argued. Since short term experimental hyperoxaluria does not produce CaOx crystal deposition in all the rats, we decided to investigate this issue by comparing renal and urinary changes in hyperoxaluric rats with and without renal CaOx deposits.

Methods: Male Sprague-Dawley rats were fed a diet containing 5% HLP for 28 days. 24 hour urine was collected on days 7, 14, 21 and 28. After that rats were sacrificed and their kidneys processed for various microscopic and molecular investigations.

Results: All rats, with and without renal CaOx crystals on 28th day, significantly increased their urinary Ox by day 7. Those with crystals excreted more Ox. With time however, differences in urinary Ox between the two groups decreased. It was highly significant ($p<0.001$) on day 7, less significant ($P<0.01$) on day 14 and even lesser ($p<0.05$)

on day 21. No statistically significant was seen on day 28, the last day. Urinary citrate and calcium were not significantly affected by hyperoxaluria. Significant increases were seen in urinary excretion of hydrogen peroxide (HP), kidney injury molecule-1 (KIM1), monocyte chemoattractant protein-1 (MCP1) and osteopontin (OPN) by rats with renal crystals than control rats and those without crystals. There was a highly significant increase in renal expression of ED1 and KIM1 as determined by densitometric analyses of western blots.

Conclusions: Increase in urinary excretion of MCP-1 starts as early as day 7 and only by rats with crystal deposits while HP and KIM-1are also increased by day 7 but in both group of rats. Increase in urinary OPN is seen by day 14 in rats with crystals but by 28th day both group of rats showed significant increase in OPN urinary excretion. Results presented here show that both high Ox as well as CaOx crystals are injurious to the kidneys. There are, however subtle differences indicating the possibility that Ox and CaOx crystals trigger different pathways.

Funding: NIDDK Support

PUB423

p63 Is a Candidate Gene Responsible for Angiotensin II-Induced Glomerular Endothelial Cell Injury in Progressive Glomerulonephritis

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Background: Angiotensin II (ang II) is a key mediator for development of glomerular hypertension and glomerulosclerosis. Glomerular endothelial cell (GEnC) plays a crucial role in the initiation and progression of glomerulonephritis (GN). However, little is known about gene expression for ang II-induced GEnC injury and its contribution to progression of GN.

Methods: Profiling gene expression of RNAs from immortalized human GEnCs stimulated by ang II (10^{-8}M) for 0, 4, 12, and 24 hours was performed. Progressive GN was induced in rats by injection of anti-Thy-1 antibody and unilateral nephrectomy and was treated with or without ang II receptor blocker (ARB; Valsartan). Gene analyses were performed by using RNAs in isolated glomeruli from control rats, vehicle-, and ARB-treated nephritic rats on day 14. Gene analyses were carried out using whole human genome oligo microarray (Agilent).

Results: Among 41,000 genes of GEnC, ang II upregulated 111 genes more than 1.5-fold during the course of experiments. Nephritic rats showed the increase of 2,632 glomerular genes more than 2-fold compared to control on day 14. ARB treatment attenuated 408 glomerular genes of those upregulated genes to the control level. By comparing 111 transcripts of GEnC with 193 human genes converted from 408 rat genes in GN, p63, which is considered as a cell senescence inducible- and tumor suppressor protein (Nat Cell Biol 11: 1451-1458, 2009), was identified as a candidate gene responsible for development and progression of GN. Finally, western blot and immunostaining showed that the increased level of GEnC-p63 expression was paralleled with progression of GN and was suppressed with ARB treatment.

Conclusions: p63 is a candidate gene responsible for ang II-induced GEnC injury in the development and progression of GN.

Funding: Government Support - Non-U.S.

PUB424

Inhibition of NFkB Acetylation Improves Kidney Injury in a HIVAN Mouse Model

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Background: Nuclear factor kappa B (NF-kB) pathway plays a critical role in the pathogenesis of HIV Associated Nephropathy (HIVAN). Activation of the NF-kB pathway is regulated by the acetylation of p65, a subunit of the NF-kB dimer, at the K310 residue by acetyltransferase coactivator p300. The bromodomain (BRD) is a conserved structural module found in many acetyltransferases, including p300, that recognize acetyllysine. Here, we examined whether a bromodomain inhibitor (BRDi) is able to suppress the NF-kB pathway and attenuate kidney injuries in a well-established animal model of HIVAN (Tg26).

Methods: p65 acetylation and NFkB activation in human renal tubular epithelial cells (RTEC) were determined by western blotting and reporter assay, respectively. The expression of NFkB target genes was profiled using microarrays and qRT-PCR arrays. BRDi (0.08mg/kg/day) was given to Tg26 and control littermates by daily gavage for 4 weeks. Renal function and mortality were monitored. Urine, serum, total kidney, and glomeruli were collected. Renal function, proteinuria, renal pathology and acetylation of p65 were determined.

Results: BRDi inhibited the acetylation of p65 (K310) and transcriptional activity of NFkB in human RTEC that were either treated with TNF-alpha or infected by a HIV-pseudotyped virus. Gene expression PCR arrays and microarray studies showed that BRDi treatment inhibited several NFkB-targeted genes related to apoptosis, inflammation, and oxidative stress. BRDi treatment of Tg26 mice significantly lessened proteinuria, improved kidney function, attenuated tubular injury, reduced p65 acetylation, and decreased renal expression of NF-KB target genes.

Conclusions: Our studies demonstrated that the BRDi could be developed as a potential pharmacologic agent for the treatment of patients with HIVAN.

Funding: NIDDK Support

PUB425

Lack of Flow in Dilated Renal Tubules in Murine Model of HIV-Associated Nephropathy

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Background: HIV-associated nephropathy (HIVAN) is the most common cause of end stage renal disease in HIV-infected patients. Histopathologic findings in HIVAN include collapsing glomerulosclerosis and severe tubulointerstitial disease with microcystic dilation of the renal tubules. Severity of tubulointerstitial disease is the best histologic predictor of progression to ESRD in most forms of chronic kidney disease. We therefore hypothesize that in HIVAN, renal tubular obstruction leads to increased intratubular pressure and dilatation resulting in decreased glomerular filtration and glomerular collapse. In these studies, we tested the hypothesis that dilated renal tubules in kidneys of HIV-transgenic mice, which develop a renal phenotype identical to HIVAN, do not have flow.

Methods: We injected HIV-transgenic mice and wild-type mice with 3kDa Texas Red-conjugated dextran which is freely filtered across the glomerulus and large, non-filterable, 500kDa fluoresceinated dextran. Kidneys were then analyzed for presence of fluorescent dextran in tubules, glomeruli and blood vessels. Tubules with preserved flow were detected by the presence of red fluorescence and tubules without red fluorescence were presumed to have no flow. Green fluorescence was used to identify patent blood vessels.

Results: High molecular weight dextran was detected within glomeruli and peritubular capillaries but not within tubules, confirming that it was not filtered by glomeruli. Low molecular weight dextran was detected in cells lining normal-appearing tubules in wild type and HIV-Tg mice but not in dilated tubules in HIV-Tg mice. The pattern of punctate sub-apical fluorescence suggests that the red dextran was localized to endocytic vesicles.

Conclusions: These results suggest that in HIVAN, as tubules become dilated, they lose flow. However, we have not ruled out the possibility that the lack of fluorescence in dilated tubules reflects impaired endocytosis in these cells. Further studies are needed to determine the role of loss of tubular patency in the progression of HIVAN and other chronic kidney diseases.

Funding: NIDDK Support

PUB426

In-Dwelling Urinary Catheter Reduces the Protective Effect of Tamm-Horsfall Protein Against Urinary Tract Infection

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Background: We have demonstrated previously that Tamm-Horsfall Protein-deficient ($THP^{-/-}$) mice, created in our laboratory, have increased susceptibility to experimental urinary tract infection (UTI) when compared to $THP^{+/+}$ mice. In this study we determined if the protective effect of THP against UTI persisted in presence of an in-dwelling urinary catheter.

Methods: Mouse urinary catheters were made from 2 cm. long segments of polyethylene tubing with a spiral portion at one end and a straight portion on the other end. The catheters were mounted on a stylet and introduced trans-urethrally into $THP^{+/+}$ and $THP^{-/-}$ mice (n = 7) under anesthesia, after collecting baseline urine samples. The end of the catheter outside the urethral meatus was trimmed. *E. coli* strain UTI 89 was propagated and a 10 ul aliquot of a suspension containing 1 x 10^8 bacteria /mL was introduced from the side of the indwelling catheter using a Hamilton syringe. The mice were housed in individual cages and allowed to eat and drink ad lib. On days 2 and 7, urine was collected and cultured on EMB agar. The mice were euthanized on Day 8 with CO_2 narcosis. Bladder and kidneys were removed aseptically and divided into two portions. One portion was homogenized and cultured on EMB agar; the other was processed for histological assessment.

Results: Results showed that the number of bacteria excreted in urine and the bacterial loads in bladder and kidneys were not statistically different between $THP^{+/+}$ and $THP^{-/-}$ mice. The number of bacteria bound to the catheter surface was also not statistically different between the two groups.

Conclusions: These findings imply that the protective effect of THP against UTI is lost in presence of an in-dwelling urinary catheter. Restoring the protective effect of THP against UTI in patients with in-dwelling urinary catheters may help reduce the burden of catheter-associated UTI.

Funding: NIDDK Support, Veterans Administration Support

PUB427

Cardiorenal Syndrome Type 1 May Be Immunologically Mediated

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Background: Cardiorenal syndrome type1 (CRS1) is characterized by acute worsening of cardiac function leading to AKI. CRS1 pathophysiology is complex and unclear.

An alteration of immune response has been postulated as a potential mechanism involved in CRS1, but has not been demonstrated.

The aim of this pilot study was to demonstrate that plasma of pts with CRS1 was able to trigger off a response in monocytes which resulted in apoptosis. In fact, monocytes have a central role in initiation, development and outcome of the immune response.

Methods: In this study, we compared the differential responses of U937 monocytes incubated for 24 hours with plasma (50%) from pts with CRS1, with acute heart failure(HF) and healthy control(CTR), to verify the behaviour of immunologic cells when exposed to the disease environment.

AKI and CRS1 was defined according to the current classification systems.

We evaluated apoptosis by fluorescence microscope (expressing as percent of the total cell population) and Caspase3, that is indispensable for chromatin condensation and DNA fragmentation in apoptosis.

Results: We enrolled 5 CRS1pts (3M/2F; mean age 75±11yrs; mean crea 1,1±0,2mg/dl, mean eGFR 72±13mL/min/1.73m^2), 7 HFpts (7M; 67±10yrs; 1±0,1mg/dl, 85±10mL/min/1.73m^2) and 5CTR (3M/2F; 63±8yrs).

Mean plasma-induced apoptosis in U937 was 28% for CRS1group while it was 16,5% for HF and 4% for CTR, at 24 hours.

Monocytes showed a greater apoptosis when incubated with plasma from CRS1pts compared to other 2groups.

There was marked proapoptotic activity in monocytes incubated with plasma from CRS1pts. In these preliminary results, Caspase3 was elevated: it was higher in CRS1(7,7ng/ml) when compared to HFgroup (5,6ng/ml) and CTR(4,1ng/ml).

It is likely that apoptosis of U937 cells may be due to presence of factors in CRS1 plasma (i.e. cytokines).

Conclusions: This is the first study that demonstrate a higher proapoptotic activity in plasma of CRS1pts. It suggests a possible immune-mediated process in CRS1 pathophysiology. The present study has some limitations: cohorts of pts was small and the paracrine signaling pathways and the factors involved did not analyze.

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PUB428

The Role of Mannan-Binding Lectin Pathway Activation and Interaction with Apoptosis Pathway in the Pathogenesis of Diabetic Nephropathy

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Background: To study the role of MBL pathway activation and downstream mechanisms in experimental STZ-induced diabetic nephropathy.

Methods: Diabetes was induced by a single intravenous injection of streptozotocin dissolved in citrate buffer . The end of 2rd week, 4th week, 6th week and 8th week after modeling were considered as observation time points. With ligation of proximal of abdominal aorta, a catheter was inserted into distal of abdominal aorta. One part of kidney was fixed in 4% paraformaldehyde for 48 hours, and then embedded in paraffin for histological examination and immunohistochemical staining. The other part of kidney were frozen rapidly in lipuid nitrogen used for RT-PCR examine and Western Blotting examine.

Results: (1) the 2th week after modeling, ACR and plasma glucose of DN group were significantly higher than control (P< 0.05), with modeling extended, both 24-h PRO and ACR of DN group were higher than the control; (2) It is showed by immunohistochemistry that MBL-A, MBL-C, NF-KAPPA, MAC and C3 depositions were significantly higher than the control (P <0.05); (3) It was showed by western blotting that MBL-A, MBL-C and MAC deposition than the control (P <0.05). (4) at the end of 2 week after modeling, CD26 and CD91 expression in DN kidney tissue increased at the end of 2th week after the modeling and reached the peak and the end of 8th week, and Western Blotting examine also supported the results.

Conclusions: (1)MBL pathway activation in diabetic nephropathy may be related to inflammation in diabetic nephropathy. (2) MBL possibly through CD26, CD91 and further amplified with the downstream effects; (3) MBL may be involved by identifying apoptotic cells and apoptotic cell clear, and further activation of endoplasmic reticulum stress

Funding: Government Support - Non-U.S.

PUB429

Obesity Is Associated with Increase in Glomerular Size in Renal Transplant Recipients

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Background: Obesity-related glomerulomegaly has been well described but little is known about the pathogenesis of the disease. Newly transplanted renal allograft recipients with various body mass index (BMI) and serial protocol biopsies provide a unique opportunity to examine the progression of glomerulomegaly.

Methods: Renal transplant recipients in the first year after transplant with at least 2 protocol biopsies 3-12 months apart (n=37) were studied. Recipient BMI at the time of transplant was noted and individuals were grouped as non-obese (BMI < 30, n=14) or obese (BMI >= 30, n=23). A subset was categorized as normal (<=25, n=8) or morbidly obese (BMI >=35, n=10). Surface area of three to nineteen glomeruli per biopsy was measured using the Aperio software. Only glomeruli with either the vascular or tubular pole present were analyzed. The percentage change between the average glomerular surface area of the paired biopsies was compared using the paired t-test.

Results: The change in surface area over time was as follows: Non-obese, (<=30), n=14, mean change -6.38% +/- 15.9% and Obese, (>30), n=23, mean change 6.0% +/- 25.9% (p=0.1171); Normal (<=25), n=8, mean change -7.46% +/- 12.9% and Obese (>=35), n=10, mean change 14.7%/- 25.4% (p= 0.0403).

Conclusions: Alteration in glomerular surface area is observed in renal transplants. Normal individuals had a reduction in surface area which is contrary to the expected adaptational response due either to early ischemia or immunosuppressive therapy. The extremely obese group developed glomerulomegaly which, though consistent with an adaptational response, is tempered by the same effects as seen in the normal group. The differences in the obese vs. non-obese group, while as not striking, showed the same trend.

Funding: NIDDK Support

PUB430

Atypical Case of Thrombotic Thrombocytopenic Purpura

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy disorder (TMA), characterized by thrombocytopenia, microangiopathic haemolytic anemia, neurologic abnormalities, renal impairment, and fever. TTP is caused by deficiency of the von Willebrand factor cleaving protease (VWFCP), ADAMTS-13, which cleaves the VWF multimers produced by endothelial cells. In the absence or abnormal ADAMTS-13, ultralarge VWF multimers occur, which bind platelets and promote clot formation. Different from the typical presentation of TTP, we report a case with ADAMTS-13 deficiency who presented with malignant hypertension and progressive kidney failure.

Methods: Retrospective review of the medical record.

Results: A 15 year old girl, presented with a hypertensive crisis (BP 190/110 mmHg) with features of chronic hypertension (i.e. exudative hypertensive retinopathy). Investigations revealed creatinine 191 umol/L, hemoglobin 94 g/L, platelets 113x10^9/L, and signs of hemolysis. Ultrasound demonstrated normal kidneys with no renal artery stenosis. Our impression was atypical hemolytic uremic syndrome (aHUS) and/or TTP. Renal function rapidly deteriorated. Thus, a renal biopsy was performed demonstrating TMA a diagnosis confirmed via repeat biopsy 6 months later. Hence, the patient was commenced on plasmaphersis (TPE) and maintained on intermittent hemodialysis (IHD). While complement work up was normal, ADAMTS-13 activity level was <5% with no mutations or antibodies detected. Recovery of renal function was noted at 6 months; therefore, IHD and TPE were slowly weaned off. Three months later, the patient had a mild disease recurrence coinciding with severe hypertension which was controlled by few sessions of plasma infusions.

Conclusions: This case is remarkable as a hypertensive crisis was the first manifestation of TTP in this female adolescent. Furthermore, ADAMTS-13 activity was only initially decreased but returned to normal upon TPE and remained normal even at the first disease recurrence. While the patient is currently stable off hemodialysis further TTP crises have to be anticipated – a scenario for which we may consider rituximab treatment.

PUB431

The Damage of Glomerular Podocytes and Its Manifestations Resulted from Aristolochic Acid

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Background: In our previous research work I found that some patients with acute aristolochic acid nephropathy presented massive proteinuria, so we designed this study to investigate whether aristolochic acid can damage glomerular podocytes and, if so, what are its manifestations?

Methods: Eighteen male SD rats were equally divided into the following 2 groups: model group in which the rats received the extract of *Aristolochia manshuriensis Kom (AmK)* by gavage; control group received tap water only by gavage. 24h urinary protein excretion was measured and urinary protein composition was analyzed with SDS-PAGE electrophoresis at the end of the 1st and 4th week, respectively. At the end of the 4th week, all the rats were sacrificed and their kidney tissue was collected for electron microscopic analysis and laser capture microdissection of glomeruli. The average widths of glomerular foot process were measured by electron microscopy and image analysis. The mRNA expression of nephrin, podocin, CDA2P, podocalyxin and podoplanin in microdissected glomeruli was determined by real time RT-PCR.

Results: At the end of the 4th week, 24h urinary protein excretion in the model group was significantly higher than that in the control group (13.8±2.4mg/d vs 10.4±1.8mg/d, *P*<0.01) and the urinary protein composition in the model group was presented as mixed pattern of glomerular and tubular protein. The segmental effacement of glomerular foot process in the model group was observed, and the average widths of glomerular foot process in the model group were significantly larger than those in the control group (0.36±0.06mm vs 0.26±0.02mm, *P*<0.01). The mRNA expression of nephrin, podocin, CDA2P, podocalyxin and podoplanin in glomeruli was significantly down-regulated in the model group compared with the control group. Their mRNA expression was reduced 34%, 62%, 56%, 50% (*P*<0.01) and 27% (*P*<0.05), respectively.

Conclusions: Aristolochic acid can damage the glomerular podocytes, leading to the down-regulation of nephrin, podocin, CD2AP, podoplanin and podocalyxin mRNA expression in podocytes, the segmental effacement of foot process, and proteinuria.

PUB432

Generation of Heme Oxygenase (HO)-1 Deficient Rats Using Zinc Finger Nuclease (ZFN)-Mediated HO-1 Gene Disruption

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Background: We have previously shown that, compared to renal tubules, the ability of rat glomeruli to upregulate the cytoprotective enzyme, HO-1, in response to natural HO-1 inducers or injury is limited, the least capable cell being the terminally differentiated GEC (J Lab Clin Med, 147[3], 150, 2006). Since the role of GEC in regulating glomerular

filtration and permeability to protein can best be studied in the rat, we generated rats with HO-1 gene disruption using a zinc finger nuclease (ZFN)-mediated gene disruption system. The ZFN reagents employed targets the HO-1 sequence 5'-GGT GGC CCA CGC ATA tac ccg cTA CCT GGG TGA CCT CTC AG-3' within Exon 3 of the rat hmox1 gene. Underlined sequences are recognized by the right and left ZFNs, respectively, and are separated by 7-bp spacer sequences where the nuclease domains interact to cause a double strand break. 149 Sprague Dawley (SD) embryos were microinjected with hmox1 specific ZFN messenger RNAs. 78 out of 149 embryos were transferred to pseudopregnant SD females and 18 live pups were obtained. Screening for target gene (hmox1) disruption revealed three distinct founders; HO1-m1, HO1-m2 and HO1-m3. The HO-m3 founder was further characterized and found to contain a 10-bp frameshift deletion. We then obtained a backcross generation (N2) of heterozygotes (hmox+/-) from this founder, with five individual pups inheriting the HO1-m3 frameshift mutation among a litter of eleven. By cross-mating these 5 heterozygotes, we have since obtained a total of 31 N3 pups, of which two were homozygotes (hmox-/-) for the 10 bp frame-shift mutation as confirmed by PCR-based genotyping coupled with Surveyor nuclease mutation detection assay. Further, this mutation was stably heritable and the absence of the hmox1 gene and protein in tissues was validated. The N3 homozygotes are surviving to-date, and this represents a 6% yield that is superior to the one achieved using the sequence-mediated gene disruption approach previously reported in HO-1 knock-out mice (PNAS, 94[20], 10919, 1997; J Clin Invest. 103[8], R23, 1999).

Conclusions: The HO-1 deficient rat can enhance our understanding on the role of HO-1 in nephron physiology.

Funding: Other NIH Support - AHA-SDG

PUB433

Angiotensin II Induces Mesangial Cell Apoptosis Via TLR4/MyD88 Pathway

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Background: Angiotensin II(Ang II) could induce mesangial cell(MC) apoptosis both in vivo and in vitro, but the precise mechanisms are far from well understood. Toll-like receptors (TLRs) have been identified to be functional receptors for response to innate immune events and present in the kidney. It is reported recently that Ang II contributes to the TLR4 expression on MC. However, evidence for the role of TLR4 in the Ang II mediated MCs apoptosis and the direct effect of candesartan on TLR4 expression in MCs is paucity. The aims of the present study were to investigate the involvement of TLR4 and its proximal adaptor myeloid differentiation factor 88(MyD88) in Ang II-induced MC early apoptosis and its possible mechanism as well as to examine whether candesartan has direct effects through this pathway.

Methods: MC was cultured in DMEM medium and treated with candesartan and/or Ang II. Apoptosis was determined by Hoechst staining and flow cytometry with annexinV-FITC and propidium iodide. The intracellular formation of reactive oxygen species(ROS) was detected by confocal microscopy with fluorescent probe CM-H2DCFDA. TLR4 and MyD88 mRNA expression were determined by reverse transcription-polymerase chain reaction. TLR4 protein was evaluated by western blotting.

Results: Ang II induces MC oxidative stress and apoptosis in a time-dependent manner. Ang II upregulates TLR4/MyD88 mRNA expression as well as TLR4 protein synthesis significantly,and causes intercellular ROS acumulation, and subsequently apoptosis on MCs. Candesartan inhibits the above effects dramaticly.

Conclusions: These results support our hypotheses firstly that TLR4/MyD88 pathway is involved in the process of Ang II-induced apoptosis through the up-regulation of intracellular ROS formation, candesartan suppresses MC apoptosis via a direct action that depends on this pathway.

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PUB434

Localization of the G-Protein-Coupled Receptor 40 (GPR40) in the Kidney

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Background: GPR40 is a seven transmembrane G protein coupled receptor that is activated by long chain fatty acids. Although GPR40 has been shown to play a role in insulin secretion in pancreatic islets, its role in other organs, including the kidney, is still undetermined. As a first step in understanding potential roles in the kidney, our initial studies were directed at determining the sites of expression along the nephron.

Methods: Expression of GPR40 was determined in male Balb/C mice by semi-quantitative RT-PCR, immunoblotting, and immunohistochemical and double- or triple-labeling immunofluorescent staining with nephron-segment specific markers

Results: Initial experiments detected GPR40 mRNA expression in the kidney. Immunoblotting analysis was used to screen for an antibody specifically recognizing for a single band of GPR40 in the homogenates of renal cortex/the outer stripe of the outer medulla (OSOM). Immunohistochemical staining with the identified specific GPR40 antibody revealed the strongest immunolabeling of GPR40 in the majority of renal tubules in OSOM as well as in a subset of cortical tubules. Double- or triple-labeling immunofluorescent staining confirmed that in the cortex, GPR40 was expressed in the THP-, DBA-, and AQP2-positive tubules, with minimal expression in the LTA-positive tubules. However, in the medulla, GPR40 staining was the highest in the LTA-positive tubules of the OSOM but very weak in the THP-, DBA-, and AQP2-positive tubules. The labeling of GPR40 was also prominent in the calbindin-D28K-positive tubules but still relatively weaker than that in the LTA-positive tubules of the OSOM.

Conclusions: Our studies have determined that in the kidney, GPR40 is expressed at the highest levels in the S3 segment of proximal tubule, with an apparent expression in the distal convoluted tubule, connecting tubule, cortical collecting duct and cortical thick ascending limb.

Funding: NIDDK Support

PUB435

HIV-Induced Down Regulation of Vitamin D Receptor (VDR) Increases Activation of Renin Angiotensin System in Tubular Cells

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Background: Renin angiotensin system (RAS) has been demonstrated to play an important role in the development of HIV-associated nephropathy (HIVAN). Vitamin D receptor (VDR) has been reported to be a negative regulator of renin transcription. Since the activation of the RAS plays an important role in the progression of HIVAN, we hypothesized that HIV infection may be activating renal cell RAS by dowreguating renal cell VDR expression.

Methods: To have a model of tubular cell HIV infection, mouse tubular cells (MCT) were transduced with either vector only or HIV (NL4-3) constructs. Vector/MCTs and HIV/MCTs were incubated in serum-free media (SFM) fro 24 hours and then followed by protein and RNA extraction. Immunoblotting and real time PCR studies were conducte for protein and mRNA expression for VDR, angiotensinogen (Agt) and renin. Ang II ELISA was carried out on cells prepared under similar conditions. To establish a causal relationship between VDR and the RAS, MCTs were silenced for VDR by transfection of siRNA-VDR and then evaluated for the RAS activation. To confirm relationship between VDR and the RAS, vector/MCCs and HIV/MCTs were treated with vitamin D2 analogue (calcitriol, 25 nM) for 24 hours and evaluated for VDR expression and the activation of the RAS as mentioned above.

Results: HIV/MCTs showed attenuated ($P<0.05$) expression of VDR when compared to control and vector/MCTs. HIV/MCTs also displayed 2-fold enhanced expression of renin. Moreover, HIV/MCTs showed 2.5 fold increase in Ang II (intracellular) when compared to vector/MCTs. Similarly, siRNA-VDR/MCTs displayed activation of the RAS. On the the other hand, Vitamin D treated HIV/MCTs not only showed upregulation (<$P<0.01$) of VDR but also displayed attenuated ($P<0.01$) expression of renin and reduction ($P<0.01$) in intracellular Ang II production.

Conclusions: These findings indicated HIV-induced VDR down regulation promotes RAS activation in tubular cells. The present study provides a mechanistic insight into the RAS activation in HIVAN patients.

Funding: NIDDK Support

PUB436

Calcineurin Correlates with Endoplasmic Reticulum (ER) Stress in Podocyte In Vivo and In Vitro

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Background: Podocyte injury is an early phenomenon in diabetic nephropathy. Saturated free fatty acid palmitate was key in causing insulin resistance by inducing ER stress in pancreatic -cells. Our previous in vitro work showed it can also induce podocyte apoptosis via ER stress. If ER stress with altered calcium homeostasis could activate calcineurin, which mediates dephosphorylation of synaptopodin and disrupts the stabilization of cytoskeleton in podocytes, was explored.

Methods: Six, nine, twelve-week-old male C57BLKS/J db/db mice (n=7) and age-matched db/m control mice (n=5) were studied. Twenty-four hour urine protein output (24UP) were assayed. The intensity of Glucose-regulated protein 78(GRP78), calcineurin was co-localized with synaptopodin in renal samples by immunofluorescence(IF) stain. In podocyte treated by palmitate with or without ursodeoxycholic acid, the changes in protein and mRNA expressions of calcineurin were assayed by western blot and real-time PCR. Calcineurin and synaptopodin were co-localized by IF stain.

Results: 24UP of all studied db/db mice were significantly higher than each age matched control ($p<0.05$), and it was significantly higher in 9w and 12w db/db mice compared with that of 6w db/db mice ($p<0.05$). Confocal microscopy showed steadly increased expression of calcineurin and GRP78 in podocytes only in db/db mice with age increase. Palmitate significantly increased cultured murine podocytes calcineurin protein and mRNA expressions time-dependently when loading 0.5 mM (3h, 5h compared with 0h, $p<0.05$) and dose-dependently when loading 0.25-0.5 mM for 5h (compared with control, $p<0.05$). Addition of 10μM ursodeoxycholic acid inhibited calcineurin increase (compared with palmitate treatment group, $p<0.05$).

Conclusions: There has calcineurin activation at the onset of albuminuria in DN animal model. Calcineurin correlates with ER stress in podocytes induced by palmitate in vitro. The inhibitory effect of ursodeoxycholic acid suggests activation of calcineurin, podocyte injury and consequent proteinuria could be ameliorated by relief of ER stress.

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PUB437

Clinicopathological Features and Treatment Discussion on HBV-Associated Endocapillary Proliferative Glomerulonephritis Jian Chen, Yinghao Yu. *Dept. of Nephrology, Dong Fang Hospital, Fuzhou, Fujian, China.*

Background: To discuss the clinicopathological features and treatment on HBV-associated endocapillary proliferative glomerulonephritis.

Methods: A total of 29387 biopsies were included from Jan. 2001 to Oct. 2009. 25 cases were diagnosed as HBV-associated endocapillary proliferative glomerulonephritis by renal biopsy. 10 cases of relatively comprehensive information have been done a retrospective analysis.

Results: 9 cases of acute nephritic syndrome, 4 cases of gross hematuria and 3 of hypertension among the above 9 cases; 5 cases of Hypoproteinemia, 1 case of NS, 4 of oliguria, 3 of acute kindey injury(AKI), 1 of abnormal liver function. Diffuse endocapillary proliferative and neutrophilic leukocyte infiltration could be seen in the diseased glomeruli;Immunoglobulin like IgG, IgM, IgA and C_3, C_4, C_{1q}, Fib deposited in many parts, in which three cases are of "full house" phenomenon;9 cases of HBsAg deposited in the renal tissue, 3 of HBcAg, 2 of HBsAg and HBcAg, Many of them, clinical symptoms disappeared and renal function recovered ,two of these cases proteinuria turned negative ,two had no change after 16 days on average of treatment in the way of treating as acute nephritis. One patient of NS was treated with Lamivudine (100mg qd) and prednisone (30mg qd) and 26 days later the level of proteinuria went down under the nephrotic level proteinuria; one of AKI with high serum HBV-DNA and abnormal liver function was given treatment of Lamivudine (100mg qd) and HD and 26 days later clinical symptoms disappeared, liver and renal function returned normal and the number of serum HBV-DNA decreased.

Conclusions: The main clinical manifestation of HBV-associated endocapillary proliferative glomerulonephritis is acute nephritic syndrome and is worse to primarily endocapillary proliferative glomerulonephritis.In terms of immunopathology,it is more complicate. The principle of treatment is same as that of acute nephritis. Anti-virus therapy works better. Patients of NS can be treated by anti-virus therapy combined with small dose corticosteroid. But the long-term efficacy needs further observation.

Funding: Government Support - Non-U.S.

PUB438

Association of Podocytopathy and Proteinuria in Membranous Lupus Nephritis Niraj B. Desai,[1] David J. Cimbaluk,[2] William Whittier,[1] Edmund J. Lewis.[1] *[1]Section of Nephrology, Department of Medicine, Rush University Medical Center, Chicago, IL; [2]Department of Pathology, Rush University Medical Center, Chicago, IL.*

Background: In patients (pts) with lupus nephritis, a wide range of proteinuria exists irrespective of the presence of peripheral or mesangial immune aggregate deposition. Biopsies from pts with membranous lupus nephritis (MLN) often exhibit evidence of glomerular involvement overlapping multiple pathogenetic mechanisms. Podocytopathy in pts with MLN may represent an independent pathogenetic process underlying the development of nephrotic range proteinuria.

Methods: We conducted a retrospective clinicopathologic study of pts with a histologic diagnosis of MLN (WHO Va and Vb, n=20) and proteinuria. The degree of immune aggregate deposition in the capillary walls and mesangium was detailed using fluorescent (FM) and electron microscopy (EM). The degree of glomerular epithelial cell effacement and average foot process width (FPW) was detailed using EM. Baseline and follow-up clinical parameters were collected.

Results: Nine pts had subnephrotic range proteinuria (<3 grams proteinuria/gram creatinine [g/g]) and eleven demonstrated nephrotic range proteinuria (≥3g/g). Mean proteinuria (8.3 +/- 5.1 g/g vs. 1.63 +/- 0.83 g/g, p=0.001) and foot process effacement (88.6 +/- 11% vs. 48.3 +/- 36.1%, p=0.002) and average FPW (1798 +/- 736 nm vs. 1000 +/- 333 nm, p=0.008) was greater in the nephrotic group compared to subnephrotic. All biopsies from nephrotic pts demonstrated at least 75% foot process effacement. There were no other significant histopathologic differences between the groups. The nephrotic pts were younger (31.9 +/- 10.8 vs. 44.2 +/- 11.4 years, p=0.02) and demonstrated a shorter time from diagnosis of lupus to time of biopsy (28.6 +/- 26.2 vs. 106.7 +/- 62.8 months, p= 0.001).

Conclusions: The single distinguishing morphologic feature in pts with MLN and nephrotic range proteinuria was diffuse visceral epithelial cell foot process effacement. No association between immune aggregate burden and proteinuria was observed. We conclude that nephrotic range proteinuria in patients with MLN is likely a manifestation of concomitant podocyopathy.

PUB439

Monoclonal Immunoglobulin Deposition Disease without Evidence of Multiple Myeloma or Extra-Renal Involvement Treated with Bortezomib. Two Pilot Cases and Literature Review Kumar Gaurav, Manish Gera, Raj Jeevan. *Nephrology, Internal Medicine Nephrology Inc., Terre Haute, IN.*

Background: Monoclonal Immunoglobulin Deposition Disease (MIDD) can present pathologically as two broad forms: AL/AH amyloidosis, which usually presents with both renal (nephrotic syndrome) and extrarenal (postural hypotension, diarrhea, CHF) manifestations. Secondly, it may present in form of Light/Heavy Chain Deposition Disease (L/HCDD) which may further manifest as AKI, CKD, tubulointerstitial or nephrotic syndrome and less commonly with extra renal features. Incidence of MM in this patient population is about 10-15%. Data on treatment of these patients is limited. In literature, treatment has ranged from Bone Marrow Transplantation to melphalan, chlorambucil and bortezomib. Usually, these treatment regimens are used in presence of multiple organ involvement or multiple myeloma. Literature on treatment of MIDD limited to renal involvement without any extrarenal manifestations or without MM is very sparse.

Methods: We present 2 such cases of MIDD with renal involvement without extra-renal manifestations and without MM. Review of literature was done.

Results: Case A presented with Nephrotic syndrome (10g/24hrs) without any cardiovascular, liver or bone marrow involvement. Renal biopsy was consistent with AL amyloidosis. Patient was treated with bortezomib and dexamethasone. Proteinuria subsided to 1.5 g/24hrs and remains stable without any other organ involvement at 1 year of follow up. Case B presented with AKI. Diagnsotic workup and renal biopsy was consistent with cast nephropathy and LCDD, which in itself is a relatively rare presentation. Patient's AKI got worse requiring Renal Replacement Therapy (RRT). Simultaneous treatment with bortezomib and dexamethasone was initiated. Patient's renal function subsequently improved without further need for RRT and remains stable at 3 years of follow up without evidence of MM.

Conclusions: 1. Both cases of renal limited MIDD demonstrate reasonable response in terms of renal parameters and future development of MM after treatment with bortezomib.

2. Given the favorable side effect profile, bortezomib may be used as a first line of treatment in such patients.

Funding: Clinical Revenue Support

PUB440

A Rare Case of Podocytic Infolding Glomerulopathy Associated with Multiple Myeloma Makoto Harada, Yasufumi Takahashi, Yuji Kamijo, Makoto Higuchi. *Nephrology Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan.*

Background: Recently, podocytic infolding glomerulopathy (PIG), newly proposed by Joe *et al*, has attracted considerable attention for its rare pathological changes. The glomerular change is characterized by specific lesions of thickened glomerular basement membrane (GBM), which include microspheres, a microtubular structure, or podocytic infolding. Today, there are only a small number of case reports of PIG, and the clinical features and the developmental process of PIG are still unclear. To accumulate the information about PIG, we report a rare case of PIG with multiple myeloma.

Methods: A 79-year-old male was admitted to our hospital for proteinuria, hypergammaglobulinemia, hypoalbuminemia and kidney dysfunction. The patient did not exhibit any autoimmune abnormality, including anti-nuclear antibody, rheumatoid factor, and hypocomplementemia. However, serum, urine and bone marrow examinations revealed the monoclonal IgG(λ) M protein, bence jones protein, and monoclonal plasma cell proliferation, indicating a diagnosis of multiple myeloma. To determine the cause of kidney dysfunction, we conducted a kidney biopsy.

Results: Light microscopic examination showed glomeruli with irregularly thickened GBM and a bubble-like appearance of the capillary walls. There was no deposition of immunoglobulin, light chain, complement, or amyloid protein. Electron microscopic analysis demonstrated a marked unusual structure, which included podocytic infoldings as well as microspheres, in the irregularly thickened GBM, suggesting PIG. The patient was treated with corticosteroid; however, he died due to severe pulmonary infection 2 months after the introduction of steroid therapy.

Conclusions: Here we report the first rare case of PIG with multiple myeloma. The mechanism underlying the development of PIG is still unclear. Several earlier studies reported the association of autoimmunity, while the current case exhibited an obvious immunoglobulin abnormality. These findings suggest a close relation between the PIG development process and immune system abnormality. Further accumulation of case reports is necessary.

PUB441

Correlation of Chemokine Biomarkers in Urine of Patients with Renal Allograft Rejection Mark J. Lerman,[1] Afzal Nikaein,[2] Brandt Moore,[2] Sandra Hinton,[1] Cindy L. Corpier,[1] Judson M. Hunt.[1] *[1]Transplant Services, Medical City Dallas Hospital, Dallas, TX; [2]Transplant Immunology, Medical City Dallas Hospital, Dallas, TX.*

Background: Diagnosis of acute renal transplant cellular rejection or chronic allograft nephropathy is based on serum creatinine (Cr) and invasive biopsies. However, biopsies do not always show graft injuries due to sample size, or section of biopsies. Urine is produced and excreted by the kidney, and therefore, has the potential to reflect injuries to the renal allograft. We have been interested to investigate the presence of bio-markers in urine of patients with or without acute cellular rejection. The bio-markers studied include osteoprotegerin (OPG), monokine induced by interferon-y (MIG) and interferon-y induced protein of kDa (IP-10). These three biomarkers have been shown to increase in urine of majority of renal allograft recipients undergoing acute cellular rejection or BK viral nephropathy, but not in patients with chronic allograft nephropathy or stable graft function.

Methods: Twenty renal transplant recipients who were biopsied for possible rejection were included. Biopsy results were correlated with serum Cr and clinical symtoms. All biopsies were stained for C4d and BK virus. Serum specimens obtained at the time of biopsies were tested for the presence of HLA antibody by solid phase immunoassays. Urine of patients was obtained at the time of biopsies and biomarkers were quantitated in the urine by solid phase immunoassay using luminex technology.

Results: Eight patients were diagnosed with acute cellular rejection, and one with BK viral nephropathy. Level of all 3 chemokines, IP-10, MIG, and OPG was increased in 6

patients with acute cellular rejection. Level of OPG was increased in all 8 patients with acute cellular rejection. Level of 2 chemokines IP-10 and OPG was increased in the one patient with BK Nephropathy.

Conclusions: This preliminary study shows that quantitation of chemokine biomarkers in the urine of renal allograft recipients may correlate with acute cellular rejection. This noninvasive test may be used for diagnosis of rejection and in some cases would eliminate an invasive test which may cause complications with bleeding and infection.

PUB442

Glomerular Involution: A Special Pattern of Glomerular Injury, Not Limited to Patients with Relapsing Minimal Change Nephrotic Syndrome

Rutger J. Maas,[1] Henry Dijkman,[2] Linda Koster-Kamphuis,[3] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Netherlands; [2]Pathology, Radboud University Nijmegen Medical Center, Netherlands; [3]Pediatric Nephrology, Radboud University Nijmegen Medical Center, Netherlands.*

Background: Glomerular involution is a special pattern of glomerular injury (Kidney Int 2007;71:44). It is distinct from global glomerulosclerosis, and characterized by a reduction in size, the presence of vital podocytes, parietal epithelial cells and the notable absence of periglomerular fibrosis. Involuted glomeruli were detected in children with frequently relapsing minimal change nephrotic syndrome (MCNS). The percentage ranged from 0-33% and depended on the interval between disease onset and renal biopsy. In the present study, we show that glomerular involution is present in renal biopsies of patients with other proteinuric disorders.

Methods: We identified 4 patients with proteinuria and biopsy reports that suggested the presence of glomerular involution. All slides were reviewed, and evaluated for the presence of involuted glomeruli, based on the abovementioned morphologic criteria.

Results: Patient 1 is a child with a compound heterozygous *NPHS1* mutation, with a spontaneous partial remission, and a 2nd biopsy during relapse. Patient 2 has a membranous nephropathy, with 2nd biopsy during relapse. Patient 3 has a mitochondrial nephropathy with FSGS. Patient 4 has a mesangiocapillary glomerulonephritis, with 2nd biopsy during relapse. Characteristics are in the table. The percentage involuted glomeruli ranged from 5-30%. The identification of involuted glomeruli in adults was hampered by the presence of globally sclerosed glomeruli of other types, with surrounding fibrosis.

Patient	Age at disease onset (yr)	Age at biopsy (yr)	Max. number of glomeruli per section	Glomerular involution (%)
1	0	3.2	30	30
2	44.7	62.2	20	15
3	< 30?	49.4	12	8
4	20.0	29.1	24	5

Conclusions: Glomerular involution is not limited to children with frequently relapsing MCNS. Typically, involuted glomeruli were seen in patients with longstanding, limited proteinuria. Additional studies are needed to determine if involution is a significant cause of nephron depletion in adult proteinuric patients

PUB443

ANCA-Associated Systemic Vasculitis Newly Onset in Maintenance Hemodialysis Patients

Huijuan Mao, Changying Xing. *Department of Nephrology, First Affiliated Hosptial of Nanjing Medical University, Nanjing, Jiangsu Province, China.*

Background: ANCA-associated systemic vasculitis (AASV), newly onset in uremic patients maintaining renal replacement therapy for several years, has not been reported before. Here we reported two cases in order to cause the clinical concerntration.

Methods: The two cases, both of male, aged 76 and 65 years old respectively, who underwent maintenance hemodialysis were retrospectively analyzed with clinical information, including diagnosis, treatment and outcome.

Results: The two patients have both maintaining renal replacement therapy for several years because of IgA nephropathy and chronic interstitial nephritis respectively. But they had cough and asthma several months ago,accompanied by fever,fatigue, anorexia; The chest CT scan showed diffuse lung involved.The treatments such as anti-infection and improving heart function didn't get any effect. Subsequently the 2 patients both had ANCA positive results(MPO and p-ANCA both positive). After steroid and cyclophosphamide treatment, those clinical symptoms were relieved obviously and ANCA examinations were negative several month later.

Conclusions: AASV newly onset in uremic patients is so rare that it is easy to be misdiagnosed and missed diagnosed, especially in the early stage. The importance of ANCA examination in the diagnosis should be paid much attention to uremic patients.

PUB444

Mesangial Hypercellular Infantile Nephrotic Syndrome

Olivera Marsenic, Ajla T. Wasti, Zhongxin Yu, Kevin Couloures, Dwayne D. Henry, Martin A. Turman. *Pediatric Nephrology, Oklahoma University Health Sciences Center, Oklahoma City, OK.*

Background: Infantile nephrotic syndrome (NS) is mainly caused by genetic mutations and has poor treatment response and prognosis. Idiopathic diffuse mesangial hypercellularity (DMH) has a good response to treatment and prognosis. DMH is seen in young children with NS, but has not been reported as cause of infantile NS.

Methods: A nine-month-old girl presented with gross hematuria, edema, hypertension, large proteinuria, decreased renal function and hypoalbuminemia. Tests for secondary (infectious and autoimmune causes, malignancy) and primary etiology were obtained. Renal biopsy was performed.

Results: Investigations for secondary NS were negative. Karyotype was 46 XX. Genetic tests for mutations in PLCE1, LAMB2, WT1, NPHS1, and NPHS2 genes were negative for known disease-causing mutations. Histologic testing showed mesangial expansion primarily by increased mesangial cellularity, and mild mesangial sclerosis. Immunofluorescence was negative. Electron microscopy showed expanded mesangial areas by increased cell number and matrix and extensive effacement of foot processes with podocyte hypertrophy and microvillous transformation of the podocyte cytoplasm. No immune type electron dense deposits were seen. She was treated with steroids and supportive treatments. Her renal function normalized and proteinuria improved over two weeks, with gross hematuria lasting 3 weeks. Steroids did not achieve full remission, and Tacrolimus was initiated. Full remission was then achieved with normal renal function, normal blood pressure and no recurrence during 5-month follow-up period.

Conclusions: We suggest that DMH be included in differential diagnosis of infantile NS. It has good prognosis with good response to immunosuppressive treatment and therefore its recognition is important in order to start treatment. This is contrary to NS due to genetic mutations that comprises majority of cases of NS in first year of life, is resistant to immunosuppressive treatment and treatment attempts are not recommended.

PUB445

The Therapeutic Mechanism of Total Flavonoids of Ajuga on Mesangial Proliferative Glomerulonephritis

Weihua Peng. *Dept. of Nephrology, Fuzhou General Hospital, Nanjing Command PLA, Fuzhou, Fujian, China.*

Background: To observe the mechanism of total flavonoids of Ajuga (TFA) on mesangial proliferative glomerulonephritis,we prepared TFA containing serum in vitro.

Methods: The glomerular mesangial cells(GMC)synchronized in the quiescent culture were divided into different groups.After confounding factor added, the cells and medium were collected in 24h&48h to detect the proliferation of GMC by MTT assay.The expression of type IVcollagen (Col-IV)was observed by ELISA assay.Mitotic cycle ofGMC was detected by flow cytometry in 48h.

Results: After 24h&48h, the cell viability had reached more than 95% in four kinds of concentration of drug-containing serum group, respectively, with no cell toxic evidence. The proliferation of GMC and the expression of Col-IV in TFA-containing serum group at 24h&48h were obviously lower than those in LPS group.When acting on GMC for 48h, the GMC percentage in G_1 phases in TFA-containing serum group was obviously higher than those in LPS group, while lower in S phases.

Table1 The influence of TFA on GMC ($\chi\pm s$)

		IOD of GMC		Col-IV (ng/ml)		Cell phase (%)	
	n	24h	48h	24h	48h	G1	S
normal control group (10% normal rat serum)	6	0.219± 0.025	0.321± 0.020	17.60± 5.83	35.20± 19.58	81.44± 5.31	12.29± 2.20
model control group (LPS 10μg/ml and 10% normal rat serum)	6	0.325± 0.026**	0.598± 0.093**	85.95± 38.77**	140.73± 43.51**	60.88± 6.40**	33.51± 3.42**
10%TFAgroups(10μg/ml LPSand 10% concentration of TFA serum)	6	0.231± 0.028ΔΔ	0.307± 0.011ΔΔ	28.75± 13.04Δ	47.72± 14.89ΔΔ	78.15± 3.10ΔΔ	14.26± 1.74ΔΔ
5%TFAgroups(adding 10μg/ml LPS and 5% concentration of TFA serum)	6	0.262± 0.044Δ	0.388± 0.063*ΔΔ	41.54± 18.41*Δ	74.32± 27.62*Δ	70.38± 3.42**Δ	23.51± 2.39**ΔΔ
2.5%TFAgroups(adding 10μg/ml LPS and 2.5% concentration of TFA serum)	6	0.273± 0.038*Δ	0.441± 0.093*Δ	48.97± 16.98**	93.35± 19.68**Δ	68.32± 4.17**Δ	27.48± 2.45**Δ

Notes:Compared with normal control group*P<0.05,** P <0.01;Compared with model control groupΔP<0.05, ΔΔP <0.01.

Conclusions: The research indicated that the TFA might be related to regulate the expression of cell cycle, decrease the proliferation of GMC and the expression of extracellular matrix.

Funding: Government Support - Non-U.S.

PUB446

Extra Medullary Haematopoiesis in the Kidney: Two Case Reports

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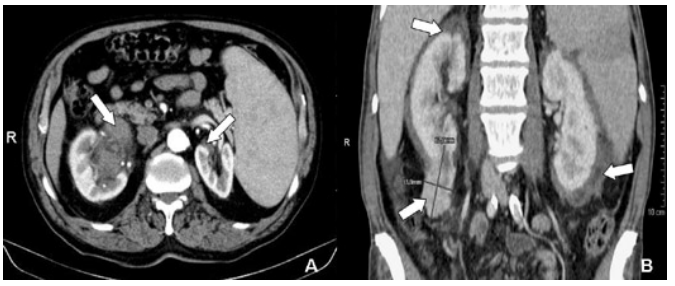
Background: The differential diagnosis of renal masses is quite wide and includes tumor, inflammation and various proliferative diseases. Often extramedullary haematopoiesis (EMH) in not considered because the involvement of other parenchymatous organs rather than liver and spleen is rare and there are only sporadic reports concerning kidney.

Methods: Case 1: An eighty-year old patient had a diagnosis of post-polycytemia myelofibrosis. The computed tomography (CT) shows bilateral parapyelic solid renal lesions that can simulate renal carcinoma.



CT guided needle biopsy was then performed. The histological exam showed positive glycophorin cellularity in the erythroid component, positive myeloperoxidases in the myeloid component with negativity for CD34 compatible with extramedullary erythropoiesis.

Case 2: In seventy-nine year old patient with perirenal infiltrating tissue, hepato-splenomegaly and idiopathic myelofibrosis, CT showed bilateral perirenal tissue with modest contrast enhancement (CE) and showed a solid right-inferior polar formation with an intense CE. The CT guided needle-biopsies revealed to the right a clear cell Adeno-Ca, while the bilateral perirenal tissue was an haematopoietic tissue.

Conclusions: Extra medullary haematopoiesis (EMH) in the kidney represents an interesting "speculative challenge" in terms of differential diagnosis in renal masses, tissue biopsy is nullifying. Furthermore the localization of hemopoietic tissue in the kidney, that is not a customary district, raises controversial questions: 1) Does the kidney possess a niche for haematopoietic stam cells (HSCs)? 2) Is the heterotopic haematopoietic tissue a true "station" of hemopoisis or just an aberrant tissue that must be cyto-reduced?

PUB447

Negative Double Stranded DNA and Anti-Smith Antibodies in Severe Lupus Nephritis Gagangeet S. Sandhu,[1] Anip Bansal,[1] Aditi Ranade,[2] James P. Jones.[1] *[1]Nephrology, St. Luke's - Roosevelt Hospital Center, Columbia University College of Physicians & Surgeons, New York, NY; [2]Pathology, St. Luke's - Roosevelt Hospital Center, Columbia University College of Physicians & Surgeons, New York, NY.*

Background: In Systemic lupus erythematosus (SLE) auto-antibodies are generated against a variety of intracellular antigens. Anti-Smith (Sm) and anti-double stranded DNA (dsDNA) antibodies are particularly considered to be nephritogenic. In addition, severe lupus nephritis (LN) may facilitate the process of Anti-Myeloperoxidase (MPO) antibody (p-ANCA) formation by promoting neutrophil degranulation. However, apart from mere seropositivity, ANCAs may have a possible pathogenetic role in LN.

We had a case where the patient had diffuse proliferative "full house" LN with very high serum anti-MPO titers, yet negative ds-DNA and anti-Sm antibodies.

Methods: Review of PubMed literature for cases of lupus nephritis with negative dsDNA antibodies.

Results: Although extremely rare, a few subsets of patients with drug-induced (hydralazine) LN have been described in the literature to have negative dsDNA and anti-Sm antibodies on serological screening. Our patient had no evidence of drug induced LN. On further review, and similar to our case, we found only 6 additional well documented cases of non-drug induced severe LN with negative dsDNA antibodies.

Six cases of non-drug induced lupus nephritis with negative dsDNA antibodies

Number	Age/Sex	Presentation	ANA	ds-DNA	Complements	p-ANCA	Renal Biopsy
1	9/F	Renal Failure	Neg.	Neg.	Normal	N/A	LN type IV
2	8/F	Nephrotic	Neg.	Neg.	Normal	N/A	LN type IV/ V
3	10/F	Nephritic	Neg.	Neg.	Normal	N/A	LN type III
4	10/F	Atypical	Neg	Neg.	Normal	Neg.	LN type III
5	22/F	Renal Failure	1:1286	Neg.	Low	+	LN type IV & ANCA GN
6	50/F	Renal Failure	1:160	Neg.	Low	+	LN type III & ANCA GN
7	29/F	Renal Failure	1:80	Neg.	Low	+	LN type IV/ V

(7) Our patient. Abbreviations: F: female; Neg: Negative; N/A: Not reported; LN: Lupus Nephritis; GN: Glomerulonephritis

Conclusions: Although considered nephritogenic, dsDNA and anti-Sm antibodies may be negative even in patients with severe proliferative LN.

PUB448

Association of Glomerular Podocytopathy and Systemic Lupus Erythematosus Roberto Savio Silva Santos, Daniela Loss Mattedi, Liliany P. Repizo, Lectícia Jorge, Rui Toledo Barros, Viktoria Woronik. *Department of Nephrology, University of Sao Paulo, SP, Brazil.*

Background: The aim of this study was to evaluate the clinicopathologic features of patients with systemic lupus erythematosus and glomerular podocytopathy.

Methods: We performed a retrospective study of 17 patients with SLE diagnosis according of the American Rheumatologic Association, proteinuria and a histologic diagnosis of MCD, mesangial proliferative glomerulonephritis or FSGS.

Results: The baseline clinical characteristics are shown in Table 1.

Clinical Features

Number	17
Age (yr)	34,4(±9,7)
Female	82,4%(14)
Serum Creatinine(mg/dl)	1,3(±0,9)
Albumin(g/dl)	1,8(±0,65)
Proteinuria(g/day)	5(±3,9)
Active urine sediment	35%(6)
C3(mg/dl)	100,5(±46,7)
ANA(>1/80)	88%(15)
Hypertension	41%(7)
Nephrotic Syndrome	88%(15)
Time since SLE diagnosis > 12 months	30,8%(4)

Results of continuous variables are mean SD.

Regarding the histological features: 2 biopsies (11,8%) were normal by light microscopy, 2 (11,8%) demonstrated mesangial proliferation alone, 9 (52,9%) had FSGS and 4 (23,5%) had GESF accompanied by mesangial proliferation. Immunofluorescence showed focal and segmental IgM and/or C3 deposits in 9 biopsies, had only mesangial deposits of IgG, IgM, C3 and C1q and was completely negative in 4 biopsies. The patients were treated with prednisone alone (12) or in association with cyclophosphamide (2), azathioprine (2) or cyclosporine (1). Complete or partial remission occurred 11 patients (65%). At the end of follow-up (82,5 ± 73,9 months) no patient progressed to end stage renal disease (creatinine 0,8 ± 0,16mg/dL; proteinuria 1,2 ± 0,9g/day; serum albumin concentration, 3,6 ± 0,6g/dL). Two patients underwent a second biopsy during nephritic flair and proliferative lupus nephritis was detected. Finally, two patients died from infection complications probably related to the immunosuppressive therapy, one patient received prednisone plus cyclophosphamide and another received prednisone plus azathioprine.

Conclusions: We report 17 cases of patients with SLE and glomerular podocytopathy, that was characterized by nephrotic syndrome, good response to steroid therapy and seems to entail low risk of progression to ESRD.

Funding: Government Support - Non-U.S.

PUB449

CT Scan Guided Percutaneous Kidney Biopsy Performed by Nephrologists: Description of the Procedure and Outcomes Jinil Yoo,[1] Roger F. Carbajal Mendoza,[2] Nripesh Pradhan,[2] Lin N. Lwin,[1] Hugo J. Villanueva.[1] *[1]Nephrology, Montefiore Medical Center, North Division, Bronx, NY; [2]Renal Service, Metropolitan Hospital Center, New York, NY.*

Background: The real-time ultrasonography has helped nephrologists to obtain adequate kidney tissue with less postbiopsy complications. Currently a CT-guided kidney biopsy is mostly performed by interventional radiologists.

Methods: The Nephrology Division [1] introduced a CT guided percutaneous kidney biopsy with an automated 18-gauge (G) biopsy-cut needle and "matched" 17–G guide-needle in 1999. The biopsies have been performed primarily by nephrology fellows under the supervision of an experienced nephrologist. First, a 17-G guide-needle is introduced and its position in the cortex is confirmed by CT scanning. Then, a 18-G biopsy needle is passed through the guide-needle to obtain 2 cores of tissue, followed by postbiopsy scanning.

Results: During the 11- year period , 107 biopsies in total were performed. 56 out of 107 biopsies were performed on an outpatient (OP) basis (discharged home after 6-hour observation at the ambulatory surgery unit after the procedure) and 51 of 107 were done for in-hospital patients (IP). 107 biopsies in all yielded enough tissues for the light-, immuno-histologic and electron microscopy. 3 out of 107 experienced gross hematuria (2 from IP, 1 from OP), not requiring transfusion, but one from OP required overnight observation. No other significant complications related to the procedure were observed. The average age of the patients: 46 (range of 20 to 87 years), the average counts of glomeruli from each biopsy: 22 (3 to 67 glomeruli) and serum creatinine level: average 3.1 mg /dL (OP: average 2.1 mg /dL with range of 0.6 to 8.4, IP: average 4.2 with range of 0.7 to 12.8 mg/dL).

Conclusions: We believe that a CT-guided percutaneous kidney biopsy technique described above can be performed safely and successfully by nephrologists for diagnostic clarity of more kidney diseases.

Funding: Clinical Revenue Support

PUB450

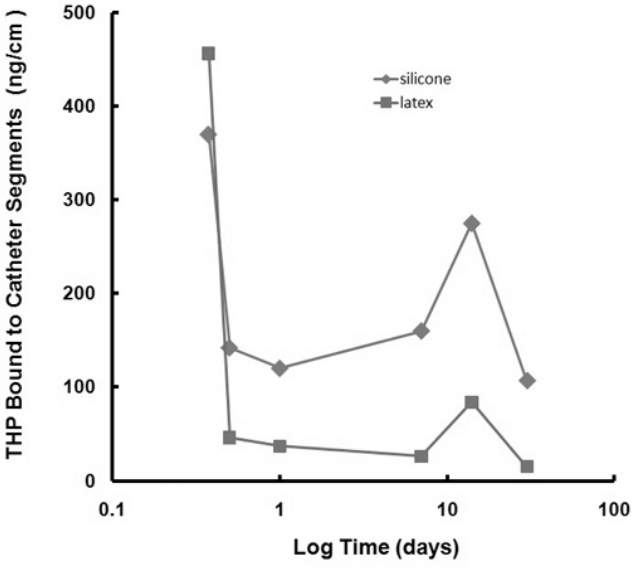
Tamm Horsfall Protein Binds to Urinary Catheters James M. Bates, Hajamohideen S. Raffi, Satish Kumar. *Medicine/Nephrology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Urinary catheters are commonly associated with bacteriuria and frequently with urinary tract infection. Tamm-Horsfall Protein (THP) is urine's most abundant protein and is known to bind to uropathogenic bacteria. We hypothesized that THP may adsorb to the surface of urinary catheters and facilitate bacterial binding. In this study, we determined the adsorption of THP to two common types of catheter in clinical use in the United States.

Methods: Bardex Infection Control (latex) and Lubri-Sil Infection Control (silicone) 20 French catheters were cut into 1-cm segments. The catheter segments were incubated with human urine at 37°C, in sterile, rotating tubes with a daily change of the solutions. Three catheter segments were removed each at 9, 12 and 24 hrs and on days 7, 14 days and 30, and rinsed in PBS. THP bound to the catheter segments was extracted in 0.5 % Triton-X 100/20 mM EDTA/pH 7.5 and quantitated by ELISA.

Results: Both silicone and latex urinary catheters were quickly and equally coated with THP from the first time point at 9 hrs (silicone, 370 ng/cm ± 65 and latex, 456 ± 155 ng/cm). At later time points, more THP was found bound to the silicone catheters than the

latex catheters: 12 hr (142 ng/cm ± 15 vs 46 ± 37 ng/cm, P = 0.001) , day 1 (120 ng/cm ± 4 vs 37 ± 5 ng/cm, P = 0.0001) , day 7 (160 ng/cm ± 33 vs 26 ± 5ng/cm, P = 0.003), day 14 (275 ng/cm ± 41 vs 84 ± 4 ng/cm, P = 0.002) and day 30 (107ng/cm ± 32 vs 15 ± 5 ng/cm, P =0.014). THP binding to urinary catheters increased further after 7 days of incubation.



Conclusions: THP binds to both latex and silicone urinary catheters, and may provide a substrate for bacterial binding. THP binding to urinary catheters begins to increase after 7 days. Catheter removal or change at 7 days may help prevent catheter associated urinary tract infection.

Funding: NIDDK Support, Veterans Administration Support

PUB451

Wnt-7a Counteracts Epithelial to Mesenchymal Transition in Mice of Unilateral Ureteral Obstruction Model Guo-Qin Wang,[1] Pei-Ling Bao,[1] Hong Cheng,[1] Ping Li,[2] Yi-Pu Chen.[1] *[1]Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China; [2]China-Japan Friendship Hospital, Beijing, China.*

Background: To investigate the protective effect of Wnt protein on renal interstitial fibrosis in unilateral ureteral obstruction(UUO)mice model.

Methods: Eighteen male C57BL/6 mice were randomly divided into 3 groups: sham-operation group, the UUO model group and treatment group; the body weight of mice were measured everyday.All mice were sacrificed at the7th day after the operation.The left kidney was taken for histology evaluation and molecular biology assay. Masson'S stain was performed as an main indicator of interstitial fibrosis. The expressions of vimentin, a-smooth muscle actin(a-SMA), and E-cadherin in renal tissue were detected by immunohistochemistry staining and Western-blot, respectively.

Results: Compared with sham-operation group, body weight of the model group was significantly lower(P<0.05) , and the relative area of interstitial fibrosis was significantly larger(P<0.05) . Furthermore, the expressions of vimentin and a-SMA were significantly up-regulated(P<0.05), and E-cadherins were significantly down-regulated(P<0.05). Compared with model group, all the above-mentioned abnormalities were restored to some extent and showed significant difference (P<0.05) in treatment group.

Conclusions: Wnt protein could decrease the interstitial fibrosis by counteracting epithelial to mesenchymal transition in UUO mice.

Funding: Government Support - Non-U.S.

PUB452

Examining the Patient-Centered Decision Making Attributes Towards Blood Transfusion among Individuals with Chronic Kidney Disease Ahmad B. Naim,[1] Danielle Walls,[2] Jan Gollins,[3] Chuck Reynolds.[4] *[1]HECOR, Centocor Ortho Biotech Services, LLC, Horsham, PA; [2]Project Manager, BDJ Solutions, Medford, MA; [3]Principal & Co-founder, Delta Modelling Group, LTD, Mount Prospect, IL; [4]President, Employer Practice, The Benfield Group, St. Louis, MO.*

Background: Examine patient engagement towards blood transfusion among individuals with chronic kidney disease (CKD) currently not on dialysis.

Methods: An online survey was conducted from a nationally representative patient panel in 1Q2011. All respondents were ≥18 years and diagnosed with CKD by a physician. Participants were asked about their blood transfusion history, information seeking behaviors, and knowledge about blood transfusion.

Results: Of 416 respondents: 59%(n=246) female; 40%(n=165) were >65 years. 35%(n=144) had stage4 and 58%(n=240) stage3 CKD. 54%(n=226) were anemic. 43%(n=179) had received blood transfusion, whereas, 57%(n=237) had no transfusions. Among previously transfused, only 50% indicated they shared in treatment decision with their doctor, whereas 40% indicated their doctor or someone else had made the decision for them. Among those who indicated someone else made the decision, 82% indicated that they like to make a shared decision. Among not transfused, only 40% are clear about their treatment choice for blood transfusion and over 75% would like to share decision to have blood transfusion with their doctor. Among not transfused, 30% agree that they are unsure about receiving blood transfusion and less than two-thirds (60%) are likely to stick with their decision to get a blood transfusion. About 39% of not transfused said it is hard to decide if benefits outweigh risks and 38% said that decision is hard for them to make.

Conclusions: There is a substantial lack of patient engagement towards shared-decision making in blood transfusion. Individuals most likely to receive blood transfusion expressed the most uncertainty about their decisions and are least informed about choices, benefits, and risks. These findings suggest that a significant portion of individuals facing a blood transfusion feel disempowered and are interested in engaging in shared-decision making with their physicians.

Funding: Pharmaceutical Company Support

PUB453

Examining Knowledge and Information Seeking Behaviors Towards Blood Transfusion among Individuals with Chronic Kidney Disease Ahmad B. Naim,[1] Danielle Walls,[2] Jan Gollins,[3] Chuck Reynolds.[4] *[1]HECOR, Centocor Ortho Biotech Services, LLC, Malvern, PA; [2]Project Manager, BDJ Solutions, Medford, MA; [3]Principal & Co-founder, Delta Modelling Group, LTD, Mount Prospect, IL; [4]President, Employer Practice, The Benfield Group, St. Louis, MO.*

Background: Examine knowledge and information seeking behaviors towards blood transfusions among individuals with chronic kidney disease (CKD) currently not on dialysis.

Methods: An online survey was conducted from a nationally representative patient panel in 1Q2011. Respondents were ≥18years and diagnosed with CKD by a physician. Participants were asked about blood transfusion history, information seeking behaviors, and knowledge about blood transfusion.

Results: Of 416 respondents: 59%(n=246) female; 40%(n=165) >65 years; 35%(n=144) had stage4 and 58%(n=240) stage3 CKD. 54%(n=226) were anemic, 43%(n=179) had received blood transfusion, 57%(n=237) had no transfusions. Top 2 sources of information were doctor(93.8%) and Internet(80.5%). Among previously transfused, 62% received right amount of information, 34.6% received too little information, and 3.4% reported receiving too much information. More than 80% of transfused indicated knowing reasons for and benefits of getting a blood transfusion. Less than two-thirds received information about effects, risks, and time it would take; only 26% knew the costs. Over 60% said it is extremely important to know right blood type, screening techniques and quality of blood, and risks of infections. Among previously transfused, only 50% agreed they made an informed choice about receiving blood transfusions. Among previously transfused, 77% agree they knew the benefits vs 49% not transfused. Among previously transfused, 69% agreed they knew the risks of blood transfusion vs 51% with no transfusion history.

Conclusions: Doctor and Internet are primary sources of information about blood transfusions. Gaps in knowledge exist about benefits, risks, and costs of blood transfusions. A significant number feel they need more information about blood transfusion to make an informed choice. Providers should consider adopting shared-decision making with their patients.

Funding: Pharmaceutical Company Support

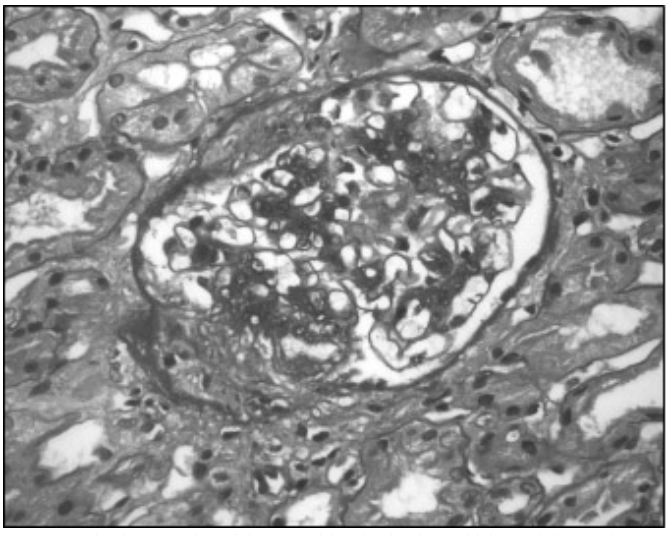
PUB454

Treatment of IgA Myeloma Results in Remission of Henoch-Schonlein Purpura Sandip Sandilya, Coral Parikh, Katherine Rausa, Ladan Golestaneh. *Nephrology, Montefiore Medical Center, Bronx, NY.*

Background: The pathogenesis of IgA nephropathy involves overproduction of IgA and deposition in mesangium, decreased clearance of IgA protein, and inflammatory response to IgA deposition. There are reported cases of the abnormally glycosylated IgA in IgA myeloma.

Methods: We present a patient with recurrent HSP who was diagnosed with IgA-producing myeloma.

Results: A 28 year old man presented with abdominal and joint pain, purpura, recurrent GI bleeds and hematuria over several months. A skin biopsy showed early leukocytoclastic vasculitis with IgA positive immunofluorescence. He was diagnosed with HSP, and flares were managed with high dose steroids. Immunofixation showed an IgA lambda monoclonal protein, and the results of a bone marrow biopsy were consistent with myeloma. His kidney biopsy showed mesangioproliferative glomerulonephritis.



He received two cycles of therapy with velcade, doxorubicin, and dexamethasone, consolidation with cytoxan, and an autologous stem cell transplantation. The patient's hematuria and proteinuria resolved after the stem cell transplant. Subsequent SPEP, quantitative immunoglobulins, and bone marrow biopsy have shown no signs of recurrence of IgA myeloma.

Patient Data

	Before	After
Bone Marrow Biopsy	Hypercellular marrow (60-70%), 20% atypical plasmacytosis consistent with plasma cell myeloma	Normocellular marrow
IgA level	2040 (68-243)	356 (68-423)
Urine Analysis	Total protein 3393 (50-100), RBCs 4261 (0-4)	Total protein 33, RBCs 0

normal values in parentheses

The patient has had no more flares of HSP over the last three years.

Conclusions: Resolution of both HSP and nephropathy are documented after treatment of IgA myeloma with simultaneous reduction in IgA levels. The resolution of nephropathy with treatment of this myeloma argues for overproduction of the IgA subtype as the main pathogenic factor in this case.

PUB455

Lack of Awareness in Future Medical Professionals of Risk of Consuming Hidden Phosphate-Containing Diet Yoshiko Shutto,[1] Michiko Shimada,[1] Hideaki Yamabe,[1] Mohammed S. Razzaque.[2] *[1]Nephrology, Hirosaki University School of Medicine, Hirosaki, Japan; [2]Oral Medicine, Harvard School of Dental Medicine, Boston.*

Background: Hyperphosphatemia is the single most important determinant of mortality in chronic kidney disease (CKD) patients receiving hemodialysis. CKD patients are advised to take low phosphate diet with phosphate lowering drugs. However, phosphate-containing ingredients are widely used as preservative in processed foods/soda drinks, and thereby can affect to total phosphate intake. As patients seek advice from medical professionals on phosphate-containing diet, we conducted a survey to determine the level of awareness in future medical professionals of diet containing artificial phosphate ingredients.

Methods: We randomly selected 190 medical and nursing students (average age: 21.6 years) at Hirosaki University School of Medicine in Japan and asked them to fill out a questionnaire.

Results: While 99% of students are aware of increased sugar-content in soda drinks, only 7% are aware of presence of phosphate (phosphoric acid) in such drinks. Similarly, only 12% of students are aware of presence of phosphate-containing ingredients in processed foods, including burgers/pizzas, etc. More importantly, 68% of the surveyed students are unaware of possible harmful effects of unrestricted consumption of phosphate-containing foods/drinks. Furthermore, 28% of surveyed students consume "fast food" at least once/week, while another 36% take such food once/month. However, after realizing long-term risks of consuming excessive phosphate, 41% of students want to reduce their phosphate-intake by minimizing consumption of fast foods/soda drinks, while another 48% showed interest in getting more information.

Conclusions: The survey highlights two important points: medical/nursing students, the future medical professionals, who will soon assume the role of patient management **1**) are not thoroughly aware of the risk related to prolonged high-phosphate intake; **2**) are not fully aware of the foods/drinks that contain hidden phosphate ingredients. This survey exposes the need for an education initiative to raise the awareness of risk posed by diet with hidden phosphate-containing ingredients.

Funding: NIDDK SupportR01-DK077276

PUB456

Interdialytic Weight Gain and Food Intake in Hemodialysis Patients, Users of a Private Clinic in Sao Paulo, Brazil Carmen B. Tzanno-Martins,[12] Camila Machado de Barros,[1] Bárbara Margareth Menardi Biavo,[1] Jacqueline Santos,[1] Elzo R. Junior.[12] *[1]Nephrology, Home Dialysis Center, São Paulo, SP, Brazil; [2]Nephrology, Integrated Center of Nephrology, Guarulhos, São Paulo, Brazil.*

Background: Individuals with chronic kidney disease (CKD) on dialysis treatment can present disorders of nutritional status and inadequate dietary intake. The purpose of this study was to evaluate interdialytic weight gain and food intake in hemodialysis patients, users of a private clinic in Sao Paulo.

Methods: This is a cross-sectional study which evaluated 97 patients. Clinical and socio-demographic data were collected, weight and height were measured and food intake was evaluated by three 24 hour-recalls. Study variables were: pre and post-dialysis weight, interdialytic weight gain, body mass index (BMI), energy intake, carbohydrates, proteins, lipids, sodium, phosphorus and potassium. It was also conducted qualitative analysis of the diet through the consumption of different food groups.

Results: According to BMI, the majority of elderly and adult had normal weight, but the elderly had a higher prevalence of malnutrition (25.0%) compared to younger patients (5.3%). The analysis of interdialytic weight gain showed that 48.5% had adequate weight gain (3 to 5% of "dry weight"), and only 15.5% of subjects had excessive weight gain. The intake of oils, sugar, beans and meat, fish and eggs proved to be excessive. Energy intake, as well as the intake of carbohydrates and lipids, presented below requirements values for most individuals. The consumption of protein and sodium was high for most individuals. Potassium intake was below the recommended values and phosphorus proved to be adequate.

Conclusions: The interdialytic weight gain was adequate in most of the population studied but it was identified inadequate food consumption, characterized by an imbalance of intake of macro and micronutrients. Therefore, it is important for individuals with CKD the adoption of proper diet to maintain their health.

PUB457

Treatment of Late Antibody Mediated Rejection in Kidney Transplant Recipients: The Johns Hopkins Experience Bassam G. Abu Jawdeh,[1] Gaurav Gupta,[1] Robert Avery Montgomery,[2] Brandon L. Trollinger,[3] Edward S. Kraus,[1] Niraj Desai,[2] Nada Alachkar.[1] *[1]Division of Nephrology, Johns Hopkins University; [2]Division of Transplant Surgery, Johns Hopkins University; [3]Department of Pharmacy, Johns Hopkins University, Baltimore, MD.*

Background: Several strategies for treating early antibody mediated rejection (AMR) have been investigated, however, evidence is sparse on the utilization and success of these therapies in late AMR. In this study, we present data from 13 patients who were treated for late AMR at our institution.

Methods: We collected data from 13 patients who developed biopsy-proven AMR after 6 months from the time of transplantation. AMR was defined as having at least two of the following pathologic changes: glomerulitis (g-score), peritubular capillary margination (ptc-score) and C4d staining (C4d-score). All patients had class I and/or class II donor specific antibodies (DSA) and were treated with plasmapheresis followed by low dose Cytogam +/- Rituximab +/- Bortezomib +/- high dose IVIg. 11 patients had at least one follow up allograft biopsy. Parameters followed through and post-treatment were serum creatinine (SCr), estimated GFR (eGFR), DSA levels and histopathologic scores.

Results: The median follow up from the time of biopsy proven AMR was 4 months (range: 1 to 15 months). The mean rate of change in SCr was -0.07 mg/dl/month (range: -0.24 to +0.11 mg/dl/month). The mean change in eGFR since diagnosis of AMR was +0.55 ml/min/1.73m2 (range: -1.71 to +3.33 ml/min/1.73m2). DSA levels of 8 patients, all with class II, did not decrease with treatment; the other 5 patients responded partially, however continued to have low level DSA. Only 2 patients had reduction in their g-scores, 2 in their ptc-scores and 5 in their C4d-scores.

Conclusions: We infer that treatment of late AMR is not associated with significant improvement in SCr, eGFR, DSA levels or histopathologic scores.

PUB458

Nested Case Control Study with Follow up Survey for Polyoma Virus among Kidney Transplant Recipients Ahmed G. Adam,[1] Nagwa Farouk,[1] Mona Salem,[2] Doaa Hashad,[3] Hala S. Elwakil.[1] *[1]Internal Medicine - Nephrology, Dialysis & Transplantation Unit, Faculty of Medicine; [2]Pathology, Faculty of Medicine; [3]Clinical Pathology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt.*

Background: Polyoma virus associated nephropathy is an increasingly recognized cause of graft dysfunction among kidney transplant recipients. It is related somehow to modern potent immunosuppression aimed at reducing acute rejection. Asymptomatic viremia and /or nephritis with or without worsing renal function may be present. Biopsy remains the gold standard for diagnosis. Untreated cases lead to allograft dysfunction or loss. There is no safe and effective antiviral therapy, so prevention based upon a screening and preemptive strategy is superior to an approach that relies upon therapy of established disease

Methods: 74 transplant recipients in University of Alexandria, Egypt were included. Urine decoy cells, PCR for BKV and Biopsy were done. Positive cases of cytology or PCR are followed up within 1-3 months.

Results: 7 +ve for decoy cells (10%), 3 viremia by PCR (4%), only one nephropathy (1.4%) presented with tubulointerstitial nephritis with intranuclear inclusions of Polyoma JC virus rather than BK, CMV or Herpes. Cases with stable renal function and viruria with or without viremia cleared the virus spontaneously during the period of follow up without the need of intervention. A biopsy proven nephropathy and deteriorating graft function lost the graft and was suggestive of JC Nephropathy (the 11th reported so far) rather than BK.

Case #	Decoy cells	PCR	Renal Biopsy
1	+/=	-/-	TIN-Inclusion
2	-/-/-	500/50/0	
3	+/-	30/0	
4	+/-	-/-	
5	+/-	-/-	
6	+/=	-/-	
7	+/-	-/-	
8	+/-	-/-	
9	Inflammtory	45	Rejection

Follow up of Decoy, PCR based on Therapy

Conclusions: The trend in quantitative rather than qualitative PCR for BKV is more important than single value especially when considering therapeutic plane. JC as a cause of nephropathy post transplantation do happen and must be considered as part of Polyoma related nephropathy; however more rare than BKV nephropathy, and possibly more aggressive. Decoy cell, viruria and viremia are neither synonymous nor interchangable and all are necessary for proper management.

PUB459

Therapeutic Options for Recurrent Focal Segmental Glomerulosclerosis after Kidney Transplantation- The Need for Multicenter Trials Vaqar Ahmed, Sarat C. Kuppachi, Beje S. Thomas, Anita Sultan, Maria F. Egidi. *Nephrology, Medical University of South Carolina, Charleston, SC.*

Background: No controlled trials have been performed to address the management of recurrent FSGS post KTX. Most studies reported single center experience with enrollment of few patients and discrepant results. Purpose of the study is to compare our management of recurrent FSGS with other experiences (not shown) and to address the need for collaborative approach.

Methods: Analysis of 7 KTX recipients with recurrent FSGS who received plasmapheresis (PP). Additionally 5 patients received 3 doses of rituximab (RTX) at 375 mg/meter square. Two living donor recipients received PP and RTX pre-KTX.

Results: One patient had complete remission while 3 had partial remission. Mean follow up 463 days. No graft losses or initiation of dialysis.

Table 1: Summarizes patients demographics, treatment of recurrent FSGS and outcomes in our institution.

Induction post Tx	Maintenance IS	Scr at diagnosis of FSGS recurrence	Treatment of recurrence	Proteinuria [in grams] before PP	Proteinuria [in grams] after last PP cycle	Duration of follow up after relapse [days]	Outcome
Thymoglobulin	Prednisone, MMF, Tacrolimus	3.8	PP	9.4	2.4	240	Scr 2.8, PCR 5.71
Thymoglobulin	Prednisone, MMF, Sirolimus	4.3	PP and RTX	5.4	0.79	285	Scr 2.9, PCR 0.8
Basiliximab	Prednisone, MMF, Tacrolimus, Everolimus	4.9	PP and RTX	20	5.8	365	Scr 1.3, PCR 8.6
Daclizumab	Prednisone, MMF, Tacrolimus	1.8	PP and RTX	3.7	3.7	1380	Scr 2.1, PCR 4.8
Thymoglobulin	Prednisone, MMF, Everolimus	1.2	PP and RTX	7.73	3.8	150	Scr 1.4, PCR 4.6
Basiliximab	Prednisone, MMF, Tacrolimus	4.7	PP	4.8	6.7	90	Scr 2.1, PCR 6.7
Daclizumab	Prednisone, MMF, Tacrolimus	1.1	PP and RTX	7.4	4.4	730	Scr 2.1, PCR 4.1

RTX= Rituximab, PP= Plasmapheresis

Conclusions: PP was effective only in temporary reduction of proteinuria. Whether RTX had an impact on patient outcomes could not been established. Multicenter trials are warranted to assess optimal strategies for recurrent FSGS. As RTX seems to have a role in FSGS treatment, question should be arisen about its consideration for an early introduction or perhaps to be used as an induction agent in this patient population.

PUB460

Urinary Tract Infections in Renal Transplant Patients: The Emergence of ESBL Mamdouh N. Albaqumi, Lutfi Alkorbi, Saad Alghamdi. *KFSHRC, Riyadh, Saudi Arabia.*

Background: UTI affects 5%- 36% of kidney transplant patients. The typical organisms causing post-transplant UTI are the enteric gram negative bacilli and enterococci. However, there is an increased rate of ESBL infections among renal transplant patients which might carry a significant morbidity. We report our center experience with UTI and ESBL- UTI impact in renal transplantation

Methods: We retrospectively reviewed electronic data and medical charts of 165 renal transplant patients followed up by nephrology team of KFSH & RC from 2003 until 2007

Results: A total of 165 patients with renal transplant were included in the analysis. 65 out of 165 (39%) patients develop UTI. Median time interval of developing UTI was 8 months. Females tend to develop more UTI as compared to males, 38(62.3%) vs. 23(37.7%) $p<0.05$. The episodes of acute rejection at 1 year were 11 (17.0%) in the UTI group as compared to 22 (22.0%) in the group without UTI with no statistical difference. There was no difference in graft and patient survival between the two groups at 1 and 2 years follow up. Both groups received similar immunosuppression. Among the UTI group, 9 patients (13.8%) were found to have ESBL positive UTI. Upon subgroup analysis of those developing ESBL, we found that the most frequent β-lactamase mediated resistant gram negative enteric bacilli was E. coli 14 (87.5%) followed by Klebsiella pneumonia 2 (12.5%). All patients in ESBL group 9 (100%) had documented recurrent UTI as compared to 40 (71.4%) in the non ESBL UTI group. Univariate analysis of the risk factors for β-lactamase-mediated resistant gram negative bacilli infection, showed that living related donor, recurrent UTI and number of UTI (>3 episodes) were significantly associated with ESBL UTI.

Conclusions: We found that UTI has no significant impact on renal function, graft function, and patient survival in the first two years. In our study 13.8% developed one or more infections due to ESBL producing gram negative bacilli. We found that recurrent UTI and number of UTI (>3 episode) are the most important risk factors for development of ESBL UTI. Efforts should be made to prevent first episode of UTI in this population of patients which might have a positive impact on long term outcome.

PUB461

Transplant Biopsy for the Early Diagnosis of Graft Dysfunction Secondary to Haemolytic Uraemic Syndrome Mansoor N. Ali, Sunil Bhandari. *Renal Medicine, Hull and East Yorkshire Hospitals NHS Trust, Hull, East Yorkshire, United Kingdom.*

Background: Haemolytic uraemic syndrome, either recurrent or de novo is uncommon post transplantation and is usually diagnosed from haematological changes including thrombocytopenia, anaemia and fragments on blood film. Confirmation with the transplant biopsy is helpful.

Methods: We highlight a series of three patients with acute transplant dysfunction which presented without clinical or haematological clues to the aetiology. Subsequent transplant biopsy revealed Haemolytic uremic syndrome (HUS); two of which had HUS as primary renal disease and one had de novo HUS secondary to possible use of tacrolimus therapy

Results: Early transplant biopsy prior to development of classical haematological features of low platelets and fragments should be undertaken to confirm the diagnosis. In this series biopsies were performed early prior to the fall in platelets and appearance of fragments.

Treatment, using plasma exchange and fresh frozen plasma in combination, intravenous immunoglobulin and switching immunosuppression, led to recovery of haematological parameters but salvage of only one graft function. Screening for complement and ADAMT13 mutations were negative.

De novo and recurrent HUS may occur post transplantation. Classical haematological changes of thrombocytopenia, anaemia (microangiopathic haemolytic anaemia) are a late phenomenon, occurring in only 50% of patients.

Conclusions: Transplant biopsy should be considered early to institute early therapy and recovery of haematological parameters and potentially transplant function thereby avoiding toxic therapy and complications.

PUB462

Cutaneous Calciphylaxsis in a Renal Allograft Recipient Amarpali Brar, Carmencita Yudis, Nabil Sumrani, Fasika M. Tedla. *SUNY Downstate Medical Center, Brooklyn, NY.*

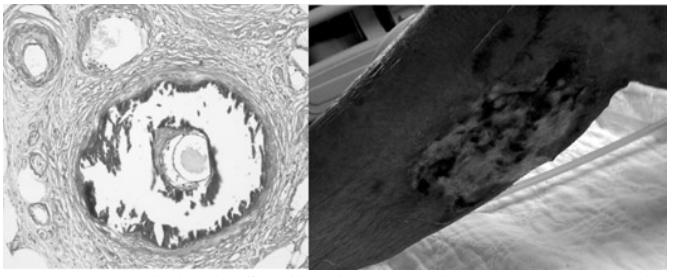
Background: Calcific uremic arteriolopathy is a disease first described in patients on dialysis. It involves calcium deposition in the walls of small and medium sized arteries with consequent ischemic necrosis and gangrene.

Methods: We describe a case of cutaneous calciphylaxsis in a patient with renal allograft transplant.

Results: A 52-year-old African American woman with end stage renal disease due to lupus nephritis who was on hemodialysis for 16 years received a deceased donor kidney transplant in 2002. She received thymoglobulin induction; and tacrolimus and prednisone for maintenance immunosuppresion. Both pre and post transplant serum calcium and phosphorus levels were normal.

Eight years after transplantation, patient was admitted with skin lesions bilaterally on the medial aspect of her thighs. She then had 6 cm X 3cm lesion in left lower extremity after trauma. The lesion was well demarcated, gangrenous in appearance and foul smelling. Peripheral pulses were intact. Laboratory data showed serum creatinine of 2.5 mg/dl, calcium of 8.9 mg/dl, phosphorus of 3.7 mg/dl and PTH of 439 pg/ml which improved over the next two months to 180 pg/ml.

Wound swabs grew Pseudomonas aeruginosa. Intravenous antibiotics were started. A skin biopsy of the lesion confirmed the diagnosis of calciphylaxis.



Later treatment included sodium thiosulfate 25 grams intravenously every other day and cinacalcet 60 mg twice a day.

She was lost to follow up and then returned four months later with worsened leg lesions with purulent discharge. Despite aggressive intervention, patient had multiple episodes of infection at the site of lesions and died due to complications of sepsis.

Conclusions: Calcific uremic arteriolopathy is a rare entity in patients with renal transplant, it is associated with high mortality and an evidence based strategy for management needs to be evaluated.

PUB463

Risk Factors and Outcomes in Renal Transplant Recipients with Hyperuricemia Amarpali Brar, Mary C. Mallappallil, Candace D. Grant, Fasika M. Tedla, Nabil Sumrani, Melissa Rampal, Moro O. Salifu. *SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Hyperuricemia is a common finding post-transplant. However risk factors for development of hyperuricemia and its impact on allograft function remain unclear.

Methods: To determine risk factors for our predominantly African American kidney transplant patients, we analyzed 208 renal transplant recipients in our center from 1998-2007, whose data set for serum uric acid was complete.

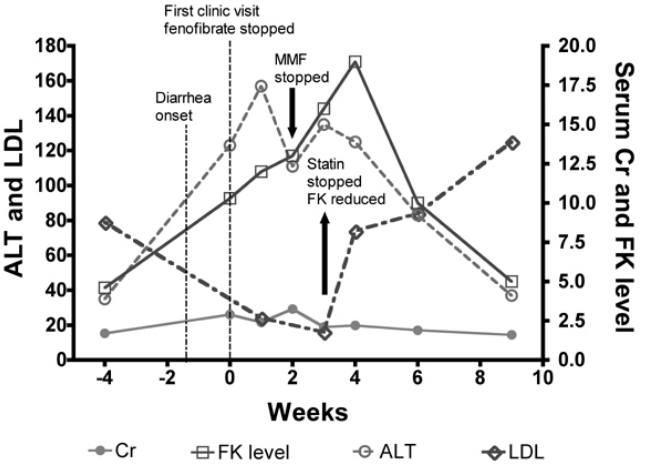
Results: During this period, immunosuppression consisted of antithymocyte globulin induction plus tacrolimus, mycophenolic acid and steroids maintenance immunosuppression. Only 7 patients received cyclosporine. Hyperuricemia was defined as uric acid level over 7 mg/dL. Patients were divided into 2 groups based on their uric acid levels; hyperuricemia (>7mg/dl, 61.5%) and normal (<7mg/dl, 38.5%). Mean age (48.8 13.1 vs. 46.6 14), black race (70.3% vs. 60%), BMI (25.9 5.7 vs. 24.8 4.9), HLA mismatch, tacrolimus levels, cholesterol, donor age, diabetes and race were not significantly different between the groups. Male gender (63.3% vs. 36.3%, p=0.0001) and DGF (18.9% vs. 7.6%, p=0.026) were significantly higher in hyperuricemic patients. Patients with hyperuricemia had significantly higher discharge creatinine (3.2 2.0 vs. 2.3 1.5, p=0.001), 6 weeks creatinine (1.95 1.5 vs. 1.49 0.91, p=0.22), 6 months creatinine (1.6 0.5 vs. 1.3 0.3, p=0.001) and 1 year creatinine (1.6 0.5 vs. 1.2 0.3, 0.001) compared to recipients with uric acid less than 7. However using logistic regression, we did not identify any independent predictors of hyperuricemia in this population. There was also no difference in acute rejection or overall graft survival between the two groups.

Conclusions: Demographics and clinical characteristics did not predict the development of hyperuricemia in this population. Hyperuricemia may be secondary to the high cell turnover in this patient population. This study may be limited by sample size and future studies are needed to elucidate the mechanism of hyperuricemia posttransplant.

PUB464

Mycophenolate Mofetil Induced Duodenal Villous Atrophy in a Renal-Transplant Recipient and Its Effect on Tacrolimus and Lipid Levels Monique E. Cho,[1] Stefanie Glenn,[1] Christine Chris Chamberlain,[2] David Kleiner,[3] Yuen-Yi Hon.[2] *[1]Kidney Disease Branch, NIDDK, NIH, Bethesda, MD; [2]Clinical Center Pharmacy, Clinical Center, NIH, Bethesda, MD; [3]Department of Laboratories, NCI, NIH, Bethesda, MD.*

Background: Mycophenolate mofetil (MMF) commonly causes diarrhea, typically early following drug initiation. We report an atypical case of MMF-associated diarrhea in a 28 year-old kidney transplant recipient, starting after 41 months of maintenance therapy with tacrolimus (FK) and MMF. Infectious etiologies were excluded and the anti-motility agents failed to improve his symptoms. He developed sharp increase in FK levels from 5 to 19 ng/mL, despite having his dose reduced. His LDL decreased from 79 to 16 mg/dL in the setting of stable statin therapy and 4-fold increase in transaminases.



Esophago-gastroduodenoscopic biopsies revealed marked shortening of duodenal villi with repair and regenerative changes, consistent with drug effect and MMF was stopped. Within a few days, his diarrhea improved and completely resolved within 4 weeks. His FK level, lipid profile and transaminases returned to baseline values within 8 weeks. Genotyping revealed that the patient is a homozygous variant for CYP3A5 6986A>G and ABCB1 1236C>T, 2677G>T/A, and 3435C>T, indicating that CYP3A4 is the enzyme primarily involved in drug bioavailability and metabolism. While few case reports have described duodenal villous atrophy with MMF use recently, it is important to consider the effect of intestinal barrier disruption on drug absorption and consequent renal and hepatic toxicities. Possible underlying mechanisms include reduced CYP3A4 expression/activity due to duodenal villi shortening, complex interplay between p-glycoprotein and CYP3A genotype and phenotype, and potential FK-statin interaction.

Funding: NIDDK Support

PUB465

Clinical Usefulness of BK Virus Monitoring by Plasma BKV Quantitative PCR in Renal Transplant Recipients Byung Ha Chung, Yu Ah Hong, Hyun Gyung Kim, In O Sun, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea.*

Background: Because of the lack of effective therapy for BK virus associated nephropathy (BKVAN), early diagnosis of BKV replication is the basis for the prevention of BKVAN. Present study analyzed the clinical usefulness of BK viremia monitoring by plasma real time PCR to prevent the development of BKVAN.

Methods: First, we compared plasma PCR with urine decoy cell and urine BKV real time PCR (urine PCR) for the diagnosis of BKVAN in comparison. Second, we prospectively monitored plasma PCR at 1, 3, 6, 9, 12 months after kidney transplantation (KT). We analyzed the kinetics of BKV replication and investigated the effectiveness and safety of preemptive immune suppressant (IS) reduction in BKV viremia.

Results: From Oct. 2006 to Oct. 2008, the prevalence of BKVAN was 3.0 % in the study population (6 / 200). The sensitivity and negative predictive value for BKVAN by decoy cell, urine and plasma BKV real time PCR was 100 % respectively. However, plasma BKV real time PCR was superior to urine real time PCR and urine decoy cell in specificity and positive predictive value. In the prospective monitoring of BKV real time PCR, BK viremia developed in 8.3 % (12 / 145) within 1 year after transplantation and the median interval from KT to the development of viremia was 163 days (29 – 685). After reduction of immune suppressant, viremia was successfully cleared out in 91.6 % (11 / 12) and it took 103 days (25 – 254). BKVAN developed in only one patient, who took intensive desensitization before KT because of high grade sensitization and ABO mismatch to donor. In comparison between patients with viremia and without viremia, allograft function and the frequency of acute rejection did not differ significantly up till post-transplant 1 year ($P > 0.05$, respectively).

Conclusions: Plasma PCR is reliable method for the diagnosis of presumptive BKVAN and regular monitoring of it is useful to prevent the development of BKVAN without the risk for acute rejection.

PUB466

Sirolimus-Induced Granulomatous Lung Disease in a Kidney Transplant Recipient Abdelaziz A. Elsanjak, Rishi Raj, Kamonpun Ussavarungsi, Vineeta Sood, Melvin E. Laski. *Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX.*

Background: Pulmonary complications of sirolimus immunosuppression include pneumonitis, bronchiolitis obliterans, organizing pneumonia, and alveolar hemorrhage. We here present a case of granulomatous lung disease, a rare side effect of sirolimus treatment.

Methods: Clinical case report and literature review

Results: The patient is 54 year old Hispanic female s/p DDKT for ESRD due to ADPKD. The patient presented with dyspnea, dry cough, fatigue and low grade fever

for two weeks. Her initial regimen included mycophenolate mofetil 750 mg BID, and tacrolimus 3 mg BID, but she had been switched to sirolimus 3 mg daily six weeks earlier because of post transplant DM. CXR showed bilateral infiltrates; WBC was normal. She was treated initially with ceftriaxone, azithromycin, bactrim, and diflucan, but five days later she nonetheless became hypoxic. Blood culture, sputum culture, and fungal and viral serology were negative. Spiral chest CT showed bilateral lower lobe ground glass infiltrates. Bronchoscopy with bronchoalveolar lavage and transbronchial biopsy revealed granulomatous lung disease. Tissue was sent for cytology, AFB, PCP, and fungal and viral culture. No infectious cause was found. Videoassisted thoracoscopy and wedge lung biopsy again demonstrated granulomatous lung disease. Tissue for cytology, AFB, CMV, and PCP again came back negative. Sirolimus induced granulomatous lung disease was suspected. Sirolimus was therefore discontinued and prednisone 60 mg po daily was started. The patient's symptoms subsequently rapidly resolved. A follow up spiral chest CT in two months showed complete resolution of lung infiltrates.

Conclusions: That sirolimus was the cause of the granulomatous lung disease in our patient is based on the occurrence of symptoms after patient was started on sirolimus, the exclusion of other causes, and the resolution of symptoms and infiltrates after discontinuation of sirolimus and treatment with steroids.

Pulmonary granulomas due to sirolimus use are rare but should be included in the differential diagnosis of transplant patients who develop respiratory symptoms and lung granulomas while on sirolimus.

Funding: Clinical Revenue Support

PUB467

De Novo Glomerulonephritis (GN) in Renal Transplants (Tx): Are We Spuriously Counting the Number of Cases of De Novo GN Post Transplant by Presuming Diabetic Nephropathy (DN) as the Cause of End Stage Renal Failure Pre Transplant?

Swarupa R. Eskapalli, Michael C. Chobanian. *Dept. of Medicine and Pediatrics, Dartmouth Medical School, Lebanon, NH.*

Background: De novo and recurrent GN are important causes of renal allograft failure that account for as many as 5-10% of allograft failures.Presumed DN as the cause for ESRD in patients with long standing uncontrolled diabetes mellitus is commonplace. Without a proper diagnosis,recurrent or de novo disease other than DN might either be under or over diagnosed in grafts.

Objective: To determine whether the incidence of presumed DN as the cause of ESRD without a native kidney biopsy prior to renal transplant inadvertently exaggerates or underestimates the diagnosis of de novo GN cases in renal allografts.

Methods: We retrospectively reviewed the charts of Tx patients undergoing 225 renal allograft biopsies performed over a 12 year period at our center from January 1997 to December 2009.The review included determining the biopsy diagnosis of the failing allograft,the etiology of ESRD based upon Form CMS-2728-U3 and whether a native renal biopsy was performed to confirm the cause of ESRD

Results: Among 225 post Tx biopsies reviewed, only 33.3%(n=75) had a native renal biopsy and 66.6%(n=150)had no prior renal biopsy.Of the 150 patients reviewed, 33.3%(n=50) had the diagnosis of presumed DN and were included in analysis.Only 6%(n=3)of those 50 patients had de novo GN diagnosed as the cause of allograft failure without features of DN, whereas 22%(n=11) had DN alone in their post biopsy specimens. The remaining 36 patients had other diagnoses including acute cellular(1%),or antibody mediated(0.6%) rejection, chronic transplant glomerulopathy(0.8%), acute TIN(12%), and CNI toxicity(14%)among others

Conclusions: De novo GN is an infrequent cause of renal allograft failure in patients presumed to have DN as the cause for ESRD.Recurrent DN is the most common form of recurrent disease in transplanted kidneys in our center.The diagnosis of DN as the presumed cause of ESRD does not significantly increase the number of de novo GN cases and thus would not alter the immunosuppressive approach for these transplant recipients because of the risk for recurrent GN.

PUB468

Incidence of Asymptomatic Elevated Uric Acid Levels and Gout after Kidney Transplantation in Inner City African American Patients

Candace D. Grant, Amarpali Brar, Mary C. Mallappallil, Moro O. Salifu. *Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: We determined the incidence of clinical gout 652 kidney transplant patients between 1997 to 2010 in a predominantly African American population in the inner city. We found of the 652 patients, serum uric acid levels in 253 patients. Of these 253, 208 had information about serum uric acid and clinical gout. Of the 208 patients, 128 (61.5%) had elevated serum uric acid levels and 80 (38.5%) had normal serum uric acid levels.

In the group with elevated serum uric acid levels (128 patients), 16.4% had gout (HUG) and 83.6% had no clinical gout (HUNG). The large number of patients with elevated serum uric acid without clinical gout (HUNG) could not be explained on the basis of immunosuppression. In the (HUNG) group there was no significant difference in uric acid levels in patients on cyclosporine or those on tacrolimus.

No correlation was found between elevated uric acid levels (HUNG) and the following :age, diabetics as the etiology of kidney failure, gender, race, mycophenolate use, **HLA**, Body Mass Index, serum creatinine levels six months and at one year, serum cholesterol at 6 months or delayed graft function.

The incidence of asymptomatic serum uric acid levels (HUNG) in the African American inner city population is high (16.4%) and the clinical significance of this has to be further studied.

HUG: High Uric acid with Gout

HUNG: High Uric acid No Gout

PUB469

Eculizumab in Acute Recurrence of Thrombotic Microangiopathy Associated with Anti-Phospholipid Antibodies after Renal Transplantation

Karine Hadaya, Pierre-Yves F. Martin. *Nephrology, University Hospital of Geneva, Switzerland.*

Background: Renal thrombotic microangiopathy (TMA) is a severe complication of systemic lupus erythematosus (SLE), associated with the presence of anti-phospholipid antibodies (aPL). In its most fulminant form, TMA leads to a rapid and irreversible end-stage renal failure. Eculizumab, an anti-C5 monoclonal antibody, is the therapy of choice for patients with paroxysmal nocturnal hemoglobinuria and a promising therapy to cure and prevent recurrences of atypical hemolytic uremic syndrome (aHUS).

Methods: We report the case of a 27-year-old woman, with past history of one abortion, which underwent a kidney biopsy for end-stage renal failure.

Results: Severe TMA, complete glomerular scarring and diffuse tubule-interstitial fibrosis were diagnosed. The presence of aPL antibodies (lupus anticoagulant, IgG anti cardiolipin and IgG anti B2 glycoprotein type I), anti-nuclear and anti-nucleosome antibodies at high titer and a reduce level of C3 level was compatible with the diagnosis of fulminant TMA in a SLE patient in presence of aPL. No evidence of genetic or biological abnormalities, similar to those described in aHUS, were detectable. After 10 months of dialysis, the patient underwent living related kidney transplantation. Immunosuppressive therapy was based on rabbit anti-thymocytes globulin induction, mycophenolate mofetil, methylprednisolone and rituximab. The graft produced urine immediately but as serum creatinine remained at 172µmol/L at day 6, a graft biopsy was performed. Isolated diffuse glomerular and arteriolar TMA, C4d negative, was detected. Despite daily plasma exchange, performed from day 7 to 10, the patient developed oligoanuria leading to the administration of weekly eculizumab perfusion under penicillin prophylaxis. Three months post transplant, serum creatinine is 100µmol/L without proteinuria, C3 level is within the normal range and aPL antibodies are undetectable. Graft biopsy revealed complete resolution of TMA without sequel.

Conclusions: This case report demonstrates for the first time the benefit of eculizumab therapy in a fulminant recurrence of TMA related to aPL antibodies and resistant to classical therapy after kidney transplantation.

PUB470

The Effect of Changes of Body Composition on Graft Function after Kidney Transplantation

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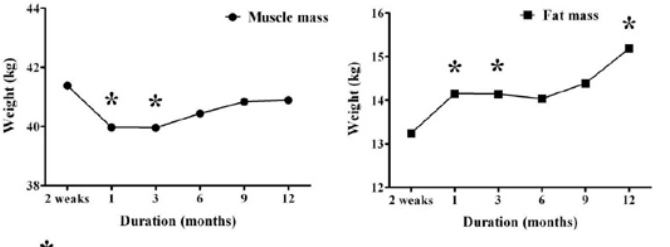
Background: Kidney transplantation and accompanying medical conditions including immunosuppressive agents may result in the changes of body composition. But the changes and its effects on graft function are not well delineated, especially in Asian recipients. In this study, we prospectively observed the changes of body composition in 50 consecutive kidney recipients and evaluated its effect on graft function.

Methods: A total of 50 Korean recipients (age, 47 ± 11.2 years; living donor, 60%; body mass index, 21.8 ± 2.64 kg/m^2) were enrolled as a prospective cohort. Body composition (muscle and fat mass) was assessed 2 weeks, 1, 3, 6, 9, and 12 months after kidney transplantation by the bioelectrical impedance analysis. Body composition in 2 weeks of transplantation was used as baseline for reducing the effect of excessive water after operation.

Results: All the patients had good graft function during the study period (last serum creatinine (SCr), 1.15 ± 0.28 mg/dL). The muscle mass decreased within 6 months (41.4 to 40.4 kg, $P = 0.056$), but was regained after 6 months. The fat mass continuously increased over time (13.2 to 15.2 kg, $P = 0.004$). The waist circumference had a similar trend to the muscle mass (81.7 to 78.9 cm in 6 months, $P = 0.043$; to 80.5 cm in 1 year, $P = 0.313$).



But BMI did not changed during the study period. High increase group (n = 25) in fat mass showed the trend of higher serum creatinine level than low increase group (n = 25); Δ SCr level (change in SCr between baseline and 1 year) was 0.15 mg/dL in high increase group and 0.01 mg/dL in low increase group, $P = 0.077$.

Conclusions: Body composition in the Asian transplant recipients changes over time and this change is associated with graft function.

PUB471

Proteinuria and the Risk of End Stage Renal Disease in Kidney Transplant Recipients Allyson Hart,[1] Liangxing Zou,[2] James Hodges,[2] Hassan N. Ibrahim.[1] *[1]Medicine - Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]Coordinating Center for Biometric Research - Biostatistics, University of Minnesota, Minneapolis, MN.*

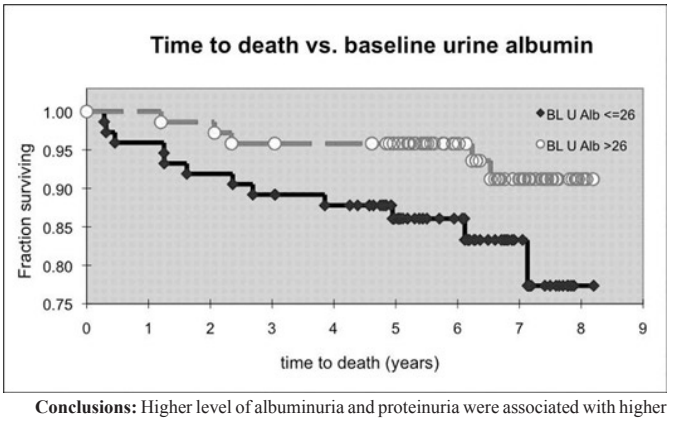
Background: Proteinuria is associated with increased risk of progression to end stage renal disease (ESRD), decreased graft survival, and death. We describe the association between proteinuria, graft survival and all-cause death in a prospective cohort of 153 kidney transplant recipients.

Methods: 153 kidney transplant recipients were followed prospectively with annual 24-hour urine protein and albumin measurements, with the first measurement occurring on average 58 days after transplantation. Analyses of time to death, ESRD, or the first of death or ESRD used Cox regression and Kaplan-Meier estimates.

Results: 19 (12%) of the 153 subjects died and 19 (12%) progressed to ESRD. Median baseline 24-hour urine protein and albumin were 0.34 g/g and 26 mg/g, respectively. Higher urine albumin was associated with higher risk of ESRD, but also with *lower* risk of death. Results were similar for proteinuria. The combined outcome of death or ESRD was not associated with baseline albuminuria or proteinuria. The results did not change notably when adjusted for age, sex, systolic blood pressure, donor source, or presence of acute rejection in the first 3 months after transplantation.

	Hazard Ratio*	(95%CI; p-value)
Outcome	BL Urine Albumin	BL Urine Protein
Death	0.42 (0.22-0.77; p=0.006)	0.38 (0.11-1.30; p=0.12)
ESRD	1.83 (0.91-3.70; p=0.09)	3.68 (1.10-12.3; p=0.034)
Death or ESRD	0.91 (0.55-1.48; p=0.69)	1.27 (0.50-3.20; p=0.62)

* For 10-fold increase in urine albumin or protein



Conclusions: Higher level of albuminuria and proteinuria were associated with higher risk of ESRD but lower risk of death in kidney transplant recipients. This result differs from what has been described in other populations, and needs to be confirmed.

Funding: NIDDK Support

PUB472

Terminal Complement Blockade by Eculizumab Effectively Reverses Recurrent Atypical Hemolytic Uremic Syndrome after Kidney Transplantation Nils Heyne,[1] Marcus Weitz,[2] Martina Guthoff,[1] Mark Dominik Alscher,[3] Hans-Ulrich Haering,[1] Silvio Nadalin.[4] *[1]Dept. of Diabetes and Endocrinology, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Germany; [2]Dept. of Pediatrics, University of Tuebingen, Germany; [3]Robert-Bosch Hospital, Stuttgart, Germany; [4]Dept. of General, Visceral and Transplantation Surgery, University of Tuebingen, Germany.*

Background: Recurrence of atypical hemolytic uremic syndrome (aHUS) is frequent after kidney transplantation, limiting transplant options for these patients. The reported incidence of 15 - 90% is largely dependent upon the underlying dysfunction of the complement system. Plasmapheresis is current standard therapy, yet of limited efficacy. The humanized C5b-antibody eculizumab is a novel therapeutic option, blocking terminal complement activation. We report eculizumab to effectively reverse recurrent aHUS in kidney transplantation.

Methods: A 43 year old patient with a history of postpartal aHUS presented for second kidney transplantation. Mutation of complement factor H had been ruled out. On day 7 after transplantation, the patient developed severe recurrent aHUS with systemic hemolysis, thrombopenia and acute kidney injury under calcineurin inhibitor-free immunosuppression. Complement C5b-9 (membrane attack complex, MAC) was highly detectable.

Results: Eculizumab was administeredand continued weekly, with subsequent prolongation of intervals. Hemolysis and thrombopenia ceased quickly and allograft function fully recovered. No unwanted side effects were observed. Under continuous monitoring of SC5b-9, dosing intervals of eculizumab were successively tapered to every other month, currently. 6 months after transplantation, allograft function is excellent with an eGFR of 41 ml/min/1.73 m² (serum creatinine 1.4 mg/dl) and C5b-9 within reference range.

Conclusions: Complement C5b blockade by eculizumab is highly effective in reversing recurrent aHUS in kidney transplantation. Pharmacodynamic monitoring of C5b-9 may guide adaptation of eculizumab dose and interval. As novel treatment option, eculizumab may facilitate access to transplantation for patients with aHUS.

PUB473

Acute Rejections (ARs) Associated with Subsequent or Concurrent Chronic Graft Dysfunction (CGD) in Kidney Transplants (Tx) Ajay K. Israni,[1,4] Robert Leduc,[2] David P. Schladt,[2] William S. Oetting,[3] Pamala A. Jacobson.[3] *[1]Medicine, HCMC, Univ. of MN, Minneapolis, MN; [2]Biostatistics, Univ. of MN, Minneapolis, MN; [3]School of Pharmacy, Univ. of MN, Minneapolis, MN; [4]DeKAF Investigators.*

Background: AR is a risk factor for CGD and allograft failure. However not all ARs lead to CGD. Therefore, we examined the severity of AR and response to AR treatments, for ARs that are association with CGD.

Methods: We enrolled 2,366 at the time of tx between 2006 - 2010. CGD was defined as ≥25% rise in serum creatinine (SCr) after 3 months post-tx, that resulted in a biopsy. The rise in SCr is defined relative to a baseline SCr that was initially set at 3 months post-tx and re-set after AR.

Results: CGD occurred in 366 recipients at 509 (± 387) days from the 3 month baseline. All CGD biopsies had chronic changes. AR was more likely among recipients with CGD than without CGD (p< 0.0001).

AR among recipients with versus without CGD

	Recipients with CGD (n=366)	Recipients without CGD (n=1,970)	p-value
No ARs	171 (46.7%)	1,744 (88.5%)	<0.0001
One AR only	136 (37.2%)	197 (10.0%)	
Two or more ARs	59 (16.1%)	29 (1.5%)	
At least one AR before 3 months post-tx	49 (13.4%)	151 (7.7%)	0.0072
At least one AR after 3 months post-tx*	160 (43.7%)	89 (4.5%)	<0.0001
Characteristics of First AR After 3 months post-tx*			
	Recipients with CGD (n=160)	Recipients without CGD (n=89)	p-value
Biopsy Findings:			
Local pathology C4d positive	60 (42.6%)	19 (33.3%)	0.010
Treatment:			
Steroids only	86 (53.8%)	67 (76.1%)	0.0023
Antibodies only	6 (3.8%)	0	
Steroids & Antibodies	30 (18.8%)	7 (8.0%)	
Steroids then Antibodies	35 (21.9%)	11 (12.5%)	
None	3 (1.2%)	3 (3.4%)	
Response to treatment:			
SCr (in mg/dl) before AR	3.0 ± 2.2	2.0 ± 1.8	0.0004
SCr returned to baseline	22 (15.3%)	27 (32.5%)	<0.0001
SCr partially improved	88 (61.1%)	19 (22.9%)	
No improvement in SCr	34 (23.6%)	37 (44.6%)	

* not including AR after CGD

Even after excluding the 141 (39%) of ARs that occurred concomitantly with CGD, AR was associated with CGD

Conclusions: AR events associated with CGD appear to be different than those without CGD: more often C4d positive, reoccurred frequently, less likely to respond despite more intensive therapy.

Funding: Other NIH Support - NIAID

PUB474

Prophylactic Ganciclovir for Gastrointestinal Cytomegalovirus Infection in Renal Transplant Recipients Hyun Chul Kim, Eun-Ah Hwang, Sung Bae Park, Seung Yeup Han. *Internal Medicine, Keimyung University School of Medicine, Daegu, Korea.*

Background: Cytomegalovirus(CMV) can cause morbidity in renal transplant recipients, and the gastrointestinal (GI) tract is a major target for CMV disease. Currently, there is no report concerning CMV prophylaxis to prevent GI CMV infection in CMV intermediate-risk patients(R(+) patients). The aim of this study was to evaluate the benefit of ganciclovir prophylaxis on GI CMV infection in R(+) patients.

Methods: In 41 patients who received renal transplantation after January 2009, intravenous ganciclovir(5mg/kg, twice daily) was started for 14days just after transplantation. The historical control group consisted of 45 patients received renal transplantation between January 2007 and December 2008. For evaluating effect of prophylaxis on GI CMV infection, routine endoscopic examination with mucosal biopsy was performed at the time of pretransplantation and then 1,3 and 6 months posttransplant.

Results: The average age of 86 studied patients was 43.7±10.6 (14-63) years and male to female ratio was 1:1.3. 43 (50%) patients received deceased donor transplantation and 84(97.7%) patients were seropositive for CMV IgG at the time of transplantation. The incidence of GI CMV infection was significantly lower in prophylaxis group than in historical control group (24.4% vs. 48.9%, p=0.026). The patient age, numbers of deceased donor and trough levels of tacrolimus at 1, 3months posttransplant were significantly lower in prophylaxis group than those of historical control group. In multivariate analysis for risk factors associated with GI CMV infection, ganciclovir prophylaxis was the only significant risk factor.

Conclusions: Prophylactic treatment with ganciclovir decreased the incidence GI CMV infection in seropositive renal transplant recipients.

PUB475

BKV Infection Stimulates Alloimmune Response in Kidney Transplant Patients Kosuke Masutani,[1] Parmjeet S. Randhawa.[1] *[1]Department of Pathology, University of Pittsburgh Medical Center, PA; [2]Department of Surgery, University of Pittsburgh Medical Center, PA.*

Background: Earlier stages of BK virus infection characterized by asymptomatic viruria are considered to be of no clinical significance.

Methods: To critically examine this widespread assumption, we identified 230 patients with sustained viruria (SVU) in whom multiple samples taken at a median 877 days (24-2739) after onset of viruria showed no progression to viremia or nephropathy.

Results: Compared to no viruria, SVU was associated with greater incidence of T-cell mediated acute rejection (30.9% versus 23.9%), increased number of rejection episodes (0.62 versus 0.33 per patient), and more frequent steroid resistance (36.2 versus 19.6% episodes). Most rejection episodes (52.1%) occurred concurrently with viral excretion, while a minority occurred prior to (7.8%) or after clearance of (40.1%) viruria. Lack of steroid response was seen more frequently in rejection occurring concurrently with viruria (48.6%), compared to rejection prior to onset of (9.1%) or subsequent to clearance of viruria (26.0%). The last serum creatinine, graft loss and death rates were not demonstrably affected.

Conclusions: These observations provide evidence that BKV infection stimulates the alloimmune response in kidney transplant recipients: its effect on development of tolerance deserves further investigation.

PUB476

Collapsing FSGS – A Significant Risk Factor for Allograft Failure Sumit Mohan, Leal C. Herlitz, Nicole M. Ali, Philip Imus, Russell J. Crew, Geoffrey K. Dube, Bekir Tanriover, Michael B. Stokes, Glen S. Markowitz, Vivette D. D'Agati, David J. Cohen, Ali G. Gharavi, Jai Radhakrishnan. *Columbia University.*

Background: Collapsing variant of FSGS (cFSGS) is an uncommon finding in the renal allograft that is associated with a high rate of graft loss (Stokes et al. AJKD 33:658-666, 1999). Transplant patients with cFSGS are a heterogeneous group underscoring the probability of diverse mechanisms leading to this pattern of allograft injury. Cases of cFGSG in allografts have been attributed to recurrent disease, ischemia, chronic allograft nephropathy and viral infections (including parvovirus B19). Literature on cFSGS in allografts is limited to case reports/small series.

Methods: We identified 17 patients (pts) with features of cFSGS on for-cause renal allograft biopsy (2 for DGF, 7 for rising creatinine, 3 for proteinuria, 5 for proteinuria and rising creatinine).

Results: Twelve pts were female(6 black, 6 Hispanic, 4 white) with a mean age of 41.8±9.8 yrs. There were 6 female and 11 male donors (7 white, 4 black, 5 Hispanic donors). Of the 17 pts who developed cFSGS in the allograft, 3 had FSGS as their native renal disease (including 1 pt with cFSGS). Four patients were seropositive for CMV; 2 pts developed CMV disease, 1 was seropositive for parvovirus B19 and 1 developed HIV post-transplant. Additionally, 4 pts had a history of lupus, 3 had prominent microvascular disease and chronic allograft nephropathy and 7 were treated with sirolimus prior to biopsy. Two pts (1 black, 1 white) received organs from the same donor and developed CMV infection after transplantation. Three pts had no identifiable risk factor for developing cFSGS. The diagnosis of cFSGS was made 3.6±4.6 yrs after transplantation with a creatinine of 3.7±1.8mg/dL (range 1.5-7.3 mg/dL) and protein/creatinine ratio of 4±3.5g/g (range 0.3-11.6g/g). Eight pts had nephrotic range proteinuria at the time of biopsy. Median allograft survival after diagnosis was 1.5 yrs (range 0.3–5.8) and appeared to be worse in those with nephrotic range proteinuria.

Conclusions: Collapsing FSGS in renal transplant is an uncommon cause of graft failure that has a poor prognosis when associated with heavy proteinuria.

PUB477

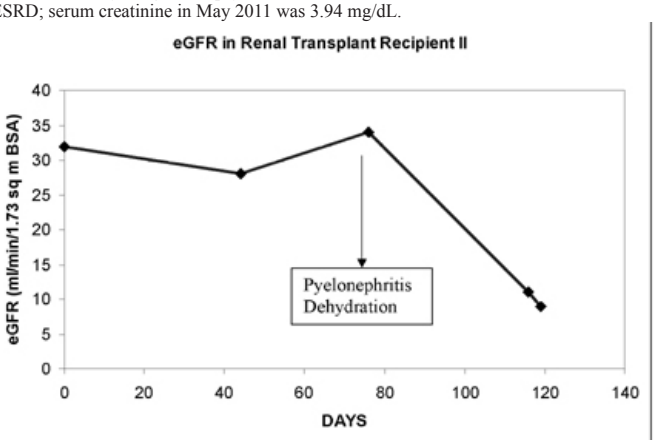
Syndrome of Rapid Onset End-Stage Renal Disease (SORO-ESRD) Described in Two Consecutive Renal Transplant Recipients over a Period of Six Months in a Mayo Clinic Renal Unit Macaulay A. Onuigbo.[1,2] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI.*

Background: We described a new syndrome of rapid onset end-stage renal disease (SORO-ESRD) in CKD patients in 2010. This is the unpredictable and unanticipated accelerated progression from a priori stable CKD to permanent ESRD requiring RRT, after AKI. Whether this syndrome similarly affects renal transplant recipients (RTR) is uncertain and unknown. Recently, over six months, two renal transplant recipients demonstrated SORO-ESRD.

Methods: Case Reports

Results: Patient I is a 56 year old Caucasian female diabetic patient who received a simultaneous pancreas/kidney (SPK) transplant in 1991 for ESRD. Transplant CKD was stable, serum creatinine was 1.8 mg/dL in June 2010. She suffered AKI from pneumonia in July 2010; serum creatinine reached 4.8 mg/dL, requiring RRT for uremic symptoms, oliguria and volume overload. She remains on HD for ESRD; serum creatinine in May 2011 was 3.68 mg/dL.

Patient II, is a Caucasian female type 1diabetic with an SPK transplant in 2000 for ESRD. CKD was stable; serum creatinine was 1.6 mg/dL in November 2010. She developed AKI from pyelonephritis with dehydration; serum creatinine quickly reached 5.16 mg/dL on January 6, 2011, requiring RRT for uremic symptoms, acidosis, oliguria and volume overload. Kidney allograft biopsy revealed acute tubular necrosis. She remains on HD for ESRD; serum creatinine in May 2011 was 3.94 mg/dL.



Conclusions: This is the first report of SORO-ESRD among renal transplant recipients. Multicenter studies are mandated to accurately identify the extent this syndrome contributes to renal allograft losses. More research into reno-prevention strategies in RTR is warranted. This may well call for critical process re-engineering of several current accepted paradigms and norms in transplant nephrology practice.

PUB478

Incidence, Risk Factors and Clinical Characteristics of Delayed Graft Function in Living Donor Renal Transplantation Hoon Suk Park, Yu Ah Hong, Sun Ryoung Choi, In O Sun, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: Delayed graft function(DGF) is the need for dialysis during the first week even after renal transplantation. DGF can be seen in deceased donor renal transplantation(DDRT) with relatively longer ischemic time. But DGF in living donor renal transplantation(LDRT) is rare, so its incidence and risk factors have not yet been established. We investigated the incidence, risk factors and clinical characteristics of DGF of LDRT for the last 10 years.

Methods: The 429 cases of LDRT for the last 10 years were analyzed. We compared recipent and donor's characteristics, HLA mismatch numbers, the frequency of non-related donors, gender matching status, total ischemic time and graft weight between DGF and non DGF group.We also reviewed the biopsy findings and clinical courses in the cases with DGF.

Results: The incidence of DGF in LDRT is 7/429(1.6%) and is significantly lower than the incidence of DGF in DDRT(13.7%) during the same period($p<0.05$). In univariate analysis, numbers in HLA mismatch were significantly increased in DGF group($4.43>2.88$, $P<0.05$), the frequencies of female recipients and non-related donors were also increased in DGF group($85.7\%>36.5\%$, $P<0.05$). The biopsy findings during DGF were available in six out of seven cases except the one in which exploratory laparotomy was done. Two cases were related to rejection, one case was related to acute pyelonephritis and the other three cases were related to ATN. The shortest duration of dialysis in DGF is just 1 day and the longest one is 97 days, whereas in DDRT, the shortest duration of dialysis in DGF is 1 day and the longest one is 55 days and most of them were within 7 days. The two cases related to rejection resulted in graft failure within three years after transplantation. But the other cases not related to rejection have been followed up with favorable graft function.

Conclusions: We conclude that the strenuous strategies including biopsy should be done when DGF developed in LDRT because DGF related to rejection in LDRT shows poor prognosis.

PUB479

Incidence, Risk Factors and Clinical Characteristics of Recurrent Focal Segmental Glomerular Sclerosis in Adult Renal Transplantation Hoon Suk Park, Yu Ah Hong, Hyun Gyung Kim, Sun Ryoung Choi, In O Sun, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: Recurrence of Focal segmental glomerular sclerosis(FSGS) is known as being relatively common compared with the recurrence of the other primary diseases in renal transplantation. Furthermore, it's recurrence tends to result in graft failure. We investigated the incidence, risk factors and clinical characteristics of recurrent FSGS in renal transplantation for the last 20 years.

Methods: The cases of which the primary renal diseases were FSGS in renal transplantation were 25 for the last 20 years. We investigated the subtypes of recurrent FSGS(early recurrence or late recurrence) and compared the possible factors that may cause recurrence between the group with recurrence and the other group without recurrence.

Results: The rate of recurrence in FSGS was 40%(10/25). The eight cases recurred early and the other two cases recurred late. There was no variable significantly different between the group with recurrence and the other group without recurrence. Five year graft survival rate(38%) in the group with recurrence was significantly lower than 80% in the other group without recurrence(p=0.01). Univariate Cox regression according to the subgroups showed the only early recurrence influenced the graft survival significantly(p=0.01, 1.91<β<53.49). There were 7 cases in 25 cases with FSGS, who underwent plasmapharesis before transplantation to prevent the recurrence of FSGS or rejection. FSGS recurred in 2 cases out of them. Eight out of 10 patients with the recurrence of FSGS were treated with plasmapharesis. Six cases resulted in graft failure and the one case with the amount of proteinuria less than 1g/day has been closely monitored and the other case with the amount of proteinuria more than 1g/day has been treated with the subsequent plasmapharesis.

Conclusions: We conclude the graft survival in FSGS with recurrence was poor, so the more intensive protocol of plasamapharesis should be done.

PUB480

Successful Use of Plasma Exchange To Prevent Recurrence of Type I Membranoproliferative Glomerulonephritis after Kidney Transplantation: A Case Report Lissa Pipeleers,[1] Jacques Sennesael,[1] Caroline Geers,[3] Robert Hilbrands,[1] Siddhartha Lieten,[1] Christian L. Tielemans,[1] Patrick Stordeur,[2] Karl M. Wissing.[1] *[1]Department.of Nephrology and Hypertension, Universitair Ziekenhuis Brussel, Brussels, Belgium; [2]Immunochemistry Laboratory - Immunobiology Clinic, Erasme Hospital, Brussels, Belgium; [3]Department of Pathology, Universitair Ziekenhuis Brussel, Brussels, Belgium.*

Background: Membranoproliferative glomerulonephritis (MPGN) with constitutional activation of the alternative complement pathway relapses in about 80% of patients with a history of disease recurrence on a previous graft. There is so far no efficient strategy to prevent recurrence.

Methods: Case report on the use of intermittent plasma exchange to prevent recurrence of MPGN in a second kidney transplant, after loss of a previous graft due to disease recurrence.

Results: The 26 year-old patient with end stage renal disease due to MPGN received a first renal transplant in 2007. She developed aggressive disease recurrence with strong mesangial C3 positivity, and electrondense deposits causing rapid graft failure. Activation of the alternative complement pathway (low C3, high C3d and normal C4) was persistent before, during and after transplantation, although the underlying pathogenic mechanism could not be documented by extensive evaluation. After a second kidney transplantation, in January 2011, standard immunosuppression was combined with plasma exchange (40mL/kg body weight per treatment) at decreasing frequency, with a maintenance schedule of 1 exchange every two weeks after the first month. The patient maintained good graft function (creatinine 1.3 mg/dL) without signs of complement activation, microscopic hematuria and proteinuria during the whole follow-up. A protocol biopsy 3 months after transplantation showed no signs of disease recurrence on immunofluorescence and electron microscopy.

Conclusions: Prophylactic plasma exchange might correct the underlying pathogenic mechanism in patients with idiopathic MPGN, thereby preventing complement activation and recurrence of disease after renal transplantation.

PUB481

Erythropoetin Requirements for Anemia in Renal Transplant Recipients Compared to Chronic Kidney Disease Patients Ashish V. Regulagadda, Shirley Shwu-Shiow Chang, Rocco C. Venuto. *Department of Nephrology, University at Buffalo, Buffalo, NY.*

Background: The risk of developing post transplant anemia (PTA) increases as renal function declines. Immunosuppression, infections, and inflammation purportedly increase the risk for PTA and may impair response to erythropoesis-stimulating agents (ESA).

Methods: We compared 39 renal transplant (RT) recipients (26 decreased and 13 living) with PTA to 41 anemic chronic kidney disease (CKD) patients receiving ESAs. All patients had hemoglobin (Hb) less than 11 gm/dl at time of initiation. Exclusion criteria were recent hospitalization and active bleeding. Data collected included age, gender, race, and medication use, specifically renin-angiotensin-aldosterone system (RAAS) blockers, immunosuppressants, prophylactic anti-viral and anti-bacterial agents.

Results: The mean Hb at ESA initiation was similar (9.08 ± 0.79 gm/dl in transplant vs. 9.5 ± 0.81 gm/dl in CKD, p=ns). Deceased RT recipients (Hb 8.95 ± 0.89 gm/dl) tend to be more anemic at baseline than living donor RT recipients (Hb 9.34 ± 0.47gm/dl)(p=0.09). The 30% of RT recipients who were not Caucasian (Hb 8.73 ± 0.75 gm/dl) were more anemic than their Caucasian counterparts (9.23 ± 0.77 gm/dl(p=0.015) despite similar eGFR (p=0.86). Transplant recipients appear to need larger doses of darbepoetin (954.7 ± 1424 mcg) than CKD patients (517.0 ± 502 mcg) (p=0.08) but did not take longer to reach the target 11 gm/dl (mean 2.66 vs. 2.57 months). This was true even though transplant recipients (31.4 ml/min) had eGFRs that were at least as high as their (p=0.84) CKD counterparts (33.4 ml/min). There was no difference in the rate of iron deficiency (defined as Tsat<20% and/or Ferritin <200ng/ml) before ESA initiation. Use of RAAS blockers was lower in RT *vs*. CKD patients (23% *vs. 39*%, p=0.01<XX< b>) respectively.

Conclusions: Renal transplant recipients developed anemia requiring ESA when their eGFRs were at least as high as race and gender matched CKD patients. Transplant recipients did not require significantly higher doses of ESA or take longer to reach target compared to CKD patients.

PUB482

Successful Treatment of Pseudallescheria Boydii Brain Abscesses in a Renal Transplant Recipient Dawinder S. Sohal, Melvin E. Laski, Vineeta Sood. *Internal Medicine/Nephrology, Texas Tech University Health Sceince Center, Lubbock, TX.*

Background: Psuedallescheria boydii (Scedosporium apoiospermum), is a ubiquitous, saprophytic fungus found in still waters. It usually causes skin infections. It can cause CNS infections with high mortality even if diagnosed and treated promptly and may be fatal in immunosuppressed patientsl. Here we present a case demonstrating successful treatment of Pseudallescheria boydii lung infection and brain abscesses in a renal transplant recipient.

Methods: Case report and literature review

Results: A 63 year old woman was hospitalized for altered mental status 6 weeks after kidney transplant. Her post transplant course had been marked by poor diabetes control but no episodes of rejection. Past medical history was positive for DM2, HTN, and Bell's palsy. Immunosuppression included tacrolimus, everolimus, and prednisone. She also took prophylactic TMP/SMX and valganciclovir. After admission, mental status rapidly worsened and respiratory failure developed requiring mechanical ventilation. Brain MRI revealed multiple hypodense ring enhancing lesions with surrounding edema. Chest CT demonstrated a right lower lobe infiltrate. Lab: WBC 7,400, Hct 34%, platelets 134,000, creatinine 0.4 mg/dl. Tacrolimus level was 15.6 ng/ml. Transbronchial biopsy and brain biopsy were both performed within 48 hours.

Brain biopsy revealed fungal abscess, and lung and brain biopsy cultures grew Psuedallescheria boydii. Three brain abscesses were each surgically drained and voriconazole was administered with resulting clinical improvement. The patient was eventually discharged to a rehabilitation facility without neurological impairment.

Conclusions: Pseudallescheria boydii is a neurotrophic pathogen which has been rarely reported to infect immunosuppressed individuals. It is sometimes confused with Aspergillus sp and Fusarium sp on pathology, which may lead to inappropriate treatment with amphotericin. As shown by this case, modification of immune suppression, aggressive drainage of abscesses, and voriconazole therapy may result in cure. Early, accurate diagnosis, aggressive surgical drainage, and specific treatment are necessary.

Funding: Clinical Revenue Support

PUB483

Clinical Characteristics of Renal Cell Carcinoma of Native Kidney in Renal Transplant Recipients In O Sun, Hoon Suk Park, Sun Ryoung Choi, Yu Ah Hong, Hyun Gyung Kim, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: The aim of this study is to investigate the incidence and clinical characteristics of renal cell carcinoma (RCC) in native kidney of renal transplant recipients

Methods: Between 1991 and 2010, 1425 patients underwent kidney transplantation at our institution. We retrospectively evaluated the clinical features and outcomes of five patients of RCC in the native kidney following transplantation.

Results: The patients included three men and two women, with a mean age of 63 years (range, 52-74). The incidence of RCC was 0.35%. The median interval between renal transplantation and occurrence of RCC was 16.2 years (range, 9 to 20). In our center, all patients with RCC had acquired renal cysts before (N=3) and after renal transplantation (N=2). The mean duration of dialysis was 12 months (range, 2 to 39), and most patients had duration of dialysis within 8 months except one. Four patients were asymptomatic and one patient presented a vague abdominal discomfort. And all patients received standard immunosuppressive treatment at the time of RCC diagnosis. Of the 5 patients, 4 received dual therapy with cyclosporine and prednisolone. And one patient received triple immunosuppression including cyclosporine, azathioprine and prednisolone. The mean tumor size was 3.2 cm (range, 0.5-5.1), and the stage of all RCCs was low grade at diagnosis. Two patients had clear cell carcinoma, another two papillary RCC and the last one multilocular cyst RCC. Radical nephrectomy was performed in all case except one who refused operation. Four patients who received radical nephrectomy showed no evidence of local recurrence or distant metastasis during median follow-up of 2.9 years. However, the patient who didn't have surgical treatment experienced the spinal metastasis of RCC six years later.

Conclusions: Follow-up period seems to be an important factor for development of RCC in renal transplant recipients.

PUB484

Delayed Graft Function Due to Early Onset Renal Transplant Artery Stenosis Si-Yen Tan,[1] David Cumberland,[1] Shahrin Merican,[1] Heinz Regele,[2] Mohan Rao,[3] Tee Chau Keng.[1] *[1]Nephrology, Prince Court Medical Centre, Kuala Lumpur, WP, Malaysia; [2]Department of Pathology, Medical University of Vienna, Austria; [3]Department of Surgery, Queen Elizabeth Hospital, Adelaide, Australia.*

Background: Transplant renal artery stenosis (TRAS) is a late transplant complication which characteristically presents with impaired graft function and hypertension. TRAS is however a rare cause of delayed graft function (DGF).

Methods: We report here 2 cases of DGF due to TRAS.

Results: The first patient had a living related renal transplant with donor renal artery anastomosed end to side into the internal iliac artery. This was complicated by DGF with

poor perfusion on imaging studies. Graft biopsy however confirmed healthy viable kidney and MRA 2 weeks post transplant suggested severe TRAS. Subsequent angioplasty and stenting of TRAS was accompanied by immediate diuresis and full recovery of graft function. The second patient had the donor renal artery anstomosed end to side to the external iliac artery. There was DGF and suspicion for injury to the initima during surgery was confirmed by CT angiogram which showed severe TRAS at the site of anastomosis. Graft biopsy confirmed healthy viable kidney with no evidence for ATN. Angioplasty and stenting of the TRAS was postponed till day 14 post surgery to minimize risk of anastomotic rupture during angioplasty. Angioplasty balloons could be fully inflated at low pressure suggesting a "soft" lesion which reappeared rapidly upon deflation and this was felt to be consistent with an intimal flap. Two stents were implanted in a "double barrel shot gun" fashion over the same lesion with one stent into the renal transplant artery whilst the second stent maintained perfusion downstream into the external iliac artery. This was accompanied by immediate diuresis within hours of stenting and subsequent full recovery of graft function.

Conclusions: Early TRAS post transplant must therefore be considered and excluded as a potential cause of DGF which is amenable to early intervention angiographically. The pathophysiology of early TRAS is likely to be different from the more common later onset TRAS and related to tissue injury during surgical anastomosis.

PUB485

A Case Series of Endothelial Tubuloreticular Inclusions in Transplant Kidney Biopsies Kawin Tangdhanakanond, Daranee Chewaproug, Eric J. Bloom, Rasib Raja. *Department of Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

Background: Tubuloreticular inclusions (TRIs) are unique subcellular structures that arise from the membranes of the rough endoplasmic reticulum of endothelial cells. TRIs have been associated with viral infections and autoimmune diseases, particularly human immunodeficiency virus (HIV) infection and lupus nephritis, as well as administration of exogenous interferon. However, it is not clear whether some other underlying illnesses or disease processes are associated with TRI expression in transplant kidney biopsies.

Methods: During the three-year period (2008 to 2011), TRIs were observed in five post-transplant kidney biopsies on ultrastructural examination. Donor and recipient records were reviewed for demographics, underlying diseases, and laboratory data including pertinent serologic tests as well as kidney biopsy findings.

Results: All donors and recipients were HIV-negative and had no clinical or serologic evidence of autoimmune diseases. Four of five recipients had hepatitis C virus (HCV) infection. Three out of these four recipients had never received any specific treatment for HCV infection; the other one received pegylated interferon 2 years prior to the kidney transplantation. All recipients were biopsied to evaluate for rejection. Borderline cellular rejection was found in two recipients. A biopsy from another recipient was focally positive for C4d (C4d 1). Biopsies from the other recipients showed no evidence of acute cellular rejection or antibody-mediated change. All recipients were on prednisone, tacrolimus, and mycophenolate as the maintenance immunosuppressive regimen.

Conclusions: Calcineurin inhibitor (CNI), has been demonstrated to increase alpha-interferon expression in patients with HCV infection. All recipients in our study were on CNI as a part of their immunosuppressive medication, and four of them had HCV infection. The TRIs seen in our biopsies may be a result of the HCV infection and/or the elevation of alpha-interferon secondary to CNI therapy; however, larger studies along with alpha- and beta-interferon measurement are necessary for further investigation.

PUB486

Pancreatic Anastomosis Leak 15 Years after Simultaneous Pancreas-Kidney Transplantion from Late-Onset Allograft Cytomegalovirus Duodenal Ulcers Presenting with Gross Hematuria Ekamol Tantisattamo,[1] Roland C.K. Ng,[1] Heath Chung,[1] Manami Okado.[2] *[1]Department of Medicine; [2]Surgery, University of Hawaii.*

Background: Cytomegalovirus (CMV) infection is one of the most important causes of mortality in solid organ transplantation. It is an important cause of hematuria, the most common urological complication in the early post-simultaneous pancreas-kidney (SPK) transplant period. Negative CMV viremia may not correlate with, and delay, diagnosis of tissue invasive disease. We report a case of a man with bladder-drained SPK transplant presenting with recurrent gross hematuria from CMV infected duodenal graft ulcers 15 years after preserved well-functioning grafts.

Results: A 70-year-old male with SPK transplantation in 1995 presented with recurrent gross hematuria for 1 month. Initial cystoscopy revealed bleeding from the duodenovesical orifice, but biopsy was not performed. He was admitted with septic shock and peritonitis from uncontrolled pancreatic ascites leaking from the duodenocystostomy suture line. Continued leakage of pancreatic fluid into the peritoneal cavity necessitated explant of the functioning pancreatic graft. Pathology revealed erosive ulcers at the duodenal graft segment. He also developed acute kidney injury from tacrolimus toxicity; transplant kidney biopsy showed no evidence of rejection. Serum quantitative PCR for CMV and BK virus were negative. The hospital course was complicated by disseminated candidiasis, pulmonary aspergillosis, and *Clostridium difficile* colitis. To reduce immunosuppression, tacrolimus was discontinued. Insulin therapy and intermittent hemodialysis were started. Despite our efforts, he expired due to uncontrolled sepsis. Postmortem duodenal graft staining for CMV was positive, and confirmed the cause of the inciting ulcers.

Conclusions: Early detection of CMV disease even in the late post-transplant period is important especially with recurrent hematuria from duodenal graft ulcers. Even though CMV antigenemia may be negative, tissue biopsy should be obtained with initial cystoscopy. Moreover, presumptive treatment for CMV disease in a SPK recipient presenting with recurrent hematuria should be considered in this at-risk population.

PUB487

Role of Immune Cell Function Assay in Predicting Risk of BK Viremia in Pediatric Renal Transplant Recipients Wee Song Yeo,[1] Yew Weng Perry Lau,[1] Kar Hui Ng,[1,2] Ching Ching Seah,[2] Yiong Huak Chan,[2] Hui Kim Yap.[1,2] *[1]Shaw-NKF Children's Kidney Center, University Children's Medical Institute, National University Health System, Singapore; [2]Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.*

Background: BK virus nephropathy is one of the significant causes of allograft dysfunction and graft loss in renal transplant recipients. Prompt diagnosis and early intervention in terms of immunosuppressant reduction is vital for optimal outcome. This study aims to determine the usefulness of the Cylex®ImmuKnow™ assay, an immune function assay based on measurement of intracellular adenosine triphosphate (ATP) levels, in predicting risk of BK viremia.

Methods: This is a prospective study involving 29 pediatric transplant recipients with 69 concomitant Cylex®ImmuKnow™ assay and plasma BK viral DNA quantification performed over the period from February 2010 to May 2011. BK viremia is defined as plasma viral titre >10,000 copies/ml. Statistical analysis was performed using Student's *t*-test to compare mean ATP values between BK viremic and non-BK viremic patients. Receiver-operating characteristic (ROC) curve analysis was used to determine the ATP threshold that would predict BK viremia.

Results: There were 5 episodes of BK viremia amongst these 29 patients. Mean ATP level was significantly lower during episodes of BK viremia as compared to episodes without BK viremia (213.8±51.6 ng/ml vs 463.2±197.4, respectively, $p<0.001$). Using ROC curve analysis, the area under the ROC curve was 0.93 (95% CI: 0.85 -1.00), and using a threshold ATP level <256.5 ng/ml gave a sensitivity of 80%, specificity of 87.5% for BK viremia. An ATP level <256.5 ng/ml also yielded a positive likelihood ratio of 6.4 (95% CI: 2.9-14.0) for BK viremia.

Conclusions: In conclusion, these results suggest over-immunosuppression as a contributing factor of BK viremia. Our results also suggest that adjustment of immunosuppression therapy towards a target ATP level >256.5 ng/ml may reduce the risk of developing BK viremia. Further prospective studies are, however, required to validate the above findings.

Funding: Private Foundation Support

PUB488

Screening of BK Viraemia and Nephropathy in Kidney Transplant Recipients Vivian W. Yiu, Rui Gao, Miriam Rose Berry, Yisu Yisu Gu, Afzal N. Chaudhry, Sharon Mulroy. *Nephrology, Addenbrookes Hospital, Cambridge, United Kingdom.*

Background: BK viraemia affects 15% of renal transplant recipient (8% develop nephropathy). A protocol to detect and treat BK viraemia was set up in our hospital.We aim to assess the effectiveness of the protocol on BK viraemia and graft outcome.

Methods: A database was set up from electronic and paper records on patients receiving a kidney transplant or simultaneous kidney-pancreas(SPK) graft from May 2009-Jan 2011.

Results: Of 263 transplant recipients,39(14.8%) developed BK viraemia. Baseline demographics of those with viraemia(Gp 1) and those without(Gp 2) were similar. Compliance with screening was 86%. Onset of viraemia was mostly between 5-20 wks. Risk factors for viraemia include Campath induction,a deceased donor, SPK graft, acute rejection(AR) and Mycophenolate(MMF) use. Urine testing was performed in 28% patients and biopsy in 40% patients with a high BK viral load(>10,000copies/ml). Immunosuppression reduction occurred in 50.0% of Gp1. The only additional treatment was Ciprofloxacin, used in 10.2%. 75.0% of Gp1 patients had more intense screening for 6 months after the resolution of viraemia. No patients died or lost their grafts as a result of BKV. Investigations beyond protocol requirements were performed in 19.0%.

Baseline characteristics

	BKV positive (gp 1)	BKV negative (gp 2)
Mean Age (yrs)	52.0	48.8
Gender (M:F)	30:9	143:81
Mean follow up (mths)	12.1	7.8
Kidney graft	31	188
SPK graft	8	34
Deceased donor	31	162
Live donor	8	62
0 AR	23	187
≥1 AR	16	37
Kidney recipients only:		
MMF immunosuppression	20	96
Azathioprine immunosuppression	11	92

Outcomes for Gp1

Mean onset of viraemia(wks)	14.4
Mean length of viraemia(wks)	14.1
BKV PCR>10,000copies/ml	20(51%)
Total biopsied	12(30.7%)
BK nephritis on biopsy	10(25.6%)
Decoy cells in urine	6(15.3%)
Median pre-viraemia creat (µmol/l)	140
Median Post viraemia creat (µmol/l)	144.5

Conclusions: Outcomes for patients with BK viraemia were good.The protocol was refined by reducing excess screening,starting intense screening for patients treated for AR and highlighting need for biopsy in those with high viral loads. Risk stratification can tailor screening for individual patients to improve efficiency and cost.

PUB489

Outcome of Renal Transplantation in Lupus Nephritis — Kuwait Expierience Narayanan Nampoory,[12345] Torkii Al-Otaibi,[12345] Praasad Nair,[12345] Nawas Nawas,[12345] Tarek Saeed,[12345] Medhat Haleem,[12345] Osama Gheith.[12345] *[1]Nephrology, OTC, Kuwait; [2]Nephrology, OTC, Kuwait; [3]Nephrology, OTC; [4]OTC, Kuwait; [5]OTC.*

Background: The major cause of morbidity and mortality in systemic lupus erythematosus (SLE) is Lupus Nephritis (LN). The outcome of renal transplantation in LN is a matter of concern especially with respect to the recurrence of the original disease in the allograft. The objective of this retrospective study is to determine the long term outcome of renal transplantation among lupus patients in Hamed Al-Essa Organ Transplant Centre of Kuwait

Results: Amongst 884 renal transplantations done in our centre between the year 1993 and 2008; 28 candidates (3%) were with LN. The clinical and laboratory data of these patients were reviewed for an average follow up period of six years and compared with age and sex matched controls (90 candidates).

Results: The recurrence of LN in the allograft was observed in 3 cases (10.7%). Serological flares of Lupus activity was noted in 2 patients (7%). The overall patient and graft survival rates on an average 6 years follow up was similar to the control group (100% and 89.3% Vs 100% and 90%). The graft rejection rates were found to be higher in the lupus group than the controls (53% Vs 33%,p<0.05) with the acute cellular rejection rates being significantly higher than the acute antibody mediated rejection (AMR) (35% Vs 24%,p<0.05) respectively. However, the Acute Antibody mediated rejection (AMR) rates were comparable (7.1% Vs 6.6).

Conclusions: The overall outcome of renal transplantation in LN is encouraging, especially with regard to the patient and graft survival rates as well as recurrence of the disease in the allograft.

PUB490

The Key to Successful Renal Transplantation in Patients with Sickle Cell Disease Sana R. Akbar,[1] Sadanand S. Palekar,[1] Ismael Ismael Shaukat,[2] Alice Joy Cohen.[2] *[1]Department of Renal and Pancreas Transplantation, Newark Beth Israel Medical Center, Newark, NJ; [2]Department of Hematology and Oncology, Newark Beth Israel Medical Center, Newark, NJ.*

Background: Renal failure in Sickle Cell Disease (SCD) occurs in 5-18% of patients and is associated with a poor prognosis. Survival of patients on hemodialysis(HD) has been shown to be equivalent to other nondiabetic ESRD patients.

Methods: We review a case in which an elderly patient with ESRD 2°to SCD underwent a successful renal transplant with no morbidity and mortality on follow up.

Results: A 69 year old African American male with SCD, homozygous for hemoglobin (Hb)S,HbF(1.2%) with ESRD on HD for 2 yrs underwent a living unrelated renal transplant. Prior to transplant he received routine HD followed by exchange transfusion with 6 Units of PRBCs to a preoperative hematocrit (HCT)of 30%.The Panel Reactive Antigen was 3% and it was a 2-2-2 mismatch transplant.Induction immunosuppression was with Daclizumab and Solumedrol.There was immediate graft function manifested by a good urine output.The maintenance immunosuppression consisted of Tacrolimus, prednisone and mycophenolate mofetil.He continued to receive simple transfusions post operatively to maintain HbS<30% then every 6 weeks for symptomatic anemia or Hb<6.5g/dl.Hydroxyurea was added to increase HbF levels and reduce transfusion requirement.The patient continues to do well one year post transplant with good allograft function.There have been no hospitalizations, episodes of vaso-occlusive crisis (VOC) or infections.

Conclusions: Sickle cell patients with ESRD rarely receive renal transplantation as a treatment modality.An increase in the frequency of VOC's has been demonstrated in patients who undergo renal transplantation.This appears to be due to increased blood viscosity stemming from endogenous hematopoiesis leading to VOC.To our knowledge,our patient is the oldest successful renal transplant recipient without evidence of VOC postoperatively. We recommend a protocol of pre-transplant exchange transfusion to a goal HCT of 30% with close follow-up of HbS along with concurrent use of Hydroxyurea to increase HbF levels thus reducing the frequency of crisis.

PUB491

Lower Dose of Mycophenolate Mofetil Is Enough for Rituximab Treated Renal Transplant Patients Chung Hee Baek,[1] Kyung Sun Park,[1] Duck Jong Han,[2] Jae Berm Park,[2] Tae Young Kim,[1] Su-Kil Park.[1] *[1]Internal Medicine, Asan Medical Center, Seoul, Republic of Korea; [2]Transplant Surgery, Asan Medical Center, Seoul, Republic of Korea.*

Background: Rituximab, anti-CD 20 antibody, enabled HLA-sensitized and ABO incompatible renal transplantations possible without splenectomy. As rituximab has a good suppressive effect on B lymphocytes, we would like to know the differences of the conventional immunosuppressive regimen without any harmful effect on graft in rituximab-treated patients compared to usual patients.

Methods: We investigated 69 patients who underwent rituximab treated (200 mg or 500 mg) living donor renal transplantation between January 2009 and March 2011 (group 1). The outcomes of seventy-two renal transplant recipients who did not require rituximab were compared as controls (group 2). All patients except 11 patients were treated with a combination of tacrolimus (FK506), mycophenolate mofetil (MMF) and methylprednisolone (mPD) after two doses of basiliximab for induction therapy in group 1.

Results: Graft survival was 98.3% in group 1 and 100% in group 2 (p=0.446). Renal function and incidence of infection including cytomegalovirus and BK virus were not significantly different between the two groups. Acute cellular rejection episodes occurred in 5.2% in group 1 and 9.7% in group 2 (p=0.511). Hyperacute rejection and antibody-mediated rejection episode was absent. The drug levels of FK506 and the doses of mPD after 1 year and 2 years after transplantation showed no difference between the two groups (p=0.237 and 0.625 at 1 year, p=1.000 and 0.667 at 2 years). The required dose of MMF (g/day, mean ± S.D.) was lower in rituximab-treated group post operative 1 month, 3 months, 6 months and 1 year (1.25 ± 0.45 g vs. 1.42 ± 0.39 g at 1 month; p=0.035, 1.15 ± 0.50 g vs. 1.38 ± 0.34 g at 3 months; p=0.006, 1.07 ± 0.51 g vs. 1.30 ± 0.42 g at 6 months; p=0.015, 0.92 ± 0.57 g vs. 1.22 ± 0.42 g at 1 year; p= 0.017).

Conclusions: These results suggest that lower dose of MMF is enough for successful immunosuppressive effect in rituximab-treated renal transplantation.

PUB492

Women Are Extremely Disproportionately Affected by High Panel Reactive Antibody Levels Prior to Kidney Transplantation Anthony J. Bleyer, Patricia L. Adams, Gregory B. Russell. *Nephrology, Wake Forest Medical School, Winston-Salem, NC.*

Background: High panel reactive antibody (PRA) levels are obstacles to kidney transplantation. The epidemiology of high PRA levels has not been well studied.

Methods: Data from the United Network of Organ Sharing on individuals placed on the transplant list for a first kidney transplant were analyzed from 1990 until 2010.

Results: 240,938 individuals between 18 and 60 yrs were placed on the waiting list. Women were 7.5 times(95% CI 6.99-7.94) more likely to have a high PRA (≥85%) than men. Of 7,721 individuals on the waiting list with high PRA levels , 6384 (82.3%) were women. Of 20,180 individuals with a medium PRA (15 to 85%), 13,253 (66%) were women. Table 1 shows the effect of pregnancy on PRA. Findings remained consistent after excluding individuals with sickle cell anemia, lupus, or transfusion history. In a multivariate logistic model with PRA (high vs. medium or low) as the outcome, odds ratios for variables associated with high PRA included gender (2.6 (95%CI 2.3-3.0)), 1 pregnancy vs. 0 (1.96 (1.7-2.3)), 2 pregnancies vs. 0 (2.9 (2.5-3.3)), 3 pregnancies vs. 0 (3.3 (2.9-3.8)), ≥4 pregnancies vs. 0 (3.8 (3.3-4.4)), sickle cell (3.0 (1.6-6.2)) lupus (2.1 (1.9-2.4)), African American race (1.2 (1.1-1.3)). Individuals with a high PRA were 1.8 times as likely not to receive a transplant (51.1% vs 28.5%, p<0.001) and 2.5 times more likely to be removed from the waiting list due to death or worsening health (33.1% vs. 13.6%, p<0.001).

Conclusions: Women are highly disproportionately affected with high PRA prior to kidney transplantation. High PRA individuals are much more likely not to receive a transplant or die while on the transplant list. Women at risk for end-stage kidney disease should be counseled about the effect of pregnancy on future transplant.

Percentage with medium or high PRA according to number of pregnancies

	Male	0	1	2	3	4 or more
All	6	12	18	22	26	29
Exclusive*	5	9	15	18	23	28

*Individuals with transfusion sickle cell anemia, lupus excluded

Funding: Clinical Revenue Support

PUB493

Early Versus Late Conversion to Sirolimus from Calcineurin Inhibitor Based Immunosuppression in Adult Kidney Transplant Recipients: A Single Center Retrospective Study Mariann D. Churchwell,[1] Rose Jung,[1] Shobha Ratnam.[2] *[1]Pharmacy Practice, University of Toledo,-College of Medicine, OH; [2]Medicine, University of Toledo-College of Medicine, OH.*

Background: To compare the efficacy and safety of conversion from calcineurin inhibitors in adult kidney transplant recipients to sirolimus as a component of their immunosuppression therapy.

Methods: We conducted a single center retrospective study of adult kidney transplant recipients who received initial immunosuppressive therapy with either cyclosporine (CYA) or tacrolimus (TAC) and were then switched to sirolimus (SIR) after 3-6 months (early conversion) or >6 months (late conversion) or remained on CYA or TAC. Patient

records were reviewed for patient demographics, medications, adverse reactions, graft rejection in addition to serum creatinine, urine protein and triglycerides for up to 5 years post transplant.

Results: We identified 220 adult kidney transplant recipients: CYA (n=73), TAC (n=70) and SIR (n=77) and mycophenolate as part of their immunosuppressive regimen. There was no difference in overall demographics between the three treatment groups. Patients receiving SIR were more likely to also receive HMG CoA Reductase Inhibitor (statin) compared to CYA and TAC patients (p=<0.0001). There was not a statistical difference in graft survival between patients converted from calcineurin inhibitors to SIR either early versus late (p= 0.83). The incidence of transplant rejection was not statistically different between the three groups (p=0.74) nor were the number of transplant failures between the groups (p=0.88). The adverse events included hypertriglyceridemia (p=0.12) and proteinuria (p=0.004) in the SIR converted patients as compared to those remaining on calcineurin inhibitors, but overall did not require a change in their immunosuppressive regimen.

Conclusions: From our single center experience we did not observe a statistical difference in the occurrence of rejection or graft failure in our adult kidney transplant recipients on a calcineurin inhibitor compared with those converted to sirolimus.

PUB494

Association of Marital Status with Access to Renal Transplantation Muhammad W. Khattak,[1] Gurprataap Singh Sandhu,[2] Bhanu K. Patibandla,[2] Akshita Narra,[2] Robert S. Woodward,[3] Alexander S. Goldfarb-Rumyantzev.[2] *[1]Hahnemann University Hospital Drexel University, Philadelphia, PA; [2]Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical Center, Boston, MA; [3]University of New hampshire.*

Background: Disparities in access to renal transplantation have been defined on the basis of age, race, gender and diabetic status. In this report we evaluated the association of marital status with access to renal transplantation.

Methods: We analyzed data from the USRDS. In patients with ESRD aged ≥27 (mean age of first marriage in the US), we analyzed the association of marital status with two outcomes: (1) likelihood of being placed on the waiting list for renal transplantation or first transplant, (2) likelihood of receiving kidney transplant in patients already listed. We analyzed marital status as a categorical variable: (1) not married (including never been married and widowed); (2) divorced or separated; and (3) currently married. Subgroups based on age, race, sex, donor type and diabetic status were also analyzed.

Results: After adjustments for the included independent variables and compared to individuals never married or widowed, those who were divorced/separated (HR 1.55, p < 0.001) and currently married (HR 1.54, p < 0.001) had a higher likelihood of being placed on the transplant waiting list. Once listed, married individuals had higher chances of getting transplanted as well (HR 1.28, p = 0.033). This trend was consistent in most of the subgroups studied.

Conclusions: We demonstrated that being married is associated with better access to renal transplantation compared to those who were never married/widowed.

PUB495

Hypotension Cured by Renal Transplant Sarath Kolluru, M.J. Barchman. *Nephrology and Hypertenstion, East Carolina University, Greenville, NC.*

Background: A 59 year old male with End Stage Renal Disease secondary to polycystic kidney disease who has been on hemodialysis for 5 years presented for deceased donor kidney transplant. Patient had bilateral nephrectomies four years prior due to uncontrolled bilateral flank pain caused by nephromegally. Before the bilateral nephrectomies, patient had a history of hypertension and took the medications of furosemide, diltiazem, and amlodipine.

Methods: At presentation for surgery in November 2006, his blood pressure was 158/92. Ever since the bilateral nephrectomies, patient has had very low blood pressures. The range for his blood pressure in clinic visits and hospital encounters during this time period range from systolic blood pressure of 80-97 and diastolic blood pressure of 52-65. Due to patient's hypotension, he has had multiple arterovenous graft complications including collapse and thrombosis requiring interventions on 5 separate occasions since his nephrectomies. Work up done by his primary nephrologist and ECU Cardiology determined that his hypotension was due to low renin levels. At the time of his renal transplant, patient's arteriovenous graft was nonfunctional and a tunneled catheter was serving as vascular access for hemodialysis.

Results: On presentation for kidney transplantation, his blood pressure was found to be 88/55. Patient claimed to be asymptomatic and denied complaints of lightheadedness, dizziness, weakness or blurry vision. There were no electrolyte abnormalities. The first postoperative day, patient's blood pressure was 107/59. On the sixth postoperative day it was 136/81. Six months after transplant, patient's blood pressure in clinic was 140/71.

Conclusions: Despite being dialysis dependant ESRD, kidneys continue to produce renin contributing to blood pressure. After removal of both kidneys, this patient developed hypotension from renin deficiency. His blood pressure was so low that long term vascular access was unsustainable. After receiving a kidney allograft, his blood pressure rose even prior to initiation of calcineurin inhibitors or any other agents known to cause elevated blood pressure. Normal blood pressure maintenance was reestablished after transplanting a new source of renin production.

PUB496

Affective Disorders among Patients on Different Renal Replacement Therapy Modalities: A Systematic Review Julio Lamprea,[1] Priscilla Auguste,[1] Patti Ephraim,[1] Deidra C. Crews,[1] Johanna Sheu,[1] Temitope Olufade,[1] Tanjala S. Purnell,[1] Raquel Greer,[1] Neil R. Powe,[2] Hamid Rabb,[1] L. Ebony Boulware.[1] *[1]Johns Hopkins Medical Institutions; [2]University of California, San Francisco.*

Background: The relation between patient's use of various renal replacement therapies (RRTs) and their development of affective disorders, has been poorly explored. We performed a systematic review of published studies to identify differences in rates of depressive and anxiety disorders among patients treated with different RRTs.

Methods: We searched Pubmed and performed a hand-bibliographic search to identify studies comparing rates of affective disorders among patients receiving different RRTs (hemodialysis-HD, peritoneal dialysis-PD and renal transplant-TX). Two reviewers abstracted outcome data and assessed article quality. We calculated standardized effect size estimates (cohen's d) of outcomes among patients on different RRTs. We considered effects with a p-value of less than 0.05 statistically significant.

Results: Among 91 studies identified as potentially relevant, 11 studies met the eligibility criteria. All studies had observational (cross-sectional) designs. Most studies reported outcomes based on validated questionnaires. However, no studies reported differences in outcomes while adjusting for potential confounders. A majority of studies (8 of 9) comparing patients on HD to patients on PD demonstrated no significant differences in rates of disorders, while most (5 of 7) studies comparing patients on HD to patients with a TX reported lower rates of disorders among patients on TX.

Table 1. Studies comparing clinical outcomes between RRT treatments

Outcome	Comparison	Number of studies	Treatment favored
Symptoms of anxiety	HD Vs PD	2	1 HD 1 Neither RRT
	HD Vs TX	2	2 TX
	PD Vs TX	1	1 Neither RRT
Symptoms of depression	HD Vs PD	5	5 Neither RRT
	HD Vs TX	4	3 TX 1 Neither RRT
	PD Vs TX	1	1 Neither RRT
Diagnosis of depression	HD Vs PD	2	2 Neither RRT
	HD Vs TX	1	1 Neither RRT

Conclusions: Cross-sectional studies suggest patients receiving transplants may have lower rates of affective disorders compared to patients on hemodialysis.However,the evidence is of variable quality, limiting definitive inferences. Prospective studies with well-balanced treatment groups are needed.

PUB497

Successful Renal Transplantation Despite Preexisting HLA-DQ or -DP Donor Specific Antibodies (DSA) Trung T. Luong,[1] Chantale Lacelle,[2] Bhavna A. Lavingia,[2] Meelie Debroy,[3] Juan Arenas,[3] Christopher Y. Lu.[1] *[1]Division of Nephrology; [2]Department of Pathology; [3]Division of Surgical Transplantation, all from UT Southwestern Medical Center, Dallas, TX.*

Background: The clinical significance of HLA-DQ and -DP DSA detected by single antigen Luminex beads remains unclear. The ability to transplant in the presence of preformed -DQ and -DP DSA may be advantageous for highly sensitized patients, however the risk of rejection versus the increased morbidity and mortality of remaining on the waitlist must be considered.

Methods: We followed 5 patients who received a kidney transplant in the presence of preformed HLA-DQ or -DP DSA from 07/2005 to 10/2010. We monitored their allograft function and DSA level using Luminex single antigen beads (One Lambda).

Results: The characteristics of the 5 kidney recipients transplanted in the presence of preformed HLA-DQ or -DP DSA and a positive B cell (pronase) flow cytometry crossmatch (FCXM) are shown in Table 1. No episodes of rejection were observed and all patients have had good allograft function.

TABLE 1: PATIENT DEMOGRAPHIC AND CHARACTERISTICS

Patient	1	2	3	4	5
Age [a]	54	55	52	48	53
Gender	F	M	F	M	F
Ethnicity	Black	White	Hispanic	White	Black
Cause of ESRD [b]	HTN	IgA	HTN	GN	HTN
Prior transplant	1	1	1	1	0
Prior transfusion	Yes	Yes	Yes	No	Yes
Pregnancy	1	_	3	_	4
T Cell FCXM dMESF (cutoff value) [c]	2105 (3227)	-689 (2250)	153 (2250)	38 (2169)	32 (2250)
B cell FCXM dMESF (cutoff value) [c]	38908 (3227)	7394 (2250)	20477 (2250)	2308 (2169)	2444 (2250)
DSA	DQ4	DP1	DQ5	DQ5	DQ5
DSA dMFI [d]	13199	6713	7713	12763	2887
DSA dMFI (PTD) [e]	13063 (278)	461 (339)	6247 (314)	389 (856)	445 (235)
Cr (PTD) [f]	1.2 (991)	1.35 (372)	1.15 (672)	1.17 (2095)	0.98 (235)

[a] Age at transplant [b] Cause of ESRD: HTN = hypertensive nephrosclerosis, IgA = IgA nephropathy, GN = proliferative glomerulonephritis [c] Immediately pre-transplant, dMESF = delta molecule of equivalent soluble fluorochrome [d] dMFI = delta median fluorescence intensity, pre-transplant [e] dMFI with PTD = post-transplant day [f] Cr = creatinine in mg/dL

Conclusions: Renal transplantation in the presence of preexisting HLA-DQ or -DP DSA may be considered as an option for highly sensitized patients.

Funding: Other NIH Support - UT Southwestern O'Brien Kidney Research Core (NIH P30DK079328)

NIH UT Southwestern Clinical Translational Service Award (NIH UL1-RR024982)

PUB498

Factors Predicting Graft and Patient Survival in Living Related Kidney Transplantation Pinaki Mukhopadhyay,[1] Manish Rathi,[2] Harbir Singh Kohli,[2] Vivekanand Jha,[2] Vinay Sakhuja.[2] *[1]Nephrology, NRS Medical College, Kolkata, West Bengal, India; [2]Nephrology, PostGraduate Institute of Medical Education and Research, Chandigarh, India.*

Background: Analysis of the factors affecting graft and patient survival in living donor kidney transplantation.

Methods: About 554 transplant recipient from Jan 2002 to Dec 2006 were studied retrospectively. Outcomes measures were overall patient and graft survival. Demographic, laboratory and perioperative variables were analyzed. Multivariate statistical analysis was undertaken using log-rank test and Cox's proportional hazards model.

Results: Of the 554 recipient 86.1% were male and 13.9% were female. The average recipient and donor age was 33.6 ± 10.3 and 42.36 ± 11.27 years. Recipient transplanted among related group76.4% and spouse 23.6% cases. Commonest primary diagnosis of ESRD was chronic glomerulonephritits(78.4%).Overall death censored graft survival at 1, 3 and 5 years were 94%, 90%, 79%. Mean time of graft loss was 33 ± 11.05 months (range 17-56 months). Commonest cause of graft loss was chronic allograft nephropathy (CAN) in 47.8 % . Significant factors predicting graft loss included episodes of rejection ($p < 0.001$), BKV infection ($p < 0.0001$), presence of CAN ($p= 0.06$). Overall patient survival at 1, 3, and 5 year was 92%, 87% and 83% respectively. Mean time to death was 18 ±19.05 months (range 0-76 months). Commonest cause of death was septicemia (66.7%). Significant factors predicting death were elderly recipient (≥ 50 year) ($p = 0.02$), unrelated transplant ($p =0.01$), presence of opportunistic infection ($p = 0.03$), CMV infection ($p < 0.0001$),disseminated fungal infection ($p = 0.002$), presence of multiple co-morbid illness ($p = 0.004$), post transplant hepatitis C infection ($p = 0.05$), pre-transplant diabetes ($p = 0.02$), Post transplant tuberculosis ($p = 0.03$).

Conclusions: Episodes of rejection, BKV infection, and presence of CAN were significant factors negatively impacting graft survival where as elderly age, opportunistic infection, CMV infection, disseminated fungal infection, diabetes, multiple co-morbid illness was independent prognostic factors of patient survival.

PUB499

Kidney Transplant Function after Early Conversion from Tacrolimus to Sirolimus in Mexican Patients Hildelisa Ordaz Solis,[1] M. Cecilia Fernandez-Luna,[1] Trinidad Orlando Lugo Lopez,[1] Benjamin Gomez-Navarro,[1] Alfonso M. Cueto-Manzano.[2] *[1]Nephrology and Organ Transplantation, IMSS, Hospital de Especialidades Centro Médico Nacional de Occidente, Guadalajara, Jalisco, Mexico; [2]Nephrology and Organ Transplantation, IMSS Hospital de Especialidades Centro Médico Naccional de Occidente, Guadalajara, Jalisco, Mali.*

Background: Currently, it is known that calcineurin inhibitor-free immunosuppression is safe after 6 months of kidney transplantation; however, there are few data about its use before that time. ***Aim:*** To evaluate the effect of early conversion from tacrolimus (TAC) to sirolimus (SIR) on graft function of kidney recipients.

Methods: Retrospective cohort of 72 Mexican patients with living-donor kidney transplant who were converted from TAC to SIR before 6 months after transplantation. All patients received prednisone and MMF. Clinical and biochemical variables were evaluated quarterly during 1 year of follow-up. Kidney function was evaluated as estimated glomerular filtration rate by means of the simplified MDRD formula. Urinary protein excretion was evaluated with 24-h collection.

Results: Sixty-one men and 11 women included with 3.7±1.1 months of transplantation at the time of conversion. Fifty-three percent had basiliximab induction. Before conversion, 87% had biopsy indicated by graft dysfunction (all biopsies documented TAC toxicity, additionally, 8% had acute rejection). After conversion, 62% had allograft biopsy showing acute rejection in 25% (80% of the latter diagnosed as borderline). Main results are shown in the table.

RESULTS

	PRECONVERSION	POSTCONVERSION	p
IMC	22.06 ± 3.48	21.93 ± 6.32	0.000
TAS	120 ± 12.7	122 ± 15	0.171
TAD	76 ± 9	75 ± 9	0.448
Leucocytes	7.77 ± 2.93	7.48 ± 2.09	0.277
Lymphocytes	1.99 ± 1.13	2.47 ± 1.27	0.040
Hb	12.8 ± 1.8	13.8 ± 1.8	0.000
Glucose	92.1 ± 17.7	84.2 ± 8.9	0.000
Urea	42.4 ± 19.9	35.5 ± 11.5	0.002
Creatinine	1.5 ± 0.4	1.3 ± 0.3	0.001
Cholesterol	177.5 ± 43.4	195.9 ± 34.5	0.033
Tryglicerides	178 (128 – 217)	173 (139 – 217)	0.606
MDRD	62.07 ± 18.09	71.52 ± 17.57	0.000
Proteinuria	240 (0 – 583)	500 (290 – 665)	0.007

Conclusions: Early conversion of TAC to SIR in patients with kidney transplantation is safe and improved kidney graft function.

PUB500

Expanded Criteria Donor Kidney Transplant Recipients: One Year Analyses from the Mycophenolic Acid Observational Renal Transplant Registry V. Ram Peddi,[1] Kimi Ueda Stevenson,[1] Kevin M. McCague,[2] Anne Wiland.[2] *[1]California Pacific Medical Center; [2]Novartis.*

Background: The Mycophenolic Acid Observational Renal Transplant (MORE) Registry, a prospective study of de novo renal transplant recipients (RTRs) receiving mycophenolic acid (MPA) therapy, is designed to determine effectiveness, tolerability and safety of enteric-coated mycophenolate sodium (EC-MPS) versus mycophenolate mofetil (MMF) regimens. The objective of this analysis was to compare 12-month outcomes in RTRs who received Expanded Criteria Donor (ECD) kidneys to RTRs who received non-ECD kidneys.

Methods: Based on local practices at 40 US sites, outcomes analyzed included: graft survival (GS), patient survival (PS), first biopsy-proven acute rejection (BPAR), adverse event (AE) rates, serum creatinine (SCr) and proportion of RTRs maintained on at least full recommended MPA dose (1.44/2.0 g/day, EC-MPS/MMF). A total of 102 ECD (73 EC-MPS/29 MMF) and 832 non-ECD (557 EC-MPS/275 MMF) RTRs were included.

Results: Donor (60.7 v. 39.0 yrs, $p=<0.01$) and recipient ages were higher (61.6 v. 50.1 yrs, $p=0.03$) in the ECD group. More African American RTRs received ECD kidneys (32.3 v. 23.6%, $p=0.07$). The majority of RTRs received induction therapy (99% ECD, 97% non-ECD), tacrolimus (97% ECD, 96% non-ECD) and steroids (69% ECD, 66% non-ECD) for maintenance therapy. At 1, 3, 6 and 12 months, more non-ECD RTRs received full MPA doses (non-ECD/ ECD: 78.2/ 71.0%, $p=0.12$; 67.4/ 53.6%, $p=0.01$; 51.9/ 42.3%, $p=0.14$; 45.7/41.0%, $p=0.50$). Comparable 12-month effectiveness, tolerability and safety outcomes were achieved in both groups whether they received EC-MPS or MMF. Comparing 12-month outcomes in ECD to non-ECD RTRs regardless of MPA type, BPAR (10.0/9.1%, $p=0.91$), GS (94.5/97.5%, $p=0.08$) and PS (98.8/98.9%) were similar whereas mean SCr (1.75/1.46 mg/dL, $p=<0.01$) was higher in the ECD RTRs. There were no differences in reported AEs (infections including BK and CMV, diabetes, gastrointestinal, neoplasms, hematological) between the groups.

Conclusions: Graft survival and BPAR were similar between RTRs who received ECD and non-ECD kidneys in the short-term. However, as expected, RTRs who received ECD kidneys exhibited higher mean SCrs.

Funding: Pharmaceutical Company Support

PUB501

Impact of Substance Abuse on Access to Renal Transplantation Gurpratap Singh Sandhu,[1] Muhammad W. Khattak,[2] Bhanu K. Patibandla,[1] Akshita Narra,[1] Martha Pavlakis,[1] Noelle C. Dimitri,[3] Alexander S. Goldfarb-Rumyantzev.[1] *[1]Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [2]Hahneman Hospital, Drexel University, Philadelphia, PA; [3]Transplant Institute, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: With an ever-increasing demand for kidneys and limited supply pool, it is essential to understand the balance between utility and equity in transplant access. The goal of this project was to evaluate the association between recipient's substance abuse and renal transplant access in patients with end-stage renal disease (ESRD).

Methods: We used data from the United States Renal Data System. The primary variables of interest were abuse of alcohol, tobacco, or illicit drugs based on information from Centers for Medicare & Medicaid Services form 2728. We analyzed three outcomes in Cox model: (1)being placed on the waiting list for renal transplantation or transplanted (whichever occurred first); (2)first transplant in patients who were placed on the waiting list; and (3) graft loss or mortality after transplant. In addition, we performed subgroup analysis based on age, race, sex, diabetic status, and donor type.

Results: We analyzed 1,077,699 patients (age of ESRD onset 62.9±15.5 years, 54.1% males, 64.2% white, and 29.7% African American). When compared with those with no substance abuse, abusing all three substances was associated with reduced transplant

access (hazard ratio 0.39, P<0.001 for wait listing/transplant; hazard ratio 0.67, P=0.019 for transplant). This trend was similar in most subgroups studied.

Conclusions: We demonstrated that patients with ESRD abusing or dependent on tobacco, alcohol, or illicit drugs are less likely to be placed on the waiting list for kidney transplant; and once on the list are less likely to be transplanted. The possible utility justifications for such disparity and potential interventions are discussed.

PUB502

Renal Transplantation from HBs Antigen Positive Donor to Antigen Negative Recipient Immunized with Intradermal Hepatitis B Vaccine: First American Experience Gurmukteshwar Singh, Andrea C. Hsia, Daniel Skiest, Michael J. Germain, Gregory Lee Braden. *Baystate Medical Center/ Tufts University School of Medicine, Springfield, MA.*

Background: The US renal transplant waiting list has 88,972 patients & grows by 4200 every year. Donor pool expansion is imperative to meet the demand-supply mismatch. Inclusion of HBs antigen positive (HBsAg+) donors has been recommended in endemic countries. We present the first American case of a successful HBsAg+ renal transplant into a newly immunized recipient.

Methods: May 2000: A 58 year-old man with mesangiocapillary glomerulonephritis needed pre-emptive renal transplant. His wife was a living unrelated donor match. She had chronic hepatitis B, was HBsAg+ with positive anti-HBc antibody. She had normal liver function & was HBe antigen negative & had undetectable Hepatitis B DNA (HBV DNA).

The recipient (negative for all hepatitis antigens & antibodies) did not mount an antibody response to 3 doses of monthly intramuscular recombinant hepatitis B vaccine (40 mcg). Intra-dermal vaccine was administered (5 mcg) every 2 weeks for 6 months. By the 4th dose, anti-HBs antibody titers reached immune levels (>10 mIU/mL). After the intradermal course, his antibody titer was 160 mIU/mL. This tapered to <10 mIU/mL at 14 months. An intramuscular vaccine booster (40mcg) resulted in an anamnestic response with titer >150 mIU/mL in a month. The patient underwent a successful living-unrelated donor transplant from his wife in August 2002 utilizing prednisone, mycophenolate mofetil & tacrolimus.

Results: Over 9 years, the patient had no evidence of hepatitis B infection. Anti-HBs antibodies slowly declined to 5.8 mIU/mL in May 2011. He has continued to be HBsAg negative with undetectable HBV DNA. Allograft function has been well preserved with a 24 hour creatinine clearance of 78.4 mL/min in December 2010. Immunosuppression was switched to prednisone+ sirolimus 3 years post-transplant.

Conclusions: Our successful long-term experience, using intradermal Hepatitis B vaccination with booster doses led to sustained anti-HBs antibody response allowing transplantation from a HBsAg+ donor. Larger studies are warranted given this strategy's potential to expand the US donor pool.

PUB503

Transient Elastography To Assess Hepatic Fibrosis in Renal Transplant Recipients Claudia Sommerer,[1] Christoph Seitz,[1] Michael Scharf,[1] Gunda Millonig,[2] Martin G. Zeier,[1] Sebastian Mueller.[2] *[1]Nephrology, University Hospital Heidelberg, Heidelberg, Germany; [2]Internal Medicine, Salem Medical Center, Heidelberg, Germany.*

Background: Transient elastography (TE) is a nonivasive, reliable and valide tool for the assessment of hepatic fibrosis. There are some limitations in certain patient groups, for example in patients with ascites or increased central venous pressure. Until now, TE has not been evaluated in renal allograft recipients. We evaluated TE in renal allograft recipients, a patient cohort who often shows hypervolemia and high central venous pressure especially in the early posttransplant period.

Methods: In 109 renal transplant patients liver stiffness measurement (LSM) was performed by transient elastography (TE). The severity of hepatic fibrosis was staged by Forns-Index.

Results: In 91/109 patients (n=29 women, age 47±8 years, 68±59 months post transplantation, BMI 25.5±3.7 kg/m²) it was possible to assess hepatic fibrosis by TE. The mean stiffness was 7.2±3.5 kPa with a mean interquartile range (IQR) of 0.9 kPa. TE and Forns-Indices correlated significantly (p=0.002) with an area under the ROC curve of 0.722. The sensitivity and specificity for detection of fibrosis was 34% and 92% with a cut-off of 8 kPa. Failure of LSM occurred in 61% of the patients with BMI > 28 kg/m². Liver stiffness was significantly higher in the early post-transplant period (p<0.05) indicating significant influence of hypervolemia on the assessment of liver stiffness by TE.

Conclusions: TE might be a rapid, non-invasive tool to assess and identify renal allograft recipients with liver fibrosis. However, several confounders including obesity and hypervolemia leading to increased central venous pressure have to be considered.

PUB504

Management of Transplanted Patients Stage 3B and 4 in France. ANTICIPE Study Paul Stroumza, Malik Touam, Eric Daugas, Georges J. Mourad, Patrick Henri, Bertrand Dussol. *Scientific Council, Amgen, France.*

Background: Describe the follow-up of renal transplanted patients in stage 3b and 4 in France

Methods: During one week of consultations for grafted patients, in a national, prospective and observational study conducted among nephrologists , we recruited 1497 patients. After exclusion of incomplete records or improperly included, we studied 1446 patients. 546 patients with renal insufficiency were on stages 3b and 4 (GFR Cockcroft-Gault).

Results: Age 52,8 + / - 13,8, 60% men, 40% women, weight 70,9kg + / - 14,4, height 167,9 cm + / -9,6, BMI <25kg/cm ²: 53,8% between 25 and 30: 33.6%, Blood pressure 138/78.9 + / -18 and + / -11.3. Initial nephropathy was: Glomerular 1st 26,8%, glomerular 2nd 5,4%, diabetic7,4% , tubulointerstitial 12,4%, vascular 10,1%, polycystic 14,4%, other 11,3% and 12,4% undetermined. Time between transplantation and the study was 1 year to 14.7%, from 2 to 3 years for 24.5%, 4 to 5 years 16.7% ,higher than 10 years to 21.9%. Before the graft: 85.7% were hypertensive, 13.7% diabetics and 69.8% never smokers. In the history, we found 13,4% of ischemic heart disease, 7% heart failure, 3.3% for stroke. 92.1% of patients were on dialysis (HD 85.5%) before transplantation. The average time between first dialysis and transplantation was 3.9 years. The graft is 94,5% of deceased donors, living 4,4%, 1,1% cœur arrested. Only 0.9% of bi-transplantation. HLA mismatch was: 0: 2,6%, 1: 8,9%, 2: 8,9%, 3: 23,9%, 4: 25,8%, 5: 66%, > 5: 6,7%. Cold ischemia was <6 hours: 4,4%,> 24 hours: 21,3%. 23.4% of patients were dialyzed after transplantation. 24.1% had a rejection and 1.9% had more than 2. Among the complications related to transplantation, we have: 33,9% of hospitalized patients over 7 days for infections. The treatment is 65.9% corticosteroids, 87.5%, calcineurin inhibitors 10.8% azathioprine, 73.4%, mycophenolate mofetil, 9,7%, inhibitor of mTOR: another immunosuppressive 1,3 %. Other treatments are: 80% antihypertensive non RAS blockers and 59,5%, blockers, 29,9% ESA, 59.7% statins, 44.1% vitamin D.

Conclusions: Nephrological monitoring of 147 investigators was used to develop this photograph of the transplanted patients stage 3b and 4 .

Funding: Pharmaceutical Company Support

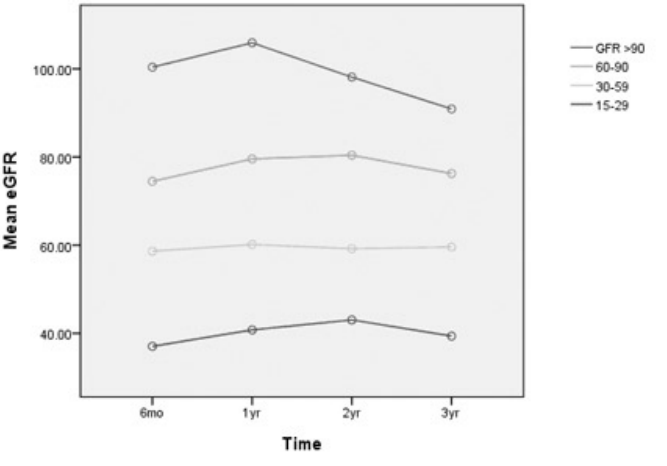
PUB505

Three-Year Graft Function Is Preserved in the Absence of Rejection or Delayed Graft Function Fasika M. Tedla, Amarpali Brar, Angella Brown, Thin Maw, Subodh J. Saggi, Moro O. Salifu. *Renal Diseases Division, Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: It is generally accepted that hyperfiltration results in progressive renal damage in native kidneys. Although graft function generally deteriorates over time, the natural course of progression in renal transplant recipients (RTRs) at different levels of renal function has not been studied longitudinally in patients free of acute rejection (AR) or delayed graft function (DGF). We conducted a single-center study of graft function over a three-year period in patients that did not have AR or DGF.

Methods: 275 patients were classified into stages of chronic kidney disease (CKD) based on serum creatinine at six weeks after transplant. Glomerular filtration rate (GFR) was estimated using the abbreviated MDRD formula at 6 weeks (6wk), 6 months (6m), and 1, 2, and 3 years (1yr, 2yr, 3yr). Temporal change in mean eGFR across and within CKD stages was analyzed using repeated-measures ANOVA. CKD stages were compared for other variables by ANOVA, t-test or χ^2 as appropriate.

Results: 123 of 275 RTRs had complete data at 3yr. The mean eGFR of stages 1-4 CKD at 6 weeks were 111, 74, 46 and 21 ml/min. The groups were similar in mean age, hemoglobin, albumin, blood pressure at baseline (6wks) and tacrolimus levels at all time points. There was no significant difference in number of total or HLA DR mismatch, or proportion of African-Americans, retransplants, diabetics, or positive flow-cytometry crossmatches. Female RTRs had higher eGFR than male RTRs at all time points (mean difference 17-21 ml/min, p<0.001). There was no significant change in mean eGFR in each CKD stage over 3 years (p=0.34)



Conclusions: In the absence of AR or DGR, graft function remained stable over three years irrespective of baseline eGFR.

PUB506

Treatment Strategy for BK Nephropathy in Renal Transplant Recipients: Five Years Follow Up Ying Wang,[1] Mona Razavian,[2] Kate Wyburn,[1] Josette M. Eris,[1] Steven J. Chadban.[1] *[1]Renal Medicine, Royal Prince Alfred Hospital, NSW, Australia; [2]Renal Medicine, Concord Repatriation General Hospital, NSW, Australia.*

Background: BK nephropathy (BKN) is a significant cause of graft loss. There is no single defined treatment algorithm established and usual management consists of a substantial reduction in immunosuppression.

Methods: We retrospectively investigated renal transplant recipients with biopsy proven BKN in our centre from 2003 to 2011. They were followed up over 1-8 years after treatment with a protocol of reduction in immunosuppression and intravenous cidofovir, with or without ciprofloxacin or leflunomide.

Results: 7 cases of BKN were diagnosed with incidence of 1 %.

Demographic characteristics of the patients with BKN

Age&gender	Months post-Tx	Additinal BKN Therapy	Outcome
60M	9	Cipro	Recovery
40M	17	Cipro	Graft loss
81M	16	Cipro	Graft loss
35M	7	No	Recovery
62M	32	LEF&IVIG	Graft loss
53M	2	LEF	Recovery
65M	3	Cipro	Recovery

All were male and 6 had received treatment for acute rejection prior to the diagnosis. The median time to diagnosis was 10 months post transplantation. A reduction in immunosuppression and commencement of cidofovir as an initial treatment strategy were applied to all cases. In addition, 4 patients received ciprofloxacin 250 mg daily concurrently. 2 out of 7 patients were switched from mycophenolate to leflunomide after failure to respond to the initial therapy. 1 patient received intravenous immunoglobulin. The median follow-up was 5.1 years after the treatment. Of 7 patients, 4 showed sustained clinical and virological improvement while 3 incurred graft loss, one due to ongoing BK nephropathy. Two graft losses occurred after a reduction in viral load and initial improvement in graft function: one due to transplant artery thrombosis and one due to rejection following non-adherence to immunosuppression. An overall BK nephropathy related graft loss was 14.3%.

Conclusions: BK nephropathy in renal transplant setting remains a serious complication. Reducing immunosuppression and administration of cidofovir produced sustained remissions for the majority of our cases, however, the optimal therapeutic strategy remains unclear and warrants prospective randomized studies.

PUB507

A Positive Flow Cross Match in HIV Patients May Not Necessitate Desensitization Sana R. Akbar,[1] Sadanand S. Palekar,[1] Prakash N. Rao,[2] Cecil L. Rhodes.[2] *[1]Department of Renal and Pancreas Transplantation, Newark Beth Israel Medical Center, Newark, NJ; [2]Laboratory Operations and Business Development, NJ Tissue and Organ Sharing Network, New Providence, NJ.*

Background: Renal transplantation in HIV positive patients is becoming common place. Advances in the patho-physiology and treatment of HIV have made it possible for these individuals with end stage renal disease, to undergo successful renal transplantation. Solid phase antibody testing, and donor specific cross-matching by complement dependent cytotoxicity (CDC) and flow cytometry (FCXM) enable the assignment of risk, and determination of the necessity for desensitization therapy with intravenous immunoglobulin or plasmapheresis.

Methods: We would like to report on a series of three HIV positive recipients being evaluated for possible living donor renal transplants.

Results: All three patients had a PRA of < 20%. Solid phase antibody testing revealed the presence of a few HLA antibodies, none of which were donor specific. Upon testing all recipients demonstrated positive T and B cell flow cross-matches with their donors. Positive Flow cytometric auto cross-match testing indicated the presence of auto-antibodies. These results would indicate that the initial donor specific positive flow cytometry cross-matches were caused by auto-antibodies.

Conclusions: Therefore, though the preliminary FCXM made these 3 patients appear to be incompatible with their donors, the autocross match revealed otherwise. Our study would suggest that the presence of positive donor specific flow cross-match in HIV positive recipients should not automatically impute a high risk situation requiring desensitization protocols. These patients can indeed be successfully transplanted without the need for desensitization.

PUB508

Effective Therapy for Resistant Acute Antibody-Mediated Rejection: Case Report and Review of Literature Narayanan Nampoory,[12345] Torkii Al-Otaibi,[12345] Osama Gheith,[12345] Medhat Haleem,[12345] Praasad Nair.[12345] *[1]OTC , Kuwait; [2]OTC , Kuwait; [3].*

Background: In an effort to reduce the long-term toxicities of immunosuppressant drugs, corticosteroid and calcineurin inhibitor (CNI) sparing immunosuppression protocols have become increasingly popular in managing kidney transplant recipients. The most vexing clinical condition caused by antibodies in organ transplants is antibody-mediated rejection.Limitations of the current AMR therapies include (1) AMR reversal tends to be gradual than prompt, (2) expense, (3) rejection reversal rates below 80%,(4) common appearance of chronic rejection after AMR treatment, and (5) long-term persistence of donor specific antibodies after therapy. Since these limitations may be due to the lack of effects on mature plasma cells, bortezomib's effects on mature plasma cells may represent a quantum advance in anti-humoral therapy.

The initial experience described herein represented the first clinical use of bortezomib as an anti-humoral agent in renal allograft recipients in Kuwait. We aimed to present 2 cases with resistant acute antibody mediated rejection to the standard therapies, and were managed successfully with bortezomib therapy.

Methods: Therefore, we were confronted by two cases of resistant acute antibody mediated rejection with mild chronic changes. The available therapies for antibody mediated rejection lack direct effects on the major antibody producing cell (the mature plasma cell).

Results: Although B lymphocyte specificity has been provided with rituximab, yet it does not deplete plasmablasts or mature plasma cells, which represent the major source of antibody production [(4)]. Each case was managed by one cycle of bortezomib with partial response and satisfactory 1-year graft survival in spite of sustained anti-HLA antibodies.

Conclusions: Immunosuppression minimization carried risk of resistant acute antibody mediated rejection and renal grafts can be rescued by earlier bortezomib use especially in the absence of chronic changes.

AUTHOR INDEX

The number refers to the location of the body of the abstract in the publication section.

SUBJECT INDEX

The number refers to the location of the body of the abstract in the publication section.